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Role of BDNF Expression Through Microglial Polarization in Adult Hippocampal Neurogenesis in Depression and the Therapeutics

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Abstract: The present study tests the proposition that enhancement of BDNF by polarizing microglial cells from M1 to M2 contribute to the phenomenon of hippocampal neurogenesis and synaptic plasticity, thus reducing depressive-like behaviors. The experimental design covers both in vitro and in vivo, with BACE1-modified microglial culture, co-culture with neural progenitors, and a mouse model of depressed state. Thus, the present study intends to understand exactly how microglial polarization is connected to the alteration in BDNF and adult hippocampal neurogenesis in depression. It is possible that the results obtained will shed light on the new therapeutic approaches to influencing microglial polarization and BDNF receptor expression in depressive disorders and improving the efficacy of the therapies. The present study adds to the current knowledge about the neuroinflammation process in psychiatric diseases, as well as possibly offering hope for better biology-driven treatment options for depression in the future.

Keywords: depression, microglial polarization, BDNF expression, neurogenesis, BACE1 inhibition.

1. Introduction

Depression is one of the most prevalent mental health disorders worldwide, causing profound impairment and imposing an important economic burden on patients and society as a whole. Recent investigations have focused in particular on a type of depression called postoperative depression (POD), usually occurring in the wake of cardiac surgery. POD is a common postoperative complication, the adverse effects of which go far beyond the initial phase of recovery into long-term outcomes for a substantial number of surgical patients. An important cog in the wheel leading to POD is the occurrence of preoperative sleep disruption. This pre-operative sleep disruption has indeed been proven to exaggerate microglial M1 polarization and thus may underlie the pathophysiology of POD. The surgical intervention itself might interrupt microglial BDNF-TrkB signaling, further paving the way for the gradual build-up of depressive symptoms [4].

As the immune sentinels of the central nervous system (CNS), microglia play an indispensable role in not only fostering brain health and promoting immune responses targeting pathological deviations. These cells are capable of differentiating into two main phenotypes, conventionally called the pro-inflammatory M1 and the anti-inflammatory M2 states [1]. In short, M1 phenotypic microglial cells are directly cytotoxic and show profound inhibitory effects on neuronal precursor cell

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proliferation, whilst secreting injurious proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1β. In marked contrast, alternatively activated (M2) microglia phagocytose cellular debris and produce a wide range of cytoprotective and trophic factors that favor preservation/repair.

An inflammation-triggered dysbalance along this phenotypic microglia polarization continuum has indeed been confirmed in the pathogenesis of depression, with an enhanced skewing towards the M1 proinflammatory and neurotoxic phenotype. This dyshomeostasis plays an intimate role in the pathophysiology of depression. Member of the neurotrophic family, but thought to have perhaps the widest range of action, is brain-derived neurotrophic factor (BDNF). It is a pivotal master regulator patterning microglial population dynamics and consequent states that affect neurogenesis under homeostatic and regenerative, but also pathological, deleterious conditions. BDNF then binds to and activates the cell-surface TrkB receptor, which in turn initiates intracellular signal transduction pathways with pro-neurogenic properties. In fact, BDNF levels are markedly reduced in depressed patients, where this reduction seems to occur specifically in the hippocampus.

The above hypothetical enhanced cross-talk between neuronal networks driven by BDNF up-regulation carries a number of clinically important neuroscience connotations that go as far as reaching neuroplastic consequences with potential therapeutic relevance. Some studies have even implicated adult hippocampal neurogenesis in the pathophysiology and perpetuation of depressive symptoms [5]. Nevertheless, the bidirectional nature of the neurobiologically-based cross-talk between neurogenesis and depression is apparent. That is, where findings imply that lowered neurogenesis leads to depressive states, interventions which are directed against stimulating neurogenesis would produce antidepressant effects. These results contribute to a growing body of evidence suggesting that the manipulation of neurogenesis as a putative treatment for depression has considerable promise [9].

Although the growing body of experiments in microglial polarization, BDNF expression, adult hippocampal neurogenesis, and depression begins to finely sketch out these cascade of etiopathogenetic events or abnormal combinations of putatively related neuropathological phenomena, the intrinsic nature of these relationships remains to be elucidated. To address these considerations, a clearly defined hypothesis is postulated that: *Modulating BDNF expression through microglial polarization (shifting from M1 to M2) enhances hippocampal neurogenesis and synaptic plasticity, alleviating depressive-like behaviors*. Specifically, promoting the BDNF-TrkB signaling pathway in M2-polarized microglia can restore neurogenesis and cognitive function in depression. More specifically, it is postulated that activation of the BDNF-TrkB signaling pathway in M2-polarized microglia may be able to at least partially reverse the deficits in neurogenesis and the cognitive impairment that constitute the hallmark of depression.

Exciting new avenues also suggest BACE-1 inhibitors as attractive candidate molecules to influence microglial polarization and skew them to a more beneficial pro-M2 phenotype with increased, chemically extractable BDNF [6, 13]. By normalizing this hypoactive state, these inhibitors have also been effective in animal models of Alzheimer's disease and depression. Based on these results and suggesting a causal role, the present study aims to investigate the potential therapeutic avenues of selective-M2 polarization of microglia and modulation of BDNF expression in depression.

2. Experiments

The translational investigation reaches from in vitro to in vivo studies, investigating the presence of phenotypic microglial activation-mediated BDNF expression and their impact on adult hippocampal neurogenesis as well as depressive-like behavior. A temporal cascade construct-based investigational

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and monitoring approach from microglial activation down to the final psycho-behavioral and molecular endpoints in a mouse model of depression is presented.

2.1. Generation and phenotypic characterization of BACE1-edited microglia

The culture purification procedure will start with 8-10 week-old adult C57BL/6 primary microglia. Refined isolation procedures based on a combination of mechanical dissociation followed by CD11b+ microbead-positive selection will be used, resulting in a far purer cellular fraction, which will act as a baseline cellular model in which to manipulate BACE1 activity [13]. Lentiviral transduction methods will be employed to modulate BACE1 expression. Two distinct lentiviral constructs will be produced, one ferrying BACE1 shRNA for knockdown and the other for BACE1 overexpression. A non-targeting scrambled shRNA control lentivirus will also be employed.

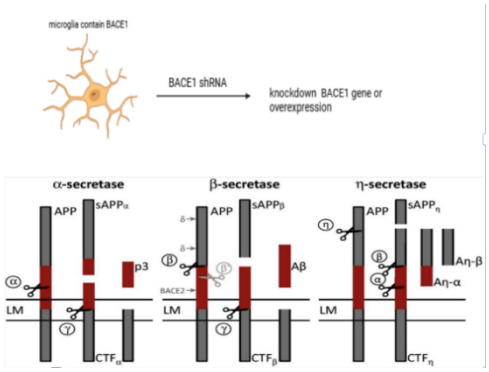

Figure 1: Schematic representation of lentiviral constructs for BACE1 knockdown and overexpression

Human microglial cells will be seeded in 24-well plates and transduced by lentiviral particles using a Multiplicity of Infection (MOI) of 10 in an overnight process. The success of the strategy to systematically modulate BACE1 will be validated using a selection of orthogonal approaches. BACE1 mRNA will be measured by quantitative real-time polymerase chain reaction (qRT-PCR), BACE1 protein expression by Western blot analysis on whole-cell lysates prepared from HER cells, and catalytic activity by a fluorescence-based enzyme assay. These protocols will ensure the desired levels of expression of BACE1 in the manipulated microglia for their particular use in the follow-up experiments [6].

2.2. In vitro microglia culture and polarization

To determine how modulation of BACE1 affects activation of microglia cells, microglia cells with altered levels of BACE1 will be activated to M1 or M2 phenotypes alongside respective control cells. M1 polarization will be accomplished with lipopolysaccharide (LPS, 100 ng/ml) treatment for 24 h, and M2 polarization with interleukin-4 (IL-4, 20 ng/ml) treatment over the same time period.

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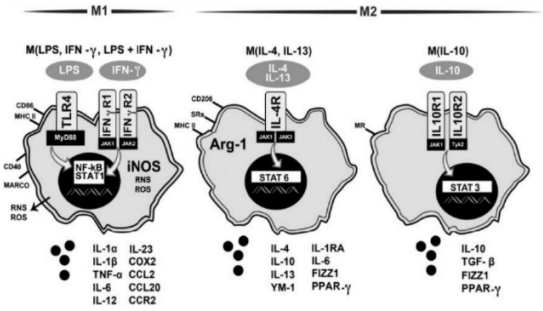

Figure 2: Illustration showing the distinct M1 and M2 phenotypes with their characteristic surface markers and secreted factors

Verification of the induced polarization states will be carried out by analyzing the expression of key markers. For M1 polarization, evaluation will be based on expression of iNOS and CD86, and for M2 polarization, Arg1 and CD206 will be diagnostic phenotypic markers. The latter markers will be assessed through quantitative reverse transcriptase-PCR and flow cytometry. In addition, to further corroborate the microglial polarized states, the respective specifically M1 (TNF-α and IL-1β) or M2-specific (IL-10 and TGF-β) secreted cytokines levels will be also assessed by ELISA in order to serve as biomarkers of the corresponding microglial polarized states.

2.3. Co-culture with neural progenitor cells

To specifically examine the direct impact of soluble mediators produced by BACE1-modified microglia upon neurogenesis, a co-culture system with neural progenitor cells (NPCs) which are isolated from the subventricular zone and the hippocampal dentate gyrus of 6–8-week-old adult C57BL/6 mice using an enzymatic dissociation technique and further purified by a density gradient [8].

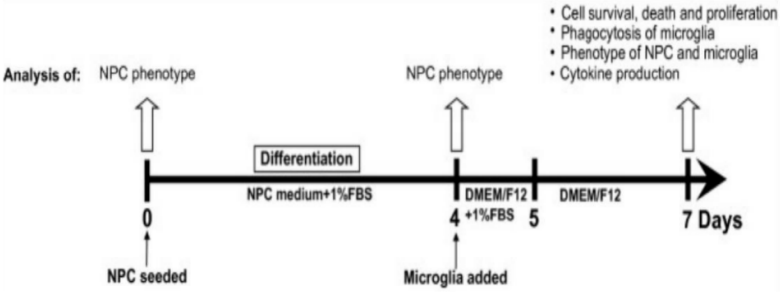

Figure 3: Schematic showing the experimental timeline for NPC seeding, microglia addition, and analysis timepoints over 7 days

The co-culture will be carried out using transwell plates that separate the neuronal and epithelial compartments, but allow for paracrine signaling without physical cell–cell contact. NPCs will be seeded in the lower compartment with microglia, either engineered to overexpress BACE1 or as unmodified controls, added to the upper chamber. This approach will enable dissection of the relative contribution of microglial-secreted factors promoting versus antagonizing NPC proliferation and

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differentiation. Co-cultures will then be kept in a 5% CO2 incubator for 1 week in NPC growth medium supplemented with N2 and B27. To assess the rate at which NPCs are dividing, BrdU incorporation experiments will be performed by exposing cells to BrdU for the 24 h period immediately before culture termination. In particular, differentiation will be evaluated by immunostaining for doublecortin (DCX), a marker of immature neurons, and by the expression of well-characterized markers of mature neurons, including MAP2 and NeuN [9].

Neurogenesis will be quantified by fluorescence microscopy imaging of at least 10 randomly selected fields per well. The fraction of BrdU- and DCX-positive cells relative to the total number of DAPI-stained nuclei will be stated as a percentage. Neurite length and branching of DCX-positive cells will also be measured using ImageJ software, providing a quantitative estimate of the extent of maturation that subsequently occurs in the nascent neurons [9].

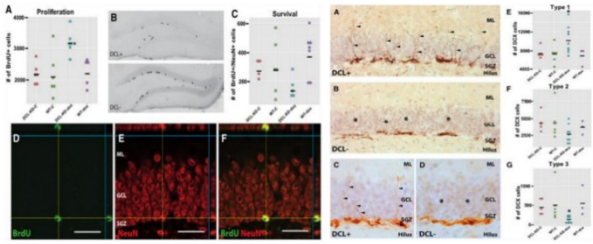

Figure 4: Representative immunofluorescence images and quantification of BrdU incorporation, DCX expression, and neurite outgrowth measurements

2.4. In vivo studies

Efficacy of the BACE-modulated microglial therapeutic approach against depression will therefore be explored using an experimental research design specifically manipulating molecular targets within the neuroimmune context in a developmental mouse model of this condition mimicked by chronic unpredictable mild stress (CUMS). Male, 8- to 10-week-old, C57BL/6 mice will be exposed to a chronic restraint procedure for six weeks (consisting of cycles of food and water deprivation, cage tilt, restraint stress), as well as forced swim and finally a resultant perturbation of the light–dark cycle in order to make detailed analyses of resulting physiological end-points. Consistent with the tenets of the CUMS protocol, cells will be engrafted by stereotactic injection. Vehicle or non-engineered microglia injections will be given to control cohorts. The latter will allow a quantitative evaluation of the contribution of the transplanted microglia towards the development of depression-like behaviors and newly formed hippocampal neurons [4].

2.5. Behavioral analysis

Functional assessment will take place at 1 and 4 weeks post-transplantation and will involve a series of behavioral tests:

- Behavioral despair will be measured by the Forced Swim Test (FST) and Tail Suspension Test (TST).
- Anhedonia will be evaluated using the Sucrose Preference Test.
- The Open Field Test (OFT) will be used to assess levels of anxiety-like behavior and locomotor activity patterns.

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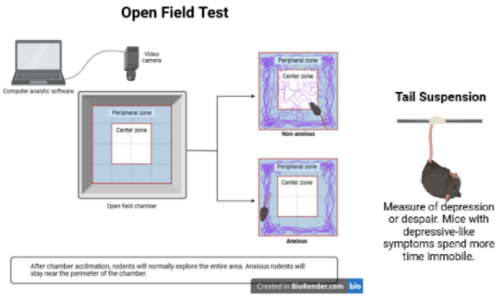
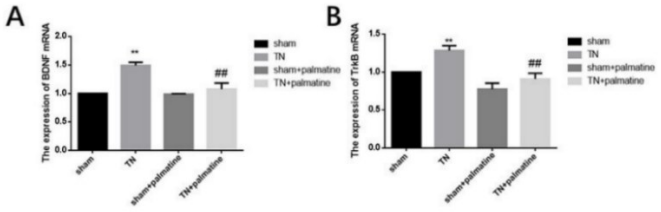


Figure 5: Diagrams showing the experimental setup for open field test and tail suspension test used to assess depression-like behaviors in mice

The mice will be humanely killed within 30 min of the termination of the 72-h time point of the final behavioral experiment; the brains will then be quickly removed, dissected on ice-cooled metal blocks, and discrete representative tissue cubes of the hippocampus collected for subsequent molecular analysis. BDNF expression will be studied at appropriate mRNA and protein levels using the methodologies of quantitative real-time polymerase chain reaction (qRT-PCR) in conjunction with enzyme-linked immunosorbent assays. TrkB activation will be measured by Western blot and immunohistochemistry for levels of phosphorylated TrkB. Moreover, immunohistochemical as well as qRT-PCR analyses will be used to measure the expression of neurogenesis markers (DCX and BrdU) and the microglial polarization states.



qPCR analysis. BDNF(A) and TrkB(B) mRNA levels in the TG of TN rats were higher than the shams. Palmatine treatment noticeably decreased these changes. Data are presented as mean ± SD, n = 6. **p < 0.01 vs sham group; ## p < 0.01 vs TN group.

Figure 6: (A) Progesterone, (B) Estradiol, and (C) Testosterone concentrations measured in control and experimental conditions

Throughout this investigation, the code of animal experimentation as well as advice on animal welfare will be adhered to.

3. Expected results

The a priori hypothesis is largely about the effect size of BACE1 manipulation on microglial polarization. More specifically, it is expected that BACE1 knockdown would promote M2 polarization, whereas BACE1 overexpression may push towards M1 polarization. It is therefore not beyond the realms of a possibility that the upregulation of BDNF by M2 microglia will lead to net improvement of NPC proliferative potential and subsequent differentiation within the co-culture system [2, 6].

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In the NPC co-cultures, the hypotheses are:

- Increased proliferation also with BrdU-positive ratio will prevail in co-cultures of NPCs with M2-polarised as well as BACE1-knockdown microglia.
- Increased neuronal differentiation, indicated by a higher percentage of cells double stained for DCX and a more developed neurite outgrowth, is also anticipated in NPCs co-cultured with M2-polarized, BACE1-knockdown microglia.
- When co-cultured alongside M1-polarized or BACE1-overexpressing microglia, proliferation and differentiation of NPCs are expected to be compromised. It has even been speculated, based on some

Some in vitro studies suggest that it might be possible to promote neurogenesis by skewing microglial activation towards enhanced BDNF expression.

4. In vivo results

In the CUMS mouse model, it is expected that the transplantation of BACE1-engineered, M2-polarized microglia will lead to:

- A decrease in immobility time in the FST and TST, thus improving behavioral despair.
- Increased preference for sucrose in the SPT, reflecting reduced anhedonia.
- Increased time spent and distance traveled in a more open region of the arena, reflecting a reduction in anxiety-like behavior.
- Increased numbers of DCX- and BrdU-labeled cells in the hippocampal dentate gyrus, indicative of increased neurogenesis.
- Increased dendritic elaboration and the presence of more spines on newly formed neurons [9].
- Increased mRNA expression and protein levels of BDNF in the hippocampus accompanied by increased phosphorylation of TrkB receptors, indicating an enhancement of the capacity of BDNF-mediated signaling.

A widespread constructive association between changes in behavior, up-regulation of markers of neurogenesis, up-regulation of BDNF and native receptors including activation and TrkB is expected. This correlation would provide the needed increase in microglial expression of BDNF, increased neurogenesis, and dampened expression of depressive-like behaviors [5].

5. Discussion

The predicted directions of effect would, if found, indeed represent an important advance in the current conceptualization of the underlying pathophysiology of depression. The evidence would be compelling if it also could then show that these BACE1-modulated/M2-polarized microglia are indeed capable of upregulating the expression of BDNF and thus activating neurogenesis, which then represents in effect an almost unbroken mechanistic chain starting with the microglial activation all the way to the corresponding endpoints. Therefore, the expected elevation of BDNF expression and the subsequent up-regulation of neurogenesis in concert with a microglial phenotype biased almost exclusively toward the M2 pole would further embody the pivotal role of these cells as orchestrators of a neurogenesis-permissive milieu. This has elegant parallels with the recent “microglia → modulators of mood and cognition” paradigm as opposed to being just proinflammatory entities [1, 7].

As such, these findings may signal a significant cognitive crossroad purported to conclusively ascribe the clinical importance of targeting microglial polarization as a novel avenue for treatment. Indeed, arguing that successful polarization of microglia towards the M2 phenotype may attenuate

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depressive-like behaviors helps build up the "micropsychiatric" rationale, hence, providing a basis for exploring novel modalities of interventions modulating the neuroimmune milieu [5, 15]. The observed BDNF modulation by microglial manipulation suggests an important additional layer of specificity and fine-tuning in the amplification of neurotrophin signaling hypothesized to take place in depression. If successful, this approach may avoid some of the limitations associated with other less targeted methods of delivery of BDNF, including poor blood-brain barrier penetration and off-target effects [2, 3].

6. Limitations and future directions

While the present experimental design has led to several important findings regarding the microglial polarization crosstalk, BDNF production, and ultimately the pathophysiology of depression, a number of limitations must also be acknowledged. Of course, any mouse model will be reductionistic by nature and thus unable to represent the full range in question. Moreover, long-term consequences of microglial transplantation and any subsequently elicited immune responses remain to be fully investigated [11]. The study reported has implications for several important future directions or approaches.

Future directions include:

- Additional follow-up investigations focusing on the contribution of BACE1-regulated microglia needed in other animal models of depression, but the identification.
- Identification of possible small molecule-based approaches to finding and skewing microglial polarization in situ.
- Advancing such findings to more directly clinically relevant corollaries of human research, such as post-mortem analyses of the brain or in vivo cerebral neuroimaging modalities able to separate states of microglial activation [12, 14].

7. Conclusion

These findings have the potential not only to dramatically refine current theoretical constructs regarding the pathophysiology of depression and pave the way for novel therapies. Indeed, by fully characterizing the contribution of applicable centrally acting therapeutic targets activated in pathways that drive the modulation of the microglial phenotype, perfectly focusing their downstream effects on BDNF expression and neurogenesis, novel antidepressant treatments may still be found that are far ahead of current neurotransmitter-based therapies [5, 15].

In this case, the inhibitory activity of the BACE1 inhibitors on microglial polarization and BDNF expression represent, respectively, the cutting edge of in silico pharmacology for alternative splicing and macrophage intervention [6, 13]. If successful, such line of treatment might open the avenue to treatments that do not ablatively remove depressive (-like) symptoms per se, but which instead halt or ameliorate the neuropathophysiologic dysregulatory trajectory that is the substrate of the disorder. Finally, these findings have clear methodological and conceptual implications pertinent to the broader discourse on the importance of neuroinflammation in neuropsychiatric disorders. The connection between microglia, BDNF, and neurogenesis as depicted by the current study may indicate the beginning of a new avenue of research that can be systematically expanded along branched pathways of interest to include a range of different neuropsychiatric illnesses underlain by impaired neuroplasticity and immune physiology [1, 7]. In the long term, studies like these might intervene further downstream and help in translational attempts to modernize the clinic, with the development of innovative treatment options for depression – a megaproblem of unmeet medical need in contemporary mental health care – as an outlook. As knowledge about the implication of increasingly sophisticated biological pathways in depression accumulates, studies like this are paramount as they

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lay the groundwork for the future development of personalized, biology-based interventions that might truly change the course for the countless many struck by this devastating disorder.

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