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Ciclo XXXVI

Giuseppe Costantino

Unravelling neuroinflammatory pathways by using in vitro and in vivo models

PhD Thesis

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Preface

My work during my PhD course in “Neuroscience” at the University of Foggia is divided in two different parts. During the first period I carried out an *in vitro* study, pointing my attention on the analysis of the role of metabotropic glutamate receptors (mGlu) belonging to class II, mGlu2 and mGlu3 receptors, on the function of the blood brain barrier (BBB) under inflammatory conditions. To this purpose, we used cell populations that are components of the BBB of human origin. More specifically, we focused on the role of microglia in the modulation of BBB function. This part of the research was carried out at the University of Catania, in the Department of Biomedical and Biotechnological Sciences, laboratory of Pharmacology under the supervision of Prof. MA Sortino and SF Spampinato. The results obtained will be the object of the first part of this thesis.

During the second part of my PhD I moved to *in vivo* studies, being involved in a project in which we analyzed the complex mechanisms underlying ASD behavioural dysfunctions, by using the BTBR strain, an idiopathic model of ASD. In this context, we firstly investigated neurochemical and biomolecular alterations underlying such behavioural impairments and, subsequntely effects in brain regions crucially involved in ASD, and after we evaluated the effect of a possible preventive treatment, on the core ASD symptoms and on the modulation of neuroinflammatory components of ASD by using β-carotene administration during pregnancy.

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1. *In Vitro* studies

1.1 Introduction

1.1.1 Blood Brain Barrier (BBB)

The blood-brain barrier (BBB) is a highly specialized structure with the main role of preserving the central nervous system (CNS). The BBB is composed of specialized endothelial cells that, together with other components of the neurovascular unit (NVU), astrocytes, pericytes, neurons and basement membrane, preserve the correct environment in the CNS, limiting the entry of potentially damaging blood-borne agents, metabolites, drugs and immune cells (Zenaro et al., 2017).

Changes in the BBB have been documented in different CNS pathological states. These include brain tumors (Wolburg et al., 2012), brain trauma (Chodobski et al., 2011), ischemia (Gursoy-Ozdemir et al., 2012) and epilepsy (Fabene et al., 2013). Furthermore, various conditions related to neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (Bartels et al., 2008) and amyotrophic lateral sclerosis (ALS) (Garbuzova-Davis et al., 2011) are characterized by structural and functional changes of the BBB.

The endothelial cells are the primary cellular component of the BBB forming a continuous endothelium barrier. They are organized as a continuous intercellular layer forming tight junctions at cell-cell contacts that lack fenestration. For these reasons molecules and solutes movements through the BBB are selective (Garcia et al., 2014; Garg et al., 2008). The entry of water-soluble solutes, such as glucose or amino acids, is guaranteed by the presence of specific transporters that regulate their access, while their expulsion from the CNS is warranted by the presence of efflux pumps. Many BBB transporters (such as those for glucose) are facilitated diffusion systems, which allow molecules following their concentration gradient. Others, however, prevent the entry of exogenous substances into the CNS; among these, one of the most relevant is the P glycoprotein. Changing the activity of these transporters may be a possible approach in some pathologies.

The BBB is in close contact with some components of the immune system and reduces the entry of peripheral cellular components into the CNS. Endothelial cells in physiological conditions express a low level of adhesion molecules for leukocytes, which reduces the access of immune cells into the CNS, while preserving a certain level of immuno-surveillance. This is guaranteed by the few immune cells that pass the barrier but remain in the perivascular space, separated from the CNS by the presence of glia (Beurel et al., 2014). In pathological conditions, the expression of adhesion molecules increases and the connections between endothelial cells decrease, facilitating the entry of immune cells into the CNS. It is therefore clear that a completely efficient barrier is essential to preserve the CNS.

1.1.2 Endothelial cells: Tight Junctions and Adherens Junctions

Endothelial cells interact among each other through two important types of junctions: the tight junctions (TJs) and the adherens junctions (AJs), that regulate the paracellular permeability. TJs and AJs are involved in maintaining junctional integrity, limiting the permeability only to small lipophilic molecules and gases. The TJs constitute the "diffusion barrier" between adjacent endothelial cells and therefore have the task of regulating the flow of ions and mainly hydrophilic molecules through the paracellular space. In physiological conditions, molecules that have a molecular weight (MW) greater than 180 kDa cannot pass through TJs (Mitic & Anderson, 1998). If observed with the electron microscope, the TJs appear as a set of linear, long and parallel fibrils, which surround the cell (Pinto da Silva & Kachar, 1982). The assembly of these fibrils, important for the adequate functioning of TJs, is a very dynamic phenomenon, and seems to increase when endothelial cells are subjected to harmful stimuli (Gumbiner, 1993). Preservation of TJs is regulated by specific transmembrane proteins and among these, claudins, occludin and junctional adhesion molecules (JAMs) are particularly important. They are associated with cytoplasmic proteins (ZO-1, ZO-2 and ZO-3) connecting the TJs to the actin of the cytoskeleton and to the AJs. Alteration of specific phosphorylation of amino acid residues and their

translocation from the cell membrane lead to a loss of junctional integrity and a consequent increase in paracellular permeability. Furthermore, functional modifications of these proteins are observed in case of alteration of specific phosphorylation of amino acid residues (such as serine, threonine and tyrosine) (Sandoval & Witt, 2008). One of the most important AJs that contributes to the impermeability of the BBB is represented by the protein VE-cadherin.

Occludin (MW: 60 kDa) is present at the level of TJs in its phosphorylated form (Sakakibara A. et al., 1997). It binds to zonula occludens proteins (ZO-1, ZO-2 and ZO-3) and therefore interacts with the actin cytoskeleton (Fanning et al., 1998; Furuse et al., 1994; Haskins et al., 1998). This event has been shown to be important for maintaining the functionality and properties of the barrier (Balda et al., 1996). Accordingly, the presence of occludin at the membrane level is correlated with an increase in electrical resistance and a reduction in paracellular permeability (Balda et al., 1996).

Claudins (MW 20 to 27 kDa) are a family of membrane proteins that are among the major structural components of TJs. In the cerebral endothelium, the most represented are claudin-3 and claudin-5 (Morita et al., 1999; Wolburg et al., 2012), that are recognized to give an important contribution in the formation of TJs and in maintaining the integrity of the barrier. Claudins support the efficiency of TJs thanks to their ability to homodimerize and heterodimerize the two cis- and trans-forms (Piehl et al., 2010; Piontek et al., 2008; J. Zhang et al., 2010). Claudin-5 plays an important role in the organization of the barrier, which derives from its ability to associate with other claudins of neighboring endothelial cells (Greene et al., 2019).

The ZO-1 (zonula occludens-1) protein is a protein of the MAGUK (membrane-associated guanylate kinase) family together with ZO-2 and ZO-3. In TJs it acts as a scaffold for the organization of transmembrane proteins, thus connecting them to the actin cytoskeleton. It is also able to recruit various signal transduction pathways (González-Mariscal et al., 2000). ZOs share three main regions among

themselves: an SH3 domain, which is responsible for binding to signaling proteins and cytoskeletal elements, a guanylate kinase, which has the task of catalyzing the ATP-dependent transformation of GMP (guanosine-mono-phosphate) in GDP (guanosine-di-phosphate), and finally a PDZ domain, which binds the carboxy-terminal regions of transmembrane proteins, controlling their correct distribution in space (Zlokovic, 2008b). The localization and interactions of these proteins are essential for the assembly of claudin, occludin and JAMs filaments in TJs and for the anchoring of the entire complex to the actin cytoskeleton (Fanning et al., 2007; Utepbergenov et al., 2006). ZO-1 is a phosphoprotein and its phosphorylation state influences its localization and interactions with other TJs.

The adherence junctions have an important role in the immunosurveillance. The quote of leukocyte infiltration depends on the expression of AJs, in particular VE-cadherin, and adhesion molecules, such as ICAM-1, VCAM-1 and P- and E-selectins. In physiological conditions, ECs at the BBB express very low levels of these leukocyte adhesion molecules (Daneman, Zhou, Agalliu, et al., 2010; Wettschureck et al., 2019). Their expression is regulated at transcriptional levels, and can be induced by soluble inflammatory mediators, such as cytokines and chemokines (Wettschureck et al., 2019). When highly expressed, adhesion molecules and AJs may be more easily engaged by their leukocyte counterpart, leading to a signaling cascade that terminates with the transendothelial migration and leukocyte access into the CNS.

Endothelial cells are supported by astrocytes and pericytes that, releasing trophic factors, induce specific BBB properties (Hill et al., 2014). The components of the NVU are assisted by two extracellular matrix structures, recognized as basement membranes (BMs) (named also “basal lamina”). The inner vascular BM surrounds the endothelial cells, separating them from pericytes, while the outer parenchymal BM is interposed between the pericytes and the astrocytes endfeet (Balabanov & Dore-Duffy, 1998; del Zoppo et al., 2006; Sorokin, 2010). The BMs play an important role in the maintenance of the close connection between the elements of NVU (Yurchenco & Patton, 2009), constitute a source of growth

factors and adhesion receptors and contribute to the regulation of the BBB permeability (Daneman & Prat, 2015; Zlokovic, 2008a).

1.1.3 Pericytes

Pericytes are contractile cells that are responsible for the production of the extracellular matrix and proteins that contribute to the regulation of BBB homeostasis (Bohannon et al., 2021; Gautam et al., 2020). They contribute to the stability of the BBB through the release of trophic factors that help maintain vascular stability and regulate the diameter of the capillaries, thus also indirectly controlling blood flow (Winkler et al., 2011). The interaction between pericytes and endothelial cells contributes to the formation of TJs and counteracts transcytosis and the expression of cell adhesion molecules. It has been recently reported that the claudin-5, the glucose transporter Glut-1 and the efflux proteins (P-glycoprotein) were still expressed in animals increased BBB leakage, increased ICAM-1 expression and focal leukocyte extravasation (Mäe et al., 2021). Accordingly, others reported that pericytes control TJs alignment and transcytosis across the BBB (Bell et al., 2010; Daneman, Zhou, Agalliu, et al., 2010; Quaegebeur et al., 2010). Pericytes seem to be also involved in the regulation of the rapid control of neurovascular coupling. Thank to their contractile activity, they can actively relax or contract to modify the cerebral blood flow (CBF) in response to neuronal activity [29,30]. Pericytes are essential in BBB development and in the maintenance of brain microcirculation, as they directly contribute to the formation of the BM (Bergers & Song, 2005) and secrete angiogenic-promoting factors (such as VEGF, angiopoietin-1, TGF-β, PDGF-BB) that stabilize BBB functions (Ribatti et al., 2011). A lack of pericytes leads to hyperplasia, abnormal vascular morphogenesis (Hellström et al., 2001) and an incomplete function of the BBB during embryogenesis, as they inhibit the expression of proteins that increase vascular permeability, such as Angiopoietin 2 and the plasmalemma-vesicle-associated protein (Plvap), and leukocyte adhesion molecules on ECs (Daneman, Zhou, Kebede, et al., 2010). There are important interactions between pericytes and astrocytes, the absence of pericytes

influences astrocyte polarization, so affecting astrocytic endfeet/endothelial interaction (Geranmayeh et al., 2019). On their side, astrocytes facilitate pericyte migration, proliferation and contact with the endothelial layer (Nakagawa et al., 2009; Y. Yao et al., 2014).

1.1.4 Astrocytes

Astrocytes have an important role in supporting neuronal activity, regulate the balance of extracellular K+, and control the recovery and recycling of neurotransmitters. They support the activity of the BBB, surrounding the endothelial cells with their peduncles and releasing trophic factors (Capaldo & Nusrat, 2009; Garg et al., 2008). Astrocyte interaction with endothelium is important for the functions of the BBB. While pericytes are more important in the early stages of BBB property induction, astrocytes are more involved in the maturation and maintenance of barrier properties (Obermeier et al., 2016; Spampinato et al., 2019). Some studies have tried to explore how the depletion of astrocytes may affect BBB properties in animal models. The laser ablation of a single astrocyte may be not sufficient to induce BBB damage (Kubotera et al., 2019), while a complete astrocyte ablation in adult mice induced an early and sustained BBB dysfunction, consequent to alterations in TJs functions (Heithoff et al., 2021). Astrocytes regulate many functions of the barrier, modulate TJs, induce the expression and location of transporters such as P-glycoprotein and GLUT1 glucose transporter and influence the expression of specific enzymes (Cheslow & Alvarez, 2016). The barrier functions are modulated by factors released by astrocytes, such as angiopoietin 1, fibroblast growth factor (FGF), and neurotrophic factor derived from glial cells (GDNF), useful for promoting vascular stabilization (Broux et al., 2015). Astrocytes are highly secretory cells, and their secretome include many molecules (Dowell et al., 2009; Harada et al., 2015). For example, the morphogen sonic hedgehog (Shh) and retinoic acid strengthen endothelial junctional tightness (S. Liu et al., 2019; Mizee et al., 2013). Astrocytes are the main productor of Angiotensinogen (Stornetta et al., 1988), that is a central player in the regulation of blood pressure, although its role in the

reinforcing or weaking of BBB integrity is controversial: it can both reduce (Wosik et al., 2007) and increase barrier permeability (Takane et al., 2017). Astrocytes produce also the main vascular trophic factor, VEGF-A, that promotes angiogenesis and endothelial cell proliferation, differentiation, and survival during brain development (Esser et al., 1998). On the other hand, high levels of VEGF-A may induce BBB disruption in pathological conditions(Spampinato et al., 2019). In general, factors produced by astrocytes may both induce and disrupt barrier properties, depending on the disease context, age, and surrounding microenvironment. Astrocytes are in direct physical contact with endothelial cells; astrocytic terminal processes, called endfeet, cover the external surface of the endothelium and surround the vasculature (Abbott, 2002). Endfeet are thought to cover up to 99% of the cerebrovascular surface (Mathiisen et al., 2010), participate in the regulation of angiogenesis and the formation of cell-to-cell junctions (Cabezas et al., 2014). The importance of astrocyte support in BBB function varies during different pathological conditions of the CNS. In fact, when glial cells are involved, astrocytes can lose their functions or modify their secreting activity to produce inflammatory cytokines. All these events contribute to a change in the interaction between glia and neurons which leads to a modification of the functionality of the endothelial barrier. Finally, the interactions between endothelial and immune cells can also modify the activity of astrocytes, for example reactive astrocytes in inflammatory conditions can form their own junctions to limit leukocyte infiltration into the CNS, supplying the BBB barrier when endothelial functions are compromised (Horng et al., 2017).

1.1.5 Microglia and Neuroinflammation

Neuroinflammation is an important event that influences the severity and progression of neurodegenerative and psychiatric disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), stroke, and others (Lyman et al., 2014). Numerous cytokines such as interleukin-23 (IL-23), IL-12 (Bennett, 2013), IL-1β, and IL-6 are involved in neurodegenerative processes. Several studies in the past years have

demonstrated that different cell types such as oligodendrocytes, microglia, astrocytes (Domingues et al., 2016), both local and circulating lymphocytes, different dendritic cell subsets (Colton, 2013), and endothelial cells (Combes et al., 2012) are involved in neuroinflammation.

More recent evidence suggests a role for microglia in the control of BBB function. Microglia cells, the resident immune cells in the CNS, pathological mediators or traumatic events may be quickly activated in response to CNS insult. The nature of microglial responses can be different: they can remove cellular debris in an attempt to contribute to cellular repair or can accelerate the induction of the neuroinflammatory response (G. I. Caruso et al., 2021; Merlo et al., 2022; Merlo, Luaces, et al., 2020).

Microglia cells were described for the first time by the neuroanatomist Pio Del Rio-Hortega in 1919, who also noticed the mesodermal origin and phagocytic capability of these cells (Sierra et al., 2016). In the last few years, various studies in mice confirmed that microglia are mesodermal-derived cells, originating from erythromyeloid progenitors of the yolk-sac, which begin to colonize the developing CNS before the formation of the cerebrovascular network, from embryonic day 8.5 until the complete development of the BBB (Ginhoux et al., 2010; Gomez Perdiguero et al., 2015). In a similar way, microglial precursors in humans infiltrate the CNS primordium starting at around 4.5 gestational weeks (Menassa & Gomez-Nicola, 2018). The differentiation of progenitors in microglia is induced by two transcription factors, Pu.1 and Irf8, and their expression distinguishes microglia from other tissue-resident macrophages (Hagemeyer et al., 2016; Kierdorf et al., 2013). In the adult brain, microglia maintenance depends on the constant activation on their surface of Colony-stimulating Factor-1 Receptor (CSF-1R) by interleukin (IL)-34 and CSF-1, which are continuously released by neurons and astrocytes, respectively. Consequent to the pharmacological blockade of CSF-1R there is a rapid microglia depletion (Chitu et al., 2016; Elmore et al., 2014). Microglia are long-living and self-renewing cells that, in inflammatory conditions, can proliferate quickly though a selected clonal

expansion (Tay et al., 2017). When there is a BBB damage in CNS diseases, circulating monocytes can infiltrate the CNS and acquire a microglial-like phenotype (Ginhoux et al., 2013). In physiological condition, in brain and spinal cord, microglial cells take on a ramified morphology with long and motile processes that continuously scan the surrounding environment (Nimmerjahn et al., 2005). Beyond immune surveillance, microglia are involved in several and different functions in the CNS: microglia activity is in fact essential for a correct synaptic pruning during development (Paolicelli et al., 2011), synaptic plasticity (George et al., 2016; Schafer et al., 2013; Sipe et al., 2016), neuronal programmed cell death and neurogenesis in adults (Bachstetter et al., 2011; Sierra et al., 2010) as well as learning and memory processes (Parkhurst et al., 2013).

After an environmental negative stimulus or injury, microglia acquire an ameboid morphology, with much shorter ramifications and larger cell bodies (Morrison et al., 2017). In several pathological conditions, microglia clear the CNS through phagocytosis of protein aggregates, e.g., amyloid-β (Hu et al., 2023; Mandrekar et al., 2009), pathogens (Cockram et al., 2019), immune cells that have crossed the BBB and damaged or stressed-but-viable neurons in a process named "phagoptosis" (G. C. Brown & Neher, 2014). Activated microglial cells are capable to release a multitude of pro-inflammatory cytokines (IL-1α and IL-1β, Tumor Necrosis Factor (TNF)-α), chemokines (CCL2, CCL5, CXCL1, MIP-1), proteases (e.g., matrix metalloproteases, MMPs) and reactive oxygen species (ROS), which, while participating to the immunological response to injury, can also compromise BBB integrity if their secretion is protracted for a long time. Similarly, microglial cells are also able to secrete molecules that promote tissue healing and reduce inflammation (e.g., VEGF, TGFβ-1, IL-10) (L.-R. Liu et al., 2020). Consequently, over the years, it has become increasingly clear that the behavior of microglia shows a high phenotypic plasticity and that microglia can modify the activity over time during disease. Starting from the early 2000s, microglial functional status has been classified as pro- or anti-inflammatory, named M1 and M2, respectively

(Paolicelli et al., 2022). This classification derives from *in vitro* studies on T-helper cells that, after the administration of the appropriate stimuli, acquire a pro- (Th1) or anti- (Th2) inflammatory activity (Paolicelli et al., 2022). In a similar way, microglia have been divided into these two categories, based on the expression of specific markers that indirectly implied a detrimental (M1) or protective (M2) phenotype (Paolicelli et al., 2022). Nowadays, the use of single-cell-transcriptomic and proteomic techniques demonstrated that the M1/M2 definition is not completely suitable for microglia, whose dynamic behavior is strongly dependent on the pathological and environmental conditions (Paolicelli et al., 2022).

1.1.6 Microglia role in BBB

As highlighted previously, the influence of microglial cells on physiological BBB functions has been only recently explored. During embryogenesis, microglial cells seem to be associated with the developing blood vessels in the retina and during neurogenesis in the cortex and contribute to vascular development (Penna et al., 2021). The absence of microglia in PU.1-/-PU mice or pharmacological depletion with the CSF-1R inhibitor PLX5622 cause a choroidal vascular atrophy, the disorganization and dysfunction of retinal pigment epithelial cells and an altered expression of angiogenic growth factors (Dixon et al., 2021; Fantin et al., 2010). Confirming this, Csf1op/op mice, which produce a mutated inactive form of CSF-1, show an early postnatal defective retinal vasculature, with altered arterial–venous patterning and reduced ramification (Y. Kubota et al., 2009).

Microglia as part of the NVU interact with endothelial cells of the BBB, and although do not seem to play an essential role in BBB formation and stability, they modulate BBB functions both in physiological and pathological conditions (da Fonseca et al., 2014). In the adult CNS, microglia establish transient and dynamic physical contact with the neuro-vasculature; accordingly, it has been recently described a specific microglial population, called capillary-associated microglia (CAM), that interacts with microvessels in a continuous way (Bisht et al., 2021). However, the existence of a “juxtavascular” microglial population had

been described much earlier (Grossmann et al., 2002). CAMs represent about 30% of the whole microglial population in adult mice and are localized preferentially along the vessels where astrocyte endfeet coverage is absent, thus suggesting the need for further investigations on their direct contribution to the regulation of microvessel diameter and, as a consequence, of CBF (Bisht et al., 2021; Mondo et al., 2020). It was shown that microglia depletion through the CSF-1R inhibitor PLX3397 results in an increased capillary diameter by 15% (Bisht et al., 2021), and a recent study by Csaszar and colleagues demonstrated that microglia make simultaneous contact with vascular elements (ECs, mural cells and astrocytes) and neurons to regulate neurovascular responses (Császár et al., 2022). Moreover, it has been demonstrated *in vitro* that the presence of microglia in co-culture with ECs promotes barrier properties in the endothelium, inducing the expression of the protein of TJs, like ZO-1 and occludin (Mehrabadi et al., 2017).

When neuroinflammation underlies the pathological condition, both astrocytes and microglia affect BBB stability.

Recruitment and migration of immune cells through the BBB is in fact facilitated by cytokines and chemokines released by reactive astrocytes (Spampinato, Merlo, et al., 2022), whereas microglial cells may promote BBB breakdown through the release and production of inflammatory cytokines, ROS and NO (Mahad & Ransohoff, 2003) that induce endothelial ICAM-1 expression (Huber et al., 2006) and destabilize the BBB by damaging supporting astrocytes (Haruwaka et al., 2019). In addition, reactive microglia contribute to the leaky BBB observed in these diseases through downregulation of TJs such as Claudin-5, occludin, and ZO-1 (Kleinberger et al., 2017; Obermeier et al., 2013).

Interestingly, however, both astrocytes and microglia can stabilize endothelial cells at the BBB, releasing trophic factors and molecules like IL-4, IL-13 and arginase implied in angiogenesis and barrier tightness (Z. Chen & Trapp, 2016; Spampinato et al., 2019). In particular, the involvement of microglia in increased

TJs stability in early phases of inflammatory conditions has been demonstrated (Haruwaka et al., 2019). Furthermore, microglia depletion can facilitate BBB permeability (Halder & Milner, 2019). Instead, during a sustained inflammation microglia can induce BBB damage (Haruwaka et al., 2019). In fact, the increased cyclooxygenase 2 activity in reactive microglia can facilitate the release of inflammatory cytokines (TNF, IL1, IL6) and the activation of pathways that contribute to BBB destabilization (Akundi et al., 2005; Ronaldson & Davis, 2020; Y. Yang & Rosenberg, 2011). Endothelium-derived factors including nitric oxide are known to suppress microglial activation and exert anti-inflammatory actions suggesting a bidirectional control between endothelium and microglia (Katusic & Austin, 2016). From this it follows that interacting with the cellular constituents of the BBB, microglia may play a dual role, initially protecting BBB integrity, but enabling its enhanced permeability during prolonged inflammation (Haruwaka et al., 2019).

A recent study investigated the role of microglia in a BBB model *in vitro*. Microglial cells were co-cultured with astrocytes and endothelial cells and the modulation of S1P receptor after an inflammatory stimulus was evaluated. S1P receptors were stimulated with BAF-312 (siponimod) a drug commonly used for MS. The drug facilitated microglial migration towards endothelial/astrocyte co-cultures, through the activity of the metalloprotease 2 (MMP2). Microglia actively cooperated with astrocytes in the maintenance of endothelial barrier stability: in the triple co-culture, selective treatment of microglial cells with BAF-312 significantly prevented cytokines' effects on the endothelial barrier, suggesting a potential additional effect of the drug in its pharmacological efficacy when used in the treatment of multiple sclerosis (Spampinato, Costantino, et al., 2022).

1.1.7 Microglia in a Chronic Inflammatory Condition: Alzheimer's Disease

Alzheimer's disease (AD) is the principal cause of memory loss and cognitive decline in the world. The pathogenic markers of the disease are Aβ plaques and hyperphosphorylated tau tangles, which lead to neuronal degeneration, synaptic loss and gliosis. The main target in AD are neurons. The progressive cognitive decline and memory impairment are caused by the reduction in synaptic functions and neuronal degeneration. Aβ have a direct action on neurons, where it modifies the activity of NMDA glutamate receptors, NA+/K+ ATPases, insulin receptors and integrins (Abbott, 2002; DiChiara et al., 2017; Texidó et al., 2011; Zhao et al., 2008) and can also modify neuronal membrane structure, altering its permeability and excitability (Merlo, Spampinato, et al., 2020). Amyloid-β (Aβ) has long been shown to be critical in Alzheimer's disease pathophysiology. Microglia contributes to the earliest responses to Aβ buildup, by direct interaction through multiple receptors. Microglial cells operate Aβ clearance and trigger inflammatory/regenerative processes that take place in the long years of silent disease progression that precede symptomatic appearance. But in time and with aging, the fine balance between pro- and anti-inflammatory activity of microglia deranges, negatively impacting its Aβ-clearing ability. Furthermore, in recent years, microglial activation has proven to be much more complex than the mere dichotomic pro/anti-inflammatory polarization previously accepted. Microglia can display a wide spectrum of phenotypes, which can even be mixed. On these bases, it is evident that while pharmacological intervention aiding microglia to prolong its ability to cope with Aβ buildup could be extremely relevant, its feasibility is hampered by such high complexity, which still needs to be completely understood (Merlo, Spampinato, et al., 2020). Furthermore, Aβ oligomers can be internalized in neurons, directly contributing to the generation of ROS (G. Caruso et al., 2019; Cheignon et al., 2018). In addition, also BBB impairment has been proposed as one of the principal elements in the pathogenesis of AD. Cerebrovascular diseases that cause an altered vascular perfusion, cerebral hemorrhage and hypoxia, can be also common comorbidities

in AD, often they constitute the first event (first hit) conducing to progressive Aβ accumulation (second hit) (Brkic et al., 2015; Sagare et al., 2012).

Usually a compromised BBB, associated with increased permeability, can be considered as a biomarker of the aging process (Fang et al., 2023). In AD patients, it has been demonstrated that the damage of the BBB is already present in the early stages of the disease: imaging studies and postmortem tissues had shown the leakage of blood-borne proteins and immune cells through the BBB in various brain areas, such as the hippocampus, entorhinal cortex, and prefrontal cortex (Nation et al., 2019; Sweeney et al., 2019; van de Haar et al., 2016). Confirming this, brain ECs derived from induced pluripotent stem cells of AD patients showed altered properties, including a dysregulated expression of TJ proteins and an increased barrier permeability (Oikari et al., 2020). Often in AD postmortem brain through Immunohistochemistry, is observed an increased interaction between neuronal and non-neuronal cells, in particular among neurons, ECs, astrocytes and microglia (Kirabali et al., 2020). In AD, the modification occurring at the BBB exasperate Aβ accumulation in the brain. In ECs of the BBB there are expressed specific transporters that regulate the movement of molecules between the brain and the blood (Ramanathan et al., 2015). Among these transporters, the Receptor for Advanced Glycated End products (RAGE) and the low-density lipoprotein receptor-related protein 1 (LRP-1), are responsible of bind and transport Aβ (Alemi et al., 2016). In AD their activity is dysregulated (Deane et al., 2004; Deane & Zlokovic, 2007), that lead to Aβ accumulation in the brain (Ramanathan et al., 2015). The expression of glucose transporter Glut-1 is also severely compromised in microvessels in the brain of AD patients and mouse models (Hooijmans et al., 2007; Merlini et al., 2011), inducing early endothelial dysfunction and barrier leakage (Winkler et al., 2015). The neurovascular uncoupling, described in the disease, conduces to increased permeability to blood-borne solutes and to immune cells, that cause a CNS inflammatory response (Huang et al., 2023). Furthermore, Aβ may directly modify the BBB functions described: in *in vitro* BBB models, ECs exposed to Aβ oligomers

undergo to a TJs redistribution (Kook et al., 2012; Wan et al., 2015) and increase the expression of the adhesion molecules ICAM-1 and VCAM-1 (Gonzalez-Velasquez et al., 2008; Marco & Skaper, 2006; Nagababu et al., 2009). Various human postmortem studies indicate that the levels of occludin, ZO-1 and claudin-5 are reduced in the cerebral blood vessels of AD patients (Carrano et al., 2012; Keaney et al., 2015), although exist conflicting results on animal models (Lochhead et al., 2020). The weaking of BBB junctions, which may be directly correlated not just with the decreased levels of the proteins but also with the loss of the balance among all junctional proteins (Schlingmann et al., 2016), can facilitate the access of plasma proteins that get worse inflammation and neurotoxicity (Chodobski et al., 2011). Also, the endothelial overexpression of adhesion molecules such as ICAM-1 and VCAM-1 facilitates leukocyte transmigration, conducing to the aggravation of the inflammatory response. Supporting this, preventing the access of neutrophils to the CNS has been associated with improving of cognitive performance and reduced AD-related pathology in AD mouse models (Zenaro et al., 2015).

BBB properties are strongly influenced by endothelial interaction with the other component of the NVU, in particular with glial cells. In AD, glial cells may both directly and indirectly participate in neuronal loss and in the setup of neuroinflammation, that also influences BBB properties.

Microglia have a time-dependent role in BBB function during development of AD (Gullotta et al., 2023). In the early stages of AD, microglial cells surround and incorporate Aβ aggregates to limit their deposition (Condello et al., 2015, 2018) and participate actively in the disassembly and digestion of senile plaques (Keren-Shaul et al., 2017). Using genetically modified mouse it has been demonstrated that the complement factors C1q and C3 released by microglia are involved in Aβ homeostasis (Hong et al., 2016); confirming this, C3R ablation in APP mice have as result a reduction of Aβ accumulation (Czirr et al., 2017). Also, the triggering receptor expressed on myeloid cells 2 (TREM2) expressed by microglia is involved in Aβ degradation (P. Yuan et al., 2016).

At later stages Aβ is accumulated in the brain and, by binding to CD33 receptor on microglia, can reduce his clearance, accordingly, knocking out CD33 in APP/PS1 mice attenuated Aβ plaque deposition and cognitive decline (Griciuc et al., 2013; Perea et al., 2020). Activation of microglia for long periods have as a consequence the secretion of pro-inflammatory cytokines and the promotion of neurodegeneration (Merlo, Spampinato, et al., 2020), which can be, in fact, the consequence of Aβ-induced reactivity on glial cells. An important aspect of the microglia role in AD is an abnormal phagocytosis that has been described in AD (Hong et al., 2016). Microglial phagocytosis is regulated by various mechanisms, including the CX3CR1/CX3CL1 interaction and the complement. Alterations in the complement pathway are described already in the early stages of AD (Hong et al., 2016). Aβ in fact can directly bind and activate the complement system (Webster et al., 1995) inducing the microglia release of the C1p complement factor, which increases the astrocytic release of C3, conducing to synaptic phagocytosis (Hong et al., 2016; Huffels et al., 2023). A constant microglial activation causes an upregulation of inflammatory mediators and cytokines, conducing to an important neuroinflammation and neuronal death (Merlo, Spampinato, et al., 2020; Sajja et al., 2016). Aβ can directly bind to the microglial TLR4 receptor (Okun et al., 2009) and increase the stimulation of the NLRP3 inflammasome (Hanslik & Ulland, 2020), leading to the activation of caspase-1 and the consequent cleavage and secretion of IL-1β and IL-18 (Freeman et al., 2017; Gustin et al., 2015).

The crosstalk between microglia and astrocytes is modified in AD (L.-R. Liu et al., 2020). Aβ induces the microglial release of complement factors and cytokines, which affect astrocytic response. The C1q complement component, released by reactive microglia, induces the astrocytic release of the C3 complement factor (Liddelow et al., 2017), which is responsible for neuronal damage, and affects EC functions at the BBB (Propson et al., 2021). In AD animal models, has been showed that microglia release TNFα and TGF-β that modulate astrocytic calcium-ion-dependent signaling and glutamate release (Domercq et al., 2006),

responsible for the beginning of excitotoxicity (Rossi et al., 2005). Microglia-derived IL-1β stimulates astrocytes to release VEGF, which lead to the degradation of TJ proteins and BBB permeability, in rats, IL-1β, released by microglia, induced the astrocytes to release VEGF (Q.-S. Wang et al., 2020), while in *in vitro* studies, IL-1β, derived from co-cultured microglia, suppressed the astrocytic release of Shh (Y. Wang et al., 2014), a factor essential for endothelial properties at the BBB. Finally, microglia-derived TNFα and IL-1β suppress the expression of astrocytic gap junctions, inducing an altered astrocytic communication, thus facilitating the reactive gliosis observed in AD (Même et al., 2006).

In AD patients and mice models endfeet of astrocytes surrounding Aβ plaques bear structural alterations (Saavedra et al., 2022). Astrocytes are involved in the generation and clearance of Aβ (Iliff et al., 2012; Ries & Sastre, 2016; Saavedra et al., 2022), the accumulation of Aβ in the brain activate the NF-kB pathway in astrocytes, that lead to the complement activation and the release of inflammatory cytokines, chemokines and factors that modify neuron–glia communication and synaptic transmission contributing to the progression of neuroinflammation and BBB damage (Frost & Li, 2017; Garwood et al., 2017; Lian et al., 2015; Liddelow et al., 2017). *In vitro*, astrocytes exposed to Aβ together with inflammatory cytokines, release VEGF, that contribute to the degradation of the junctional protein claudin-5 and endothelial barrier permeability (Spampinato et al., 2017).

Astrocytes may also have a protective role depending on the disease's phase, they participate in the elimination of neuritic dystrophies in AD mouse models and in AD patients (Gomez-Arboledas et al., 2018) and release factors, such as TGF-β, that increase microglial phagocytic activity and reduce neuronal damage (Gomez-Arboledas et al., 2018; Iram et al., 2016). Astrocytes can also support BBB properties in AD. In response to exogenous trigger stimulus, such as Aβ accumulation, astrocytes activate heat-shock response, such as Dnajb1, Hspa1a and Hspa1b (Lau et al., 2020), in this way they maintain BBB functions (Thuringer

& Garrido, 2019). The upregulation of the gene Nr4a1 prevents astrocyte inflammatory response, by reducing NF-kB activity, and contributes to the maintenance of BBB integrity (Pan et al., 2021). Astrocytes modulate also the access in the perivascular space of the leukocytes (Horng et al., 2017). They can increase the release of protease inhibitors, such as Pcsk1n, that protect the endothelial barrier from the degradative enzymes released by infiltrating leukocytes (Pan et al., 2021). In *in vitro* model astrocytes, after a short Aβ exposure, regulate the endothelial expression of the adhesion molecule ICAM-1, contrasting leukocyte transmigration (Spampinato et al., 2017). ON the other hand, when the barrier is compromised, astrocytes may directly interact with infiltrated leukocytes, regulating their functions, lymphocytes exposed to Aβ in the presence of astrocytes showed a Th2 phenotype, increasing the release of BDNF and IL-4 that reduced endothelial barrier damage (Spampinato et al., 2020).

Regarding the interaction with neurons, microglia also have a dual role in the modulation of BBB properties in AD. Microglia activated by a constant inflammatory stimulus, can contribute to BBB leakage (McKim et al., 2018; Zenaro et al., 2017; H. Zhou et al., 2006); on the other hand, in the early phases of the insult, in AD, such as in other CNS pathological conditions, microglia can support the BBB (Haruwaka et al., 2019; Spampinato, Costantino, et al., 2022). In an *in vitro* model, the presence of microglia reduced endothelial permeability induced by an inflammatory stimulus (Spampinato, Costantino, et al., 2022). Accordingly, also in a mouse model, reactive microglia migrated toward the endothelial barrier, promoting the expression of the endothelial junctional protein, claudin-5, thus supporting ECs in reducing BBB leakiness (Haruwaka et al., 2019). Finally, the neurovascular coupling mediated by microglia through the purinergic receptor P2Y12R is involved in AD, thus affecting the cerebrovascular response to neuronal activity, and reducing endothelial barrier properties (Császár et al., 2022; Kenkhuis et al., 2022).

After Aβ exposure, microglia can also cause a pericyte loss (Huang et al., 2023; Takata et al., 2021). In AD, also the pericyte dysfunction participate in BBB disruption, and a pericyte loss has been described in both AD patients and mice models (Sagare et al., 2013; Sengillo et al., 2013), caused by Aβ toxicity (Alcendor, 2020). In AD patients, the diameter of small capillaries, in particular in those in the proximity of senile plaques, are restricted and irregular (Kitaguchi et al., 2007), a recent study proposed the involvement of contracted pericytes as the cause of this phenomenon (Nortley et al., 2019). The loss of pericytes may also induce the expression of VEGF, contributing to barrier weakness (Hellström et al., 2001). At the end, Aβ-activated microglia may upregulate MMP9 released by pericyte (Takata et al., 2011) which, is one of the principal causes of TJs degradation and BBB disruption.

1.1.8 Glutamate Receptors Classification

Glutamate is the most important excitatory neurotransmitter of the mammalian central nervous system (CNS), it has a central role in memory, synaptic plasticity and neuronal development. Glutamate has also a negative role, because a glutamate overstimulation is implicated in neurodegeneration (Ferraguti et al., 2008; Nakanishi & Masu, 1994). Two types of glutamate receptors are described: ionotropic and metabotropic receptors. Ionotropic glutamate receptors are N-Methyl-d-Aspartate (NMDA), α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite receptors, all ligand-gated ion channels that promote a rapid excitatory neurotransmission (Dingledine et al., 1999) Metabotropic glutamate receptors (mGluRs) belong to family C G protein-coupled receptors (GPCRs) that, after stimulation, promote Gα-βγ uncoupling, conducing to Gα-mediated increase in intracellular second messenger levels, βγ regulation of ion channels, and furthermore the stimulation of G protein-independent pathways (Gerber et al., 2007; J.-P. Pin et al., 2003). mGluRs may form dimers composed of two subunits cross-linked by a disulphide bridge and dimer formation is important for mGluRs function (Kniazeff et al., 2011). Up to now, eight mGluR subtypes have been identified and characterized and are divided into three

subgroups based on sequence homology and cell signaling activation (Conn & Pin, 1997; J. P. Pin & Duvoisin, 1995). Group I mGluRs are composed by mGluR1 and mGluR5, these receptors are coupled to $G\alpha_{q/11}$, promote the release of Ca^{2+} from intracellular stores (Abdul-Ghani et al., 1996; Dhami & Ferguson, 2006), and are functionally linked to polyphosphoinositide hydrolysis and negatively coupled with KC channels (Abdul-Ghani et al., 1996; Nicoletti et al., 2011). Group II mGluRs are composed by mGluR2 and mGluR3. Finally, group III mGluRs comprises mGluR4, mGluR6, mGluR7 and mGluR8 (Gerber et al., 2007). Both Group II and Group III mGluRs negatively regulate adenylyl cyclase via $G\alpha_i$ but can also activate MAP kinase and PI-3-kinase pathways (Iacovelli et al., 2002; Nicoletti et al., 2011; Niswender & Conn, 2010). Both groups are principally localized presynaptically, and work as autoreceptors to inhibit glutamate or gamma-aminobutyric acid (GABA) release (Schoepp, 2001). Group I mGluRs are mostly located at postsynaptic elements in a perisynaptic zone surrounding the ionotropic receptors, where they modulate neuronal excitability (Lujan et al., 1996; Shigemoto et al., 1997). mGlu receptor subtypes form homo- and heterodimers among themself (Kammermeier, 2012; Vafabakhsh et al., 2015; Yin et al., 2014), but mGlu receptors dimerize with also other receptors coupled to Gq such as 5-HT2A, β1-adrenergic, and GABAB receptors (J.-P. Pin & Bettler, 2016). Finally, evidence exists of functional interactions between mGlu receptors and other membrane G protein-coupled receptors such as estrogen receptors in neurons (Spampinato et al., 2012).

All component of CNS, neurons, astrocytes, microglia, oligodendrocytes, endothelial cells, and other circulating immune cells have a role in response to both acute and subacute injury and in chronic neurodegeneration. Some multifunctional treatment strategies can involve also targeting mGluRs, which are expressed in several cell types commonly distributed throughout the CNS (Ferraguti & Shigemoto, 2006). Glial cells express both, ionotropic glutamate receptors and mGluRs, and also glutamate transporters. The different and heterogeneous locations of mGluRs in the CNS constitute an interesting

opportunity to investigate selectivity different drug's target depending by receptor subtypes. A good number of studies have demonstrated that mGluRs are expressed in lymphocytes and in antigen-presenting cells, like dendritic cells, microglia, and macrophages (Fallarino et al., 2010; Pacheco et al., 2006). Furthermore, mGluRs regulate cell migration, glutamate release, and the induction of the inflammatory phenotype in microglia (Barker-Haliski & White, 2015). mGluRs can also modulate immune responses, the kind of the modulation depending on the specific subtype of mGluRs that is involved. (Boldyrev et al., 2005).

The Intracellular signaling stimulated by mGlu receptors has been predominantly studied in neuronal cells, (Gerber et al., 2007). Group I mGlu receptors activate MAP kinase signal that have an important role in protein synthesis-dependent neuronal plasticity (Gerber et al., 2007; Hellyer et al., 2017). Following mGlu receptors activation, the MAPK cascade have several translation and transcription factors as target, that have been well described (Gerber et al., 2007). Group I mGlu receptors dependent phosphorylation of JNKs increases transcription mediated by activator protein- 1 (L. Yang et al., 2006), meanwhile activation of p38 regulates NF-kB (O'Riordan et al., 2006). Usually G-protein coupled receptor can activate canonical pathway mediated by the Ga subunit but also non-canonical pathways (e.g., MAPK activation) mediated by scaffolding proteins such as β-arrestin (Hathaway et al., 2015; Iacovelli et al., 2014). The recruitment of β-arrestin-dependent signaling pathways occurs in response to G-protein coupled Receptor Kinase (GRK)-dependent phosphorylation and is strictly ligand-dependent (Hellyer et al., 2017). The expression of mGlu receptors is highly regulated during the development. mRNA levels for mGlu1, mGlu2, and mGlu4 receptors are low at birth and increase during postnatal development (Luján et al., 2005). For mGluR5, the expression of the shorter mGlu5a receptor isoform is higher in prenatal stages, and mainly localized in cortex, hippocampus and subventricular zone, where it colocalizes with neural progenitors (Boer et al.,

2010), astrocytes and microglia. However, in mature brain, mGlu5b receptor is the main isoform expressed (Luján et al., 2005; Romano et al., 2002).

In glial cells, mGlu1, mGlu3, and mGlu5 receptors are found in astrocytes while mGlu2, mGlu3, and mGlu5 receptors are expressed in microglial cells. In oligodendrocytes, mGlu1 and mGlu4 are highly expressed (Ribeiro et al., 2017), while mGlu5a and mGlu2/3 receptors are present in early developmental stages and downregulated in mature MBPC oligodendrocytes (Deng et al., 2004; Luyt et al., 2003; Spampinato et al., 2015).

In glial cells mGlu receptors regulate cell proliferation (Ciccarelli et al., 1997), the release of growth factors, cytokines (Aronica et al., 2005; Ciccarelli et al., 1999), and neurotransmitters including glutamate, ATP and adenosine, which propagate Ca^{2+} signaling between astrocytes and other glial cells (Hamilton et al., 2010). Glial mGlu receptors can also modulate the activity and the expression of glutamate transporters, participating in this way to the regulation of synaptic function (Aronica, Gorter, Jansen, et al., 2003; Vermeiren et al., 2005).

1.1.9 Group II mGluRs

mGluRs constitute a class of membrane receptors associated with G proteins. Three different receptor classes are distinguished based on their structure, their binding to G proteins and their pharmacological profile. In particular, group 2 is made up of the mGluR2 and mGluR3 receptors, highly similar from a structural point of view, capable of stimulating the Gi protein, but with different distribution on the various cell types and capable of stimulating different, sometimes opposite, responses (Spampinato et al., 2018). Both have been studied as a relevant pharmacological target in neurodegenerative disorders (Bruno et al., 2017).

Regarding the cerebral localization of the Group II receptors, mGluR2 has been identified in only a few brain regions, like the olfactory bulb and cerebellar cortex. As cellular distribution, mGluR2 is mainly concentrated in neurons, more present in the pre-terminal region of axons, far from the sites of

neurotransmitter release (Tamaru et al., 2001). The presynaptic mGluR2/mGluR3 can be activated by an excess of glutamate coming from an over-release of synaptic glutamate or from the glutamate released from astrocytes via the cystine–glutamate membrane antiporter (Kalivas, 2009). Modifications of the expression and activity of the cysteine–glutamate antiporter may influence the function of mGluR2 and mGluR3 in brain areas involved in drug dependence (Crupi et al., 2019; Schmidt & Pierce, 2010). Presynaptic mGluR2/mGluR3 have the main role of reducing the release of neurotransmitters. Both receptor types play a role in the modulation of synaptic plasticity, particularly in stimulating LTD of excitatory synaptic transmission (Altinbilek & Manahan-Vaughan, 2009; Grueter & Winder, 2005; Nicholls et al., 2006).

In the case of mGluR3 distribution in the CNS, it is primarily expressed in the olfactory tubercle, dentate gyrus, cerebral cortex, nucleus accumbens, lateral septal nucleus, striatum, amygdaloid nuclei, cerebellar cortex, and substantia nigra pars reticulata (Petralia et al., 1996; Tamaru et al., 2001; Tanabe et al., 1993). Expression of mGluR3 is also reported presynaptically, postsynaptically, and on glial cells (Ferraguti & Shigemoto, 2006; Ohishi et al., 1993).

Group II mGlu receptors, have been studied as a possible pharmacological target in neurodegenerative disorders (Bruno et al., 2017).

Group II mGluRs were studied in Parkinson Disease, where it has been shown that the selective mGlu2/3 receptor agonist LY379268 improved rotarod performance in animal models of the disease but did not modify akinesia in 6-OHDA-lesioned rats and might even worsen motor symptoms (Johnson et al., 2009). Hence, Group II mGluR agonists were suggested as a possible treatment for neuropsychiatric symptoms associated with PD (J. S. Han et al., 2006).

The stress-related psychiatric conditions, among which major depressive disorder (MDD), anxiety, and drug abuse are considered a relevant problem concerning health worldwide (Cryan & Holmes, 2005). The L-glutamate system represents the primary excitatory neurotransmitter system involved in the circuits of

emotion and cognition and has a central role in the pathophysiology and persistence of disorders related to mental health. Clinical neuroimaging studies have reported a consistent volumetric alteration in brain areas where glutamatergic neurons predominate, among which there are amygdala, hippocampus, and several cortical regions (Lorenzetti et al., 2009). The expression of mGlu2Rs in the hippocampus has been strongly correlated with the mechanisms underlying resilience (or non-resilience) to stress, which have a central role in the pathophysiology of MDD and other stress-related disorders (McEwen et al., 2015).

The potential use of mGluR2/3 agonists in the treatment of schizophrenia was heavily tested in pre-clinical studies using different animal models (Kryszkowski & Boczek, 2021). In a phencyclidine (PCP)-induced model, LY379268 reversed certain behavioral phenotypes and pre-pulse inhibition (Cartmell et al., 2000) to a degree comparable with atypical antipsychotic clozapine. Other studies carried out on mGluR3 and mGluR2 knockout mice demonstrated that mGluR2, but not mGluR3, mediated the effects of LY379268 in experimental models predictive of antipsychotic activity (Woolley et al., 2008). LY379268 also showed promising effects in other schizophrenia models based on pharmacological blockage of NMDA receptor (ketamine and MK-801) (Sokolenko et al., 2019).

The first drug targeting mGluR2/3 receptors that was clinically tested in humans for the treatment of schizophrenia was LY-2140023, based on the assumption that it could normalize hyperactive cortical pyramidal neurons in the thalamus, prefrontal cortex and limbic system (Patil et al., 2007). In subsequent years more selective mGluR2/3 PAMS were tested in clinical trials and they can still be considered promising drugs in schizophrenia treatment to be used alone or in combination with other antipsychotics (Kryszkowski & Boczek, 2021).

Among studies carried out on mGluR2/3 in NVU, it has been shown that under conditions of diabetes complicated by depression, hippocampal neurovascular units were damaged, leading to decreased barrier function and also to other

disfunctions such as elevated Glutamate levels, upregulation of vesicular glutamate transporter 3 (VGLUT-3) and metabotropic glutamate receptor 2/3 (mGluR2/3) expression, downregulation excitatory amino acid transporter 1 (EAAT-1) expression, and finally alterations in the balance of key proteins associated with the extracellular signal-regulated kinase (ERK)/glial cell-derived neurotrophic factor (GDNF)/PI3K signaling pathway. Consequently the results of the study suggested that the Glu/mGluR2/3/PI3K pathway, induced by glucocorticoid receptor activation in the hippocampal neurovascular unit, may be associated with diabetes-related depression. (J. Liu et al., 2021).

As mentioned above, both receptors are expressed in glial cells, astrocytes express only mGlu3 receptor that is present at all developmental stages (Sun et al., 2013), while microglial cells express both mGlu2 and mGlu3 receptors (J. J. G. Geurts, 2003).

1.1.10 mGluR2-3 role in Astrocytes

Stimulation of Group II mGluRs increases nerve growth factor (NGF) and BDNF production by glial cells (Ciccarelli et al., 1997; Matarredona et al., 2001). Elevated Glu levels stimulated the over-expression of the Glu receptor mGluR2/3 (Losonczy et al., 2003) that can also be increased in astrocytes in response to FGF and EGF (Aronica, Gorter, Ijlst-Keizers, et al., 2003) or after exposure to pro-inflammatory cytokines (TNF-α and IL-1β), (Berger et al., 2012). mGlu3 receptor participates in the control of extracellular glutamate by increasing the expression of the transporter GLAST and GLT-1 (Aronica, Gorter, Ijlst-Keizers, et al., 2003; Gegelashvili et al., 2000; H. Yao et al., 2005; F. Zhou et al., 2006). On these bases, the use of mGlu3 receptor agonists and/or PAMs has been proposed in the treatment of ALS in which a defect of GLT-1 has been described (Battaglia et al., 2015; Rothstein et al., 1995).

In astrocytes, the stimulation of mGluR3 activate MAPK and PI3K pathways, increasing the production of neurotrophic factors (Bruno et al., 1998; Caraci et

al., 2011; Durand et al., 2017) that promote neuroprotection against different toxic insults (Ribeiro et al., 2017).

mGlu5 and mGlu3 receptor activation may control, either in cooperation or independently, various astrocyte functions, such as glutamate transporter activity, astrocyte–arteriolar and astrocyte–neuronal interactions (Bradley & Challiss, 2012). Among group I mGlu receptors, mGlu5 is the mostly expressed in astrocytes. Both mGluR3 and mGluR5 can have at the same time a positive and a negative influence on cell proliferation, and they are also both highly expressed in cultured oligodendrocyte progenitor cells (Aronica, Gorter, Ijlst-Keizers, et al., 2003).

In addition, astrocytic mGlu3 receptors, through activation of MAPK and PI3K pathways, contribute to neuroprotection by increasing synthesis and secretion of neurotrophic factors (Bruno et al., 2017). These include TGF-β1, that prevents both NMDA- and Aβ-induced toxicity on neurons (Bruno et al., 1998; Caraci et al., 2011; Corti et al., 2007) and GDNF, that is considered an important neurotrophic agent for nigral dopaminergic neurons and has shown neuroprotective and restorative activity in several preclinical models of parkinsonism (Ibáñez & Andressoo, 2017). Accordingly, the pharmacological activation of mGlu3 receptor in mice increases GDNF mRNA and protein levels in striatal neurons (Battaglia et al., 2009). GDNF has also a key role in the protection of neurons from excitotoxicity also in cultured spinal motor neurons (Battaglia et al., 2015). Thus, selective mGlu3 receptor stimulation may have a role in slowing neuronal degeneration in different conditions such as ALS (Battaglia et al., 2015) and PD (Bruno et al., 2017). The role of astrocytic mGlu3 receptors and the interaction astrocyte-neuron has been particularly claimed in this case. Accordingly, studies have shown that mGlu2/3 receptors agonists protect cortical neurons against excitotoxic death only in the presence of astrocytes (Bruno et al., 2017; Caraci et al., 2012). These data have been reinforced by studies carried out in cultured astrocytes from mGlu3 knockout mice (Battaglia et al., 2015; Caraci et al., 2011; Corti et al., 2007) demonstrating the key role of astrocytic mGlu3 receptor in the

mediation of the neuroprotective effects of mGlu2/3 receptor agonists. Activation of mGlu3 receptor activity also protects astrocytes from OGD (Ciccarelli et al., 2007) and nitric oxide damage, due to the reduction of cAMP content and consequent activation of PI3K/Akt pathway (Durand et al., 2010, 2013). It has also been suggested that mGlu3 receptor might represent a relevant pharmacological target to develop disease-modifying drugs in AD (Caraci, Nicoletti, et al., 2018). Even if no clear data are available in human AD brains, mGlu3 receptor expression seems reduced in several animal models of AD (Cha et al., 2001; Dewar et al., 1991; Durand et al., 2014; Knezevic & Mizrahi, 2018). When treated with the mGlu2/3 receptor agonist LY379268, astrocytes *in vitro* reduced neuronal Aβ toxicity through the release of neuroprotective factors such as TGF-β1 (Caraci et al., 2011) and BDNF (Durand et al., 2017). This is in line with the observation that TGF-β1 has anti-inflammatory and neuroprotective effects in experimental models of AD (J.-H. Chen et al., 2015), and a deficit of TGF-β1 signaling has been found in the early phases of AD and appears to contribute to neuroinflammation and cognitive decline in AD (Caraci, Spampinato, et al., 2018). Activation of mGlu3 receptor can also have a relevance in other steps of AD pathogenesis by reducing Aβ production (Durand et al., 2014) or increasing Aβ clearance (Durand et al., 2017). Astrocytic mGlu3 receptors can stimulate the activity of α-secretase, the enzyme that cleaves APP downstream of the N-terminus domain of $A\beta_{(1-42)}$ (Durand et al., 2014). When astrocytes were exposed to LY379268, they reduced the levels of β-secretase, while increasing the expression of sAPPα, with a consequent decrease of neurotoxic Aβ. It has been also demonstrated that LY379268 can increase Aβ uptake in astrocytes and microglia, promoting Aβ clearance from the extracellular space (Durand et al., 2017). The contribution of mGlu3 receptor seems not completely clear, because Aβ phagocytosis was not prevented by LY2389575, a selective mGlu3 receptor NAM, suggesting that the effects observed after LY379268 stimulation can also involve mGlu2 receptor activation (Durand et al., 2017).

1.1.11 mGluR2-3 Role in Microglia

mGlu2 and mGlu3 receptors can differentially modulate microglial function. Activation of mGlu2 receptors causes neuroinflammation by stimulating the production of pro-inflammatory cytokines (Pinteaux-Jones et al., 2008; Taylor et al., 2002, 2005). In contrast, activation of mGlu3 receptors drives microglia to an anti-inflammatory function (Pinteaux-Jones et al., 2008). Group II stimulation with the agonist DCGIV also induces BDNF expression in microglia (Venero et al., 2002).

The role of Group II mGluRs in microglia has been investigated in AD models. In response to Aβ, microglial cells increase their glutamate release (Barger & Basile, 2001). The exposure of microglial cells to the active fragment $A\beta_{(25-35)}$ induces also mGlu2 receptor activation, that can drive an increased neurotoxicity (Taylor et al., 2002, 2005). In fact, selective activation of mGlu2 receptors, promotes a pro-inflammatory and neurotoxic phenotype that releases TNF-α and FAS-L, and potentiates microglial reactivity in response to chromogranin-A, up-regulated in AD (Taylor et al., 2002, 2005).

It is well known that microglial activation plays a key role in the pathogenesis of MS (Strachan-Whaley et al., 2014). *In vitro*, exposure of microglial cells to myelin fragments induces microglia activation, increasing the release of glutamate and TNF-α, that cause neuronal death. In this context, activation of microglial mGlu2 receptor worsen myelin-evoked neurotoxicity, whereas activation of mGlu3 receptor is protective (Pinteaux-Jones et al., 2008).

A good neuroprotective effects of orthosteric mGlu2/3 receptor agonists have been observed in animal models of global and focal brain ischemia (Bond et al., 1998, 2000), probably as a consequence of the involvement of mGlu2 receptors expressed in neurons (Corti et al., 2007; Mastroiacovo et al., 2017; Motolese et al., 2015). However, the role of microglial mGlu2 receptor in stroke ischemia has not been completely clarified. mGlu2 and mGlu3 receptors are expressed by microglia in the ischemic penumbra, where apoptotic neuronal death progresses

slowly, making this area more open to therapeutic intervention and moreover microglial cells mediate neurotoxicity in the stroke penumbra (Kaushal & Schlichter, 2008). In experimental models of ischemia, it has been demonstrated that glutamate, released by "ischemic" neurons, activates microglia through group II mGlu receptors with the following activation of NF-kB, induction of TNF-α, and subsequent neuronal death (Kaushal & Schlichter, 2008).

A recent study demonstrated that the microglial mGlu3 receptor is highly downregulated in a rat model of perinatal brain injury. Selective pharmacological activation of mGlu3 receptors during early postnatal life might mitigate neuroinflammation associated with perinatal adverse conditions (Zinni et al., 2021). In particular, after exposure to LPD (low protein diet) together IL-1β, Grm3 gene expression results significantly downregulated in the developing microglia (Zinni et al., 2021). The study demonstrated that pharmacological blockade of mGlu3 receptors induced a pro-inflammatory phenotype in microglia sorted from rat pups. Finally microglia reactivity to inflammatory response induced by LPD/IL-1β exposure was reduced by an mGlu3 receptor agonist (Zinni et al., 2021).

In mice, it has been demonstrated that the activation of mGlu3 receptors has a protective activity against ischemic brain damage and associated neuroinflammation (Mastroiacovo et al., 2021). In fact, in mGlu3$^{-/-}$ mice, there is increased expression of several pro-inflammatory genes such as those encoding for IL-1β, COX-2, TNF-α, CD86, and IL-6; these genes were more present in the peri-infarct region of mGlu3$^{-/-}$ mice. On the other hand, following the medial cerebral artery occlusion, the expression of two genes associated with the anti-inflammatory phenotype of microglia (those encoding the mannose-1-phosphate receptor and the a-subunit of interleukin-4 receptor) and the gene encoding for the neuroprotective factor, GDNF, were enhanced in the peri-infarct region of wild-type mice, but not mGlu3$^{-/-}$ mice (Mastroiacovo et al., 2021).

1.2 Aims of the study

The main aims of the first part of the study were to define the role of microglia in BBB function using an *in vitro* cellular model that allowed identifying the specific interplay among different cell types of the NVU and to assess the effect of pharmacological intervention. Among the various possible targets expressed in glial cells, group 2 metabotropic receptors were investigated. The specific aims of the study were:

- to evaluate whether, in an *in vitro* BBB model, modulation of mGlu2 and mGlu3 receptors plays a role in maintaining barrier properties following an inflammatory stimulus;
- to assess the role of microglia in modulating BBB properties and to evaluate whether this function can be affected by stimulation of mGlu2/3 receptors;
- to analyze the role of pericytes in the control of BBB function in inflammatory conditions.

1.3 Materials and Methods

1.3.1 Cells cultures of BBB *in vitro* model

To simulate the BBB *in vitro* model were used three adult human cell lines: TY-10 cells, brain microvascular endothelial cells; hAST, astrocytic cells; and HMC3 human microglial cell line (ATTC, LGC Standards, Manassas, VA, USA). Both TY-10 and hAst are transfected with a plasmid expressing temperature sensitive Simian virus-40 large T-antigen and the catalytic subunit of human telomerase, as previously described (Haruki et al., 2013). Both cell lines were developed at Yamaguchi University (Japan), in the labs of Dr. Sano and Kanda. TY-10 cells were grown in MCDB-131 media (Thermo Fisher Scientific, Milan, Italy), supplemented with EGM-2 SingleQuots (Lonza, Basel, Switzerland) and 20% heat inactivated FBS (Thermo Fisher Scientific). hAST were grown in astrocyte medium containing 3% heat-inactivated Fetal Bovine Serum (FBS), astrocyte growth supplement and

penicillin/ streptomycin solution, as provided with the Astrocyte media kit (Clinisciences, Nanterre, France). HMC3 was grown in Eagle's Minimum Essential Medium (EMEM, Sigma Aldrich) supplemented with 10% FBS (Thermofisher Scientific), Non-essential amino acids, sodium Pyruvate and penicillin (100 U/mL)/streptomycin (100 ug/mL). hAst and TY-10 were grown at 33 °C and 5% CO2 for two days and then were transferred to 37 °C, where they exhibited growth arrest and differentiation. HMC3 were grown at 37 °C and 5% of CO2 for 1 to 3 days. For the study, TY-10 were cultured alone or in co-culture with hAst or hAst and HMC3. For the endothelium/astrocytes were used specific insert with a polycarbonate membrane according to experiment protocol and for the triple-culture was also used a coverslip placed between the cell culture plate and the insert for the third cell type. Both hAst and TY10 were exposed to treatments after two days at 37 °C simultaneously at HMC3 for the triple-culture. All treatments were done in astrocyte medium supplemented with 2% FBS. Treatment with cytokines (TNFα, 1 ng/mL, and IFNγ, 1 ng/mL; T&I) was maintained either for 5 or 36 h, according to the experimental protocol. Endothelial monocultures, endothelial/astrocytes co-cultures, or endothelial/astrocytes/microglia co-cultures when required by experimental design, were pretreated with a negative allosteric modulator of mGluR2, (VU 6001966, 3 µM, VU) for 20 min and with an agonist of mGluR2/3 (LY379268, 1 µM, LY37) or an mGluR3 positive allosteric modulator LY2794193 (LY27, 5 µM) for 40 min.

1.3.2 Western Blot

For western Blot analisys, Endothelial cell monocultures (5×10^5 cells for each well) were plated on 6 wells MW plates. When cocultured with endothelial cells, hAST (3×10^5 cells for each well) were plated on 0.4 µm pores poly-carbonate membrane transwell inserts (Falcon) and when cocultured with endothelial cells and microglia, were plated on the downside of a glass coverslip (3×10^5 cells for each coverslip). In the triple colture, microglial cells were plated on the transwell insert (4×10^5 cells for each well). For double and triple colture, endothelial cells

and hAst were put together when the cells ware passed to 37°C. For the triple-culture. Microglia was added in the culture before the treatments. This setting allowed the passage of soluble factors between the different cell types without direct physical contact, thus also facilitating their isolation for western blot investigations. All the treatments were performed in Astrocyte medium suppled with 2% FBS. After 36 hours of treatments, pellets were collected and lysed in RIPA lysis buffer (Sigma-Aldrich) supplemented with protease and phosphatase inhibitors for 5 hours at 4°C. Samples were sonicated and centrifuged at high speed for 5 min at 4 °C, and protein concentration was determined by a Bradford reagent (Sigma-Merck, Darmstadt, Germany), and absorbance was measured with a VarioskanTM Flash Multimode Reader. Sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE) was performed by loading 40 ug of protein extracts per experiment on pre-cast “any-kDa” or 4–20% gradient gels (Bio-Rad, Hercules, CA, USA) followed by transfer to nitrocellulose membrane (Hybond ECL, Amersham Biosciences Europe GmbH, Milan, Italy) using a Transblot semidry transfer cell (Bio-Rad, Hercules, CA, USA). Membranes were blocked with a Blocker FL Fluorescent Blocking buffer (Thermofisher Scientific) and incubated with primary antibodies: mouse anti-GAPDH (1:800; Millipore), rabbit anti-Flotillin-1 (1:700; Santa Cruz), and rabbit anti-Claudin-5 (1:300; Thermo Fisher Scientific) overnight at 4 °C. Membranes were then washed and exposed to secondary antibodies: anti-mouse AlexaFluor 488 Plus-conjugated secondary antibody (ThermoFisher Scientific) and anti-rabbit IRdye 800 secondary antibody (Licor) one hour at RT. The detection of specific bands was carried out using the iBright FL1500 Imaging System (Thermofisher Scientific). Band intensity was analyzed using the ImageJ software, developed by the National Institutes of Health (NIH) and in the public domain.

1.3.3 Immunocytochemistry

For immunocytochemical analysis, endothelial cells will be fixed in ice cold acetone (10 minutes) and then in ice-cold methanol (15 minutes). Cells are incubated with primary antibody: rabbit anti-Claudin-5 (1:100; Thermo Fisher

Scientific) and rabbit anti-ZO-1 (1:100, Thermo Fisher Scientific), in the presence of 0.1% Triton X-100 at 4 °C overnight. The secondary antibody: anti-rabbit AlexaFluor 488 Plus-conjugated secondary antibody (ThermoFisher Scientific) is incubated for 1 hour at room temperature. Slides will be observed using an epifluorescent microscope (Zeiss Observer.Z1) equipped with the Apotome.2 acquisition system connected to a digital camera.

1.3.4 FITC-dextran permeability assay

For the test endothelial cells were plated on a 3 uM pores poly-carbonate membrane transwell insert (Corning) (15 x 10^4 cells for each well). Endothelial cells could be mono-cultured, co-cultured with hAst plated in 24 MW plate (9 x 10^4 cells for each well), cocultured with hAst and HMC3, in this case, HMC3 were plated on the well (15 x 10^4 cells for each well) and hAst were plated on a glass coverslip. Also in this experiment protocol, TY-10 and hAst were put together when cells were passed to 37 °C, and HMC3 before the treatments. After 36 hours of treatments inserts were equilibrated in the phenol red free DMEM media supplemented with 1% FBS. Solute permeability was assessed using 10 kDa FITC-conjugated dextran (1 mg/mL). Conjugated dextran was applied to the luminal compartment. One hundred microliters of sample will be collected from the abluminal compartment after 60 minutes and fluorescence of the sample measured at 485/520 nm (excitation/emission) using a Varioskan TM LUX multimode microplate reader (ThermoFisher Scientific).

1.3.5 Trans-endothelial electrical resistance analysis (TEER)

For TEER analysis were used the same insert and the same model of the permeability assay. The measurements were performed in real time, without causing damage to cells. The electrical resistance, in Ohm (Ω), was measured by means of an electrode which is positioned so that one end was in contact with the culture medium inside the insert and the other with the culture medium outside of it. The cells are subjected to various measurements at different times, before and during treatments. Various measurements were performed, and the average TEER value was calculated. For the TEER evaluation, was used the

specific device Millicell®-ERS (Electrical Resistance System) Voltohmeter (Millipore).

1.3.6 Quantitative Real-Time Polymerase Chain Reaction (PCR)

The PCR experiments were performed using the same model previously described in western blot analysis for the co-cultures and a microglial cells monoculture (5 x 10^5 cells for each well) plated on a 6 MW plate.

Total RNA was extracted from cell cultures using the RNeasy plus Mini Kit (Qiagen). One mg of RNA was used for cDNA synthesis, using the Superscript-VILO kit (Invitrogen) according to manufacturer's instruction. Quantitative real-time PCR was performed with Rotor Gene Q using QuantiNova SYBR Green PCR Kit (Qiagen). The melting curves obtained after each PCR amplification reaction confirm the specificity of the 2-[N-(3-dimethylaminopropyl)-N-propylamino]-4-[2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methyli-dene]-1phenylquinolinium (SYBR Green assays). The following pairs of primers were used: human CCL2F (CCC CAG TCA CCT GCT GTT AT), human CCL2R (AGA TCT CCT TGG CCA CAA TG), human GRM2F (CCA GGA GCT GGG TCC CTT), human GRM2R (AAG TCT CCC TCC AGG GTC AG), human GRM3F (CGC TTT GCA CAA AAT GCA GC) and human GRM3R (AAC ACG TTG TAT CGC CCC AT) from Invitrogen (Thermo Fisher Scientific), and the following primeQuantitec primers (Qiagen) were used: human IL-6 (QT00083720), human BDNF (QT00235368), human IL-1β (QT00021385) and human RPLP0 (QT00075012) that was used as an endogenous control. Expression fold changes were calculated by applying the 2 – ΔCt method**.**

1.3.7 Human Brain Pericytes (HBP) cell culture and treatment

Human brain pericytes (HBP) utilized in another part of the study (performed at "Research Center Blood-Brain Barrier Laboratory, LBHE", University of Artois, France) were originally isolated from a patient who had unexpectedly died from a heart attack (Shimizu et al., 2011). Study protocol for human tissue was approved by the ethics committee of the Medical Faculty (IRB#: H18-033-6), University of Yamaguchi Graduate School, and was conducted in accordance with

the Declaration of Helsinki, as amended in Somerset West in 1996. The protocol was approved by the French Ministry of Higher Education and Research (CODECOH Number DC2011-1321). All experiments were performed in accordance with the approved protocol. Briefly, these pericytes were transfected and immortalized using retro-virus vectors holding human temperature-sensitive SV40 T antigen (tsA58) and human telomerase (Htert). HBP were then amplified and cultured at 33 °C in high glucose (4.5 g/L) Dulbecco's modified Eagles' medium (DMEM/HG), supplemented with 10% non-heat-inactivated fetal calf serum (FCS, Sigma-Aldrich, Saint-Louis, MS, USA), 1% L-glutamine (Merck chemicals, Darmstadt, Germany) and 1% penicillin–streptomycin (Sciencell, Carlsbad, CA, USA). Before plating, well plates were coated with collagen I (Corning, NY, USA). Then, HBP were seeded in 12-well plates (for fluorescence experiments) or on a coverslip (for immunofluorescence experiments) at a density of 50,000 cells/well. Only brain pericytes at passage 11 were used for the experiments of this study. Cells were cultured at 37 °C in DMEM/HG supplemented with 1% L-glutamine (Merck KGaA, Darmstadt, Germany) and 1% penicillin–streptomycin (Sciencell) during 48 h to reach 80 to 90% of confluence. For the treatment performed, TNF-α (Sigma-Aldrich) was dissolved in DMEM/HG supplemented with 0.1% bovine albumin serum (BSA, Sigma-Aldrich) at a concentration of 10 ng/mL. When ready, HBP were rinsed once with warm DMEM/HG/0.1% BSA and then incubated in DMEM/HG/0.1%.BSA supplemented or not with 10 ng/mL of TNF-α.

1.3.8 Quantification of $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ fluorescence uptake experiments

For these experiments the HBP treated or not with TNF-α 10 ng/mL for 24H. Then cells were rinsed with warm DMEM/HG/0.1%.BSA and incubated with the solution of β-Amyloid HiLyte™ Fluor 488-labeled (1-40) (AS-60492-01, ANASPEC, Fremont CA, USA) or β-Amyloid (1-42) HiLyte™ Fluor 488-labeled (Cod: AS-60479-01, ANASPEC, Fremont CA, USA), both to a concentration of 100 nM in DMEM/HG/0.1%.BSA for 3 hours at 37 °C. After the treatment the cells were rinsed with Ringer Hepes (RH; 150 mM NaCl, 5.2 mM KCl, 2.2 mM $CaCl_2$, 0.2 mM

$MgCl_2$–6H2O, 6 mM $NaHCO_3$, 5mM HEPES, 2.8mM glucose; pH: 7.4) for three times and lysed with RIPA buffer addition of protease inhibitors to assess the quantity fluorescence peptides were taken from cells. The lysates were collected, and an aliquot was taken in a dark 96-wells plate for measuring the fluorescence. The fluorescence quantification was quantified to values of λ: Aβ-40 (503-532) for Aβ-40 and (550-576) Aβ-42, excitation/emission with a spectrofluorometer (Synergy H1, Biotek, Colmar, France). The values of fluorescence obtained were normalized on the total amount of proteins measured with Bradford assay for each sample. Experiments were performed on at least three independent experiments in triplicate for each condition.

1.3.9 Immunofluorescence of $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ uptake

The HBP were treated as described above. First with TNF-α and then with Aβ-40 or Aβ-42 at the same concentrations and times. At the end of the incubation, cells were washed with RH buffer for three times and were treated briefly with Trypan Blue (0,2%) for 5 minutes used as autofluorescence quenching. After three washing with PBS the cells were fixed with Ice-cold Methanol for 1 minute, followed by three washing with PBS for 5 minutes. Lately the coverslips with the fixed cells were mounted on a microscope slide with a mounting solution with DAPI. Images were acquired using a Leica microscope (DMRD; Leica Microsystems, Wetzlar, Germany) and processed using the ImageJ software. Experiments were performed on at least three independent experiments in triplicate for each condition.

1.3.10 Statistical Analysis

All data are expressed as mean ±SEM of 3–5 different experiments, each run in duplicate or in triplicate, as specified in the figure legends. Data were analyzed by one-way ANOVA, followed by the Newman–Keuls test for significance or by Unpaired Student's t-test two-sided. $p < 0.05$ was taken as the criterion for statistical significance.

1.4 Results

1.4.1 GRM2 and GRM3 expression

To evaluate the effects of the modulation of BBB's properties by mGluR2 and mGluR3, we first verified the expression of the two receptors in the three different cells lines with real time PCR analysis. Endothelial cells, astrocytes and microglia expressed both genes GRM2 and GRM3. (Tab.1).

	take off	amplification			take off	amplification
mGluR2_TY10	29,7	1,66		mGluR3_TY10	41,4	1,73
mGluR2_hAST	26,5	1,61		mGluR3_hAST	29,5	1,71
mGluR2_HMC3	25,5	1,59		mGluR3_HMC3	30,8	1,76

Tab. 1. mRNA expression levels for mGluR2 and mGluR3 in the cell lines utilized in the study.

1.4.2 mGlu3 receptor activation modifies endothelial function

After, to assess the effect of mGlu2/3 activation on the endothelium, a monoculture of endothelial cells grown alone was exposed to the inflammatory stimulus of TNFα (1 ng/mL) and IFNγ (1 ng/mL) (T&I) for 24-36 hours. This treatment caused impairment of barrier properties as measured by increased permeability to FITC-conjugated dextran; pretreatment with the mGluR2/3 agonist LY379268 (LY37, 1 µM) contrasted the effect of T&I, significantly reducing permeability (Fig 1a). In contrast, the addiction in the pretreatment of a mGluR2 NAM, VU6001976, did not significantly modify the effects of LY37 (Fig 1a). At 24 h there was also a decrease of Trans-endothelial electrical resistance (TEER) and pre-treatment with LY37 rescued TEER at basal values (Fig 1b). Endothelial permeability is dependent also on changes in the expression of endothelial tight junctions (TJs) like Claudin-5 and Zo-1. Expression of claudin-5 was significantly reduced by the inflammatory insult in the membrane fraction and mostly redistributed to the cytosolic compartment. Treatment with LY37 rescued claudin-5 localization to the membrane, whereas VU did not modify LY37 effect (Fig. 2a.) Furthermore, immunocytochemical analysis showed that both claudin-5

(Fig. 2b) and Zo-1 (Fig. 2c) were clearly reduced on the membrane and internalized in the cytosol and LY37 contrasted these effects.

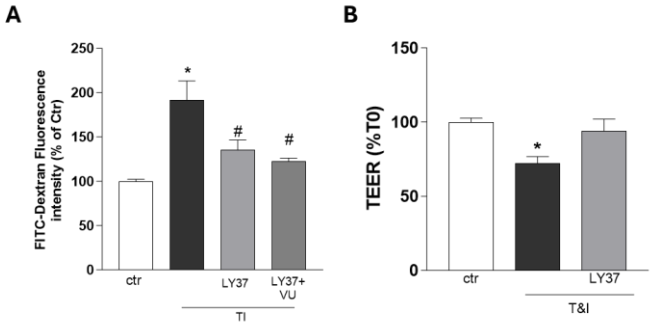

Fig. 1. Evaluation of Barrier function in Endothelial cells monolayer. A) FITC-Dextran Fluorescence Permeability assay on Endothelial cells monolayer after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37, # $p < 0.05$ T&I vs LY37+VU. **B)** Measure of Trans Endothelial Electrical Resistance (TEER) on Endothelial cells monolayer after 24H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I.

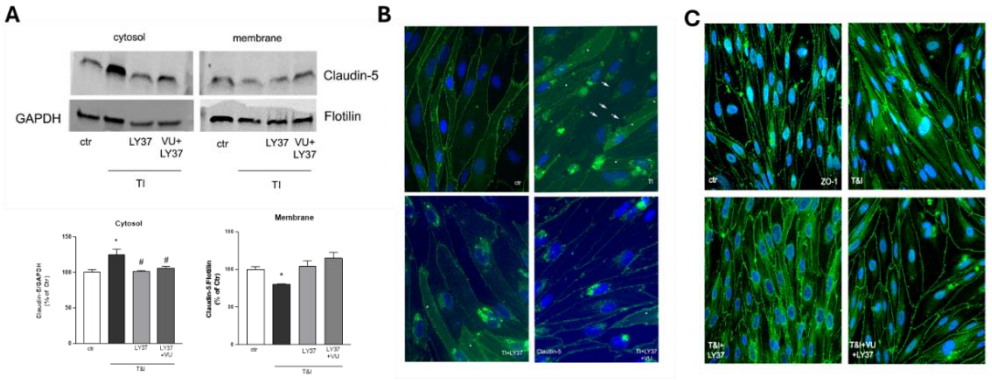

Fig. 2. Tight Junction (TJs) proteins expression in Endothelial cells monolayer. A) Claudin-5 expression levels in cytosolic and membrane compartment after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, # $p < 0.05$ T&I vs LY37+VU+T&I. **B)** Immunocytochemistry fluorescence image of claudin-5 expression after 36H of T&I treatment, Green: claudin-5, Blue: DAPI. **C)** Immunocytochemistry fluorescence image of claudin-5 expression after 36H of T&I treatment, Green: claudin-5, Blue: DAPI.

1.4.3 mGlu3 mediates the protective effect on BBB function in endothelium/astrocyte co-cultures

Exposure of the endothelium/astrocytes co-culture to T&I treatment (both at 1 ng/mL for 24-36 h) induced an enhancement of FITC-dextran fluorescence permeability measured at 36 h, LY37 prevented this effect, that was not modified by VU (Fig. 3a). T&I caused an increase of TEER already significant at 24 h, that was contrasted by pre-treatment with LY37 (Fig. 3b). Furthermore T&I caused also a reduction in the expression of the junction protein claudin-5, as detected by western blot analysis of the whole protein extract. LY37 prevented the effects of T&I on claudin-5 expression, that was not significantly modified when the NAM of the mGluR2 receptor VU was added (Fig. 4). We then selectively stimulated the mGluR3 receptor using the positive allosteric modulator LY2794193 (LY27, 5 µM) and observed a significant effect on the increased permeability induced by T&I (Fig 5a). We also analyzed the inflammatory response on astrocytes grown in an endothelium/astrocytes co-culture following treatment with T&I and pretreatment with LY27. The latter completely prevented the gene expression of the pro-inflammatory cytokine IL-6, induced by a 5 h-exposure to T&I (Fig. 5b). In contrast, mGluR3 stimulation with LY27 increased the expression of transforming growth factor-β (TGF-β), that was not modified by the inflammatory challenge of T&I (Fig. 5c).

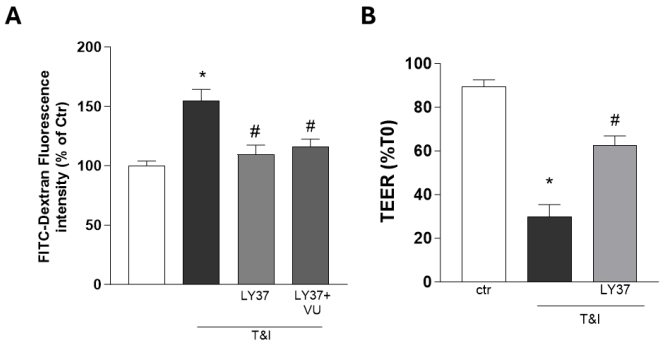

Fig. 3. Evaluation of Barrier function in Endothelial cells of Endothelium/Astrocytes co-cultures. A) FITC-Dextran Fluorescence Permeability assay on Endothelial cells from endothelium/astrocytes co-cultures after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, # $p < 0.05$ T&I vs LY37+VU+T&I. **B)** Measure of Trans Endothelial Electrical Resistence (TEER) on Endothelial cells from endothelium/astrocytes co-cultures after 24H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I.

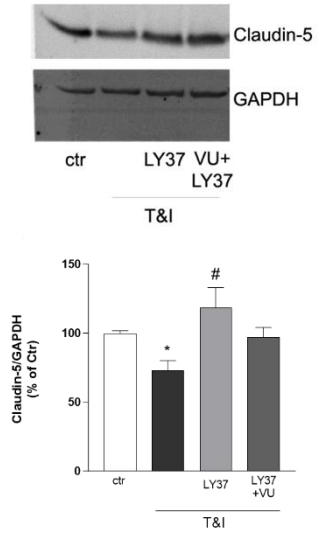

Fig. 4. Tight Junction (TJs) proteins expression in Endothelial cells from Endothelium/Astrocytes Co-cultures. Claudin-5 representative bands and expression levels in Endothelial cells from Endothelium/Astrocytes Co-cultures after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I.

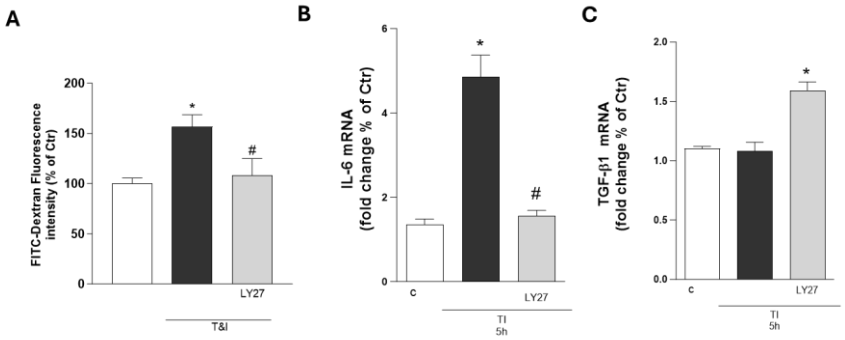

Fig. 5. Effects of selective mGluR3 stimulation on endothelial barrier properties and Astrocytes mRNA expression levels in Endothelium/Astrocytes Co-cultures. **A)** FITC-Dextran Fluorescence Permeability assay on Endothelial cells monolayer from Endothelium/astrocytes co-cultures after 36H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY27+T&I. **B)** IL-6 mRNA expression levels in Astrocytes from Endothelium/astrocytes co-cultures after 5H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY27+T&I. B) TGF-β1 mRNA expression levels in Astrocytes from Endothelium/astrocytes co-cultures after 5H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ T&I vs LY27+T&I.

1.4.4 mGluR2 cooperates to protect BBB function in a triple endothelial-astrocyte-microglia co-culture

In the triple endothelial-astrocyte-microglia co-culture, the increased endothelial permeability to dextran induced by T&I, was reduced by LY37 (1 µM) and this effect was contrasted by treatment with the mGlu2 NAM, VU (3 µM); (Fig 6a). Similarly, T&I-reduced expression of claudin-5 was restored by pre-treatment with LY37, and this effect was again counteracted by VU (Fig. 6b).

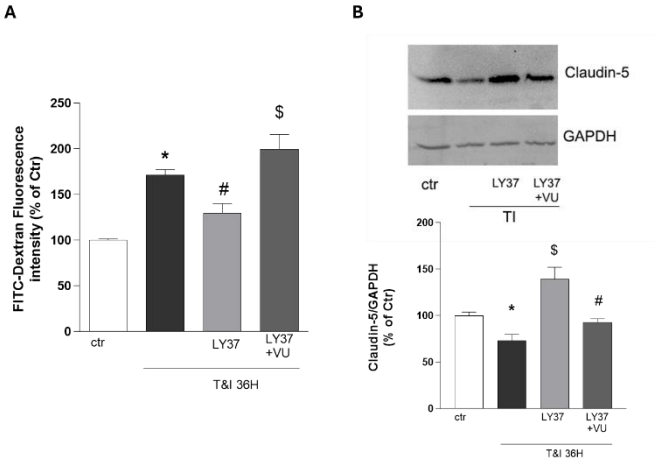

Fig. 6. Evaluation of Barrier function and TJs Protein expression in Endothelial cells of Endothelium/Astrocytes/Microglia triple co-cultures. A) FITC-Dextran Fluorescence Permeability assay on Endothelial cells from endothelium/astrocytes/microglia triple co-cultures after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, $ $p < 0.05$ LY37+T&I vs LY37+VU+T&I. **B)** Claudin-5 representative bands and expression levels in Endothelial cells from Endothelium/Astrocytes/Microglia triple Co-cultures after 36H of T&I treatment. One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, $ $p < 0.05$ T&I vs LY37+T&I, # $p < 0.05$ LY37+T&I vs LY37+VU+T&I.

1.4.5 mGluR2 modulates microglia inflammatory response

To better understand the role of microglia, we therefore evaluated the expression of selected factors also in a microglial monoculture exposed to the same treatments via real time PCR. LY37 attenuated the expression of the inflammatory cytokines IL-6 and IL-1β and the chemokine CCL2 in microglia cells activated by T&I (Fig. 7A-C) Blockade of mGluR2 with the NAM VU reduced these effects for IL-6 (Fig. 7A) and CCL2 (Fig. 7C) while prevented the effects of LY37 for IL-1β (Fig. 7B). Treatment of microglia with LY37 also increased the expression of the neurotrophic factor BDNF, and this effect was blocked by VU (Fig. 7D).

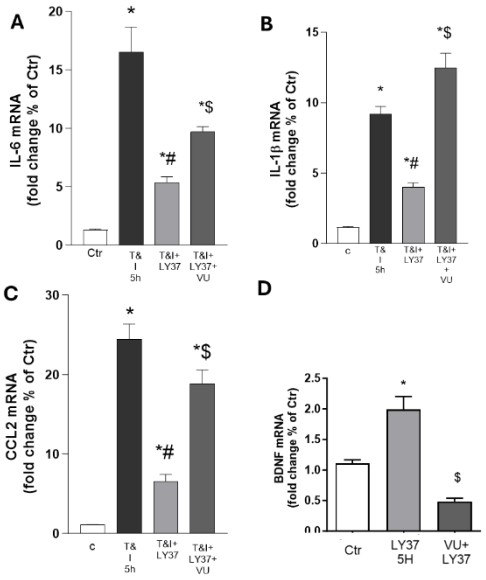

Fig. 7. Microglia mRNA expression levels in Microglia monocolture. **A**) IL-6 mRNA expression levels in Microglia after 5H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, $ $p < 0.05$ LY37+T&I vs LY37+VU+T&I **B)** IL-1β mRNA expression levels in Microglia after 5H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, $ $p < 0.05$ LY37+T&I vs LY37+VU+T&I **C)** CCL2 mRNA expression levels in Microglia after 5H of T&I treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs T&I, # $p < 0.05$ T&I vs LY37+T&I, $ $p < 0.05$ LY37+T&I vs LY37+VU+T&I **D)** BDNF mRNA expression levels in Microglia after 5H of LY37 treatment, One-way ANOVA followed by Newman–Keuls test. * $p < 0.05$ CTR vs LY37, $ $p < 0.05$ LY37 vs LY37+VU.

1.4.6 TNF-α treatment reduced the Aβ-peptides uptake on Human Brain Pericytes

To assess the function of brain pericytes in favoring transport and clearance at the BBB, we analyzed the uptake of the Aβ-peptides in cultured Human Brain Pericytes (HBP) utilizing fluorescent-conjugated forms of both $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ peptides. After TNF-α treatment, cells were incubated for 3 hours with one of the two peptides. At the end of incubation, the cells were washed to eliminate as much possible the external quote of Aβ-peptides and the uptake quotes were determined by quantifying fluorescence in the cell lysate or qualitatively with immunofluorescence on fixed cells. Regarding the fluorescence quantification, results showed that the TNF-α treatment was able to reduce fluorescence (normalized on the total protein amount), compared to the control cells, for both

Aβ $_{(1-40)}$ and Aβ $_{(1-42)}$ peptides and consequently the cells internalized less Aβ-peptides (Fig. 8 A-B). For Aβ $_{(1-40)}$ data were confirmed also by immunofluorescence images where TNF-α treatment showed less internalization compared to control (Fig. 8C).

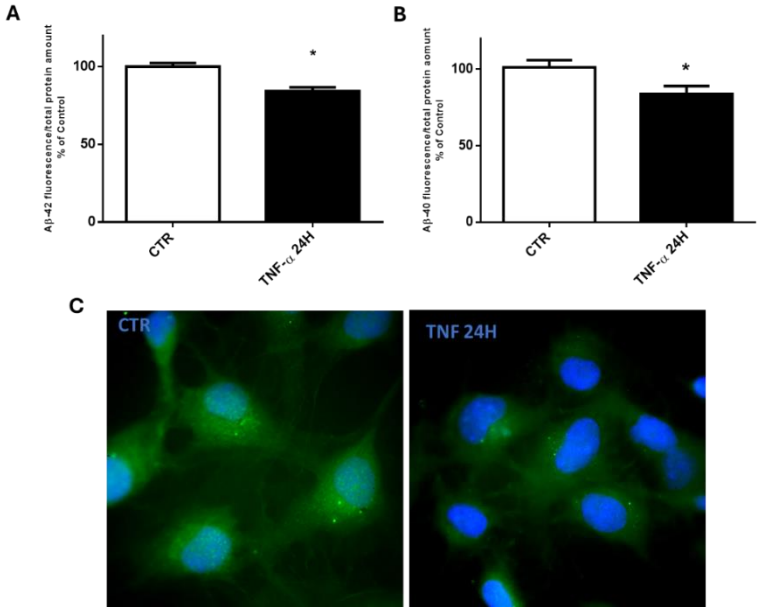

Fig. 8. Aβ-peptides uptake on Human Brain Pericytes. A) Aβ $_{(1-42)}$ peptide fluorescence normalized on total protein amount after 3H of exposition to the peptide in human brain pericytes. Unpaired Student's t-test, $P < 0.05$ CTR vs TNF-α 24H. **B)** Aβ $_{(1-40)}$ peptide fluorescence normalized on total protein amount after 3H of exposition to the peptide in human brain pericytes. Unpaired Student's t-test, $P < 0.05$ CTR vs TNF-α 24H **C)** Aβ $_{(1-40)}$ peptide immunofluorescence after 3H of exposition to the peptide in human brain pericytes. Aβ $_{(1-40)}$: green, DAPI: blue.

Although these are only preliminary results, they confirm that an inflammatory condition can affect also the behavior of pericytes that reduce their ability to internalize Aβ-peptides. These results suggest a main role also for this cellular component in BBB function. Further studies are needed to assess which BBB transporters, including LRP1, ABCA1, ABCB1 (P-gp), can be modified following treatment with TNF-α.

1.5 Discussion

In this study we investigated BBB properties in an in-vitro model, utilizing a single, double- and triple-co-culture system. The objective was to understand the influence of the mGluR2-3 receptors in the presence of an inflammatory insult, focusing in particular on the role of microglia cells. It is well known from literature that microglia can have a role in the modulation of BBB function in neuroinflammatory conditions that follow an acute event, such as stroke, but also participate in slow neurodegenerative conditions, such as Alzheimer's disease or multiple sclerosis (Gullotta et al., 2023; Spampinato, Costantino, et al., 2022). Attention has been paid to these conditions, taking into account the well-recognized role of neuroinflammation in the slow events that characterize neurodegenerative diseases. In response to an insult, the behavior of microglia can be different: they can remove cellular debris in an attempt to contribute to cellular repair or can accelerate the induction of the neuroinflammatory response (G. I. Caruso et al., 2021; Merlo et al., 2022; Merlo, Luaces, et al., 2020).

Regarding their role on BBB, microglia cells interact with endothelial cells of the barrier, and even though they do not seem to play an essential role in BBB formation and stability, they modulate BBB functions, both in physiological and pathological conditions (da Fonseca et al., 2014). In particular, in inflammatory conditions, microglia demonstrated to play a dual role. Microglial cells may promote BBB breakdown through the release and production of inflammatory cytokines, ROS and NO (Mahad & Ransohoff, 2003) that induce endothelial ICAM-1 expression (Huber et al., 2006) and weaken the BBB by damaging supporting astrocytes (Haruwaka et al., 2019). In addition, reactive microglia contribute to a leaking BBB that can be observed in neuroinflammatory conditions through downregulation of TJs such as Claudin-5, occludin, and ZO-1 (Kleinberger et al., 2017; Obermeier et al., 2013). On the other hand, the involvement of microglia in increasing TJs stability in early phases of inflammatory conditions has also been demonstrated (Haruwaka et al., 2019). Furthermore, endothelium-derived factors including nitric oxide are known to suppress microglial activation and

exert anti-inflammatory actions, suggesting a bidirectional control between endothelium and microglia (Katusic & Austin, 2016).

In this context, we investigated the role of mGluR2 and mGluR3, two receptors that are considered relevant pharmacological targets in neurodegenerative disorders (Bruno et al., 2017). Both receptors are expressed in glial cells: astrocytes express principally mGlu3 receptor that is present at all developmental stages (Sun et al., 2013). Alternatively, microglial cells express both mGlu2 and mGlu3 receptors (J. J. G. Geurts, 2003). The protective role of mGluR3 in astrocytes in well established (Spampinato et al., 2018) and it is largely known that stimulation of mGluR3 activates MAPK and PI3K pathways, increasing the production of neurotrophic factors (Bruno et al., 1998; Caraci et al., 2011; Durand et al., 2017). In contrast, the role of these receptors on microglia appears more uncertain. Activation of mGlu2 receptors causes neuroinflammation by stimulating the production of pro-inflammatory cytokines (Pinteaux-Jones et al., 2008; Taylor et al., 2002, 2005), whereas activation of mGlu3 receptors drives microglia to an anti-inflammatory function (Pinteaux-Jones et al., 2008). More recent evidence suggests that selective pharmacological activation of mGlu3 receptors in microglia during early postnatal life might mitigate neuroinflammation associated with perinatal adverse conditions (Zinni et al., 2021). In mice, it has also been demonstrated that the activation of mGlu3 receptors has a protective activity against ischemic brain damage and associated neuroinflammation (Mastroiacovo et al., 2021).

We examined the endothelial barrier function in the three different culture conditions. Starting from monoculture, endothelial cells were exposed to the inflammatory stimulus of TNFα and IFNγ (T&I), a treatment that caused a profound impairment of barrier properties, as demonstrated by increased membrane permeability and reduced endothelial electrical properties. Our results also showed that treatment with T&I caused a translocation of claudin-5 from membrane to cytosol. Regarding the modulation of mGluR2/3, results showed that pre-treatment with LY37 was able to prevent the effects of

inflammatory cytokines on permeability, TEER and claudin expressions, while the addition of VU did not modify these effects. These results suggest that mGluR3 is the receptor subtype more involved in the modulation of endothelial properties.

Similarly, in the endothelium/astrocyte co-cultures, T&I impaired the BBB properties, as measured by increased permeability, reduction in TEER and of total claudin-5 expression. Stimulation of mGlu2/3 receptors again prevented these effects and the lack of changes observed in the presence of blockade of mGlu2 receptor led us to hypothesize that in endothelium/astrocytes co-culture, the effect of LY37 was more linked to the activity of the mGlu3 receptor which, as is known from the literature (Spampinato et al., 2018), is highly expressed at astrocytic level. To analyze in more details this aspect, we utilized also the mGluR3 positive allosteric modulator LY2794193. As expected, in the endothelium-astrocyte co-cultures, LY27 prevented the increase of permeability, and the gene expression of the pro-inflammatory cytokine IL-6, both caused by T&Y, and increased the expression of transforming growth factor-β (TGF-β). This last result is in line with what is reported in literature where mGluR3 stimulation led to an increase of TGF-β in astrocytes (Caraci et al., 2011). Therefore, also in the endothelium/astrocytes co-culture, mGluR3 has a major role in the modulation of BBB properties.

Interesting results were obtained when we analyzed microglia influence on BBB properties in the triple endothelial-astrocyte-microglia co-culture. In this case, in fact, the effects of LY37, that were similar to those described above for the endothelial monoculture and the endothelial/astrocyte co-culture, were counteracted by VU that caused blockade of mGlu2 receptor. So, in contrast to what observed before, these results suggest that mGluR2 has a role in the protection of barrier function in the triple endothelial-astrocyte-microglia co-culture. To investigate in more details the specific role of microglia and the selective involvement of mGlu2 and mGlu3 receptors, we decided to use a monolayer of microglia cells challenged with T&I to assess expression of inflammatory cytokines and chemokines. The results obtained showed that LY37

attenuated the microglial inflammatory activation by T&I. All these effects were modified in the presence of blockade of mGluR2, confirming a role for mGlu2 receptor in this anti-inflammatory microglial response. Stimulation of mGlu2 and mGlu3 also increased the expression of the neurotrophic factor BDNF, an effect already reported in literature (Venero et al., 2002). These data are in apparent contrast with mGluR2 mediating a pro-inflammatory effect. Data from the literature report in fact that activation of 2 mGlu receptors stimulated the production of pro-inflammatory cytokines (Pinteaux-Jones et al., 2008; Taylor et al., 2002, 2005). One important aspect of mGlu2 and mGlu3 behavior is their capability to form homo- and heterodimers (Kammermeier, 2012; Vafabakhsh et al., 2015; Yin et al., 2014), a phenomenon particularly important for mGlu receptor function (Kniazeff et al., 2011). Although it is hard to conclude from the present data, we cannot rule out that the results we observed might be influenced by a change in the homo- and hetero-dimers proportion on microglial cells. The poor availability of very selective mGlu2 and mGlu3 receptor agonists/antagonists did not allow a clear identification of the specific role of each receptor subtype.

In conclusion, our results showed that mGluR3 and mGluR2 protect BBB functions in the presence of an inflammatory insult. In particular, the role of mGluR3 is prevalent in endothelial cells and astrocytes while mGluR2 appears to be involved in microglia. The effects observed are direct on the endothelial component where TEER, expression of tight junctions and consequently permeability are modified, but seem to be also indirect, through modulation of the inflammatory response on astrocytes and microglia.

2. *In Vivo* studies

2.1 Introduction

2.1.1 Animal models of Autism Spectrum Disorders

The Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders generally diagnosed by three core behavioural symptoms, *i.e.* stereotyped repertoire, communication impairments and social dysfunctions (Hodges et al., 2020). However, besides the symptoms listed above there is an extensive range of additional symptoms, including cognitive dysfunctions, restricted interests, hyperactivity and impulsivity, that are often overlapping with different neuropsychiatric disorders, such as schizophrenia, attention-deficit/hyperactivity disorder, anxiety and obsessive-compulsive disorder (Guo & Commons, 2017; Murray, 2010).

ASD incidence has increased intensely during the last decade, by reaching the 1% estimation from the World Health Organization, that only accounts for approximately 16% of the global pediatric population; while European and US reports estimated around 1.4-2.5% in children of 8 years old on average based on population studies (Hodges et al., 2020; Sacco et al., 2023; Scattoni et al., 2023; Zeidan et al., 2022). The etiopathogenesis underlying the development of this type of diseases is very complex and still largely unknown, at the base genetic and environmental factors are hypothesized as the most involved. Although there is indication for a solid influence of genetic factors, with 60–90% concordance in monozygotic twins and 4–10% in dizygotic twins, (Muhle et al., 2004) with a study suggesting an incidence near to 30% for the seconds (Hallmayer et al., 2011), ASD seems to be polygenic, with hundreds of contributing loci (Bncur, 2011), among which the majority seems to be *de novo* mutations and copy number variants (Huguet et al., 2013; Iossifov et al., 2014; Jeste & Geschwind, 2014). ASD is more frequently diagnosed in boys, with the sex ratio of 4.3:1 (Newschaffer et al., 2007) for classic autism and 11:1 in Asperger Syndrome (Gillberg et al., 2006),thus suggesting that some of the genes involved are X-

linked. Moreover, only 10–15% of ASD cases can be directly associated with monogenic disorders, such as Rett syndrome, Fragile X syndrome or tuberous sclerosis complex (Chévere-Torres et al., 2012; Hagerman et al., 2011; Zoghbi, 2005), while most cases are described as idiopathic.

Recently, multiple factors are emerging as possibly implicated in this pathological picture, such as immune system, diet, metabolic system, and gastrointestinal system.(Napolitano et al., 2022; Samsam et al., 2014). In addition, an overgrowth of cortical gray matter and decreased migration of neurons during early development with consequences in heterotopias, thinning of the corpus callosum and malformations of the ventricular system have been described in ASD patients (Courchesne et al., 2011; Ecker et al., 2012; Wegiel et al., 2010). However, the presence of such alterations in the population of ASD patients is not enough to consider them main causes of the behavioural phenotype related to ASD. Other proposed impairments include imbalance in excitatory and inhibitory neurotransmissions, atypical formation of dendritic spines, dysfunctions in secondary messenger systems, extracellular matrix and synaptic protein and neuroinflammation during fetal development and later in life (Persico & Bourgeron, 2006). Furthermore, around 30% of autistic individuals also suffer from other mental health conditions, such as epilepsy (Tuchman & Rapin, 2002), anxiety disorders (van Steensel et al., 2011), obsessive compulsive disorders or attention deficit hyperactivity disorder (Leyfer et al., 2006).

Considering that, studies involving humans are frequently influenced by willful variables and biases and some differing results and inconsistencies in clinical evaluations have also been noted. In this regard, animal models can be really helpful to longitudinally study and understand behavioural alterations mimicking human symptoms in a translational way and to investigate the underpinning neurobiological mechanisms (Grosso, Galvano, et al., 2014; Grosso, Pajak, et al., 2014). To this purpose, the BTBR T+ Itpr3tf/J (BTBR) mice are an inbred mouse strain that well reproduces the principal behavioural deficits of ASD (Blanchard et al., 2012; Guo & Commons, 2017). Among the different ASD mice models, the

BTBR mouse strain shows strong and evident deficits in reciprocal social interactions, altered ultrasonic vocalization and repetitive stereotyped repertoire (Scattoni et al., 2013; Wöhr et al., 2011). Furthermore, BTBR mice show cognitive and emotional deficits typical of the psychiatric comorbidities of ASD (Chao et al., 2018; Chao, Marron Fernandez de Velasco, et al., 2020).

Regarding the social behaviour, in the three-chamber test for social approach, BTBR males failed to show a preference for the social chamber (Meyza et al., 2013). In addition, in the Visible burrow system (VBS) test, BTBR mice showed reductions in all interactive behaviours, including approach, follow and grooming another mouse, while they showed increases in non-interactive behaviours, like self-grooming and being alone (Bove et al., 2018; Pobbe et al., 2010). In social proximity test (Defensor et al., 2011) performed involving same-strain pairs, BTBR male mice showed decreased nose tip-to-nose tip, and upright behaviours, therefore suggesting that BTBR mice have an aversion for reciprocal frontal (face-to-face) orientations. In the Social conditioned place preference test, BTBR mice conditioned for 10 days didn't show a preference for the social chamber during testing, while a preference for this chamber was retrieved in C57BL/6 (BL6) mice, hence highlighting that BTBR mice showed social motivation deficits(Pearson et al., 2012). Indeed, BL6 mice are commonly used as control in several studies involving sociability, due to their normo-social behaviour.

Moreover, BTBR mice also displayed communication impairments, by emitting an uncommon pattern of vocalizations. Indeed, BTBR adult mice emitted a smaller number of ultrasonic vocalizations (USVs) in male-to-male, male-to-female, and female-to-female encounters (Scattoni et al., 2011). Furthermore, BTBR male mice also deposited few odor marks in the presence of female urine (Roullet et al., 2011; Wöhr et al., 2011). In this regard, some studies suggested that elevated USVs in BTBR pups may resemble the high levels of inconsolable crying in infants that are later diagnosed with autism (Scattoni et al., 2008); while reduced USVs and olfactory marking in BTBR adults are paralleling to communication difficulties in autistic children (Roullet et al., 2011; Scattoni et al., 2011; Wöhr et al., 2011).

Regarding the repeated and stereotyped behaviours, numerous studies have shown that BTBR mice displayed elevated levels of general self-grooming (Amodeo et al., 2012; McFarlane et al., 2008; Pearson et al., 2011; Silverman, Tolu, et al., 2010). BTBR mice also showed high levels of bar biting (Pearson et al., 2011), and marble burying (Amodeo et al., 2012), that are two additional indicative parameters of stereotyped motor behaviour in laboratory rodents (Londei et al., 1998; Nevison et al., 1999). In the hole board test, BTBR mice showed a preference for a particular corner hole (Moy et al., 2008). The same was retrieved in the novel object test, where BTBR mice introduced in a box containing four novel objects in each corner showed increased preference for individual objects, by significantly spent more time exploring the first and second most investigated objects and reduced time exploring the smallest preferred object compared to controls. Moreover, BTBR mice showed more episodes of visiting the objects in a specific order (Pearson et al., 2011) due to the cognitive inflexibility characterized by resistance to change that has been correlated with stereotypies (Tanimura et al., 2008). Nonetheless, cognitive inflexibility manifestation in autism is highly variable (H. M. Geurts et al., 2009; Zandt et al., 2007), maybe because tests for resistance to change require that mice first have to learn an established routine and then to start a new routine. A relevant paradigm to study cognitive inflexibility is the Morris water maze test. This test is commonly used for studying spatial learning by measuring the time necessary to find a hidden, submerged platform, first during an acquisition phase, then in a new location in a reversal phase. In this test, BTBR mice had normal results in the acquisition phase, but showed reduced performance in finding the platform (M. Yang et al., 2012) and did not show a preference for the quadrant containing the platform (Moy et al., 2007) during the reversal phase. Furthermore, in the spatial reversal learning test (Amodeo et al., 2012), BTBR mice showed normal acquisition, but reduced results in reversal phase. However they performed normally in both phases when in one arm a reinforcement was continuously present (Amodeo et al., 2012; Moy et al., 2007), hence indicating that

diminished reversal learning and cognitive flexibility deficits in BTBR mice are dependent on the type of reinforcement and the percentage of reinforcement.

Regarding anxiety-related and stress-related behaviours, in the elevated plus maze tests and in the light/dark exploration test used for evaluating anxiety-like behaviours, results on BTBR mice have been contradictory. The elevated plus maze (EPM) is a test generally used for evaluating general anxiety by measuring the number of entries and duration of time spent in an open arm compared to a closed arm, with more open arm activity usually indicating less anxiety. In the EPM, the results reported are different and variables, indeed BTBR mice showed normal (Moy et al., 2007), high (M. Yang et al., 2009), and low (Pobbe et al., 2011) number of entries into the open arms, and showed, at the same time, normal (Benno et al., 2009; Moy et al., 2007; M. Yang et al., 2009) and low (Pobbe et al., 2011) open arm durations. While in the elevated zero maze, where the maze has a "zero" form, several studies reported that BTBR mice spent more time in the open quadrants (McFarlane et al., 2008; Pobbe et al., 2011). The light/dark exploration test is also utilized for evaluating anxiety-related behaviours and is based on the aversion of mice to intensely lighted areas. In this test, the BTBR mice did not showed difference compared to BL6 mice in the number of transitions between the light and dark compartments, the time spent in the dark chamber or in the latency to enter the dark chamber.

The mouse defense test battery is another test where is evaluated the anxiety-like behaviour, by using a hand-handheld anesthetized rat to evaluate behaviours in response to predator exposure in various contexts (Blanchard et al., 2003). In the MDTB, BTBR mice showed more vocalizations when the contact with the predator was forced, meanwhile BTBR mice also allowed the predator to approach within shorter distances before escaping in an approach/avoidance setting (Pobbe et al., 2011). Moreover, BTBR mice revealed increased locomotion, increased wall jumping and defensive standing behaviours after, but not before, the predator was introduced, suggesting increased predator-induced anxiety.

Regarding the stress-reactive behaviours, BTBR mice seem to respond differently depending on the stressor stimulus. Indeed, they showed normal reactivity to an acoustic alarm stimulus and tail flick, but they showed also a normal prepulse inhibition and low reactivity to a hot plate stimulus (Silverman, Yang, et al., 2010). Furthermore, whether the EPM test was preceded by tail suspension, BTBR mice showed increased anxiety-like behaviour (Benno et al., 2009). Lastly, in tests for evaluate depressive-like behaviours, BTBR mice showed low levels of immobility in the forced swimming test and the tail suspension test, suggesting a decreased tendency to depressive-like behaviour (Silverman, Yang, et al., 2010).

2.1.2 Neurochemical and biomolecular pathways underlying ASD symptoms

Considering their very specific behavioural profile, BTBR mice may represent a helpful model to better understand the neurobiological alterations that are the base of the complex heterogeneity of ASD symptoms, to identify possible biomarkers involved and, finally, to develop possible pharmacological treatments (Kas et al., 2019). Going into specifics, concerning repetitive and restricted behaviours, BTBR mice showed both “lower-order” motor stereotypies and “higher-order” cognitive stereotypies (Pearson et al., 2011), giving to this animal model the characteristics for being used to study the neurobiological pathways related to these behavioural dysfunctions. Pharmacological treatments to reduce repetitive behaviours are principally focused on impulsivity and irritability, by using typical and atypical antipsychotics, consequently they are utilized in limited cases. Nowadays, there are no drugs approved for an effective treatment of ASD repetitive symptoms (Aishworiya et al., 2022; McPheeters et al., 2011; Sharma & Shaw, 2012; Whitehouse & Lewis, 2015). Therefore, to discover more details on mechanisms related to stereotypies would offer a significant step forward to the development of novel pharmacological treatments for ASD repetitive repertoire. Although the pathophysiology of ASD is still not completely clear, a wide variety of imaging and post-mortem studies described the key role of corticostriatal and limbic circuits, by showing cortical, hippocampal and amygdalar anatomical

anomalies and functional alterations (Haznedar et al., 2000; Hollander et al., 2005; Kemper & Bauman, 1998; Langen et al., 2011; Zilbovicius et al., 2000).

Regarding neuroanatomic structure, BTBR mice show severe anomalies of the corpus callosum, often accompanied with a substantial reduction of the hippocampal commissure (HC) (Kusek et al., 2007; Wahlsten et al., 2003), a collapse of lateral ventricles (LV)(Meyza et al., 2012) with lateral displacement of the hippocampi (Mercier et al., 2011). The length of the subventricular zone (SVZ) is decreased in BTBR mice and both the concentration of laminin and heparan sulfate (HS) therein are diminished (Meyza et al., 2012). This is probably an effect of downregulation of exostosin-1 Ext1 expression in BTBR mice, because Ext1 is involved in synthesis and elongation of HS polysaccharides, that in the appropriately sulfated form is considered a guidance molecules crucial for the crossing of corpus callosum (CC) neuronal fibers across the midline during development (E14-E17) (Busse et al., 2007). Indeed the Ext1 KO and Hs6st1−/− and Hs2st−/− mice, missing sulfotransferases responsible for sulfation of the polysaccharide HS chain, showed a lack of CC, and altered guidance of other neuronal tracts in the brain (Conway et al., 2011; Inatani et al., 2003). Although the intact CC is a prerequisite for development of normal social behaviours in mice, there are evidence that in postnatal day (PND) 7 there was a lesion of CC in BL6, but mice social behaviour was not affected (M. Yang et al., 2009). Unfortunately, it is difficult to understand the effect of earlier disruption of CC, and also Ext1 KO, Hs6st1−/− and Hs2st−/− mice did not postnatally show any social behaviour deficits. Probably, it seems that the absence of the guidance factors influencing the CC genesis, more than the functioning of the CC itself, could be implicated in the behaviours of BTBR mice. Indeed, conditional knockouts of Ext1 (Ext1CKO, CaMKII-Cre2834; Ext1flox/flox, (Irie et al., 2012), in which Ext1 is disrupted from PND21, exhibited abnormal social interactions, improved repetitive behaviours and reduced ultrasonic vocalizations. The Ext1CKO animals also showed decreased neuronal activation to social stimuli in the amygdala and damaged excitatory synaptic transmission therein, suggesting

that HS is involved in correct functioning of glutamatergic synapses. As regarding BTBR mice, it has been reported decreased neurogenesis in the dentate gyrus of the hippocampus (Stephenson et al., 2011) and such characteristic has been linked to the autism-like behavioural phenotype (L. Wei et al., 2011), proving that youthful neurogenesis is crucial for development of normal social repertoire. It has also showed that BTBR mice also have reduced general levels of plasma sulfates (Corley et al., 2012), which is in accordance with clinical data from ASD patients (Adams et al., 2011) and could indicate that atypical sulfate metabolism or excretion may be affecting HS levels in these mice.

For their metabolism, BTBR mice were studied many years before the knowledge of their social behaviour impairment. Indeed, BTBR leptin ob/ob mice are susceptible to develop type-2 insulin resistant diabetes and were studied as an animal model for this disease. Even in the absence of leptin, ob/ob BTBR mice have been shown to have elevated plasma insulin levels compared to BL6 mice (Stoehr et al., 2000). Male BTBR mice are prone to develop abdominal obesity, hypertriglyceridemia and hypercholesterolemia, with a decreasing insulin-stimulated uptake of glucose to white adipose tissue (Flowers et al., 2007; Ranheim et al., 1997). Insulin resistance in BTBR males is correlated with increased expression of several genes in the adipose tissue, including cytoskeletal and focal adhesion molecules (Actb, Acta2, Flna, Diap, Rhoa), proteins involved in inflammation (Pir's, Fos, Ptgs2, Syk, Cflar, H2ab1) and phosphatases (Ptprs, Pp2r4, Pp6c) (Flowers et al., 2007)

Regarding the immune system, BTBR mice have a different immune response from those of BL6 mice on both humoral and cellular levels. In particular, they showed higher basal levels of plasma IgG, IgE, anti-brain antibodies (Abs), and also of brain tissue deposited IgG and IgE (Heo et al., 2011), together with higher expression of proinflammatory cytokines, such as IL-33, IL-18 and IL-1b. Moreover, the brain–blood barrier permeability measured by Evans blue was comparable in BTBR and BL6 mice and, the number of mast cells in the brain was significantly higher in BTBR mice, thus suggesting a constant neuroinflammation.

In addition, the microglia of BTBR differed from BL6 for the number of MHC class II-expressing cells. However, these immune differences could not be related to differences in NF-kB signaling, the studies showed that the phosphorylation of NF-kB p65 are not significantly altered in the cerebellum and cortex of BTBR mice and in a similar way both the expression and phosphorylation of NF-kB p65 are not significantly altered in the cerebellum and cortex of autistic subjects as compared with the control subjects.(Malik et al., 2011; Manzardo et al., 2012).

From a genetical point of view, BTBR is an inbred strain developed originally at Columbia University, by crossing mice carrying the wildtype T (brachyury) gene with the stock carrying tufted (tf) mutation. Subsequently the strain was outcrossed to c129 mice and then maintained by inbreeding. Several studies aimed to investigate the genes involved in the behavioural differences, including social behaviour of these mice. Investigations performed in large scale compared to gene expression in 10 inbred mouse strains, including BTBR and BL6 mice, tried to correlate this expression with motor coordination in the rotarod test. The results indicated a correlation of motor activity with the expression of several transcripts in the cerebellum, hippocampus and amygdala, but did not focus on between strain differences and only provided a general statement regarding a cluster analysis in which BTBR mice were placed closest to BALB/cByJ mice, in terms of overall expression patterns (Nadler et al., 2006). In another study performed analyzing the entire genome on BTBR mice backcrossed to obtain an obese phenotype (ob/ob), 3 main loci that differentiated BTBR from BL6 mice and influence plasma glucose and/or insulin levels were identified; the first of these was situated on chromosome 2 and was related to insulin sensitivity and the other two were situated on chromosomes 16 and 19 and influenced quickly glucose and insulin levels (Clee et al., 2005). Analysis of 124 assumed autism candidate genes showed that there are four genes with nonsynonymous coding single nucleotide polymorphisms (SNPs) that differentiate BL6 from BTBR mice. Two of them, Smo (smoothened, signaling protein regulated by Sonic hedgehog) and Pkd1 (polycystic kidney disease 1) were common for other inbred strains.

Regarding the other two, Slc6a4 encoding serotonin transporter was different in BL6 mice, while Kmo encoding kynurenine 3-hydroxylase was different in BTBR mice. This gene is constituted by 3 different SNPs situated on chromosomes 9 and 13, among which the one situated on chromosome 13 is a cytosine/tymine substitution in a sequence that code for a transmembrane part of the protein. This part is a conserved domain identical in mice and humans (McFarlane et al., 2008) and may constitute a relevant biomarker, because Kynurenine 3-hydroxylase is involved in synthesis of kynurenic acid, a potent antagonist of glutamate and nicotinic receptors, involved in neuroprotection, dendritic spine formation and dopamine release (Alkondon et al., 2004; H.-Q. Wu et al., 2007; Yu et al., 2004). Further analysis showed that BTBR mice also carry a Disrupted in Schizophrenia (Disc1) mutation, a 25 bp deletion causing a frame shift and a premature termination of translation of the protein. It is located on chromosome 7 [http://jaxmice.jax.org/strain/002282.html].

Interestingly, a large study collected a wide range of transcriptomic and proteomic data indicating a different expression of a big variety of genes and proteins in the hippocampus and cortex of BTBR and BL6 mice. Among those genes and proteins, brain derived neurotrophic factor [BDNF], p21-activated kinase type1 [Pak1] and cortistatin [Cort] were downregulated in BTBR hippocampus and cortex(Daimon et al., 2015), while some members of MAPK signaling pathway, were both up and downregulated. (Daimon et al., 2015) These data were in line with previous studies that have described a BDNF deficiency in adult BTBR mice (Scattoni et al., 2013; Stephenson et al., 2011), MAPK signaling interruption (Faridar et al., 2014; Seese et al., 2014) and an increased p-ERK levels (Seese et al., 2014). Such high levels of p-ERK in the prefrontal cortex was correlated with deficits in juvenile sociability (Faridar et al., 2014) and in adult memory formation (Seese et al., 2014).

Furthermore, Wei et colleagues in a proteomic study showed that BTBR mice have down-regulated levels of the stable tubule only polypeptide protein (STOP) and myelin-related proteins, such as myelin basic protein (MBP) and myelin

associated glycoprotein (MAG) (H. Wei, Ma, Ding, et al., 2016). Accordingly, histopathological examination of BTBR brain tissue showed a reduction of myelin markers, such as CNPase and MBP and an increase in the oligodendrocyte precursor NG2 (Stephenson et al., 2011). Analysis of fetal brain proteins demonstrated reduced levels of glial fibrillary acidic protein (GFAP) and increased BDNF and MBP levels in BTBR compared to FVB/NJ mice. Concerning BDNF, the upregulation of its expression at an early developmental stage might be confirmed by clinical data showing increased plasma's level of BDNF in children with ASD (M. Wang et al., 2015).

An interesting study performed proteomic analysis in hippocampi and cortices in aged BTBR mice, by identifying elevated levels of some synaptic proteins, including spinophilin, Synapsin 1 (and p-Synapsin 1), PSD95 and NeuN in the hippocampus and spinophilin, p-Synapsin 1 and NeuN in the cortex (Jasien et al., 2014), together with reduced BDNF levels in these aged animals.

In addition, some genes related to ASD are known to modify the neural structure, function and connectivity, but also influencing neurotransmission and neurotropism (Gandhi & Lee, 2020; Rubenstein, 2010). Among the neurotrophins family, particularly BDNF and Nerve Growth Factor (NGF) play an essential role in neurodevelopmental processes which are atypical in ASD pathology (Theoharides et al., 2015). BDNF is crucial for the appropriate cerebral development and significantly associated to synaptic plasticity. It has been recently proposed as a possible diagnostic marker in children suffering from ASD since different line of evidence have reported its involvement in ASD onset and development (Barbosa et al., 2020; Crespi, 2019; Hellings et al., 2017). On the other hand NGF has a key role in the regulation of nerve-cell growth, survival and differentiation, being mainly expressed in the central nervous system, particularly in the cerebral cortex, hippocampus (HIPP) and amygdala (AMY) (von Richthofen et al., 2003).Recently, few studies linked NGF and ASD, by describing an involvement of the NGF signaling pathway in the pathogenesis of ASD (Lu et al., 2013; Theoharides et al., 2015), also considering the central role of this neurotrophins

in the immune modulation and in the induction of the release of different neuropeptides and neurotransmitters (Minnone et al., 2017), such as glutamate and dopamine (DA) (Paredes et al., 2007). On the other side, catecholamines are notoriously known to increase NGF content (Furukawa et al., 1986), thus resulting in a constant crosstalk between these two pathways. Concerning this, catecholamines, such as DA and noradrenaline (NA) are known to play a fundamental role in the modulation of executive functions, attention, impulsivity, and emotional state, which are all processes disturbed in ASD (Aston-Jones, 2005; Sara, 2009). In this context, recent studies have reported a possible implication of DA neurotransmission and metabolism in ASD development (DiCarlo et al., 2019; Mandic-Maravic et al., 2021; Pavăl & Micluția, 2021), whereas several evidence demonstrated that neuropathological changes associated to the noradrenergic system often happen in ASD (Beversdorf, 2020; M. Kubota et al., 2020; Leshem et al., 2021). Moreover, an imbalance of the excitatory-inhibitory synaptic transmission has also been related to ASD pathology (Yizhar et al., 2011). In support of this, alterations of glutamate and gamma-aminobutyric acid (GABA) receptors expression have been described in *post-mortem* brains of ASD patients (Blatt & Fatemi, 2011) and decreased GABA levels have been detected in different brain regions of ASD children (Horder et al., 2018). In addition, brain regions with a massive cholinergic innervation, such as prefrontal cortex (PFC), hippocampus (HIPP) and amygdala (AMY), are linked to social cognition, thus resulting remarkably involved in ASD pathophysiology (Tanimizu et al., 2017). Indeed, lower basal levels of extracellular acetylcholine were described in BTBR prefrontal cortex (McTighe et al., 2013). In this regard, it has been showed that low acetylcoline (ACh) levels in mice presented reduced social interactions and that, by treating with acetylcholinesterase inhibitors such as Donepezil, this impairment might be contrasted (Kljakic et al., 2021) and both social approach and social novelty preference in the three chamber social approach task were improved(Karvat & Kimchi, 2014). Although there were no differences in acetylcholinesterase (AChE) expression in the forebrain of BTBR

and BL6 mice (Stephenson et al., 2011). Regarding drugs acting on Ach transmission, such as Oxotremorine, an antagonist of muscarinic receptor, it was able to reduced repetitive behaviours (grooming and marble burying) in BTBR mice (Amodeo, Yi, et al., 2014), while concerning nicotinic receptor agonist, nicotine administered in low doses enhanced sociability and in high doses reduced grooming (L. Wang et al., 2015).

As reported above, BTBR mice present impairments in monoaminergic (5HT, DA) and cholinergic transmission that have been related with the seriousness of symptoms in ASD patients (Cartier et al., 2015; Gangi et al., 2016; Muller et al., 2016). BTBR mice showed higher hippocampal $5HT_{1A}$ receptor presence (Gould et al., 2011, 2014), as well as lower [(3)H] cyanoimipramine and citalopram binding to the serotonin transporter SERT(Gould et al., 2011). Increased 8-OH-DPAT-stimulated (8-OH-DPAT is a full agonist of $5HT_{1A}$ receptor) GTPγS binding in the BTBR hippocampus indicated an elevated capacity of $5HT_{1A}$ receptors to stimulate G-proteins (Gould et al., 2011). At the same time, less serotonergic cells were detected in the hippocampus of BTBR compared to BL6 mice (Guo & Commons, 2017). On the other hand, this was compensated by improved number of serotonergic cells in brain areas projecting to the hippocampus, especially in the median raphe. Moreover, dopaminergic neurotransmission is also altered in the BTBR mice. In particular, D2 receptor function seems to be compromised and the result is a minor activation of the reward system in a functional magnetic resonance imaging (fMRI) scan after administration of a dopamine reuptake inhibitor. (Squillace et al., 2014). Several drugs acting on monoaminergic pathways have been investigated for their efficacy in ameliorating autism-like behaviours in the BTBR mouse (Meyza & Blanchard, 2017). For instance, risperidone, that act on both serotonergic and dopaminergic transmission had no effect on sociability (Chadman, 2011; Gould et al., 2011) and anxiety (Chadman, 2011), but it showed a reduction of repetitive behaviours (Gould et al., 2011) and an enhancement of reversal learning in the probabilistic learning paradigm (Amodeo, Jones, et al., 2014). The reduction in grooming was also observed after

intraperitoneal administration of a $5HT_{2A}$ antagonist, M100907(Amodeo et al., 2016, 2017; Amodeo, Jones, et al., 2014). In opposite way, fluoxetine, a SERT blocker, increased social approach, but had no effect on the repetitive behaviour (Gould et al., 2011) and anxiety level (Chadman, 2011). Similar effects were described for buspirone, a 5HT1A receptor partial agonist (Gould et al., 2011). Moreover, administration of tryptophan from diet was able to enhance social approach without influencing social novelty preference, it was also able to reduce the excessive grooming and the number of marbles buried in the BTBR mice (W. Q. Zhang et al., 2015). Furthermore, paracetamol, beyond its analgesic effect, also acts as 5HT agonist, and it was able to improve social approach in the three-chamber apparatus (Gould et al., 2012). In addition, different psychostimulant drugs that act on the dopaminergic circuitry, are able to affect the behaviour of BTBR mice, such as the D-amphetamine that increased the number but reduced the duration of social sniffing and reduced the excessive grooming in BTBR mice (Silverman et al., 2013), the, while (−)-trans-9-tetrahydrocannabinol (THC) was able to reduce spontaneous, repetitive wheel running (S. Onaivi et al., 2011).

Regarding the serotonergic system, drug enhancing such neurotransmission, including citalopram (Crowley et al., 2005), fluoxetine and buspirone, demonstrated a reduction of depressive-like behaviour and an increase in sociability (Chadman, 2011; Gould et al., 2011). Moreover, In SERT and 5HT1a receptors binding were observed some differences between BTBR and BL6 mice, but there was no difference in the 5HT2a receptor binding to (Tanimura et al., 2008). Indeed, it has been demonstrated that risperidone a DA/5HT2a,c antagonist/blocker had no effects on social behaviour (Chadman, 2011; Gould et al., 2011), although it decreased marble burying (Gould et al., 2011). In ASD patients' risperidone has been utilized to repress aggression, but had no effect in enhancing the social behaviour (West et al., 2009). Regarding glutamatergic neurotransmission, a decrease in repetitive self-grooming behaviours was detected in BTBR mice after administration of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a mGluR5 receptor antagonist (Burket et al., 2011), which might

suggest that alterations in NMDA signaling may cause excessive self-grooming in these mice. Supporting this, the administration of GRN-529, a GluR5 antagonist, was able to decrease repetitive behaviours in BTBR mice and increase different parameters related to social approach and social interactions (Silverman et al., 2012), while an improvement of GABA neurotransmission induced by diazepam moderated BTBR jump escape, vertical position and crawl under behaviours in the social proximity test (Defensor et al., 2011).

Going deeper to the Excitatory/Inhibitory (E/I) neurotransmission imbalance, it has been proposed as pivotal mechanism in autism-like behaviours (Uzunova et al., 2016). In this direction it was reported abnormalities in glutamate release in BTBR mice, specifically were reported decreased baseline and KCl-evoked glutamate release levels from the cortical synaptoneurosome in both young adult (8 weeks-old) and aged BTBR mice. (H. Wei et al., 2015; H. Wei, Ma, Liu, et al., 2016). Moreover, during the early postnatal period, in the CA3 region of BTBR mice hippocampi, an increase in GABAergic and a reduction in glutamatergic currents was observed (Cellot et al., 2016). Conversely, the frequency of spontaneous inhibitory post-synaptic current was significantly reduced, whereas the amplitude and the frequency of spontaneous excitatory post-synaptic current was increased in BTBR hippocampal slices of adult mice. Both currents were restabilized to a normal function by the treatment with clonazepam, a GABA-A receptor agonist (S. Han et al., 2014). In support of this hypothesis, different pharmacological studies reported that by acting on E/I balance was possible to reverse at least one characteristic of autism-relevant behaviours in the BTBR mouse (Meyza & Blanchard, 2017)

Regarding their hormonal situation, BTBR mice showed increased basal plasma level of corticosterone (Benno et al., 2009; Silverman, Yang, et al., 2010)Although it was not associated by an increase in CRF peptide levels or its mRNA expression after exposure to stressors (Silverman, Yang, et al., 2010), but they had higher levels of glucocorticoid receptors in the CA1 (but not CA2) field of the hippocampus, thus possibly indicating a modified negative feedback loop for the

hypothalamo-pituitary-adrenal axis (HPA axis) (Silverman, Yang, et al., 2010). The study also suggested that elevated basal corticosterone levels might be linked to insulin resistance previously observed in BTBR and to higher levels of plasmatic oxytocin compared to BL6 mice (Silverman, Yang, et al., 2010). Moreover, BTBR female mice also expressed increased circulating progesterone levels and enhanced 5αpregnan-3α-ol-20-one levels in both plasma and hypothalamus (Frye & Llaneza, 2010), suggesting that the higher basal CORT described in these mice may be a consequence of an reduced feedback of 5αpregnan-3α-ol-20-one on the HPA axis. In a similar way, BTBR male mice had higher levels of plasmatic testosterone (Flowers et al., 2007) that from one side may be responsible for their insulin resistance, since testosterone could inhibit the production of adiponectin (A. Xu et al., 2005), and on the other side might stimulate the expression of TNF-α in macrophages (Ashcroft & Mills, 2002), by modulating their immune reactivity.

Furthermore, it was demonstrated that an Oxytocin (OT) signaling deficiency (observed in oxytocin knockout mice, OTKO; oxytocin receptor knockout mice, OTRKO and CD38KO mice where the CD38 enzyme that is a multifunctional enzyme involved in calcium signaling and Nicotinamide Adenine Dinucleotide (NAD+) metabolism, regulates the Ca2+ dependent secretion of oxytocin, was associated to social behaviour impairments. Such deficiency could be counteracted by intranasal supplementation of oxytocin, or by a non-peptide agonists of OTRs or by OT release promoting factors (Modi & Young, 2012).

Oxytocin exerts also a central role to maintain E/I imbalance due to its capability to promote the formation of GABAergic synapses (Theodosis et al., 2006). Indeed, it was reported that mice deficient of oxytocin receptors had a reduced number of GABAergic synapses in hippocampus that led to an increased rate of glutamatergic over GABAergic synapses and an increased convulsion susceptibility, together with impaired social behaviour and cognitive flexibility (Sala et al., 2011). The administration of a single dose of oxytocin was able to enhance the social information maintenance and to decrease repetitive

behaviours in ASD patients (Hollander et al., 2007). In addition, intranasal OT administration had positive effects on empathic accuracy (Guastella et al., 2010) and trust (Andari et al., 2010).However, chronic intranasal oxytocin administration in BTBR mice did not show an improvement in juvenile reciprocal social interactions and it did not influence repetitive self-grooming, open-field exploratory activity or fear-conditioned learning and memory (Bales et al., 2014).

To sum up, several molecular alterations, in terms of neurotrophins, catecholamines and aminoacids, have been described in both animal and human ASD studies, and, from an anatomical point of view, tendency towards overgrowth of PFC and AMY have been found in individuals with ASD compared to neurotypical controls (Bellani et al., 2013b, 2013a; Pardo & Eberhart, 2007). Furthermore, data from structural magnetic resonance imaging highlighted common cerebral abnormalities in ASD patients, that involved total brain volume, fronto-parieto-temporal and cerebellar regions(J. K. Lee et al., 2022).

2.1.3 The role of neuroinflammation in ASD

As widely reported in the first part of the study, neuroinflammation is a well-coordinated inflammatory response within the brain or spinal cord mediated by various groups of glial cells, particularly astrocytes and microglia, that acting releasing inflammatory mediators, namely cytokines, chemokines, reactive oxygen species, and secondary messengers, together with peripherally derived immune cells (DiSabato et al., 2016).

Inflammation and dysregulation of the immune system are clinical signs of ASD (Ashwood et al., 2011; Ashwood & Wakefield, 2006). Several studies theorized that chronic inflammation and neuroinflammation during the period of early brain development could trigger behavioural and cognitive losses, ultimately affecting the etiology of ASD (Ashwood et al., 2011; Liao et al., 2020; Sciara et al., 2020). In ASD children, higher plasma levels of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-17, IL-12p40, and TNF-α, were described (Inga Jácome et al., 2016). Indeed, elevated cytokine levels in plasma were correlated with

seriousness of ASD symptoms, in particular, increasing cytokine levels were related with reduced communications and unusual behaviours (Ashwood et al., 2011; Inga Jácome et al., 2016).

Chronic neuroinflammation might be implicated in the biogenesis of behavioural and cognitive impairments during the early brain development. Indeed, neuroinflammation plays a key role in developing and maintaining the dendritic spines in glutamatergic and GABAergic neurotransmission (Alabdali et al., 2014; El-Ansary & Al-Ayadhi, 2014). In this regard, cytokines can influence the length, location, and organization of dendritic spines on excitatory and inhibitory neurons and recruit and impact glial cell function around the neurons (Eroglu & Barres, 2010), with consequences on the development that may contribute to the ASD behavioural and cognitive symptoms.

It has been reported that acute or chronic maternal immune activation could modify proinflammatory cytokine levels in the fetal environment, by affecting neuroinflammation mechanisms, developing brain, and possibly led ASD development. (Toscano et al., 2021). Both Clinical and preclinical studies have suggested a link between maternal immune activation during pregnancy and the development of ASD (Atladóttir et al., 2009, 2010; A. S. Brown et al., 2014; Choi et al., 2016; B. K. Lee et al., 2015; Rudolph et al., 2018), by reporting a positive correlation between maternal autoimmunity and ASD development (Atladóttir et al., 2009). This association could be related to a mixture of a common genetic background and a possible prenatal mother's antibodies exposure or an alteration in the fetal environment during pregnancy (Atladóttir et al., 2009) that could cross the placenta and affect fetal brain development (Braunschweig et al., 2012; Dalton et al., 2003; Zimmerman et al., 2007).

In ASD patients, astrocytes reactivity was identified in several brain regions. A significant increase in GFAP was described in the *postmortem* superior frontal cortex, parietal cortex, prefrontal cortex, and cerebellum (Edmonson et al., 2014; Laurence & Fatemi, 2005). In addition, GFAP levels were also increased in fresh-

frozen brain tissue of the cerebellum, middle frontal gyrus, and anterior cingulate gyrus from ASD patients (Vargas et al., 2005).

Interestingly, children with ASD may also suffer of autoimmune disorders (Ormstad et al., 2018). It has been suggested that autism symptoms might originate from central nervous system imbalances due to chronic inflammatory reactions, with activation of microglial cells (Bjorklund et al., 2016; Vargas et al., 2005). In support of this hypothesis, ASD children had improved numbers of circulating monocytes, essential precursors for macrophages, dendritic, and microglial cell activation (Rodriguez & Kern, 2011; Sweeten et al., 2003). Microglial cells are the brain immune residence cells and it was described an increase in microglial density in cortices of ASD patients (Morgan et al., 2010; Tetreault et al., 2012; Vargas et al., 2005) and increase activated microglia in the striatum (Morgan et al., 2014), accompanied by no changes in the number of microglia cells in the amygdala (Morgan et al., 2014). Moreover, some methylated genes involved in microglial cell differentiation and synaptic pruning during brain development were affected in ASD (Nardone et al., 2014; The DDD Study et al., 2014). Confirming this, the progenies of the autoimmune ASD mice model showed hypermethylated DNA regions in numerous transcription factor subjects critical for early microglial development and immune activation, and in the cytokines IL-6, IL4, IL-8, and Jak-STAT, TNF, and mTOR signaling (Vogel Ciernia et al., 2018).

Furthermore, samples from cerebrospinal fluid, blood, and *postmortem* brain tissue from ASD patients showed increased level in pro-inflammatory cytokine, together with reduced levels of anti-inflammatory cytokines and activation of microglia with changes in the microglia-neuronal spatial organization (A. S. Lee et al., 2017; Morgan et al., 2010; Rodriguez & Kern, 2011). A major density of microglial cells were observed in the fronto-insular and visual cortex of the autopsy brains of ASD patients compared to control brains (Tetreault et al., 2012). In ASD patients, by using the microglia marker IBA-1 through optical fractionator analysis, it was described a major microglia density in the brain sections of the

dorsolateral prefrontal cortex, with evidently morphological alterations of microglia, including expansion of soma, retraction, and thickening of processes and extension of filopodia (Morgan et al., 2010). The same study had also showed a major microglial cell density in the gray-matter and an increased somal volume of microglia in the white-matter of ASD patients (Morgan et al., 2010), accompanied by signs of excessive microglia activation in amygdala (Morgan et al., 2014).

It usually happens that changes in microglia density can be accompanied by changes in microglia phenotype and morphology. In this regard, in ASD *postmortem* brain temporal cortex, six structurally and functionally microglial phenotypes were identified and quantified with IBA-1 immuno-staining. These results showed a minor density of ramified microglia and a major density of primed microglia in ASD subjects compared with controls (A. S. Lee et al., 2017). In another study using positron emission tomography and a radiotracer for microglia, an enhanced microglia activity in the cerebellum, fusiform gyri, anterior cingulate and orbitofrontal cortices was founded (Suzuki et al., 2013). In addition, a marked activation of microglia and astroglia was described in the cerebral cortex, white matter and in the cerebellum of autistic patients and also the cerebrospinal fluid reported an important increase in the proinflammatory cytokine MCP-1 (Vargas et al., 2005).

Furthermore, some microglial-specific markers, such as TREM2, DAP12, and CX3CR1, had a higher expression in the prefrontal cortex of autistic people compared to controls. Iin particular, the expression of TREM2 was the highest of all microglial markers in brain tissue from ASD patients, although it was lower in autistic than in controls when analyzed in the *postmortem* cerebellum (Edmonson et al., 2014). Another confirming result of neuroinflammatory profile of microglia activation in postmortem brain of ASD patients is the increased levels of proinflammatory cytokines (such as IL-1β, IL-6, IL-8, INF-γ, and TNF-α) described in both brain and blood samples of autistic patients (X. Li et al., 2009; Vargas et al., 2005; H. Wei et al., 2011). In the middle frontal gyrus of ASD

patients an increase in TGF- β1, increased MCP-1, IL-6, and IL-10 in the anterior cingulate gyrus were showed (Vargas et al., 2005). Whereas, regarding the dorsolateral prefrontal cortex of ASD patients, there were no changes in characteristic markers of microglial activation, such as IL-6, IL-1 β, and TNF- α (Chana et al., 2015). Elevated levels of TNF-α have been associated with altered pineal melatonin release and sleep dysfunction in ASD, since it was proposed that circadian dysregulation in ASD is strongly linked to amplified immune-inflammatory activity, that can lead to sleep disorders, cognitive and behavioural alterations (da Silveira Cruz-Machado et al., 2021). Coming back to the proinflammatory cytokines, IL-6 was increased in the frontal cortex and in the cerebellum (X. Li et al., 2009; H. Wei et al., 2011).Moreover, it was described an important increase in Th1 cytokine (IFN-γ), but not in Th2 cytokines (IL-4, IL-5, and IL-10) in brain tissues of ASD, thus indicating that ASD brain can excessively adapt its response through activation of the Th1 pathway compared to the activation of the Th2 pathway (X. Li et al., 2009). Both excitatory and inhibitory synapse formation and transmission were altered by increased levels of IL-6 in shape, length, and distribution of dendritic spines (H. Wei et al., 2012). These studies suggested an unusual microglial-specific gene expression in autistic brains, indicating as a consequence the microglial activation, that might play an important role in ASD development.

Regarding BTBR mice, in this strain they were demonstrated enhanced brain cytokine levels, such as IL-33, IL-18 and IL-1ß, an increased number of CD4+ T cells in the spleen, mesenteric lymph nodes and peripheral blood, and an increased proportion of microglial cells compared to controls (Heo et al., 2011).It has to be taken into account that when there is a Chronic neuro-inflammation, BBB allows the entrance to peripheral cytokines and immune cells in the CNS. Although the effects of peripheral cytokines on the CNS immune environment in ASD has to be fully explained yet, they were described some alteration in gene expression associated with BBB integrity and function (Fiorentino et al., 2016).

2.1.4 The role of oxytocin and microglia in ASD

As previously described, neuroinflammation plays an important role in mechanisms of ASD and other early brain developmental diseases. In addition, oxytocinergic system impairment appears to be crucially involved in ASD and other neurodevelopmental diseases development, particularly such disorders that implicate social behaviours deficits. Recently, possible novel functions of OT for the developing brain are progressively emerging. Indeed, OT seems be able to modulate glial activity in neuroinflammatory states, in particular via the activation of astrocytes and microglia state (Knoop et al., 2022). A meta-analysis of 18 studies, comprising a total of 1422 patients, found lower levels of endogenous OT in the blood, plasma or saliva of children with ASD compared to neurotypical controls (Moerkerke et al., 2021). In this regard, changes in OT system during brain development were reported also in ASD animal model(Knoop et al., 2022).

Furthermore, studies on neuroinflammation, by using interleukin-1β (IL-1β) or lipopolysaccharide (LPS) administration to adult male rats, have demonstrated that these cytokines caused an increase in plasmatic OT levels, and a major activity of the immediate early gene c-FOS in all regions where OT was synthesized (Matsunaga et al., 2000). Similar increase in OT activity were described also in MG6 microglia cells following LPS stimulation (Maejima et al., 2022). Moreover, in a rat model of fetal growth restriction, increased pro-inflammatory cytokine levels and microglial activation were described, accompanied by a downregulation in hypothalamic OT levels in the neonatal pups (Mairesse et al., 2019). These results support a developmental and functional alteration of the OT system in inflammation-related neurodevelopment diseases. This hypothesis piloted some researchers to study whether the social deficits seen in ASD models, could be due to a lack in the hippocampal OT system (Bertoni et al., 2021; Meziane et al., 2015). To this purpose, several studies tested neonatal OT as a therapy in neuroinflammatory and neuroinflammation-related models. (Knoop et al., 2022).

Focusing on OT role on astrocyte and microglia state, the expression of OTR was described in several brain areas including hypothalamus, hippocampus, frontal cortex and amygdala (Baudon et al., 2022). Concerning astrocytes, electrophysiological experiments described a depolarization of the astrocyte membrane potential and an increase in calcium release after activation of the OT-receptor (OTR) (Kuo et al., 2009; P. Wang et al., 2017). In addition, OTR activation in astrocytes could possibly increase local astrocyte network activity (Wahis et al., 2021). Furthermore, the reactive astrocyte phenotype is associated with a decrease in the expression of OTR (Guttenplan et al., 2020; Hasel et al., 2021), indeed, it was reported that OT administration reduced astrocyte-specific GFAP immunoreactivity in the supraoptic nucleus (SON) of healthy adult male rat (P. Wang et al., 2017), and that such effect was related with the inhibition of ERK1/2 kinase and with the activation of protein kinase A (PKA), thus suggesting that the reduced effects of OT in astrocytes might involve the MAPK pathway (Baudon et al., 2022). Subsequently, a possible target for OT modulation of reactive Astrocytes was identified as TGF-β. A link between TGF-β and OT has been found in astrocytes purified from E16 rat embryos and cultured for 12 days, where TGF-β increased the binding of astrocyte OTR and astrocyte OTR mRNA levels (Mittaud et al., 2002). However, OT effects on astrocytes could aim to reduce their neurotoxic effects, since it was showed that OT application to astrocytes was able to cause the retraction of their processes from the synapse in healthy conditions in the rodent hypothalamus (Baudon et al., 2022). This OT-mediated changes in astrocyte cytoskeleton is probably due to an effect on microtubule/actin dynamics (Baudon et al., 2022; Y.-F. Wang & Hatton, 2007). These results might let hypothesize that in neuroinflammatory states, OT-mediated retraction of astrocyte processes from the synapse/gap-junction could form a spatial obstacle in order to avoid astrocytes neurotoxic effect (Baudon et al., 2022).

Regarding microglial response, several studies described that OT treatment was able to induce a shift in microglial phenotype and reactivity during the

inflammatory response (Knoop et al., 2022). Different *in vitro* studies showed that OT pre-treatment decreased the LPS-induced increase in the production of pro-inflammatory cytokines from primary microglia and macrophages (Szeto et al., 2017; L. Yuan et al., 2016). In addition, in OTR knock-out mice an improved microglial activation in the medial amygdala compared to WT mice was reported, as demonstrated by an increased Iba1-immunoreactivity, and a solider presence of amoeboid-shaped cells (Miyazaki et al., 2016). In this regard, there are several possible mechanisms underlying the effect of OT on microglial reactivity during neuroinflammation. Firstly, a possible mechanism could be linked to the reduction of phagocytic activity via NADPH/ROS signaling, since the neurotoxicity mediated by activated microglia is extremely dependent on NADPH oxidase (Block et al., 2007). Supporting this, it was showed that the OT treatment in macrophages and THP-1 monocytes markedly reduced the production of NADPH oxidase-dependent superoxide (Szeto et al., 2008). Secondly, another possible mechanism is represented by the regulation of cytokines secretion via the NF-kB/MAPK pathway. Indeed, the OT-induced reduction in pro-inflammatory cytokines expression in cultured BV-2 murine microglial cell line and primary microglia was related with decreased phosphorylated ERK1/2 and p38 MAPK protein levels (L. Yuan et al., 2016). Strictly related to MAPK protein, there is the NF-kB protein, a transcription factor that regulates pro-inflammatory cytokine production in microglia. Concerning this transcription factor, in a neonatal maternal separation rat model, it was showed that OT pre-treatment was able to reverse the increase in GFAP signaling and TLR4/NF-kB signaling compared to vehicles (S. Xu et al., 2018). Furthermore, in a mouse model of early stage Alzheimer's disease, in which there were an increased in neuroinflammatory levels, it was found that OT release inhibited microglial activation and cytokine secretion by blocking the ERK/p38 MAPK and COX2/iNOS NF-kB signaling pathways (Ye et al., 2022). Taken together, these data suggest that the NF-kB/MAPK pathway could be an important factor contributing to the neuroprotective effect of OT on microglia.

Although OT might be able to influence the reactivity of microglia in neuroinflammation, it has not been clarified yet whether it can influence the phenotype. The presence of OTR expression on microglia are not completely described, but, although not in the neonatal age, several studies described microglial OTR expression predominantly in *in vitro* experiments (Knoop et al., 2022). To report some examples, LPS stimulation of P5 primary microglia induced a reduction in OTR mRNA levels, as assessed with RNA sequencing (Guttenplan et al., 2020), Moreover, LPS stimulation of P1-P2 primary microglia triggered an increase in OTR mRNA and protein levels (L. Yuan et al., 2016). A hypothesis about the effect of OT on microglia could involve the modulation of neurotransmitter systems, such as serotonin. It has been demonstrated in mice that serotonin can modulate microglial reactivity via the microglial 5-HT2b receptor, and that restricted knock-out of this gene in microglia caused a prolongation of the inflammatory response 4 and 24 h post LPS injection (Béchade et al., 2021). Therefore, such modulation could be mediated by OT, by having a positive correlation with serotonin levels and ultimately cause the release of serotonin in several brain regions of the limbic system, including the amygdala (Dölen et al., 2013; Nishimura et al., 2022). OT also upregulates the availability of the 5-HT1A receptor (Lefevre et al., 2017) and this effect is due to the presence of OTR expression on serotonergic cells (Yoshida et al., 2009). These data imply that the serotonergic system is another potential, indirect, pathway of effect between OT and microglial activity in neonatal neuroinflammation. In support of this hypothesis, a study showed only the consequences of 5-HTR2B knockout on neuroinflammation without studying the possible protective effect via increased upregulation of this receptor. (Béchade et al., 2021).

OT may also influence the crosstalk between astrocytes and microglia. In this regard, it has been reported that inflammation caused by LPS injections in adult mice increased TNF-α reactivity levels of both astrocytes and microglia, although pre-treatment with OT was able to reverse only the TNF-α immunoreactivity of microglia and did not affect astrocytes, thus suggesting that the anti-

inflammatory effect of OT may be independent of astrocytic reactivity in adults (L. Yuan et al., 2016).Therefore, it could be hypothesized that OT treatment during inflammatory states might affect the number of astrocytes, but not their phenotype. However, it has been showed that OT injections between P2 and P6 in healthy rats enhanced the expression of hippocampal GFAP mRNA and protein two months later, without reporting changes in CD68 levels, a marker of microglial phagocytic activity (Havránek et al., 2017), hence hypothesizing that the effects of OT on astrocytes and microglia are dependent on the presence of inflammation (Bordt et al., 2019). Furthermore, positive link between TGF-β and OT has been suggested (Mittaud et al., 2002)., since TGF-β can also have a role on microglial cells by binding to the TGF-β receptor type II, which phosphorylates TGF-β receptor type I (Zöller et al., 2018). In addition, it was described that TGF-β secretion reduced microglial activation by decreasing microglial production of pro-inflammatory cytokines, NO and oxygen-free radicals (W. Liu et al., 2011). Moreover, an *in vitro* study reported that, in baseline conditions, astrocytes involved TGF-β2 to exert an immune-regulatory control on microglia, which repressed the microglial response to low inflammatory stimuli (Baxter et al., 2021).

2.1.5 β-Carotene impact on neuroinflammation and microglia

β-carotene belongs to the category of carotenoids, plant pigments that are precursors of vitamin A (retinol). As it has been well shown, carotenoids exert many beneficial effects. Indeed, they are principally known for their antioxidant properties, being main scavengers of ROS (Saini et al., 2015). B-carotene exhibits different pharmacological effects, such as anti-inflammatory, antioxidant and anti-tumoral (Liebler et al., 1997; Y. Yang et al., 2021; Y. Zhang et al., 2016). Several *in vitro* and *in vivo* studies reported that carotenoids can be involved in a variety of processes associated to the immune inflammatory response. In this regard, it has been described that these compounds influence both cellular and humoral mechanisms of immunity (J. S. Park et al., 2010; Seyedzadeh et al., 2014).Concerning anti-oxidant properties, it has been shown that β-carotene was

able to prevent the upregulation of heme oxygenase 1 expression in human skin fibroblasts (FEK4) exposed to UV-A (El-Agamey et al., 2004; Jomova & Valko, 2013), although beingless effective in preventing lipid peroxidation (El-Agamey et al., 2004). In addition, β-carotene was able to counteract the production of NO, prostaglandin E2 and superoxide dismutase, while downregulating the expression of iNOS/cox-/NADPH oxidase proteins and mRNA, associated by inhibition TNF-α, thus showing important anti-inflammatory properties(Hadad & Levy, 2012). Moreover, β-carotene treatment reduced the oxidation level in porcine intestinal epithelial cells induced by LPS, by reducing the expression of Caspase-3 and mitigating the inflammatory response through the inhibition of NF-kB, JK2/STAT3 and JNK2/p38MAPK signaling pathways in macrophages (R. Li et al., 2019). Interestingly, a study of network pharmacology, by using molecular docking analysis, identified potential targets and possible signal pathways of the anti-inflammatory effects of β-carotene, by showing a good affinity with TNF, IL1B, and Leptin (LEP) (S. Wu et al., 2023).

Furthermore, the protective role of β-carotene against oxidative stress and neuroinflammation was demonstrated in a rat model of spinal cord injury, where β-carotene reduced the production of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-18 and COX-2, and repressed the activation of astrocyte in the spinal cord, acting a strong inhibition of the NF-κB pathway activation (L. Zhou et al., 2018). In addition, β-carotene also showed a protective effect against myeloperoxidase-mediated oxidative stress and inflammation in a rat ischemic brain injury model, by reducing NF-kB and myeloperoxidase activity in the brain. Moreover, β-carotene was able to significantly block Bcl-2-associated X protein and caspase-3 expression, while upregulated B-cell lymphoma-2 expression, suggesting a neuroprotective role (Althurwi et al., 2022).

Several studies reported that antioxidant and anti-inflammatory properties of carotenoids might improve the efficiency of cognitive function, by increasing neuronal efficiency or stabilizing the lipid-protein bonds in neuronal membranes (Kaulmann & Bohn, 2014; Mohammadzadeh Honarvar et al., 2017; Sorrenti et al.,

2020; Sujak et al., 1999). In addition,other neuroprotective mechanisms of carotenoids, such as improvement of communication between gaps and modulation of the functional properties of synaptic membranes (Stahl & Sies, 2001; Sujak et al., 1999), have been described. Interestingly, carotenoids might play a protective role in the development of depression through various mechanisms (Rasmus & Kozłowska, 2023), including a possible allosteric activation of BDN (S.-J. Park et al., 2021). Indeed, concerning depressive context, low levels of BDNF probably influenced by pro-inflammatory cytokines, such as IL-6 and TNF-α ,might have a key role in the onset of such diseases (Videbech & Ravnkilde, 2004). Although further studies are necessarily required, it has been hypothesized that carotenoid could promote the BDNF activation and, considering their antioxidant and anti-inflammatory effects, they might ultimately exert a promising antidepressant effect.(Rasmus & Kozłowska, 2023).

Recently, different studies revealed that patients with ASD have reduced levels of oxytocin and his regulatory ectoenzyme CD38. Since β-carotene is an inducer of the CD38 enzyme (Avraham et al., 2019), thereduced CD38 expression, and consequent reduced OT levels, observed in ASD might be counteracted by β-carotene treatment, suggesting its use as a new therapeutic strategy in the treatment of ASD (Ebstein et al., 2011, 2012; Riebold et al., 2011). All trans-retinoic acids are potent CD38 inducers, but they are also toxic and teratogenic. Hence, β-carotene, being not toxic and not teratogenic, could represent a good safe and preventive strategy for ASD.

2.2 Aims of the Study

In this study, we used the BTBR strain, an idiopathic model of ASD, to better understand the complex mechanisms underlying ASD behavioural dysfunctions and, subsequently, to develop a possible preventive strategy.

In the first part of the study, we focused on BTBR behavioral abnormalities resembling human ASD symptoms and we investigated neurochemical and

biomolecular alterations underlying such behavioural alterations in brain regions crucially involved in ASD, such as PFC and HIPP.

In the second part of the study, we aimed to assess the effect of a possible preventive treatment, by using β-carotene administration during pregnancy, on the core ASD symptoms, such as stereotyped repertoire and social impairments, occurring in BTBR offspring. In addition, we evaluated the effects of β-carotene treatment on the modulation of neuroinflammatory components of ASD.

2.3 Materials and Methods

2.3.1 Animals

The study was focused on male sex since ASD shows a male prevalence, being males three times more diagnosed than females (Loomes et al., 2017). In the first part of the study, a total of 20 (2-3 per litter, randomly distributed) 10-weeks-old male mice, C57/BL6J (BL6) (Envigo, San Pietro al Natisone, Italy) and BTBR (Charles-River Italia, Milan, Italy), were used in this experimental study. They were raised at constant room temperature (22 ± 1°C) with relative humidity (55 ± 5%), under a 12 h light/dark cycle (lights on from 7:00 AM to 7:00 PM) and free access to water and food. All experiments on animals and their care were carried out in accordance with the institutional guidelines of the Italian Ministry of Health (D.Lgs. n. 26/2014), the Guide for the Care and Use of Laboratory Animals: Eight Edition, the Guide for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council, 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific intents, as defined by Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines 2.0. The experimental protocol was approved by the Italian Ministry of Health (protocol nr. B2EF8.24). Animals' health state was checked daily during experimental period. Moreover, to accomplish the 3R's principles, we performed the behavioural test battery on the same group of animals; in order to reduce as

much as possible, the number of animals used, and every procedure was performed with the aim to minimize their suffering.

2.3.2 β-Carotene Treatment

In the second part of the study was performed a treatment with β-carotene on BTBR mice. The treatment with β-carotene was performed through prenatal supplementation ant it was carried out at different doses 8, 16, 32 and 64 mg/kg subcutaneously in seed oil in pregnant mothers twice a week during the three weeks of pregnancy on five female BTBR mice for each dosage and vehicle respectively.

2.3.3 Battery of behavioural tests

2.3.4 Marble burying test

During day 1, The marble burying test was performed in an unfamiliar standard polycarbonate cage (26 x 48 x 20 cm) filled with unscented bedding material distributed in an even layer that was 5 cm deep. Twenty-four marbles were positioned inside the cage without food and water. Each mouse was allowed to explore the cage for 30 min and then returned to its home cage. At the end of the test, an experimenter counted marbles buried, scoring only those buried by 2/3 or more of its surface.

2.3.5 Hole board test

During day 2, animals performed the Hole Board task. This test was carried out in a wooden box (40 x 40 x 35 cm) having 16 holes with a diameter of 3 cm on the ground and placed 5 cm from the floor, as described in literature (Souza et al., 2016). The test lasted 10 min and an automatic counter registered the number of times the animals poking the hole for a duration of at least 1 sec, reported as number of poking holes.

2.3.6 Open Field test

During day 3, the Open Field test was performed. The test was executed according the literature (Carola et al., 2002). Briefly, the animals were left to explore an open field arena (40 x 40 x 35 cm) for 5 minutes. ANY-maze tracking

software version 7 (Ugo Basile-Varese, Gemonio, Italy) recorded and analyzed the locomotory activity of each mouse by measuring the distance travelled, the duration and the frequency of freezing behaviour and the time spent in the center and in the wall of the arena. Between one test and another, a solution at 70% of ethanol was used to clean arena floor and avoid inter-assay bias.

2.3.7 Social Interaction test

During day 4, the Social Interaction test was performed, as described in literature (Bove et al., 2022). The animals, after 2 days of individual housing, were left in the same large box used for the Open Field arena and let free to explore one plastic object, one paper cylinder, one ball and an unfamiliar stimulus mouse (same strain, sex and age of subject mouse) for 5 minutes. A camera recorded the test and a blind observer scored the frequency and duration of social behaviours from an investigative and affiliative point of view and also frequency and duration of non-social behaviour, considering the attitude towards objects, like sniffing, exploring and playing with the objects. (Kraeuter et al., 2019; Laviola et al., 2004).

2.3.8 Elevated Zero Maze test

During day 5, the Elevated Zero Maze test was performed, as described in literature(Tucker & McCabe, 2017). Precisely, a maze built in black acrylic in a circular track 10 cm wide, 105 cm in diameter and 72 cm high was used. Two opposed closed quadrants and two opposed open quadrants with black acrylic walls 28 cm high composed the maze. On the test day the animal was placed at a casually chosen boundary between an open and a closed zone, facing the closed area. After each trial, the maze was cleaned with a 70% ethanol solution. The test lasted 5 minutes and a blind observer evaluated the time spent in the open and in the closed corridors, expressed in seconds.

2.3.9 Post-mortem tissues analyses

After behavioural tests, the mice were sacrificed by cervical dislocation and brains were randomly divided for ex vivo analysis. PFC and HIPP were removed

from brains, in accordance with the mouse brain atlas of Paxinos and Franklin, and frozen, stored at -80°C for subsequent analyses. To perform biomolecular studies, the tissues were homogenated and diluted 1:10 w/v in PBS buffer with 1:100 protease and phosphatase inhibitor (HALT inhibitors, Thermo Fisher Scientific, Cleveland, OH, USA) at 4 °C; while for neurochemical analyses, the samples were homogenated and diluted 1:10 w/v in perchloric acid 0.1M at 4 °C. In both cases, after dilution, a centrifuge at 10.000 x g at 4 °C for 10 min was carried out and the supernatants were analyzed.

2.2.10 Western blotting quantification

In this procedure, protein extracts were generated from fresh frozen PFC and HIPP tissues following samples homogenization with a Halt™ Protease and Phosphatase Inhibitor Single-Use Cocktail, EDTA-Free (Thermo Fisher Scientific, Cleveland, OH, USA). Subsequently, lysates were measured for total protein concentration using a Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Cleveland, OH, USA) and the Multiskan™ FC Microplate spectrophotometer (Thermo Fisher Scientific, USA) at 570 nm. For SDS-PAGE the total amount of samples protein (40 µg) loaded in to the 4–15% Mini-PROTEAN™ TGX Stain-Free™ Protein Gels (Bio-Rad Laboratories Inc, Segrate (MI), Italy) for electrophoresis and then transferred on the Nitrocellulose membrane (Bio-Rad Laboratories Inc, Segrate (MI), Italy) by Pierce™ Power Blotter (Thermo Fisher Scientific, Cleveland, OH, USA). Then, the membranes were blocked in the 5% skimmed milk for 1 h at room temperature followed the incubation with rabbit monoclonal antibodies against NGF (ab52918; 1:1000, Abcam, Cambridge, UK), BDNF (ab226843; 1:1000, Abcam, Cambridge, UK), NF-kB (Ab16502; 1:1000, Abcam, Cambridge, UK), Occludin (Ab216327; 1:750, Abcam, Cambridge, UK), CD11-β (Ab133357; 1:1000, Abcam, Cambridge, UK), IBA-1 (Ab178846; 1:500, Abcam, Cambridge, UK), and mouse monoclonal antibodies against β-actin (ab8226; 1:1000, Abcam, Cambridge, UK), GFAP (Ab279290; 1:1000, Abcam, Cambridge, UK); overnight at 4°C. In add to this, the incubation (1 h at room temperature) with horseradish peroxidase-conjugated specific (Goat anti-rabbit (ab6721; 1:5000, Abcam, UK)

and goat anti-mouse (ab205719; 1:5000, Abcam, UK)) secondary antibodies were used. Clarity™ Western ECL Substrate (Bio-Rad Laboratories Inc, Segrate (MI), Italy) was used for protein bands visualization. ChemiDoc™ XRS+ system (Bio-Rad Laboratories Inc, Segrate (MI), Italy) was used to detect chemiluminescence and ImageJ software (version 1.52a; National Institutes of Health, USA) was utilized to quantify the optical densities of the bands that were then normalized versus bands of β-actin.

2.3.11 Neurochemical quantifications

The neurochemical quantifications were carried out in PFC and HIPP of BTBR and BL6 animals. In particular DA, NA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and Kynurenine (KYN) were measured by using high-performance liquid chromatography coupled with an electrochemical detector (Ultimate ECD, Dionex Scientific, Milan, Italy). LC18 reverse phase column (Kinetex, 150mm x 3.0 mm, ODS 5 µm; Phenomenex, Castel Maggiore-Bologna, Italy) was utilized to separate the catecholamines that were detected by a thin-layer amperometric cell (Dionex, ThermoScientific, Milan, Italy) with a 5-mm diameter glassy carbon electrode by using 400 or 750 mV as working potential vs. Pd. An aqueous buffer (pH 3.0) composed of 75 mM NaH2PO4, 1.7 mM octane sulfonic acid, 0.3 mM EDTA, acetonitrile 10%, was used as mobile phase and an isocratic pump (Shimadzu LC-10 AD, Kyoto, Japan) worked at 0.7 ml·min-1 as flow rate. To perform the data acquisition and integration, Chromeleon software (version 6.80, Dionex, Thermo Scientific, San Donato Milanese, Italy) was utilized. Furthermore, GABA and Glutamate amounts were quantified by high-performance liquid chromatography with fluorescence detection, after derivatization with ophthalaldehyde/mercaptopropionic acid (emission length, 460 nm; excitation length, 340 nm) by using an OD column (Kinetex, 150mm x 3.0 mm, ODS 5 µm; Phenomenex, Castel Maggiore-Bologna, Italy). The mobile phase used was a gradient phase of 50 mM sodium acetate buffer, pH 6.95, with methanol increasing linearly from 2 to 30% (v/v) over 40 minutes. A pump (JASCO, Tokyo, Japan) maintained the flow rate at 0.5 ml/min and the Borwin

software (version 1.50; Jasco) was used to analyze the results. Results, after being normalized for total area weight, were expressed as concentration/mg of tissue.

2.3.12 Acetylcholine (ACh) assay

The levels of ACh were quantified in tissue homogenized by using the commercially available kit (Catalog Number MAK435, Sigma-Aldrich, Milano, Italy), following the manufacturer's instructions. Tissue has been prepared by homogenization in cold 1× PBS and centrifugation (5 minutes at 14,000 × g). In the assay utilized, acetylcholine is hydrolyzed by acetylcholinesterase to choline which is oxidized by choline oxidase to betaine and H_2O_2. H_2O_2 reacts with a specific dye to form a colored product. The color intensity was analysed by the Multiskan™ FC Microplate spectrophotometer (Thermo Fisher Scientific, USA) at 570 nm and it is directly proportional to the acetylcholine concentration in the sample. Results were expressed in accordance to the linear detection range for the acetylcholine assay method (10-200 µM). Each sample analysis was performed in duplicate to avoid intra-assay variations.

2.3.13 Enzyme-Linked Immunosorbent Assays (ELISA)

Plasma samples were collected after centrifuge to 10000g at 4 °C of fresh blood samples and were stored at -80 °C until the analysis were performed. Commercially available ELISA kits were used for measurement of Oxytocin (EIAM-OXT-1, RayBiotech, Peachtree Corners, GA, USA), Prolactin (ELM-Prolactin-1, RayBiotech, Peachtree Corners, GA, USA) and Vasopressin (EIAM-VAS-1, RayBiotech, Peachtree Corners, GA, USA) levels, according to the manufacturer's instructions. In order to avoid intra-assay variations, all samples and standards were analyzed in duplicate.

2.3.14 Blindness of the study

For each test, scoring process and analysis, the experimenters were blind with respect to the experimental groups.

2.3.15 Statistical analyses

Sample size calculation has been performed a priori by using G power software. Statistical analyses were carried out by GraphPad Prism software (version 9.5.0; San Diego, CA, USA). In particular, Shapiro-Wilk test for normality and ROUT method to identify statistical outliers were performed for each group. Subsequently, Unpaired Student's t-test two-sided, with Welch's correction when needed, was used to analyze data that were expressed as mean ± standard error of the mean (SEM). When the group were more than two, was performed One-way ANOVA followed by Tukey's multiple comparisons test.

2.4 Results

2.4.1 BTBR mice showed increased repetitive behaviour and novelty-induced hyperlocomotion.

To assess repetitive behaviours, the Hole Board test was performed on BTBR and BL6 mice. The results obtained showed that BTBR mice reported a significant increase in the number of poking holes respect to control mice (Fig. 1A). Moreover, the locomotor activity in a novel environment was explored in the Open Field paradigm. We observed an increase of the distance travelled in the arena (Fig. 1B), in BTBR mice compared with control animals. Additionally, BTBR mice also showed a reduction in freezing duration (Fig. 1D) and freezing frequency (Fig. 1C) compared with BL6 animals.

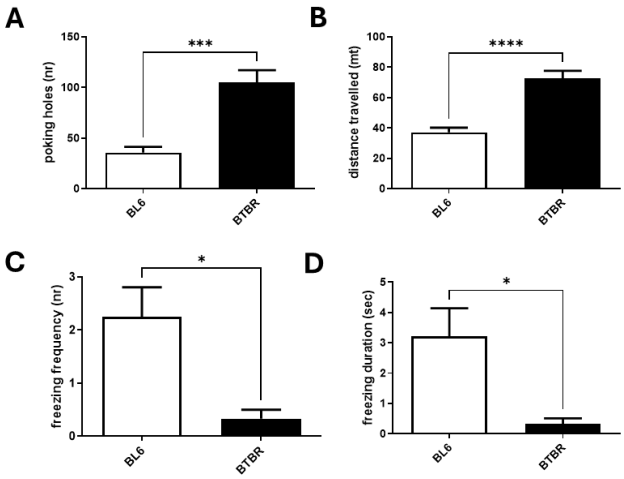

Fig. 1. Repetitive and stereotyped behaviours of BTBR mice. A) Number of pooking holes in Hole Board task, Unpaired Student's t-test, P<0.001 BTBR vs. BL6. **B)** Distance travelled in the Open Field paradigm, Unpaired Student's t-test, P < 0.0001 BTBR vs. BL6. **C)** Freezing duration in Open Field paradigm, Unpaired Student's t-test, P < 0.05 BL6 vs. BTBR **D)** Freezing frequency in Open Field paradigm, Unpaired Student's t-test, P < 0.05 BL6 vs. BTBR.

2.4.2 BTBR mice showed reduced social behaviours and increased non-social behaviours

The Social Interaction test was performed to explore social dysfunctions. During the test, BTBR mice demonstrated significantly less duration of social interaction (sec) compared to BL6 mice (Fig. 2A). Furthermore, the time employed (sec) performing non-social interactions in BTBR animals was increased against controls (Fig. 2B). Observing the social frequency time, measurements of BTBR mice were significantly decreased (Fig. 2C), while there was a significant improvement in non-social frequency vis-a-vis control (Fig. 2D).

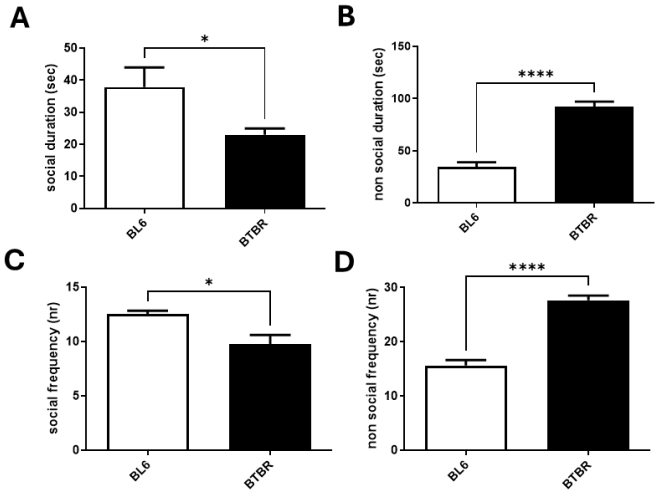

Fig. 2. Social-related behaviours of BTBR mice. A) Time spent (sec) in Social Interaction test, Unpaired Student's t-test, $P < 0.05$ BTBR vs. BL6. **B)** liTime spent (sec) in non-social interactions, Unpaired Student's t-test, $P < 0.0001$ BTBR vs. BL6. **C)** Social frequency measurements in social interaction Test, Unpaired Student's t-test, $P < 0.05$ BL6 vs. BTBR. **D)** Non-social frequency, Unpaired Student's t-test, $P < 0.0001$ BTBR vs. BL6.

2.4.3 BTBR mice did not show anxiety-like behaviours

To evaluate the anxiety-like behaviours, were performed the Elevated Zero Maze and the Open Field tests. In contrast to control animals, BTBR animals showed an increase in the time spent exploring the open arms compared to controls (Fig. 3A). In addition, results from Open field test showed that the time spent in the center in BTBR animals was significantly augmented respect to control animals (Fig. 3B), whereas the time spent in the wall was a significantly reduced (Fig. 3C), respectively.

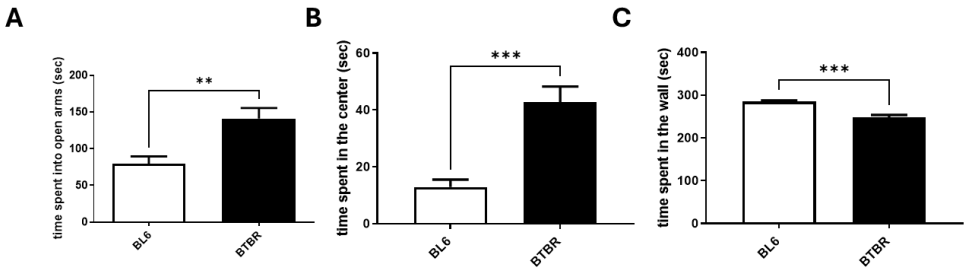

Fig. 3. Anxiety-related behaviours of BTBR mice. A) Time spent (sec) exploring the open corridors in Elevated Zero Maze, Unpaired Student's t-test, $P < 0.01$ BTBR vs. BL6. **B)** Time spent (sec) in the center in Open field test, Unpaired Student's t-test, $P < 0.001$ BTBR vs. BL6. **C)** Time spent (sec) in the wall in Open Field test, Unpaired Student's t-test, $P < 0.001$ BTBR vs. BL6).

2.4.4 BTBR mice showed reduced NA, ACh and GABA levels, and increased Kyn levels in PFC

The results report a significantly reduction of NA levels in the PFC of BTBR animals related to BL6 (Fig. 4A), similarly also ACh (Fig. 4E) and GABA (Fig. 5B) levels was reduced in the BTBR mice. Concerning Kyn, its levels were increased in BTBR mice respect to the control animals (Fig. 5C), whereas levels of DA (Fig. 4B), DOPAC (Fig. 4C), HVA (Fig. 4D), NGF (Fig. 6A), BDNF (Fig. 6B) and glutamate (Fig. 5A), were not significantly different between the two groups.

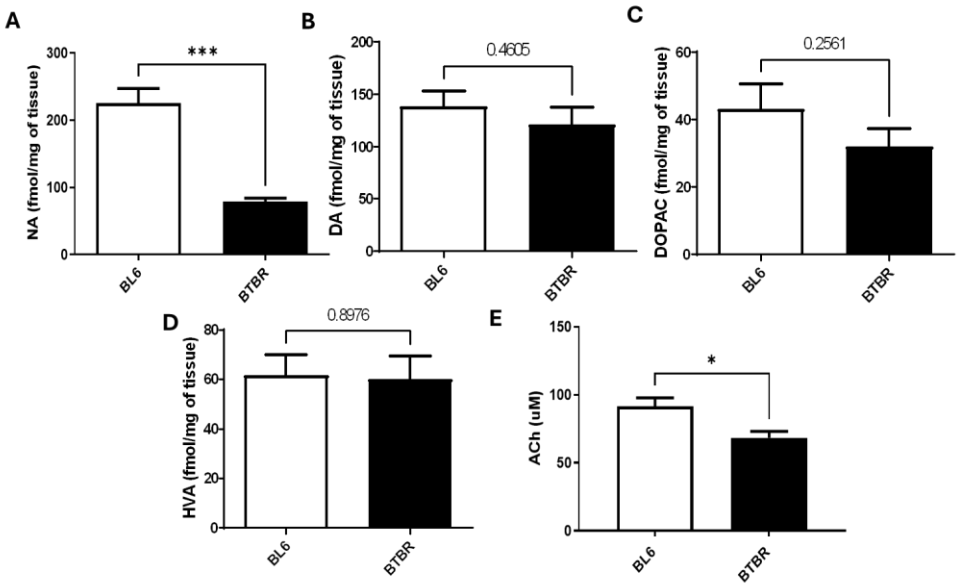

Fig. 4. **Catecholamines and Acetylcholine levels in PFC of BTBR and BL6 mice**. **A)** NA levels, Unpaired Student's t-test, P < 0.0001 BTBR vs. BL6. **B)** DA levels, Unpaired Student's t-test, n.s. **C)** DOPAC levels, Unpaired Student's t-test, n.s. **D**) HVA levels, Unpaired Student's t-test, n.s. **E)** Achlevels, Unpaired Student's t-test, P < 0.05 BTBR vs. BL6

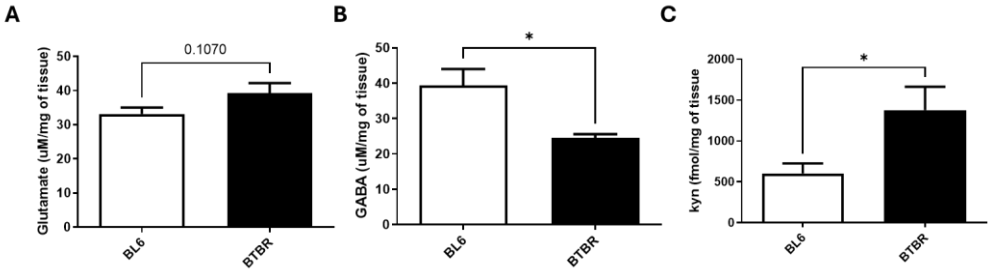

Fig. 5. **Glutamate, GABA and Kyn levels in PFC of BTBR and BL6 mice. A)** Glutamate levels, Unpaired Student's t-test, n.s. **B)** GABA levels, Unpaired Student's t-test, P < 0.05 BTBR vs. BL6. **C)** Kyn levels, Unpaired Student's t-test, P < 0.05 BTBR vs. BL6.

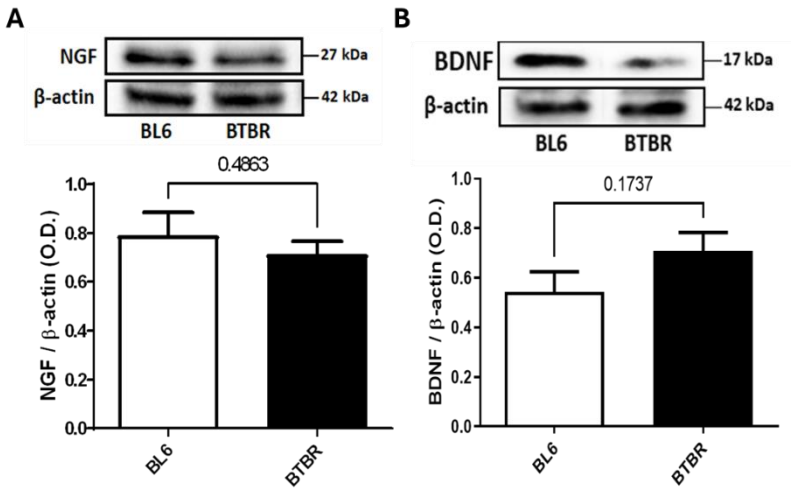

Fig. 6. **NGF and BDNF expression levels in PFC of BTBR and BL6 mice. A)** NGF representative bands and expression levels, Unpaired Student's t-test, n.s. **B)** BDNF representative bands and expression levels, Unpaired Student's t-test, n.s.

2.4.5 BTBR mice showed enhanced DOPAC and Kyn levels, and decreased NGF, BDNF and GABA levels in HIPP

In HIPP, the results did not show any difference in NA (Fig. 7A), DA (Fig. 7B), HVA (Fig. 7D), Ach (Fig. 7E) and glutamate (Fig. 8A) levels between BTBR and the control groups. Furthermore, analysis of DOPAC levels reported a significant increase in BTBR animals against controls (Fig. 7C). Whereas GABA levels were decreased (Fig. 8B). Regarding neurotrophins, both NGF and BDNF expression levels were reduced (Fig 9 A-B). Lately, Kyn levels showed an increase in BTBR mice respect to the control mice (Fig. 8C).

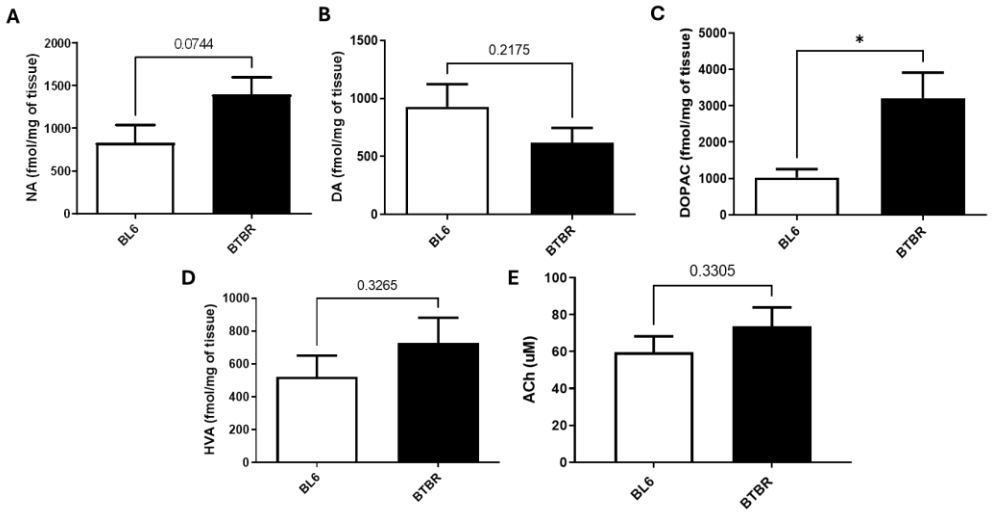

Fig. 7. **Catecholamines and Acetylcholine levels in HIPP of BTBR and BL6 mice**. **A)** NA levels, Unpaired Student's t-test, n.s. **B)** DA levels, Unpaired Student's t-test, n.s. **C)** DOPAC levels, Unpaired Student's t-test, P < 0.05 BTBR vs. BL6. **D**) HVA levels, Unpaired Student's t-test, n.s. **E)** Ach levels, Unpaired Student's t-test, n s.

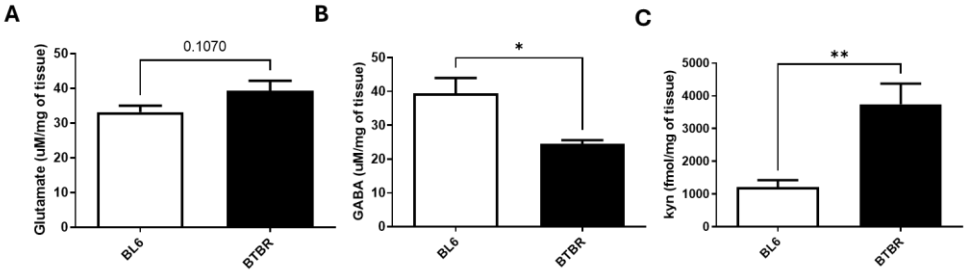

Fig. 8. Glutamate, GABA and Kyn levels in HIPP of BTBR and BL6 mice. A) Glutamate levels, Unpaired Student's t-test, n.s. **B)** GABA levels, Unpaired Student's t-test, P < 0.05 BTBR vs. BL6. **C)** Kyn levels, Unpaired Student's t-test, P < 0.01 BTBR vs. BL6.

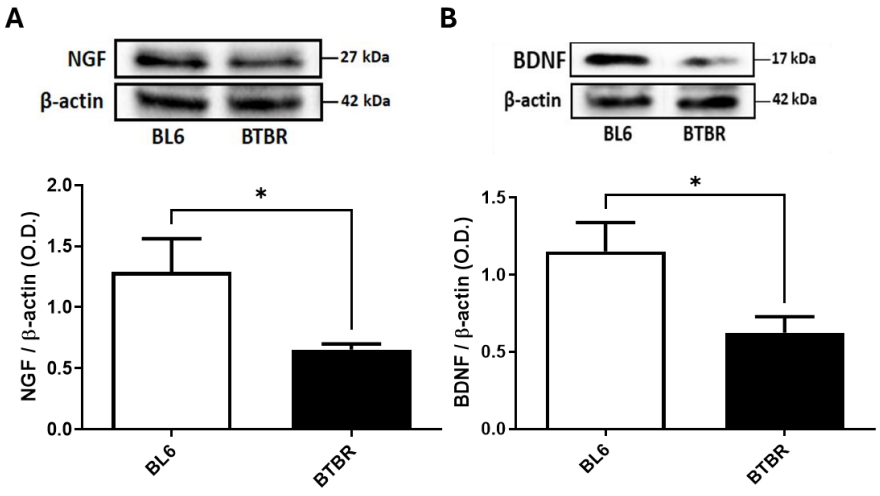

Fig. 9. NGF and BDNF expression levels in HIPP of BTBR and BL6 mice. A) NGF representative bands and expression levels, Unpaired Student's t-test, $P < 0.05$ BTBR vs. BL6. **B)** BDNF representative bands and expression levels, Unpaired Student's t-test, $P < 0.05$ BTBR vs. BL6.

BTBR mice showed decreased Oxytocin plasma levels.

Oxytocin levels were measured in plasma using an ELISA kit. The results demonstrated that control indicators of Oxytocin plasma levels in the BTTR group exceeded those of the control group.

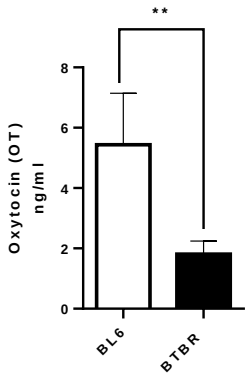

Fig. 10. Oxytocin levels in Plasma of BTBR and BL6 mice. Oxytocin plasma levels, Unpaired Student's t-test, $P < 0.01$ BTBR vs. BL6.

2.4.6 β-Carotene treatment was able to reduce repetitive and stereotyped behaviours of BTBR mice

In order to evaluate the effect of β-Carotene administration on repetitive and stereotyped behaviours, the Marble Burying test, the Hole Board test and the Open Field test were performed in the offspring of treated and vehicle dams. The results obtained showed that the administration of β-carotene in pregnant mice at a dose of 16 mg/kg was able to significantly reduce the duration (Fig. 11A) and frequency of grooming (Fig. 11C), the number of buried marbles (Fig. 11B) and the number of poking holes (Fig. 11D) in male offspring compared to offspring of pregnant mice treated with vehicle (seed oil) alone. Regarding the other dosages, the administration of β-carotene at a dose of 32 mg/kg led to a significant reduction in the frequency of grooming (Fig. Fig. 11C) and the 8 mg/kg dose significantly reduced the number of poking holes (Fig. 11D). Therefore, supplementation with β-carotene at a dose of 16 mg/kg was the most effective in preventing repetitive behaviours in BTBR mice. In addition, in the Open Field test, the total distance travelled, an index of locomotor activity, was measured, the results showed that in male offspring treated with β-carotene at a dose of 16 mg/kg was able to significantly reduce the distance traveled (Fig. 11E) compared to controls and doses of 32 mg/kg and 64 mg/kg.

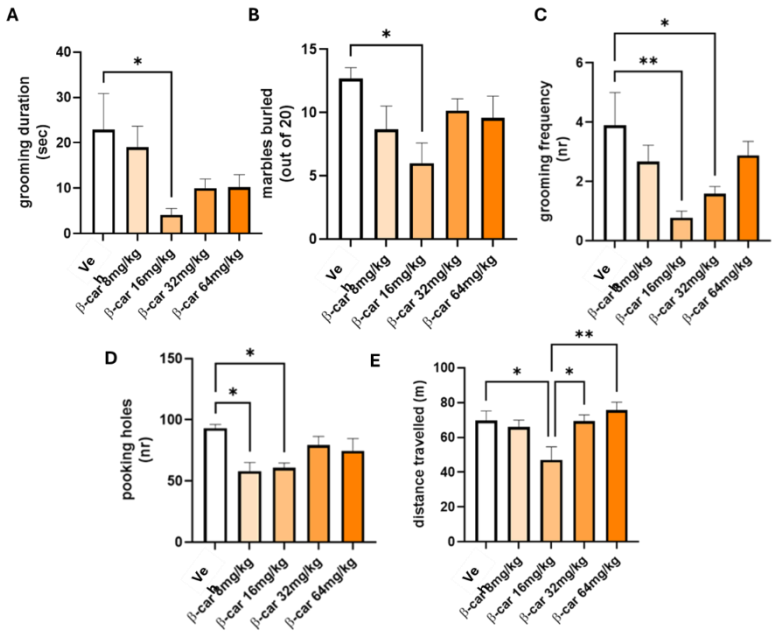

Fig. 11. Repetitive and stereotyped behaviours in BTBR dams treated with vehicle or β-carotene. A) Grooming duration, One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 16 mg/kg vs VEH. **B)** Number of marbles buried, One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 16 mg/kg vs VEH). **C)** Grooming Frequency, One-way ANOVA followed by Tukey's multiple comparisons test, P<0.01 β-car 16 mg/kg vs VEH and One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 32 mg/kg vs VEH. **D)** Number of Pooking holes in hole board test, One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 8 mg/kg vs VEH and One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 16 mg/kg vs VEH. **E)** Distance travelled in Open Field paradigm, One-way ANOVA followed by Tukey's multiple comparisons test, P<0.05 β-car 16 mg/kg vs VEH.

2.4.7 β-Carotene Treatment enhanced social related behaviours of BTBR mice

To evaluate the social related behaviours the social interaction test was performed. The results obtained showed that supplementation with β-carotene at a dose of 16 mg/kg was able to increase the frequency of social interactions in male offspring compared to control mice and the other three doses (Fig. 12B). Regarding the duration of the non-social interactions with the three objects, β-carotene doses of 32 mg/kg and 64 mg/kg administered to pregnant mice were able to decrease this parameter in male offspring compared to control progeny (Fig. 12C).

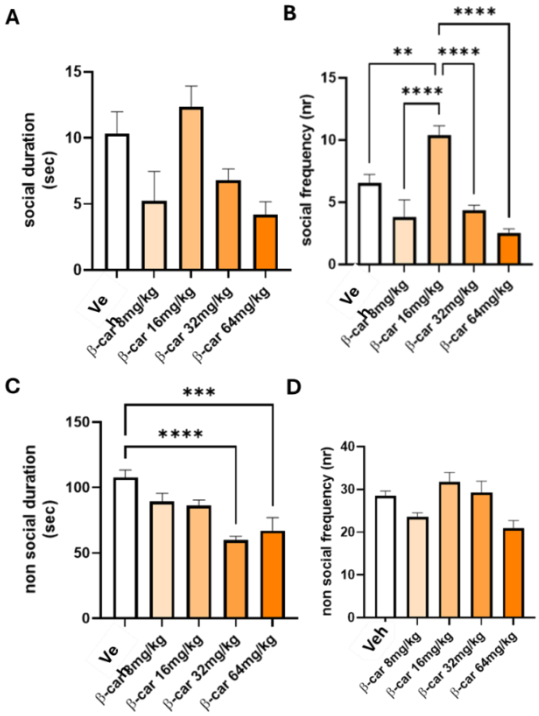

Fig. 12. BTBR dams treated with vehicle or β-carotene in the social interaction test. A) Time spent (sec) in social interactions, One-way ANOVA followed by Tukey's multiple comparisons test β-car 16 mg/kg vs VEH n.s. **B**) Frequency of social interactions, One-way ANOVA followed by Tukey's multiple comparisons test, ****P<0.0001 β-car 16 mg/kg vs β-car 8 mg/kg, β-car 32 mg/kg and β-car 64 mg/kg; **P<0.01 β-car 16 mg/kg vs VEH. **C)** Time spent (sec) on non social interactions, One-way ANOVA followed by Tukey's multiple comparisons test, ****P<0.0001 β-car 32 mg/kg vs CTRL; ***P<0.001 β-car 64 mg/kg vs VEH. **D)** Frequency of non social interactions, One-way ANOVA followed by Tukey's multiple comparisons test β-car 16 mg/kg vs VEH n.s.

2.4.8 β-Carotene Treatment increased plasmatic Oxytocin levels BTBR mice

Based on behaivoural results we choose the dose of 16 mg/kg to perform *ex-vivo* analysis. In particular we performed analysis of oxytocin, prolactin and Vasopressin on plasma samples. Among the factors examined the B-Carotene treated dams did not show differences in Prolactin and Vasopressin levels compared to the Vehicle treated dams (Fig. 13 B-C). At the same time, treatment with β-carotene produced an increase in oxytocin levels in the male offspring from β-carotene treated dams compared to the offspring from vehicle treated dams (Fig. 13A).

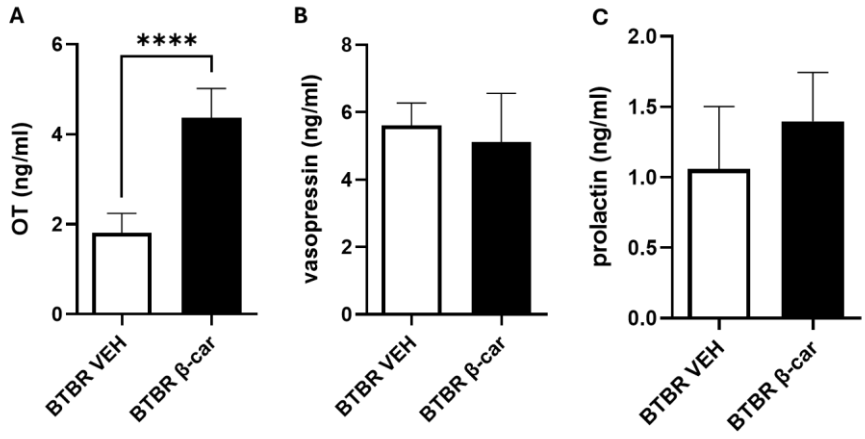

Fig. 13. Effects of β-carotene treatment on Oxytocin, Vasopressin and Prolactin levels of BTBR mice. A) Oxytocin plasmatic levels, Unpaired Student's t-test, P < 0.0001 β-car 16 mg/kg vs VEH. **B)** Vasopressin plasmatic levels, Unpaired Student's t-test n.s**. C)** Prolactin plasmatic levels, Unpaired Student's t-test n.s**.**

2.4.9 β-Carotene treatment decreased the Kynurenine levels in PFC of BTBR mice

To evaluate the effect of β-Carotene treatment on some markers related to BBB function and neuroinflammation in PFC, NF-kB, BDNF, Kynurenine, Occludin, GFAP, CD11-b and IBA-1 were quantified. The results obtained did not show any substantial differences in the expression levels of NF-kB (Fig. 14A) and BDNF (Fig. 14B) between offspring from β-Carotene treated dams and offspring of vehicle treated dams. Regarding instead the kynurenine in PFC the offspring of the β-carotene treated dams showed a significant decrease in the kynurenine levels compared to offspring of vehicle treated dams (Fig. 14C). Furthermore, the results obtained did not show any substantial differences in the expression levels of Occludin (Fig. 15A), GFAP (Fig. 15B), CD11-b (Fig. 15C) and IBA-1 (Fig. 15D) in the offspring of β-carotene from treated dams compared to the offspring from vehicle treated dams.

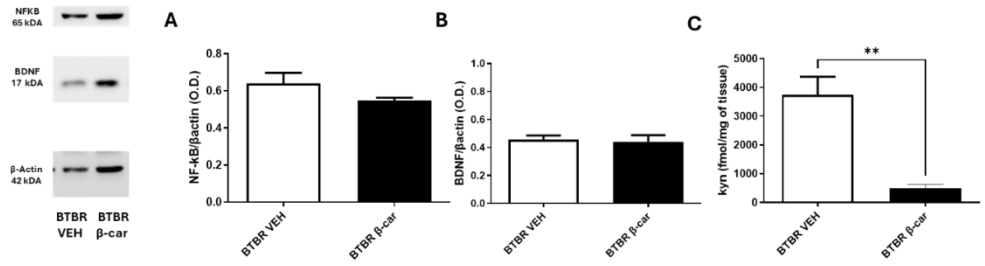

Fig. 14. Effects of β-carotene treatment on NF-kb and BDNF expression levels and on Kyn Levels in PFC of BTBR mice. A) NF-kb expression levels, Unpaired Student's t-test, n.s. **B)** BDNF expression levels, Unpaired Student's t-test, n.s. **C)** Kyn levels, Unpaired Student's t-test, P < 0.01 β-car 16 mg/kg vs VEH

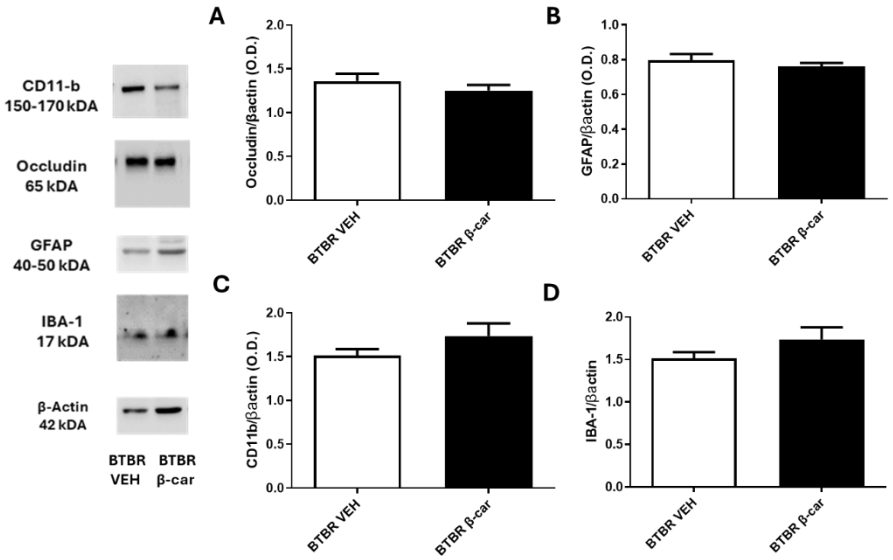

Fig. 15. Effects of β-carotene treatment on Occludin, GFAP, CD11-b and IBA-1 expression levels in PFC of BTBR mice. A) Occludin expression levels, Unpaired Student's t-test, n.s. **B)** GFAP expression levels, Unpaired Student's t-test, n.s. **C)** CD11-b expression levels, Unpaired Student's t-test, n.s. **D)** IBA-1 expression levels, Unpaired Student's t-test, n.s.

2.4.10 β-Carotene Treatment reduced Kynurenine and NF-kB levels and increased the BDNF, Occludin and IBA-1 levels in HIPP of BTBR mice

To evaluate the effect of β-Carotene treatment on some markers related to BBB function and neuroinflammation in HIPP, NF-kB, BDNF, Kynurenine, Occludin, GFAP, CD11-β and IBA-1 were quantified. The treatment effect showed a reduction in the expression levels of NF-kB (Fig. 16A) and an increase in the expression levels BDNF (Fig. 16B) in HIPP for the offspring from β-Carotene 16/mg treated dams compared to the offspring of the vehicle treated dams.

Moreover, the content of kynurenine in HIPP was significantly reduced in the offspring from β-Carotene Treated dams compared to the offspring from controls (Fig. 16C). Lastly the offspring from dams treated with B Carotene 16/mg showed increased levels in the expression of Occludin (Fig. 17A) and IBA-1 (Fig. 17D), while did not show any substantial differences in the expression levels of CD11-β and GFAP (Fig. 17 B-C) compared to offspring from controls.

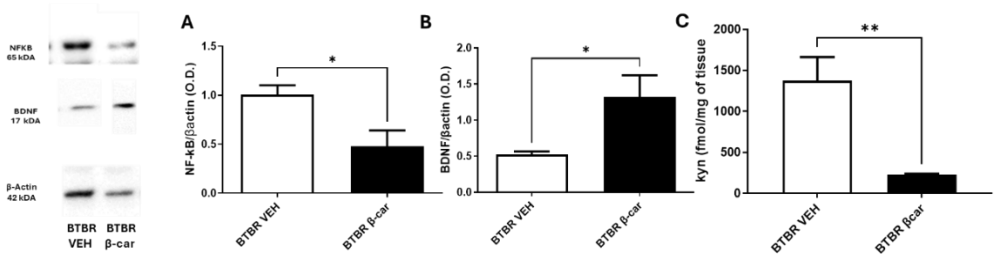

Fig. 16. Effects of β-carotene treatment on NF-kb and BDNF expression levels and on Kyn Levels in HIPP of BTBR mice. A) NF-kb expression levels, Unpaired Student's t-test, P < 0.05 β-car 16 mg/kg vs VEH. **B)** BDNF expression levels, Unpaired Student's t-test, P < 0.05 β-car 16 mg/kg vs VEH. **C)** Kyn levels, Unpaired Student's t-test, P < 0.01 β-car 16 mg/kg vs VEH.

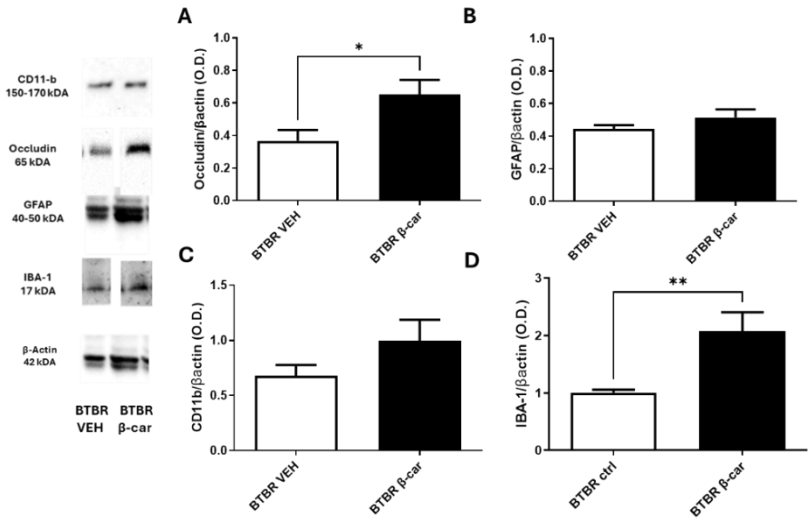

Fig. 17. Effects of β-carotene treatment on Occludin, GFAP, CD11-β and IBA-1 expression levels in HIPP of BTBR mice. A) Occludin expression levels, Unpaired Student's t-test, P < 0.05 β-car 16 mg/kg vs VEH. **B)** GFAP expression levels, Unpaired Student's t-test, n.s. **C)** CD11-β expression levels, Unpaired Student's t-test, n.s. **D)** IBA-1 expression levels, Unpaired Student's t-test, P < 0.05 β-car 16 mg/kg vs VEH.

2.5 Discussion

Although ASD are exclusively human diseases, mice models able to resemble the wide range of ASD behavioural phenotypes represent a useful instrument to understand the underlying neurobiological alterations (Bove et al., 2022).

The results obtained in this part of the thesis confirmed that the BTBR mice model of ASD reported stereotyped repertoire and social interaction deficits, by performing the Hole Board task and a modified version of the Social Interaction test, in which we evaluated social duration and frequency, together with frequency and time spent performing exploration of non-socially related objects. Interestingly, despite the well-identified reduction of social interactions in BTBR mice (Blanchard et al., 2012; Bove et al., 2022; Karvat & Kimchi, 2014; Nadeem et al., 2019), results also showed an increase of the time spent performing non-social exploration, together with frequency of this behaviour. Regarding this, the exploration of three objects non socially related might have similarities with the absence of interests in people and in developing relationships, an important behavioural feature of ASD patients (Davidson et al., 2015). Furthermore, results showed an increase in the distance travelled and a reduction in the duration and frequency of freezing in the Open Field test, both considered as indexes of hyperactivity, an important ASD collateral symptom (Aman & Langworthy, 2000). Behavioural results are in agreement with the previous literature on this animal model, in particular regarding repetitive behaviours (Brierley et al., 2021; McFarlane et al., 2008; Moy et al., 2007) and higher initial activity in the open field test (Silverman, Tolu, et al., 2010). Regarding the anxiety-related behaviours, BTBR mice showed a reduction of these behaviours in the Elevated Plus Maze and in the Open Field tests, as already reported in literature (Pobbe et al., 2011). Interestingly, spending more time in the center and in the open areas might be correlated to an increase in risk taking behaviours.In addition, the reduction in the anxiety-related behaviours could also be interpreted as an enhanced hyperactivity and impulsiveness, important traits typical of ASD patients (Rodrigues et al., 2021).

From a molecular point of view, different neuroanatomical abnormalities shared across BTBR mice and ASD subpopulations have been described (Chao, Pathak, et al., 2020; Ellegood et al., 2013). This study focused on two different brain areas (PFC and HIPP) that, together with AMY, are centrally involved in ASD and that are strictly interplaying among each other. In this study dopaminergic, noradrenergic, and excitatory-inhibitory neurotransmissions were investigated, together with kynurenine, BDNF and NGF levels, in both PFC and HIPP. Concerning DA, results did not show differences in PFC and HIPP, but there was an increase in the levels of its metabolite DOPAC in PFC. In this regard, several studies reported a dopaminergic modulation in widespread forebrain areas of ASD patients (Chadman et al., 2012; Garnier et al., 1986; Ghanizadeh & Moghimi-Sarani, 2013; Launay et al., 1987; Martineau et al., 1992; Nguyen et al., 2014; Squillace et al., 2014). Moreover, we found a decrease in cortical NA levels in BTBR mice, with no differences in the hippocampal area. In this regard, it has been reported that stereotyped behaviours, considered as derivatives of poor adaptive behaviours, are principally mediated by the noradrenergic system and that the administration of an alpha-2-receptor agonist might be helpful to improve ASD behavioural dysfunctions (Nanjappa et al., 2022). In line with our results, another model of ASD, the Engrailed-2 knock-out (EN2KO) mice, also reported NA neurotransmission alterations in the ventral hindbrain (Brielmaier et al., 2014). In addition, it has been shown that the administration of atomoxetine, a NA reuptake inhibitor, could be effective for treating hyperactivity in children with ASD (Arnold et al., 2006). In regards to ACh content, results showed a decrease in PFC according to neurochemical alterations in the cholinergic pathway observed in a postmortem study involving ASD patients (Brielmaier et al., 2014). Moreover, it has been reported that ACh increase could conduce to an improvement of cognitive deficits in ASD and other neuropsychiatric disorders (L. Wang et al., 2015). Interestingly, reduced ACh in the basal forebrain has been linked to decreased social interactions and social memory dysfunctions in mice (Kljakic et al., 2021).

In our results, we also found impairments of excitatory-inhibitory neurotransmissions, in particular, regarding PFC and HIPP, we only reported a decrease in GABA levels, while Glu levels remained unchanged. Therefore, BTBR animals demonstrated a perturbation of the excitatory-inhibitory balance, by showing disruptions in both PFC and HIPP. As regarding GABA, the reduction in its levels in HIPP has been associated with decreased sociability, especially considering that HIPP is a crucial component of the social brain (Paine et al., 2017). Regarding kynurenine levels, our results showed an increase both in PFC and HIPP, thus confirming the neuroinflammatory component of ASD (DiSabato et al., 2016). Accordingly, a tryptophan-kynurenine pathway dysregulation was reported in inflammation and immune activation contest (Q. Wang et al., 2015).

Furthermore, our results showed a decrease in the expression levels of the neurotrophins NGF and BDNF in HIPP, a brain region in which such neurotrophins are mainly present (Dinçel et al., 2013). To this purpose, different studies reported an interplay between NA neurotransmission and NGF expression (Benitez et al., 2021; Counts & Mufson, 2010; Naoi & Maruyama, 2010). In particular, it has been shown that, in cellular models, monoamine oxidase inhibitors increased NGF expression (Naoi & Maruyama, 2010) and that NA can exert neuroprotective properties by inducing NGF expression (Counts & Mufson, 2010). In line with our results, hippocampal BDNF and NGF deficiency has been associated with ASD development (Dingsdale et al., 2022; C. Liu et al., 2023), however results are contrasting, since BDNF brain levels fluctuations have been reported in response to several known, such as brain areas and age, and unknown factors (Kasarpalkar et al., 2014).

Furthermore, our results showed a decrease in the oxytocin plasmatic levels of BTBR mice. Accordingly, it has been demonstrated that an impairment of OT signaling could be associated to social behaviour impairments (Modi & Young, 2012). Concerning the treatment used in this study, β carotene subchronic administration (at the dose of 16 mg/kg, 2 times/week for the whole pregnancy) was able to increase OT plasmatic levels but did not affect the levels of

vasopressin and prolactin in the offspring of BTBR mice after β-carotene or vehicle treatment. In line with this, it has been demonstrated that in patients with ASD there were reduced levels of oxytocin and his CD38 regulatory ectoenzyme. Interestingly, β-carotene is a potent inducer of the CD38 enzyme (Avraham et al., 2019). Considering the role of OT in the social behaviours, results obtained showed that supplementation with β-carotene during pregnancy was able to increase the frequency of social interactions in male BTBR offspring compared to vehicles. In particular, among the four doses tested (8, 16, 32 and 64 mg/Kg), the dose of 16/kg appeared to be the most effective. Moreover, such dose was also able to reduce repetitive and stereotyped behaviours of BTBR mice, in particular regarding grooming, numbers of marbles burled, numbers of head dipping in hole board task and distance travelled in open field test. In line with our results, pups treated with β-carotene injection reported a reduction of repetitive and stereotyped behaviours (Avraham et al., 2019). Behavioural results were corroborated by *ex vivo* analyses. Indeed, cortical and hippocampal neurochemical and biomolecular measurements between the BTBR vehicle and the BTBR treated with 16 mg/kg of β-carotene group were performed. By focusing on the possible role of β-carotene to counteract neuroinflammatory components of ASD, we quantified kynurenine, NF-Kb and BDNF in HIPP and PFC. Indeed, beyond its effects on the sociability and on the stereotyped repertoire, β carotene owns also anti-inflammatory and anti-oxidant effects(Liebler et al., 1997; Y. Yang et al., 2021; Y. Zhang et al., 2016). In this regard, our results showed that β carotene was able to reduce kynurenine levels in both HIPP and PFC and the NF-kB expression levels in HIPP, but not in PFC, thus supporting its role in contrasting neuroinflammation, In line with our results, different studies reported that β-carotene strongly inhibited the NF-kB pathway activation in a rat model of spinal cord injury (L. Zhou et al., 2018) and that the β-carotene treatment reduced NF-kB expression in a model of rat ischemic brain injury (Althurwi et al., 2022). Furthermore, the results obtained showed that β-carotene administration was able to enhance the expression levels of BDNF in

HIPP. Accordingly, recert studies on BDNF and carotenoid docking may indicate the possibility of an allosteric activation of BDNF by carotenoids (S.-J. Park et al., 2021).

Concerning BBB and glia-related markers, our results showed different interesting effects of β-carotene administration in HIPP. In particular, β-carotene treatment increased the expression levels of Occludin and IBA-1, while the expression levels of GFAP and CD11-b did not show any difference between β-carotene treated and vehicle-treated BTBR in HIPP. As regarding PFC, no difference was detected between the treated BTBR group compared to the vehicle one. The hippocampal effects could be due to the increase in OT levels induced by β carotene treatment. Indeed, OT can modulate the inflammatory response of microglia cells (Knoop et al., 2022) maybe acting through NF-kB/MAPK pathway (Ye et al., 2022). Although it is not clear how OT influences the microglial phenotype, the increase we observed after β carotene treatment in microglial cell expression, via the IBA-1 marker, could be linked to the increase in OT induced by β-carotene administration. Moreover, enhanced the Occludin expression levels could be linked to a BBB strengthening following the less inflammatory conditions induced by β-carotene treatment.

In conclusion, the molecular characterization of the BTBR strain suggested that such idiopathic animal model might be useful to understand neurobiological and neurochemical correlates underlying ASD behavioural dysfunctions, highlighting the important role of neurotransmitter and neurotrophins alterations in a specific brain region, such as HIPP and PFC. Furthermore, our results showed also the promising preventive effect of β-carotene treatment during pregnancy to ameliorate ASD behavioural dysfunctions in offspring and also to counteract neuroinflammatory components of these neurodevelopmental pathologies.

3. Bibliography

Abbott, N. J. (2002). Astrocyte–endothelial interactions and blood–brain barrier permeability*. *Journal of Anatomy*, *200*(6), 629–638. https://doi.org/10.1046/j.1469-7580.2002.00064.x

Abdul-Ghani, M. A., Valiante, T. A., Carlen, P. L., & Pennefather, P. S. (1996). Metabotropic glutamate receptors coupled to IP3 production mediate inhibition of IAHP in rat dentate granule neurons. *Journal of Neurophysiology*, *76*(4), 2691–2700. https://doi.org/10.1152/jn.1996.76.4.2691

Adams, J. B., Audhya, T., McDonough-Means, S., Rubin, R. A., Quig, D., Geis, E., Gehn, E., Loresto, M., Mitchell, J., Atwood, S., Barnhouse, S., & Lee, W. (2011). Nutritional and metabolic status of children with autism vs. Neurotypical children, and the association with autism severity. *Nutrition & Metabolism*, *8*(1), 34. https://doi.org/10.1186/1743-7075-8-34

Aishworiya, R., Valica, T., Hagerman, R., & Restrepo, B. (2022). An Update on Psychopharmacological Treatment of Autism Spectrum Disorder. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *19*(1), 248–262. https://doi.org/10.1007/s13311-022-01183-1

Akundi, R. S., Candelario-Jalil, E., Hess, S., Hüll, M., Lieb, K., Gebicke-Haerter, P. J., & Fiebich, B. L. (2005). Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia. *Glia*, *51*(3), 199–208. https://doi.org/10.1002/glia.20198

Alabdali, A., Al-Ayadhi, L., & El-Ansary, A. (2014). Association of social and cognitive impairment and biomarkers in autism spectrum disorders. *Journal of Neuroinflammation*, *11*, 4. https://doi.org/10.1186/1742-2094-11-4

Alcendor, D. J. (2020). Interactions between Amyloid-B Proteins and Human Brain Pericytes: Implications for the Pathobiology of Alzheimer's Disease. *Journal of Clinical Medicine*, *9*(5), 1490. https://doi.org/10.3390/jcm9051490

Alemi, M., Gaiteiro, C., Ribeiro, C. A., Santos, L. M., Gomes, J. R., Oliveira, S. M., Couraud, P.-O., Weksler, B., Romero, I., Saraiva, M. J., & Cardoso, I. (2016). Transthyretin participates in beta-amyloid transport from the brain to the liver—Involvement of the low-density lipoprotein receptor-related protein 1? *Scientific Reports*, *6*, 20164. https://doi.org/10.1038/srep20164

Alkondon, M., Pereira, E. F. R., Yu, P., Arruda, E. Z., Almeida, L. E. F., Guidetti, P., Fawcett, W. P., Sapko, M. T., Randall, W. R., Schwarcz, R., Tagle, D. A., & Albuquerque, E. X. (2004). Targeted deletion of the kynurenine aminotransferase ii gene reveals a critical role of endogenous kynurenic acid in the regulation of

synaptic transmission via alpha7 nicotinic receptors in the hippocampus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *24*(19), 4635–4648. https://doi.org/10.1523/JNEUROSCI.5631-03.2004

Althurwi, H. N., Abdel-Rahman, R. F., Soliman, G. A., Ogaly, H. A., Alkholifi, F. K., Abd-Elsalam, R. M., Alqasoumi, S. I., & Abdel-Kader, M. S. (2022). Protective Effect of Beta-Carotene against Myeloperoxidase- Mediated Oxidative Stress and Inflammation in Rat Ischemic Brain Injury. *Antioxidants (Basel, Switzerland)*, *11*(12), 2344. https://doi.org/10.3390/antiox11122344

Altinbilek, B., & Manahan-Vaughan, D. (2009). A specific role for group II metabotropic glutamate receptors in hippocampal long-term depression and spatial memory. *Neuroscience*, *158*(1), 149–158. https://doi.org/10.1016/j.neuroscience.2008.07.045

Aman, M. G., & Langworthy, K. S. (2000). Pharmacotherapy for hyperactivity in children with autism and other pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *30*(5), 451–459. https://doi.org/10.1023/a:1005559725475

Amodeo, D. A., Jones, J. H., Sweeney, J. A., & Ragozzino, M. E. (2012). Differences in BTBR T+ tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors. *Behavioural Brain Research*, *227*(1), 64–72. https://doi.org/10.1016/j.bbr.2011.10.032

Amodeo, D. A., Jones, J. H., Sweeney, J. A., & Ragozzino, M. E. (2014). Risperidone and the 5- HT $_{2A}$ Receptor Antagonist M 100907 Improve Probabilistic Reversal Learning in BTBR T + $_{TF}$ / J Mice. *Autism Research*, *7*(5), 555–567. https://doi.org/10.1002/aur.1395

Amodeo, D. A., Rivera, E., Cook, E. H., Sweeney, J. A., & Ragozzino, M. E. (2017). 5HT $_{2A}$ receptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice. *Genes, Brain and Behavior*, *16*(3), 342–351. https://doi.org/10.1111/gbb.12343

Amodeo, D. A., Rivera, E., Dunn, J. T., & Ragozzino, M. E. (2016). M100907 attenuates elevated grooming behavior in the BTBR mouse. *Behavioural Brain Research*, *313*, 67–70. https://doi.org/10.1016/j.bbr.2016.06.064

Amodeo, D. A., Yi, J., Sweeney, J. A., & Ragozzino, M. E. (2014). Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice. *Frontiers in Synaptic Neuroscience*, *6*. https://doi.org/10.3389/fnsyn.2014.00017

Andari, E., Duhamel, J.-R., Zalla, T., Herbrecht, E., Leboyer, M., & Sirigu, A. (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences*, *107*(9), 4389–4394. https://doi.org/10.1073/pnas.0910249107

Arnold, L. E., Aman, M. G., Cook, A. M., Witwer, A. N., Hall, K. L., Thompson, S., & Ramadan, Y. (2006). Atomoxetine for hyperactivity in autism spectrum disorders: Placebo-controlled crossover pilot trial. *Journal of the American Academy of Child and Adolescent Psychiatry*, *45*(10), 1196–1205. https://doi.org/10.1097/01.chi.0000231976.28719.2a

Aronica, E., Gorter, J. A., Ijlst-Keizers, H., Rozemuller, A. J., Yankaya, B., Leenstra, S., & Troost, D. (2003). Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: Opposite regulation of glutamate transporter proteins. *European Journal of Neuroscience*, *17*(10), 2106–2118. https://doi.org/10.1046/j.1460-9568.2003.02657.x

Aronica, E., Gorter, J. A., Jansen, G. H., Van Veelen, C. W. M., Van Rijen, P. C., Ramkema, M., & Troost, D. (2003). Expression and Cell Distribution of Group I and Group II Metabotropic Glutamate Receptor Subtypes in Taylor-type Focal Cortical Dysplasia. *Epilepsia*, *44*(6), 785–795. https://doi.org/10.1046/j.1528-1157.2003.54802.x

Aronica, E., Gorter, J. A., Rozemuller, A. J., Yankaya, B., & Troost, D. (2005). Activation of metabotropic glutamate receptor 3 enhances interleukin (IL)-1β-stimulated release of IL-6 in cultured human astrocytes. *Neuroscience*, *130*(4), 927–933. https://doi.org/10.1016/j.neuroscience.2004.10.024

Ashcroft, G. S., & Mills, S. J. (2002). Androgen receptor-mediated inhibition of cutaneous wound healing. *The Journal of Clinical Investigation*, *110*(5), 615–624. https://doi.org/10.1172/JCI15704

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., & Van de Water, J. (2011). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, Behavior, and Immunity*, *25*(1), 40–45. https://doi.org/10.1016/j.bbi.2010.08.003

Ashwood, P., & Wakefield, A. J. (2006). Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *Journal of Neuroimmunology*, *173*(1–2), 126–134. https://doi.org/10.1016/j.jneuroim.2005.12.007

Aston-Jones, G. (2005). Brain structures and receptors involved in alertness. *Sleep Medicine*, *6 Suppl 1*, S3-7. https://doi.org/10.1016/s1389-9457(05)80002-4

Atladóttir, H. O., Pedersen, M. G., Thorsen, P., Mortensen, P. B., Deleuran, B., Eaton, W. W., & Parner, E. T. (2009). Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics*, *124*(2), 687–694. https://doi.org/10.1542/peds.2008-2445

Atladóttir, H. O., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *40*(12), 1423–1430. https://doi.org/10.1007/s10803-010-1006-y

Avraham, Y., Berry, E. M., Donskoy, M., Ahmad, W. A., Vorobiev, L., Albeck, A., & Mankuta, D. (2019). Beta-carotene as a novel therapy for the treatment of 'Autistic like behavior' in animal models of Autism. *Behavioural Brain Research*, *364*, 469–479. https://doi.org/10.1016/j.bbr.2017.09.041

Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., Hudson, C. E., Cole, M. J., Harrison, J. K., Bickford, P. C., & Gemma, C. (2011). Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiology of Aging*, *32*(11), 2030–2044. https://doi.org/10.1016/j.neurobiolaging.2009.11.022

Balabanov, R., & Dore-Duffy, P. (1998). Role of the CNS microvascular pericyte in the blood-brain barrier. *Journal of Neuroscience Research*, *53*(6), 637–644. https://doi.org/10.1002/(SICI)1097-4547(19980915)53:6<637::AID-JNR1>3.0.CO;2-6

Balda, M. S., Whitney, J. A., Flores, C., González, S., Cereijido, M., & Matter, K. (1996). Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *The Journal of Cell Biology*, *134*(4), 1031–1049. https://doi.org/10.1083/jcb.134.4.1031

Bales, K. L., Solomon, M., Jacob, S., Crawley, J. N., Silverman, J. L., Larke, R. H., Sahagun, E., Puhger, K. R., Pride, M. C., & Mendoza, S. P. (2014). Long-term exposure to intranasal oxytocin in a mouse autism model. *Translational Psychiatry*, *4*(11), e480–e480. https://doi.org/10.1038/tp.2014.117

Barbosa, A. G., Pratesi, R., Paz, G. S. C., Dos Santos, M. A. A. L., Uenishi, R. H., Nakano, E. Y., Gandolfi, L., & Pratesi, C. B. (2020). Assessment of BDNF serum levels as a diagnostic marker in children with autism spectrum disorder. *Scientific Reports*, *10*(1), 17348. https://doi.org/10.1038/s41598-020-74239-x

Barger, S. W., & Basile, A. S. (2001). Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. *Journal of Neurochemistry*, *76*(3), 846–854. https://doi.org/10.1046/j.1471-4159.2001.00075.x

Barker-Haliski, M., & White, H. S. (2015). Glutamatergic Mechanisms Associated with Seizures and Epilepsy. *Cold Spring Harbor Perspectives in Medicine*, *5*(8), a022863. https://doi.org/10.1101/cshperspect.a022863

Bartels, A. L., Willemsen, A. T. M., Kortekaas, R., de Jong, B. M., de Vries, R., de Klerk, O., van Oostrom, J. C. H., Portman, A., & Leenders, K. L. (2008). Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *Journal of Neural Transmission (Vienna, Austria: 1996)*, *115*(7), 1001–1009. https://doi.org/10.1007/s00702-008-0030-y

Battaglia, G., Molinaro, G., Riozzi, B., Storto, M., Busceti, C. L., Spinsanti, P., Bucci, D., Di Liberto, V., Mudò, G., Corti, C., Corsi, M., Nicoletti, F., Belluardo, N., & Bruno, V. (2009). Activation of mGlu3 Receptors Stimulates the Production of GDNF in Striatal Neurons. *PLoS ONE*, *4*(8), e6591. https://doi.org/10.1371/journal.pone.0006591

Battaglia, G., Riozzi, B., Bucci, D., Di Menna, L., Molinaro, G., Pallottino, S., Nicoletti, F., & Bruno, V. (2015). Activation of mGlu3 metabotropic glutamate receptors enhances GDNF and GLT-1 formation in the spinal cord and rescues motor neurons in the SOD-1 mouse model of amyotrophic lateral sclerosis. *Neurobiology of Disease*, *74*, 126–136. https://doi.org/10.1016/j.nbd.2014.11.012

Baudon, A., Clauss Creusot, E., Althammer, F., Schaaf, C. P., & Charlet, A. (2022). Emerging role of astrocytes in oxytocin-mediated control of neural circuits and brain functions. *Progress in Neurobiology*, *217*, 102328. https://doi.org/10.1016/j.pneurobio.2022.102328

Baxter, P. S., Dando, O., Emelianova, K., He, X., McKay, S., Hardingham, G. E., & Qiu, J. (2021). Microglial identity and inflammatory responses are controlled by the combined effects of neurons and astrocytes. *Cell Reports*, *34*(12), 108882. https://doi.org/10.1016/j.celrep.2021.108882

Béchade, C., D'Andrea, I., Etienne, F., Verdonk, F., Moutkine, I., Banas, S. M., Kolodziejczak, M., Diaz, S. L., Parkhurst, C. N., Gan, W. B., Maroteaux, L., & Roumier, A. (2021). The serotonin 2B receptor is required in neonatal microglia to limit neuroinflammation and sickness behavior in adulthood. *Glia*, *69*(3), 638–654. https://doi.org/10.1002/glia.23918

Bell, R. D., Winkler, E. A., Sagare, A. P., Singh, I., LaRue, B., Deane, R., & Zlokovic, B. V. (2010). Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron*, *68*(3), 409–427. https://doi.org/10.1016/j.neuron.2010.09.043

Bellani, M., Calderoni, S., Muratori, F., & Brambilla, P. (2013a). Brain anatomy of autism spectrum disorders I. Focus on corpus callosum. *Epidemiology and Psychiatric Sciences*, *22*(3), 217–221. https://doi.org/10.1017/S2045796013000139

Bellani, M., Calderoni, S., Muratori, F., & Brambilla, P. (2013b). Brain anatomy of autism spectrum disorders II. Focus on amygdala. *Epidemiology and Psychiatric Sciences*, *22*(4), 309–312. https://doi.org/10.1017/S2045796013000346

Benitez, A., Riquelme, R., Del Campo, M., Araya, C., & Lara, H. E. (2021). Nerve Growth Factor: A Dual Activator of Noradrenergic and Cholinergic Systems of the Rat Ovary. *Frontiers in Endocrinology*, *12*, 636600. https://doi.org/10.3389/fendo.2021.636600

Bennett, D. A. (2013). Plasma multianalyte profiling in mild cognitive impairment and Alzheimer disease. *Neurology*, *80*(7), 690–691. https://doi.org/10.1212/01.wnl.0000427396.91304.3d

Benno, R., Smirnova, Y., Vera, S., Liggett, A., & Schanz, N. (2009). Exaggerated responses to stress in the BTBR T+tf/J mouse: An unusual behavioral phenotype. *Behavioural Brain Research*, *197*(2), 462–465. https://doi.org/10.1016/j.bbr.2008.09.041

Berger, J. V., Dumont, A. O., Focant, M. C., Vergouts, M., Sternotte, A., Calas, A.-G., Goursaud, S., & Hermans, E. (2012). Opposite regulation of metabotropic glutamate receptor 3 and metabotropic glutamate receptor 5 by inflammatory stimuli in cultured microglia and astrocytes. *Neuroscience*, *205*, 29–38. https://doi.org/10.1016/j.neuroscience.2011.12.044

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology*, *7*(4), 452–464. https://doi.org/10.1215/S1152851705000232

Bertoni, A., Schaller, F., Tyzio, R., Gaillard, S., Santini, F., Xolin, M., Diabira, D., Vaidyanathan, R., Matarazzo, V., Medina, I., Hammock, E., Zhang, J., Chini, B., Gaiarsa, J.-L., & Muscatelli, F. (2021). Oxytocin administration in neonates shapes hippocampal circuitry and restores social behavior in a mouse model of autism. *Molecular Psychiatry*, *26*(12), 7582–7595. https://doi.org/10.1038/s41380-021-01227-6

Betancur, C. (2011). Etiological heterogeneity in autism spectrum disorders: More than 100 genetic and genomic disorders and still counting. *Brain Research*, *1380*, 42–77. https://doi.org/10.1016/j.brainres.2010.11.078

Beurel, E., Harrington, L. E., Buchser, W., Lemmon, V., & Jope, R. S. (2014). Astrocytes modulate the polarization of CD4+ T cells to Th1 cells. *PloS One*, *9*(1), e86257. https://doi.org/10.1371/journal.pone.0086257

Beversdorf, D. Q. (2020). The Role of the Noradrenergic System in Autism Spectrum Disorders, Implications for Treatment. *Seminars in Pediatric Neurology*, *35*, 100834. https://doi.org/10.1016/j.spen.2020.100834

Bisht, K., Okojie, K. A., Sharma, K., Lentferink, D. H., Sun, Y.-Y., Chen, H.-R., Uweru, J. O., Amancherla, S., Calcuttawala, Z., Campos-Salazar, A. B., Corliss, B., Jabbour, L., Benderoth, J., Friestad, B., Mills, W. A., Isakson, B. E., Tremblay, M.-È., Kuan, C.-Y., & Eyo, U. B. (2021). Capillary-associated microglia regulate vascular structure and function through PANX1-P2RY12 coupling in mice. *Nature Communications*, *12*(1), 5289. https://doi.org/10.1038/s41467-021-25590-8

Bjorklund, G., Saad, K., Chirumbolo, S., Kern, J. K., Geier, D. A., Geier, M. R., & Urbina, M. A. (2016). Immune dysfunction and neuroinflammation in autism spectrum disorder. *Acta Neurobiologiae Experimentalis*, *76*(4), 257–268. https://doi.org/10.21307/ane-2017-025

Blanchard, D. C., Defensor, E. B., Meyza, K. Z., Pobbe, R. L. H., Pearson, B. L., Bolivar, V. J., & Blanchard, R. J. (2012). BTBR T+tf/J mice: Autism-relevant behaviors and reduced fractone-associated heparan sulfate. *Neuroscience and Biobehavioral Reviews*, *36*(1), 285–296. https://doi.org/10.1016/j.neubiorev.2011.06.008

Blanchard, D. C., Griebel, G., & Blanchard, R. J. (2003). The Mouse Defense Test Battery: Pharmacological and behavioral assays for anxiety and panic. *European Journal of Pharmacology*, *463*(1–3), 97–116. https://doi.org/10.1016/s0014-2999(03)01276-7

Blatt, G. J., & Fatemi, S. H. (2011). Alterations in GABAergic biomarkers in the autism brain: Research findings and clinical implications. *Anatomical Record (Hoboken, N.J.: 2007)*, *294*(10), 1646–1652. https://doi.org/10.1002/ar.21252

Block, M. L., Zecca, L., & Hong, J.-S. (2007). Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. *Nature Reviews. Neuroscience*, *8*(1), 57–69. https://doi.org/10.1038/nrn2038

Boer, K., Encha-Razavi, F., Sinico, M., & Aronica, E. (2010). Differential distribution of group I metabotropic glutamate receptors in developing human cortex. *Brain Research*, *1324*, 24–33. https://doi.org/10.1016/j.brainres.2010.02.005

Bohannon, D. G., Long, D., & Kim, W.-K. (2021). Understanding the Heterogeneity of Human Pericyte Subsets in Blood-Brain Barrier Homeostasis and Neurological Diseases. *Cells*, *10*(4), 890. https://doi.org/10.3390/cells10040890

Boldyrev, A. A., Carpenter, D. O., & Johnson, P. (2005). Emerging evidence for a similar role of glutamate receptors in the nervous and immune systems. *Journal of Neurochemistry*, *95*(4), 913–918. https://doi.org/10.1111/j.1471-4159.2005.03456.x

Bond, A., Jones, N. M., Hicks, C. A., Whiffin, G. M., Ward, M. A., O'Neill, M. F., Kingston, A. E., Monn, J. A., Ornstein, P. L., Schoepp, D. D., Lodge, D., & O'Neill, M. J. (2000). Neuroprotective effects of LY379268, a selective mGlu2/3 receptor

agonist: Investigations into possible mechanism of action in vivo. *The Journal of Pharmacology and Experimental Therapeutics*, *294*(3), 800–809.

Bond, A., O'Neill, M. J., Hicks, C. A., Monn, J. A., & Lodge, D. (1998). Neuroprotective effects of a systemically active Group II metabotropic glutamate receptor agonist LY354740 in a gerbil model of global ischaemia: *NeuroReport*, *9*(6), 1191–1193. https://doi.org/10.1097/00001756-199804200-00042

Bordt, E. A., Smith, C. J., Demarest, T. G., Bilbo, S. D., & Kingsbury, M. A. (2019). Mitochondria, Oxytocin, and Vasopressin: Unfolding the Inflammatory Protein Response. *Neurotoxicity Research*, *36*(2), 239–256. https://doi.org/10.1007/s12640-018-9962-7

Bove, M., Ike, K., Eldering, A., Buwalda, B., De Boer, S. F., Morgese, M. G., Schiavone, S., Cuomo, V., Trabace, L., & Kas, M. J. H. (2018). The Visible Burrow System: A behavioral paradigm to assess sociability and social withdrawal in BTBR and C57BL/6J mice strains. *Behavioural Brain Research*, *344*, 9–19. https://doi.org/10.1016/j.bbr.2018.02.003

Bove, M., Schiavone, S., Tucci, P., Sikora, V., Dimonte, S., Colia, A. L., Morgese, M. G., & Trabace, L. (2022). Ketamine administration in early postnatal life as a tool for mimicking Autism Spectrum Disorders core symptoms. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *117*, 110560. https://doi.org/10.1016/j.pnpbp.2022.110560

Bradley, S. J., & Challiss, R. A. J. (2012). G protein-coupled receptor signalling in astrocytes in health and disease: A focus on metabotropic glutamate receptors. *Biochemical Pharmacology*, *84*(3), 249–259. https://doi.org/10.1016/j.bcp.2012.04.009

Braunschweig, D., Duncanson, P., Boyce, R., Hansen, R., Ashwood, P., Pessah, I. N., Hertz-Picciotto, I., & Van de Water, J. (2012). Behavioral correlates of maternal antibody status among children with autism. *Journal of Autism and Developmental Disorders*, *42*(7), 1435–1445. https://doi.org/10.1007/s10803-011-1378-7

Brielmaier, J., Senerth, J. M., Silverman, J. L., Matteson, P. G., Millonig, J. H., DiCicco-Bloom, E., & Crawley, J. N. (2014). Chronic desipramine treatment rescues depression-related, social and cognitive deficits in Engrailed-2 knockout mice. *Genes, Brain, and Behavior*, *13*(3), 286–298. https://doi.org/10.1111/gbb.12115

Brierley, N. J., McDonnell, C. G., Parks, K. M. A., Schulz, S. E., Dalal, T. C., Kelley, E., Anagnostou, E., Nicolson, R., Georgiades, S., Crosbie, J., Schachar, R., Liu, X., & Stevenson, R. A. (2021). Factor Structure of Repetitive Behaviors Across Autism Spectrum Disorder and Attention-Deficit/Hyperactivity Disorder. *Journal of*

Autism and Developmental Disorders, *51*(10), 3391–3400. https://doi.org/10.1007/s10803-020-04800-0

Brkic, M., Balusu, S., Van Wonterghem, E., Gorlé, N., Benilova, I., Kremer, A., Van Hove, I., Moons, L., De Strooper, B., Kanazir, S., Libert, C., & Vandenbroucke, R. E. (2015). Amyloid β Oligomers Disrupt Blood-CSF Barrier Integrity by Activating Matrix Metalloproteinases. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *35*(37), 12766–12778. https://doi.org/10.1523/JNEUROSCI.0006-15.2015

Broux, B., Gowing, E., & Prat, A. (2015). Glial regulation of the blood-brain barrier in health and disease. *Seminars in Immunopathology*, *37*(6), 577–590. https://doi.org/10.1007/s00281-015-0516-2

Brown, A. S., Sourander, A., Hinkka-Yli-Salomäki, S., McKeague, I. W., Sundvall, J., & Surcel, H.-M. (2014). Elevated maternal C-reactive protein and autism in a national birth cohort. *Molecular Psychiatry*, *19*(2), 259–264. https://doi.org/10.1038/mp.2012.197

Brown, G. C., & Neher, J. J. (2014). Microglial phagocytosis of live neurons. *Nature Reviews Neuroscience*, *15*(4), 209–216. https://doi.org/10.1038/nrn3710

Bruno, V., Battaglia, G., Casabona, G., Copani, A., Caciagli, F., & Nicoletti, F. (1998). Neuroprotection by Glial Metabotropic Glutamate Receptors Is Mediated by Transforming Growth Factor-β. *The Journal of Neuroscience*, *18*(23), 9594–9600. https://doi.org/10.1523/JNEUROSCI.18-23-09594.1998

Bruno, V., Caraci, F., Copani, A., Matrisciano, F., Nicoletti, F., & Battaglia, G. (2017). The impact of metabotropic glutamate receptors into active neurodegenerative processes: A “dark side” in the development of new symptomatic treatments for neurologic and psychiatric disorders. *Neuropharmacology*, *115*, 180–192. https://doi.org/10.1016/j.neuropharm.2016.04.044

Burket, J. A., Herndon, A. L., Winebarger, E. E., Jacome, L. F., & Deutsch, S. I. (2011). Complex effects of mGluR5 antagonism on sociability and stereotypic behaviors in mice: Possible implications for the pharmacotherapy of autism spectrum disorders. *Brain Research Bulletin*, *86*(3–4), 152–158. https://doi.org/10.1016/j.brainresbull.2011.08.001

Busse, M., Feta, A., Presto, J., Wilén, M., Grønning, M., Kjellén, L., & Kusche-Gullberg, M. (2007). Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation. *The Journal of Biological Chemistry*, *282*(45), 32802–32810. https://doi.org/10.1074/jbc.M703560200

Cabezas, R., Avila, M., Gonzalez, J., El-Bachá, R. S., Báez, E., García-Segura, L. M., Jurado Coronel, J. C., Capani, F., Cardona-Gomez, G. P., & Barreto, G. E. (2014). Astrocytic modulation of blood brain barrier: Perspectives on Parkinson’s disease.

Frontiers in Cellular Neuroscience, *8*, 211. https://doi.org/10.3389/fncel.2014.00211

Capaldo, C. T., & Nusrat, A. (2009). Cytokine regulation of tight junctions. *Biochimica Et Biophysica Acta*, *1788*(4), 864–871. https://doi.org/10.1016/j.bbamem.2008.08.027

Caraci, F., Molinaro, G., Battaglia, G., Giuffrida, M. L., Riozzi, B., Traficante, A., Bruno, V., Cannella, M., Merlo, S., Wang, X., Heinz, B. A., Nisenbaum, E. S., Britton, T. C., Drago, F., Sortino, M. A., Copani, A., & Nicoletti, F. (2011). Targeting Group II Metabotropic Glutamate (mGlu) Receptors for the Treatment of Psychosis Associated with Alzheimer's Disease: Selective Activation of mGlu2 Receptors Amplifies β-Amyloid Toxicity in Cultured Neurons, Whereas Dual Activation of mGlu2 and mGlu3 Receptors Is Neuroprotective. *Molecular Pharmacology*, *79*(3), 618–626. https://doi.org/10.1124/mol.110.067488

Caraci, F., Nicoletti, F., & Copani, A. (2018). Metabotropic glutamate receptors: The potential for therapeutic applications in Alzheimer's disease. *Current Opinion in Pharmacology*, *38*, 1–7. https://doi.org/10.1016/j.coph.2017.12.001

Caraci, F., Spampinato, S. F., Morgese, M. G., Tascedda, F., Salluzzo, M. G., Giambirtone, M. C., Caruso, G., Munafò, A., Torrisi, S. A., Leggio, G. M., Trabace, L., Nicoletti, F., Drago, F., Sortino, M. A., & Copani, A. (2018). Neurobiological links between depression and AD: The role of TGF-β1 signaling as a new pharmacological target. *Pharmacological Research*, *130*, 374–384. https://doi.org/10.1016/j.phrs.2018.02.007

Caraci, F., Spampinato, S., Sortino, M. A., Bosco, P., Battaglia, G., Bruno, V., Drago, F., Nicoletti, F., & Copani, A. (2012). Dysfunction of TGF-β1 signaling in Alzheimer's disease: Perspectives for neuroprotection. *Cell and Tissue Research*, *347*(1), 291–301. https://doi.org/10.1007/s00441-011-1230-6

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural Brain Research*, *134*(1–2), 49–57. https://doi.org/10.1016/s0166-4328(01)00452-1

Carrano, A., Hoozemans, J. J. M., van der Vies, S. M., van Horssen, J., de Vries, H. E., & Rozemuller, A. J. M. (2012). Neuroinflammation and blood-brain barrier changes in capillary amyloid angiopathy. *Neuro-Degenerative Diseases*, *10*(1–4), 329–331. https://doi.org/10.1159/000334916

Cartier, E., Hamilton, P. J., Belovich, A. N., Shekar, A., Campbell, N. G., Saunders, C., Andreassen, T. F., Gether, U., Veenstra-Vanderweele, J., Sutcliffe, J. S., Ulery-Reynolds, P. G., Erreger, K., Matthies, H. J. G., & Galli, A. (2015). Rare Autism-Associated Variants Implicate Syntaxin 1 (STX1 R26Q) Phosphorylation and the

Dopamine Transporter (hDAT R51W) in Dopamine Neurotransmission and Behaviors. *EBioMedicine*, *2*(2), 135–146. https://doi.org/10.1016/j.ebiom.2015.01.007

Cartmell, J., Monn, J. A., & Schoepp, D. D. (2000). Attenuation of specific PCP-evoked behaviors by the potent mGlu2/3 receptor agonist, LY379268 and comparison with the atypical antipsychotic, clozapine. *Psychopharmacology*, *148*(4), 423–429. https://doi.org/10.1007/s002130050072

Caruso, G. I., Spampinato, S. F., Costantino, G., Merlo, S., & Sortino, M. A. (2021). SIRT1-Dependent Upregulation of BDNF in Human Microglia Challenged with Aβ: An Early but Transient Response Rescued by Melatonin. *Biomedicines*, *9*(5), 466. https://doi.org/10.3390/biomedicines9050466

Caruso, G., Spampinato, S. F., Cardaci, V., Caraci, F., Sortino, M. A., & Merlo, S. (2019). β-amyloid and Oxidative Stress: Perspectives in Drug Development. *Current Pharmaceutical Design*, *25*(45), 4771–4781. https://doi.org/10.2174/1381612825666191209115431

Cellot, G., Maggi, L., Di Castro, M. A., Catalano, M., Migliore, R., Migliore, M., Scattoni, M. L., Calamandrei, G., & Cherubini, E. (2016). Premature changes in neuronal excitability account for hippocampal network impairment and autistic-like behavior in neonatal BTBR T+tf/J mice. *Scientific Reports*, *6*(1), 31696. https://doi.org/10.1038/srep31696

Cha, J.-H. J., Farrell, L. A., Ahmed, S. F., Frey, A., Hsiao-Ashe, K. K., Young, A. B., Penney, J. B., Locascio, J. J., Hyman, B. T., & Irizarry, M. C. (2001). Glutamate Receptor Dysregulation in the Hippocampus of Transgenic Mice Carrying Mutated Human Amyloid Precursor Protein. *Neurobiology of Disease*, *8*(1), 90–102. https://doi.org/10.1006/nbdi.2000.0330

Chadman, K. K. (2011). Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. *Pharmacology Biochemistry and Behavior*, *97*(3), 586–594. https://doi.org/10.1016/j.pbb.2010.09.012

Chadman, K. K., Guariglia, S. R., & Yoo, J. H. (2012). New directions in the treatment of autism spectrum disorders from animal model research. *Expert Opinion on Drug Discovery*, *7*(5), 407–416. https://doi.org/10.1517/17460441.2012.678828

Chana, G., Laskaris, L., Pantelis, C., Gillett, P., Testa, R., Zantomio, D., Burrows, E. L., Hannan, A. J., Everall, I. P., & Skafidas, E. (2015). Decreased expression of mGluR5 within the dorsolateral prefrontal cortex in autism and increased microglial number in mGluR5 knockout mice: Pathophysiological and neurobehavioral implications. *Brain, Behavior, and Immunity*, *49*, 197–205. https://doi.org/10.1016/j.bbi.2015.05.009

Chao, O. Y., Marron Fernandez de Velasco, E., Pathak, S. S., Maitra, S., Zhang, H., Duvick, L., Wickman, K., Orr, H. T., Hirai, H., & Yang, Y.-M. (2020). Targeting inhibitory cerebellar circuitry to alleviate behavioral deficits in a mouse model for studying idiopathic autism. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *45*(7), 1159–1170. https://doi.org/10.1038/s41386-020-0656-5

Chao, O. Y., Pathak, S. S., Zhang, H., Dunaway, N., Li, J.-S., Mattern, C., Nikolaus, S., Huston, J. P., & Yang, Y.-M. (2020). Altered dopaminergic pathways and therapeutic effects of intranasal dopamine in two distinct mouse models of autism. *Molecular Brain*, *13*(1), 111. https://doi.org/10.1186/s13041-020-00649-7

Chao, O. Y., Yunger, R., & Yang, Y.-M. (2018). Behavioral assessments of BTBR T+Itpr3tf/J mice by tests of object attention and elevated open platform: Implications for an animal model of psychiatric comorbidity in autism. *Behavioural Brain Research*, *347*, 140–147. https://doi.org/10.1016/j.bbr.2018.03.014

Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C., & Collin, F. (2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology*, *14*, 450–464. https://doi.org/10.1016/j.redox.2017.10.014

Chen, J.-H., Ke, K.-F., Lu, J.-H., Qiu, Y.-H., & Peng, Y.-P. (2015). Protection of TGF-β1 against Neuroinflammation and Neurodegeneration in Aβ1–42-Induced Alzheimer's Disease Model Rats. *PLOS ONE*, *10*(2), e0116549. https://doi.org/10.1371/journal.pone.0116549

Chen, Z., & Trapp, B. D. (2016). Microglia and neuroprotection. *Journal of Neurochemistry*, *136 Suppl 1*, 10–17. https://doi.org/10.1111/jnc.13062

Cheslow, L., & Alvarez, J. I. (2016). Glial-endothelial crosstalk regulates blood-brain barrier function. *Current Opinion in Pharmacology*, *26*, 39–46. https://doi.org/10.1016/j.coph.2015.09.010

Chévere-Torres, I., Maki, J. M., Santini, E., & Klann, E. (2012). Impaired social interactions and motor learning skills in tuberous sclerosis complex model mice expressing a dominant/negative form of tuberin. *Neurobiology of Disease*, *45*(1), 156–164. https://doi.org/10.1016/j.nbd.2011.07.018

Chitu, V., Gokhan, Ş., Nandi, S., Mehler, M. F., & Stanley, E. R. (2016). Emerging Roles for CSF-1 Receptor and its Ligands in the Nervous System. *Trends in Neurosciences*, *39*(6), 378–393. https://doi.org/10.1016/j.tins.2016.03.005

Chodobski, A., Zink, B. J., & Szmydynger-Chodobska, J. (2011). Blood-brain barrier pathophysiology in traumatic brain injury. *Translational Stroke Research*, *2*(4), 492–516. https://doi.org/10.1007/s12975-011-0125-x

Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., Hoeffer, C. A., Littman, D. R., & Huh, J. R. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science (New York, N.Y.)*, *351*(6276), 933–939. https://doi.org/10.1126/science.aad0314

Ciccarelli, R., D'Alimonte, I., Ballerini, P., D'Auro, M., Nargi, E., Buccella, S., Di Iorio, P., Bruno, V., Nicoletti, F., & Caciagli, F. (2007). Molecular Signalling Mediating the Protective Effect of A $_1$ Adenosine and mGlu3 Metabotropic Glutamate Receptor Activation against Apoptosis by Oxygen/Glucose Deprivation in Cultured Astrocytes. *Molecular Pharmacology*, *71*(5), 1369–1380. https://doi.org/10.1124/mol.106.031617

Ciccarelli, R., Di Iorio, P., Bruno, V., Battaglia, G., D'Alimonte, I., D'Onofrio, M., Nicoletti, F., & Caciagli, F. (1999). Activation of A1 adenosine or mGlu3 metabotropic glutamate receptors enhances the release of nerve growth factor and S-100? Protein from cultured astrocytes. *Glia*, *27*(3), 275–281. https://doi.org/10.1002/(SICI)1098-1136(199909)27:3<275::AID-GLIA9>3.0.CO;2-0

Ciccarelli, R., Sureda, F. X., Casabona, G., Di Iorio, P., Caruso, A., Spinella, F., Filippo Condorelli, D., Nicoletti, F., & Caciagli, F. (1997). Opposite influence of the metabotropic glutamate receptor subtypes mGlu3 and -5 on astrocyte proliferation in culture. *Glia*, *21*(4), 390–398. https://doi.org/10.1002/(SICI)1098-1136(199712)21:4<390::AID-GLIA6>3.0.CO;2-7

Clee, S. M., Nadler, S. T., & Attie, A. D. (2005). Genetic and genomic studies of the BTBR ob/ob mouse model of type 2 diabetes. *American Journal of Therapeutics*, *12*(6), 491–498. https://doi.org/10.1097/01.mjt.0000178781.89789.25

Cockram, T. O. J., Puigdellívol, M., & Brown, G. C. (2019). Calreticulin and Galectin-3 Opsonise Bacteria for Phagocytosis by Microglia. *Frontiers in Immunology*, *10*, 2647. https://doi.org/10.3389/fimmu.2019.02647

Colton, C. A. (2013). Immune Heterogeneity in Neuroinflammation: Dendritic Cells in the Brain. *Journal of Neuroimmune Pharmacology*, *8*(1), 145–162. https://doi.org/10.1007/s11481-012-9414-8

Combes, V., Guillemin, G. J., Chan-Ling, T., Hunt, N. H., & Grau, G. E. R. (2012). The crossroads of neuroinflammation in infectious diseases: Endothelial cells and astrocytes. *Trends in Parasitology*, *28*(8), 311–319. https://doi.org/10.1016/j.pt.2012.05.008

Condello, C., Yuan, P., & Grutzendler, J. (2018). Microglia-Mediated Neuroprotection, TREM2, and Alzheimer's Disease: Evidence From Optical Imaging. *Biological Psychiatry*, *83*(4), 377–387. https://doi.org/10.1016/j.biopsych.2017.10.007

Condello, C., Yuan, P., Schain, A., & Grutzendler, J. (2015). Microglia constitute a barrier that prevents neurotoxic protofibrillar Aβ42 hotspots around plaques. *Nature Communications*, *6*, 6176. https://doi.org/10.1038/ncomms7176

Conn, P. J., & Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annual Review of Pharmacology and Toxicology*, *37*, 205–237. https://doi.org/10.1146/annurev.pharmtox.37.1.205

Conway, C. D., Howe, K. M., Nettleton, N. K., Price, D. J., Mason, J. O., & Pratt, T. (2011). Heparan sulfate sugar modifications mediate the functions of slits and other factors needed for mouse forebrain commissure development. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(6), 1955–1970. https://doi.org/10.1523/JNEUROSCI.2579-10.2011

Corley, M. J., Meyza, K. Z., Blanchard, D. C., & Blanchard, R. J. (2012). Reduced sulfate plasma concentrations in the BTBR T+tf/J mouse model of autism. *Physiology & Behavior*, *107*(5), 663–665. https://doi.org/10.1016/j.physbeh.2012.04.010

Corti, C., Battaglia, G., Molinaro, G., Riozzi, B., Pittaluga, A., Corsi, M., Mugnaini, M., Nicoletti, F., & Bruno, V. (2007). The Use of Knock-Out Mice Unravels Distinct Roles for mGlu2 and mGlu3 Metabotropic Glutamate Receptors in Mechanisms of Neurodegeneration/Neuroprotection. *The Journal of Neuroscience*, *27*(31), 8297–8308. https://doi.org/10.1523/JNEUROSCI.1889-07.2007

Counts, S. E., & Mufson, E. J. (2010). Noradrenaline activation of neurotrophic pathways protects against neuronal amyloid toxicity. *Journal of Neurochemistry*, *113*(3), 649–660. https://doi.org/10.1111/j.1471-4159.2010.06622.x

Courchesne, E., Campbell, K., & Solso, S. (2011). Brain growth across the life span in autism: Age-specific changes in anatomical pathology. *Brain Research*, *1380*, 138–145. https://doi.org/10.1016/j.brainres.2010.09.101

Crespi, B. J. (2019). Comparative psychopharmacology of autism and psychotic-affective disorders suggests new targets for treatment. *Evolution, Medicine, and Public Health*, *2019*(1), 149–168. https://doi.org/10.1093/emph/eoz022

Crowley, J. J., Blendy, J. A., & Lucki, I. (2005). Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology*, *183*(2), 257–264. https://doi.org/10.1007/s00213-005-0166-5

Crupi, R., Impellizzeri, D., & Cuzzocrea, S. (2019). Role of Metabotropic Glutamate Receptors in Neurological Disorders. *Frontiers in Molecular Neuroscience*, *12*, 20. https://doi.org/10.3389/fnmol.2019.00020

Cryan, J. F., & Holmes, A. (2005). The ascent of mouse: Advances in modelling human depression and anxiety. *Nature Reviews Drug Discovery*, *4*(9), 775–790. https://doi.org/10.1038/nrd1825

Császár, E., Lénárt, N., Cserép, C., Környei, Z., Fekete, R., Pósfai, B., Balázsfi, D., Hangya, B., Schwarcz, A. D., Szabadits, E., Szöllősi, D., Szigeti, K., Máthé, D., West, B. L., Sviatkó, K., Brás, A. R., Mariani, J.-C., Kliewer, A., Lenkei, Z., … Dénes, Á. (2022). Microglia modulate blood flow, neurovascular coupling, and hypoperfusion via purinergic actions. *The Journal of Experimental Medicine*, *219*(3), e20211071. https://doi.org/10.1084/jem.20211071

Czirr, E., Castello, N. A., Mosher, K. I., Castellano, J. M., Hinkson, I. V., Lucin, K. M., Baeza-Raja, B., Ryu, J. K., Li, L., Farina, S. N., Belichenko, N. P., Longo, F. M., Akassoglou, K., Britschgi, M., Cirrito, J. R., & Wyss-Coray, T. (2017). Microglial complement receptor 3 regulates brain Aβ levels through secreted proteolytic activity. *The Journal of Experimental Medicine*, *214*(4), 1081–1092. https://doi.org/10.1084/jem.20162011

da Fonseca, A. C. C., Matias, D., Garcia, C., Amaral, R., Geraldo, L. H., Freitas, C., & Lima, F. R. S. (2014). The impact of microglial activation on blood-brain barrier in brain diseases. *Frontiers in Cellular Neuroscience*, *8*, 362. https://doi.org/10.3389/fncel.2014.00362

da Silveira Cruz-Machado, S., Guissoni Campos, L. M., Fadini, C. C., Anderson, G., Markus, R. P., & Pinato, L. (2021). Disrupted nocturnal melatonin in autism: Association with tumor necrosis factor and sleep disturbances. *Journal of Pineal Research*, *70*(3), e12715. https://doi.org/10.1111/jpi.12715

Daimon, C. M., Jasien, J. M., Wood, W. H., Zhang, Y., Becker, K. G., Silverman, J. L., Crawley, J. N., Martin, B., & Maudsley, S. (2015). Hippocampal Transcriptomic and Proteomic Alterations in the BTBR Mouse Model of Autism Spectrum Disorder. *Frontiers in Physiology*, *6*. https://doi.org/10.3389/fphys.2015.00324

Dalton, P., Deacon, R., Blamire, A., Pike, M., McKinlay, I., Stein, J., Styles, P., & Vincent, A. (2003). Maternal neuronal antibodies associated with autism and a language disorder. *Annals of Neurology*, *53*(4), 533–537. https://doi.org/10.1002/ana.10557

Daneman, R., & Prat, A. (2015). The blood-brain barrier. *Cold Spring Harbor Perspectives in Biology*, *7*(1), a020412. https://doi.org/10.1101/cshperspect.a020412

Daneman, R., Zhou, L., Agalliu, D., Cahoy, J. D., Kaushal, A., & Barres, B. A. (2010). The mouse blood-brain barrier transcriptome: A new resource for understanding the development and function of brain endothelial cells. *PloS One*, *5*(10), e13741. https://doi.org/10.1371/journal.pone.0013741

Daneman, R., Zhou, L., Kebede, A. A., & Barres, B. A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature*, *468*(7323), 562–566. https://doi.org/10.1038/nature09513

Davidson, C., O'Hare, A., Mactaggart, F., Green, J., Young, D., Gillberg, C., & Minnis, H. (2015). Social relationship difficulties in autism and reactive attachment disorder: Improving diagnostic validity through structured assessment. *Research in Developmental Disabilities*, *40*, 63–72. https://doi.org/10.1016/j.ridd.2015.01.007

Deane, R., Wu, Z., Sagare, A., Davis, J., Du Yan, S., Hamm, K., Xu, F., Parisi, M., LaRue, B., Hu, H. W., Spijkers, P., Guo, H., Song, X., Lenting, P. J., Van Nostrand, W. E., & Zlokovic, B. V. (2004). LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron*, *43*(3), 333–344. https://doi.org/10.1016/j.neuron.2004.07.017

Deane, R., & Zlokovic, B. V. (2007). Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Current Alzheimer Research*, *4*(2), 191–197. https://doi.org/10.2174/156720507780362245

Defensor, E. B., Pearson, B. L., Pobbe, R. L. H., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2011). A novel social proximity test suggests patterns of social avoidance and gaze aversion-like behavior in BTBR T+ tf/J mice. *Behavioural Brain Research*, *217*(2), 302–308. https://doi.org/10.1016/j.bbr.2010.10.033

del Zoppo, G. J., Milner, R., Mabuchi, T., Hung, S., Wang, X., & Koziol, J. A. (2006). Vascular matrix adhesion and the blood–brain barrier. *Biochemical Society Transactions*, *34*(6), 1261–1266. https://doi.org/10.1042/BST0341261

Deng, W., Wang, H., Rosenberg, P. A., Volpe, J. J., & Jensen, F. E. (2004). Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. *Proceedings of the National Academy of Sciences*, *101*(20), 7751–7756. https://doi.org/10.1073/pnas.0307850101

Dewar, D., Chalmers, D. T., Graham, D. I., & McCulloch, J. (1991). Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: An autoradiographic study of the hippocampus. *Brain Research*, *553*(1), 58–64. https://doi.org/10.1016/0006-8993(91)90230-S

Dhami, G. K., & Ferguson, S. S. G. (2006). Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. *Pharmacology & Therapeutics*, *111*(1), 260–271. https://doi.org/10.1016/j.pharmthera.2005.01.008

DiCarlo, G. E., Aguilar, J. I., Matthies, H. J., Harrison, F. E., Bundschuh, K. E., West, A., Hashemi, P., Herborg, F., Rickhag, M., Chen, H., Gether, U., Wallace, M. T., & Galli, A. (2019). Autism-linked dopamine transporter mutation alters striatal

dopamine neurotransmission and dopamine-dependent behaviors. *The Journal of Clinical Investigation*, *129*(8), 3407–3419. https://doi.org/10.1172/JCI127411

DiChiara, T., DiNunno, N., Clark, J., Bu, R. L., Cline, E. N., Rollins, M. G., Gong, Y., Brody, D. L., Sligar, S. G., Velasco, P. T., Viola, K. L., & Klein, W. L. (2017). Alzheimer's Toxic Amyloid Beta Oligomers: Unwelcome Visitors to the Na/K ATPase alpha3 Docking Station. *The Yale Journal of Biology and Medicine*, *90*(1), 45–61.

Dinçel, N., Ünalp, A., Kutlu, A., Öztürk, A., Uran, N., & Ulusoy, S. (2013). Serum nerve growth factor levels in autistic children in Turkish population: A preliminary study. *The Indian Journal of Medical Research*, *138*(6), 900–903.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews*, *51*(1), 7–61.

Dingsdale, H., Garay, S. M., Tyson, H. R., Savory, K. A., Sumption, L. A., Kelleher, J. S., Langley, K., Van Goozen, S., & John, R. M. (2022). Cord serum brain-derived neurotrophic factor levels at birth associate with temperament outcomes at one year. *Journal of Psychiatric Research*, *150*, 47–53. https://doi.org/10.1016/j.jpsychires.2022.03.009

DiSabato, D. J., Quan, N., & Godbout, J. P. (2016). Neuroinflammation: The devil is in the details. *Journal of Neurochemistry*, *139 Suppl 2*(Suppl 2), 136–153. https://doi.org/10.1111/jnc.13607

Dixon, M. A., Greferath, U., Fletcher, E. L., & Jobling, A. I. (2021). The Contribution of Microglia to the Development and Maturation of the Visual System. *Frontiers in Cellular Neuroscience*, *15*, 659843. https://doi.org/10.3389/fncel.2021.659843

Domercq, M., Brambilla, L., Pilati, E., Marchaland, J., Volterra, A., & Bezzi, P. (2006). P2Y1 receptor-evoked glutamate exocytosis from astrocytes: Control by tumor necrosis factor-alpha and prostaglandins. *The Journal of Biological Chemistry*, *281*(41), 30684–30696. https://doi.org/10.1074/jbc.M606429200

Domingues, H. S., Portugal, C. C., Socodato, R., & Relvas, J. B. (2016). Oligodendrocyte, Astrocyte, and Microglia Crosstalk in Myelin Development, Damage, and Repair. *Frontiers in Cell and Developmental Biology*, *4*. https://doi.org/10.3389/fcell.2016.00071

Dowell, J. A., Johnson, J. A., & Li, L. (2009). Identification of Astrocyte Secreted Proteins with a Combination of Shotgun Proteomics and Bioinformatics. *Journal of Proteome Research*, *8*(8), 4135–4143. https://doi.org/10.1021/pr900248y

Durand, D., Carniglia, L., Beauquis, J., Caruso, C., Saravia, F., & Lasaga, M. (2014). Astroglial mGlu3 receptors promote alpha-secretase-mediated amyloid precursor

protein cleavage. *Neuropharmacology*, *79*, 180–189. https://doi.org/10.1016/j.neuropharm.2013.11.015

Durand, D., Carniglia, L., Caruso, C., & Lasaga, M. (2013). mGlu3 receptor and astrocytes: Partners in neuroprotection. *Neuropharmacology*, *66*, 1–11. https://doi.org/10.1016/j.neuropharm.2012.04.009

Durand, D., Carniglia, L., Turati, J., Ramírez, D., Saba, J., Caruso, C., & Lasaga, M. (2017). Amyloid-beta neurotoxicity and clearance are both regulated by glial group II metabotropic glutamate receptors. *Neuropharmacology*, *123*, 274–286. https://doi.org/10.1016/j.neuropharm.2017.05.008

Durand, D., Caruso, C., Carniglia, L., & Lasaga, M. (2010). Metabotropic glutamate receptor 3 activation prevents nitric oxide-induced death in cultured rat astrocytes. *Journal of Neurochemistry*, *112*(2), 420–433. https://doi.org/10.1111/j.1471-4159.2009.06469.x

Ebstein, R. P., Knafo, A., Mankuta, D., Chew, S. H., & Lai, P. S. (2012). The contributions of oxytocin and vasopressin pathway genes to human behavior. *Hormones and Behavior*, *61*(3), 359–379. https://doi.org/10.1016/j.yhbeh.2011.12.014

Ebstein, R. P., Mankuta, D., Yirmiya, N., & Malavasi, F. (2011). Are retinoids potential therapeutic agents in disorders of social cognition including autism? *FEBS Letters*, *585*(11), 1529–1536. https://doi.org/10.1016/j.febslet.2011.05.004

Ecker, C., Suckling, J., Deoni, S. C., Lombardo, M. V., Bullmore, E. T., Baron-Cohen, S., Catani, M., Jezzard, P., Barnes, A., Bailey, A. J., Williams, S. C., Murphy, D. G. M., & MRC AIMS Consortium. (2012). Brain anatomy and its relationship to behavior in adults with autism spectrum disorder: A multicenter magnetic resonance imaging study. *Archives of General Psychiatry*, *69*(2), 195–209. https://doi.org/10.1001/archgenpsychiatry.2011.1251

Edmonson, C., Ziats, M. N., & Rennert, O. M. (2014). Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Molecular Autism*, *5*(1), 3. https://doi.org/10.1186/2040-2392-5-3

El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G., & Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Archives of Biochemistry and Biophysics*, *430*(1), 37–48. https://doi.org/10.1016/j.abb.2004.03.007

El-Ansary, A., & Al-Ayadhi, L. (2014). GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders. *Journal of Neuroinflammation*, *11*, 189. https://doi.org/10.1186/s12974-014-0189-0

Ellegood, J., Babineau, B. A., Henkelman, R. M., Lerch, J. P., & Crawley, J. N. (2013). Neuroanatomical analysis of the BTBR mouse model of autism using magnetic resonance imaging and diffusion tensor imaging. *NeuroImage*, *70*, 288–300. https://doi.org/10.1016/j.neuroimage.2012.12.029

Elmore, M. R. P., Najafi, A. R., Koike, M. A., Dagher, N. N., Spangenberg, E. E., Rice, R. A., Kitazawa, M., Matusow, B., Nguyen, H., West, B. L., & Green, K. N. (2014). Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*, *82*(2), 380–397. https://doi.org/10.1016/j.neuron.2014.02.040

Eroglu, C., & Barres, B. A. (2010). Regulation of synaptic connectivity by glia. *Nature*, *468*(7321), 223–231. https://doi.org/10.1038/nature09612

Esser, S., Lampugnani, M. G., Corada, M., Dejana, E., & Risau, W. (1998). Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *Journal of Cell Science*, *111*(13), 1853–1865. https://doi.org/10.1242/jcs.111.13.1853

Fabene, P. F., Laudanna, C., & Constantin, G. (2013). Leukocyte trafficking mechanisms in epilepsy. *Molecular Immunology*, *55*(1), 100–104. https://doi.org/10.1016/j.molimm.2012.12.009

Fallarino, F., Volpi, C., Fazio, F., Notartomaso, S., Vacca, C., Busceti, C., Bicciato, S., Battaglia, G., Bruno, V., Puccetti, P., Fioretti, M. C., Nicoletti, F., Grohmann, U., & Di Marco, R. (2010). Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. *Nature Medicine*, *16*(8), 897–902. https://doi.org/10.1038/nm.2183

Fang, Y.-C., Hsieh, Y.-C., Hu, C.-J., & Tu, Y.-K. (2023). Endothelial Dysfunction in Neurodegenerative Diseases. *International Journal of Molecular Sciences*, *24*(3), 2909. https://doi.org/10.3390/ijms24032909

Fanning, A. S., Jameson, B. J., Jesaitis, L. A., & Anderson, J. M. (1998). The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *The Journal of Biological Chemistry*, *273*(45), 29745–29753. https://doi.org/10.1074/jbc.273.45.29745

Fanning, A. S., Little, B. P., Rahner, C., Utepbergenov, D., Walther, Z., & Anderson, J. M. (2007). The unique-5 and -6 motifs of ZO-1 regulate tight junction strand localization and scaffolding properties. *Molecular Biology of the Cell*, *18*(3), 721–731. https://doi.org/10.1091/mbc.e06-08-0764

Fantin, A., Vieira, J. M., Gestri, G., Denti, L., Schwarz, Q., Prykhozhij, S., Peri, F., Wilson, S. W., & Ruhrberg, C. (2010). Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial

tip cell induction. *Blood*, *116*(5), 829–840. https://doi.org/10.1182/blood-2009-12-257832

Faridar, A., Jones-Davis, D., Rider, E., Li, J., Gobius, I., Morcom, L., Richards, L. J., Sen, S., & Sherr, E. H. (2014). Mapk/Erk activation in an animal model of social deficits shows a possible link to autism. *Molecular Autism*, *5*(1), 57. https://doi.org/10.1186/2040-2392-5-57

Ferraguti, F., Crepaldi, L., & Nicoletti, F. (2008). Metabotropic glutamate 1 receptor: Current concepts and perspectives. *Pharmacological Reviews*, *60*(4), 536–581. https://doi.org/10.1124/pr.108.000166

Ferraguti, F., & Shigemoto, R. (2006). Metabotropic glutamate receptors. *Cell and Tissue Research*, *326*(2), 483–504. https://doi.org/10.1007/s00441-006-0266-5

Fiorentino, M., Sapone, A., Senger, S., Camhi, S. S., Kadzielski, S. M., Buie, T. M., Kelly, D. L., Cascella, N., & Fasano, A. (2016). Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Molecular Autism*, *7*, 49. https://doi.org/10.1186/s13229-016-0110-z

Flowers, J. B., Oler, A. T., Nadler, S. T., Choi, Y., Schueler, K. L., Yandell, B. S., Kendziorski, C. M., & Attie, A. D. (2007). Abdominal obesity in BTBR male mice is associated with peripheral but not hepatic insulin resistance. *American Journal of Physiology. Endocrinology and Metabolism*, *292*(3), E936-945. https://doi.org/10.1152/ajpendo.00370.2006

Freeman, L., Guo, H., David, C. N., Brickey, W. J., Jha, S., & Ting, J. P.-Y. (2017). NLR members NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes. *The Journal of Experimental Medicine*, *214*(5), 1351–1370. https://doi.org/10.1084/jem.20150237

Frost, G. R., & Li, Y.-M. (2017). The role of astrocytes in amyloid production and Alzheimer's disease. *Open Biology*, *7*(12), 170228. https://doi.org/10.1098/rsob.170228

Frye, C. A., & Llaneza, D. C. (2010). Corticosteroid and neurosteroid dysregulation in an animal model of autism, BTBR mice. *Physiology & Behavior*, *100*(3), 264–267. https://doi.org/10.1016/j.physbeh.2010.03.005

Furukawa, Y., Furukawa, S., Satoyoshi, E., & Hayashi, K. (1986). Catecholamines induce an increase in nerve growth factor content in the medium of mouse L-M cells. *The Journal of Biological Chemistry*, *261*(13), 6039–6047.

Furuse, M., Itoh, M., Hirase, T., Nagafuchi, A., Yonemura, S., Tsukita, S., & Tsukita, S. (1994). Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. *The Journal of Cell Biology*, *127*(6 Pt 1), 1617–1626. https://doi.org/10.1083/jcb.127.6.1617

Gandhi, T., & Lee, C. C. (2020). Neural Mechanisms Underlying Repetitive Behaviors in Rodent Models of Autism Spectrum Disorders. *Frontiers in Cellular Neuroscience*, *14*, 592710. https://doi.org/10.3389/fncel.2020.592710

Gangi, D. N., Messinger, D. S., Martin, E. R., & Cuccaro, M. L. (2016). Dopaminergic variants in siblings at high risk for autism: Associations with initiating joint attention. *Autism Research*, *9*(11), 1142–1150. https://doi.org/10.1002/aur.1623

Garbuzova-Davis, S., Rodrigues, M. C. O., Hernandez-Ontiveros, D. G., Louis, M. K., Willing, A. E., Borlongan, C. V., & Sanberg, P. R. (2011). Amyotrophic lateral sclerosis: A neurovascular disease. *Brain Research*, *1398*, 113–125. https://doi.org/10.1016/j.brainres.2011.04.049

Garcia, K. O., Ornellas, F. L. M., Martin, P. K. M., Patti, C. L., Mello, L. E., Frussa-Filho, R., Han, S. W., & Longo, B. M. (2014). Therapeutic effects of the transplantation of VEGF overexpressing bone marrow mesenchymal stem cells in the hippocampus of murine model of Alzheimer's disease. *Frontiers in Aging Neuroscience*, *6*, 30. https://doi.org/10.3389/fnagi.2014.00030

Garg, S. K., Banerjee, R., & Kipnis, J. (2008). Neuroprotective immunity: T cell-derived glutamate endows astrocytes with a neuroprotective phenotype. *Journal of Immunology (Baltimore, Md.: 1950)*, *180*(6), 3866–3873. https://doi.org/10.4049/jimmunol.180.6.3866

Garnier, C., Comoy, E., Barthelemy, C., Leddet, I., Garreau, B., Muh, J. P., & Lelord, G. (1986). Dopamine-beta-hydroxylase (DBH) and homovanillic acid (HVA) in autistic children. *Journal of Autism and Developmental Disorders*, *16*(1), 23–29. https://doi.org/10.1007/BF01531575

Garwood, C. J., Ratcliffe, L. E., Simpson, J. E., Heath, P. R., Ince, P. G., & Wharton, S. B. (2017). Review: Astrocytes in Alzheimer's disease and other age-associated dementias: a supporting player with a central role. *Neuropathology and Applied Neurobiology*, *43*(4), 281–298. https://doi.org/10.1111/nan.12338

Gautam, J., Cao, Y., & Yao, Y. (2020). Pericytic Laminin Maintains Blood-Brain Barrier Integrity in an Age-Dependent Manner. *Translational Stroke Research*, *11*(2), 228–242. https://doi.org/10.1007/s12975-019-00709-8

Gegelashvili, G., Dehnes, Y., Danbolt, N. C., & Schousboe, A. (2000). The high-affinity glutamate transporters GLT1, GLAST, and EAAT4 are regulated via different signalling mechanisms. *Neurochemistry International*, *37*(2–3), 163–170. https://doi.org/10.1016/S0197-0186(00)00019-X

George, J., Cunha, R. A., Mulle, C., & Amédée, T. (2016). Microglia-derived purines modulate mossy fibre synaptic transmission and plasticity through P2X4

and A1 receptors. *The European Journal of Neuroscience*, *43*(10), 1366–1378. https://doi.org/10.1111/ejn.13191

Geranmayeh, M. H., Rahbarghazi, R., & Farhoudi, M. (2019). Targeting pericytes for neurovascular regeneration. *Cell Communication and Signaling: CCS*, *17*(1), 26. https://doi.org/10.1186/s12964-019-0340-8

Gerber, U., Gee, C. E., & Benquet, P. (2007). Metabotropic glutamate receptors: Intracellular signaling pathways. *Current Opinion in Pharmacology*, *7*(1), 56–61. https://doi.org/10.1016/j.coph.2006.08.008

Geurts, H. M., Corbett, B., & Solomon, M. (2009). The paradox of cognitive flexibility in autism. *Trends in Cognitive Sciences*, *13*(2), 74–82. https://doi.org/10.1016/j.tics.2008.11.006

Geurts, J. J. G. (2003). Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis. *Brain*, *126*(8), 1755–1766. https://doi.org/10.1093/brain/awg179

Ghanizadeh, A., & Moghimi-Sarani, E. (2013). A randomized double blind placebo controlled clinical trial of N-Acetylcysteine added to risperidone for treating autistic disorders. *BMC Psychiatry*, *13*, 196. https://doi.org/10.1186/1471-244X-13-196

Gillberg, C., Cederlund, M., Lamberg, K., & Zeijlon, L. (2006). Brief report: 'the autism epidemic'. The registered prevalence of autism in a Swedish urban area. *Journal of Autism and Developmental Disorders*, *36*(3), 429–435. https://doi.org/10.1007/s10803-006-0081-6

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., & Merad, M. (2010). Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. *Science*, *330*(6005), 841–845. https://doi.org/10.1126/science.1194637

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., & Huber, T. (2013). Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience*, *7*, 45. https://doi.org/10.3389/fncel.2013.00045

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., De Bruijn, M. F., Geissmann, F., & Rodewald, H.-R. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*, *518*(7540), 547–551. https://doi.org/10.1038/nature13989

Gomez-Arboledas, A., Davila, J. C., Sanchez-Mejias, E., Navarro, V., Nuñez-Diaz, C., Sanchez-Varo, R., Sanchez-Mico, M. V., Trujillo-Estrada, L., Fernandez-Valenzuela, J. J., Vizuete, M., Comella, J. X., Galea, E., Vitorica, J., & Gutierrez, A. (2018).

Phagocytic clearance of presynaptic dystrophies by reactive astrocytes in Alzheimer's disease. *Glia*, *66*(3), 637–653. https://doi.org/10.1002/glia.23270

González-Mariscal, L., Betanzos, A., & Avila-Flores, A. (2000). MAGUK proteins: Structure and role in the tight junction. *Seminars in Cell & Developmental Biology*, *11*(4), 315–324. https://doi.org/10.1006/scdb.2000.0178

Gonzalez-Velasquez, F. J., Kotarek, J. A., & Moss, M. A. (2008). Soluble aggregates of the amyloid-beta protein selectively stimulate permeability in human brain microvascular endothelial monolayers. *Journal of Neurochemistry*, *107*(2), 466–477. https://doi.org/10.1111/j.1471-4159.2008.05618.x

Gould, G. G., Burke, T. F., Osorio, M. D., Smolik, C. M., Zhang, W. Q., Onaivi, E. S., Gu, T.-T., DeSilva, M. N., & Hensler, J. G. (2014). Enhanced novelty-induced corticosterone spike and upregulated serotonin 5-HT1A and cannabinoid CB1 receptors in adolescent BTBR mice. *Psychoneuroendocrinology*, *39*, 158–169. https://doi.org/10.1016/j.psyneuen.2013.09.003

Gould, G. G., Hensler, J. G., Burke, T. F., Benno, R. H., Onaivi, E. S., & Daws, L. C. (2011). Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior: Central 5-HT regulation and social behavior in BTBR mice. *Journal of Neurochemistry*, *116*(2), 291–303. https://doi.org/10.1111/j.1471-4159.2010.07104.x

Gould, G. G., Seillier, A., Weiss, G., Giuffrida, A., Burke, T. F., Hensler, J. G., Rock, C., Tristan, A., McMahon, L. R., Salazar, A., O'Connor, J. C., Satsangi, N., Satsangi, R. K., Gu, T.-T., Treat, K., Smolik, C., & Schultz, S. T. (2012). Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *38*(2), 260–269. https://doi.org/10.1016/j.pnpbp.2012.04.011

Greene, C., Hanley, N., & Campbell, M. (2019). Claudin-5: Gatekeeper of neurological function. *Fluids and Barriers of the CNS*, *16*(1), 3. https://doi.org/10.1186/s12987-019-0123-z

Griciuc, A., Serrano-Pozo, A., Parrado, A. R., Lesinski, A. N., Asselin, C. N., Mullin, K., Hooli, B., Choi, S. H., Hyman, B. T., & Tanzi, R. E. (2013). Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron*, *78*(4), 631–643. https://doi.org/10.1016/j.neuron.2013.04.014

Grossmann, R., Stence, N., Carr, J., Fuller, L., Waite, M., & Dailey, M. E. (2002). Juxtavascular microglia migrate along brain microvessels following activation during early postnatal development. *Glia*, *37*(3), 229–240. https://doi.org/10.1002/glia.10031

Grosso, G., Galvano, F., Marventano, S., Malaguarnera, M., Bucolo, C., Drago, F., & Caraci, F. (2014). Omega-3 fatty acids and depression: Scientific evidence and biological mechanisms. *Oxidative Medicine and Cellular Longevity*, *2014*, 313570. https://doi.org/10.1155/2014/313570

Grosso, G., Pajak, A., Marventano, S., Castellano, S., Galvano, F., Bucolo, C., Drago, F., & Caraci, F. (2014). Role of omega-3 fatty acids in the treatment of depressive disorders: A comprehensive meta-analysis of randomized clinical trials. *PloS One*, *9*(5), e96905. https://doi.org/10.1371/journal.pone.0096905

Grueter, B. A., & Winder, D. G. (2005). Group II and III Metabotropic Glutamate Receptors Suppress Excitatory Synaptic Transmission in the Dorsolateral Bed Nucleus of the Stria Terminalis. *Neuropsychopharmacology*, *30*(7), 1302–1311. https://doi.org/10.1038/sj.npp.1300672

Guastella, A. J., Einfeld, S. L., Gray, K. M., Rinehart, N. J., Tonge, B. J., Lambert, T. J., & Hickie, I. B. (2010). Intranasal Oxytocin Improves Emotion Recognition for Youth with Autism Spectrum Disorders. *Biological Psychiatry*, *67*(7), 692–694. https://doi.org/10.1016/j.biopsych.2009.09.020

Gullotta, G. S., Costantino, G., Sortino, M. A., & Spampinato, S. F. (2023). Microglia and the Blood–Brain Barrier: An External Player in Acute and Chronic Neuroinflammatory Conditions. *International Journal of Molecular Sciences*, *24*(11), 9144. https://doi.org/10.3390/ijms24119144

Gumbiner, B. M. (1993). Breaking through the tight junction barrier. *The Journal of Cell Biology*, *123*(6 Pt 2), 1631–1633. https://doi.org/10.1083/jcb.123.6.1631

Guo, Y., & Commons, K. G. (2017). Serotonin neuron abnormalities in the BTBR mouse model of autism. *Autism Research*, *10*(1), 66–77. https://doi.org/10.1002/aur.1665

Gursoy-Ozdemir, Y., Yemisci, M., & Dalkara, T. (2012). Microvascular protection is essential for successful neuroprotection in stroke. *Journal of Neurochemistry*, *123 Suppl 2*, 2–11. https://doi.org/10.1111/j.1471-4159.2012.07938.x

Gustin, A., Kirchmeyer, M., Koncina, E., Felten, P., Losciuto, S., Heurtaux, T., Tardivel, A., Heuschling, P., & Dostert, C. (2015). NLRP3 Inflammasome Is Expressed and Functional in Mouse Brain Microglia but Not in Astrocytes. *PloS One*, *10*(6), e0130624. https://doi.org/10.1371/journal.pone.0130624

Guttenplan, K. A., Weigel, M. K., Adler, D. I., Couthouis, J., Liddelow, S. A., Gitler, A. D., & Barres, B. A. (2020). Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model. *Nature Communications*, *11*(1), 3753. https://doi.org/10.1038/s41467-020-17514-9

Hadad, N., & Levy, R. (2012). The synergistic anti-inflammatory effects of lycopene, lutein, β-carotene, and carnosic acid combinations via redox-based inhibition of NF-κB signaling. *Free Radical Biology & Medicine*, *53*(7), 1381–1391. https://doi.org/10.1016/j.freeradbiomed.2012.07.078

Hagemeyer, N., Kierdorf, K., Frenzel, K., Xue, J., Ringelhan, M., Abdullah, Z., Godin, I., Wieghofer, P., Costa Jordão, M. J., Ulas, T., Yorgancioglu, G., Rosenbauer, F., Knolle, P. A., Heikenwalder, M., Schultze, J. L., & Prinz, M. (2016). Transcriptome-based profiling of yolk sac-derived macrophages reveals a role for Irf8 in macrophage maturation. *The EMBO Journal*, *35*(16), 1730–1744. https://doi.org/10.15252/embj.201693801

Hagerman, R., Au, J., & Hagerman, P. (2011). FMR1 premutation and full mutation molecular mechanisms related to autism. *Journal of Neurodevelopmental Disorders*, *3*(3), 211–224. https://doi.org/10.1007/s11689-011-9084-5

Halder, S. K., & Milner, R. (2019). A critical role for microglia in maintaining vascular integrity in the hypoxic spinal cord. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(51), 26029–26037. https://doi.org/10.1073/pnas.1912178116

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J., Fedele, A., Collins, J., Smith, K., Lotspeich, L., Croen, L. A., Ozonoff, S., Lajonchere, C., Grether, J. K., & Risch, N. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry*, *68*(11), 1095–1102. https://doi.org/10.1001/archgenpsychiatry.2011.76

Hamilton, N., Vayro, S., Wigley, R., & Butt, A. M. (2010). Axons and astrocytes release ATP and glutamate to evoke calcium signals in NG2-glia. *Glia*, *58*(1), 66–79. https://doi.org/10.1002/glia.20902

Han, J. S., Fu, Y., Bird, G. C., & Neugebauer, V. (2006). Enhanced Group II mGluR-Mediated Inhibition of Pain-Related Synaptic Plasticity in the Amygdala. *Molecular Pain*, *2*, 1744-8069-2–18. https://doi.org/10.1186/1744-8069-2-18

Han, S., Tai, C., Jones, C. J., Scheuer, T., & Catterall, W. A. (2014). Enhancement of Inhibitory Neurotransmission by GABA A Receptors Having α 2,3 -Subunits Ameliorates Behavioral Deficits in a Mouse Model of Autism. *Neuron*, *81*(6), 1282–1289. https://doi.org/10.1016/j.neuron.2014.01.016

Hanslik, K. L., & Ulland, T. K. (2020). The Role of Microglia and the Nlrp3 Inflammasome in Alzheimer's Disease. *Frontiers in Neurology*, *11*, 570711. https://doi.org/10.3389/fneur.2020.570711

Harada, K., Kamiya, T., & Tsuboi, T. (2015). Gliotransmitter Release from Astrocytes: Functional, Developmental, and Pathological Implications in the Brain. *Frontiers in Neuroscience*, *9*, 499. https://doi.org/10.3389/fnins.2015.00499

Haruki, H., Sano, Y., Shimizu, F., Omoto, M., Tasaki, A., Oishi, M., Koga, M., Saito, K., Takahashi, T., Nakada, T., & Kanda, T. (2013). NMO sera down-regulate AQP4 in human astrocyte and induce cytotoxicity independent of complement. *Journal of the Neurological Sciences*, *331*(1–2), 136–144. https://doi.org/10.1016/j.jns.2013.05.035

Haruwaka, K., Ikegami, A., Tachibana, Y., Ohno, N., Konishi, H., Hashimoto, A., Matsumoto, M., Kato, D., Ono, R., Kiyama, H., Moorhouse, A. J., Nabekura, J., & Wake, H. (2019). Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. *Nature Communications*, *10*(1), 5816. https://doi.org/10.1038/s41467-019-13812-z

Hasel, P., Rose, I. V. L., Sadick, J. S., Kim, R. D., & Liddelow, S. A. (2021). Neuroinflammatory astrocyte subtypes in the mouse brain. *Nature Neuroscience*, *24*(10), 1475–1487. https://doi.org/10.1038/s41593-021-00905-6

Haskins, J., Gu, L., Wittchen, E. S., Hibbard, J., & Stevenson, B. R. (1998). ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. *The Journal of Cell Biology*, *141*(1), 199–208. https://doi.org/10.1083/jcb.141.1.199

Hathaway, H. A., Pshenichkin, S., Grajkowska, E., Gelb, T., Emery, A. C., Wolfe, B. B., & Wroblewski, J. T. (2015). Pharmacological characterization of mGlu1 receptors in cerebellar granule cells reveals biased agonism. *Neuropharmacology*, *93*, 199–208. https://doi.org/10.1016/j.neuropharm.2015.02.007

Havránek, T., Lešťanová, Z., Mravec, B., Štrbák, V., Bakoš, J., & Bačová, Z. (2017). Oxytocin Modulates Expression of Neuron and Glial Markers in the Rat Hippocampus. *Folia Biologica*, *63*(3), 91–97.

Haznedar, M. M., Buchsbaum, M. S., Wei, T. C., Hof, P. R., Cartwright, C., Bienstock, C. A., & Hollander, E. (2000). Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *The American Journal of Psychiatry*, *157*(12), 1994–2001. https://doi.org/10.1176/appi.ajp.157.12.1994

Heithoff, B. P., George, K. K., Phares, A. N., Zuidhoek, I. A., Munoz-Ballester, C., & Robel, S. (2021). Astrocytes are necessary for blood-brain barrier maintenance in the adult mouse brain. *Glia*, *69*(2), 436–472. https://doi.org/10.1002/glia.23908

Hellings, J. A., Arnold, L. E., & Han, J. C. (2017). Dopamine antagonists for treatment resistance in autism spectrum disorders: Review and focus on BDNF

stimulators loxapine and amitriptyline. *Expert Opinion on Pharmacotherapy*, *18*(6), 581–588. https://doi.org/10.1080/14656566.2017.1308483

Hellström, M., Gerhardt, H., Kalén, M., Li, X., Eriksson, U., Wolburg, H., & Betsholtz, C. (2001). Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *The Journal of Cell Biology*, *153*(3), 543–553. https://doi.org/10.1083/jcb.153.3.543

Hellyer, S., Leach, K., & Gregory, K. J. (2017). Neurobiological insights and novel therapeutic opportunities for CNS disorders from mGlu receptor allosteric and biased modulation. *Current Opinion in Pharmacology*, *32*, 49–55. https://doi.org/10.1016/j.coph.2016.10.007

Heo, Y., Zhang, Y., Gao, D., Miller, V. M., & Lawrence, D. A. (2011). Aberrant immune responses in a mouse with behavioral disorders. *PloS One*, *6*(7), e20912. https://doi.org/10.1371/journal.pone.0020912

Hill, J., Rom, S., Ramirez, S. H., & Persidsky, Y. (2014). Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *9*(5), 591–605. https://doi.org/10.1007/s11481-014-9557-x

Hodges, H., Fealko, C., & Soares, N. (2020). Autism spectrum disorder: Definition, epidemiology, causes, and clinical evaluation. *Translational Pediatrics*, *9*(Suppl 1), S55–S65. https://doi.org/10.21037/tp.2019.09.09

Hollander, E., Anagnostou, E., Chaplin, W., Esposito, K., Haznedar, M. M., Licalzi, E., Wasserman, S., Soorya, L., & Buchsbaum, M. (2005). Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biological Psychiatry*, *58*(3), 226–232. https://doi.org/10.1016/j.biopsych.2005.03.040

Hollander, E., Bartz, J., Chaplin, W., Phillips, A., Sumner, J., Soorya, L., Anagnostou, E., & Wasserman, S. (2007). Oxytocin Increases Retention of Social Cognition in Autism. *Biological Psychiatry*, *61*(4), 498–503. https://doi.org/10.1016/j.biopsych.2006.05.030

Hong, S., Beja-Glasser, V. F., Nfonoyim, B. M., Frouin, A., Li, S., Ramakrishnan, S., Merry, K. M., Shi, Q., Rosenthal, A., Barres, B. A., Lemere, C. A., Selkoe, D. J., & Stevens, B. (2016). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science (New York, N.Y.)*, *352*(6286), 712–716. https://doi.org/10.1126/science.aad8373

Hooijmans, C. R., Graven, C., Dederen, P. J., Tanila, H., van Groen, T., & Kiliaan, A. J. (2007). Amyloid beta deposition is related to decreased glucose transporter-1 levels and hippocampal atrophy in brains of aged APP/PS1 mice. *Brain Research*, *1181*, 93–103. https://doi.org/10.1016/j.brainres.2007.08.063

Horder, J., Petrinovic, M. M., Mendez, M. A., Bruns, A., Takumi, T., Spooren, W., Barker, G. J., Künnecke, B., & Murphy, D. G. (2018). Glutamate and GABA in autism spectrum disorder-a translational magnetic resonance spectroscopy study in man and rodent models. *Translational Psychiatry*, *8*(1), 106. https://doi.org/10.1038/s41398-018-0155-1

Horng, S., Therattil, A., Moyon, S., Gordon, A., Kim, K., Argaw, A. T., Hara, Y., Mariani, J. N., Sawai, S., Flodby, P., Crandall, E. D., Borok, Z., Sofroniew, M. V., Chapouly, C., & John, G. R. (2017). Astrocytic tight junctions control inflammatory CNS lesion pathogenesis. *Journal of Clinical Investigation*, *127*(8), 3136–3151. https://doi.org/10.1172/JCI91301

Hu, J., Chen, Q., Zhu, H., Hou, L., Liu, W., Yang, Q., Shen, H., Chai, G., Zhang, B., Chen, S., Cai, Z., Wu, C., Hong, F., Li, H., Chen, S., Xiao, N., Wang, Z., Zhang, X., Wang, B., … Mo, W. (2023). Microglial Piezo1 senses Aβ fibril stiffness to restrict Alzheimer's disease. *Neuron*, *111*(1), 15-29.e8. https://doi.org/10.1016/j.neuron.2022.10.021

Huang, W., Xia, Q., Zheng, F., Zhao, X., Ge, F., Xiao, J., Liu, Z., Shen, Y., Ye, K., Wang, D., & Li, Y. (2023). Microglia-Mediated Neurovascular Unit Dysfunction in Alzheimer's Disease. *Journal of Alzheimer's Disease: JAD*, *94*(s1), S335–S354. https://doi.org/10.3233/JAD-221064

Huber, J. D., Campos, C. R., Mark, K. S., & Davis, T. P. (2006). Alterations in blood-brain barrier ICAM-1 expression and brain microglial activation after lambda-carrageenan-induced inflammatory pain. *American Journal of Physiology. Heart and Circulatory Physiology*, *290*(2), H732-740. https://doi.org/10.1152/ajpheart.00747.2005

Huffels, C. F. M., Middeldorp, J., & Hol, E. M. (2023). Aß Pathology and Neuron-Glia Interactions: A Synaptocentric View. *Neurochemical Research*, *48*(4), 1026–1046. https://doi.org/10.1007/s11064-022-03699-6

Huguet, G., Ey, E., & Bourgeron, T. (2013). The Genetic Landscapes of Autism Spectrum Disorders. *Annual Review of Genomics and Human Genetics*, *14*(1), 191–213. https://doi.org/10.1146/annurev-genom-091212-153431

Iacovelli, L., Bruno, V., Salvatore, L., Melchiorri, D., Gradini, R., Caricasole, A., Barletta, E., De Blasi, A., & Nicoletti, F. (2002). Native group-III metabotropic glutamate receptors are coupled to the mitogen-activated protein kinase/phosphatidylinositol-3-kinase pathways. *Journal of Neurochemistry*, *82*(2), 216–223. https://doi.org/10.1046/j.1471-4159.2002.00929.x

Iacovelli, L., Felicioni, M., Nisticò, R., Nicoletti, F., & De Blasi, A. (2014). Selective regulation of recombinantly expressed mGlu7 metabotropic glutamate receptors

by G protein-coupled receptor kinases and arrestins. *Neuropharmacology*, *77*, 303–312. https://doi.org/10.1016/j.neuropharm.2013.10.013

Ibáñez, C. F., & Andressoo, J.-O. (2017). Biology of GDNF and its receptors—Relevance for disorders of the central nervous system. *Neurobiology of Disease*, *97*, 80–89. https://doi.org/10.1016/j.nbd.2016.01.021

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., Benveniste, H., Vates, G. E., Deane, R., Goldman, S. A., Nagelhus, E. A., & Nedergaard, M. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Science Translational Medicine*, *4*(147), 147ra111. https://doi.org/10.1126/scitranslmed.3003748

Inatani, M., Irie, F., Plump, A. S., Tessier-Lavigne, M., & Yamaguchi, Y. (2003). Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. *Science (New York, N.Y.)*, *302*(5647), 1044–1046. https://doi.org/10.1126/science.1090497

Inga Jácome, M. C., Morales Chacòn, L. M., Vera Cuesta, H., Maragoto Rizo, C., Whilby Santiesteban, M., Ramos Hernandez, L., Noris García, E., González Fraguela, M. E., Fernandez Verdecia, C. I., Vegas Hurtado, Y., Siniscalco, D., Gonçalves, C. A., & Robinson-Agramonte, M. de L. A. (2016). Peripheral Inflammatory Markers Contributing to Comorbidities in Autism. *Behavioral Sciences (Basel, Switzerland)*, *6*(4), 29. https://doi.org/10.3390/bs6040029

Iossifov, I., O'Roak, B. J., Sanders, S. J., Ronemus, M., Krumm, N., Levy, D., Stessman, H. A., Witherspoon, K. T., Vives, L., Patterson, K. E., Smith, J. D., Paeper, B., Nickerson, D. A., Dea, J., Dong, S., Gonzalez, L. E., Mandell, J. D., Mane, S. M., Murtha, M. T., … Wigler, M. (2014). The contribution of de novo coding mutations to autism spectrum disorder. *Nature*, *515*(7526), 216–221. https://doi.org/10.1038/nature13908

Iram, T., Trudler, D., Kain, D., Kanner, S., Galron, R., Vassar, R., Barzilai, A., Blinder, P., Fishelson, Z., & Frenkel, D. (2016). Astrocytes from old Alzheimer's disease mice are impaired in Aβ uptake and in neuroprotection. *Neurobiology of Disease*, *96*, 84–94. https://doi.org/10.1016/j.nbd.2016.08.001

Irie, F., Badie-Mahdavi, H., & Yamaguchi, Y. (2012). Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(13), 5052–5056. https://doi.org/10.1073/pnas.1117881109

Jasien, J. M., Daimon, C. M., Wang, R., Shapiro, B. K., Martin, B., & Maudsley, S. (2014). The effects of aging on the BTBR mouse model of autism spectrum

disorder. *Frontiers in Aging Neuroscience*, *6*. https://doi.org/10.3389/fnagi.2014.00225

Jeste, S. S., & Geschwind, D. H. (2014). Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nature Reviews Neurology*, *10*(2), 74–81. https://doi.org/10.1038/nrneurol.2013.278

Johnson, K., Conn, P., & Niswender, C. (2009). Glutamate Receptors as Therapeutic Targets for Parkinsons Disease. *CNS & Neurological Disorders - Drug Targets*, *8*(6), 475–491. https://doi.org/10.2174/187152709789824606

Jomova, K., & Valko, M. (2013). Health protective effects of carotenoids and their interactions with other biological antioxidants. *European Journal of Medicinal Chemistry*, *70*, 102–110. https://doi.org/10.1016/j.ejmech.2013.09.054

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience*, *10*(8), 561–572. https://doi.org/10.1038/nrn2515

Kammermeier, P. J. (2012). Functional and Pharmacological Characteristics of Metabotropic Glutamate Receptors 2/4 Heterodimers. *Molecular Pharmacology*, *82*(3), 438–447. https://doi.org/10.1124/mol.112.078501

Karvat, G., & Kimchi, T. (2014). Acetylcholine Elevation Relieves Cognitive Rigidity and Social Deficiency in a Mouse Model of Autism. *Neuropsychopharmacology*, *39*(4), 831–840. https://doi.org/10.1038/npp.2013.274

Kas, M. J., Penninx, B., Sommer, B., Serretti, A., Arango, C., & Marston, H. (2019). A quantitative approach to neuropsychiatry: The why and the how. *Neuroscience and Biobehavioral Reviews*, *97*, 3–9. https://doi.org/10.1016/j.neubiorev.2017.12.008

Kasarpalkar, N. J., Kothari, S. T., & Dave, U. P. (2014). Brain-Derived Neurotrophic Factor in children with Autism Spectrum Disorder. *Annals of Neurosciences*, *21*(4), 129–133. https://doi.org/10.5214/ans.0972.7531.210403

Katusic, Z. S., & Austin, S. A. (2016). Neurovascular Protective Function of Endothelial Nitric Oxide—Recent Advances. *Circulation Journal: Official Journal of the Japanese Circulation Society*, *80*(7), 1499–1503. https://doi.org/10.1253/circj.CJ-16-0423

Kaulmann, A., & Bohn, T. (2014). Carotenoids, inflammation, and oxidative stress—Implications of cellular signaling pathways and relation to chronic disease prevention. *Nutrition Research (New York, N.Y.)*, *34*(11), 907–929. https://doi.org/10.1016/j.nutres.2014.07.010

Kaushal, V., & Schlichter, L. C. (2008). Mechanisms of Microglia-Mediated Neurotoxicity in a New Model of the Stroke Penumbra. *The Journal of*

Neuroscience, *28*(9), 2221–2230. https://doi.org/10.1523/JNEUROSCI.5643-07.2008

Keaney, J., Walsh, D. M., O'Malley, T., Hudson, N., Crosbie, D. E., Loftus, T., Sheehan, F., McDaid, J., Humphries, M. M., Callanan, J. J., Brett, F. M., Farrell, M. A., Humphries, P., & Campbell, M. (2015). Autoregulated paracellular clearance of amyloid-β across the blood-brain barrier. *Science Advances*, *1*(8), e1500472. https://doi.org/10.1126/sciadv.1500472

Kemper, T. L., & Bauman, M. (1998). Neuropathology of infantile autism. *Journal of Neuropathology and Experimental Neurology*, *57*(7), 645–652. https://doi.org/10.1097/00005072-199807000-00001

Kenkhuis, B., Somarakis, A., Kleindouwel, L. R. T., van Roon-Mom, W. M. C., Höllt, T., & van der Weerd, L. (2022). Co-expression patterns of microglia markers Iba1, TMEM119 and P2RY12 in Alzheimer's disease. *Neurobiology of Disease*, *167*, 105684. https://doi.org/10.1016/j.nbd.2022.105684

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., Itzkovitz, S., Colonna, M., Schwartz, M., & Amit, I. (2017). A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell*, *169*(7), 1276-1290.e17. https://doi.org/10.1016/j.cell.2017.05.018

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., Wieghofer, P., Heinrich, A., Riemke, P., Hölscher, C., Müller, D. N., Luckow, B., Brocker, T., Debowski, K., Fritz, G., Opdenakker, G., Diefenbach, A., Biber, K., Heikenwalder, M., … Prinz, M. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience*, *16*(3), 273–280. https://doi.org/10.1038/nn.3318

Kirabali, T., Rust, R., Rigotti, S., Siccoli, A., Nitsch, R. M., & Kulic, L. (2020). Distinct changes in all major components of the neurovascular unit across different neuropathological stages of Alzheimer's disease. *Brain Pathology (Zurich, Switzerland)*, *30*(6), 1056–1070. https://doi.org/10.1111/bpa.12895

Kitaguchi, H., Ihara, M., Saiki, H., Takahashi, R., & Tomimoto, H. (2007). Capillary beds are decreased in Alzheimer's disease, but not in Binswanger's disease. *Neuroscience Letters*, *417*(2), 128–131. https://doi.org/10.1016/j.neulet.2007.02.021

Kleinberger, G., Brendel, M., Mracsko, E., Wefers, B., Groeneweg, L., Xiang, X., Focke, C., Deußing, M., Suárez-Calvet, M., Mazaheri, F., Parhizkar, S., Pettkus, N., Wurst, W., Feederle, R., Bartenstein, P., Mueggler, T., Arzberger, T., Knuesel, I., Rominger, A., & Haass, C. (2017). The FTD-like syndrome causing TREM2 T66M mutation impairs microglia function, brain perfusion, and glucose metabolism.

The EMBO Journal, *36*(13), 1837–1853. https://doi.org/10.15252/embj.201796516

Kljakic, O., Al-Onaizi, M., Janíčková, H., Chen, K. S., Guzman, M. S., Prado, M. A. M., & Prado, V. F. (2021). Cholinergic transmission from the basal forebrain modulates social memory in male mice. *The European Journal of Neuroscience*, *54*(6), 6075–6092. https://doi.org/10.1111/ejn.15400

Knezevic, D., & Mizrahi, R. (2018). Molecular imaging of neuroinflammation in Alzheimer's disease and mild cognitive impairment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *80*, 123–131. https://doi.org/10.1016/j.pnpbp.2017.05.007

Kniazeff, J., Prézeau, L., Rondard, P., Pin, J.-P., & Goudet, C. (2011). Dimers and beyond: The functional puzzles of class C GPCRs. *Pharmacology & Therapeutics*, *130*(1), 9–25. https://doi.org/10.1016/j.pharmthera.2011.01.006

Knoop, M., Possovre, M.-L., Jacquens, A., Charlet, A., Baud, O., & Darbon, P. (2022). The Role of Oxytocin in Abnormal Brain Development: Effect on Glial Cells and Neuroinflammation. *Cells*, *11*(23), 3899. https://doi.org/10.3390/cells11233899

Kook, S.-Y., Hong, H. S., Moon, M., Ha, C. M., Chang, S., & Mook-Jung, I. (2012). $A\beta_{1-42}$-RAGE interaction disrupts tight junctions of the blood-brain barrier via Ca^{2+}-calcineurin signaling. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*(26), 8845–8854. https://doi.org/10.1523/JNEUROSCI.6102-11.2012

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). Free Dyadic Social Interaction Test in Mice. *Methods in Molecular Biology (Clifton, N.J.)*, *1916*, 93–97. https://doi.org/10.1007/978-1-4939-8994-2_8

Kryszkowski, W., & Boczek, T. (2021). The G Protein-Coupled Glutamate Receptors as Novel Molecular Targets in Schizophrenia Treatment-A Narrative Review. *Journal of Clinical Medicine*, *10*(7), 1475. https://doi.org/10.3390/jcm10071475

Kubota, M., Fujino, J., Tei, S., Takahata, K., Matsuoka, K., Tagai, K., Sano, Y., Yamamoto, Y., Shimada, H., Takado, Y., Seki, C., Itahashi, T., Aoki, Y. Y., Ohta, H., Hashimoto, R.-I., Zhang, M.-R., Suhara, T., Nakamura, M., Takahashi, H., … Higuchi, M. (2020). Binding of Dopamine D1 Receptor and Noradrenaline Transporter in Individuals with Autism Spectrum Disorder: A PET Study. *Cerebral Cortex (New York, N.Y.: 1991)*, *30*(12), 6458–6468. https://doi.org/10.1093/cercor/bhaa211

Kubota, Y., Takubo, K., Shimizu, T., Ohno, H., Kishi, K., Shibuya, M., Saya, H., & Suda, T. (2009). M-CSF inhibition selectively targets pathological angiogenesis and

lymphangiogenesis. *The Journal of Experimental Medicine*, *206*(5), 1089–1102. https://doi.org/10.1084/jem.20081605

Kubotera, H., Ikeshima-Kataoka, H., Hatashita, Y., Allegra Mascaro, A. L., Pavone, F. S., & Inoue, T. (2019). Astrocytic endfeet re-cover blood vessels after removal by laser ablation. *Scientific Reports*, *9*(1), 1263. https://doi.org/10.1038/s41598-018-37419-4

Kuo, J., Hariri, O. R., & Micevych, P. (2009). An interaction of oxytocin receptors with metabotropic glutamate receptors in hypothalamic astrocytes. *Journal of Neuroendocrinology*, *21*(12), 1001–1006. https://doi.org/10.1111/j.1365-2826.2009.01922.x

Kusek, G. K., Wahlsten, D., Herron, B. J., Bolivar, V. J., & Flaherty, L. (2007). Localization of two new X-linked quantitative trait loci controlling corpus callosum size in the mouse. *Genes, Brain, and Behavior*, *6*(4), 359–363. https://doi.org/10.1111/j.1601-183X.2006.00264.x

Langen, M., Durston, S., Kas, M. J. H., van Engeland, H., & Staal, W. G. (2011). The neurobiology of repetitive behavior: …and men. *Neuroscience and Biobehavioral Reviews*, *35*(3), 356–365. https://doi.org/10.1016/j.neubiorev.2010.02.005

Lau, S.-F., Cao, H., Fu, A. K. Y., & Ip, N. Y. (2020). Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(41), 25800–25809. https://doi.org/10.1073/pnas.2008762117

Launay, J. M., Bursztejn, C., Ferrari, P., Dreux, C., Braconnier, A., Zarifian, E., Lancrenon, S., & Fermanian, J. (1987). Catecholamines metabolism in infantile autism: A controlled study of 22 autistic children. *Journal of Autism and Developmental Disorders*, *17*(3), 333–347. https://doi.org/10.1007/BF01487064

Laurence, J. A., & Fatemi, S. H. (2005). Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *Cerebellum (London, England)*, *4*(3), 206–210. https://doi.org/10.1080/14734220500208846

Laviola, G., Adriani, W., Rea, M., Aloe, L., & Alleva, E. (2004). Social withdrawal, neophobia, and stereotyped behavior in developing rats exposed to neonatal asphyxia. *Psychopharmacology*, *175*(2), 196–205. https://doi.org/10.1007/s00213-004-1800-3

Lee, A. S., Azmitia, E. C., & Whitaker-Azmitia, P. M. (2017). Developmental microglial priming in postmortem autism spectrum disorder temporal cortex. *Brain, Behavior, and Immunity*, *62*, 193–202. https://doi.org/10.1016/j.bbi.2017.01.019

Lee, B. K., Magnusson, C., Gardner, R. M., Blomström, Å., Newschaffer, C. J., Burstyn, I., Karlsson, H., & Dalman, C. (2015). Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain, Behavior, and Immunity*, *44*, 100–105. https://doi.org/10.1016/j.bbi.2014.09.001

Lee, J. K., Andrews, D. S., Ozturk, A., Solomon, M., Rogers, S., Amaral, D. G., & Nordahl, C. W. (2022). Altered Development of Amygdala-Connected Brain Regions in Males and Females with Autism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *42*(31), 6145–6155. https://doi.org/10.1523/JNEUROSCI.0053-22.2022

Lefevre, A., Richard, N., Jazayeri, M., Beuriat, P.-A., Fieux, S., Zimmer, L., Duhamel, J.-R., & Sirigu, A. (2017). Oxytocin and Serotonin Brain Mechanisms in the Nonhuman Primate. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(28), 6741–6750. https://doi.org/10.1523/JNEUROSCI.0659-17.2017

Leshem, R., Bar-Oz, B., Diav-Citrin, O., Gbaly, S., Soliman, J., Renoux, C., & Matok, I. (2021). Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin Norepinephrine Reuptake Inhibitors (SNRIs) During Pregnancy and the Risk for Autism spectrum disorder (ASD) and Attention deficit hyperactivity disorder (ADHD) in the Offspring: A True Effect or a Bias? A Systematic Review & Meta-Analysis. *Current Neuropharmacology*, *19*(6), 896–906. https://doi.org/10.2174/1570159X19666210303121059

Leyfer, O. T., Folstein, S. E., Bacalman, S., Davis, N. O., Dinh, E., Morgan, J., Tager-Flusberg, H., & Lainhart, J. E. (2006). Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *Journal of Autism and Developmental Disorders*, *36*(7), 849–861. https://doi.org/10.1007/s10803-006-0123-0

Li, R., Hong, P., & Zheng, X. (2019). β-carotene attenuates lipopolysaccharide-induced inflammation via inhibition of the NF-κB, JAK2/STAT3 and JNK/p38 MAPK signaling pathways in macrophages. *Animal Science Journal = Nihon Chikusan Gakkaiho*, *90*(1), 140–148. https://doi.org/10.1111/asj.13108

Li, X., Chauhan, A., Sheikh, A. M., Patil, S., Chauhan, V., Li, X.-M., Ji, L., Brown, T., & Malik, M. (2009). Elevated immune response in the brain of autistic patients. *Journal of Neuroimmunology*, *207*(1–2), 111–116. https://doi.org/10.1016/j.jneuroim.2008.12.002

Lian, H., Yang, L., Cole, A., Sun, L., Chiang, A. C.-A., Fowler, S. W., Shim, D. J., Rodriguez-Rivera, J., Taglialatela, G., Jankowsky, J. L., Lu, H.-C., & Zheng, H. (2015). NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. *Neuron*, *85*(1), 101–115. https://doi.org/10.1016/j.neuron.2014.11.018

Liao, X., Liu, Y., Fu, X., & Li, Y. (2020). Postmortem Studies of Neuroinflammation in Autism Spectrum Disorder: A Systematic Review. *Molecular Neurobiology*, *57*(8), 3424–3438. https://doi.org/10.1007/s12035-020-01976-5

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., Bennett, M. L., Münch, A. E., Chung, W.-S., Peterson, T. C., Wilton, D. K., Frouin, A., Napier, B. A., Panicker, N., Kumar, M., Buckwalter, M. S., Rowitch, D. H., Dawson, V. L., Dawson, T. M., … Barres, B. A. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*, *541*(7638), 481–487. https://doi.org/10.1038/nature21029

Liebler, D. C., Stratton, S. P., & Kaysen, K. L. (1997). Antioxidant actions of beta-carotene in liposomal and microsomal membranes: Role of carotenoid-membrane incorporation and alpha-tocopherol. *Archives of Biochemistry and Biophysics*, *338*(2), 244–250. https://doi.org/10.1006/abbi.1996.9822

Liu, C., Liu, J., Gong, H., Liu, T., Li, X., & Fan, X. (2023). Implication of Hippocampal Neurogenesis in Autism Spectrum Disorder: Pathogenesis and Therapeutic Implications. *Current Neuropharmacology*, *21*(11), 2266–2282. https://doi.org/10.2174/1570159X21666221220155455

Liu, J., Han, Y.-S., Liu, L., Tang, L., Yang, H., Meng, P., Zhao, H.-Q., & Wang, Y.-H. (2021). Abnormal Glu/mGluR2/3/PI3K pathway in the hippocampal neurovascular unit leads to diabetes-related depression. *Neural Regeneration Research*, *16*(4), 727–733. https://doi.org/10.4103/1673-5374.296418

Liu, L.-R., Liu, J.-C., Bao, J.-S., Bai, Q.-Q., & Wang, G.-Q. (2020). Interaction of Microglia and Astrocytes in the Neurovascular Unit. *Frontiers in Immunology*, *11*, 1024. https://doi.org/10.3389/fimmu.2020.01024

Liu, S., Chang, L., & Wei, C. (2019). The sonic hedgehog pathway mediates Tongxinluo capsule-induced protection against blood-brain barrier disruption after ischaemic stroke in mice. *Basic & Clinical Pharmacology & Toxicology*, *124*(6), 660–669. https://doi.org/10.1111/bcpt.13186

Liu, W., Tang, Y., & Feng, J. (2011). Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. *Life Sciences*, *89*(5–6), 141–146. https://doi.org/10.1016/j.lfs.2011.05.011

Lochhead, J. J., Yang, J., Ronaldson, P. T., & Davis, T. P. (2020). Structure, Function, and Regulation of the Blood-Brain Barrier Tight Junction in Central Nervous System Disorders. *Frontiers in Physiology*, *11*, 914. https://doi.org/10.3389/fphys.2020.00914

Londei, T., Valentini, A. M., & Leone, V. G. (1998). Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour. *Behavioural Brain Research*, *94*(2), 249–254. https://doi.org/10.1016/s0166-4328(97)00162-9

Loomes, R., Hull, L., & Mandy, W. P. L. (2017). What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, *56*(6), 466–474. https://doi.org/10.1016/j.jaac.2017.03.013

Lorenzetti, V., Allen, N. B., Fornito, A., & Yücel, M. (2009). Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. *Journal of Affective Disorders*, *117*(1–2), 1–17. https://doi.org/10.1016/j.jad.2008.11.021

Losonczy, A., Somogyi, P., & Nusser, Z. (2003). Reduction of excitatory postsynaptic responses by persistently active metabotropic glutamate receptors in the hippocampus. *Journal of Neurophysiology*, *89*(4), 1910–1919. https://doi.org/10.1152/jn.00842.2002

Lu, A. T.-H., Yoon, J., Geschwind, D. H., & Cantor, R. M. (2013). QTL replication and targeted association highlight the nerve growth factor gene for nonverbal communication deficits in autism spectrum disorders. *Molecular Psychiatry*, *18*(2), 226–235. https://doi.org/10.1038/mp.2011.155

Lujan, R., Nusser, Z., Roberts, J. D., Shigemoto, R., & Somogyi, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *The European Journal of Neuroscience*, *8*(7), 1488–1500. https://doi.org/10.1111/j.1460-9568.1996.tb01611.x

Luján, R., Shigemoto, R., & López-Bendito, G. (2005). Glutamate and GABA receptor signalling in the developing brain. *Neuroscience*, *130*(3), 567–580. https://doi.org/10.1016/j.neuroscience.2004.09.042

Luyt, K., Varadi, A., & Molnar, E. (2003). Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. *Journal of Neurochemistry*, *84*(6), 1452–1464. https://doi.org/10.1046/j.1471-4159.2003.01661.x

Lyman, M., Lloyd, D. G., Ji, X., Vizcaychipi, M. P., & Ma, D. (2014). Neuroinflammation: The role and consequences. *Neuroscience Research*, *79*, 1–12. https://doi.org/10.1016/j.neures.2013.10.004

Mäe, M. A., He, L., Nordling, S., Vazquez-Liebanas, E., Nahar, K., Jung, B., Li, X., Tan, B. C., Chin Foo, J., Cazenave-Gassiot, A., Wenk, M. R., Zarb, Y., Lavina, B., Quaggin, S. E., Jeansson, M., Gu, C., Silver, D. L., Vanlandewijck, M., Butcher, E. C., … Betsholtz, C. (2021). Single-Cell Analysis of Blood-Brain Barrier Response to Pericyte Loss. *Circulation Research*, *128*(4), e46–e62. https://doi.org/10.1161/CIRCRESAHA.120.317473

Maejima, Y., Yokota, S., Ono, T., Yu, Z., Yamachi, M., Hidema, S., Nollet, K. E., Nishimori, K., Tomita, H., Yaginuma, H., & Shimomura, K. (2022). Identification of oxytocin expression in human and murine microglia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *119*, 110600. https://doi.org/10.1016/j.pnpbp.2022.110600

Mahad, D. J., & Ransohoff, R. M. (2003). The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). *Seminars in Immunology*, *15*(1), 23–32. https://doi.org/10.1016/S1044-5323(02)00125-2

Mairesse, J., Zinni, M., Pansiot, J., Hassan-Abdi, R., Demene, C., Colella, M., Charriaut-Marlangue, C., Rideau Batista Novais, A., Tanter, M., Maccari, S., Gressens, P., Vaiman, D., Soussi-Yanicostas, N., & Baud, O. (2019). Oxytocin receptor agonist reduces perinatal brain damage by targeting microglia. *Glia*, *67*(2), 345–359. https://doi.org/10.1002/glia.23546

Malik, M., Tauqeer, Z., Sheikh, A. M., Wen, G., Nagori, A., Yang, K., Brown, W. T., & Li, X. (2011). NF-κB signaling in the brain of autistic subjects. *Mediators of Inflammation*, *2011*, 785265. https://doi.org/10.1155/2011/785265

Mandic-Maravic, V., Grujicic, R., Milutinovic, L., Munjiza-Jovanovic, A., & Pejovic-Milovancevic, M. (2021). Dopamine in Autism Spectrum Disorders-Focus on D2/D3 Partial Agonists and Their Possible Use in Treatment. *Frontiers in Psychiatry*, *12*, 787097. https://doi.org/10.3389/fpsyt.2021.787097

Mandrekar, S., Jiang, Q., Lee, C. Y. D., Koenigsknecht-Talboo, J., Holtzman, D. M., & Landreth, G. E. (2009). Microglia Mediate the Clearance of Soluble Aβ through Fluid Phase Macropinocytosis. *The Journal of Neuroscience*, *29*(13), 4252–4262. https://doi.org/10.1523/JNEUROSCI.5572-08.2009

Manzardo, A. M., Henkhaus, R., Dhillon, S., & Butler, M. G. (2012). Plasma cytokine levels in children with autistic disorder and unrelated siblings. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *30*(2), 121–127. https://doi.org/10.1016/j.ijdevneu.2011.12.003

Marco, S., & Skaper, S. D. (2006). Amyloid beta-peptide1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells. *Neuroscience Letters*, *401*(3), 219–224. https://doi.org/10.1016/j.neulet.2006.03.047

Martineau, J., Roux, S., Adrien, J. L., Garreau, B., Barthélémy, C., & Lelord, G. (1992). Electrophysiological evidence of different abilities to form cross-modal associations in children with autistic behavior. *Electroencephalography and*

Clinical Neurophysiology, *82*(1), 60–66. https://doi.org/10.1016/0013-4694(92)90183-i

Mastroiacovo, F., Moyanova, S., Cannella, M., Gaglione, A., Verhaeghe, R., Bozza, G., Madonna, M., Motolese, M., Traficante, A., Riozzi, B., Bruno, V., Battaglia, G., Lodge, D., & Nicoletti, F. (2017). Genetic deletion of mGlu2 metabotropic glutamate receptors improves the short-term outcome of cerebral transient focal ischemia. *Molecular Brain*, *10*(1), 39. https://doi.org/10.1186/s13041-017-0319-6

Mastroiacovo, F., Zinni, M., Mascio, G., Bruno, V., Battaglia, G., Pansiot, J., Imbriglio, T., Mairesse, J., Baud, O., & Nicoletti, F. (2021). Genetic Deletion of mGlu3 Metabotropic Glutamate Receptors Amplifies Ischemic Brain Damage and Associated Neuroinflammation in Mice. *Frontiers in Neurology*, *12*, 668877. https://doi.org/10.3389/fneur.2021.668877

Matarredona, E. R., Santiago, M., Venero, J. L., Cano, J., & Machado, A. (2001). Group II metabotropic glutamate receptor activation protects striatal dopaminergic nerve terminals against MPP+-induced neurotoxicity along with brain-derived neurotrophic factor induction. *Journal of Neurochemistry*, *76*(2), 351–360. https://doi.org/10.1046/j.1471-4159.2001.00056.x

Mathiisen, T. M., Lehre, K. P., Danbolt, N. C., & Ottersen, O. P. (2010). The perivascular astroglial sheath provides a complete covering of the brain microvessels: An electron microscopic 3D reconstruction. *Glia*, *58*(9), 1094–1103. https://doi.org/10.1002/glia.20990

Matsunaga, W., Miyata, S., Takamata, A., Bun, H., Nakashima, T., & Kiyohara, T. (2000). LPS-induced Fos expression in oxytocin and vasopressin neurons of the rat hypothalamus. *Brain Research*, *858*(1), 9–18. https://doi.org/10.1016/s0006-8993(99)02418-x

McEwen, B. S., Bowles, N. P., Gray, J. D., Hill, M. N., Hunter, R. G., Karatsoreos, I. N., & Nasca, C. (2015). Mechanisms of stress in the brain. *Nature Neuroscience*, *18*(10), 1353–1363. https://doi.org/10.1038/nn.4086

McFarlane, H. G., Kusek, G. K., Yang, M., Phoenix, J. L., Bolivar, V. J., & Crawley, J. N. (2008). Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes, Brain, and Behavior*, *7*(2), 152–163. https://doi.org/10.1111/j.1601-183X.2007.00330.x

McKim, D. B., Weber, M. D., Niraula, A., Sawicki, C. M., Liu, X., Jarrett, B. L., Ramirez-Chan, K., Wang, Y., Roeth, R. M., Sucaldito, A. D., Sobol, C. G., Quan, N., Sheridan, J. F., & Godbout, J. P. (2018). Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. *Molecular Psychiatry*, *23*(6), 1421–1431. https://doi.org/10.1038/mp.2017.64

McPheeters, M. L., Warren, Z., Sathe, N., Bruzek, J. L., Krishnaswami, S., Jerome, R. N., & Veenstra-Vanderweele, J. (2011). A systematic review of medical

treatments for children with autism spectrum disorders. *Pediatrics*, *127*(5), e1312-1321. https://doi.org/10.1542/peds.2011-0427

McTighe, S. M., Neal, S. J., Lin, Q., Hughes, Z. A., & Smith, D. G. (2013). The BTBR Mouse Model of Autism Spectrum Disorders Has Learning and Attentional Impairments and Alterations in Acetylcholine and Kynurenic Acid in Prefrontal Cortex. *PLoS ONE*, *8*(4), e62189. https://doi.org/10.1371/journal.pone.0062189

Mehrabadi, A. R., Korolainen, M. A., Odero, G., Miller, D. W., & Kauppinen, T. M. (2017). Poly(ADP-ribose) polymerase-1 regulates microglia mediated decrease of endothelial tight junction integrity. *Neurochemistry International*, *108*, 266–271. https://doi.org/10.1016/j.neuint.2017.04.014

Même, W., Calvo, C.-F., Froger, N., Ezan, P., Amigou, E., Koulakoff, A., & Giaume, C. (2006). Proinflammatory cytokines released from microglia inhibit gap junctions in astrocytes: Potentiation by beta-amyloid. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *20*(3), 494–496. https://doi.org/10.1096/fj.05-4297fje

Menassa, D. A., & Gomez-Nicola, D. (2018). Microglial Dynamics During Human Brain Development. *Frontiers in Immunology*, *9*, 1014. https://doi.org/10.3389/fimmu.2018.01014

Mercier, F., Cho Kwon, Y., & Kodama, R. (2011). Meningeal/vascular alterations and loss of extracellular matrix in the neurogenic zone of adult BTBR T+ tf/J mice, animal model for autism. *Neuroscience Letters*, *498*(3), 173–178. https://doi.org/10.1016/j.neulet.2011.05.014

Merlini, M., Meyer, E. P., Ulmann-Schuler, A., & Nitsch, R. M. (2011). Vascular β-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAβ mice. *Acta Neuropathologica*, *122*(3), 293–311. https://doi.org/10.1007/s00401-011-0834-y

Merlo, S., Caruso, G. I., Bonfili, L., Spampinato, S. F., Costantino, G., Eleuteri, A. M., & Sortino, M. A. (2022). Microglial polarization differentially affects neuronal vulnerability to the β-amyloid protein: Modulation by melatonin. *Biochemical Pharmacology*, *202*, 115151. https://doi.org/10.1016/j.bcp.2022.115151

Merlo, S., Luaces, J. P., Spampinato, S. F., Toro-Urrego, N., Caruso, G. I., D'Amico, F., Capani, F., & Sortino, M. A. (2020). SIRT1 Mediates Melatonin's Effects on Microglial Activation in Hypoxia: In Vitro and In Vivo Evidence. *Biomolecules*, *10*(3), 364. https://doi.org/10.3390/biom10030364

Merlo, S., Spampinato, S. F., Caruso, G. I., & Sortino, M. A. (2020). The Ambiguous Role of Microglia in Aβ Toxicity: Chances for Therapeutic Intervention. *Current Neuropharmacology*, *18*(5), 446–455. https://doi.org/10.2174/1570159X18666200131105418

Meyza, K. Z., & Blanchard, D. C. (2017). The BTBR mouse model of idiopathic autism – Current view on mechanisms. *Neuroscience & Biobehavioral Reviews*, *76*, 99–110. https://doi.org/10.1016/j.neubiorev.2016.12.037

Meyza, K. Z., Blanchard, D. C., Pearson, B. L., Pobbe, R. L. H., & Blanchard, R. J. (2012). Fractone-associated N-sulfated heparan sulfate shows reduced quantity in BTBR T+tf/J mice: A strong model of autism. *Behavioural Brain Research*, *228*(2), 247–253. https://doi.org/10.1016/j.bbr.2011.11.004

Meyza, K. Z., Defensor, E. B., Jensen, A. L., Corley, M. J., Pearson, B. L., Pobbe, R. L. H., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2013). The BTBR T+ tf/J mouse model for autism spectrum disorders-in search of biomarkers. *Behavioural Brain Research*, *251*, 25–34. https://doi.org/10.1016/j.bbr.2012.07.021

Meziane, H., Schaller, F., Bauer, S., Villard, C., Matarazzo, V., Riet, F., Guillon, G., Lafitte, D., Desarmenien, M. G., Tauber, M., & Muscatelli, F. (2015). An Early Postnatal Oxytocin Treatment Prevents Social and Learning Deficits in Adult Mice Deficient for Magel2, a Gene Involved in Prader-Willi Syndrome and Autism. *Biological Psychiatry*, *78*(2), 85–94. https://doi.org/10.1016/j.biopsych.2014.11.010

Minnone, G., De Benedetti, F., & Bracci-Laudiero, L. (2017). NGF and Its Receptors in the Regulation of Inflammatory Response. *International Journal of Molecular Sciences*, *18*(5), 1028. https://doi.org/10.3390/ijms18051028

Mitic, L. L., & Anderson, J. M. (1998). Molecular architecture of tight junctions. *Annual Review of Physiology*, *60*, 121–142. https://doi.org/10.1146/annurev.physiol.60.1.121

Mittaud, P., Labourdette, G., Zingg, H., & Guenot-Di Scala, D. (2002). Neurons modulate oxytocin receptor expression in rat cultured astrocytes: Involvement of TGF-beta and membrane components. *Glia*, *37*(2), 169–177. https://doi.org/10.1002/glia.10029

Miyazaki, S., Hiraoka, Y., Hidema, S., & Nishimori, K. (2016). Prenatal minocycline treatment alters synaptic protein expression, and rescues reduced mother call rate in oxytocin receptor-knockout mice. *Biochemical and Biophysical Research Communications*, *472*(2), 319–323. https://doi.org/10.1016/j.bbrc.2016.02.109

Mizee, M. R., Wooldrik, D., Lakeman, K. A. M., van het Hof, B., Drexhage, J. A. R., Geerts, D., Bugiani, M., Aronica, E., Mebius, R. E., Prat, A., de Vries, H. E., & Reijerkerk, A. (2013). Retinoic acid induces blood-brain barrier development. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(4), 1660–1671. https://doi.org/10.1523/JNEUROSCI.1338-12.2013

Modi, M. E., & Young, L. J. (2012). The oxytocin system in drug discovery for autism: Animal models and novel therapeutic strategies. *Hormones and Behavior*, *61*(3), 340–350. https://doi.org/10.1016/j.yhbeh.2011.12.010

Moerkerke, M., Peeters, M., de Vries, L., Daniels, N., Steyaert, J., Alaerts, K., & Boets, B. (2021). Endogenous Oxytocin Levels in Autism-A Meta-Analysis. *Brain Sciences*, *11*(11), 1545. https://doi.org/10.3390/brainsci11111545

Mohammadzadeh Honarvar, N., Saedisomeolia, A., Abdolahi, M., Shayeganrad, A., Taheri Sangsari, G., Hassanzadeh Rad, B., & Muench, G. (2017). Molecular Anti-inflammatory Mechanisms of Retinoids and Carotenoids in Alzheimer's Disease: A Review of Current Evidence. *Journal of Molecular Neuroscience: MN*, *61*(3), 289–304. https://doi.org/10.1007/s12031-016-0857-x

Mondo, E., Becker, S. C., Kautzman, A. G., Schifferer, M., Baer, C. E., Chen, J., Huang, E. J., Simons, M., & Schafer, D. P. (2020). A Developmental Analysis of Juxtavascular Microglia Dynamics and Interactions with the Vasculature. *The Journal of Neuroscience*, *40*(34), 6503–6521. https://doi.org/10.1523/JNEUROSCI.3006-19.2020

Morgan, J. T., Barger, N., Amaral, D. G., & Schumann, C. M. (2014). Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. *PloS One*, *9*(10), e110356. https://doi.org/10.1371/journal.pone.0110356

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., & Everall, I. P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry*, *68*(4), 368–376. https://doi.org/10.1016/j.biopsych.2010.05.024

Morita, K., Sasaki, H., Furuse, M., & Tsukita, S. (1999). Endothelial claudin: Claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *The Journal of Cell Biology*, *147*(1), 185–194. https://doi.org/10.1083/jcb.147.1.185

Morrison, H., Young, K., Qureshi, M., Rowe, R. K., & Lifshitz, J. (2017). Quantitative microglia analyses reveal diverse morphologic responses in the rat cortex after diffuse brain injury. *Scientific Reports*, *7*(1), 13211. https://doi.org/10.1038/s41598-017-13581-z

Motolese, M., Mastroiacovo, F., Cannella, M., Bucci, D., Gaglione, A., Riozzi, B., Lütjens, R., Poli, S. M., Celanire, S., Bruno, V., Battaglia, G., & Nicoletti, F. (2015). Targeting type-2 metabotropic glutamate receptors to protect vulnerable hippocampal neurons against ischemic damage. *Molecular Brain*, *8*(1), 66. https://doi.org/10.1186/s13041-015-0158-2

Moy, S. S., Nadler, J. J., Poe, M. D., Nonneman, R. J., Young, N. B., Koller, B. H., Crawley, J. N., Duncan, G. E., & Bodfish, J. W. (2008). Development of a mouse test for repetitive, restricted behaviors: Relevance to autism. *Behavioural Brain Research*, *188*(1), 178–194. https://doi.org/10.1016/j.bbr.2007.10.029

Moy, S. S., Nadler, J. J., Young, N. B., Perez, A., Holloway, L. P., Barbaro, R. P., Barbaro, J. R., Wilson, L. M., Threadgill, D. W., Lauder, J. M., Magnuson, T. R., & Crawley, J. N. (2007). Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains. *Behavioural Brain Research*, *176*(1), 4–20. https://doi.org/10.1016/j.bbr.2006.07.030

Muhle, R., Trentacoste, S. V., & Rapin, I. (2004). The genetics of autism. *Pediatrics*, *113*(5), e472-486. https://doi.org/10.1542/peds.113.5.e472

Muller, C. L., Anacker, A. M. J., & Veenstra-VanderWeele, J. (2016). The serotonin system in autism spectrum disorder: From biomarker to animal models. *Neuroscience*, *321*, 24–41. https://doi.org/10.1016/j.neuroscience.2015.11.010

Murray, M. J. (2010). Attention-deficit/Hyperactivity Disorder in the context of Autism spectrum disorders. *Current Psychiatry Reports*, *12*(5), 382–388. https://doi.org/10.1007/s11920-010-0145-3

Nadeem, A., Ahmad, S. F., Al-Harbi, N. O., Attia, S. M., Bakheet, S. A., Ibrahim, K. E., Alqahtani, F., & Alqinyah, M. (2019). Nrf2 activator, sulforaphane ameliorates autism-like symptoms through suppression of Th17 related signaling and rectification of oxidant-antioxidant imbalance in periphery and brain of BTBR T+tf/J mice. *Behavioural Brain Research*, *364*, 213–224. https://doi.org/10.1016/j.bbr.2019.02.031

Nadler, J. J., Zou, F., Huang, H., Moy, S. S., Lauder, J., Crawley, J. N., Threadgill, D. W., Wright, F. A., & Magnuson, T. R. (2006). Large-scale gene expression differences across brain regions and inbred strains correlate with a behavioral phenotype. *Genetics*, *174*(3), 1229–1236. https://doi.org/10.1534/genetics.106.061481

Nagababu, E., Usatyuk, P. V., Enika, D., Natarajan, V., & Rifkind, J. M. (2009). Vascular endothelial barrier dysfunction mediated by amyloid-beta proteins. *Journal of Alzheimer's Disease: JAD*, *17*(4), 845–854. https://doi.org/10.3233/JAD-2009-1104

Nakagawa, S., Deli, M. A., Kawaguchi, H., Shimizudani, T., Shimono, T., Kittel, A., Tanaka, K., & Niwa, M. (2009). A new blood-brain barrier model using primary rat brain endothelial cells, pericytes and astrocytes. *Neurochemistry International*, *54*(3–4), 253–263. https://doi.org/10.1016/j.neuint.2008.12.002

Nakanishi, S., & Masu, M. (1994). Molecular diversity and functions of glutamate receptors. *Annual Review of Biophysics and Biomolecular Structure*, *23*, 319–348. https://doi.org/10.1146/annurev.bb.23.060194.001535

Nanjappa, M. S., Voyiaziakis, E., Pradhan, B., & Mannekote Thippaiah, S. (2022). Use of selective serotonin and norepinephrine reuptake inhibitors (SNRIs) in the treatment of autism spectrum disorder (ASD), comorbid psychiatric disorders and ASD-associated symptoms: A clinical review. *CNS Spectrums*, *27*(3), 290–297. https://doi.org/10.1017/S109285292000214X

Naoi, M., & Maruyama, W. (2010). Monoamine oxidase inhibitors as neuroprotective agents in age-dependent neurodegenerative disorders. *Current Pharmaceutical Design*, *16*(25), 2799–2817. https://doi.org/10.2174/138161210793176527

Napolitano, A., Schiavi, S., La Rosa, P., Rossi-Espagnet, M. C., Petrillo, S., Bottino, F., Tagliente, E., Longo, D., Lupi, E., Casula, L., Valeri, G., Piemonte, F., Trezza, V., & Vicari, S. (2022). Sex Differences in Autism Spectrum Disorder: Diagnostic, Neurobiological, and Behavioral Features. *Frontiers in Psychiatry*, *13*, 889636. https://doi.org/10.3389/fpsyt.2022.889636

Nardone, S., Sams, D. S., Reuveni, E., Getselter, D., Oron, O., Karpuj, M., & Elliott, E. (2014). DNA methylation analysis of the autistic brain reveals multiple dysregulated biological pathways. *Translational Psychiatry*, *4*(9), e433. https://doi.org/10.1038/tp.2014.70

Nation, D. A., Sweeney, M. D., Montagne, A., Sagare, A. P., D'Orazio, L. M., Pachicano, M., Sepehrband, F., Nelson, A. R., Buennagel, D. P., Harrington, M. G., Benzinger, T. L. S., Fagan, A. M., Ringman, J. M., Schneider, L. S., Morris, J. C., Chui, H. C., Law, M., Toga, A. W., & Zlokovic, B. V. (2019). Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nature Medicine*, *25*(2), 270–276. https://doi.org/10.1038/s41591-018-0297-y

Nevison, C. M., Hurst, J. L., & Barnard, C. J. (1999). Why do male ICR(CD-1) mice perform bar-related (stereotypic) behaviour? *Behavioural Processes*, *47*(2), 95–111. https://doi.org/10.1016/s0376-6357(99)00053-4

Newschaffer, C. J., Croen, L. A., Daniels, J., Giarelli, E., Grether, J. K., Levy, S. E., Mandell, D. S., Miller, L. A., Pinto-Martin, J., Reaven, J., Reynolds, A. M., Rice, C. E., Schendel, D., & Windham, G. C. (2007). The epidemiology of autism spectrum disorders. *Annual Review of Public Health*, *28*, 235–258. https://doi.org/10.1146/annurev.publhealth.28.021406.144007

Nguyen, M., Roth, A., Kyzar, E. J., Poudel, M. K., Wong, K., Stewart, A. M., & Kalueff, A. V. (2014). Decoding the contribution of dopaminergic genes and

pathways to autism spectrum disorder (ASD). *Neurochemistry International*, *66*, 15–26. https://doi.org/10.1016/j.neuint.2014.01.002

Nicholls, R. E., Zhang, X., Bailey, C. P., Conklin, B. R., Kandel, E. R., & Stanton, P. K. (2006). mGluR2 acts through inhibitory Gα subunits to regulate transmission and long-term plasticity at hippocampal mossy fiber-CA3 synapses. *Proceedings of the National Academy of Sciences*, *103*(16), 6380–6385. https://doi.org/10.1073/pnas.0601267103

Nicoletti, F., Bockaert, J., Collingridge, G. L., Conn, P. J., Ferraguti, F., Schoepp, D. D., Wroblewski, J. T., & Pin, J. P. (2011). Metabotropic glutamate receptors: From the workbench to the bedside. *Neuropharmacology*, *60*(7–8), 1017–1041. https://doi.org/10.1016/j.neuropharm.2010.10.022

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. *Science*, *308*(5726), 1314–1318. https://doi.org/10.1126/science.1110647

Niswender, C. M., & Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annual Review of Pharmacology and Toxicology*, *50*, 295–322. https://doi.org/10.1146/annurev.pharmtox.011008.145533

Nortley, R., Korte, N., Izquierdo, P., Hirunpattarasilp, C., Mishra, A., Jaunmuktane, Z., Kyrargyri, V., Pfeiffer, T., Khennouf, L., Madry, C., Gong, H., Richard-Loendt, A., Huang, W., Saito, T., Saido, T. C., Brandner, S., Sethi, H., & Attwell, D. (2019). Amyloid β oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. *Science (New York, N.Y.)*, *365*(6450), eaav9518. https://doi.org/10.1126/science.aav9518

Obermeier, B., Daneman, R., & Ransohoff, R. M. (2013). Development, maintenance and disruption of the blood-brain barrier. *Nature Medicine*, *19*(12), 1584–1596. https://doi.org/10.1038/nm.3407

Obermeier, B., Verma, A., & Ransohoff, R. M. (2016). The blood-brain barrier. *Handbook of Clinical Neurology*, *133*, 39–59. https://doi.org/10.1016/B978-0-444-63432-0.00003-7

Ohishi, H., Shigemoto, R., Nakanishi, S., & Mizuno, N. (1993). Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: An in situ hybridization study. *Journal of Comparative Neurology*, *335*(2), 252–266. https://doi.org/10.1002/cne.903350209

Oikari, L. E., Pandit, R., Stewart, R., Cuní-López, C., Quek, H., Sutharsan, R., Rantanen, L. M., Oksanen, M., Lehtonen, S., de Boer, C. M., Polo, J. M., Götz, J., Koistinaho, J., & White, A. R. (2020). Altered Brain Endothelial Cell Phenotype from a Familial Alzheimer Mutation and Its Potential Implications for Amyloid

Clearance and Drug Delivery. *Stem Cell Reports*, *14*(5), 924–939. https://doi.org/10.1016/j.stemcr.2020.03.011

Okun, E., Griffioen, K. J., Lathia, J. D., Tang, S.-C., Mattson, M. P., & Arumugam, T. V. (2009). Toll-like receptors in neurodegeneration. *Brain Research Reviews*, *59*(2), 278–292. https://doi.org/10.1016/j.brainresrev.2008.09.001

O'Riordan, K. J., Huang, I.-C., Pizzi, M., Spano, P., Boroni, F., Egli, R., Desai, P., Fitch, O., Malone, L., Jin Ahn, H., Liou, H.-C., Sweatt, J. D., & Levenson, J. M. (2006). Regulation of Nuclear Factor κB in the Hippocampus by Group I Metabotropic Glutamate Receptors. *The Journal of Neuroscience*, *26*(18), 4870–4879. https://doi.org/10.1523/JNEUROSCI.4527-05.2006

Ormstad, H., Bryn, V., Saugstad, O. D., Skjeldal, O., & Maes, M. (2018). Role of the Immune System in Autism Spectrum Disorders (ASD). *CNS & Neurological Disorders Drug Targets*, *17*(7), 489–495. https://doi.org/10.2174/1871527317666180706123229

Pacheco, R., Oliva, H., Martinez-Navío, J. M., Climent, N., Ciruela, F., Gatell, J. M., Gallart, T., Mallol, J., Lluis, C., & Franco, R. (2006). Glutamate Released by Dendritic Cells as a Novel Modulator of T Cell Activation. *The Journal of Immunology*, *177*(10), 6695–6704. https://doi.org/10.4049/jimmunol.177.10.6695

Paine, T. A., Swedlow, N., & Swetschinski, L. (2017). Decreasing GABA function within the medial prefrontal cortex or basolateral amygdala decreases sociability. *Behavioural Brain Research*, *317*, 542–552. https://doi.org/10.1016/j.bbr.2016.10.012

Pan, J., Ma, N., Zhong, J., Yu, B., Wan, J., & Zhang, W. (2021). Age-associated changes in microglia and astrocytes ameliorate blood-brain barrier dysfunction. *Molecular Therapy. Nucleic Acids*, *26*, 970–986. https://doi.org/10.1016/j.omtn.2021.08.030

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., & Gross, C. T. (2011). Synaptic Pruning by Microglia Is Necessary for Normal Brain Development. *Science*, *333*(6048), 1456–1458. https://doi.org/10.1126/science.1202529

Paolicelli, R. C., Sierra, A., Stevens, B., Tremblay, M.-E., Aguzzi, A., Ajami, B., Amit, I., Audinat, E., Bechmann, I., Bennett, M., Bennett, F., Bessis, A., Biber, K., Bilbo, S., Blurton-Jones, M., Boddeke, E., Brites, D., Brône, B., Brown, G. C., … Wyss-Coray, T. (2022). Microglia states and nomenclature: A field at its crossroads. *Neuron*, *110*(21), 3458–3483. https://doi.org/10.1016/j.neuron.2022.10.020

Pardo, C. A., & Eberhart, C. G. (2007). The neurobiology of autism. *Brain Pathology (Zurich, Switzerland)*, *17*(4), 434–447. https://doi.org/10.1111/j.1750-3639.2007.00102.x

Paredes, D., Granholm, A.-C., & Bickford, P. C. (2007). Effects of NGF and BDNF on baseline glutamate and dopamine release in the hippocampal formation of the adult rat. *Brain Research*, *1141*, 56–64. https://doi.org/10.1016/j.brainres.2007.01.018

Park, J. S., Chyun, J. H., Kim, Y. K., Line, L. L., & Chew, B. P. (2010). Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutrition & Metabolism*, *7*, 18. https://doi.org/10.1186/1743-7075-7-18

Park, S.-J., Jaiswal, V., & Lee, H.-J. (2021). Dietary Intake of Flavonoids and Carotenoids Is Associated with Anti-Depressive Symptoms: Epidemiological Study and In Silico-Mechanism Analysis. *Antioxidants (Basel, Switzerland)*, *11*(1), 53. https://doi.org/10.3390/antiox11010053

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., Lafaille, J. J., Hempstead, B. L., Littman, D. R., & Gan, W.-B. (2013). Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor. *Cell*, *155*(7), 1596–1609. https://doi.org/10.1016/j.cell.2013.11.030

Patil, S. T., Zhang, L., Martenyi, F., Lowe, S. L., Jackson, K. A., Andreev, B. V., Avedisova, A. S., Bardenstein, L. M., Gurovich, I. Y., Morozova, M. A., Mosolov, S. N., Neznanov, N. G., Reznik, A. M., Smulevich, A. B., Tochilov, V. A., Johnson, B. G., Monn, J. A., & Schoepp, D. D. (2007). Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: A randomized Phase 2 clinical trial. *Nature Medicine*, *13*(9), 1102–1107. https://doi.org/10.1038/nm1632

Pavăl, D., & Micluția, I. V. (2021). The Dopamine Hypothesis of Autism Spectrum Disorder Revisited: Current Status and Future Prospects. *Developmental Neuroscience*, *43*(2), 73–83. https://doi.org/10.1159/000515751

Pearson, B. L., Bettis, J. K., Meyza, K. Z., Yamamoto, L. Y., Blanchard, D. C., & Blanchard, R. J. (2012). Absence of social conditioned place preference in BTBR T+tf/J mice: Relevance for social motivation testing in rodent models of autism. *Behavioural Brain Research*, *233*(1), 99–104. https://doi.org/10.1016/j.bbr.2012.04.040

Pearson, B. L., Pobbe, R. L. H., Defensor, E. B., Oasay, L., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2011). Motor and cognitive stereotypies in the BTBR T+tf/J mouse model of autism. *Genes, Brain, and Behavior*, *10*(2), 228–235. https://doi.org/10.1111/j.1601-183X.2010.00659.x

Penna, E., Mangum, J. M., Shepherd, H., Martínez-Cerdeño, V., & Noctor, S. C. (2021). Development of the Neuro-Immune-Vascular Plexus in the Ventricular

Zone of the Prenatal Rat Neocortex. *Cerebral Cortex (New York, N.Y.: 1991)*, *31*(4), 2139–2155. https://doi.org/10.1093/cercor/bhaa351

Perea, J. R., Bolós, M., & Avila, J. (2020). Microglia in Alzheimer's Disease in the Context of Tau Pathology. *Biomolecules*, *10*(10), 1439. https://doi.org/10.3390/biom10101439

Persico, A. M., & Bourgeron, T. (2006). Searching for ways out of the autism maze: Genetic, epigenetic and environmental clues. *Trends in Neurosciences*, *29*(7), 349–358. https://doi.org/10.1016/j.tins.2006.05.010

Petralia, R. S., Wang, Y.-X., Niedzielski, A. S., & Wenthold, R. J. (1996). The metabotropic glutamate receptors, MGLUR2 and MGLUR3, show unique postsynaptic, presynaptic and glial localizations. *Neuroscience*, *71*(4), 949–976. https://doi.org/10.1016/0306-4522(95)00533-1

Piehl, C., Piontek, J., Cording, J., Wolburg, H., & Blasig, I. E. (2010). Participation of the second extracellular loop of claudin-5 in paracellular tightening against ions, small and large molecules. *Cellular and Molecular Life Sciences: CMLS*, *67*(12), 2131–2140. https://doi.org/10.1007/s00018-010-0332-8

Pin, J. P., & Duvoisin, R. (1995). The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology*, *34*(1), 1–26. https://doi.org/10.1016/0028-3908(94)00129-g

Pin, J.-P., & Bettler, B. (2016). Organization and functions of mGlu and GABAB receptor complexes. *Nature*, *540*(7631), 60–68. https://doi.org/10.1038/nature20566

Pin, J.-P., Galvez, T., & Prézeau, L. (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacology & Therapeutics*, *98*(3), 325–354. https://doi.org/10.1016/s0163-7258(03)00038-x

Pinteaux-Jones, F., Sevastou, I. G., Fry, V. A. H., Heales, S., Baker, D., & Pocock, J. M. (2008). Myelin-induced microglial neurotoxicity can be controlled by microglial metabotropic glutamate receptors. *Journal of Neurochemistry*, *106*(1), 442–454. https://doi.org/10.1111/j.1471-4159.2008.05426.x

Pinto da Silva, P., & Kachar, B. (1982). On tight-junction structure. *Cell*, *28*(3), 441–450. https://doi.org/10.1016/0092-8674(82)90198-2

Piontek, J., Winkler, L., Wolburg, H., Müller, S. L., Zuleger, N., Piehl, C., Wiesner, B., Krause, G., & Blasig, I. E. (2008). Formation of tight junction: Determinants of homophilic interaction between classic claudins. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *22*(1), 146–158. https://doi.org/10.1096/fj.07-8319com

Pobbe, R. L. H., Defensor, E. B., Pearson, B. L., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2011). General and social anxiety in the BTBR T+ tf/J mouse strain. *Behavioural Brain Research*, *216*(1), 446–451. https://doi.org/10.1016/j.bbr.2010.08.039

Pobbe, R. L. H., Pearson, B. L., Defensor, E. B., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2010). Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system. *Behavioural Brain Research*, *214*(2), 443–449. https://doi.org/10.1016/j.bbr.2010.06.025

Propson, N. E., Gedam, M., & Zheng, H. (2021). Complement in Neurologic Disease. *Annual Review of Pathology*, *16*, 277–298. https://doi.org/10.1146/annurev-pathol-031620-113409

Quaegebeur, A., Segura, I., & Carmeliet, P. (2010). Pericytes: Blood-brain barrier safeguards against neurodegeneration? *Neuron*, *68*(3), 321–323. https://doi.org/10.1016/j.neuron.2010.10.024

Ramanathan, A. M., Nelson, A. R. P., Sagare, A. P. P., & Zlokovic, B. V. M. (2015). Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: The role, regulation and restoration of LRP1. *Frontiers in Aging Neuroscience*, *7*. https://doi.org/10.3389/fnagi.2015.00136

Ranheim, T., Dumke, C., Schueler, K. L., Cartee, G. D., & Attie, A. D. (1997). Interaction between BTBR and C57BL/6J genomes produces an insulin resistance syndrome in (BTBR x C57BL/6J) F1 mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *17*(11), 3286–3293. https://doi.org/10.1161/01.atv.17.11.3286

Rasmus, P., & Kozłowska, E. (2023). Antioxidant and Anti-Inflammatory Effects of Carotenoids in Mood Disorders: An Overview. *Antioxidants (Basel, Switzerland)*, *12*(3), 676. https://doi.org/10.3390/antiox12030676

Ribatti, D., Nico, B., & Crivellato, E. (2011). The role of pericytes in angiogenesis. *The International Journal of Developmental Biology*, *55*(3), 261–268. https://doi.org/10.1387/ijdb.103167dr

Ribeiro, F. M., Vieira, L. B., Pires, R. G. W., Olmo, R. P., & Ferguson, S. S. G. (2017). Metabotropic glutamate receptors and neurodegenerative diseases. *Pharmacological Research*, *115*, 179–191. https://doi.org/10.1016/j.phrs.2016.11.013

Riebold, M., Mankuta, D., Lerer, E., Israel, S., Zhong, S., Nemanov, L., Monakhov, M. V., Levi, S., Yirmiya, N., Yaari, M., Malavasi, F., & Ebstein, R. P. (2011). All-trans Retinoic Acid Upregulates Reduced CD38 Transcription in Lymphoblastoid Cell Lines from Autism Spectrum Disorder. *Molecular Medicine*, *17*(7–8), 799–806. https://doi.org/10.2119/molmed.2011.00080

Ries, M., & Sastre, M. (2016). Mechanisms of Aβ Clearance and Degradation by Glial Cells. *Frontiers in Aging Neuroscience*, *8*, 160. https://doi.org/10.3389/fnagi.2016.00160

Rodrigues, R., Lai, M.-C., Beswick, A., Gorman, D. A., Anagnostou, E., Szatmari, P., Anderson, K. K., & Ameis, S. H. (2021). Practitioner Review: Pharmacological treatment of attention-deficit/hyperactivity disorder symptoms in children and youth with autism spectrum disorder: a systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *62*(6), 680–700. https://doi.org/10.1111/jcpp.13305

Rodriguez, J. I., & Kern, J. K. (2011). Evidence of microglial activation in autism and its possible role in brain underconnectivity. *Neuron Glia Biology*, *7*(2–4), 205–213. https://doi.org/10.1017/S1740925X12000142

Romano, C., Smout, S., Miller, J. K., & O'Malley, K. L. (2002). Developmental regulation of metabotropic glutamate receptor 5b protein in rodent brain. *Neuroscience*, *111*(3), 693–698. https://doi.org/10.1016/S0306-4522(02)00042-8

Ronaldson, P. T., & Davis, T. P. (2020). Regulation of blood-brain barrier integrity by microglia in health and disease: A therapeutic opportunity. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *40*(1_suppl), S6–S24. https://doi.org/10.1177/0271678X20951995

Rossi, D., Brambilla, L., Valori, C. F., Crugnola, A., Giaccone, G., Capobianco, R., Mangieri, M., Kingston, A. E., Bloc, A., Bezzi, P., & Volterra, A. (2005). Defective tumor necrosis factor-alpha-dependent control of astrocyte glutamate release in a transgenic mouse model of Alzheimer disease. *The Journal of Biological Chemistry*, *280*(51), 42088–42096. https://doi.org/10.1074/jbc.M504124200

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology*, *38*(1), 73–84. https://doi.org/10.1002/ana.410380114

Roullet, F. I., Wöhr, M., & Crawley, J. N. (2011). Female urine-induced male mice ultrasonic vocalizations, but not scent-marking, is modulated by social experience. *Behavioural Brain Research*, *216*(1), 19–28. https://doi.org/10.1016/j.bbr.2010.06.004

Rubenstein, J. L. R. (2010). Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. *Current Opinion in Neurology*, *23*(2), 118–123. https://doi.org/10.1097/WCO.0b013e328336eb13

Rudolph, M. D., Graham, A. M., Feczko, E., Miranda-Dominguez, O., Rasmussen, J. M., Nardos, R., Entringer, S., Wadhwa, P. D., Buss, C., & Fair, D. A. (2018). Maternal IL-6 during pregnancy can be estimated from newborn brain

connectivity and predicts future working memory in offspring. *Nature Neuroscience*, *21*(5), 765–772. https://doi.org/10.1038/s41593-018-0128-y

S. Onaivi, E., Benno, R., Halpern, T., Mehanovic, M., Schanz, N., Sanders, C., Yan, X., Ishiguro, H., Liu, Q.-R., L. Berzal, A., P. Viveros, M., & F. Ali, S. (2011). Consequences of Cannabinoid and Monoaminergic System Disruption in a Mouse Model of Autism Spectrum Disorders. *Current Neuropharmacology*, *9*(1), 209–214. https://doi.org/10.2174/157015911795017047

Saavedra, J., Nascimento, M., Liz, M. A., & Cardoso, I. (2022). Key brain cell interactions and contributions to the pathogenesis of Alzheimer's disease. *Frontiers in Cell and Developmental Biology*, *10*, 1036123. https://doi.org/10.3389/fcell.2022.1036123

Sacco, R., Camilleri, N., Eberhardt, J., Umla-Runge, K., & Newbury-Birch, D. (2023). The Prevalence of Autism Spectrum Disorder in Europe. In M. Carotenuto (Ed.), *Autism Spectrum Disorders—Recent Advances and New Perspectives*. IntechOpen. https://doi.org/10.5772/intechopen.108123

Sagare, A. P., Bell, R. D., Zhao, Z., Ma, Q., Winkler, E. A., Ramanathan, A., & Zlokovic, B. V. (2013). Pericyte loss influences Alzheimer-like neurodegeneration in mice. *Nature Communications*, *4*, 2932. https://doi.org/10.1038/ncomms3932

Sagare, A. P., Bell, R. D., & Zlokovic, B. V. (2012). Neurovascular dysfunction and faulty amyloid β-peptide clearance in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(10), a011452. https://doi.org/10.1101/cshperspect.a011452

Saini, R. K., Nile, S. H., & Park, S. W. (2015). Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. *Food Research International (Ottawa, Ont.)*, *76*(Pt 3), 735–750. https://doi.org/10.1016/j.foodres.2015.07.047

Sajja, V. S. S. S., Hlavac, N., & VandeVord, P. J. (2016). Role of Glia in Memory Deficits Following Traumatic Brain Injury: Biomarkers of Glia Dysfunction. *Frontiers in Integrative Neuroscience*, *10*, 7. https://doi.org/10.3389/fnint.2016.00007

Sala, M., Braida, D., Lentini, D., Busnelli, M., Bulgheroni, E., Capurro, V., Finardi, A., Donzelli, A., Pattini, L., Rubino, T., Parolaro, D., Nishimori, K., Parenti, M., & Chini, B. (2011). Pharmacologic Rescue of Impaired Cognitive Flexibility, Social Deficits, Increased Aggression, and Seizure Susceptibility in Oxytocin Receptor Null Mice: A Neurobehavioral Model of Autism. *Biological Psychiatry*, *69*(9), 875–882. https://doi.org/10.1016/j.biopsych.2010.12.022

Samsam, M., Ahangari, R., & Naser, S. A. (2014). Pathophysiology of autism spectrum disorders: Revisiting gastrointestinal involvement and immune

imbalance. *World Journal of Gastroenterology*, *20*(29), 9942–9951. https://doi.org/10.3748/wjg.v20.i29.9942

Sandoval, K. E., & Witt, K. A. (2008). Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiology of Disease*, *32*(2), 200–219. https://doi.org/10.1016/j.nbd.2008.08.005

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews. Neuroscience*, *10*(3), 211–223. https://doi.org/10.1038/nrn2573

Scattoni, M. L., Fatta, L. M., Micai, M., Sali, M. E., Bellomo, M., Salvitti, T., Fulceri, F., Castellano, A., Molteni, M., Gambino, G., Posada, M., Romano, G., & Puopolo, M. (2023). Autism spectrum disorder prevalence in Italy: A nationwide study promoted by the Ministry of Health. *Child and Adolescent Psychiatry and Mental Health*, *17*(1), 125. https://doi.org/10.1186/s13034-023-00673-0

Scattoni, M. L., Gandhy, S. U., Ricceri, L., & Crawley, J. N. (2008). Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PloS One*, *3*(8), e3067. https://doi.org/10.1371/journal.pone.0003067

Scattoni, M. L., Martire, A., Cartocci, G., Ferrante, A., & Ricceri, L. (2013). Reduced social interaction, behavioural flexibility and BDNF signalling in the BTBR T+tf/J strain, a mouse model of autism. *Behavioural Brain Research*, *251*, 35–40. https://doi.org/10.1016/j.bbr.2012.12.028

Scattoni, M. L., Ricceri, L., & Crawley, J. N. (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes, Brain, and Behavior*, *10*(1), 44–56. https://doi.org/10.1111/j.1601-183X.2010.00623.x

Schafer, D. P., Lehrman, E. K., & Stevens, B. (2013). The “quad-partite” synapse: Microglia-synapse interactions in the developing and mature CNS. *Glia*, *61*(1), 24–36. https://doi.org/10.1002/glia.22389

Schlingmann, B., Overgaard, C. E., Molina, S. A., Lynn, K. S., Mitchell, L. A., Dorsainvil White, S., Mattheyses, A. L., Guidot, D. M., Capaldo, C. T., & Koval, M. (2016). Regulation of claudin/zonula occludens-1 complexes by hetero-claudin interactions. *Nature Communications*, *7*, 12276. https://doi.org/10.1038/ncomms12276

Schmidt, H. D., & Pierce, R. C. (2010). Cocaine-induced neuroadaptations in glutamate transmission: Potential therapeutic targets for craving and addiction. *Annals of the New York Academy of Sciences*, *1187*, 35–75. https://doi.org/10.1111/j.1749-6632.2009.05144.x

Schoepp, D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *The Journal of Pharmacology and Experimental Therapeutics*, *299*(1), 12–20.

Sciara, A. N., Beasley, B., Crawford, J. D., Anderson, E. P., Carrasco, T., Zheng, S., Ordway, G. A., & Chandley, M. J. (2020). Neuroinflammatory Gene Expression Alterations in Anterior Cingulate Cortical White and Gray Matter of Males With Autism Spectrum Disorder. *Autism Research: Official Journal of the International Society for Autism Research*, *13*(6), 870–884. https://doi.org/10.1002/aur.2284

Seese, R. R., Maske, A. R., Lynch, G., & Gall, C. M. (2014). Long-Term Memory Deficits are Associated with Elevated Synaptic ERK1/2 Activation and Reversed by mGluR5 Antagonism in an Animal Model of Autism. *Neuropsychopharmacology*, *39*(7), 1664–1673. https://doi.org/10.1038/npp.2014.13

Sengillo, J. D., Winkler, E. A., Walker, C. T., Sullivan, J. S., Johnson, M., & Zlokovic, B. V. (2013). Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathology (Zurich, Switzerland)*, *23*(3), 303–310. https://doi.org/10.1111/bpa.12004

Seyedzadeh, M. H., Safari, Z., Zare, A., Gholizadeh Navashenaq, J., Razavi, S. A., Kardar, G. A., & Khorramizadeh, M. R. (2014). Study of curcumin immunomodulatory effects on reactive astrocyte cell function. *International Immunopharmacology*, *22*(1), 230–235. https://doi.org/10.1016/j.intimp.2014.06.035

Sharma, A., & Shaw, S. R. (2012). Efficacy of risperidone in managing maladaptive behaviors for children with autistic spectrum disorder: A meta-analysis. *Journal of Pediatric Health Care: Official Publication of National Association of Pediatric Nurse Associates & Practitioners*, *26*(4), 291–299. https://doi.org/10.1016/j.pedhc.2011.02.008

Shigemoto, R., Kinoshita, A., Wada, E., Nomura, S., Ohishi, H., Takada, M., Flor, P. J., Neki, A., Abe, T., Nakanishi, S., & Mizuno, N. (1997). Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *17*(19), 7503–7522. https://doi.org/10.1523/JNEUROSCI.17-19-07503.1997

Shimizu, F., Sano, Y., Abe, M.-A., Maeda, T., Ohtsuki, S., Terasaki, T., & Kanda, T. (2011). Peripheral nerve pericytes modify the blood-nerve barrier function and tight junctional molecules through the secretion of various soluble factors. *Journal of Cellular Physiology*, *226*(1), 255–266. https://doi.org/10.1002/jcp.22337

Sierra, A., De Castro, F., Del Río-Hortega, J., Rafael Iglesias-Rozas, J., Garrosa, M., & Kettenmann, H. (2016). The "Big-Bang" for modern glial biology: Translation and comments on Pío del Río-Hortega 1919 series of papers on microglia. *Glia*, *64*(11), 1801–1840. https://doi.org/10.1002/glia.23046

Sierra, A., Encinas, J. M., Deudero, J. J. P., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., Tsirka, S. E., & Maletic-Savatic, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*, *7*(4), 483–495. https://doi.org/10.1016/j.stem.2010.08.014

Silverman, J. L., Babineau, B. A., Oliver, C. F., Karras, M. N., & Crawley, J. N. (2013). Influence of stimulant-induced hyperactivity on social approach in the BTBR mouse model of autism. *Neuropharmacology*, *68*, 210–222. https://doi.org/10.1016/j.neuropharm.2012.07.042

Silverman, J. L., Smith, D. G., Rizzo, S. J. S., Karras, M. N., Turner, S. M., Tolu, S. S., Bryce, D. K., Smith, D. L., Fonseca, K., Ring, R. H., & Crawley, J. N. (2012). Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. *Science Translational Medicine*, *4*(131), 131ra51. https://doi.org/10.1126/scitranslmed.3003501

Silverman, J. L., Tolu, S. S., Barkan, C. L., & Crawley, J. N. (2010). Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *35*(4), 976–989. https://doi.org/10.1038/npp.2009.201

Silverman, J. L., Yang, M., Turner, S. M., Katz, A. M., Bell, D. B., Koenig, J. I., & Crawley, J. N. (2010). Low stress reactivity and neuroendocrine factors in the BTBR T+tf/J mouse model of autism. *Neuroscience*, *171*(4), 1197–1208. https://doi.org/10.1016/j.neuroscience.2010.09.059

Sipe, G. O., Lowery, R. L., Tremblay, M.-È., Kelly, E. A., Lamantia, C. E., & Majewska, A. K. (2016). Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nature Communications*, *7*, 10905. https://doi.org/10.1038/ncomms10905

Sokolenko, E., Hudson, M. R., Nithianantharajah, J., & Jones, N. C. (2019). The mGluR2/3 agonist LY379268 reverses NMDA receptor antagonist effects on cortical gamma oscillations and phase coherence, but not working memory impairments, in mice. *Journal of Psychopharmacology (Oxford, England)*, *33*(12), 1588–1599. https://doi.org/10.1177/0269881119875976

Sorokin, L. (2010). The impact of the extracellular matrix on inflammation. *Nature Reviews. Immunology*, *10*(10), 712–723. https://doi.org/10.1038/nri2852

Sorrenti, V., Davinelli, S., Scapagnini, G., Willcox, B. J., Allsopp, R. C., & Willcox, D. C. (2020). Astaxanthin as a Putative Geroprotector: Molecular Basis and Focus on Brain Aging. *Marine Drugs*, *18*(7), 351. https://doi.org/10.3390/md18070351

Souza, L. S., Silva, E. F., Santos, W. B., Asth, L., Lobão-Soares, B., Soares-Rachetti, V. P., Medeiros, I. U., & Gavioli, E. C. (2016). Lithium and valproate prevent methylphenidate-induced mania-like behaviors in the hole board test. *Neuroscience Letters*, *629*, 143–148. https://doi.org/10.1016/j.neulet.2016.06.044

Spampinato, S. F., Bortolotto, V., Canonico, P. L., Sortino, M. A., & Grilli, M. (2019). Astrocyte-Derived Paracrine Signals: Relevance for Neurogenic Niche Regulation and Blood-Brain Barrier Integrity. *Frontiers in Pharmacology*, *10*, 1346. https://doi.org/10.3389/fphar.2019.01346

Spampinato, S. F., Copani, A., Nicoletti, F., Sortino, M. A., & Caraci, F. (2018). Metabotropic Glutamate Receptors in Glial Cells: A New Potential Target for Neuroprotection? *Frontiers in Molecular Neuroscience*, *11*, 414. https://doi.org/10.3389/fnmol.2018.00414

Spampinato, S. F., Costantino, G., Merlo, S., Canonico, P. L., & Sortino, M. A. (2022). Microglia Contributes to BAF-312 Effects on Blood–Brain Barrier Stability. *Biomolecules*, *12*(9), 1174. https://doi.org/10.3390/biom12091174

Spampinato, S. F., Merlo, S., Chisari, M., Nicoletti, F., & Sortino, M. A. (2015). Glial metabotropic glutamate receptor-4 increases maturation and survival of oligodendrocytes. *Frontiers in Cellular Neuroscience*, *8*. https://doi.org/10.3389/fncel.2014.00462

Spampinato, S. F., Merlo, S., Costantino, G., Sano, Y., Kanda, T., & Sortino, M. A. (2022). Decreased Astrocytic CCL2 Accounts for BAF-312 Effect on PBMCs Transendothelial Migration Through a Blood Brain Barrier in Vitro Model. *Journal of Neuroimmune Pharmacology*, *17*(3–4), 427–436. https://doi.org/10.1007/s11481-021-10016-5

Spampinato, S. F., Merlo, S., Fagone, E., Fruciano, M., Sano, Y., Kanda, T., & Sortino, M. A. (2020). Reciprocal Interplay Between Astrocytes and CD4+ Cells Affects Blood-Brain Barrier and Neuronal Function in Response to β Amyloid. *Frontiers in Molecular Neuroscience*, *13*, 120. https://doi.org/10.3389/fnmol.2020.00120

Spampinato, S. F., Merlo, S., Sano, Y., Kanda, T., & Sortino, M. A. (2017). Astrocytes contribute to Aβ-induced blood-brain barrier damage through activation of endothelial MMP9. *Journal of Neurochemistry*, *142*(3), 464–477. https://doi.org/10.1111/jnc.14068

Spampinato, S. F., Molinaro, G., Merlo, S., Iacovelli, L., Caraci, F., Battaglia, G., Nicoletti, F., Bruno, V., & Sortino, M. A. (2012). Estrogen Receptors and Type 1 Metabotropic Glutamate Receptors Are Interdependent in Protecting Cortical Neurons against β-Amyloid Toxicity. *Molecular Pharmacology*, *81*(1), 12–20. https://doi.org/10.1124/mol.111.074021

Squillace, M., Dodero, L., Federici, M., Migliarini, S., Errico, F., Napolitano, F., Krashia, P., Di Maio, A., Galbusera, A., Bifone, A., Scattoni, M. L., Pasqualetti, M., Mercuri, N. B., Usiello, A., & Gozzi, A. (2014). Dysfunctional dopaminergic neurotransmission in asocial BTBR mice. *Translational Psychiatry*, *4*(8), e427–e427. https://doi.org/10.1038/tp.2014.69

Stahl, W., & Sies, H. (2001). Effects of carotenoids and retinoids on gap junctional communication. *BioFactors (Oxford, England)*, *15*(2–4), 95–98. https://doi.org/10.1002/biof.5520150209

Stephenson, D. T., O'Neill, S. M., Narayan, S., Tiwari, A., Arnold, E., Samaroo, H. D., Du, F., Ring, R. H., Campbell, B., Pletcher, M., Vaidya, V. A., & Morton, D. (2011). Histopathologic characterization of the BTBR mouse model of autistic-like behavior reveals selective changes in neurodevelopmental proteins and adult hippocampal neurogenesis. *Molecular Autism*, *2*(1), 7. https://doi.org/10.1186/2040-2392-2-7

Stoehr, J. P., Nadler, S. T., Schueler, K. L., Rabaglia, M. E., Yandell, B. S., Metz, S. A., & Attie, A. D. (2000). Genetic obesity unmasks nonlinear interactions between murine type 2 diabetes susceptibility loci. *Diabetes*, *49*(11), 1946–1954. https://doi.org/10.2337/diabetes.49.11.1946

Stornetta, R. L., Hawelu-Johnson, C. L., Guyenet, P. G., & Lynch, K. R. (1988). Astrocytes synthesize angiotensinogen in brain. *Science (New York, N.Y.)*, *242*(4884), 1444–1446. https://doi.org/10.1126/science.3201232

Strachan-Whaley, M., Rivest, S., & Yong, V. W. (2014). Interactions Between Microglia and T Cells in Multiple Sclerosis Pathobiology. *Journal of Interferon & Cytokine Research*, *34*(8), 615–622. https://doi.org/10.1089/jir.2014.0019

Sujak, A., Gabrielska, J., Grudziński, W., Borc, R., Mazurek, P., & Gruszecki, W. I. (1999). Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. *Archives of Biochemistry and Biophysics*, *371*(2), 301–307. https://doi.org/10.1006/abbi.1999.1437

Sun, W., McConnell, E., Pare, J.-F., Xu, Q., Chen, M., Peng, W., Lovatt, D., Han, X., Smith, Y., & Nedergaard, M. (2013). Glutamate-Dependent Neuroglial Calcium Signaling Differs Between Young and Adult Brain. *Science*, *339*(6116), 197–200. https://doi.org/10.1126/science.1226740

Suzuki, K., Sugihara, G., Ouchi, Y., Nakamura, K., Futatsubashi, M., Takebayashi, K., Yoshihara, Y., Omata, K., Matsumoto, K., Tsuchiya, K. J., Iwata, Y., Tsujii, M., Sugiyama, T., & Mori, N. (2013). Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry*, *70*(1), 49–58. https://doi.org/10.1001/jamapsychiatry.2013.272

Sweeney, M. D., Zhao, Z., Montagne, A., Nelson, A. R., & Zlokovic, B. V. (2019). Blood-Brain Barrier: From Physiology to Disease and Back. *Physiological Reviews*, *99*(1), 21–78. https://doi.org/10.1152/physrev.00050.2017

Sweeten, T. L., Posey, D. J., & McDougle, C. J. (2003). High blood monocyte counts and neopterin levels in children with autistic disorder. *The American Journal of Psychiatry*, *160*(9), 1691–1693. https://doi.org/10.1176/appi.ajp.160.9.1691

Szeto, A., Nation, D. A., Mendez, A. J., Dominguez-Bendala, J., Brooks, L. G., Schneiderman, N., & McCabe, P. M. (2008). Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *American Journal of Physiology. Endocrinology and Metabolism*, *295*(6), E1495-1501. https://doi.org/10.1152/ajpendo.90718.2008

Szeto, A., Sun-Suslow, N., Mendez, A. J., Hernandez, R. I., Wagner, K. V., & McCabe, P. M. (2017). Regulation of the macrophage oxytocin receptor in response to inflammation. *American Journal of Physiology. Endocrinology and Metabolism*, *312*(3), E183–E189. https://doi.org/10.1152/ajpendo.00346.2016

Takane, K., Hasegawa, Y., Lin, B., Koibuchi, N., Cao, C., Yokoo, T., & Kim-Mitsuyama, S. (2017). Detrimental Effects of Centrally Administered Angiotensin II are Enhanced in a Mouse Model of Alzheimer Disease Independently of Blood Pressure. *Journal of the American Heart Association*, *6*(4), e004897. https://doi.org/10.1161/JAHA.116.004897

Takata, F., Dohgu, S., Matsumoto, J., Takahashi, H., Machida, T., Wakigawa, T., Harada, E., Miyaji, H., Koga, M., Nishioku, T., Yamauchi, A., & Kataoka, Y. (2011). Brain pericytes among cells constituting the blood-brain barrier are highly sensitive to tumor necrosis factor-α, releasing matrix metalloproteinase-9 and migrating in vitro. *Journal of Neuroinflammation*, *8*, 106. https://doi.org/10.1186/1742-2094-8-106

Takata, F., Nakagawa, S., Matsumoto, J., & Dohgu, S. (2021). Blood-Brain Barrier Dysfunction Amplifies the Development of Neuroinflammation: Understanding of Cellular Events in Brain Microvascular Endothelial Cells for Prevention and Treatment of BBB Dysfunction. *Frontiers in Cellular Neuroscience*, *15*, 661838. https://doi.org/10.3389/fncel.2021.661838

Tamaru, Y., Nomura, S., Mizuno, N., & Shigemoto, R. (2001). Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: Differential location

relative to pre- and postsynaptic sites. *Neuroscience*, *106*(3), 481–503. https://doi.org/10.1016/S0306-4522(01)00305-0

Tanabe, Y., Nomura, A., Masu, M., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1993). Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *The Journal of Neuroscience*, *13*(4), 1372–1378. https://doi.org/10.1523/JNEUROSCI.13-04-01372.1993

Tanimizu, T., Kenney, J. W., Okano, E., Kadoma, K., Frankland, P. W., & Kida, S. (2017). Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social Recognition Memory. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(15), 4103–4116. https://doi.org/10.1523/JNEUROSCI.3451-16.2017

Tanimura, Y., Yang, M. C., & Lewis, M. H. (2008). Procedural learning and cognitive flexibility in a mouse model of restricted, repetitive behaviour. *Behavioural Brain Research*, *189*(2), 250–256. https://doi.org/10.1016/j.bbr.2008.01.001

Tay, T. L., Mai, D., Dautzenberg, J., Fernández-Klett, F., Lin, G., Sagar, null, Datta, M., Drougard, A., Stempfl, T., Ardura-Fabregat, A., Staszewski, O., Margineanu, A., Sporbert, A., Steinmetz, L. M., Pospisilik, J. A., Jung, S., Priller, J., Grün, D., Ronneberger, O., & Prinz, M. (2017). A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nature Neuroscience*, *20*(6), 793–803. https://doi.org/10.1038/nn.4547

Taylor, D. L., Diemel, L. T., Cuzner, M. L., & Pocock, J. M. (2002). Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease. *Journal of Neurochemistry*, *82*(5), 1179–1191. https://doi.org/10.1046/j.1471-4159.2002.01062.x

Taylor, D. L., Jones, F., Kubota, E. S. F. C. S., & Pocock, J. M. (2005). Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *25*(11), 2952–2964. https://doi.org/10.1523/JNEUROSCI.4456-04.2005

Tetreault, N. A., Hakeem, A. Y., Jiang, S., Williams, B. A., Allman, E., Wold, B. J., & Allman, J. M. (2012). Microglia in the cerebral cortex in autism. *Journal of Autism and Developmental Disorders*, *42*(12), 2569–2584. https://doi.org/10.1007/s10803-012-1513-0

Texidó, L., Martín-Satué, M., Alberdi, E., Solsona, C., & Matute, C. (2011). Amyloid β peptide oligomers directly activate NMDA receptors. *Cell Calcium*, *49*(3), 184–190. https://doi.org/10.1016/j.ceca.2011.02.001

The DDD Study, Homozygosity Mapping Collaborative for Autism, UK10K Consortium, The Autism Sequencing Consortium, De Rubeis, S., He, X., Goldberg, A. P., Poultney, C. S., Samocha, K., Ercument Cicek, A., Kou, Y., Liu, L., Fromer, M., Walker, S., Singh, T., Klei, L., Kosmicki, J., Fu, S.-C., Aleksic, B., … Buxbaum, J. D. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*, *515*(7526), 209–215. https://doi.org/10.1038/nature13772

Theodosis, D. T., Koksma, J.-J., Trailin, A., Langle, S. L., Piet, R., Lodder, J. C., Timmerman, J., Mansvelder, H., Poulain, D. A., Oliet, S. H. R., & Brussaard, A. B. (2006). Oxytocin and estrogen promote rapid formation of functional GABA synapses in the adult supraoptic nucleus. *Molecular and Cellular Neuroscience*, *31*(4), 785–794. https://doi.org/10.1016/j.mcn.2006.01.006

Theoharides, T. C., Athanassiou, M., Panagiotidou, S., & Doyle, R. (2015). Dysregulated brain immunity and neurotrophin signaling in Rett syndrome and autism spectrum disorders. *Journal of Neuroimmunology*, *279*, 33–38. https://doi.org/10.1016/j.jneuroim.2014.12.003

Thuringer, D., & Garrido, C. (2019). Molecular chaperones in the brain endothelial barrier: Neurotoxicity or neuroprotection? *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *33*(11), 11629–11639. https://doi.org/10.1096/fj.201900895R

Toscano, C. V. A., Barros, L., Lima, A. B., Nunes, T., Carvalho, H. M., & Gaspar, J. M. (2021). Neuroinflammation in autism spectrum disorders: Exercise as a 'pharmacological' tool. *Neuroscience and Biobehavioral Reviews*, *129*, 63–74. https://doi.org/10.1016/j.neubiorev.2021.07.023

Tuchman, R., & Rapin, I. (2002). Epilepsy in autism. *The Lancet. Neurology*, *1*(6), 352–358. https://doi.org/10.1016/s1474-4422(02)00160-6

Tucker, L. B., & McCabe, J. T. (2017). Behavior of Male and Female C57BL/6J Mice Is More Consistent with Repeated Trials in the Elevated Zero Maze than in the Elevated Plus Maze. *Frontiers in Behavioral Neuroscience*, *11*, 13. https://doi.org/10.3389/fnbeh.2017.00013

Utepbergenov, D. I., Fanning, A. S., & Anderson, J. M. (2006). Dimerization of the scaffolding protein ZO-1 through the second PDZ domain. *The Journal of Biological Chemistry*, *281*(34), 24671–24677. https://doi.org/10.1074/jbc.M512820200

Uzunova, G., Pallanti, S., & Hollander, E. (2016). Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics.

The World Journal of Biological Psychiatry, *17*(3), 174–186. https://doi.org/10.3109/15622975.2015.1085597

Vafabakhsh, R., Levitz, J., & Isacoff, E. Y. (2015). Conformational dynamics of a class C G-protein-coupled receptor. *Nature*, *524*(7566), 497–501. https://doi.org/10.1038/nature14679

van de Haar, H. J., Burgmans, S., Jansen, J. F. A., van Osch, M. J. P., van Buchem, M. A., Muller, M., Hofman, P. A. M., Verhey, F. R. J., & Backes, W. H. (2016). Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology*, *281*(2), 527–535. https://doi.org/10.1148/radiol.2016152244

van Steensel, F. J. A., Bögels, S. M., & Perrin, S. (2011). Anxiety disorders in children and adolescents with autistic spectrum disorders: A meta-analysis. *Clinical Child and Family Psychology Review*, *14*(3), 302–317. https://doi.org/10.1007/s10567-011-0097-0

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology*, *57*(1), 67–81. https://doi.org/10.1002/ana.20315

Venero, J. L., Santiago, M., Tomás-Camardiel, M., Matarredona, E. R., Cano, J., & Machado, A. (2002). DCG-IV but not other group-II metabotropic receptor agonists induces microglial BDNF mRNA expression in the rat striatum. Correlation with neuronal injury. *Neuroscience*, *113*(4), 857–869. https://doi.org/10.1016/s0306-4522(02)00232-4

Vermeiren, C., Najimi, M., Vanhoutte, N., Tilleux, S., De Hemptinne, I., Maloteaux, J., & Hermans, E. (2005). Acute up-regulation of glutamate uptake mediated by mGluR5a in reactive astrocytes. *Journal of Neurochemistry*, *94*(2), 405–416. https://doi.org/10.1111/j.1471-4159.2005.03216.x

Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: A meta-analysis of MRI studies. *The American Journal of Psychiatry*, *161*(11), 1957–1966. https://doi.org/10.1176/appi.ajp.161.11.1957

Vogel Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521. https://doi.org/10.1002/glia.23261

von Richthofen, S., Lang, U. E., & Hellweg, R. (2003). Effects of different kinds of acute stress on nerve growth factor content in rat brain. *Brain Research*, *987*(2), 207–213. https://doi.org/10.1016/s0006-8993(03)03338-9

Wahis, J., Baudon, A., Althammer, F., Kerspern, D., Goyon, S., Hagiwara, D., Lefevre, A., Barteczko, L., Boury-Jamot, B., Bellanger, B., Abatis, M., Da Silva

Gouveia, M., Benusiglio, D., Eliava, M., Rozov, A., Weinsanto, I., Knobloch-Bollmann, H. S., Kirchner, M. K., Roy, R. K., … Charlet, A. (2021). Astrocytes mediate the effect of oxytocin in the central amygdala on neuronal activity and affective states in rodents. *Nature Neuroscience*, *24*(4), 529–541. https://doi.org/10.1038/s41593-021-00800-0

Wahlsten, D., Metten, P., & Crabbe, J. C. (2003). Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. *Brain Research*, *971*(1), 47–54. https://doi.org/10.1016/s0006-8993(03)02354-0

Wan, W., Cao, L., Liu, L., Zhang, C., Kalionis, B., Tai, X., Li, Y., & Xia, S. (2015). Aβ(1-42) oligomer-induced leakage in an in vitro blood-brain barrier model is associated with up-regulation of RAGE and metalloproteinases, and down-regulation of tight junction scaffold proteins. *Journal of Neurochemistry*, *134*(2), 382–393. https://doi.org/10.1111/jnc.13122

Wang, L., Almeida, L. E. F., Spornick, N. A., Kenyon, N., Kamimura, S., Khaibullina, A., Nouraie, M., & Quezado, Z. M. N. (2015). Modulation of social deficits and repetitive behaviors in a mouse model of autism: The role of the nicotinic cholinergic system. *Psychopharmacology*, *232*(23), 4303–4316. https://doi.org/10.1007/s00213-015-4058-z

Wang, M., Chen, H., Yu, T., Cui, G., Jiao, A., & Liang, H. (2015). Increased serum levels of brain-derived neurotrophic factor in autism spectrum disorder. *NeuroReport*, *26*(11), 638–641. https://doi.org/10.1097/WNR.0000000000000404

Wang, P., Qin, D., & Wang, Y.-F. (2017). Oxytocin Rapidly Changes Astrocytic GFAP Plasticity by Differentially Modulating the Expressions of pERK 1/2 and Protein Kinase A. *Frontiers in Molecular Neuroscience*, *10*, 262. https://doi.org/10.3389/fnmol.2017.00262

Wang, Q., Liu, D., Song, P., & Zou, M.-H. (2015). Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. *Frontiers in Bioscience (Landmark Edition)*, *20*(7), 1116–1143. https://doi.org/10.2741/4363

Wang, Q.-S., Ding, H.-G., Chen, S.-L., Liu, X.-Q., Deng, Y.-Y., Jiang, W.-Q., Li, Y., Huang, L.-Q., Han, Y.-L., Wen, M.-Y., Wang, M.-Q., & Zeng, H.-K. (2020). Hypertonic saline mediates the NLRP3/IL-1β signaling axis in microglia to alleviate ischemic blood-brain barrier permeability by downregulating astrocyte-derived VEGF in rats. *CNS Neuroscience & Therapeutics*, *26*(10), 1045–1057. https://doi.org/10.1111/cns.13427

Wang, Y., Jin, S., Sonobe, Y., Cheng, Y., Horiuchi, H., Parajuli, B., Kawanokuchi, J., Mizuno, T., Takeuchi, H., & Suzumura, A. (2014). Interleukin-1β induces blood-

brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *PloS One*, *9*(10), e110024. https://doi.org/10.1371/journal.pone.0110024

Wang, Y.-F., & Hatton, G. I. (2007). Interaction of extracellular signal-regulated protein kinase 1/2 with actin cytoskeleton in supraoptic oxytocin neurons and astrocytes: Role in burst firing. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(50), 13822–13834. https://doi.org/10.1523/JNEUROSCI.4119-07.2007

Webster, S., Glabe, C., & Rogers, J. (1995). Multivalent binding of complement protein C1Q to the amyloid beta-peptide (A beta) promotes the nucleation phase of A beta aggregation. *Biochemical and Biophysical Research Communications*, *217*(3), 869–875. https://doi.org/10.1006/bbrc.1995.2852

Wegiel, J., Kuchna, I., Nowicki, K., Imaki, H., Wegiel, J., Marchi, E., Ma, S. Y., Chauhan, A., Chauhan, V., Bobrowicz, T. W., de Leon, M., Louis, L. A. S., Cohen, I. L., London, E., Brown, W. T., & Wisniewski, T. (2010). The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathologica*, *119*(6), 755–770. https://doi.org/10.1007/s00401-010-0655-4

Wei, H., Chadman, K. K., McCloskey, D. P., Sheikh, A. M., Malik, M., Brown, W. T., & Li, X. (2012). Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. *Biochimica Et Biophysica Acta*, *1822*(6), 831–842. https://doi.org/10.1016/j.bbadis.2012.01.011

Wei, H., Ding, C., Jin, G., Yin, H., Liu, J., & Hu, F. (2015). Abnormal glutamate release in aged BTBR mouse model of autism. *International Journal of Clinical and Experimental Pathology*, *8*(9), 10689–10697.

Wei, H., Ma, Y., Ding, C., Jin, G., Liu, J., Chang, Q., Hu, F., & Yu, L. (2016). Reduced Glutamate Release in Adult BTBR Mouse Model of Autism Spectrum Disorder. *Neurochemical Research*, *41*(11), 3129–3137. https://doi.org/10.1007/s11064-016-2035-5

Wei, H., Ma, Y., Liu, J., Ding, C., Hu, F., & Yu, L. (2016). Proteomic analysis of cortical brain tissue from the BTBR mouse model of autism: Evidence for changes in STOP and myelin-related proteins. *Neuroscience*, *312*, 26–34. https://doi.org/10.1016/j.neuroscience.2015.11.003

Wei, H., Zou, H., Sheikh, A. M., Malik, M., Dobkin, C., Brown, W. T., & Li, X. (2011). IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *Journal of Neuroinflammation*, *8*, 52. https://doi.org/10.1186/1742-2094-8-52

Wei, L., Meaney, M. J., Duman, R. S., & Kaffman, A. (2011). Affiliative Behavior Requires Juvenile, But Not Adult Neurogenesis. *The Journal of Neuroscience*, *31*(40), 14335–14345. https://doi.org/10.1523/JNEUROSCI.1333-11.2011

West, L., Waldrop, J., & Brunssen, S. (2009). Pharmacologic treatment for the core deficits and associated symptoms of autism in children. *Journal of Pediatric Health Care: Official Publication of National Association of Pediatric Nurse Associates & Practitioners*, *23*(2), 75–89. https://doi.org/10.1016/j.pedhc.2008.12.001

Wettschureck, N., Strilic, B., & Offermanns, S. (2019). Passing the Vascular Barrier: Endothelial Signaling Processes Controlling Extravasation. *Physiological Reviews*, *99*(3), 1467–1525. https://doi.org/10.1152/physrev.00037.2018

Whitehouse, C. M., & Lewis, M. H. (2015). Repetitive Behavior in Neurodevelopmental Disorders: Clinical and Translational Findings. *The Behavior Analyst*, *38*(2), 163–178. https://doi.org/10.1007/s40614-015-0029-2

Winkler, E. A., Bell, R. D., & Zlokovic, B. V. (2011). Central nervous system pericytes in health and disease. *Nature Neuroscience*, *14*(11), 1398–1405. https://doi.org/10.1038/nn.2946

Winkler, E. A., Nishida, Y., Sagare, A. P., Rege, S. V., Bell, R. D., Perlmutter, D., Sengillo, J. D., Hillman, S., Kong, P., Nelson, A. R., Sullivan, J. S., Zhao, Z., Meiselman, H. J., Wendy, R. B., Soto, J., Abel, E. D., Makshanoff, J., Zuniga, E., De Vivo, D. C., & Zlokovic, B. V. (2015). GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nature Neuroscience*, *18*(4), 521–530. https://doi.org/10.1038/nn.3966

Wöhr, M., Roullet, F. I., & Crawley, J. N. (2011). Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. *Genes, Brain, and Behavior*, *10*(1), 35–43. https://doi.org/10.1111/j.1601-183X.2010.00582.x

Wolburg, H., Noell, S., Fallier-Becker, P., Mack, A. F., & Wolburg-Buchholz, K. (2012). The disturbed blood-brain barrier in human glioblastoma. *Molecular Aspects of Medicine*, *33*(5–6), 579–589. https://doi.org/10.1016/j.mam.2012.02.003

Woolley, M. L., Pemberton, D. J., Bate, S., Corti, C., & Jones, D. N. C. (2008). The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. *Psychopharmacology*, *196*(3), 431–440. https://doi.org/10.1007/s00213-007-0974-x

Wosik, K., Cayrol, R., Dodelet-Devillers, A., Berthelet, F., Bernard, M., Moumdjian, R., Bouthillier, A., Reudelhuber, T. L., & Prat, A. (2007). Angiotensin II Controls Occludin Function and Is Required for Blood–Brain Barrier Maintenance:

Relevance to Multiple Sclerosis. *The Journal of Neuroscience*, *27*(34), 9032–9042. https://doi.org/10.1523/JNEUROSCI.2088-07.2007

Wu, H.-Q., Rassoulpour, A., & Schwarcz, R. (2007). Kynurenic acid leads, dopamine follows: A new case of volume transmission in the brain? *Journal of Neural Transmission (Vienna, Austria: 1996)*, *114*(1), 33–41. https://doi.org/10.1007/s00702-006-0562-y

Wu, S., Chen, R., Chen, J., Yang, N., Li, K., Zhang, Z., & Zhang, R. (2023). Study of the Anti-Inflammatory Mechanism of β-Carotene Based on Network Pharmacology. *Molecules (Basel, Switzerland)*, *28*(22), 7540. https://doi.org/10.3390/molecules28227540

Xu, A., Chan, K. W., Hoo, R. L. C., Wang, Y., Tan, K. C. B., Zhang, J., Chen, B., Lam, M. C., Tse, C., Cooper, G. J. S., & Lam, K. S. L. (2005). Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *The Journal of Biological Chemistry*, *280*(18), 18073–18080. https://doi.org/10.1074/jbc.M414231200

Xu, S., Qin, B., Shi, A., Zhao, J., Guo, X., & Dong, L. (2018). Oxytocin inhibited stress induced visceral hypersensitivity, enteric glial cells activation, and release of proinflammatory cytokines in maternal separated rats. *European Journal of Pharmacology*, *818*, 578–584. https://doi.org/10.1016/j.ejphar.2017.11.018

Yang, L., Mao, L., Chen, H., Catavsan, M., Kozinn, J., Arora, A., Liu, X., & Wang, J. Q. (2006). A Signaling Mechanism from Gαq-Protein-Coupled Metabotropic Glutamate Receptors to Gene Expression: Role of the c-Jun N-Terminal Kinase Pathway. *The Journal of Neuroscience*, *26*(3), 971–980. https://doi.org/10.1523/JNEUROSCI.4423-05.2006

Yang, M., Abrams, D. N., Zhang, J. Y., Weber, M. D., Katz, A. M., Clarke, A. M., Silverman, J. L., & Crawley, J. N. (2012). Low sociability in BTBR T+tf/J mice is independent of partner strain. *Physiology & Behavior*, *107*(5), 649–662. https://doi.org/10.1016/j.physbeh.2011.12.025

Yang, M., Clarke, A. M., & Crawley, J. N. (2009). Postnatal lesion evidence against a primary role for the corpus callosum in mouse sociability. *The European Journal of Neuroscience*, *29*(8), 1663–1677. https://doi.org/10.1111/j.1460-9568.2009.06714.x

Yang, Y., Li, R., Hui, J., Li, L., & Zheng, X. (2021). β-Carotene attenuates LPS-induced rat intestinal inflammation via modulating autophagy and regulating the JAK2/STAT3 and JNK/p38 MAPK signaling pathways. *Journal of Food Biochemistry*, *45*(1), e13544. https://doi.org/10.1111/jfbc.13544

Yang, Y., & Rosenberg, G. A. (2011). Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. *Stroke*, *42*(11), 3323–3328. https://doi.org/10.1161/STROKEAHA.110.608257

Yao, H., Ding, J., Zhou, F., Wang, F., Hu, L., Sun, T., & Hu, G. (2005). Enhancement of glutamate uptake mediates the neuroprotection exerted by activating group II or III metabotropic glutamate receptors on astrocytes. *Journal of Neurochemistry*, *92*(4), 948–961. https://doi.org/10.1111/j.1471-4159.2004.02937.x

Yao, Y., Chen, Z.-L., Norris, E. H., & Strickland, S. (2014). Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nature Communications*, *5*, 3413. https://doi.org/10.1038/ncomms4413

Ye, C., Cheng, M., Ma, L., Zhang, T., Sun, Z., Yu, C., Wang, J., & Dou, Y. (2022). Oxytocin Nanogels Inhibit Innate Inflammatory Response for Early Intervention in Alzheimer's Disease. *ACS Applied Materials & Interfaces*, *14*(19), 21822–21835. https://doi.org/10.1021/acsami.2c00007

Yin, S., Noetzel, M. J., Johnson, K. A., Zamorano, R., Jalan-Sakrikar, N., Gregory, K. J., Conn, P. J., & Niswender, C. M. (2014). Selective Actions of Novel Allosteric Modulators Reveal Functional Heteromers of Metabotropic Glutamate Receptors in the CNS. *The Journal of Neuroscience*, *34*(1), 79–94. https://doi.org/10.1523/JNEUROSCI.1129-13.2014

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., Sohal, V. S., Goshen, I., Finkelstein, J., Paz, J. T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J. R., Hegemann, P., & Deisseroth, K. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*, *477*(7363), 171–178. https://doi.org/10.1038/nature10360

Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L. J., Onaka, T., & Nishimori, K. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(7), 2259–2271. https://doi.org/10.1523/JNEUROSCI.5593-08.2009

Yu, P., Di Prospero, N. A., Sapko, M. T., Cai, T., Chen, A., Melendez-Ferro, M., Du, F., Whetsell, W. O., Guidetti, P., Schwarcz, R., & Tagle, D. A. (2004). Biochemical and phenotypic abnormalities in kynurenine aminotransferase II-deficient mice. *Molecular and Cellular Biology*, *24*(16), 6919–6930. https://doi.org/10.1128/MCB.24.16.6919-6930.2004

Yuan, L., Liu, S., Bai, X., Gao, Y., Liu, G., Wang, X., Liu, D., Li, T., Hao, A., & Wang, Z. (2016). Oxytocin inhibits lipopolysaccharide-induced inflammation in microglial cells and attenuates microglial activation in lipopolysaccharide-treated mice.

Journal of Neuroinflammation, *13*(1), 77. https://doi.org/10.1186/s12974-016-0541-7

Yuan, P., Condello, C., Keene, C. D., Wang, Y., Bird, T. D., Paul, S. M., Luo, W., Colonna, M., Baddeley, D., & Grutzendler, J. (2016). TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. *Neuron*, *90*(4), 724–739. https://doi.org/10.1016/j.neuron.2016.05.003

Yurchenco, P. D., & Patton, B. L. (2009). Developmental and pathogenic mechanisms of basement membrane assembly. *Current Pharmaceutical Design*, *15*(12), 1277–1294. https://doi.org/10.2174/138161209787846766

Zandt, F., Prior, M., & Kyrios, M. (2007). Repetitive behaviour in children with high functioning autism and obsessive compulsive disorder. *Journal of Autism and Developmental Disorders*, *37*(2), 251–259. https://doi.org/10.1007/s10803-006-0158-2

Zeidan, J., Fombonne, E., Scorah, J., Ibrahim, A., Durkin, M. S., Saxena, S., Yusuf, A., Shih, A., & Elsabbagh, M. (2022). Global prevalence of autism: A systematic review update. *Autism Research: Official Journal of the International Society for Autism Research*, *15*(5), 778–790. https://doi.org/10.1002/aur.2696

Zenaro, E., Piacentino, G., & Constantin, G. (2017). The blood-brain barrier in Alzheimer's disease. *Neurobiology of Disease*, *107*, 41–56. https://doi.org/10.1016/j.nbd.2016.07.007

Zenaro, E., Pietronigro, E., Della Bianca, V., Piacentino, G., Marongiu, L., Budui, S., Turano, E., Rossi, B., Angiari, S., Dusi, S., Montresor, A., Carlucci, T., Nanì, S., Tosadori, G., Calciano, L., Catalucci, D., Berton, G., Bonetti, B., & Constantin, G. (2015). Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nature Medicine*, *21*(8), 880–886. https://doi.org/10.1038/nm.3913

Zhang, J., Piontek, J., Wolburg, H., Piehl, C., Liss, M., Otten, C., Christ, A., Willnow, T. E., Blasig, I. E., & Abdelilah-Seyfried, S. (2010). Establishment of a neuroepithelial barrier by Claudin5a is essential for zebrafish brain ventricular lumen expansion. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(4), 1425–1430. https://doi.org/10.1073/pnas.0911996107

Zhang, W. Q., Smolik, C. M., Barba-Escobedo, P. A., Gamez, M., Sanchez, J. J., Javors, M. A., Daws, L. C., & Gould, G. G. (2015). Acute dietary tryptophan manipulation differentially alters social behavior, brain serotonin and plasma corticosterone in three inbred mouse strains. *Neuropharmacology*, *90*, 1–8. https://doi.org/10.1016/j.neuropharm.2014.10.024

Zhang, Y., Zhu, X., Huang, T., Chen, L., Liu, Y., Li, Q., Song, J., Ma, S., Zhang, K., Yang, B., & Guan, F. (2016). β-Carotene synergistically enhances the anti-tumor effect of 5-fluorouracil on esophageal squamous cell carcinoma in vivo and in vitro. *Toxicology Letters*, *261*, 49–58. https://doi.org/10.1016/j.toxlet.2016.08.010

Zhao, W.-Q., De Felice, F. G., Fernandez, S., Chen, H., Lambert, M. P., Quon, M. J., Krafft, G. A., & Klein, W. L. (2008). Amyloid beta oligomers induce impairment of neuronal insulin receptors. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *22*(1), 246–260. https://doi.org/10.1096/fj.06-7703com

Zhou, F., Yao, H.-H., Wu, J.-Y., Yang, Y.-J., Ding, J.-H., Zhang, J., & Hu, G. (2006). Activation of Group II/III metabotropic glutamate receptors attenuates LPS-induced astroglial neurotoxicity via promoting glutamate uptake. *Journal of Neuroscience Research*, *84*(2), 268–277. https://doi.org/10.1002/jnr.20897

Zhou, H., Lapointe, B. M., Clark, S. R., Zbytnuik, L., & Kubes, P. (2006). A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. *Journal of Immunology (Baltimore, Md.: 1950)*, *177*(11), 8103–8110. https://doi.org/10.4049/jimmunol.177.11.8103

Zhou, L., Ouyang, L., Lin, S., Chen, S., Liu, Y., Zhou, W., & Wang, X. (2018). Protective role of β-carotene against oxidative stress and neuroinflammation in a rat model of spinal cord injury. *International Immunopharmacology*, *61*, 92–99. https://doi.org/10.1016/j.intimp.2018.05.022

Zilbovicius, M., Boddaert, N., Belin, P., Poline, J. B., Remy, P., Mangin, J. F., Thivard, L., Barthélémy, C., & Samson, Y. (2000). Temporal lobe dysfunction in childhood autism: A PET study. Positron emission tomography. *The American Journal of Psychiatry*, *157*(12), 1988–1993. https://doi.org/10.1176/appi.ajp.157.12.1988

Zimmerman, A. W., Connors, S. L., Matteson, K. J., Lee, L.-C., Singer, H. S., Castaneda, J. A., & Pearce, D. A. (2007). Maternal antibrain antibodies in autism. *Brain, Behavior, and Immunity*, *21*(3), 351–357. https://doi.org/10.1016/j.bbi.2006.08.005

Zinni, M., Mairesse, J., Pansiot, J., Fazio, F., Iacovelli, L., Antenucci, N., Orlando, R., Nicoletti, F., Vaiman, D., & Baud, O. (2021). mGlu3 receptor regulates microglial cell reactivity in neonatal rats. *Journal of Neuroinflammation*, *18*(1), 13. https://doi.org/10.1186/s12974-020-02049-z

Zlokovic, B. V. (2008a). New therapeutic targets in the neurovascular pathway in Alzheimer's disease. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *5*(3), 409–414. https://doi.org/10.1016/j.nurt.2008.05.011

Zlokovic, B. V. (2008b). The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders. *Neuron*, *57*(2), 178–201. https://doi.org/10.1016/j.neuron.2008.01.003

Zoghbi, H. Y. (2005). MeCP2 dysfunction in humans and mice. *Journal of Child Neurology*, *20*(9), 736–740. https://doi.org/10.1177/08830738050200090701

Zöller, T., Schneider, A., Kleimeyer, C., Masuda, T., Potru, P. S., Pfeifer, D., Blank, T., Prinz, M., & Spittau, B. (2018). Silencing of TGFβ signalling in microglia results in impaired homeostasis. *Nature Communications*, *9*(1), 4011. https://doi.org/10.1038/s41467-018-06224-y