The gut-brain axis, BDNF, NMDA and CNS disorders

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ABSTRACT

Gastro-intestinal (GI) microbiota and the 'gut-brain axis' are proving to be increasingly relevant to early brain development and the emergence of psychiatric disorders. This review focuses on the influence of the GI tract on Brain-Derived Neurotrophic Factor (BDNF) and its relationship with receptors for N-methyl-D-aspartate (NMDAR), as these are believed to be involved in synaptic plasticity and cognitive function. NMDAR may be associated with the development of schizophrenia and a range of other psychopathologies including neurodegenerative disorders, depression and dementias. An analysis of the routes and mechanisms by which the GI microbiota contribute to the pathophysiology of BDNF-induced NMDAR dysfunction could yield new insights relevant to developing novel therapeutics for schizophrenia and related disorders. In the absence of GI microbes, central BDNF levels are reduced and this inhibits the maintenance of NMDAR production. A reduction of NMDAR input onto GABA inhibitory interneurons causes disinhibition of glutamatergic output which disrupts the central signal-to-noise ratio and leads to aberrant synaptic behaviour and cognitive deficits. Gut microbiota can modulate BDNF function in the CNS, via changes in neurotransmitter function by affecting modulatory mechanisms such as the kynurenine pathway, or by changes in the availability and actions of short chain fatty acids (SCFAs) in the brain. Interrupting these cycles by inducing changes in the gut microbiota using probiotics, prebiotics or antimicrobial drugs has been found promising as a preventative or therapeutic measure to counteract behavioural deficits and these may be useful to supplement the actions of drugs in the treatment of CNS disorders.

Key-words: Microbiota; BDNF; schizophrenia; NMDA; kynurenine;

INTRODUCTION

The gut-brain axis

In humans the influence of the gastrointestinal (GI) tract on the brain has been noted since the nineteenth century. Recent analysis indicates a bidirectional route of communication between the GI tract and the CNS, termed the 'gut-brain axis' [1]which may have profound effects on CNS development and on aspects of behaviour relevant to normal and pathological cognitive function.

There are neuronal and hormonal contributions to the gut-brain axis [2,3]. Although the vagus nerve has both sensory and motor components, the majority of these are afferent, highlighting the importance of gut feedback by the vagus nerve in gut-to-brain communication.

The hormonal component is mediated via the hypothalamic-pituitary-adrenal axis which affects the gut through its mediation of stress responses and which can be programmed in early life [4-6]. The intestinal microbiota can be altered in response to psychological and physical stressors and this correlates with an increased susceptibility to disease [7-9]. In response to stress, corticotrophin-releasing hormone, produced in the paraventricular nucleus of the hypothalamus, initiates a cascade of events culminating in the release of cortisol from the adrenal glands. Cortisol can affect gut permeability by modulating the function and composition of the intestinal mucosal barrier, [10,11] altering the composition of the microbiome and causing dysbiosis [12,13]. The intestinal barrier is normally considered to comprise the microbiota, lining mucus layer, epithelial columnar cells together with the goblet cells admixed amongst them, and the internal lamina propria which provides a home to several types of immune system cells [14]. Between the epithelial cells (enterocytes) there are junctional complexes consisting of tight junctions and desmosomes which provide a

selectively permeable barrier restricting the passage of unwanted compounds [14,15]. These narrow paracellular spaces are expanded when intestinal permeability becomes compromised (e.g. in response to cytokines) allowing larger molecules, dietary antigens and even microbes to cross the epithelium, [16,17] potentially triggering an immune reaction.

The increased bacterial translocation can have deleterious effects on the host and has been associated with depression [17]. Exposure to stress at an early stage, such as through maternal separation has revealed a long-term alteration in the microbiota and an increase in the activity of the hypothalamic-pituitary-adrenal axis [5,18,19]. This exaggerated stress response is also produced in germ-free (GF) mice and, interestingly, can be normalised by bacterial colonisation [4]. Compositional changes in gut microbiota (the relative amounts of various bacterial species) are also observed in adulthood on exposure to chronic stress, accompanied by an increase in cytokines [20].

Indeed, much of the influence of the GI tract on behaviour may be the result of its content of micro-organisms – primarily over 100 species of bacteria - which account for around 90% of the cells in adult mammals [21]. Increasingly it appears that these prokaryotes can modulate human brain development and function [22-25]. Advances in sequencing technology and metagenomics have highlighted the significance of the enteric micro-organisms, giving rise to the nomenclature 'microbiota-gut-brain axis' which is now often used synonymously with the former ‘gut-brain axis’. The highest density of microbes is found on the mucosal surface of the large intestine where a symbiotic relationship is forged between the host and bacteria at an early stage of postnatal development [25-27].

Dominant bacterial phylotypes in the gut include *Bacteroidetes and Firmicutes* [28] and it is bacteria such as these which provide a range of benefits to the host including, but not limited to: degradation of otherwise indigestible fibres, protection from pathogenic bacteria (e.g. through physical barrier formation), ‘training’ of the innate immune system [28] and

synthesising essential nutrients (e.g. vitamin K). A particularly important nutrient is tryptophan, an amino acid essential for protein synthesis but which also sits at the head of pathways generating 5-hydroxytryptamine, melatonin and the multi-functional kynurenine metabolites including kynurenic acid, quinolinic acid and nicotinamide [29-31].

Many of the tasks carried out by these bacteria are critical to normal body function and involve mechanisms which have not evolved in humans themselves [32]. While humans are unable to synthesise tryptophan, for example, it is generated in many bacteria [33]. Indeed there are also synthetic enzymes in bacteria for key metabolites of tryptophan with marked activity at NMDARs (quinolinic and kynurenic acids, discussed below). The tryptophan-kynurenine pathway is therefore a strong candidate for a mechanism by which the GI microbiota can influence CNS function.

GI microbiota and embryonic development

Embryological development can be highly influenced by maternal factors such as stress, diet and infection [34-40]. Therefore, any disruption in normal maternal behaviour may lead to alterations in the maternal gut microbiota during pregnancy resulting in the release of atypical metabolites which can in turn be detrimental to the developing foetus [41, 42].

As prenatal life was thought to constitute a period of sterility, the dynamic population of GI microbiota has been thought to be acquired postnatally, shortly after parturition [4, 43] from when it develops continuously throughout life with a compositional profile reflecting that of the mother as a result of mother-infant proximity and lactation [44]. Postnatal microbial colonisation is influenced by a variety of factors including type of delivery [45] and the surrounding environment. Vaginal delivery exposes the foetus to faecal and vaginal bacteria, whereas a Caesarean section would expose the foetus to skin bacteria. The initially maternal

signature of the neonatal microbiome develops into a unique population after approximately one year.

The discovery of bacteria in the pre-term uterus was shown to be linked to complications in pregnancy (such as preterm labour), often contributing to neonatal morbidity and mortality [46]. However, several studies have concluded that foetuses may come into contact with commensal microbes before birth [47] and this premature exposure could be pivotal in priming the immature immune system and brain development in healthy neonates. This modification to the prevalent dogma of exclusively postnatal colonisation has arisen from studies of the maternal placenta and foetal meconium. DNA originating from *Lactobacillus* and *Bifidobacterium* species was found in a significant majority of human placentae tested by Satokari et al. [47] which suggested maternal to foetal horizontal transfer *in utero*. Bacterial DNA contains unmethylated motifs which, if transferred to the foetus, could trigger an immune response and affect early development.

Thus, although the exact timing of initial exposure is controversial, it remains clear that pre- and postnatal microbial colonisation of the GI tract is a highly significant event. The peri-natal period is a critical one in neurodevelopment and the composition of the microbiota at this stage, predominantly *Proteobacteria* and *Actinobacteria* [48], is involved in developmental programming which can have life-long effects [49-53], especially since the late pre- and early post-natal period is known to contain several critical developmental windows, in which NMDA receptors play a key role [54-58].

The ultimate composition of the gut microbiota is influenced by a number of factors including genetics [59], diet, antibiotic use and disease [60,61]. Although the microbial profile of an individual is unique, the relative abundance and distribution of microbes is similar in most healthy individuals, supporting the view that significant perturbations from normal gut flora could lead to disease.

Gut Microbiota and psychiatric disorders

There is a growing recognition of the influence of GI microbiota in depression, anxiety and autistic spectrum disorders [8, 62-69] as well as cognitive function [70]. Around 70-90% of patients with inflammatory bowel disease experience clinical depression and anxiety [71-73]. A study utilising the maternal separation model in GF rats assessed the animals for depressive behaviour with a forced swim test [74]. In comparison to controls, these rats were found to possess heightened levels of corticotrophin-releasing hormone mRNA in the amygdala (associated with stress and anxiety) and displayed increased immobility during the test (indicative of depression). In addition, administration of a probiotic reversed these behavioural changes, implying that bacteria were able to modify the psychiatric state.

Children diagnosed with autism spectrum disorders have been found to possess an altered gut microbial composition, with a decrease in species of *Bifidobacteria* and an increase in *Lactobacilli* [75] and *Bacteroidetes* [76] species. It can be difficult to distinguish whether these modifications are the cause of the condition, a direct result of the condition, or whether they can be attributed to the atypical eating behaviour exhibited by many autistic children, but the result emphasises the potentially important changes of behaviour that can be dependent on GI factors.

Schizophrenia is classified as a major psychotic disorder which causes significant deviations in the perception, emotions and behaviour of an affected individual. Symptoms most commonly present during early adulthood are classified as positive (hallucinations and delusions), negative (social withdrawal and lack of motivation) or cognitive (learning deficits and impaired working memory). Research using genome wide association studies have uncovered thousands of single nucleotide polymorphisms which together are thought to account for approximately 30% of the risk of developing schizophrenia [77]. However, the

exact aetiology of schizophrenia remains elusive and it is clear that both genetic and environmental factors contribute to development of the condition. Many schizophrenic patients also present with associated GI complaints, raising questions about whether the gut could be involved in the pathophysiology of the disorder which is generally attributed to aberrations of neurodevelopment and synaptic plasticity [78-80].

A number of studies have focused on the immunological pathway in schizophrenia as immune dysregulation is frequently seen in these patients [81-86] and genome wide association findings have identified a link between the risk of developing the condition and immune-related genes [87]. The microbiota interact with gut mucosal cells to induce the release of pro- and anti-inflammatory cytokines [20] and several studies have discovered an up-regulation of pro-inflammatory cytokines in individuals with schizophrenia. In chronic schizophrenia, pro-inflammatory interleukin-6 (IL-6) levels were found to be elevated and anti-inflammatory interleukin 10 (IL-10) levels were found to be reduced in comparison to controls [88]. This was corroborated by Song et al. [89] who also found an increase in IL-6, as well as raised serum levels of interleukin 1β (IL-1β) and tumour necrosis factor α (TNFα). The elevation of these pro-inflammatory cytokines demonstrates an up-regulated inflammatory status in schizophrenia patients and represents an additional mode of immunological, bi-directional brain-gut communication, with enteric microbes having the ability to affect brain function indirectly by altering circulating cytokine levels.

There are many links between the presence of obesity or diabetes and psychiatric disorders. One study suggested that an elevated white blood cell count could be utilised as a marker for the systemic inflammation which is characteristic of schizophrenia, with a higher count being related to a greater risk of developing metabolic syndrome, as well as predicting an increased severity of psychiatric symptoms [90]. Furthermore, anti-psychotic drugs have been shown to produce metabolic symptoms in rats. As shown by Davey et al. [91], gender-

specific (female only) rapid weight gain and up-regulation of inflammatory cytokines were observed in rats treated with olanzapine. The observed increase in weight is a well-known side effect of anti-psychotic medication in humans which often negatively impacts patient compliance and, therefore, this is an important aspect of pharmaceuticals which must be targeted and improved. The effects of this weight gain could subsequently lead to metabolic syndrome, particularly the development of insulin resistance. The microbial profiles of both male and female rats in this study were also significantly altered, with an increase in *Firmicutes* and a decrease in *Bacteroidetes* species.

Glutamate, NMDA and schizophrenia

The dominant theory in schizophrenia research for several decades involved abnormal dopamine levels and metabolism in the brain, particularly within the ventral striatal mesolimbic area and prefrontal cortex. This hypothesis arose from the discovery of the potent anti-psychotic action of dopamine antagonists which suggested that a hyper-dopaminergic state was involved in the aetiology of schizophrenia especially since atypical dopamine levels can influence processes involved in schizophrenia such as synaptic plasticity and associative learning [92]. More recently there has been a shift in research focus towards the fusion of genetic and environmental factors which subsequently affect the glutamatergic system during development, resulting in NMDAR dysfunction [81].

NMDAR are involved in many aspects of early brain development and they provide a site at which a range of modulatory factors could influence brain formation and maturation [55-58]. One example of this is the ability of tryptophan metabolites such as quinolinic acid (an agonist at NMDAR [93]) and kynurenic acid (an antagonist at all glutamate receptors [94]) to alter neuronal excitability via their effects on glutamate receptors [30, 31]. Inhibiting the

kynurenine pathway during pregnancy results in substantial changes to the structure, electrophysiological functionality and protein expression of the postnatal offspring [95-98].

It has been claimed that kynurenic acid is also able to block α7-nicotinic receptors at low concentrations [99] but this has been disproved by at least three independent laboratories which have failed to reproduce any nicotinic blockade [101-102].

NMDARs are tetrameric, composed of two GluN1 sub-units (containing the glycine/D-serine binding site) and two GluN2 sub-units (containing the glutamate/NMDA binding site). The receptors exist on both pre- and post-synaptic sites [103]. The GluN2A-containing receptors have been associated especially with synaptic locations where they are believed to function during brain development, while receptors which include the GluN2B-subunit often exist at extra-synaptic locations and act in opposition to those containing GluN2A subunits. Both can modulate synaptic transmission and neuronal viability, with the GluN2A-containing receptors promoting potentiation and protection from damage, while GluN2B subunit receptors are linked to synaptic depression and cell death [104]. Other sub-units also exist including GluN2C, GluN2D, GluN3A and GluN3B. Deletion of the *Grin1* gene in hippocampal CA1 pyramidal cells results in adult mice which do not possess obvious developmental abnormalities, but lack the circuitry and long-term potentiation (LTP) mediated by NMDARs, leading to deficits in spatial memory [105]. The cognitive dysfunction seen in schizophrenia may be a result of a disrupted link between the activation of NMDARs, activity-dependent gene expression and neuroplasticity of interneurons [106].

The NMDAR hypofunction model aims to explain the cognitive problems associated with schizophrenia. Abnormalities in dopamine signalling may be secondary to NMDAR dysfunction [107], since NMDARs regulate dopaminergic neurons and glutamate and dopamine signalling interact in order to transmit sensory information [108].

NMDARs are important in GABA interneuron development and function, and abnormalities in receptors located on these interneurons are implicated in behavioural changes seen in schizophrenia [103]. Malfunction of NMDARs on fast-spiking inhibitory GABAergic interneurons within the limbic system causes a reduction in the tonic regulation of pyramidal neurons within the cerebral cortex. As a result of this disinhibition, there is increased firing and glutamatergic output. In addition, interneuron dysfunction negatively affects brain connectivity and integrative circuitry, which are compromised and lead to abnormal signal-to-noise ratios. As the ratio between the strength of desired transmission to that of unwanted interference is skewed towards an increase in background noise, there is an increase in uncoordinated impulse firing which is thought to provide a basis for the cognitive deficits observed in patients with schizophrenia [109].

Mice deficient in sub-unit GluN1 (5% of normal) display elevated motor activity and a reduction in social interactions [110]. In addition, a significant number of genes which underlie the genetic risk for developing schizophrenia impact NMDARs directly, or indirectly through signalling pathway components [54]. NMDAR antagonists, such as phencyclidine (PCP) and ketamine, cause schizophrenia-like symptoms including psychosis and cognitive dysfunction in healthy subjects, while exacerbating the symptoms of chronic schizophrenics. For example, PCP, a dissociative anaesthetic, was found to induce feelings of estrangement and hostility as well as disorganised thought in normal individuals, whereas pre-existing symptoms in schizophrenic patients were precipitated or enhanced [111]. Schizophreniform effects were exacerbated in animal models by persistent blockade of the receptors. Furthermore, a recent study investigating the effects of gut microbiota on the CNS reported a schizophrenia-like state in rats through subchronic PCP administration [112]. In response to this treatment, cognitive deficits measured through impaired novel object recognition were seen for up to three weeks in these rats, when compared to controls. These changes were

found to be associated with an altered gut microbiota population, (assessed by analysis of faecal samples), and cognitive disturbances were normalised by ampicillin treatment. These effects of a major antibiotic are consistent with the idea that specific symptoms of schizophrenia are a result of microbial disturbance and receptor malfunction. It should always be remembered, however, that the penicillin group of antibiotics have other, more direct pharmacological actions which can contribute to their effects on CNS function, including the modification of GABA-mediated neurotransmission and the actions of benzodiazepines [113-116].

In contrast, agents which enhance the activity of NMDARs are able to significantly reduce these symptoms [117]. Levin et al. [118] investigated the effects of the NMDAR co-agonist D-serine in healthy human subjects at the glycine binding site of the receptor [119]. In order to interpret the findings in relation to changes in cognition, several behavioural and cognitive tests were employed: Visual Analogue Scales; Continuous Performance Test-Identical Pairs and Rey Auditory Verbal Learning Tests. Following administration of D-serine, there was a significant decrease in feelings of depression and anxiety in comparison to baseline and placebo measurements, as indicated through Visual Analogue scores. In addition, Continuous Performance Test-Identical Pairs and Rey Auditory Verbal Learning Test scores revealed an improvement in attention and information retention respectively post D-serine treatment. These findings show that increasing agonistic activity on NMDARs produces pro-cognitive and anti-psychotic effects which might be useful for treating a range of neuropsychiatric conditions including anxiety, depression, autistic disorders, schizophrenia, strokes and degenerative cognitive dysfunction. The observations do, however, raise the question of whether NMDAR agonists or antagonists are likely to be of most use in these disorders. The problem has been contentious for many years with respect to neurodegeneration and the problem of whether acute or low dose activation of receptors is an appropriate treatment with

the risk that chronic or high dose treatments may induce calcium overload and exacerbate brain damage. In a similar fashion it is now clear that some NMDAR antagonists such as ketamine are not only anti-depressant, but are more rapidly efficacious than traditional monoamine-related treatments [120-122]. Interestingly, it has been suggested that BDNF is involved in the antidepressant activity of the NMDAR antagonist 7-chloro-kynurenic acid [123].

Brain-derived Neurotrophic Factor (BDNF)

BDNF is one of the most widely expressed and closely studied neurotrophins and is structurally related to nerve growth factor, neurotrophin-3 and neurotrophin-4, which regulate the viability and functional integrity of specific neuronal populations.

The synthesis of BDNF originates from a precursor protein, pre-pro-BDNF, which is translated in the rough endoplasmic reticulum. The signal peptide of this precursor is removed through endoproteolytic cleavage to produce pro-BDNF, and then again to produce BDNF in its mature state. Mature BDNF is then transported to post-synaptic dendrites in secretory granules or pre-synaptic terminals in vesicles [124]. There is controversy surrounding the location at which the last cleavage stage occurs, intracellularly [125] or extracellularly [126]. This is an extremely important debate to settle as pro- and mature BDNF have substantially different functions. Mature BDNF is involved in LTP induced by high frequency stimulation, whereas pro-BDNF is involved in long-term depression (LTD) induced by low-frequency stimulation [127]. The induction of LTP increases BDNF mRNA levels in CA1 hippocampal neurons whereas LTD can decrease BDNF secretion [128,129].

BDNF is involved in a plethora of functions within the CNS including neuronal survival and differentiation [130] and changes in BDNF levels may contribute to the chemical and structural imbalances associated with schizophrenia through dysfunction of synaptic

transmission and plasticity leading to cognitive deficits [78]. Hippocampus-specific deletion of BDNF in mice leads to impairments in Novel Object Recognition and spatial learning, compared to controls [131]. Participation in exercise and increased consumption of niacin (vitamin B3) have been found to affect positively the synthesis of BDNF.

BDNF production can occur through a constitutive pathway (spontaneous) or regulated pathway (in response to neural activity), with the latter being the predominant route [132]. BDNF signals through two membrane-bound receptors: p75 (the neurotrophin receptor) and TrkB (one of the tropomyosin-related kinase receptors) [133]. When activated by BDNF binding, TrkB undergoes dimerization and phosphorylation, with the resulting activation of mitogen-activated protein kinases; phospholipase C and phosphatidylinositol-3-kinase [132].

The *Bdnf* gene is expressed from early development throughout life and the diversity of roles which BDNF plays in the brain may be attributed to the complexity of its genomic structure [134] which includes at least eight 5' exons and one 3' exon with corresponding promoter regions [132]. The range of promoters leads to production of numerous BDNF transcripts which are distributed widely throughout the brain with high specificity. Long term effects of BDNF are mediated by gene expression, but interactions with effectors in the cytoplasm allow the neurotrophin to mediate short-term effects on neuron excitability and synaptic transmission [78].

There is a single nucleotide polymorphism in the human BDNF gene involving a valine to methionine amino acid substitution at position 66 (Val66Met) which impacts the packaging process of mature BDNF to secretory vesicles. This consequently decreases the activity-dependent secretion of the neurotrophin [132] and may contribute to several psychotic disorders [135]. Mice with a single copy deletion of the BDNF gene were found to have significantly impaired learning when tested in the water maze and required training for twice

as long as wild type mice [136]. In addition, homozygous BDNF knockout mice are unable to sustain life due to gross neurodevelopmental and sensory defects [137].

When compared to healthy individuals, the brains of patients with psychiatric conditions, including schizophrenia and bipolar disorder, have been found to possess decreased BDNF functionality. The full-length trkB receptor isoform trkB-TK+ is the primary mediator of the effects of BDNF on neurotrophic properties and therefore constitutes a useful measure of BDNF activity in relation to neuropsychiatric disorders. Post mortem analysis of schizophrenic brains has revealed significantly lowered trkB-TK+ mRNA levels in the hippocampus compared with control brains [138].

The microbiota and BDNF

GI microbiota may have the ability to modulate behaviour via changes in BDNF production. BDNF levels are lower in the cortex and hippocampus of GF mice compared to controls suggesting that the GI microbiota played some role in elevating brain BDNF [4]. This theory is supported by the fact that colonisation of these sterile mice with faecal matter from specific pathogen-free (SPF) mice or probiotic administration, resulted in partial and complete normalisation of behaviour and BDNF levels respectively.

Heijtz et al. [25] also found that BDNF mRNA expression in the hippocampus, amygdala and cingulate cortex (neural areas involved in anxiety and fear) of GF mice was significantly lower than in SPF mice. In addition, expression of synaptophysin and Post-Synaptic Density-95 in GF mice was higher than SPF mice in the striatum, suggesting that these proteins may also be subject to modulation by enteric bacteria. As these proteins are involved in synaptic vesicle maturation, their elevation may cause a potentiation of synaptic transmission leading to long-term changes and behavioural disorders in adults. GF mice exhibit a decrease in BDNF mRNA [139] although only male mice displayed a significant decrease in mRNA

expression in the hippocampus. This is of particular interest as epidemiological data for schizophrenia reveal that the condition is more prevalent in males than females [140,141]. Gender specificity was not evident in immune or endocrine responses which, as expected, were diminished and exaggerated respectively in all the GF mice tested.

Despite these correlations Neufeld et al., [142] found that the absence of gut microbiota in GF mice had an anxiolytic effect in an elevated plus maze. An increase in hippocampal BDNF mRNA expression, specifically in the dentate gyrus region, was also recorded in GF mice compared to SPF animals. Use of the probiotic *Bifidobacterium breve* 6330 caused an increase in BDNF total splice variants but a decrease in BDNF splice variant IV in normal rats [143]. In addition to GF animals, this study also used rats stressed by maternal separation which were found to possess increased BDNF levels in the hippocampus, although administration of the probiotic had no effect in these animals.

The variations in results may stem from the use of different measures of BDNF such as RNA message, protein expression or biological activity, all of which are required ideally for a complete assessment of function. It is also important to note that each of these parameters may differ in different brain regions, animal age or gender even within the same species.

Microbial regulation of BDNF and NMDARs

A link between stress, BDNF and NMDA receptors was described by Klug et al. [144] who observed that the administration of corticosterone, mimicking the effects of stress, on BDNF heterozygous mice (with around 50% of normal cerebral BDNF) altered the subunit composition of NMDA receptors in the hippocampus. Differential effects were seen in male and female mice with differences also between dorsal hippocampal regions concerned with learning and memory, and ventral regions involved more in fear and anxiety behaviour.

A study looking at memory dysfunction found that GF mice, on exposure to Novel Object Recognition and T-maze exploration tests, lacked non-spatial and working memory [145] which are believed to involve NMDARs in the hippocampus [146-149]. In addition, the animals exhibited a decrease in hippocampal BDNF expression compared to colonised controls consistent with evidence that the microbiota can modulate cerebral neurotrophins [81]. These interactions indicate the importance of normal gut microbiota in linking BDNF and NMDA-dependent hippocampal memory. This impairment in cognitive function, associated with a reduction in BDNF levels, may therefore be preventable or treatable through manipulation of the bacterial populations or a direct restoration of BDNF activity.

The findings by Sudo et al. [4] not only revealed altered levels of BDNF in GF mice, but also found that the expression of NMDAR sub-unit GluN2A was decreased in GF mice compared to controls. As previously discussed, receptors with these sub-units are associated with potentiation of synaptic transmission and neural protection [104] and, therefore, a decrease in these sub-units could result in reduced synaptic potentiation and vulnerability to damage. Neufeld et al. [142], also discovered a reduction in the expression of NMDAR GluN2B sub-unit mRNA in the amygdala which strengthens the case that modulation of BDNF levels affects NMDAR function.

The activation of NMDARs promotes the synthesis of BDNF. The depolarisation-induced influx of calcium through voltage-gated Ca^{2+} channels (VGCCs) in hippocampal neurons [134] triggers the binding of transcription factors such as Ca^{2+} response factor (CaRF) and cyclic AMP response element binding protein to components of the *Bdnf* gene [150]. Conversely, BDNF acts on the NMDARs to increase excitatory synaptic transmission in cortical and hippocampal areas via the phosphorylation of GluN1 and GluN2B sub-units. In hippocampal synaptoneurosomes, phosphorylation of the GluN1 subunit is demonstrable within 5 minutes [151], indicating a role for BDNF in the acute regulation of synaptic

transmission and plasticity. Additionally, on stimulation with BDNF, there is a differential up-regulation of protein and mRNA levels of GluN1, GluN2A and GluN2B sub-units in cultured hippocampal neurons [152]. BDNF stimulation also increased the cohort of sub-unit proteins associated with the plasma membrane and increased activity of the NMDAR, providing a potential explanation of why a deficiency in BDNF, as described in the brains of many schizophrenic patients [153-155] can cause dysfunction of NMDARs. A different view arises from observations demonstrating an inhibitory effect of BDNF on NMDA-mediated excitotoxicity in normal [156] or Huntington's disease model animals [157], perhaps reflecting the reduced receptor function that can occur as a result of over-stimulation and desensitisation. A lack of BDNF can certainly modify NMDA receptor function to a degree which induces depression [158], an interaction which may also account for the lower cognitive function which develops under omega-3-fatty acid deficiency [159].

There are several types of LTP in the hippocampus which can be loosely classified as associative and non-associative, both of which are influenced by BDNF. Associative forms of LTP can be potentiated by convergence of several inputs, involve NMDARs and have a postsynaptic component [124]. During induction of LTP, NMDARs are also known to be phosphorylated by a range of tyrosine kinases, one of which (the tyrosine kinase Fyn) is thought to play a major role in linking BDNF and NMDAR in the formation of spatial memory. The proposed mechanism involves BDNF stimulation of TrkB receptors which then associate with Fyn and cause it to bind to the Src homology 2 domains of NMDAR sub-units GluN2A and GluN2B [160]. This study employed the use of radial arm maze training to investigate the effects of spatial memory on phosphorylation of TrkB, Fyn, GluN2A and GluN2B in the rat hippocampus. Phosphorylation of all of these components, except sub-unit GluN2A, was stimulated by spatial learning and this was increased in trained rats. In addition, when Fyn was suppressed by administration of the tyrosine kinase inhibitor PP2 (4-

amino-3-(4-chlorophenyl)-1-(t-butyl)-1H-pyrazolo[3,4-d]-pyrimidine, which also reduced phosphorylation of GluN2B), there was a delay in memory acquisition before the rats reached the same level of learning as controls. This pharmacological blockade had no effect on TrkB phosphorylation.

Levels of BDNF can be elevated in response to exercise. The non-competitive NMDA receptor antagonist dizocilpine maleate (MK-801) induces schizophreniform symptoms in mice with changes in NMDAR expression and BDNF levels after treadmill exercise [161]. Dizocilpine treatment produced a decrease in NMDAR and BDNF, but a compensatory increase in response to treadmill exercise, raising the possibility of using exercise as a form of treatment in schizophrenia, working via BDNF and the regulation of NMDAR activity.

Underlying mechanisms

Gut microbiota can synthesise and recognise an array of neurochemicals, including neurotransmitters, neuroactive short chain fatty acids (SCFAs), secondary bile acids and other biologically active small molecules. This could be the mechanism by which gut microbiota are able to directly influence neural systems in the host in a bidirectional manner. Several biosynthetic pathways present in eukaryotic cells exist in prokaryotic cells, probably as a result of gene transfer, providing a mechanism for this inter-species signalling [2,3]. The concept is supported by the discovery that *Lactobacillus* and *Bifidobacterium* spp. generate gamma-aminobutyric acid (GABA), *Candida, Streptococcus*, *Escherichia* and *Enterococcus* spp. synthesise 5-hydroxytrypamine (5-HT, or serotonin) and *Bacillus* spp. produce dopamine [48]. The serotonergic system of the brain develops abnormally in the absence of gut microbiota [139] highlighting the importance of these bacteria in neurodevelopment and emotional responses. Other neuroactive substances including histamine and adrenaline are also produced by microbiota. The presence of these agents in the intestinal lumen may

activate neural afferents or interact with chemosensitive epithelial cells or enterochromaffin cells which can then transmit sensory information regarding the luminal environment to the brain [2]. Therefore, neurotransmitters have been established as a possible route for microbiota to communicate with the gut-brain axis, with GABA and dopamine being of particular interest for their association with schizophrenia pathophysiology.

There is some evidence which indicates the involvement of SCFAs in the microbiota-gut-brain axis. SCFAs are the end products of anaerobic fermentation of dietary fibre and starch in the large intestine and are absorbed primarily via the portal vein. Butyrate is an SCFA which acts as a colonic cell substrate and is known for its anti-inflammatory and anti-carcinogenic properties [162], therefore playing an integral role in colonic health. Several studies have shown that sodium butyrate, a histone deacetylase inhibitor, has antidepressant-like effects when administered as an epigenetic drug [163-166] and may be involved in neuropsychiatric conditions. Wei et al. [166] employed a rat model of depression to monitor the effects of sodium butyrate in relation to DNA methylation in the prefrontal cortex. These depressed mice possess decreased Ten-Eleven Translocation methylcytosine dioxygenase 1 (TET1), which is the enzyme responsible for catalysing the conversion of DNA methylation to hydroxymethylation. On administration of sodium butyrate, there was a reduction in depressive-like behaviour on the forced swim test and an elevation in TET1 levels. This positively correlated with a decrease in *Bdnf* methylation which subsequently caused an overexpression of BDNF. The findings indicate that the antidepressant effects of sodium butyrate may be caused by the associated elevation in BDNF which, as previously discussed, is thought to be diminished in schizophrenia and related mood disorders.

Another microbial fatty acid product with the potential to affect behaviour is propionic acid [69] although this has received less attention than butyrate. A wide range of compounds generated by GI tract microbiota have direct or indirect effects on the brain and behaviour

[167]. Many of these compounds can target GABA receptors [168] in addition to the glutamate receptor modulation produced by tryptophan and its kynurenine metabolites discussed earlier.

A variety of amines can be produced which could cross the intestinal-vascular barriers in their uncharged form [169-174] including 5-hydroxytryptamine (serotonin) [175]. These compounds may arise directly from bacteria or indirectly from the actions of bacteria on dietary components [172,175-180]. Particularly interesting for this review is the compound lactoferrin which can alter BDNF expression [181], as well as immune modulators [182] and complex carbohydrates with implications for cellular energy metabolism [183,184]. Some of the compounds produced, such as claudin-2 [185], can affect barrier permeability and therefore the absorption of other, less permeable microbial products. A number of peptides originating in the GI tract can affect BDNF and thus, indirectly, behaviour. Pancreatic polypeptide, for example, acts on the hypothalamic appetite control centres to promote BDNF expression in the ventromedial satiety centre, ultimately modifying behaviour for food-seeking [186]. Similarly, stimulation of synthesis and release of BDNF from intestinal cells by substance P and substance P and pituitary adenylate cyclase-activating peptide (PACAP) can indirectly affect behaviour [187]

Developing novel therapeutics for schizophrenia

The data discussed in this review are not only relevant to future research into the aetiology of schizophrenia, but also indicate the potential for developing new anti-psychotic treatments as well as an earlier diagnosis of this disorder. In addition to targeting NMDARs directly through pharmacological intervention, novel ways to manipulate enteric bacteria, including probiotics, prebiotics and antimicrobial drugs, may hold the key to future treatment for neurological and psychiatric disease.

Probiotics

Probiotics provide a resource for altering the composition of gut microbiota though ingestion of live cultures. This approach is already popular for the amelioration of inflammatory bowel disease symptoms. GF mice displayed exaggerated stress and anxiety-like behaviour compared to SPF mice [4] but, treated with *Bifidobacterium infantis*, these animals exhibited a complete normalisation of behaviour [188]. This not only reinforces the idea that the absence of gut microbiota was the cause of the stress and anxiety behaviour, but also introduces the prospect of using live cultures of healthy bacteria to maintain or restore a normal intestinal environment.

Beneficial effects of probiotics have also been reported in depression [74]. The rat maternal separation model is associated with up-regulation of the pro-inflammatory cytokine IL-6 in parallel with behavioural deficits and reduced levels of noradrenaline in the brain compared to controls. A reversal of all of these effects was observed following administration of *B. infantis*. This is highly significant as it shows that probiotics are effective in the treatment of depression in rats and this may have huge implications for human studies and in the treatment of a wide range of mood disorders, including schizophrenia.

Prebiotics

Prebiotics are soluble sugars, such as fructo-oligosaccharide and galacto-oligosaccharide (GOS), which are ingested by commensal microbiota and promote their proliferation. This in turn influences gene expression of neurotransmitters and neuromodulators in the hippocampus, subsequently modulating neurodevelopment in mice [189]. Analysis of faecal pellets revealed an approximately 25% and 80% increase in *Bifidobacteria* in fructo-oligosaccharide and GOS fed rats respectively, when compared to controls. There was also an

increase in hippocampal BDNF protein levels and expression of the NMDAR GluN1 sub-unit in response to prebiotic feeding as well as a significant increase in BDNF and GluN1 mRNA in the dentate gyrus. In addition, GOS ingestion specifically caused an increase in GluN2A sub-unit immunoreactivity and mRNA in the hippocampus with increased GluN1 expression and D-serine levels in frontal cortex. It was suggested that BDNF release may be associated with SCFAs or gut hormones such as peptide YY, as this hormone was found to be increased following GOS ingestion. Interestingly, these interactions can also be observed in vitro: a synthetic form of GOS induced BDNF release from cultured human SH-SY5Y neuroblastoma cells.

Antimicrobials

Antimicrobial agents affect the central levels of BDNF and behaviour in mice. Oral administration of non-absorbable antibiotics such as neomycin, bacitracin and pimaricin to SPF (but not GF) mice can increase hippocampal expression of BDNF [190]. This was accompanied by alterations in the composition of the gut microbiota (the putative cause of BDNF changes), including an increase in *Lactobacilli*, *Firmicutes* and *Actinobacteria* and a decrease in *Proteobacteria* and *Bacteroidetes* populations, accompanied by an increase in exploratory behaviour (the putative consequence of BDNF changes).

The altered behaviour was measured through standard step-down and light-dark preference tests in which mice treated with antibiotics had a shorter step down time and spent longer in the brighter compartment. It is important to note that these results were found to be independent of the autonomic nervous system, enteric neurotransmitters and inflammation. The microbial composition and behavioural changes were reversible and transferable: antibiotic effects were transient and normalised two weeks after withdrawal, and the

colonisation of GF mice with microbiota from different species conferred different behavioural phenotypes on the recipients.

Diet, microbiota and behaviour

One final aspect of this topic arises from all that has been noted above. An individual's natural diet will determine not only which microbiota gain entry to the alimentary canal, but also the degree of survival and balance between the different species, depending on the requirements and the extent of competition with other micro-organisms and the host. Since the microbiota clearly do play a significant role in defining the activity of the brain, it follows that there is likely to be some relationship between diet and mental health, with the possibility that manipulating dietary content could be a treatment or beneficial adjunct in the treatment of patients. It is certainly clear that patients with schizophrenia, for example, have a poor diet and low interest in dietary or culinary matters, with few unprocessed plant-based products [191-195]. The fat and calorie intake parallels the intensity of psychotic symptoms [196,197] and high-fat diets can counter aspects of schizophrenia-like behaviour in some animal models [198]. An especially instructive study by Gama et al. [199] showed that dietary supplementation with omega-3 fatty acids could prevent the behavioural, schizophrenia-like symptoms of animals treated with the NMDA antagonist ketamine.

Since BDNF is intimately involved in the regulation of food intake, dietary-induced obesity in animals alters the brain levels of BDNF as well as food-seeking behaviour [135]. Just as some probiotic bacteria can modify BDNF levels in the brain [143] and improve behavioural response to stress in parallel with increases in central BDNF levels [200-202], so specific patterns of food intake have been linked to behavioural changes [203] with altered BDNF production. The carbohydrate and fat content is again particularly important [204] and patients with schizophrenia exhibit large increases in central BDNF in response to

feeding low calorific diets. High cereal diets have also been linked with psychotic behaviour [205] and specific dietary components such as curcumin [206] and polyphenols [203] have been identified as compounds mediating some of these effects [207].

CONCLUSIONS

The studies discussed here indicate that gut microbiota harbour the ability to modulate levels of BDNF and NMDAR activity in the CNS. As BDNF is necessary for a range of neurodevelopmental and neuroprotective functions including synaptic plasticity, this has a significant impact on learning and memory, which is consistent with the reduced levels of BDNF in schizophrenia and other mood disorders [4,25,139]. LTP is thought to be the result of BDNF-mediated phosphorylation of NMDARs. A decrease in BDNF levels means less phosphorylation and a decreased ability to maintain normal levels of NMDAR activity, resulting in the receptor hypofunction that is now believed to be central to the development of schizophrenic symptoms. NMDAR hypofunction on GABA-releasing inhibitory interneurons causes disinhibition of glutamatergic output which modifies the signal-to-noise ratio and leads to abnormal patterns of network activity. This discordant firing manifests as a hyperdopaminergic state, which culminates in psychosis and cognitive deficits in schizophrenia, particularly through impairments in learning and memory. Perturbations in gut microbiota have a negative impact on BDNF levels, leading to NMDAR hypofunction and prebiotics, probiotics and antimicrobials are amongst the array of methods currently being investigated to normalise an abnormal microbial composition as potential anti-psychotic treatments.

Gaining a deeper insight into the routes by which gut microbiota are able to control the host CNS and manipulate it in a mutually beneficial way, will open new avenues into treatment for a wide range of disorders. Pinpointing the mechanisms which are disrupted in

psychiatric conditions such as schizophrenia will allow for the development of new drug targets. A normal microbial composition can be re-established following disruption by ingestion of pro- and pre-biotics, with huge implications in a world in which micro-organisms are increasingly tolerant and resistant to conventional antibiotic treatments. For example, it might be possible to treat brain disorders with a simple but effective drink containing live cultures, which has the benefit of increased patient compliance and ease of administration. The limitations which may be faced in this endeavour are primarily a result of the vast complexity of the microbiome and the fact that it is probably the overall mixture of microbiota which creates a chemical milieu which is unique to each individual patient.

It is important to note that the composition of microbiota and innervation of the GI tract are not homogeneous along the intestine. As a result, extensive research will be required to analyse the microbiome at different points along the gut to ascertain what is an overall 'normal' population at each point. Information gathered from a particular section of GI tract can then be assessed in relation to the innervation of that section and the range of signalling pathways mediating the reciprocal influences of microbes and host cell function. This will aid metagenomic studies and allow for the identification of alterations seen in patients with schizophrenia and their physiological impact. In addition, it may be found that certain neuroactive molecules are only produced by certain species at certain points, that these substances only elicit an effect at specific sites or that these agents stimulate the epithelial cells which then release further molecules to elicit the observed effects. Since it is established that diet is a major factor determining the composition of bacteria in the gut it must be taken into consideration that microbiota may require certain substrates obtained though digestion for the synthesis of various products. This could cause significant differences between results obtained *in vitro* and *in vivo* preparations, which would need to be accounted for in any analysis intended to apply realistically to everyday activity.

Overall this review has emphasised the role of GI microbiota on behaviour, BDNF levels and NMDAR function, involving bi-directional interactions between BDNF and NMDAR composition and function which together reveal a network that could underlie behavioural disorders in humans, such as depression and schizophrenia. This concept has major implications for understanding the origins and mechanisms of such disorders, but also for identifying future avenues for treatment by the modulation of the GI microbiome. On the wider landscape, many more disorders may involve changes in BDNF and could become amenable to interference with GI microbiota. Some long-standing example of how chronic diseases such as rheumatoid arthritis can be treated by dietary manipulation [208] may find explanation in these concepts. A loss of BDNF has even been demonstrated in the brains of patients with Huntington's disease, especially in the medium spiny neuron population of the striatum. Conversely, enhancing BDNF function improves brain structure and reduces behavioural deficits in mouse models of the disorder [209]. Together these data may imply that disease severity might be modulated by appropriate manipulation of the diet and GI microbiota. Similar considerations in other inherited conditions may show that even these disorders are susceptible to exacerbation or improvement by appropriate manipulation of the intestinal flora and fauna.

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ABBREVIATIONS

BDNF	brain-derived neurotrophic factor
CNS	central nervous system
FOS	fructo-oligosaccharide
GABA	gamma-aminobutyric acid
GF	germ-free mice
GI	gastrointestinal
GOS	galacto-oligosaccharide
IL-10	interleukin 10
IL-1β	interleukin 1β
IL-6	interleukin 6
LTD	long term depression
LTP	long term potentiation
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
SCFA	short-chain fatty acid
SPF	Specific pathogen-free

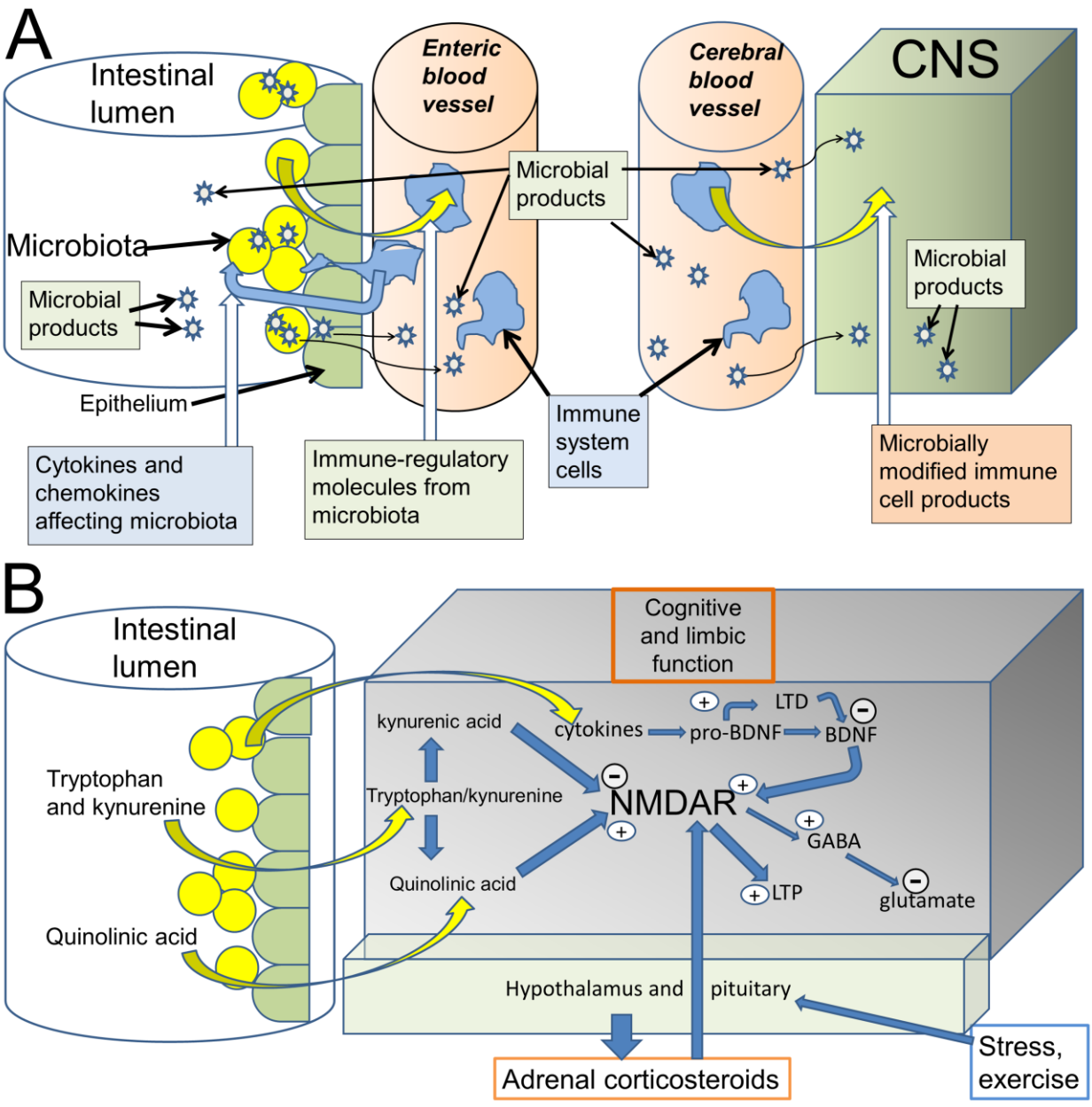
Figure legend

Figure 1. Schematic summary of the main concepts.

Panel A summarises the main premise that the microbial lining of the intestine, produce a variety of compounds which can penetrate the epithelium and affect the activity of immune cells and their production of cytokines and chemokines. Conversely cytokines from the white cells can modulate the viability and activity of the microbiota. The microbial factors ultimately reach the CNS where they are able to regulate neuronal activity and neurotransmission.

Panel B focusses on the mechanisms mediating the microbial influence on CNS function and thus cognition and behaviour. Kynurenine and quinolinic acid, as well as tryptophan itself, synthesised by bacteria can cross the epithelial barrier and reach the CNS where kynurenine is converted to quinolinic acid, an NMDAR agonist, and kynurenic acid, a glutamate receptor blocker. The interaction between microbiota, intestinal epithelium and white cells affect the overall concentrations of several cytokines, which in turn modulates the synthesis of pro-BDNF and BDNF. Pro-BDNF contributes to LTD and inhibits BDNF production, while BDNF increases NMDAR synthesis and functionality. NMDAR contribute to LTP but also depolarises GABA-releasing neurons that depress glutamatergic neurons. Superimposed on this is the effect of stress, illustrated as the induction of corticosteroid release which modulates the interaction between BDNF and NMDAR. Glucocorticoids also promote the conversion of tryptophan to kynurenine via their induction of tryptophan-2,3-dioxygenase (TDO). Exercise also increases BDNF production and so enhances cognitive function.

FIGURE 1


A
Intestinal lumen
Enteric blood vessel
Cerebral blood vessel
CNS
Microbial products
Microbiota
Microbial products
Microbial products
Epithelium
Cytokines and chemokines affecting microbiota
Immune-regulatory molecules from microbiota
Immune system cells
Microbially modified immune cell products
B
Intestinal lumen
Cognitive and limbic function
kynurenic acid
cytokines
pro-BDNF
LTD
BDNF
Tryptophan and kynurenine
Tryptophan/kynurenine
NMDAR
GABA
Quinolinic acid
LTP
glutamate
Quinolinic acid
Hypothalamus and pituitary
Stress, exercise
Adrenal corticosteroids