Microglia and BDNF at the crossroads of stressor related disorders: Towards a unique trophic phenotype

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ABSTRACT

Stressors ranging from psychogenic/social to neurogenic/injury to systemic/microbial can impact microglial inflammatory processes, but less is known regarding their effects on trophic properties of microglia. Recent studies do suggest that microglia can modulate neuronal plasticity, possibly through brain derived neurotrophic factor (BDNF). This is particularly important given the link between BDNF and neuropsychiatric and neurodegenerative pathology. We posit that certain activated states of microglia play a role in maintaining the delicate balance of BDNF release onto neuronal synapses. This focused review will address how different “activators” influence the expression and release of microglial BDNF and address the question of tropomyosin receptor kinase B (TrkB) expression on microglia. We will then assess sex-based differences in microglial function and BDNF expression, and how microglia are involved in the stress response and related disorders such as depression. Drawing on research from a variety of other disorders, we will highlight challenges and opportunities for

Abbreviations: 2-AG, 2-Arachidonoylglycerol; A2AR, Adenosine 2A receptors; ACTH, Adrenocorticotropic hormone; AD, Alzheimer’s disease; ADP, Adenosine diphosphate; Akt, Protein kinase B; ALS, Amyotrophic lateral sclerosis; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid; AMPK, Adenosine monophosphate-activated protein kinase; AR, Adrenergic receptor; arg1, Arginase 1; ATP, Adenosine Triphosphate; Axl, Tyrosine-protein kinase receptor UFO; BCP, Beta-caryophyllene; BDNF, Brain-derived neurotrophic factor; CaM, Calcium-modulated protein/CaM; CAMK, Calcium/calcmodulin-dependent protein kinase; CAMK2a, Calcium/calcmodulin-dependent protein kinase type II subunit alpha; cAMP, Cyclic adenosine monophosphate; CB1, Cannabichromene; CREB, CREB binding protein; CD11b, Cluster of differentiation 11b; CD14, Cluster of differentiation 14; CD200R, Cluster of differentiation 20 receptor; CD206, Cluster of differentiation 206/mannose receptor TYPE 1; CD45, Cluster of differentiation 45; CD68, Cluster of differentiation 68; CNS, Central Nervous System; CORT, Corticosterone; COX-2, Cyclooxygenase-2; CRE, Cyclic AMP response element; CREB, Cyclic AMP response element binding protein; CRH, Corticotropin-releasing hormone; CRF, C-reactive protein; CSF-1, Colony stimulating factor 1; CUS, Chronic unpredictable stress; CX3CR1, CX3 chemokine receptor 1; DAMP, Damage-associated molecular pattern; DHA, Docosahexaenoic acid; ERK1/2, extracellular signal-regulated kinase 1/2; FGF-2, Fibroblast growth factor 2; FTY720, 2-amino-(2-[4-octylphenyl]ethyl)-1,3-propanediol hydrochloride aka fingolimod; GABA, Gamma-aminobutyric acid; GC, Glucocorticoid; GDNF, Glial-derived neurotrophic factor; GLP-1, Glucagon-like peptide-1; HMGB1, High-mobility group box 1; HPA, Hypothalamic-pituitary-adrenal; Hsp72, Heat-shock protein 72; IBA-1, Ionised calcium binding adaptor molecule 1; IFN-β, Interferon beta; IFN-γ, Interferon gamma; IGF-1, Insulin growth factor 1; IL, Interleukin; IL-10, Interleukin 10; IL-18, Interleukin 18; IL-1β, Interleukin 1β; IL-23, Interleukin 23; IL-34, Interleukin 34; IL-6, Interleukin 6; iNOS, Inducible nitric oxide synthetase; IRF-3, IFN regulatory factor-3; JNK/pJNK, c-Jun N-terminal kinase/phosphorylated c-Jun N-terminal kinase; LPS, Lipopolysaccharide; LTP, Long-term potentiation; MAL, MyD88 adaptor-like protein; MAPK, Mitogen-activated protein kinase; M-CSF/CSF-1, Macrophage colony stimulating factor; MDD, Major depressive disorder; MeCP2, Methyl CpG binding protein 2; MerTK, Mer tyrosine kinase; MHC, Major histocompatibility complex; MIA, Maternal immune activation; MMP, Matrix metalloproteinase; mRNA, Messenger ribonucleic acid; mTOR, Mammalian target of rapamycin; MYD88, Myeloid differentiation primary response 88 protein; NE, Norepinephrine; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NGF, Nerve growth factor; NLRP3, Nod-like receptor protein 3; NO, Nitric oxide; NOS, Nitric oxide synthetase; NSAID, Non-steroidal anti-inflammatory drug; NT-3, Neurotrophin-3; NT-4, Neurotrophin-4/5; NTRK2, Gene encoding tropomyosin receptor kinase B receptors; P2X3R, P2X purinoceptor 3; P2X7R, P2X purinoceptor 7; P2Y1R, P2Y purinoceptor 12; p38MAPK, p38 mitogen-activated protein kinase; PAMP, Pathogen-associated molecular pattern; PD, Parkinson’s Disease; PGE2, Prostaglandin E2; PI3K, Phosphatidylinositol 3-kinase; PKA, Protein kinase A; Poly (I.C), Polyinosinic-polycytidylic acid; PPAR, Peroxisome proliferator-activated receptor; RAGE, Receptor for advanced glycation end products; ROCK, Rho-associated protein kinase; ROS, Reactive Oxygen Species; S1P, Sphingosine 1-phosphate; SIRPα, Signal regulatory protein α; TIRAP, Toll/interleukin-1 receptor domain-containing adaptor protein; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor alpha; TRIF, Toll-interleukin-1 receptor domain-containing adaptor inducing interferon beta; TrKB, Tropomyosin receptor Kinase B; TrkB.FL, Full-length Tropomyosin receptor Kinase B; TrkB.T1, Truncated Tropomyosin receptor Kinase B.

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1. Introduction

Besides being the immunocompetent cells of the brain, microglia play a crucial role in the plasticity of individual neurons, and, at a broader level, impact neural circuitry (Ferrini and De Koninck, 2013). In this regard, a rich array of data has accumulated to suggest that microglia not only participate in the early sculpting of the central nervous system (CNS), but also the continual modification of neural functioning and connectivity throughout adulthood. Early in development, microglia play a crucial role in phagocytosis of neural stem cells (Michell-Robinson et al., 2015) and early synapse formation and elimination (Nandi et al., 2012). Throughout the lifespan, microglia participate in processes critical for synaptic plasticity (Bar and Barak, 2019), neurogenesis (Diaz-Aparicio et al., 2020; Lorenzen et al., 2021), circuit structure and functioning (Schafer et al., 2012; Wake et al., 2013), as well as synaptic maintenance (Paolicelli et al., 2011; Paolicelli and Ferretti, 2017). Microglia mediate these activities through their well-defined neuroimmune actions, such as phagocytosis or release of cytokines, and these processes can occur even in the absence of a particular immune stimulus or threat. Further, microglia can also produce and respond to trophic factors, such as brain derived neurotrophic factor (BDNF) (Coull et al., 2005; Ferrini and De Koninck, 2013; Gomes et al., 2013; Nakajima et al., 2001), and consequently, this is another important and indirect route through which these cells can modify neuroplasticity. Hence, it is not surprising alterations in microglial function have been implicated in a host of developmental disorders, such as autism and schizophrenia, as well as neuropsychiatric conditions such as depression and anxiety (Bizollo et al., 2018; Felger, 2018; Gomes et al., 2015; Ohja et al., 2018; Singhal and Baune, 2017; Wohleb et al., 2018).

Microglia are highly plastic cells that are never truly “resting”, but rather constantly patrolling their microenvironment providing a defence against a variety of stressors that range from external insults (e.g. pathogens, toxicants) to internal disruptions (e.g. cell death or toxic metabolic by products). Since microglia are thesentinels of the brain, constantly probing for threats or perturbations in their microenvironment, they are in a perfect position to “inform” neurons of environmental challenges and hence, permit appropriate neural responses. Accordingly, during times of stressor exposures, from pathogenic to psychological to traumatic events, microglia act as a type of “first responders” that can either reinforce or inhibit neuronal activities. Indeed, microglial responses can be triggered by a spectrum on environmental stressors, ranging from neurogenic (e.g. injury, trauma, pain) to systemic (e.g. bacterial or viral threats). In recent years, it has even been observed that psychogenic stressors that do not necessarily physically harm the organism (e.g. such as social defeat or chronic mild stress) may evoke microglial responses, often referred to as “sterile inflammation” (Fleshner, 2013; Shen et al., 2013). Intriguingly, inflammatory-activated microglia might also be responsible for inciting astrocytes into toxic reactive states (Liddelow et al., 2017), which suggests some degree of glial cooperation in the face of stress in the microenvironment.

Much of the work we will discuss highlights the fact that microglia are at the front lines, responding in real time to stimuli and changes in their local microenvironment, elicited by pathogenic, traumatic or even psychogenic threats, and are charged with maintaining a homeostatic balance within intracellular, extracellular and synaptic spaces. Further, we postulate that environmental influences can alter the microglia/neuron dynamics that underpin CNS disorders and thus, enhancing microglial trophic responses may provide opportunities for new treatments that go beyond merely suppressing inflammation.

We first seek to characterize how differing types of classically identified microglial “activators” influence microglial expression of BDNF to outline important underlying pathways. Attention will also be devoted to mechanisms behind BDNF release and the evidence surrounding the still open question as to whether microglia actually express TrkB receptors. In addition to exogenously applied factors that have typically been used to modulate microglial phenotypes (such as LPS, ATP, Poly(I : C) and minocycline), we will also cover the research on psychologically relevant or psychogenic stressors, as they pertain to microglial BDNF production. Within this context we discuss microglia activation in clinical depression and how modulating of these cells towards a more trophic response might have treatment implications. In the ensuing sections, we will then address whether trophic factor regulation of microglial phenotypes is brain region-specific and delve into the mounting evidence for sex-dependent differences in microglial pathways (Rossetti et al., 2019; Salter, 2019). It is certainly important to understand how sex hormones sculpt microglial epigenetics early in development, ultimately impacting reactivity to a range of insults later in life. Hormonal factors may also interact with biological processes and environmental influences in adulthood to produce sexually dimorphic microglial phenotypes.

Finally, and most importantly, we consider the possibility of a “trophic” microglial phenotype that could potentially be exploited as a novel therapeutic approach for stressor-related disorders that involve disturbances of neuroplasticity. In this manuscript, we posit a working model that is uniquely focused upon the possibility that the microglial cell (rather than a sole focus on the neuron per se) might hold special utility in regulating the trophic homeostasis underlying emotional and cognitive states. Such unique trophic microglial phenotype(s) may be adopted in response to certain extracellular factors acting on endogenous receptors (e.g. sphingosine 1-phosphate receptors (S1PRs), peroxisome proliferator-activated receptors (PPARs), Glucagon-like peptide-1 receptors (GLP-1Rs), and cannabinoid type 2 receptors (CB2Rs)). Further still, we propose that these microglia might act as a unique source of BDNF, which upon its release, exerts trophic effects upon adjacent neurons and possibly, neighbouring astrocytes or even acts to self-regulate microglia (though microglial TrkB expression is an open issue, as discussed later). Targeting our findings to selectively manipulate a microglial trophic state might yield novel treatments for the array of stressor related disorders that involve both neurotrophic and neuro-inflammatory alterations.

2. BDNF/TrkB background

As a member of the neurotrophin family of growth factors, BDNF was first isolated from pig brains in 1982, where it was found to support the survival of embryonic sensory neurons in culture (Barde et al., 1982). In the ensuing decades, BDNF variants have been identified and there have been thousands of publications documenting the function and role of these various forms in development and pathological processes, including a significant focus on the vital role of BDNF in mood disorders (for a review see (Hempstead, 2015)).

BDNF undergoes extensive post-translational modification, with BDNF mRNA first translated into a precursor peptide, proBDNF, which is subsequently cleaved to produce the 32 kDa protein, proBDNF (Mowla et al., 2001). Further processing occurs in the trans-golgi network, where the protein may cleaved by proteases into a mature 14 kDa form of BDNF (Dieni et al., 2012). Through secretory and regulatory pathways, proBDNF can be released and bind to the promiscuous p75 receptor, which can trigger activation of apoptotic pathways and long-term depression (Barker, 2009). In contrast, the mature 14 kDa...
form binds to TrkB receptors and this signaling cascade has been linked to a multitude of neuroprotective functions (Minichiello, 2009). Within this manuscript, the vast majority of studies assessed the mature 14 kDa form of BDNF; however, in a few instances the larger “pro” form of the protein was also assessed (and we will explicitly mention this form in such cases).

BDNF transcription is controlled by multiple promoters, the most important of which is the cyclic adenosine monophosphate (cAMP)-response element (CRE). The CRE is activated by the transcription factor, cAMP-response-element-binding protein (CREB), following its response element (CRE). The CRE is activated by the transcription factor, cAMP-response-element-binding protein (CREB), following its phosphorylation through intracellular Ca

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Parallelizing the distribution of BDNF, various forms of the TrkB receptor can be found in both the CNS and tissues of the peripheral nervous system. In mammals, there are several possible isoforms of TrkB, including a full-length variant (TrkB.FL), and several truncated variants, the most common of which is TrkB.T1. Just as BDNF is critical to survival, so too is TrkB.FL, with constitutive knockout mice only surviving a few days past birth and suffering from heavy impairment in motor neuron, ganglion, and cerebellar development (Klein et al., 1993). Numerous studies have confirmed the presence of TrkB.FL on neurons, with some indication it may appear briefly on immature astrocytes (Andreska et al., 2020; Armanini et al., 1995; Klein et al., 1990; Shelton et al., 1995). The TrkB receptors are single-pass type I membrane proteins, which form dimers upon ligand binding. TrkB.FL is the only variant containing three intracellular phosphorylation sites at tyrosine residues, with downstream signalling tied to multiple pathways supporting cell survival, neuronal growth/differentiation, transcription, cytoskeletal changes, and upregulation of synaptic proteins (Gupta et al., 2013; Minichiello, 2009).

The most studied truncated variant, TrkB.T1, has an extracellular domain homologous to TrkB.FL, but lacks an intracellular kinase domain. Though present on neurons, TrkB.T1 is most abundant on astrocytes, particularly in adulthood (Fenner, 2012; Holt and Olsen, 2016; Rose et al., 2003). On neurons, TrkB.T1 can form heterodimers with TrkB.FL, where it can act as a negative regulator of TrkB.FL signaling (Li et al., 1998). The functions of TrkB.T1 on astrocytes are less well understood, but are most likely involved in regulating calcium signaling (Fenner, 2012; Rose et al., 2003). Finally, there is a high degree of homology between rodent and human forms of BDNF and TrkB, making rodent models well-suited to the study of these proteins (Klein et al., 1989).

In neurons, BDNF release is governed by regulatory mechanisms (Hyman et al., 1994; Maisonnier et al., 1990; Mowla et al., 1999), with its activity-dependent release from both pre- and post-synaptic neurons being well established (Arai et al., 1996; Balkowiec and Katz, 2002; Yang et al., 2005). Further, there is evidence of BDNF acting as an autocrine signaling factor in neurons (Harward et al., 2016; Rabacchi et al., 1999), suggesting a self-regulatory or feedback role for the trophic factor in maintaining neuronal signaling and homeostasis. As previously mentioned, BDNF/TrkB signaling involves pathways that upregulate synaptic proteins that are important for synaptic plasticity at glutamatergic synapses (Cohen-Cory et al., 2010; Lu, 2003) where it is essential for neurodevelopment, synaptic growth and maintenance, and learning and memory (Gubellini et al., 2005; Itoh et al., 2016; Ivanova and Beyer, 2001). There is also compelling evidence that BDNF governs plasticity at inhibitory synapses including mediating inhibitory circuit formation (Berghuis et al., 2004; Vaz et al., 2011; Zhu et al., 2019). BDNF might also impact neuronal functioning by acting on the truncated TrkB.T1 receptor on astrocytes where it may be involved in the sequestration of excess synaptic BDNF (Fenner, 2012; Girardet et al., 2013; Ohno et al., 2015; Parpura et al., 2012; Rose et al., 2003). Finally, as we will discuss shortly, there is emerging evidence that under the right conditions, microglia can also express BDNF (Coull et al., 2005; Ferrini and De Koninck, 2013; Nakajima et al., 2001; Trang et al., 2011).

3. Microglia and classical activators

It is well known that microglia can dynamically change in response to their microenvironment, with the literature classically referring to three primary microglial phenotypes, M0 (“resting” and extensively ramified), M1 (activated/inflammatory and either fully or partially amoeboid) and M2 (pro-regenerative/anti-inflammatory and either fully or partially amoeboid) (Franco and Fernández-Suárez, 2015). While not an exhaustive list, classical signs of the M0 state are cell surface expression of the CX3 chemokine receptor 1 (CX3CR1), cluster of differentiation 200 (CD200), signal regulatory protein α (SIRPα), and cluster of differentiation 45 (CD45) (Sajio and Glass, 2011). Typical M1 activation includes expression of pro-inflammatory markers such as interleukins 6, 1, 8, 13, 23 (IL-6; IL-1; IL-12; IL-23), nitric oxide (NO) and its catalyzing enzyme inducible nitric oxide synthetase (iNOS), cluster of differentiation 68 (CD68), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) (Rodríguez-Gómez et al., 2020). In contrast, M2 microglia typically express anti-inflammatory and/or trophic markers such as interleukin 10 (IL-10), cluster of differentiation 206/mannose receptor TYPE 1 (CD206), arginase 1 (arg1) and insulin growth factor 1 (IGF-1) (Franco and Fernández-Suárez, 2015).

Despite attempts to classify these primary phenotypes into subtypes, recent work has also highlighted a high degree of heterogeneity in these cells, particularly in early development and in injured or aged brains (Hammond et al., 2019). Thus, we recognize that microglia have significantly more nuanced and varied activation and resting phenotypes which express region and stimulus-specific markers (for a review see, Jurga et al., 2020). This makes them all the more interesting as these various intermediate phenotypes not only regulate normal immune sentinel patrolling, but also have important regulatory effects on neuroplasticity. Such a range of plastic microglial phenotypes could be recruited by differing stimulus combinations, and conceivably contribute to key symptoms in stressor-related illnesses such as depression, anxiety, developmental and/or eating disorders. This could include core features, such as disturbances of fatigue/energy, sleep, feeding/anorexia and mood/anhedonia/reward. In fact, rather than attributing microglial functions to specific disorders, it might be more fruitful to think more broadly about their involvement in specific symptom clusters that occur across all CNS pathologies. This is certainly in line with the fact that microglial involvement cuts across research areas and disease modalities. The bottom line might be that microglia activation can directly regulate neurotrophic and neurochemical signaling, with their shaping of synaptic systems indirectly modulating long-term CNS plasticity and functioning.

Regardless of the complexity of microglial intermediate states, common classically activated inflammatory phenotypes typically occur via either pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) which can signal through a variety of receptors (Morioka et al., 2015; Olson and Miller, 2004; Rodríguez-Gómez et al., 2020; Tang et al., 2015; Olson and Miller, 2004). The most relevant PAMP receptors in the CNS are the toll-like receptors (TLRs), which have been extensively assessed in response to neuro-inflammatory processes and disease (Lehnardt, 2010; Olson and Miller, 2004). Through these receptors, it is well established that microglia are early-stage mediators of virtually all threats to the CNS through their
ability to detect specific chemical signatures in their microenvironment. Once activated, microglia can remove microbial threats by phagocytosis, through the release of toxic oxidative intermediates or by enabling secondary immunological defenses.

Microglial receptors and pathways are under increasing scrutiny as potential targets for treatment of many CNS conditions, as each may support alternative activation phenotypes, including mediating the production and release of BDNF (Di Benedetto and Rupprecht, 2013; Walker and Lee, 2005; Wen et al., 2011). In our efforts to discuss potential treatment targets, it is of critical importance to identify not only activating ligands, but also to understand the necessary microenvironmental milieu and underlying signaling pathways that might support a microglial trophic state. Timing, dosage and the ligand/receptor combination all play an important role in mediating the microglial phenotype, including their morphology, proliferation, and production of BDNF and cytokines. An enormous number of soluble factors have been used to activate microglia for the study of stress and depression (for a review, see (Yirmiya et al., 2015)). Unfortunately, where BDNF was examined, most of these types of studies have resulted in a global or regional downregulation of BDNF and very few have been studied in the context of their specific impact on microglial BDNF.

Of the classical “activators” of microglia, a few that have been reported to up-regulate microglial BDNF (at least transiently and albeit in very specific conditions) are adenosine triphosphate (ATP), LPS, and to a lesser extent, poly (I:C). These factors have been used extensively in both in vitro and in vivo studies, modulating both BDNF and inflammatory cytokine release (Farber and Kettenmann, 2006; Gandhi et al., 2007; Hoogland et al., 2015). In addition, the tetracycline antibiotic, minocycline, is often used as an anti-inflammatory regulator and can have its own independent effects on microglial activity (Garrido-Mesa et al., 2013a, 2013b; Möller et al., 2016). Importantly, each of these ligands binds to unique receptors on microglia, which are, in turn, tied to distinct molecular pathways that uniquely modulate inflammatory responses and microglial phenotypes. For instance, the exogenous application of ATP has been found to upregulate BDNF production in cultured microglia, with moderate increases in TNF-α, but little impact on other inflammatory cytokines (Hu et al., 2020). In contrast, LPS or poly (I:C) strongly stimulates the release of TNF-α, IL-1β, and IL-6 (Hoogland et al., 2015), however, as will be discussed shortly, there are a few reports that LPS might also stimulate microglial BDNF production. In addition, there is also some emerging evidence that anti-inflammatory cytokines, such as IL-4 and drugs of abuse can induce microglia to produce BDNF (Cotto et al., 2018; Zhao et al., 2015). We will now individually discuss these primary microglial regulatory factors with a particular focus on studies which reported their impact on microglial BDNF production and secretion (see Table 1).

3.1. ATP/ADP

In the context of microglial functioning, the purine, ATP, plays distinctly different roles in intracellular and extracellular compartments. Within the intracellular milieu, ATP provides energy to cellular machinery as a phosphate donor, however, when released into the extracellular space it can act as a DAMP signaling molecule (Calovi et al., 2019). By acting through purinergic receptors, ATP-induced P2Y1 and P2Y2/4 receptor activation appears to be capable of producing outwardly rectifying K+ currents leading to hyperpolarization, whereas P2X receptors respond to ATP leading to some degree of depolarization (Boucsein et al., 2003). During typical neuronal firing, neurons can release small amounts of vesicular ATP into the synapse (Fields and Burnstock, 2006), and non-vesicular extra-synaptic release from axon cation channels (Fields and Ni, 2010). Astrocytes can also contribute in activity-dependent release of small quantities of ATP, through P2X receptors and hemichannels (Suadicani et al., 2006). During times of cellular distress, however, markedly increased ATP leakage into the surrounding extracellular space can promote DAMP signaling by ATP binding to G-protein-coupled P2Y receptors and ionotropic P2X purinoceptors on microglia. In fact, purinergic signaling, via ATP and ADP, appears to be a critical event that regulates BDNF production and release by microglia (Calovi et al., 2019; Lecca et al., 2012; Trang et al., 2009). Within the spinal cord, Coull et al. (2005) elegantly demonstrated that it was specifically ATP-induced microglial BDNF that caused a depolarizing shift in anion gradients in lamina I neurons, leading to the development of chronic pain.

Microglial activation is uniquely sensitive to the quantity and duration of ATP released by surrounding cells. This response appears to be dose-dependent with ATP preferentially activating P2Y receptors at low concentrations, and P2X receptors at higher concentrations (with sub-populations of microglia predominantly expressing one or the other type of receptor) (Boucsein et al., 2003). Lower concentrations of ATP also appeared to induce a more anti-inflammatory/trophic phenotype, whereas higher concentrations (which can be released from injured or dying neurons) tend to trigger an inflammatory response. The G1-coupled P2Y12R has been identified as the dominant P2Y receptor on “resting/non-activated” microglia (Haynes et al., 2006) and appears to be tied to a more inflammatory response (see Fig. 1). In contrast, activation of the G11-coupled P2Y1 receptor actually upregulates cAMP, however despite being expressed in human brains, there is no evidence to date that it is expressed in rats or mice (von Kügelgen, 2019).

While evidence of P2Y receptor involvement in BDNF secretion is lacking, ATP-driven activation of ionotropic P2X receptors on microglia can result in the synthesis and release BDNF, with the neurotrophin acting at local synapses. Under sustained exposure to ATP, both P2X4R and P2X7R-containing complexes open rapidly and become permeable to larger cations (Burnstock, 2007). Indeed, these P2X receptors can form both homomeric and heteromeric trimers, the composition of which mediates the properties of a non-selective ion channel that is permeable to Na+, K+ and Ca2+ (Hattori and Gouaux, 2012). In particular, P2X7-Rs can form a reversible transmembrane pore which may lead to signal cascades that induce a hyper-inflammatory state mediating the release of pro-inflammatory TNF-α (Boucsein et al., 2003; Suzuki et al., 2004), along with further ATP (Pellegatti et al., 2005). Interestingly, moderate P2X receptor activation on microglia is associated more with trophic responses, while P2Y receptor activation is primarily responsible for induction of chemotaxis and cell migration (Honda et al., 2001). Ultimately, ATP appears to be one of the most potent triggers of BDNF release from microglia in both homeostatic and pathogenic responses, primarily through its activation of P2X receptors.

The time course of BDNF release from primary microglial cultures appears complex, with the application of ATP initially evoking the rapid release of BDNF from existing internal pools, followed by a second burst of BDNF release into the extracellular medium after 60 min (Trang et al., 2009). In agreement with previous reports (Long et al., 2020), extracellular Ca2+ and phosphorylation of p38MAPK were essential mediators in both early and late phases of BDNF release from microglia stimulated by ATP (Trang et al., 2009) (Fig. 1). It was further found that transcriptional and translational inhibitors, including actinomycin-D and cycloheximide, blocked the second burst, implying that further de novo synthesis took place. As well, upregulation and phosphorylation of mammalian target of rapamycin (mTOR) was essential to BDNF production (Hu et al., 2020), and this was likely due to its mediating effects on protein translation (Keane et al., 2021). Whatever the case, it can reasonably be expected that ATP-driven microglial BDNF release might ultimately impart effects aligned with synaptic plasticity, including enhancing activity-dependent synapse formation (Kowianski et al., 2015).

Once released, microglial BDNF can impart downstream signaling through both pre- and post-synaptic TrkB-FL receptors on neurons, resulting in the activation of multiple pathways, including mTOR and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), which can modulate long-term potentiation (LTP) and other aspects of synaptic plasticity (Song et al., 2017). Similarly, microglial BDNF has also been
| BDNF Expression | Activator | Environment/ Species | Mechanism | Method of Measurement | Study |
|-----------------|----------|----------------------|-----------|-----------------------|-------|
| ↑               | ATP (50 μM) | Primary culture/RAT | P2RX4 / p38MAPK Ca<sup>2+</sup> | WB | (Trang et al., 2009) |
| ↑               | ATP (50 μM) | BV2 culture | P2RX4 / p38MAPK | WB | (Long et al., 2020) |
| ↑               | ATPs (100 μM) | Primary culture/RAT | P2RX4 / p38MAPK/ mTOR | (RT)-qPCR | (Hu et al., 2020) |
| ↑               | ATP (1 uM) | Spinal cord microglia/ Mouse | P2RX4 | WB, IF | (Ulmann et al., 2008) |
| ↑               | ATP (10 μM) | Primary Microglia culture/Rat (brainstem & cortex) | P2RX4/ mTOR | indirect ELISA | (Lai et al., 2012) |
| ↑               | LPS (100 ng/mL) | Primary Microglia culture/Rat (hippocampus) | P2RX4 / p38MAPK/ mTOR | (RT)-qPCR | (Hu et al., 2020) |
| ↑               | LPS (100 ng/mL) | Murine N9 microglia culture | A2AR/ COX-2/ PGE<sub>2</sub> | WB | (Gomes et al., 2013) |
| ↑               | LPS (1ug/mL) | Primary Microglia culture/Rat (brainstem, hippocampus, striatum,cortex) | A2AR | indirect ELISA | (Lai et al., 2012) |
| ↑               | LPS (1ug/mL) | Primary Microglia culture/Rat | PKG<sub>δ</sub> | WB | (Nakajima et al., 2001) |
| ↑               | LPS (100 ng/mL) | Murine N9 microglia culture | A2A | WB | (Gomes et al., 2013) |
| ↓               | LPS (100 ng/mL) | Primary Microglia culture/Rat | A2A | IHC | (Kous et al., 2019) |
| ↓               | LPS (1ug/mL) | BV2 culture | miR-142-3p/ CAMK2a | RT-PCR | (Gupta et al., 2020) |
| ↓               | LPS (100ng/mL) | Primary Microglia culture/Rat BV2 culture | P3K-AKT-CREB | WB, IF | (Hutchinson et al., 2009) |
| ↑               | histone deacetylase inhibitor (Nabu) | Primary microglia culture/Rat | P3K-AKT-CREB | WB, IF | (Saw et al., 2020) |
| ↑               | IFN-γ (ip, 10 μg/kg/day) | Mice, in vivo | P3K-AKT-CREB | WB | (Abd-El-Basset et al., 2020) |
| ↑               | Minocycline | Rats, in vivo (intracerebral hemorrhage) | human immortalized microglia culture | WB, IF | (Miao et al., 2018) |
| ↑               | Glutamate | Primary Microglia culture/Rat (Striatum) | P3K-AKT-CREB | WB | (Cotto et al., 2018) |
| ↑               | PGE<sub>2</sub> | mouse immortalized microglia culture | P3K-AKT-CREB | WB, IF | (Zhang et al., 2021) |
| ↑               | LPS (100 ng/mL) | Murine N9 microglia culture | P3K-AKT-CREB | WB, IF | (Gomes et al., 2013) |

Studies which have isolated microglia or have examined activation of microglia in cell culture to assess the impact of various molecular activators on BDNF production. Abbreviations: adeno-associated virus (AAV); enzyme-linked immunosorbent assay (ELISA); western blot (WB), immunohistochemistry (IHC); immunofluorescence (IF); reverse-transcriptase polymerase chain reaction (RT-PCR); Quantitative reverse transcription PCR (RT-qPCR); chronic mild stress (CMS).
shown to alter network excitability via regulation of gamma-aminobutyric acid (GABA) release from interneurons (Ferrini and De Koninck, 2013) and these functions can then, in turn, regulate circuitry involved in higher order processes.

A trophic microglial phenotype might be one that not only produces BDNF in response to ATP, but also plays a crucial inhibitory role by releasing adenosine into the synapse as a by-product of ATP dissolution. This was recently elegantly demonstrated by Badimon et al. (2020) in a series of experiments where they show that microglia engage a complex mechanism of sensing extracellular ATP from neurons via P2Y12, which then triggers the production and release of adenosine. The microglial adenosine can then serve to downregulate neuronal activity as part of a negative feedback loop with the effect of mitigating potential excitotoxicity. Further, a study in zebrafish observed that when “non-activated” microglia make contact with neurons, they downregulate spontaneous and evoked neuronal activity (Li et al., 2012). Indeed, this concept of microglial-mediated inhibition of neuronal firing is also supported by the fact that mice lacking microglia suffered prolonged kainic acid-induced seizures when GABA_A receptors were inhibited (Badimon et al., 2020).

ATP is also involved in microglial BDNF release via the autocrine binding of adenosine to A2A receptors (see Fig. 2). As we will report in the next section, Gomes et al. (2013) identified activation of adenosine 2A receptors (A_2A_R) as a key aspect of LPS induction of BDNF. This cascade has also been linked to the upregulation of cyclooxygenase-2 (COX-2) and production of prostaglandin E_2 (PGE_2) (Fiebich et al., 1996), which can further modify BDNF release, possibly as a function of concentration, brain region, receptor subtype activation, and chronicity (Chen et al., 2018; Milatovic et al., 2011; Luo et al., 2017). Ultimately, it appears that ATP can directly induce BDNF expression in microglia via binding to purinergic receptors, or indirectly via secondary release of adenosine binding to A_2A_R.

It is conceivable that novel adenosine related therapies may be developed that capitalize on the modulation of inflammatory and trophic processes for the treatments of neuropsychiatric disorders, such as depression (van Calker et al., 2019). Along these lines, though somewhat controversial, it is noteworthy that a single nucleotide polymorphism in the purine, P_2RX_7 gene (rs2230921), has been linked to higher incidence of both major depressive disorder (MDD) and bipolar disorder (for a review see Illes et al., 2019). Further, ATP-hypofunction, coupled with overactivation of P_2RX and P38MAPK, resulting in translocation of NFkB to the nucleus (Tatsunami et al., 2015). (b) alternative activation of P38 and AKT that upregulates integrin-p1 supporting chemotaxis (Ohsawa et al., 2010; Swiatkowski et al., 2016); and finally, (i) P2Y12R can interact with CD39 to facilitate conversion of ADP to AMP (Badimon et al., 2020), which can facilitate BDNF upregulation via autocrine binding of adenosine to A2A receptors (see Fig. 2). Created with Biorender.com.

3.2. LPS

The gram-negative endotoxin and bacterial mimic, LPS, activates microglia through toll-like-receptor 4 (TLR4) in conjunction with the cluster of differentiation 14 (CD14) receptor (Akira et al., 2006; Hug et al., 2018; Laflamme and Rivest, 2001; Parajuli et al., 2012). Subsequent protein-protein interactions can occur through the myeloid differentiation primary response 88 protein (MYD88) (Hines et al., 2013), leading to downstream activation of map kinases, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and enhanced transcription of (among other things) pro-inflammatory cytokines (Fig. 2). Alternatively, TLR4 activation can induce a MYD88-independent pathway, triggering Toll-interleukin-1 receptor domain-containing adaptor inducing interferon beta (TRIF) domain and associated adapters (Lu et al., 2008). While there have been some reports of LPS activating astrocytes in vitro, it is possible those studies suffered from microglial contamination. Indeed, some evidence suggests that murine astrocytes do not express TLR4 (Lehnaard et al., 2002) or express only low levels (Falsig et al., 2006; Mishra et al., 2006; Schroder et al., 2012; Zhang et al., 2015b), while others have found that LPS has no effect on human astrocytes, but activates murine astrocytes (Tarassishin et al., 2014). Thus, while the impact of LPS on astrocytes remains controversial (Vijay, 2018), its pro-inflammatory effects on microglia are well-established.
LPS has long been used in the study of stress and depression, wherein systemic administration in rodents results in sickness behaviour, depressive features and significant down-regulation of BDNF, particularly in the hippocampus (Frihauf-Perez et al., 2018; Ke et al., 2020; Zhao et al., 2019). Certainly, LPS robustly stimulates microglia to produce pro-inflammatory cytokines including TNF-α, IL-6, IL-1β, and a variety of oxidative stress radicals (Papageorgiou et al., 2016; Pirnese-Karhu et al., 2012; Yang et al., 2013), as well as upregulation of indoleamine 2,3-dioxygenase (IDO) shunting tryptophan metabolism to the kynurenine pathway (O’Connor et al., 2009; Salazar et al., 2012; Zhang et al., 2020b; Zhao et al., 2019). Recent work has also reported that LPS upregulates microRNA micro-RNA 142-3p (miR-142-3p), which suppresses transcription of Ca²⁺/calmodulin dependent kinase 2a (CAMK2a) – an important mediator in the expression of BDNF (Gupta et al., 2020). However, several studies examining LPS effects on microglia in vitro actually raises the possibility that some of the activation pathways downstream of TLR4 might serve as targets for the upregulation of BDNF.

Despite a plethora of studies using LPS systemically, a small number of studies have looked at its impact on the production and release of BDNF specifically from isolated microglia. On the one hand, LPS applied to primary rat microglial cultures has been reported to stimulate the increased synthesis and release of BDNF (Lai et al., 2012; Miwa et al., 1997; Nakajima et al., 2001) (Fig. 2). Similarly, work on immortalized N9 microglia (murine origin) cultures found that LPS enhanced the release of BDNF at 6 but not 24 h, and this was dependent on co-activation of A2A receptors (Gomes et al., 2013). On the other hand, some researchers have found that in primary microglial cultures, the application of LPS either downregulated BDNF synthesis (Chang et al., 2020; Gupta et al., 2020; Koss et al., 2019), or did not result in any significant changes (Elkabes et al., 1998). Interestingly, Koss et al. (2019) found that primary rat microglia cultures treated with LPS continued to have reduced BDNF expression even after recovery following exposure to the potent anti-inflammatory steroid, dexamethasone. This suggests that in some circumstances, TLR4 activation by LPS may result in a particularly enduring hypo-neurotrophic state.

Conflicting results in LPS studies has been attributed to a high degree of variability in LPS dosing and timing of measurements (Franco and Fernández-Suárez, 2015). Indeed, both dose and timing of measurements may be critical to trophic responses. When considering dosage, very high doses of LPS can induce apoptotic cell death in microglia through an IFN regulatory factor-3 (IRF-3)/interferon beta (IFN-β) pathway (Jung et al., 2005), whereas low doses may actually induce neuroprotection against subsequent trauma (Chen et al., 2012; Sangaran et al., 2021). A dose-dependent relationship was also demonstrated in a more recent set of experiments which found that while low doses of LPS induced elevated inflammatory cytokine release from microglia, only high doses of LPS impaired long-term potentiation (LTP) and inhibited overall BDNF expression in the hippocampus (Golia et al., 2019). Further, Kreisel et al. (2013) found that systemic LPS administration to already stressed mice increased latency to immobility in the forced swim test (an antidepressant-like effect), whereas it had the opposite impact when given to naïve, unstressed mice. This latter finding implies that, in further studies which found that while low doses of LPS induced elevated inflammatory cytokine release from microglia, only very high doses of LPS can induce apoptotic cell death in microglia through an IFN regulatory factor-3 (IRF-3)/interferon beta (IFN-β) pathway (Jung et al., 2005), whereas low doses may actually induce neuroprotection against subsequent trauma (Chen et al., 2012; Sangaran et al., 2021). A dose-dependent relationship was also demonstrated in a more recent set of experiments which found that while low doses of LPS induced elevated inflammatory cytokine release from microglia, only high doses of LPS impaired long-term potentiation (LTP) and inhibited overall BDNF expression in the hippocampus (Golia et al., 2019). Further, Kreisel et al. (2013) found that systemic LPS administration to already stressed mice increased latency to immobility in the forced swim test (an antidepressant-like effect), whereas it had the opposite impact when given to naïve, unstressed mice. This latter finding implies that, in addition to dose, the timing of LPS exposure relative to one’s stressor history modulates the behavioral impact.

As already mentioned, CREB is well-documented as a primary BDNF transcription factor (Esveld et al., 2020; Tao et al., 1998; Wen et al., 2010), and there is evidence that CREB can be activated by both MyD88-dependent, and to a lesser extent, MyD88-independent signaling (Fig. 2). In particular, the MyD88-dependent pathway was found to be essential to the upregulation of BDNF in response to LPS in macrophages, but only if sufficient levels of cAMP were present to activate PKA (Avni et al., 2019) and lead to enhanced transcription of (b) BDNF (Mayr and Montminy, 2001) and A2A receptors (Gomes et al., 2013). (i) Activation of A2A receptors can further promote BDNF via interaction with adenyl cyclase (AC) and upregulation of PKA (Gomes et al., 2013). Further, the (j) AP-1 transcription complex can result in the upregulation of pro- and anti-inflammatory cytokines (Shimoyama et al., 2019; Smolinska et al., 2008), along with (k) COX-2, leading to enhanced production of PGE₂, which binds in an autocrine fashion to (l) EP₂ receptors which activate PKA and eventually, CREB (Gomes et al., 2013). (m) Alternatively, LPS can upregulate BDNF, via PKCα (Nakajima et al., 2001) and promote (n) IRF3 induced modulation of cytokine expression (Doyle et al., 2002; Kawai et al., 2003; Yamanoto et al., 2003). Figure created with BioRender.com.
et al., 2010; Mellett et al., 2011; Woo et al., 2003). This finding ties into those of Gomes et al. (2013) showing that LPS-induced release of BDNF in microglia was tied to A2AR-dependent upregulation of cAMP/PKA. It is important to note that the MyD88 pathway can also reduce BDNF levels though the production of inflammatory cytokines and induction of NF-κB which can inhibit CREB (Hui et al., 2018) (Fig. 2).

Overall, the few in vitro studies reporting LPS-induced upregulation of BDNF would appear to be in direct conflict with an enormous body of work showing that systemic LPS administration reduces BDNF levels and signaling. How can we reconcile evidence from in vitro work suggesting that LPS acting on TLR4 can induce the release of BDNF from microglia, when we see a global downregulation of BDNF in models of systemic administration? Ultimately, one must bear in mind that systemic LPS activates not only microglia but also macrophages and other peripheral immune cells (Delrue-Perollet et al., 1995). These additional factors may also impact BDNF expression. It also is likely that the amount of BDNF released from microglia would be relatively small, compared to that produced by neurons. For example, neurons are known to markedly increase their release of BDNF during activity-dependent synaptic plasticity and in response to many learning tasks (Brigadski and Leßmann, 2020; Kowiański et al., 2018). In contrast, changes in microglial BDNF would be expected to be more modest; albeit there is a lack of data comparing actual concentrations of the trophic factor as a function of cell type. It is plausible that local microglial BDNF release might not necessarily be indicative of the larger global neuronal response engendered by a robust pro-inflammatory stimulus (such as LPS). This is not to say that the microglial derived BDNF is not important, but rather that in such situations, microglial BDNF might be involved in very specific tuning of functions at the synaptic level.

Based on the evidence presented here, we hypothesize that microglia may produce an initial limited trophic response to LPS as a protective measure, but that ultimately, the prolonged activation of inflammatory and kynurenine pathways, together with the release of ROS and pro-inflammatory cytokines (Arizó et al., 2019; Czech et al., 2011), tips the balance in favour of inflammation, thereby inhibiting BDNF associated trophic responses. Such biphasic responses are commonly observed in response to a variety of immune activators, and in one recent study LPS did indeed induce higher glutamine levels at 2 h post-administration, but lower than baseline after a 24 h (Fritz et al., 2020). Likewise, LPS exerts well known biphasic febrile and sickness responses, with hyperthermia often followed by hypothermia and in the most serious cases, endotoxic shock can ensue (Romanovsky et al., 1996). In fact, systemic LPS challenge often produces an initial acute sickness response (ptosis, piloerection, curled body posture and lethargy in rodents), that is followed by upregulation of inflammatory effects, including the ability to inhibit M1 polarization (Kobayashi et al., 2013). Importantly, the activation of TLR3 on astrocytes has been found to induce the release of pro-inflammatory mediators (Scott et al., 2018). It’s important to note that minocycline does not exclusively inhibit microglia – it has broad immunomodulatory impacts on neurons and other glia and in the peripheral immune system as well (Möller et al., 2016). Although minocycline has been found to be neuroprotective in many situations, there is limited direct evidence of its ability to induce a trophic phenotype in microglia. Nevertheless, its extensive use in numerous studies makes it worthy of examination as a potential regulator of microglial BDNF.

3.4. Minocycline

Minocycline is a tetracycline antibiotic known to have broad anti-inflammatory effects, including the ability to inhibit M1 polarization in microglia (Garrido-Mesa et al., 2013a, 2013b; Möller et al., 2016; Scott et al., 2018). It’s important to note that minocycline does not exclusively inhibit microglia – it has broad immunomodulatory impacts on neurons and other glia and in the peripheral immune system as well (Möller et al., 2016). Although minocycline has been found to be neuroprotective in many situations, there is limited direct evidence of its ability to induce a trophic phenotype in microglia. Nevertheless, its extensive use in numerous studies makes it worthy of examination as a potential regulator of microglial BDNF.

Minocycline has been used as a neuroprotective agent in many animal models of disease and its degree of success seems to vary across models. In a model of amyotrophic lateral sclerosis (ALS) using transgenic SOD1G93A mice, minocycline suppressed expression of inflammatory cytokines, and classic M1 activation markers, but did not alter expression of M2 markers (Kobayashi et al., 2013). Importantly, the authors found that minocycline administration before but not after disease onset prolonged survival of mice. Unfortunately, these results did not translate into the clinic, as in humans, minocycline administration, used in a phase III clinical trial, surprisingly actually accelerated deterioration of patients with ALS (Gordon et al., 2007). In a model of intracerebral hemorrhage in rats, administration of minocycline attenuated neuronal loss and behavioural deficits induced by activated microglia (Miao et al., 2018). In fact, Miao et al. (2018) reported that minocycline induced an M2-like polarization and this effect was linked to increased TrkB and BDNF mRNA expression, and was reversed by application of the small-molecule TrkB inhibitor, ANA-12. In the spinal cord however, minocycline appears to attenuate BDNF release from directionality of BDNF expression from whole tissue lysates.

Poly (I:C) is commonly used in studies of maternal immune activation and microglial priming resulting in behavioural consequences that extend into adulthood, including impaired BDNF expression in the hippocampus (Giovanoli et al., 2015; Talukdar et al., 2020), and PFC (Han et al., 2016). Further, hippocampal BDNF expression deficits were linked to robust cognitive impairments that persisted despite a lack of ongoing neuroinflammatory response (Giovanoli et al., 2015). Conversely, one study found that gestational administration of poly (I:C) to rat dams actually resulted in an upregulation of BDNF mRNA transcripts in the PFC of offspring at P60 (Hemmerle et al., 2015). These conflicting and varied results highlight the heterogeneity and complexity of differential responses to inflammatory mediators in different regions of the brain.

Many of the developmental effects of poly (I:C) on neurotrophic processes might be related to time-dependent epigenetic variations on the BDNF gene. When administered to P7 rats, Poly (I:C) upregulated BDNF production in the short term in both the hippocampus and PFC, but ultimately resulted in a reduction in BDNF mRNA relative to controls at 12 weeks of age (Baghel et al., 2018), with this change tied to alterations gene methylation (Baghel et al., 2019). Finally, in addition to developmental consequences, poly (I:C) injection in adult rodents similarly impairs cognitive performance and reduces BDNF levels within the PFC and hippocampus (Gibney et al., 2013; Kranjac et al., 2012) with reductions linked to anxious and depressive behaviors (notably anhedonia) (Gibney et al., 2013). As is often the case in many BDNF studies, the exact cellular source of the trophic factor was not determined in these reports. Nevertheless, given the robust time-dependent microglial phenotypic variations observed that correlate with BDNF expression levels, it is plausible that poly (I:C) is impacting BDNF release from microglia. Indeed, further work looking specifically at the influence of TLR3 activation on microglial trophic responses is warranted, as the activation of TLR3 on astrocytes has been found to induce the release of neuroprotective factors (Bisbi et al., 2006).
microglia in models of pain and hyperalgesia, but only if applied in the early stages (Wang et al., 2012; Zhang et al., 2012; Zhou et al., 2011).

In vitro studies using cortical microglia subjected to hypoxic conditions revealed that minocycline suppressed microglial activation and the release of pro-inflammatory cytokines, but did not alter BDNF protein levels (Lai and Todd, 2006). In contrast, another in vitro study on N2a cells (a neuroblastoma cell line) reported that minocycline induced an upregulation of BDNF protein but with no change at the mRNA level, suggesting that minocycline may be operating on a translational regulatory mechanism in neurons that is not present in microglia (Lu et al., 2018).

How minocycline exerts its effects on microglia is not completely understood, but it likely involves the modulation of a plethora of inflammatory factors, including prostaglandins and leukotrienes (Silva Bastos et al., 2011) and TLR2 (Hu et al., 2014; Mishra et al., 2020; Park et al., 2011). Minocycline inhibits NF-κB, p38MAPK and MAPK-extracellular signal-regulated kinase 1/2 (ERK1/2) (Garrido-Mesa et al., 2013a, 2013b), - some of which are also involved in the upregulation of CREB and subsequent transcription of BDNF (Fig. 2). Minocycline also suppresses the proteolytic activity of MMP9, which is important in cleavage and conversion of proBDNF into mature BDNF (Huntley, 2012; Yoo et al., 2016). Thus, there are multiple routes through which minocycline might actually inhibit BDNF production, and this might explain why so many studies have shown mixed results with respect to enhancing microglial BDNF.

Despite its well-demonstrated anti-inflammatory effects, direct and compelling evidence of minocycline positively upregulating BDNF in microglia is lacking. However, in light of its anti-inflammatory properties, the possibility has been raised that minocycline could be suitable either as a primary or adjunctive therapy for a variety of neurological or possibly in neuropsychiatric conditions (Husain et al., 2020; Rosenblat and McIntyre, 2018; Zazula et al., 2020). For instance, a meta-analysis of three clinical studies using minocycline found a large antidepressant effect, however small sample sizes and heterogeneity of study designs warrants more extensive trials (Rosenblat and McIntyre, 2018). In a more recent analysis of two randomized clinical trials assessing the use of minocycline as a potential adjunctive therapy for MDD, the authors reported statistically significant reductions in depressive and anxiety symptoms in pooled data, however the authors cautioned that more rigorous studies with a greater number of patients should be conducted to confirm these findings (Zazula et al., 2020). As well, caution should be exercised in clinical use, as application of minocycline following traumatic brain injury reduced signs of microglial activation but increased markers of neurodegeneration (Scott et al., 2018). Maternal and early neonatal administration in mice resulted in microglial activation and highly elevated cell death in offspring, suggesting that minocycline may also interfere with brain development (Strahan et al., 2017). Ultimately, the impact of minocycline upon microglia can vary by brain region, and this could be related to the fact that microglia themselves display regional differences in density, gene expression and inflammatory reactivity (Grabert et al., 2016; Lai et al., 2012). These differences are especially pronounced in comparisons with microglia from the spinal cord, which appear to have marked differences in inflammatory gene expression and response to injury, and distinct profiles for gray vs white matter microglia (for a review see (Xuan et al., 2019)).

3.5. Other modulators of microglial BDNF

Drugs of abuse have also been linked to microglial activation with concomitant influences on BDNF production. Cocaine administration, for example, is strongly associated with upregulation of BDNF in the nucleus accumbens (Anderson et al., 2017; Li et al., 2015). However, like LPS, cocaine also activates TLR4 on microglia (Brown et al., 2018; Kashima and Grueter, 2017), suggesting a possible role for microglia inflammatory and/or trophic effects in the development of addiction. Indeed, in a model of addiction in rats, cocaine self-administration induced microglial hypertrophy in the prefrontal cortex and nucleus accumbens, and this was accompanied by reduced expression of mature BDNF, and an upregulation of pro-BDNF in microglia (Cotto et al., 2018). Taken at face value, these results might imply that extended exposure to cocaine reduces BDNF, however, in vitro, Ehinger et al. (2020) noted a sharp increase in BDNF in culture media 48 h after exposure to cocaine. This increase was prevented by inhibiting phosphorylation the BDNF transcriptional regulator, Methyl CpG binding protein 2 (MeCP2), raising the possibility that cocaine might be inducing the rapid upregulation and release of microglial BDNF into the synapse.

Finally, there is data suggesting that certain cytokines can directly promote growth factor expression in microglia. The interleukins, IL-4 and IL-10, are anti-inflammatory and have also been found to alternatively activate microglia to an “M2a” sub-state increasing expression of Arg1 and CD206 (Franco and Fernández-Suárez, 2015; Gadasi et al., 2012; Ghosh et al., 2016). However, only recently was the impact of IL-4 on microglial BDNF examined in the context of chronic stress. Over-expression of IL-4 in the hippocampus (via adeno-associated virus), enhanced neurogenesis and attenuated behavioural deficits in mice exposed to chronic mild stress, and these effects were mediated by increased microglial production of BDNF as determined by BDNF-RNAScope and immunohistochemistry (Zhang et al., 2021). Importantly, these results required the presence of IL-4Rα receptors on microglia, indicating the direct impact of the cytokine on these cells.

4. How does psychological stress influence microglial BDNF?

As previously discussed, cellular stress or distress can arise from damage to a cell or dysregulation of energetic and homeostatic mechanisms. Yet, even psychological stress, when sufficiently severe or prolonged can provoke DAMP responses by impairing cellular functioning (Franklin et al., 2018) and inducing phenotypic states that are core features of sterile inflammation (Churchward et al., 2019; Fleshner, 2013). Most of the work examining the role of psychological stress in microglia has focused on inflammatory responses - where stress has been documented to mobilize the release of DAMPs such as adenosine triphosphate (ATP), heat-shock protein 72 (Hsp72) (Benison et al., 2014; Maslanik et al., 2013), uric acid crystals (Maslanik et al., 2013), and high-mobility group box 1 (HMGB1) (Frank et al., 2015). Pro-inflammatory activation of microglia and glucocorticoid-induced proliferation has been well documented in the hippocampus, nucleus accumbens and PFC in numerous studies involving psychological stressors in rodents, indicating that stress does, likely activate microglia (Calcia et al., 2016; Nair and Bonneau, 2006), but what are the mechanisms underlying its influence on microglial BDNF production?

In terms of DAMPs, several studies have found that HMGB1 activates microglia through the receptor for advanced glycation end products (RAGE) receptors (Franklin et al., 2018; Okuma et al., 2012; Weber et al., 2015; Zhang et al., 2020a) with stress-induced behavioural deficits ameliorated in RAGE-knockout mice (Franklin et al., 2018). Importantly, Tian et al. (2017) reported that RAGE activation downregulates BDNF production in microglia. The mechanism behind this loss of BDNF is likely due to overactivation of NF-κB, which has been demonstrated to suppress CREB binding to DNA promoter region of the BDNF gene (Zou and Crews, 2006). Similarly, stressors have been shown to elevate synaptic glutamate release both from microglia and neurons in the hippocampus and prefrontal cortex (McEwen et al., 2012), and excessive activation of ionotropic glutamate receptors on microglia is associated with enhanced inflammatory cytokine release including IL-1β, through NF-κB (Murugan et al., 2013). While not directly measured, it is plausible that any stimulus that provokes an imbalance between NF-κB and CREB can lead to the downregulation of BDNF in microglia.

Psychological stressors can of course stimulate the hypothalamo-pituitary-adrenal (HPA) axis leading to glucocorticoid (GC) release. Many studies have reported that GCs are mostly anti-inflammatory and
such actions can be largely attributed to their influence over transcriptional alterations in leukocytes (Coutinho and Chapman, 2011). However, GCs also have the capacity to induce inflammatory responses through interactions with TLR2 and enhanced induction of NLRP3 and P2Y receptors, the latter receptor being tied to microglial activation by way of ATP (Busillo and Cidlowski, 2013; Sorrells et al., 2009). Activation of GC receptors on microglia can also downregulate expression of CD200 receptors, which are important in suppressing inflammatory responses (Frank et al., 2018). Indeed, in their review, Busillo and Cidlowski (2013) highlight that glucocorticoid pleiotropy is complex with initial inflammatory responses tied to both GC levels and co-activation and/or crosstalk with other mediators. In this context, dose-dependent responses have been observed wherein low doses of GCs stimulated the release pro-inflammatory cytokines, but high doses suppressed such responses (Lim et al., 2007). GCs can also serve to prime microglia, as indicated by Frank et al. (2014), such that a later ex vivo challenge with LPS, produced an exaggerated expression of key pro-inflammatory molecules, NLRP3, TNFα, IL-1β, and IL-6. Similarly, LPS injected into the hippocampi of young adult rats activated microglia only if the rats were also subjected to nine days of chronic stress (Espinoza-Oliva et al., 2011). The combined deleterious effects of LPS and stress were counteracted by the glucocorticoid inhibitor RU486, implying that transactivation of glucocorticoid receptors on microglia in conjunction with an external inflammatory insult may result in a hyperinflammatory state (Espinoza-Oliva et al., 2011).

Although local brain pathology or the release of danger signals can activate microglia, some attention should be devoted to the potential impact of peripheral factors in the context of stressor exposure. For instance, various stressors have been reported to augment adhesion molecules which facilitate infiltration of immune cells into the brain, and inhibition of their migration blunted microglial priming (McKim et al., 2018; Weber et al., 2017; Wohleb et al., 2013). Similarly, others reported that social stress mobilized peripheral immune cell migration into the CNS, mediated by glucocorticoid and β-adrenergic signaling (Ataka et al., 2013; Niraula et al., 2018). There has even been a report of a unique phenotypic type of microglia that may arise from infiltrating bone marrow-derived macrophages, and is only present during non-homeostatic states, such as chronic stress, aging and degenerative diseases. Dubbed “dark microglia”, this phenotype is unique in its morphology with ultra-thin ramified processes that appear to be tightly integrated into synapses and heavily involved in synaptic remodeling (Bisht et al., 2016).

Stress can also exacerbate neurological disorders including cerebral stroke, PD and Alzheimer’s disease (Ullic and Block, 2010; Piirainen et al., 2017; Vyas et al., 2016). In a rat model of cerebral ischemia, stress exposure prior to the ischemic insult influenced the expression of trophic factors and cytokines in both the inflammatory (M1) and anti-inflammatory (M2) states. Specifically, seven days of social defeat stress in male rats stimulated microglia such that later ischemic insult resulted in enhanced M1 inflammatory markers and blunted expression of M2 and trophic factors, including BDNF (Espinoza-Garcia et al., 2017). In another stroke model that assessed depressive behaviors, rats subjected to middle cerebral artery occlusion followed by 8 weeks of chronic unpredictable mild stress resulted in reduced microglial BDNF in the amygdala which was correlated with a depressive-like behavioural phenotype (Zhu et al., 2020). Importantly, the authors saw no reduction in BDNF co-localized in neurons of the amygdala. Further, intracerebral injection of the mono-amino acid glutaminase activator which cleaves proBDNF into mature BDNF reduced depressive-like symptoms and increased expression of mature BDNF in microglia (Zhu et al., 2020).

Stress-mediated microglial dysfunction has also been implicated in the pathology of Alzheimer’s disease, particularly because microglia are essential to the elimination of amyloid-beta rich senile plaques (Bisht et al., 2018).

Only a few studies have teased apart the neuronal vs microglial contribution to BDNF modulation in chronic stress. Consequently, given the paucity of data available one may only make a general statement, that the duration and nature of the stressor in conjunction with brain location, jointly determine if BDNF is upregulated or downregulated. Though not specific to microglial BDNF, there is a wealth of data identifying stress-induced regional changes in BDNF expression (Duman and Monteggia, 2006), and it is tempting to speculate that microglia play a role in those changes. For example, chronic restraint or unpredictable stress ultimately downregulated BDNF production in the PFC and hippocampus (Bath et al., 2013; Fanous et al., 2010), yet chronic social defeat stress increased BDNF in the nucleus accumbens (Wook Koo et al., 2016), and chronic foot-shock stress, increased BDNF expression in the amygdala (Bath et al., 2013). Accordingly, regional variations in microglial BDNF expression may follow a similar pattern and be influenced by factors such as the specific decline of microglia in the hippocampus during stress (Kreisel et al., 2013), epigenetic differences in GC receptor expression and activity (Farrell and O’Keane, 2016; Oakley and Cidlowski, 2011) and differences in monoamine transmission (i.e. dopaminergic vs glutamatergic). Lastly, there may also be regional differences in BDNF processing, possibly through epigenetic modifications to protease or BDNF transcription (Miao et al., 2020).

At best we can speculate that overactivation of inflammatory pathways and factors such as NF-κB can lead to suppression of BDNF transcription (Hui et al., 2018) and a general decline in microglial BDNF production. A better understanding of how stress specifically influences microglial BDNF production at a regional level, may provide insights into microglial treatment targets. This could be especially important for the possible role or microglia in the early programming of brain substrates or as regulators of adult neuroplasticity in the face of stressors.

5. BDNF, microglia and depression

Although microglia and BDNF have been implicated in many stressor-related CNS disorders, it is beyond the scope of this paper to go over all of these; yet, some brief discussion of their role in the most ubiquitous stress disorder, clinical depression, seems warranted. It is well-established that enhancing BDNF is fundamental to effective treatment responses (Bjorkholm and Monteggia, 2015; Hayley and Litteljohn, 2013; Zhou et al., 2017). Indeed, the efficacy of antidepressants to remediate the characteristic symptoms of depression may be linked to their ability to modulate key aspects of neuroplasticity that are aligned with BDNF functions (Pittenger and Duman, 2008). Virtually all effective antidepressants modulate BDNF levels or signaling via neuronal TrkB (Castrén and Kojima, 2017; Chen et al., 2001; Lepack et al., 2014; Rantamäki, 2019; Saarelainen et al., 2003). The most notable example being the relatively rapid antidepressant effects of ketamine being linked to BDNF (Browne and Lucki, 2013). Similarly, the delayed onset of selective serotonin reuptake inhibitor (SSRI) drug efficacy also correlates well to BDNF signalling and changes in neuroplasticity (Hamon and Blier, 2013). Yet, most studies have focused on neuronal sources of BDNF and few have addressed the possible role for microglia as contributors to BDNF-related processes that might be important for major depression.

It should also be mentioned that the effects of BDNF are region-specific. The majority of studies presented here have focused on hippocampal and frontal cortical regions, wherein BDNF generally appears to have beneficial effects with regards to depression. In contrast, within the ventral tegmental area (VTA) and nucleus accumbens, BDNF might have the opposite effect, and actually promote depressive features. For example, injections of BDNF into the VTA-nucleus accumbens pathway reduced time to immobility in the forced swim test in rats, while enhanced TrkB.T1 expression on neurons in the nucleus accumbens (which acts as a negative regulator of BDNF/TrkB.FL signaling) produced a robust antidepressant-like response (Eisch et al., 2003). Further, in contrast to reductions in the cortex and hippocampus, social defeat stress in induced a protracted elevation of VTA BDNF expression and interestingly, this enhanced subsequent psychostimulant impact (Wang et al., 2000).
have been reported in a post-mortem analysis of several brain regions from Deutch, 2018). Similarly, a unique microglial phenotype has recently strongly suggests that modulating microglia plays an important role in brain and are also observed in suicides in schizophrenic patients (Mallya and brains of schizophrenic suicide completers (Steiner et al., 2008), and is implicated in the aberrant pruning in schizophrenic brains (Mallya and Deutch, 2018). Similarly, a unique microglial phenotype has recently been reported in a post-mortem analysis of several brain regions from individuals with clinical depression (MDD) (Göttcher et al., 2020).

Substantial evidence has implicated BDNF and microglia, at least separately, in the pathogenesis of depression (Jin et al., 2019). In the case of microglio, enhanced microgliosis was observed in the prefrontal and anterior cingulate cortex, along with the hippocampus of depressed individuals that died by suicide (Suzuki et al., 2019). Similarly, brain-region and sex-dependent reductions in BDNF have been reported in the depressed brain of individuals that died by suicide (Hayley et al., 2015; Pandey et al., 2008). Of course, enhanced inflammatory microglia and BDNF variations are not limited to the depressed brain and are also observed in suicides in schizophrenic patients (Reinhart et al., 2015). It might even be that suicidal ideation, or the act itself, is linked to specific biological signatures, conceivably involving disturbances of BDNF, that could trigger ruminate thoughts, ultimately fueling impulsive self-harming.

Stressor based animal models of depression (e.g. social defeat and chronic unpredictable mild stress) have also mostly provided evidence of enhanced pro-inflammatory microglial and cytokine activity, while BDNF is generally reduced in these models, and this often occurs in the presence of other signs of diminished neuroplasticity, such as neurogenesis (Clarke et al., 2016; Jha et al., 2011; Mirescu and Gould, 2006; Rudyk et al., 2019). Furthermore, direct administration of pro-inflammatory agents, such as LPS or poly (I:C), promoted depressive behaviors in the context of microglial activation and concomitantly, hippocampal and cortical BDNF and TrkB were reduced by these inflammatory agents (Gibney et al., 2013; Réus et al., 2015).

There is accumulating evidence that successful antidepressant treatment requires the resolution of inflammatory processes and the promotion of a positive trophic state. In fact, all useful antidepressant regimens promote trophic responses that to some degree include the modulation of adult hippocampal neurogenesis and synaptic activity (Castren and Rantamäki, 2010). These therapeutic agents often possess anti-inflammatory properties, which in turn, can impact trophic states. The commonly used antidepressant SSRIs, fluoxetine, and the cholesterol lowering statin, atorvastatin, both prevented the LPS-induced behavioral effects in the forced swim and tail suspension tests, along with ameliorating the reduction of hippocampal and cortical BDNF (Tangutti et al., 2019). In other instances, the tricyclic antidepressant, imipramine, reduced proinflammatory cytokine release, while elevating central BDNF expression (Réus et al., 2013). Even the more recently used rapid acting NMDA-based antidepressant, ketamine, inhibited post-surgical depression in rats by limiting pro-inflammatory cytokines and elevating brain BDNF levels (Yang et al., 2020). While these studies did not measure microglial contributions to BDNF levels, evidence strongly suggests that modulating microglia plays an important role in elevating BDNF in key mood-regulating regions.

Recent studies have further implicated BDNF in antidepressant efficacy by demonstrating that several SSRIs (and ketamine) can actually directly transactivate TrkB receptors, invoking antidepressant responses (Casarotto et al., 2021; Rantamäki, 2019; Rantamäki et al., 2011). This effect was not limited to neurons since one study using primary neuron and glial cultures found that the antidepressants clomipramine, duloxetine and fluvoxamine upregulated BDNF expression in microglia and astrocytes, but not neurons (Hisaoka-Nakashima et al., 2016). Further to this work, Kinoshita et al. (2018), demonstrated that the antidepressant fluoxetine enhances ATP efflux from astrocytes, and as we have previously discussed, both ATP and its metabolite, adenosine, have been shown to induce BDNF release from microglia (Trang et al., 2011, 2009; Ulmann et al., 2008). Recent work in HM3C cultures (a human microglial cell line) found that both ketamine and its key metabolites upregulated BDNF production via modulation of translational regulators (Ho et al., 2019). Combined, these studies suggest that glia may play a more prominent role in BDNF-mediated antidepressant responses than previously considered.

It remains to be determined whether chronically aberrant neuroplasticity and neuroinflammation might be mechanistically linked together to give rise to elements of the depressive state. One process may serially induce the other, or alternatively, it could be that the inflammation and trophic dysregulation act in parallel to produce differing depressive symptoms. Essentially, on the one hand, microglial-driven neuroinflammation could easily induce the malaise, lethargy, low motivation, feeding and sleep disturbances often evident in depression. Yet, on the other hand, reduced BDNF trophic support could diminish cortical and sub-cortical limbic circuitry that is critical for dealing with stressors and may give rise to melancholic or anhedonic features of the illness. The alternative possibility is that the combined dysregulation of inflammatory and trophic processes could act in a synergistic manner to augment the overall depressive state. Thus, it could be envisioned that reducing microglial inflammatory responses and enhancing BDNF expression might play an important role in tipping the cellular phenotype towards one that ameliorates both types of depressive symptoms.

6. Do microglia express TrkB?

Microglia have been demonstrated to release BDNF (Gupta et al., 2020; He et al., 2020; Pöyhönen et al., 2019; Ulmann et al., 2008), but do they express its cognate receptor, TrkB? This is still a controversial question. Given that BDNF is released by neurons and astrocytes, and both these cell types express TrkB receptors (Caravagna et al., 2013; Harward et al., 2016; Wei et al., 2010), it is plausible that in producing BDNF, microglia would also express TrkB receptors. Indeed, microglia have been reported to express other receptors and ligands in the NTRK family (Elkabes et al., 1998). Unfortunately, there is a dearth of research in this area and it remains controversial as to whether full-length TrkB or its truncated isoform, TrkB.T1, are present on microglia. Early work using in situ hybridization on cultured rat microglia did not detect any TrkB mRNA (Frisen et al., 1993). Similarly, in a study analyzing neurotrophin receptor expression across multiple glial cell types from whole brain rat cultures, the authors reported low levels of TrkB mRNA expression in microglia, but acknowledged that the results were inconclusive due to contamination by astrocytes (Condorelli et al., 1995).

Despite the advent of new sequencing technologies, such as single cell RNAseq, few genomics studies have identified expression of the TrkB gene, NTRK2, in microglia, raising the possibility that expression might be transient or simply not evident at all. For example, in an extensive study of mouse microglial transcriptomics, expression of NTRK2 was only detected at early developmental times of E14 and P60 (Bennett et al., 2016). But it was also notable that when examining the database for this study (published at brainrnaseq.org), one can see that mRNA for BDNF in microglia was only detected at P60. Further, recent single cell genomics assays in the Allen Brain Institute transcriptomics explorer (Yao et al., 2020) showed no murine or human expression of NTRK2 on microglia. By the same token, these assays also failed to show any expression of BDNF in microglia, which is contrary to many published studies (Gomes et al., 2013; Mohammadi et al., 2020; Parkhurst et al., 2013). This raises the possibility that perhaps activation state and a host
of other factors are crucial in stimulating the expression of BDNF and TrkB proteins in microglia. We are in fact learning that the heterogeneity of gene expression seen in microglia can be influenced by a multitude of factors including age, sex, region of the brain, priming, and of course, current inflammatory influences (Grabert et al., 2016). Given their functional role as both custodians of synaptic development and guardians of the brain, microglia have perhaps some of the most dynamic gene expression of all cell types. By necessity, they must be able to rapidly alter their transcriptional and translational responses on very short timescales. Indeed, it is perhaps precisely this kind of dynamism that is one of the sources of conflicting results in transcriptomic studies.

If TrkB is expressed on microglia, it is plausible that autocrine activation via microglial BDNF could alter microglial function and regulate its subsequent production. Though not directly measuring BDNF, the application of exogenous BDNF to N9 microglia cells significantly increased proliferation, implying that some form of BDNF/TrkB signaling took place (Gomes et al., 2013). In contrast, Trang et al. (2009) used the BDNF sequestration fusion protein TrkB-Fc on primary rat microglia cultures prior to stimulation with ATP, and found no indication that BDNF expression was regulated by autocrine feedback. These results imply the possibility of a lack of full-length TrkB receptor signaling on microglia. Similarly, Holt et al. (2019) found nominal full-length TrkB expression on murine microglia in culture through qPCR, and only slightly higher, but still extremely low levels of the truncated TrkB.T1 isoform. However, these microglia were not challenged with a BDNF-inducing activator such as ATP, and the sex of the glia in culture was not identified. This distinction is especially important in light of work showing that both sex and microglial activation state are critical mediators of BDNF protein expression (Liddelow et al., 2017).

In contrast to studies that reported no or negligible TrkB expression, emerging data are consistent with the receptor being present on microglia, albeit likely at very low levels in the absence of a significant challenge. For instance, Spencer-Segal et al. (2011) did find evidence of phosphorylated full-length TrkB (pTrkB) on microglia in hippocampal subregions. More recently it was also shown that the toxicant, kainic acid, induced the presence of phosphorylated TrkB specifically within microglia present in the CA3 region of the hippocampus (Onodera et al., 2021). Using double-labeling immunofluorescence, Matyas et al. (2017) reported that the truncated TrkB receptor, TrkB.T1, was found in mouse microglia within the spinal cord following local injury. Along these same lines, BDNF has been reported to bind to TrkB.T1 in cultured mouse microglial cells and elevate intracellular Ca2+ levels ( Mizoguchi et al., 2009), Studies probing BV2 microglia in vitro also reported expression of both TrkB and BDNF via western blot (Ye et al., 2017; Zhou et al., 2020b). In another study, Mizoguchi et al. (2014) determined that transient receptor potential 3 (TRPC3) channels on microglia were also upregulated by the exogenous application of BDNF, implying the possible presence of TrkB. Similarly, knocking down neuronal BDNF in the mossy fibers of hippocampal CA3, reduced phosphorylated pan-Trk receptors and enhanced proliferation and phagocytosis of local microglia (Onodera et al., 2021). In this same study, the authors used slice cultures from a CX3CR1GFP/+ mouse line to study the impact of BDNF on microglial dynamics. Using time-lapsed recordings, inhibition of BDNF signaling, via K252a or an anti-BDNF antibody, resulted in significant enhancement of microglial motility and process extension (Onodera et al., 2021). Further support for TrkB expression on microglia comes from a recent study where the authors reported that pre-treatment with BDNF prior to LPS application improved survivability and inhibited activation of microglial cells (Wu et al., 2020). Importantly, the authors identified both TrkB.FL and TrkB.T1 expression, via western blot, in these primary cell cultures.

These findings are corroborated in a model of spinal cord injury in rats, where the authors found TrkB significantly upregulated seven days post-injury and that application of BDNF upregulated phosphorylation of p38MAPK in spinal microglia (Zhou et al., 2011). Similarly, mouse macrophages derived from the peritoneal cavity displayed TrkB expression that was up-regulated by LPS and correlated with phagocytic activity of these cells (Asami et al., 2006), but once again, although these cells bear a striking resemblance to microglia, there are many differences as well. Of course, the possibility exists that spinal cord microglia and macrophages might express more TrkB than other discrete areas in the CNS. Whatever the case, any technique has its limitations, and immunohistochemistry suffers from challenges with antibody specificity, which make quantifying TrkB expression through these techniques difficult and fraught with problems of potential cross-reactivity.

Ultimately, the expression of TrkB on microglia is still an open question deserving of further investigation, but even in the possible absence of the receptor these cells still appear able produce and physiologically respond to BDNF. It is also conceivable that microglial might respond to BDNF through TrkB-independent mechanisms, possibly involving endocytic uptake or similar mechanisms. BDNF is in fact a “sticky” molecule that one could imagine interact with various glycoproteins associated with the microglial membrane. The trophic factor might even enter microglia and spread via membrane bound vesicles, such as exosomes. There is, in fact, some intriguing evidence that macrophage or microglial derived exosomes are able to take up BDNF (Yuan et al., 2017), and that such exosomes can facilitate cell to cell transmission of various proteins (Paolicelli et al., 2019). It is however still speculative as to the existence or physiological relevance of such alternative microglial BDNF signaling routes, and little work has been done to elucidate potential BDNF signaling pathways within microglia.

7. Sex differences in microglial BDNF

Microglia begin to show sex-differentiated characteristics early in development, including differential expression of inflammatory genes and cytokines in the preoptic area, limbic system and cortex (for a review see Lenz and McCarthy, 2015). Microglial transcriptomes at age P60 from female mice were found to be more developmentally advanced, however, male, microglia matured more rapidly in response to immune challenges (Hanamsagar et al., 2017). Guneykaya et al. (2018) also reported sex differences in the microglia of mice at 13 weeks, reporting that male microglia are more plentiful, and that their somas are larger and expressed more MHC molecules in the cortex and hippocampus than their female counterparts. In addition, males had significantly increased translation of purinergic receptors and TLR pathway proteins, perhaps giving males an advantage in responding to pathogens (Guneykaya et al., 2018). In contrast, others found few sexual differences in microglia at three developmental timepoints; E14.5, P4/5 & P100, with the only major difference being a very small cluster of genes enriched in females at P4/5 (Hammond et al., 2019). While highly cited, one should note that the Hammond et al. (2019) study examined only 4 females per time point and combined with the nature of single-cell sequencing, this may have impacted the statistical power of the results. Lastly, sex-dependent effects can also be influenced by the developing microbiome and have an enduring impact on immune response genes that persist into adulthood (Thion et al., 2018).

During adulthood there might be further sex-dependent differences in microglia. For instance, in the preoptic area of the mouse hypothalamus, a very elegant study by Lenz et al. (2013) found sex differences in the size and number of microglia. The authors also found that masculinization of the brain and later adult behaviour was mediated through prostaglandin PGE2 receptors on microglia. Remarkably, this was dependent on microglia being in a dual activation phenotype at key developmental stages: displaying both classical M1 activation markers, IL-1β and TNF-α, as well as expression of markers associated with an M2 state, arginase-1 and CD206 (Lenz et al., 2013). It was also reported that the impact of sex upon microglia appears to be brain region specific, with the density and morphology of microglia varying as a function of sex in the cortex, amygdala, and hippocampus (Han et al., 2021). In fact, male microglia responded with a more vigorous IL-1β response to LPS.
than females, but with advancing age female microglia selectively upregulated M1 markers to a greater extent than males (Han et al., 2021), suggesting sex-dependent differences in reactivity to an inflammatory stimulus, as well as maturational immune status.

Besides inflammatory markers, microglial expression of BDNF might also be altered in a sex specific manner. For example, Liu et al. (2019) found that four weeks of chronic unpredictable mild stress resulted in sex-dependent activation of hippocampal microglia and inflammatory marker changes. These variations were correlated with a pronounced downregulation of BDNF-neuronal TrkB signaling and behavioural changes specifically in females (Liu et al., 2019). In one study that used a partial BDNF knockout, the trophic factor was found to play a role in mitigating inflammatory cytokine release from microglia only in females (Rossetti et al., 2019). In another study, adult male and female mice were irradiated with high-energy particle beams to simulate radiative exposure in space travel. While not specific to microglial BDNF, the results revealed that only females expressed increased levels of the microglial inflammatory markers, CD11b and CD68, and only males displayed a reduction of cortical BDNF (Raber et al., 2019). These results are consistent with other findings that adult female microglia are, in certain situations, more reactive in the face of immune challenges (Doyle et al., 2017).

Perhaps the most prominent work in sex differences in microglial BDNF has been in the field of pain research. As previously discussed, ATP-induced release of BDNF from microglia in the spine signals certain situations, more reactive in the face of immune challenges (Beggs and Salter, 2013; Trang et al., 2011). Within this context, intriguing sex differences were reported in work by Sorge et al. (2015), such that while both male and female mice develop pain sensitivity, specifically mechanical allodynia, the microglial inhibitor, minocycline, reversed this effects only in males, and not in female mice. Similarly, they found that inhibiting p38MAPK and BDNF in microglia reversed the allodynia only in male mice. Along these same lines, following peripheral nerve injury or ischemic stroke, the microglia from male mice expressed significantly more P2X4R than that of females (Mapplebeck et al., 2016; Nguyen et al., 2020). Recent work by Paige et al. (2018), also found sexually dimorphic responses to hyperalgesia pain priming in mice, with inhibition of P2X4R receptors blocking pain priming only in male mice.

While the importance of microglial BDNF is still in the early phases of study and may be tied to specific pain modalities, the fact that P2X4R signalling pathways preferentially mediate pain hypersensitivity in a sexually dimorphic manner seems fairly clear (for a review see Halievski et al., 2020). Beyond the study of chronic pain, sexually dimorphic microglial differences in reactivity and BDNF expression could be linked to clinical variations in psychiatric illness, such as the higher incidence of depression in females.

Although the exact mechanisms responsible for sex differences in microglia and BDNF are not apparent, there certainly is reason to believe the estrogen signalling might be important. This is in keeping with the fact that BDNF and estrogen often appear to have common actions in the regulation of CNS systems (Sohrabji and Lewis, 2006). It is beyond the scope of this review to detail the many studies that have implicated estrogen effects. For example, estradiol blunted the LPS provoked cytokine response from microglia in a sex-dependent manner (Loram et al., 2012), and heightened microglial reactivity in the hypothalamus (Velez-Perez et al., 2020), with evidence that phosphorylation of estrogen receptor alpha is key to the anti-inflammatory response (Shindo et al., 2020). In terms of neurotrophic responses, in vitro work has revealed that the addition of estradiol to cultured pre-optic neurons promoted increased spine density, but most importantly, this trophic response depended upon the presence of microglia in the culture (Lenz et al., 2013). Invivo estrogen replacement in ovariectomized female rats demonstrated elevated expression of BDNF in numerous brain regions including the cortex, hippocampus, amygdala and olfactory bulb (Sohrabji and Lewis, 2006). It has even been reported that estrogen-ligand complexes may bind to a portion of the BDNF gene that has a sequence similar to that of the estrogen response element (Sohrabji et al., 1995). Accordingly, estrogen and BDNF can both activate MAP kinases, such as ERK1/2, in a similar time frame (Singh et al., 1999), reinforcing the commonality and possible cooperative signalling between these two factors.

8. Towards a unique “trophic” microglial phenotype

We suggest the possibility that in addition to the traditional M2 anti-inflammatory and neuroprotective phenotype, there are other discrete and distinctive state(s) that modulate “protective” or “recovery” processes. Indeed, microglia have been reported to modulate several different aspects of neuroplasticity, including synapse formation and maintenance, as well as the production of neurotrophic factors and even neurogenesis (Díaz-Aparicio et al., 2020; Sierra et al., 2014). When considering a trophic phenotype one most acknowledge that there never may be a “pure” trophic microglial state that exists totally independent of immune properties. It is certainly exceedingly difficult to disentangle inflammatory-immune from trophic actions. Yet, there are accumulating data to support the contention that discrete trophic, homeostatic, and reparative states exist. We suggest that a distinctive “neurotrophic” phenotype that enhances microglial BDNF/neuronal-TrkB signalling may be possible. By extension, such a state could aid in regaining a homeostatic balance and facilitate necessary dendritic and synaptic remodelling in neural circuits that are disturbed in stress-related affective, anxious and developmental disorders (Qiao et al., 2016; Vigers et al., 2012; Zhang et al., 2015a). Indeed, a non-inflammatory and pro-trophic role for microglia has been reported in the context of several animal models of disease, including that of autism and epilepsy (Edmonson et al., 2016; Zhao et al., 2018), wherein neural connectivity appears modulated by microglia.

Microglia that adopt a trophic phenotype may directly contact neuronal dendrites to facilitate synaptic sculpting. At least developmentally, newly formed microglial filopodia appear to play a role in the maturation of neuronal circuit connections (Miyanmo et al., 2016). Indeed, microglia have been shown to actively prune synapses via neuronal complement system signaling in retinal ganglion cells (Schafer et al., 2012) and eliminate dopaminergic receptors in a sex-dependent manner in the developing nucleus accumbens (Kopec et al., 2015). A similar role may be adopted in adulthood to facilitate learning and maintain homeostasis in the face of stressors. Along these lines, one incredibly important study found that selective depletion of BDNF from microglia reduced spine formation both during development and in the context of cognitively demanding tasks in adulthood (Parkhurst et al., 2013). In support of these findings, Lim et al. (2013) reported that adding microglia to cultured hippocampal neurons increased dendritic spine density. In terms of signalling mechanisms, there is evidence for the mTOR pathway in microglial mediated morphology, proliferation, and phagocytosis in the absence of any pro-inflammatory cytokine changes (Zhao et al., 2018). Further evidence was provided by the finding that inhibiting mTOR using its antagonist, rapamycin, blunted phenotypic shifts in microglia in response to both pro- and anti-inflammatory stimuli (Hu et al., 2020) While the relationship between BDNF and mTOR is well established, the coordinated role of these factors in specifically regulating microglial physiology is still evolving.
Lessons learned from studying microglial activation via ATP and LPS suggest that molecules that induce upregulation of cAMP and MAPKs with concomitant phosphorylation and nuclear translocation of the transcription factor CREB appear to be vital for microglial upregulation of BDNF (Gomes et al., 2013; Lai et al., 2012; Miwa et al., 1997; Nakajima et al., 2001; Trang et al., 2009; Ulmann et al., 2008). As it turns out, drugs and targets emerging from the fields of multiple sclerosis (MS), cardiovascular disease, pain and diabetes show promise in this area, including agonists of sphingosine-1-phosphate receptors (S1PRs), peroxisome proliferator-activated receptors (PPARs), Glucagon-like peptide-1 receptors (GLP-1Rs), and cannabinoid type 2 receptors (CB2Rs) (Fig. 3). We posit that these factors might be novel microglia regulators that could shift the cells towards a neurotrophic recovery phenotype.

8.1. Sphingosine-1-phosphate agonists

The phosphorylated lipid, sphingosine-1-phosphate (SIP) can act on five different receptor subtypes, each with varying degrees of presentation on most mammalian cells (Brinkmann, 2007). The SIP analog and receptor modulator, FTY720 (2-amino-2-(4-octylphenyl)ethyl)-1, 3-propanediol hydrochloride, aka fingolimod), has been extensively used to assess SIP receptor (S1PR) signaling. In particular, FTY720 has marked immunosuppressive consequences and has actually been used as a treatment for relapsing-remitting multiple sclerosis since its FDA approval in 2010 (Chun and Hartung, 2010; Jain and Bhatti, 2012). In the periphery, FTY720 induces the internalization and degradation of S1P₁ receptors, which inhibits lymphocyte trafficking into the CNS, and thus acting as a functional antagonist of S1P₁R (Brinkmann, 2007). Of course, such immunosuppression can promote adverse side-effects including increased risk of viral infection (Brinkmann, 2007; Epstein et al., 2018). Yet, of particular interest is the fact that in addition to abating lymphocyte migration, FTY720 and other SIP analogs also attenuate neuroinflammation and potentially upregulate BDNF in the brain (Cipriani et al., 2015).

Microglia express all five S1PR subtypes, and FTY720 was shown to act on S1P₁R suppressing the inflammatory response, while augmenting local BDNF expression (Noda et al., 2013). In primary mouse microglial cultures, FTY720 downregulated inflammatory cytokine levels in LPS-activated microglia through S1P₁R while upregulating BDNF (Noda et al., 2013). Similarly, FTY720 treatment reversed the pro-inflammatory markers, CD16 and iNOS, induced by LPS, but elevated levels of the anti-inflammatory CD206 and arginase1 and these changes were believed to stem from modulation of STAT1 signalling (Hu et al., 2021). Further, FTY720 has been found to increase BDNF and mitigate many behavioral and functional deficits induced in an MeCP2-knockout mouse model of Rett syndrome (Deogracias et al., 2012). Application of phosphorylated FTY720 (FTY720-P) also enhanced BDNF expression and dendritic spine density in primary hippocampal neuronal cultures (Patnaik et al., 2020).

Subsequent work in BV2 microglia found that activation of S1P₁R upregulates microglial ATP release, which then enhances cell motility and exocytosis (Zahiri et al., 2021). As previously discussed, autocrine binding of the ATP degradation product, ADP, to purinergic receptors is correlated with the activation PI3K/AKT and then CREB (Choi et al., 2014; Ji et al., 2010; Xing et al., 2008) (b) Similarly, PPAR-γ activation is associated with suppression of inflammatory responses via downregulation of NFκB and p38 (Cheng et al., 2014; Choi et al., 2017; Ji et al., 2010; Xing et al., 2008), together with the activation PI3K/AKT and then CREB (Bernardo and Minghetti, 2008; Xing et al., 2006). (c) Gα0-coupled CB2Rs can be activated by endogenous 2-AG or exogenous mimics resulting in the stimulation of AMPK and ERK1/2 pathways; which again, leads to phosphorylation and translocation of CREB (Carrier et al., 2004; Komorowska-Müller and Schmölz, 2020). (d) GLP-1R agonists have been shown to upregulate BDNF via multiple pathways, including PI3K/AKT and p38MAPK, as well as upregulating CAMP and the PKA pathway, all of which stimulate CREB (Spigelman et al., 2017; Wu et al., 2018) leading to upregulation of BDNF and other markers of M2 activation states. (e) Activation of S1P₁R has been shown to cause receptor internalization and degradation, and suppression of histone deacetylase (HDAC), which leads to inhibition of NFκB and downregulation of inflammatory cytokines. Additionally, S1P₁R can promote release of ATP, through RhoA kinase, once again, leading to the activation of CREB and BDNF production (Brunkhorst et al., 2014; Chun and Hartung, 2010; Huwiler and Zangemeister-Wittke, 2018; Noda et al., 2013).

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importantly, the short-chain analog, C8 ceramide, dramatically upregulated BDNF in microglial cultures and this occurred in the absence of any change in inflammatory cytokines or oxidative stress factors (Nakajima et al., 2002). As we have seen with ATP and LPS, inducing BDNF release from microglia often involves the concomitant release of inflammatory cytokines, thus finding a mechanism that does not also induce such inflammatory factors supports the possibility that a unique neutral trophic phenotype can be achieved.

Excitingly, FTY720 has demonstrated antidepressant-like effects in preclinical evaluations in both rat and mouse models of chronic unpredictable stress, and the effects were linked to a combination of enhanced BDNF/TrkB and glial-derived neurotrophic factor (GDNF) signaling, downregulation of inflammatory cytokines, and reduction in markers of oxidative stress (di Nuzzo et al., 2015; Guo et al., 2020). Also, multiple sclerosis patients who switched to oral fingolimod from injectable therapies reported statistically significant improvements in both somatic and affective depression symptoms (Hunter et al., 2016), suggesting potential broad clinical efficacy.

8.2. Peroxisome proliferator-activated receptor agonists

PPARs are part of a family of nuclear hormone receptor protein and transcription factors present in neurons and microglia. There are three primary PPAR isoforms: α, β/δ and γ. When activated, PPARs form a heterodimer with retinoid-X-receptors (RXRs), and this complex then attaches to response elements on DNA to help up-regulate or down-regulate transcription in a cell-dependent fashion (Nanjan et al., 2018). PPAR receptors are distributed throughout the body and natural activators include prostaglandins and fatty acids (Dussault and Forman, 2000; Kliewer et al., 1997). Although the bulk of PPAR expression appears to be neuronal in the human and mouse brain (Warden et al., 2016), microglia also express PPARs, and increasing evidence suggests they play a regulatory role in microglial activation and may be able to induce a trophic phenotype (Bernardo and Minghetti, 2006). All three types of PPARs have been associated with downregulation of NF-κB and suppression of inflammation (Delerive et al., 2001), however PPAR-α and PPAR-γ have shown the most promise in upregulating microglial BDNF.

PPAR-γ has been the most studied isotype to date and is comprised of two main isoforms produced by alternative splicing; the highly abundant PPAR-γ1 is broadly distributed and has been localized to neuronal and microglial cells, with conflicting evidence as to its presence in astrocytes (Warden et al., 2016). In contrast, the lesser-expressed PPAR-γ2 is found mainly in the intestinal tract and adipose tissue (Janani and Ranjitha Kumari, 2015). One class of PPAR-γ agonists, thiazolidinediones (TZD), have proven efficacy in modulating insulin signaling and glucose metabolism (Liu et al., 2018). Indeed, oral pioglitazone and rosiglitazone are approved for use as diabetes treatments in several countries (Schernthaner et al., 2013). Although PPAR-γ is most known for its regulation of glucose metabolism and lipid expression, its activation has also been shown to polarize microglia to a more M2-like state, as well as upregulate their expression of BDNF (Stahel et al., 2008).

While much research has focused on modulation of PPAR-γ in neurons (Moreno et al., 2004), recent work has begun to address its role in regulating microglial activation states that may modulate behavioral deficits. In this regard, using primary mouse microglia, upregulation of PPAR-γ expression by IL-4 was correlated with increased M2 microglial polarization, and upregulated trophic factors, including BDNF (Zheng et al., 2020a, 2020b). In the case of peripherally administered PPAR-γ agonists, a shift of microglia from an inflammatory to neuroprotective phenotype was evident, wherein BDNF was upregulated with attenuated inflammatory cytokine release (Liao et al., 2017). The PPAR-γ agonist pioglitazone, reversed the behavioural deficits in an LPS-induced model of sickness behaviour in rats with corresponding reductions in inflammatory cytokines and reactive oxygen species, and an increase in BDNF (Bebeshti et al., 2019). Pioglitazone also had antidepressant-like behavioral effects in the context of an inflammatory challenge (reversing anhedonia and other features) and these effects were related to a downregulation of inflammatory signalling (NF-κB/IL-6/STAT3) and augmentation of BDNF-CREB levels (Liao et al., 2017). A double blind clinical human study further reported that PPAR-γ agonists can elicit antidepressant effects, as evidenced by reduced Hamilton depression rating scale score and these effects were independent of any effect of the drug on insulin (Kashani et al., 2013).

Much recent evidence has implicated stressor induced PPAR-γ variations in neurological pathology (Calle et al., 2017; Qin et al., 2020; Tufano and Pinna, 2020). One study found neuroprotective effects of PPAR-γ stimulation, using pioglitazone, in a animal model of epilepsy, and most importantly, this effect was dependent upon BDNF signaling through neuronal TrkB (Sun et al., 2013). In another animal model, using surgery-induced cognitive decline, post-operative administration of pioglitazone also shifted microglia to pro-regenerative phenotype, upregulating BDNF and largely rescuing the profound cognitive losses in aged mice (Zhang et al., 2017). Other PPAR-γ agonists have also shown promise in improving cognition, including berberetone, which down-regulated inflammatory cytokine release and enhanced microglial BDNF production in rodents with traumatic head injury (He et al., 2018, 2020). The plant derivative, berberine, is a bioactive alkaloid that is also being explored for its ability to modulate both PPAR-γ and PPAR-α, and has demonstrated ability to upregulate BDNF in a model of diabetic neuropathy in rats (Zhou et al., 2019).

Endogenous lipid ligands such as palmitoylethanolamide (PAA), as well as some types of statins can upregulate PPAR-α and consequently, BDNF in microglia (Grygiel-Gorniak, 2014). Independent of its cholesterol-lowering activity, the statin, simvastatin, binds specifically to leucine and tyrosine residues on PPAR-α and has been found to potently upregulate BDNF and NT-3 in neurons, astrocytes, and microglia (Roy et al., 2015). Simvastatin was also found to upregulate BDNF while reducing IL-1β in LPS-activated microglia in a cholesterol-dependent manner (Churchward and Todd, 2014). From a behavioural perspective, simvastatin treatment improved memory in a mouse model of Alzheimer’s disease. Importantly, it was elegantly demonstrated that the neurotrophic effects of simvastatin were independent of its anti-inflammatory and lipid-lowering activity (Roy et al., 2015). While not specifically targeting microglia, other murine studies have demonstrated that PPAR-α-specific agonists such as fenofibrate, upregulate BDNF and ameliorate depressive phenotypes (Jiang et al., 2017).

It is also of interest that PPAR-γ and PPAR-α can be activated by NSAIDS and polysaturated fatty acids, like docosahexaenoic acid (DHA) (Korbecki et al., 2019; Patel et al., 2026; Robinson et al., 2019). Both of these classes of PPAR inducers have been reported to augment the remission of depressive symptoms when co-administered with antidepressants (Bai et al., 2020; Robinson et al., 2019; Smith et al., 2018).

Not all statins or PPAR agonists induce the same degree of trophic responses (Roy et al., 2015), and they can also impact inflammatory processes owing to varied interactions with the ligand binding domains of both PPAR-γ and PPAR-α (Xu et al., 2001). That being said, PPAR-γ is heavily expressed in macrophages that are drawn to white adipose tissue during metabolic stress (Olefsky and Glass, 2009), and current PPAR agonists of the thiazolidinediones class continue to have risk of serious adverse side-effects related to inflammatory processes (Gale, 2001; Botta et al., 2018; Nanjan et al., 2018). To address these safety issues, new and more targeted agonists for both PPAR-γ and PPAR-α are the focus of intense research in treating diabetes, cardiovascular, Parkinson’s and Alzheimer’s diseases (Burgaz et al., 2019; Justin et al., 2020; Khan et al., 2019; Medrano-Jimenez et al., 2019; Omeragic et al., 2020; Reich et al., 2018; Song et al., 2016), with a particular focus on developing a newer class of selective PPAR-α modulators (Fruchart et al., 2017). In addition to synthetic analogs, many naturally occurring compounds such as terpenes, carotenoids, resveratrol, and flavonoids can activate PPAR-α and PPAR-γ providing opportunities for potential "nutraceutical" therapeutics (Rigano et al., 2017; Wang et al., 2014).
Leveraging the body of established and ongoing work, analyses of these newer compounds, for their ability to induce a neurotrophic phenotype in microglia, may lead to new adjunctive therapies for depressive disorders with enhanced safety profiles.

8.3. Glucagon-like peptide-1 receptor agonists

Largely produced by intestinal enteroendocrine cells, the incretin peptide hormone, GLP-1, is best known for its ability to mediate blood sugar levels, enhance insulin production and promote weight loss. As with PPAR-γ agonists, several GLP-1R agonists have been approved for the treatment of type II diabetes, including exenatide, semaglutide and liraglutide (Chan et al., 2021). Importantly, microglia are significant contributors to GLP-1 expression in the brain (Kappe et al., 2012), and agonists have been shown to both upregulate M2 markers and expression of BDNF (Fig. 3). Indeed, work by Wu et al. (2018) determined that exenatide upregulated M2 microglial markers, including CD206 and Arg1, through activation of CREB and MAP kinase pathways. It is of note, that these are the same pathways tied to BDNF signaling.

In cell culture studies, the application of GLP-1 potently upregulated BDNF release in BV2 microglia (Spelman et al., 2017). Further still, conditioned media, taken from BV2 microglial cells that had been exposed to GLP-1, promoted recovery of damaged N2a neurons in culture and enhanced phosphorylation of CREB, suggesting the presence of trophic factors, like BDNF (Yoon et al., 2020).

In the context of neuropsychiatric disorders, work examining GLP-1R agonism is limited to only a few studies. One such study that used LPS to induce maternal immune activation in rats reported that 45 days of postnatal treatment with exenatide enhanced GABAergic and serotonegenic activity and ameliorated behavioural deficits (Solmaz et al., 2020). Another study using intracerebral injections of GLP-1 in rats found that chronic administration reduced immobility in the forced swim test, suggesting an antidepressant-like effect (Anderberg et al., 2016). In rodents subjected to long-term antidepressant treatment, three weeks of daily injections with the GLP-1 agonist, liraglutide, partially rescued behavioural deficits by enhancing mobility in the forced swim test (Sharma et al., 2015).

In humans there have been two small studies assessing the impacts of GLP-1R agonism in Alzheimer’s disease and schizophrenia, but these found no improvements in cognitive outcomes (Gejl et al., 2016; Ihsay et al., 2017). Another small open-label pilot human study assessed the impact of 4 weeks of daily treatment with the GLP-1R agonist, liraglutide, noting its demonstrated ability to cross the blood-brain barrier (Mansur et al., 2017). The authors reported that liraglutide improved cognitive scores in a small number of patients with MDD or bipolar disorder (Mansur et al., 2017). These and other small trials have spurred interest in using liraglutide to treat psychiatric disorders, however most studies to date have been small, often lacking sufficient randomization and control for confounding variables, and missing objective measures related to mood (Camkurt et al., 2018).

8.4. Cannabinoid type 2 receptor agonists

Modulation of the endocannabinoid system has shown promise in a number of conditions including pain, traumatic brain injury, autoimmune and neuropsychiatric disorders. Though initially difficult to validate due to nominal expression in brain tissue (Liu et al., 2009), CB2R are clearly found on microglia (Cabral et al., 2006), with expression tightly correlated to activation state (Mecho et al., 2016). To this point, cultured microglia that were activated by LPS displayed reduced CB2R (Carlisle et al., 2002), whereas those primed with GM-CSF (granulocyte-macrophage colony stimulating factor) and IFN-γ had augmented CB2R expression (Maresa et al., 2005).

Cannabinoids impact microglia in many ways, including their proliferative and migratory behavior (Dirikoc et al., 2007; Komorowska-Müller and Schmöle, 2020; Tolón et al., 2009) Early work found that exposure of rat microglial cell lines to the CB2R agonist, 2-AG, enhanced CB2R internalization, upregulated ERK1 signaling, and induced microglial proliferation (Carrier et al., 2004). Exploration of synthetic and targeted CB2R agonists has revealed that these receptors have multiple binding sites with activation based on brain location and occupancy level (Shoemaker et al., 2005). While mechanisms of action for CB2R on microglia are not fully mapped out, a picture is emerging that this receptor may have the capacity to induce a neurotrophic phenotype, and possibly also induce PPAR-γ, which, as previously discussed, is also associated with inducing microglial trophic responses.

Beta-caryophyllene (BCP) is a constituent of Cannabis Sativa that has been under intense investigation for its ability to selectively activate CB2Rs. Of interest, BCP has been found to co-activate PPAR-γ in mouse models of ulcerative colitis (Bento et al., 2011), high-fat diet (Youssef et al., 2019), and Alzheimer’s disease (Cheng et al., 2014). It also was reported to have the ability to upregulate BDNF production and attenuate behavioural despair in chronic restraint stress in mice (Hwang et al., 2020). Agonism of CB2Rs by pre-treatment with BCP in mice that were then subjected to forced swim testing, reduced immobility and significantly elevated hippocampal BDNF (de Oliveira et al., 2018). BCP was also found to provide neuroprotection and increase BDNF levels in mixed neuron/glial culture model of ischemia, by upregulating adeno-sine monophosphate-activated protein kinase (AMPK) and CREB (Choi et al., 2013).

Other CB2R agonists, including the synthetic agents, JWH-133 and AM1241, upregulated microglial expression of BDNF, while reducing a variety of inflammatory markers (Ma et al., 2015; Tang et al., 2017). In fact, JWH133 not only increased levels of BDNF in microglia, but also promoted an overall shift in the microglia towards a phenotype that facilitated neurogenesis and the restoration of damaged neural circuitry in a model of germinal matrix hemorrhage (Tang et al., 2017). Another study found that JWH133 promoted microglial M2 polarization resulting in improved clincial outcome in a model of intracerebral hemorrhage (Lin et al., 2017). Since CREB neutralization prevented this M2 microglial polarization, CB2R engagement appears able to foster a restorative phenotype in microglia by way of classical PKA-CREB signaling. Of course, this pathway is also fundamental for upregulating BDNF transcription. Other cannabinoid ligands, including 2-AG and AEA also increased the expression of the M2 marker, Arg-1 (Mech et al., 2015), further confirming a reciprocal relationship between M2 profile and cannabinoid signaling.

Though not yet extensively tested in humans, newer agonists of CB2 receptors including phytochemical components of cannabis, such as cannabichromene (CBC) (Udo et al., 2019) and BCP will undoubtedly open the door to more targeted options at manipulating microglial phenotypes and increasing treatment possibilities for CNS disorders.

9. Anti-inflammatory treatment considerations

In the treatment of depression, trials of broad-spectrum anti-inflammatory agents (e.g. NSAIDs, minocycline) have had mixed results, with treatment response most pronounced in individuals with higher inflammatory cytokine profiles (Husain et al., 2020; Osimo et al., 2020; Raison et al., 2013). It is likely that anti-inflammatory agents are only effective in a subset of patients that already display signs of excessive inflammatory processes. Indeed, minocycline was shown to be an effective adjuvant in treatment-resistant depression, in which inflammation is believed to be present (Husain et al., 2017). Notably however, a recent largest-scale double-blind placebo controlled clinical trial in bipolar depressed patients found no apparent benefit of 12 weeks of anti-inflammatory adjunctive treatment with minocycline or Celecoxib upon outcome measures (Hamilton Depression Rating Scale: HAMD17, Global Clinical Impression scale or Generalized Anxiety Disorder scale: GAD-7) (Husain et al., 2020). It is possible that, besides the importance of inflammation being present, only very selective measures of depression (e.g. anhedonia, as observed in stressor-based animal models) are
appreciably impacted by anti-inflammatory clinical treatments (Miller and Pariante, 2020). Whatever the case, use of such treatments must also consider possible off target effects, the particular sub-type of depression (e.g. typical vs atypical vs dysthymia vs bipolar), treatment resistance, as well as the timing of drug administration relative to the onset and duration of symptoms. For instance, minocycline was reported to only mitigate the behavioural effects of chronic stressor treatments if applied prophylactically, with no behavioural amelioration if applied post-stress (Kreisel et al., 2013) and in fact, may adversely impact trophic process if exposure occurs at certain developmental stages (Ueno et al., 2013). Ultimately, new anti-inflammatory treatments should probably be tailored to the patient’s immune profile, and may involve a combination of factors that dampen inflammatory responses while concomitantly stimulating a trophic phenotype.

It is important to consider that some degree of “background” neuroinflammatory processes might be “optimal” for CNS functioning. As with so many other factors, activating a trophic phenotype in microglia may be governed by the old adage, attributed to the Swiss physician Paracelsus, “the dose makes the poison”. For example, in culture models of neuronal hypoxia, microglia can assume a range of activation states, including those that are both inflammatory and protective, which are influenced by the amount of ATP and glutamate released by injured and dying neurons (Lai and Todd, 2008). Further, BDNF and LTP were suppressed by high doses of the broad spectrum NSAID drug, ibuprofen, leading the study authors to conclude that strong suppression of microglial activation may also have deleterious effects on neuroplasticity (Golia et al., 2019). It may not be a matter of “on or off” of microglia, but a combination of braking and gas (to use a driving analogy), that modulates the release of soluble factors that promote synaptogenesis and recovery. In point of fact, inflammatory cytokines such as IFN-γ and TNF-α, play important synaptic regulatory roles, with TNF-α mediating upregulation of AMPA receptor insertion into the post-synaptic membrane in excitatory synapses (Stellwagen and Malenka, 2006), and IFN-γ vital to neurogenesis and progenitor differentiation (Baron et al., 2008; Kim et al., 2007; Zhang et al., 2018).

Besides the importance of inflammation targeted drug therapies, non-pharmacological approaches might be a useful treatment that modulates microglial and BDNF processes. While many studies have long implicated exercise and certain dietary interventions in positive effects on CNS functioning, emerging reports are beginning to suggest the possibility that such improvements might in part, by related to microglial processes. For instance, dietary polyphenol supplementation improved cognitive performance in aged rodents and this was related to a reduction in pro-inflammatory features (microglial state and pro-inflammatory cytokine levels) in the brain (Duggan and Parikh, 2021). Similarly, aerobic exercise blunted indices of microglial activation and increased anti-inflammatory cytokine levels in aged rodents and also had beneficial effects on peripheral cytokines in human studies (Gleeson et al., 2011; Gomes da Silva et al., 2013). This is in agreement with studies showing that voluntary exercise diminished the inflammatory impact of LPS upon microglia, while also promoting a trophic pro-neurogenic phenotype in aged rodents (Littlefield et al., 2015). It was further posited that BDNF which is derived specifically from microglia might play a critical role in the beneficial effects of exercise (Duggan and Parikh, 2021), which raises the possibility of such non-pharmacological activities influencing microglial transition into or persisting in a trophic state.

As addressed in detail here, it is important to mention that any microglial trophic state would likely involve the release of other beneficial trophic factors (beyond BDNF) such as IGF-1, fibroblast growth factor 2 (FGF-2), NGF and GDNF (Koss et al., 2019; Liu et al., 1998; Minnone et al., 2017; Morioka et al., 2015; Nakajima et al., 2001; Pöyhonen et al., 2019; Suh et al., 2013). In fact, it is noteworthy that moderate neuronal injury, but not mild or severe injury, was found to enhance microglial production of both BDNF and GDNF (Lai and Todd, 2008). Finally, also of significance is consideration of the fact that the ratio of the “immature” precursor proBDNF to the mature form of BDNF might hold importance for microglia, as imbalances in this ratio can lead to cellular dysfunction or even death (Mohammadi et al., 2020; Teng et al., 2005). In recent years it has become evident that proBDNF can be released as a signalling factor that has many actions that are the opposite of the mature BDNF form (via the p75 receptor) and that microglia can respond to proBDNF with a sustained elevation of intracellular calcium levels (Mizoguchi et al., 2021). Yet, it remains to be determined how anti-inflammatory treatments that modulate trophic microglial state might impact processing of pro- to mature-BDNF.

10. Conclusions and future considerations

The role of BDNF in stressor related disorders has been a subject of intense research for decades (Björkholm and Monteggia, 2016; Duncan et al., 2013; Green et al., 2013; Jin et al., 2019; Kerman, 2012; Polyakova et al., 2015). New strategies such has low-dose ketamine treatment have underscored the fact that depression and other neuropsychiatric disorders are heavily mediated by dysregulated neuroplasticity, with enhanced BDNF expression an essential component for positive treatment responses (Duman, 2014; Izumi and Zorumski, 2014; Kerman, 2012). However, the focus has primarily been on neuronal sources of BDNF, and the contribution of microglia has largely been ignored. Combining data from multiple fields of research including pain, neurodevelopmental disorders and neurodegeneration, there is an emerging picture of microglial BDNF playing a vital role in synaptic plasticity. Indeed, we posit that microglia may be key regulators of the synapse, with an inducible trophic phenotype as a treatment target for mood disorders such as major depression.

Key receptor targets for potentially inducing a unique microglial trophic phenotype include P2X4R and P2X7R, SIP, PPAR-γ, GLP-1 and possibly CB2 receptors. It is particularly interesting that two of these potential treatment targets have emerged from work on regulating glucose homeostasis, supporting the possibility that microglia may play a greater role in mediating metabolic responses than previously identified. Indeed, a recent review highlighted that several anti-hyperglycemic agents have demonstrated antidepressant effects in a number of animal models, but human clinical data is still lacking (Essmat et al., 2020). Ultimately, whatever agent is used, the complex activation profile of microglia and rapid response to environmental cues presents challenges in identifying optimal dose-response ratios and potential co-activators.

A fast path to microglial-modulating treatments for stressor-related neuropsychiatric or possibly even neurodegenerative disorders may be through repurposing and combining existing approved drugs in novel ways. For example, a combination of the FDA-approved statin drug, simvastatin, together with BDNF delivered intranasally in nanoparticle polymersomes reduced microglial inflammation induced by systemic LPS (Manickavasagam et al., 2020). And while little work has been done to assess its influence on BDNF production, the diabetes medication, metformin, has been found to promote neurogenesis and differentiation of neural precursors cells (Ayoub et al., 2020), and attenuate inflammatory cytokine release from microglia (Labuzek et al., 2010; Pan et al., 2016; Tao et al., 2018). Similarly, fingoilomin/FTY720, which targets SIP receptors, is an orally bioavailable, approved treatment for MS, which potently downregulates expression of inflammatory cytokines, enhances BDNF production from microglia (Noda et al., 2013) and has been shown to mitigate depressive symptoms in MS patients (Hunter et al., 2016). Newer SIP modulators are also being tested both pre-clinically and in clinical trials (Dash et al., 2018; Goodman et al., 2019; Scott et al., 2016; Wenderfer et al., 2008), and may show promise in reducing side-effects, while continuing to induce a trophic phenotype in microglia.

Although we have asserted that BDNF may have a unique role in trophic properties of microglia, this proposition should be tempered in light of the scarcity of studies that actually assessed microglial specific...
BDNF. Indeed, most studies have either not specified the cellular source of BDNF or have specifically focused on neuronal sources. It is also important to underscore that those that did specifically address microglial specific BDNF were largely in vitro studies. This complicates matters since cultured cellular responses do not always perfectly map on to the in vivo situation and in fact, the very act of simply culturing microglia is known to affect their morphological state. To this point, recent work by Bohlen et al. (2019) highlighted the fact that standard serum-based media used in most glial cell culture produce amoeboid and phagocytic microglial phenotypes, whereas alternative serum-free media containing CSF-1 can produce morphology more akin to typical basal in vivo states. Nevertheless, it has become increasingly clear that microglial cells have many important functions outside of the realm of their role as immune first responders to pathogenic stimuli, wherein they also play a fundamental role in shaping neuroplasticity. Beyond their pro- and anti-inflammatory states, less is known concerning whether specific “trophic” microglial phenotypes exist. While the “M2” anti-inflammatory reparative phenotype is well characterized, we speculate that certain factors could promote selective recovery processes independent of microglial neuroinflammatory alterations through the induction of BDNF and more specific microglial trophic states. However, we acknowledge that our hypothesis regarding a specific BDNF-related trophic phenotype of microglia is still very speculative at this stage.

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Declaration of Competing Interest

The authors declare they have no competing interests.

References

Abd-El-Basset, E.M., Rao, M.S., Alasgobi, A., 2020. Interferon-gamma and Interleukin-1Beta enhance the secretion of brain-derived neurotrophic factor and promotes the survival of cortical neurons in brain injury. Neurosci. Insights 15. https://doi.org/10.1186/s40350-019-0147-8.

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell. https://doi.org/10.1016/j.cell.2006.02.015.

Albertini, G., Deneyer, L., Ottestad-Hansen, S., Zhou, Y., Ates, G., Walrave, L., Anderberg, R.H., Richard, J.E., Hansson, C., Nissbrandt, H., Bergquist, F., Skibicka, K.P., Amini, M., Courtois, A., Sartor, O., Asgharian, S., Demuyser, T., Bentea, E., Sato, H., De Bundel, D., Danbolt, N.C., Massie, A., Smolders, I., 2018. Genetic deletion of sct attenuates peripheral and central inflammation and mitigates LPS-induced sickness and depressive-like behavior in mice. Glia 66, 1845–1861. https://doi.org/10.1002/glia.23343.

Anderberg, R.H., Richard, J.E., Hansson, C., Nissbrandt, H., Amini, M., Courtois, A., Sartor, O., Asgharian, S., Demuyser, T., Bentea, E., Sato, H., De Bundel, D., Danbolt, N.C., Massie, A., Smolders, I., 2018. Genetic deletion of sct attenuates peripheral and central inflammation and mitigates LPS-induced sickness and depressive-like behavior in mice. Glia 66, 1845–1861. https://doi.org/10.1002/glia.23343.

Avery, R., Kipke, S.D., 2015. The p38alpha MAPK regulates microglial responsiveness to diffuse traumatic brain injury. J. Neurosci. 35, 6143–6153. https://doi.org/10.1523/JNEUROSCI.5399-12.2013.

Badimon, A., Strabouger, H.J., Ayata, P., Chen, X., Nair, A., Ibegani, A., Iwango, P., Chan, A.T., Groves, S.M., Uverna, J.O., Lededore, C., Katha, M.G., Wheeler, M.A., Kahan, A., Ishikawa, M., Wang, Y.C., Loh, Y.H.E., Jiang, J.X., Surmeier, D.J., Robson, S.C., Junger, W.G., Sehra, R., Calipari, E.S., Kenny, P.J., Eyo, U.B., Colonna, M., Quintana, F.J., Wake, H., Gradinaru, V., Schaefer, A., 2020. Negative feedback control of neuronal activity by microglia. Nature 586, 417–423. https://doi.org/10.1038/s41586-020-2777-y.

Baghel, M.S., Singh, B., Dhuria, Y.K., Shukla, R.K., Patro, N., Khanna, V.K., Patro, I.K., Thakur, M.K., 2018. Postnatal exposure to poly (I:C) impacts learning and memory through postnatal depression in progenitor cells. Developmental Neurobiol. Learn. Mem. 155, 379–389. https://doi.org/10.1177/1041123818809005.

Baghel, M.S., Singh, B., Patro, N., Khanna, V.K., Patro, I.K., Thakur, M.K., 2019. Poly (I: C) exposure in early life alters methylation of DNA and acetylation of histone at synaptic plasticity gene promoter in developing rat brain, leading to memory impairment. Ann. Neurosci. 26, 35–41. https://doi.org/10.1177/0218471618792513.

Bai, S., Guo, W., Feng, Y., Deng, H., Li, G., Nie, H., Gun, G., Yu, H., Mo, Y., Wang, J., Chen, J., Jing, J., Yang, Y., Tung, Y., Tang, Z., 2020. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. J. Neurol. Neurosurg. Psychiatry 91, 21–32. https://doi.org/10.1136/jnnp-2019-320912.

Balkowiec, A., Katz, D.M.D., 2002. Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. J. Neurosci. 22, 10399–10407. https://doi.org/10.1523/JNEUROSCI.22-33-10399.2002.

Bar, E., Barak, B., 2019. Microglia roles in synaptic plasticity and myelination in homeostatic conditions and neurodevelopmental disorders. Glia. https://doi.org/10.1002/glia.23677.

Barbic, M., 1994. The Trk family of neurotrophin receptors. J. Neurobiol. 25, 1386–1403. https://doi.org/10.1002/neu.480251107.

Barde, Y.A., Edgar, D., Thoenen, H., 1982. Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1, 549–553. https://doi.org/10.1002/j.1460-2076.1982.tb0207x.

Barker, P.A., 2009. Whither proBDNF? Nat. Neurosci. 12, 1056–1059. https://doi.org/10.1038/nn.2334.

Barnes, N., 2008. The effects of PPAR-alpha agonist pioglitazone on hippocampal cytokines, brain-derived neurotrophic factor, memory impairment, and oxidative stress status in lipopolysaccharide-treated rats. Iran. J. Basic Med. Sci. 22, 940–948. https://doi.org/10.22038/ijbms.2018.36155.9166.

Beninson, L.A., Brown, P.N., Loughridge, A.B., Saladien, J.P., Matasian, T., Hills, A.K., Woodworth, T., Craig, W., Amini, M., Kim, H., Flendtger, M., 2020. Intranasal BDNF downregulates TNF and modifies plasma exosome-associated heat shock protein 72 (Hsp72) and microRNA (miR-142-5p and miR-203). PLoS One 9. https://doi.org/10.1371/journal.pone.0229470.

Bennett, M.L., Bennett, F.C., Liddelow, S.A., Ajami, B., Zamanian, J.L., Fernhoff, N.B., Mulinyawe, S.B., Bohlen, C.J., Adl, A., Tucker, A., Weissman, I.L., Chang, E.F., Li, G., Grant, A.A., Hayden Gephart, M.G., Barres, B.A., 2016. New tools for studying microglia in the mouse and human CNS. Nat. Protoc. Adv. Sci. 5, 13738–13740. https://doi.org/10.1038/nprot.2015.28113.
Matyas, J.J., McMik, D.B., Weber, M.D., Niraula, A., Sawicki, C.M., Liu, X., Jarrett, B.L., Ramirez-Lull, M.E., Block, M.L., 2010. Microglial activation and chronic neurodegeneration. Luo, Y., Peng, M., Wei, H., 2017. Melatonin promotes brain-derived neurotrophic factor.

Maresz, K., Carrier, E.J., Ponomarev, E.D., Hillard, C.J., Dittel, B.N., 2005. Modulation of.

Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E.,

Mansur, R.B., Ahmed, J., Cha, D.S., Woldeyohannes, H.O., Subramaniapillai, M.,

Lorenzen, K., Mathy, N.W., Whiteford, E.R., Eischeid, A., Chen, J., Behrens, M., Chen, X.

Luo, Y., Chang, Z., Hu, J., 2018. Microarray expression of Cd274 cells via inhibition of miR-155-mediated repairation after oxygen-glucose deprivation and rexygenation. Cell Mol. Neurobiol. 38, 1305-1313. https://doi.org/10.1007/s10571-018-0599-0.

Luli, M.E., Block, M.L. 2010. Microglial activation and chronic neurodegeneration. Neurotherapeutics 7, 354–365. https://doi.org/10.1002/ntu.2010.05.014.

Luo, Y., Peng, M., Wei, H., 2017. Melatonin promotes brain-derived neurotrophic factor (BDNF) expression and anti-apoptotic effects in neuronal hemolmic hyperbilirubinemia via a phospholipase (PLC)-Mediated mechanism. J. Neurosci. Res. 50, 1023–1029. https://doi.org/10.1101/2019.03.02.06992.

Luo, B., 2003. BDNF and activity-dependent synaptic modulating. Learn. Mem. 10, 86–98. https://doi.org/10.1101/2046.

Lu, Y.-C., Yeh, W.-C., Ohashi, P.S., 2008. LPS/LR4 signal transduction pathway. Cytokine 42, 145-151. https://doi.org/10.1016/j.cyto.2008.01.006.

Lu, Y.-C., Luo, Y.-C., Huang, C.-Y., 2018. Microarray expression of BDNF mRNA expression in primary cortical cells by stimulating PI3K/AKT intracellular signaling in vitro. Mol. Biol. Rep. https://doi.org/10.1007/s10008-012-1600-8.

Macdonald, L., Shah, N., 2012. The role of microglial activation in traumatic brain injury. Neurobiol. Aging 33, 1094–1103. https://doi.org/10.1016/j.nia.2011.07.010.

Maciver, D.A., 2011. Microglial activation during neurodegeneration. J. Neuroinflammation 8, 101. https://doi.org/10.1186/1742-2094-8-101.

Macleod, R.M., Brown, P.J., 2010. Microglial activation and the regulation of brain immunity. Ann. Rev. Immunol. 28, 543–575. https://doi.org/10.1146/annurev.immunol.26.021607.163735.

Mallik, M.P., Lamm, K., Overman, M.E., Grady, W., 2009. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 211, 137–147. https://doi.org/10.1016/j.jnimm.2008.11.007.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.

Maller, S., Gaido, K., Meade, J.W., 2011. Microglial activation and its role in the regulation of brain immunity. J. Neuroimmunol. 236, 36–46. https://doi.org/10.1016/j.jneuroim.2011.02.024.
Warden, A., Merriman, M., Ponomareva, O., Jameson, K., Ferguson, L.B., Weber, M.D., Godbout, J.P., Sheridan, J.F., 2017. Repeated social defeat, proBDNF. Nat. Neurosci. 12, 113.
Yang, J., Siao, C.-J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z.-Y., Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Wenderfer, S.E., Stepkowski, S.M., Braun, M.C., 2008. Increased survival and reduced inflammation in aged mice: a role for CD11c+ proinflammatory DCs. J. Immunol. 181, 123–132.
Yoon, G., Kim, Y.-K., Song, J., 2020. Glucagon-like peptide-1 suppresses neuroinflammation and reduces neurostructural changes. Brain Res. 1724, 116464.
Ye, X., Yu, L., Zuo, D., Zhang, L., Liu, J., Huang, Y., 2018. Noninflammatory changes of microglia are sufficient to cause social isolation-induced depression-like behavioral changes. J. Neuroinflammation 15, 17.
Yoo, M.H., Kim, T.-Y., Yoon, Y.-K., Koh, J.-Y., 2016. Autism phenotypes in ZnT3 null mice: involvement of zinc homeostasis. Mol. Psychiatry 21, 1051–1058.
Yuan, Y., Zuo, Y., Banks, W.A., Bullock, K.M., Haney, M., Batra-Kovacevich, E., Kahanov, A.V., 2017. Macrophage exosomes as natural nanoparticles for protein delivery to inflamed brain. Biomaterials 142, 1–12. doi: 10.1016/j.biomaterials.2017.07.011.
Zahiri, D., Buzov, P., Grönlund, C., Müller, C.E., Klapperstrick, M., Markwardt, F., 2021. Phosphine–lipoprotein interaction promotes neuronal activation of voltage-sensitive anion channels, ATP secretion and activation of purinergic receptors. Biochim. Biophys. Acta. Mol. Cell. Res. 1868. doi: 10.1016/j.biocel.2021.10.038.
Zhu, Z., Tan, P.-H., Cheng, J.-K., Liu, Y.-C., Ji, R.-R., 2011. Microglia: a promising target for treating neoplastic and postoperative pain, and morphine tolerance. J. Formos. Med. Assoc. 110, 487–494.

Zhou, C., Zhong, J., Zou, B., Fang, L., Chen, J., Deng, X., Zhang, L., Zhao, X., Qu, Z., Lei, Y., Lei, T., 2017. Meta-analyses of comparative efficacy of antidepressant medications on peripheral BDNF concentration in patients with depression. PLoS One 12, e0172270. https://doi.org/10.1371/journal.pone.0172270.

Zhou, G., Yan, M., Guo, G., Tong, N., 2019. Ameliorative effect of Berberine on neonatally induced type 2 diabetic neuropathy via modulation of BDNF, IGF-1, PPAR-γ, and AMPK expressions. DoseResponse 17. https://doi.org/10.1177/1559325819862449.

Zhou, D., Ji, L., Chen, Y., 2020a. TSPO modulates IL-4-Induced Microglia/Macrophage M2 polarization via PPAR-γ pathway. J. Mol. Neurosci. 70, 542–549. https://doi.org/10.1007/s12031-019-01454-1.

Zhou, J., Wang, M., Deng, D., 2020b. KLF2 protects BV2 microglial cells against oxygen and glucose deprivation injury by modulating BDNF/TrkB pathway. Gene 735, 144277. https://doi.org/10.1016/j.gene.2019.144277.

Zhu, G., Sun, X., Yang, Y., Du, Y., Lin, Y., Xiang, J., Zhou, N., 2019. Reduction of BDNF results in GABAergic neuroplasticity dysfunction and contributes to late-life anxiety disorder. Behav. Neurosci. 133, 212-224. https://doi.org/10.1037/bne0000301.

Zhu, H.X., Cheng, L.J., Yang, R.W.O., Li, Y.Y., Liu, J., Dai, D., Wang, W., Yang, N., Li, Y., 2020. Reduced amygdala microglial expression of brain-derived neurotrophic factor and tyrosine kinase receptor B (TrkB) in a rat model of poststroke depression. Med. Sci. Monit. 26 https://doi.org/10.12659/MSM.926023.

Zou, J., Crews, F., 2006. CREB and NF-κB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Cell. Mol. Neurobiol. 26, 385–405. https://doi.org/10.1007/s10571-006-9045-9.