SAPIENZA
UNIVERSITÀ DI ROMA

PhD Program in Clinical/Experimental Neuroscience and Psychiatry

Curriculum Neurophysiology
XXXII Cycle

The role of microglial cells in modulating hippocampal synaptic plasticity and serotonergic antidepressant efficacy

PhD student
Maria Teresa Golia

Tutor
Prof.ssa Cristina Limatola

Coordinator
Prof. Marco Salvetti

Supervisor
Dr. Laura Maggi

Academic Year 2018/2019

INDEX

GENERAL INTRODUCTION

1. HIPPOCAMPUS

The hippocampus is a small but complex brain structure, which owes its name to the similarity with the sea horse (from the Latin *hippocampus)*. Some anatomist divides it into hippocampus proper, dentate gyrus (DG), subiculum and entorhinal cortex (EC); entire set is called as hippocampal formation. Glutamate is the main neurotransmitter in the hippocampus; the interneurons of the hippocampal formation are GABAergic. These brain regions play a pivotal role in learning, memory formation and consolidation, novelty detection and spatial navigation. Beyond these cognitive functions, the hippocampus is also critically involved in the regulation of emotion, fear, anxiety, and stress. Besides, the hippocampus is a plastic and vulnerable structure that gets affected in a variety of neurological and psychiatric disorders such as Alzheimer's disease, epilepsy, neuroinflammation, etc.[1].

1.1 Anatomical and morphological aspects

The hippocampus is located in the medial temporal lobe of the brain and lies a group of many millions of neurons organized into a network quite different from that found anywhere else in the nervous system. It is a bulb-like shape structure which protrudes over the temporal horn of the lateral ventricle and occupies the medial region of its floor. Humans and other mammals have two hippocampi, one on each side of the brain, which joined at the stems by the hippocampal commeasure that crosses the midline under the anterior corpus callosum (Fig. 1a).

The hippocampus is an elaboration of the edge of the cerebral cortex, that can be distinguished as a zone where the cortex thins from six layers to the three or four layers of densely packed neurons, which curls into a tight S shape [2]. It consists of two interlocking gray matter folds: Cornu Ammonis, hence the subdivisions CA1 through CA4, and the dentate gyrus. It is a three-layered allocortical structure that is reciprocally connected to other cortical and subcortical areas. The principal neurons of the hippocampus are organized in layers and receive unidirectional polymodal input from the EC, where layer II neurons project via the perforant path to granule cells in the DG. The trisynaptic pathway from the DG to CA3 via mossy fibers and onward to CA1 via Schaffer collaterals is the principal feed-forward circuit involved in the processing of information through the hippocampus. Additionally, layer III neurons from the EC

directly project to CA1 neurons, the major output relay neurons, which in turn project via the subicular complex back to deep layers of the EC and various subcortical and cortical areas. The CA1 fields are separated into four layers (pyramidal, stratum oriens, stratum lucidum, stratum radiatum) and use pyramidal cells as the principal excitatory cells. The DG with its three layers (molecular, granular and polymorphic layers) consists mainly of granule cells and receives polymodal input from the EC. Furthermore, the synaptic connectivity within the hippocampal formation is much more complex than the classic 'trisynaptic pathway', with several parallel input pathways and feedback pathways . Finally, the CA2 hippocampal region, which has traditionally been considered a transition zone between CA1 and CA3, has its own functions and must be regarded as a distinct computational unit on par with CA3 and CA1 [3]. The anatomy of the hippocampus is of chief importance to its function. Hippocampus receives input from and sends output via entorhinal cortex (EC), a structure located in the parahippocampal gyrus, which is strongly and reciprocally connected with many other parts of the cerebral cortex. In addition to EC, several other connections play important roles in hippocampal function. The hippocampus receives direct inputs from the perirhinal and postrhinal cortex. It also receives major subcortical inputs from the medial septum, which sends cholinergic and GABAergic fibers to all parts of the hippocampus, locus coeruleus, raphe nucleus, nucleus reuniens, and amygdala. The CA3 and CA1 regions have a major output to the lateral septum via the fornix. CA1 also projects to the nucleus accumbens, amygdala, and prefrontal cortex. This list is incomplete and is presented here as an indication that the hippocampal anatomy is more complex than the classic trisynaptic circuit. This basic layout of cells and fiber pathways holds across the full range of mammalian species, from hedgehog to human, although the details vary (Fig. 1b).

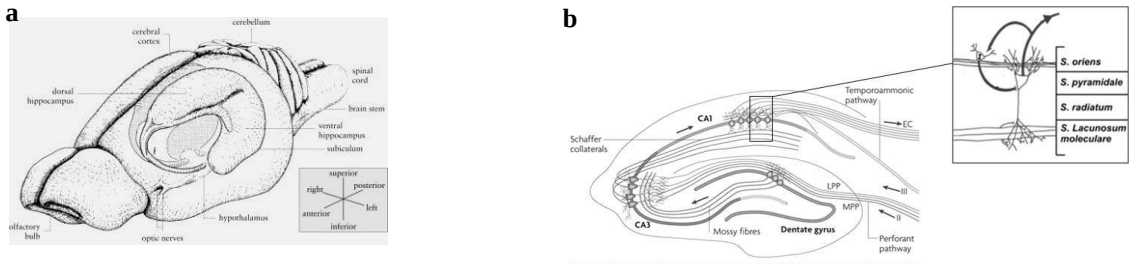


Figure 1 - Anatomy of the Hippocampus
a) The C-shaped hippocampus is shown in a trasparent shell of the rodents brain indicating the longitudinal axis (Adapted from Witter MP and Amaral DG, 2004). b)Schematic rapresentation of hippocampal excitatory trisynaptic pathway (EC–dentate gyrus–CA3–CA1–EC) is depicted by solid arrows. The axons of layer II neurons in the EC project to the dentate gyrus and directly to CA3, through the PP. CA1 receives direct input from EC layer III neurons and sends back-projections to EC. EC: entorhinal cortex ; CA1, CA2, CA3: cornu ammonis fields 1–3; PP: perforant pathway; TA: temporoammonic pathway. (Adapted from Deng W, Aimone JB and Gage FH, 2010).

1.2 Hippocampal functions

Over the years, a variety of functions have been assigned to the hippocampus including learning and memory, emotion, olfaction, and navigation [4]. Since hippocampus receives direct inputs from the olfactory bulb, it was considered to be involved in olfaction for a long time [5].

The hypothesis that hippocampus is related to memory started dominating in 1957 and was supported by studies and observations in which hippocampal removal resulted in a loss of the ability to form new memories. Although it had historical precursors, this idea derived its main impetus from a famous report by Scoville and Milner describing the results of surgical removal of portions of the medial temporal lobes, including the hippocampus, in an attempt to cure a patient H.M. of epileptic seizures [6]. Following the surgery, his cognitive functions were mostly intact; however, he lost the ability to create new memories for facts and events, a condition known as anterograde amnesia [7]. In the ensuing years, other patients with similar levels of hippocampal damage and amnesia have been studied as well, and thousands of experiments have studied the physiology of activity-driven changes in synaptic connections in the hippocampus. There is now almost universal agreement that the hippocampus plays some sort of important role in memory.

Hippocampus is important also in spatial navigation and emotional responses. The discovery of place cells in the 1970s led to a theory that the hippocampus might act as a cognitive map, a sort of device used by the brain for mapping layouts of the environment [8]. Studies conducted on freely moving rats and mice have shown that many hippocampal neurons have "place fields", that is, they fire bursts of action potentials when a rat passes through a particular part of the environment. As with the memory theory, there is now almost universal agreement that spatial coding plays an important role in hippocampal function, but the details are widely debated [9]. A neuroanatomical hypothesis proposed by Papez suggested that the hippocampus was part of a circuit called limbic system, which includes also cingulate cortex, olfactory cortex, and amygdala. Paul MacLean once suggested, as part of his triune brain theory, that the limbic structures comprise the neural basis of emotion and they are associated with the functions of feeling and reacting. Yet, the hippocampus is anatomically connected to parts of the brain that are involved with emotional behavior—the septum, the hypothalamic mammillary body, and the anterior nuclear complex in the thalamus—therefore its role as a limbic structure cannot be completely dismissed [10].

A less well-known function of the hippocampus is its role as a negative feedback regulator of the Hypothalamic Pituitary Axis (HPA). The high concentration of adrenal steroid receptors in the hippocampus and the hippocampal projections to the hypothalamus provide an indirect link between the hippocampus and regulation of the stress response [11].

Finally, the hippocampus is involved in mood disorders; this is suggested by magnetic resonance imaging studies demonstrating a small reduction in hippocampal volume in depressed patients [12].

1.2.1 Functional differentiation along the hippocampal longitudinal axis

The hippocampus is an elongated structure with a longitudinal axis extending in a C-shaped fashion. In rodents, this axis is referred to as the dorsal (septal)-ventral (temporal). The dorsal hippocampus is the most rostral part of the hippocampus. At more caudal levels, the hippocampus curves ventrally (see Fig. 1a). In primates, the entire hippocampus is arranged primarily in an anterior-posterior orientation. Although this intrinsic pattern of connectivity repeats itself along the axis, there are connectivity changes as one moves from one pole to the other. Specifically, the dorsal hippocampus is preferentially connected to the retrosplenial and posterior parietal cortices while ventral hippocampus is connected to the amygdala, prefrontal cortex (PFC), and hypothalamus [13,14]. This suggests that the dorsal and ventral parts of the hippocampus may be responsible for dissimilar and dissociable functions [15,16]. Studies based on targeted lesions, electrophysiological recording and selective pharmacological blockade suggest that dorsal region is mainly involved in cognitive and spatial processing, while ventral region is thought to be involved principally in the regulation of emotion and stress [17,18]. Several studies based on gene expression, anatomical and behavioral measurements have suggested that the hippocampus has different functional organizations along its longitudinal axis. In particular, it has been proposed that either this brain region is organized in multiple functional domains or it is structured according to a spatial gradient [19,20].

1.3 Hippocampus-associated disorders

The hippocampal formation has been implicated in a growing number of disorders, from Alzheimer's disease and cognitive aging to schizophrenia and depression, but only in rare instances, the hippocampus is the sole site of pathological damage. It is subject to the same pathologies that can affect other cortical areas, such as tumors, vascular

malformations, and cortical dysgenesis; it is also notable for its particular vulnerability to damage as a consequence of ischemia/hypoxia, trauma, and hypoglycemia. How can the hippocampal formation be involved in a range of such phenotypically and mechanistically distinct disorders? Neuroimaging findings indicate that these disorders differentially target distinct subregions of the hippocampal circuit [21].

One of the earliest studies of hippocampal dysfunction was that of "patient H.M.", which as described above, after having undergone surgical removal of the hippocampus to treat epileptic seizures, showed a condition known as anterograde amnesia. Such amnesia is typical in disorders that involve hippocampal dysfunction. For example, in Alzheimer disease, the hippocampus undergoes massive cell loss, which is associated with memory deficits that manifest in the early stages of the disease.

Notably, the hippocampus is also particularly vulnerable to neuropsychiatric disease, such as depression. It has been reported that stress and depression are associated with a loss of ability to generate new cells in the dentate gyrus, as well as a loss of dendritic spines and reduced dendritic branching throughout the hippocampus. The hippocampus contains high levels of glucocorticoid receptors, which make it more vulnerable to long-term stress than most other brain areas [22].

Besides, the involvement of the hippocampus is being increasingly recognized in schizophrenia and associated disorders. Individuals who survive a hypoxic episode, a temporary deprivation of oxygen in the brain, often sustain hippocampal damage and anterograde amnesia. Finally, the hippocampus often is the focus of epileptic seizures, which can lead to hippocampal sclerosis, a pathological loss of hippocampal cells.

2. SYNAPTIC PLASTICITY

"When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased." (Hebb, 1949)

One of the most important and fascinating properties of the mammalian brain is its plasticity. Synaptic plasticity is the ability of intrinsic or extrinsic stimuli such as changes in the environment, development or lesions, to modify neural circuit function and thereby modify subsequent thoughts, feelings, and behavior. Principally it refers to the ability of synapses to strengthen or weaken over time as a result of increases or decreases in their activity [23]. Synaptic plasticity plays a central role in early development of neural circuitry and in the capacity of the brain to incorporate transient experiences into persistent memory traces; furthermore, it is also the basis of much brain pathology as seen in various neurological disorders, and maladaptive synaptic plasticity may contribute to neuropsychiatric disorders [24].

Synaptic plasticity implies a direct regulation of pre- and/or postsynaptic neurons through alterations of the synaptic machinery such as changes (i) of the number of neurotransmitter receptors in the postsynaptic membrane, (ii) in the quantity of neurotransmitters released from the presynaptic neuron into a synapse, or (iii) in receptor sensitivity to the released neurotransmitters [25]. Synaptic plasticity has been found at synapses that convey glutamate-mediated excitation or at other synapses that mediate GABAergic inhibition, and these changes span temporal domains ranging from milliseconds to hours, days, and presumably even [26]. Therefore, it can be classified as either short term, which occurs at periods from sub-second to minutes, or long term which changes the efficacy of synapses for hours to years and is thought to form lasting memories that are stored in brain circuits.

2.1 Glutamate receptors

Synaptic plasticity has been most intensively studied at synapses that release glutamate, the major excitatory neurotransmitter in the mammalian brain. Glutamatergic terminals typically connect to postsynaptic "spines", morphologically diverse membrane protrusions, ~1 μm in diameter, that decorate complex dendritic projections from postsynaptic cell bodies [27]. A narrow neck between the heads of mature spines and the main dendritic shaft represents a significant electrical and chemical barrier to the communication of electrical and chemical changes between the two subcellular compartments.

Glutamatergic synaptic transmission is mediated by the ionotropic receptors α-amino-3-hydroxy-5-methyl-4-isoazolepropionicacid (AMPARs), N-methyl-d-aspartate (NMDARs) and kainate receptors. Furthermore, glutamate exerts a prominent modulatory role of the fast excitatory tone set by the ionotropic receptors by activation of the metabotropic glutamate receptor (mGluR) family. Ionotropic and metabotropic glutamate receptors interact in the fine-tuning of neuronal responses under different conditions of activity and their co-localisation indicates that glutamate modulates neurotransmission and neuronal excitability at the same synapses. The actions of glutamate on ionotropic receptors are responsible for numerous physiological processes, including basic neuronal communication, axonal pathfinding, mood regulation, and memory formation. It has been suggested that dysregulation of the glutamatergic system is implicated in a variety of psychiatric and neurological disorders such as schizophrenia, major depression disorder, and Parkinson's disease [28].

2.1.1 AMPA receptors

AMPA receptors (AMPARs) are the mediators of fast excitatory neurotransmission in the CNS. They occur at almost all excitatory synapses in the hippocampus and gate a cation-selective channel. At resting membrane potentials, Na^+ influx accounts for most of the current, but the channel is also permeant to other small monovalent cations, so K^+ efflux can also occur at depolarized potentials. Developmentally and activity-regulated changes in the numbers and properties of AMPARs that are localized at the postsynaptic membrane are essential for excitatory synapse formation, stabilization, synaptic plasticity and neural circuit formation [29].

AMPARs are heterotetrameric assemblies of combinations of four subunits (GluRA1-4) that are products of separate genes. Each subunit has an identical membrane topology and core structure comprising ~900 amino acids with a molecular weight of ~105 kDa. The amino terminus is extracellular, and there are three membrane-spanning domains, one re-entrant loop domain, and an intracellular carboxy (C)-terminal domain [30]. This C-terminal domain is a highly variable region that provides a platform for both the protein interactions and the post-translational modifications that regulate subunit-dependent trafficking and regulation [31]. Depending on their subunit composition, AMPA receptors can also show significant permeability to Ca^{2+} ions. This permeability is determined by the presence or absence of an arginine (R) in a pore-lining segment of the GluR2 subunit. This subunit undergoes post-transcriptional RNA editing resulting in a change of the amino acid at this position from glutamine (Q),

encoded by the genomic sequence, to arginine. The presence of the edited form of GluR2 ensures that the receptor is impermeable to Ca^{2+}; if the GluR2 subunit is absent, the receptor has significant Ca^{2+} permeability [32]. Some studies indicate that in the hippocampus and amygdala, AMPA receptors lacking edited GluR2 predominate in inhibitory interneurons, whereas in pyramidal neurons are largely non-Ca^{2+}-permeable, being GluR1-2 or GluR2-3 tetramers [33,34]. In addition, some fetal GluR2 subunits are not edited.

AMPARs have very fast binding kinetics and a high opening probability. Although most kinetic schemes assume that two glutamate molecules must bind for the receptor to open, it has also been proposed that four binding steps to a tetrameric receptor take place, and that the conductance level of an individual channel increases with the number of glutamate molecules bound [30].

Both the opening probability and the conductance of the channel can be modulated by phosphorylation, phenomena that may play an important role in synaptic plasticity [35-37]. Bound receptors do not remain open continuously but they flicker between open and closed states; even in their open state, they fluctuate among distinct preferred conductance levels. Following clearance of synaptic glutamate, native AMPA receptors deactivate rapidly. Deactivation is probably sufficient to explain the termination of AMPA receptor-mediated EPSCs because glutamate is cleared from the synaptic cleft faster than this. If glutamate is not cleared, however, AMPA receptors close rapidly and enter a desensitized state from which they recover relatively slowly. The time course of desensitization depends on the subunit composition of the receptors.

2.1.2 NMDA receptors

NMDA receptors (NMDARs) are glutamate-gated ion channels that mediate a Ca^{2+}-permeable component of excitatory neurotransmission in the CNS. They play key physiological roles in synaptic function, such as synaptic plasticity, learning, and memory, but are also implicated in numerous neurological disorders and pathological conditions [38]. NMDARs consist of subunits belonging to two relatively distinct subtypes; they exist as heteromultimers of NR1 and NR2A-D subunits [39]. The NR1 subunit is encoded by one gene but exists in several alternatively spliced isoforms. It does not bind glutamate but, instead, contains an important binding site for glycine or D-serine, which acts as a co-agonist. The NR2A-D subunits, on the other hand, contain the glutamate-binding site. They are encoded by four genes and are variably expressed in different regions of the brain and at different stages of development. NMDAR

subunits all share a common membrane topology characterized by a large extracellular N-terminus, a membrane region comprising three transmembrane segments plus a re-entrant pore loop, an extracellular loop and a cytoplasmic C-terminus, which varies in size depending upon the subunit and provides multiple sites of interaction with numerous intracellular proteins [40].

NMDA receptors show many striking properties that mark them out as quite different from AMPA receptors; they have very slow kinetics and can continue to mediate an ion flux for several hundreds of milliseconds after the glutamate pulse has terminated. That is, once glutamate molecules have become bound to NMDA receptors, they remain bound for a long time, during which time the ionophore can undergo repeated opening. In addition to their slow kinetics, NMDARs have other important features: i) their requirement for the binding of two agonists, glutamate and glycine (or D-serine). Synaptic NMDA receptors are temporally controlled by the synaptic release of glutamate for activation, because extracellular agonists are thought to be continuously present at a fairly constant concentration. The distinction of glycine or D-serine appears to depend on brain region in addition to the subcellular localization of the receptor [41-43]; ii) a highly permeability to Ca^{2+} ions and monovalent cations. Accompanying the high Ca^{2+} permeability of NMDA receptors is a relatively high single-channel conductance, which is greater than that of most AMPA receptors; iii) Mg^{2+} ions block the ionophore in a voltage-dependent manner [44]. Thus, at resting membrane potentials NMDA receptors are unable to mediate an EPSC even if glutamate and glycine (or D-serine) are present. They mediate an ion flux only when the membrane is depolarized. Ca^{2+} influx occurs only if there is a conjunction of presynaptic glutamate release and postsynaptic depolarization, a situation that arises when pre- and post-synaptic activity occur together.

2.2 Short-term plasticity

A neurotransmitter is released when an action potential invades the axon terminal causing activation of voltage-dependent Ca^{2+} channels. The ensuing Ca^{2+} influx greatly increases the probability that vesicles docked at active sites in the axon terminal will fuse with the presynaptic membrane. These use-dependent changes in the efficacy of a presynaptic terminal to liberate neurotransmitter define a synapse-specific property, known as short-term plasticity [45]. Most forms of short-term synaptic plasticity are triggered by short bursts of the activity causing a transient accumulation of calcium in presynaptic nerve terminals. This increase in presynaptic calcium, in turn, causes

changes in the probability of neurotransmitter release by directly modifying the biochemical processes that underlie the exocytosis of synaptic vesicles. Every synapse exhibits numerous different forms of short-term synaptic plasticity that play important roles in short-term adaptations to sensory inputs, transient changes in behavioral states, and short-lasting forms of memory [24].

2.2.1 Paired-Pulse ratio (PPR): a measure of the release probability

PPR, it has been used as an easy measure of the probability of transmitter release at the synapse. It consists of applying two consecutive stimuli with a variable interval time and in evaluating the ratio of the amplitude of the second response to that of the first. When pair of stimuli are delivered within a short interval (less than 20 ms) the response to the second stimulus is considerably reduced compared to the first, a phenomenon known as paired-pulse depression (PPD); on the other hand, when two stimuli are applied at longer inter-stimulus intervals (20 to 500 ms) the second response is larger than the first, and this process is known as PPF [46].

Facilitation can be attributed to the transient increase in the concentration of intraterminal calcium produced by an invading action potential. A small fraction of the Ca^{2+} that enters the terminal in response to the first action potential declines to basal values over a few hundred milliseconds, but the calcium influx at the time of the second stimulus adds to the residual calcium from the first, resulting in an enhanced calcium concentration and hence to an increase in the probability of release. Depression of synaptic efficacy also occurs to the second of a pair of stimuli. This may result from inactivation of voltage-dependent sodium or calcium channels or from a transient depletion of the synaptic vesicles that are ‘‘docked’’ adjacent to the presynaptic plasma membrane, waiting to be released. It results in a decrease in the probability of release. Whether a synapse exhibits paired-pulse facilitation or depression depends on the recent history of activation of the synapse. There is the possibility that transient modulation, by activation of protein kinases, of some of the presynaptic phosphoproteins that are involved in the control of transmitter release may play an important role in very short-term synaptic plasticity.

2.3 Long-term plasticity

The brain encodes external and internal events as complex spatio-temporal patterns of activity in large ensembles of neurons that can be conceptualized as ‘neural circuits’. A corollary to this hypothesis is that new information is stored (i.e., memories are

generated) when activity in a circuit causes a long-lasting change in the pattern of synaptic weights [24]. This simple idea was put forth by Ramon y Cajal almost 100 years ago and was further advanced in the late 1940s by Donald Hebb, who proposed that associative memories are formed in the brain by a process of synaptic modification that strengthens connections when presynaptic activity correlates with postsynaptic firing [47]. Experimental support was lacking until the early 1970s when Bliss and colleagues reported that repetitive activation of excitatory synapses in the hippocampus caused a potentiation of synaptic strength that could last for hours or even days. This phenomenon, termed long-term potentiation (LTP), has been the object of intense investigation because it is widely believed that it provides an important key to understanding some of the cellular and molecular mechanisms by which memories are formed and, more generally, by which experience modifies behavior [48,49]. LTP is only one of several different forms of long-term synaptic plasticity that exist in specific circuits in the mammalian brain; most synapses that exhibit LTP also express one or more forms of long-term depression (LTD). Thus, a key concept is that synaptic strength at excitatory synapses is bidirectionally modifiable by different patterns of activity.

2.3.1 LTP: from induction to expression

Several basic properties of LTP make it an attractive cellular mechanism for rapid information storage: i) cooperativity means that it can be induced by the coincident activation of a critical number of synapses; ii) input specificity indicates that LTP is elicited only at activated synapses and not at adjacent, inactive synapses on the same postsynaptic cell, and iii) associativity which is the capacity to potentiate a weak input (a small number of synapses) when it is activated in association with a strong input (a larger number of synapses) (Fig. 2).

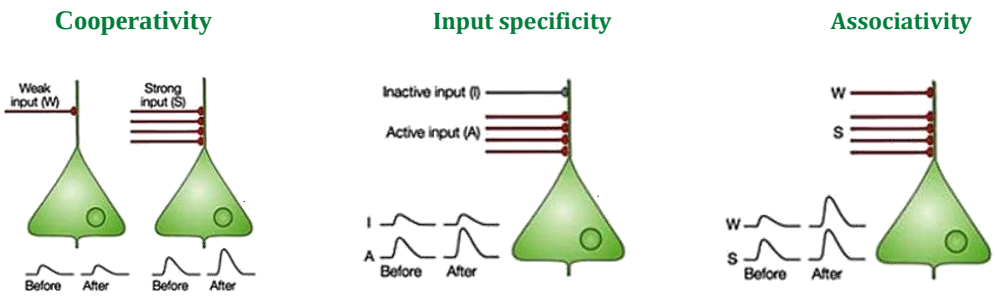


Figure 2 - Basic properties of long-term potentiation
Three key properties of long-term potentiation (LTP) were elucidated in the 1970s and early 1980s. All of these can be explained mechanistically by the biophysical properties of the NMDA receptors that are required to trigger LTP. (From Malenka RC, 2003).

LTP has been defined in many different synapses in the brain and may manifest itself in many different forms in different synapses; the most widely studied forms is NMDAR-dependent LTP because it is a suitable model for associative learning. LTP consists of a complex interplay of biophysical and cell-biological mechanisms operating at the cellular and local circuit levels. In approaching this complexity, it can be distinguished in an induction phase, which comprises the collection of short-lived events that trigger changes in synaptic weight but do not themselves affect it, and in an expression phase, which includes all those mechanisms, whether presynaptic or postsynaptic, that directly enhance synaptic efficacy. Thus, events associated with the activation of NMDA receptors, relief of the magnesium block, permeation of Ca^{2+} through the NMDA receptor and activation of Ca^{2+}-dependent protein kinases can all be considered components of induction. Expression mechanisms include increases in the probability of transmitter release, phosphorylation of AMPA receptors and their insertion into the postsynaptic density. It was already known that under normal resting membrane potential NMDARs are potently blocked by Mg^{2+} ions. Thus, two conditions are necessary for generating an NMDA receptor-mediated response: presynaptic activity to release glutamate and strong postsynaptic depolarization; when spines become strongly depolarized, the Mg^{2+} block is relieved and Ca^{2+} permeates through the receptor leading to an increase in its postsynaptic concentration. Ca^{2+} influx activates signaling pathways that ultimately lead to changes in synaptic responsiveness (Fig. 3). These conditions offer a molecular mechanism for both LTP induction and the properties of associativity and input specificity.

NMDARs activation is not, or at least not always, a sufficient condition; neither NMDA nor glutamate, when applied alone to hippocampal slices, readily induces long-lasting potentiation [50,51]. These results suggest that the expression of LTP is dependent, in part, on presynaptic mechanisms. This point garnered significant attention because it required the production of a retrograde messenger that was released by postsynaptic cells and acted on presynaptic terminals. Popular candidates, such as nitric oxide and arachidonic acid, have largely fallen by the wayside and no longer receive significant attention [52]. Neurotrophins, such as BDNF, have also been proposed to play this role, but there is evidence that a role for BDNF in LTP involves its release from presynaptic, not postsynaptic, sources [53]. Prime additional candidates for synaptic retrograde messengers during LTP include a large number of synaptic cell adhesion molecules, which physically connect and align the presynaptic transmitter release apparatus with the postsynaptic density [54]. On the other hand, numerous studies indicate as major

mechanism of LTP expression, the changes in postsynaptic neurons such as an increase in the numbers of AMPARs within the postsynaptic density (PSD) [55]. This hypothesis began to emerge with the discovery of "silent synapses" that contain only NMDARs with few or no AMPARs, such that at normal resting membrane potentials these synapses exhibit no detectable postsynaptic responses to synaptically released glutamate [56]. The "unsilencing" of these synapses during the induction of LTP is thought to occur through the incorporation of AMPARs into the postsynaptic membrane. These findings led to a large investigative effort into the molecular mechanisms regulating the trafficking of AMPARs [31,57,58]. Evidences suggest that recycling endosomes in the dendrites contain a reserve pool of AMPARs that are mobilized during LTP via a process that requires the small GTP-binding protein, Rab11a [59]. AMPARs do not appear to be inserted directly into the PSD but rather are exocytosed in the perisynaptic regions and then they laterally diffuse in the plasma membrane. PSD-95 has received the most attention and its level appears to be particularly important for controlling the number of AMPARs at individual synapses as evidenced by the findings that its overexpression increases synaptic strength and occludes LTP, whereas knockdown of PSD-95 decreases surface expression of AMPARs and synaptic strength [60-62]. The mechanism that results in AMPAR insertion into the membrane, depends on Ca^{2+} influx into the cell following NMDARs activation. The increase in intracellular calcium activates multiple signaling enzymes including the kinases calcium/calmodulin-dependent protein kinase II (CaMKII), and other Ca^{2+}-dependent enzymes such as PKA, different PKC isoforms, MAPK and tyrosine kinases. The activation of CaMKII is necessary and sufficient for the early phase of LTP in which biochemical events and trafficking of proteins begin, without de novo protein synthesis [63]. CaMKII, along with PKC, phosphorylates Ser831 in the intracellular C terminus of GluA1 subunit to an increase in single-channel conductance of AMPA receptors [64,65]. However, the exact role of Ser831 phosphorylation in vivo is still unclear, since mice lacking phosphorylation at Ser831still show CaMKII-dependent synaptic insertion and normal hippocampal LTP. Other phosphorylation sites may be important: GluA1 is phosphorylated by PKA at Ser845 to regulate the open probability of the channel and promote receptor exocytosis and anchoring at perisynaptic sites [66-68]. CaMKII also phosphorylates the AMPAR-interacting protein stargazin, one of the transmembrane AMPA receptor regulatory proteins (TARPs), that favors its interaction with the synaptic scaffold protein PSD-95, and this interaction helps anchor AMPARs at synaptic sites [69]. Although it remains unclear how CaMKII activation drives the insertion of AMPARs during LTP, it has

been reported that the molecular motor protein myosinVa is required for this effect. MyosinVa associates with AMPARs and this interaction is enhanced through activation of the small GTPase Rab11. This mediates the short-range endosomal transport of GluA1-containing receptors from pools in the dendritic shaft, to the spine head where it can be inserted at the synapse during LTP [70]. The role of phosphorylation in synaptic plasticity also extends beyond the synapse to enable these changes to persist in the long term. The persistence of LTP, called late phase of LTP (defined as the potentiation present more than 1–2 h after LTP induction) depends upon local dendritic protein synthesis and transcription in the nucleus [71]. The signaling to the nucleus depends on a number of protein kinases which activate key transcription factors that may include cAMP response element-binding protein (CREB) and immediate-early genes such as c-Fos. In particular, CREB and other transcription factors are activated via a complex kinase cascade. Calcium entry through NMDARs increases levels of Ras-GTP, which activates the protein kinase Raf; activated Raf stimulates MAPK/extracellular signal-related kinase (ERK) kinase (MEK), which activates ERK1 and ERK2, which in turn, phosphorylate the transcription factors Elk1 and CREB [72,73]. These transcriptional complexes promote expression of effector genes that are required for maintaining the synaptic enhancement and memory consolidation. Several mRNAs can be found in dendrites, including those of the AMPARs themselves and proteins which may function to regulate receptor trafficking [74].

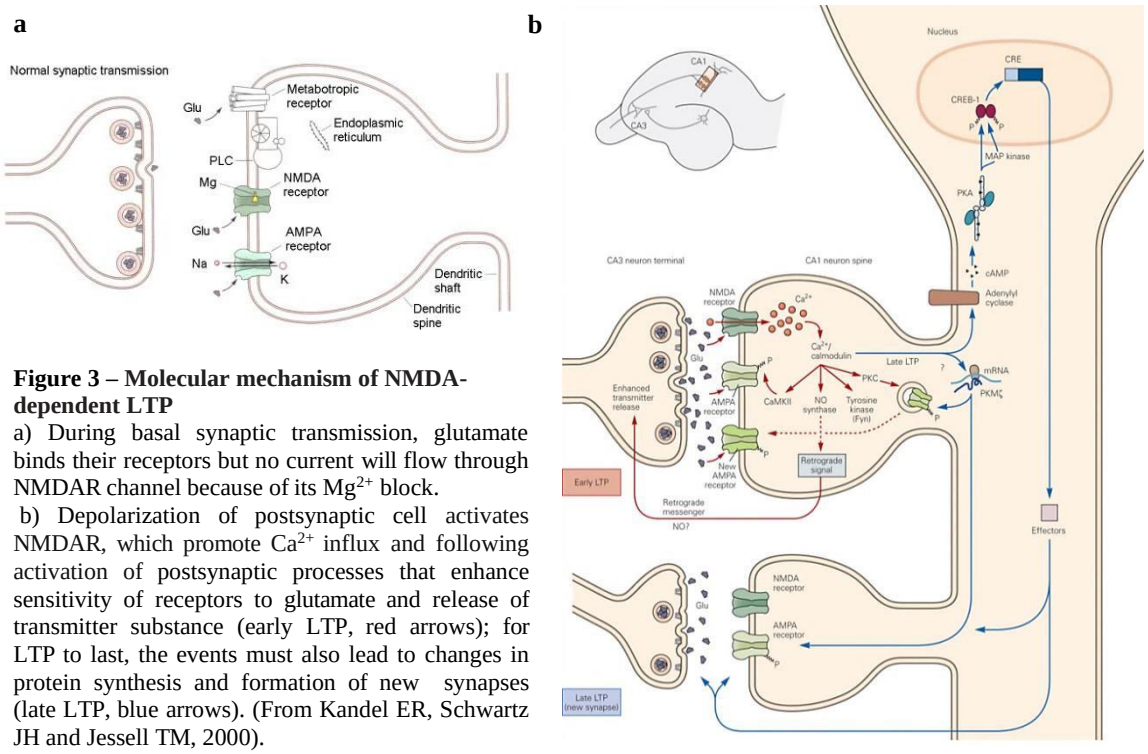


Figure 3 – Molecular mechanism of NMDA-dependent LTP

a) During basal synaptic transmission, glutamate binds their receptors but no current will flow through NMDAR channel because of its Mg^{2+} block.

b) Depolarization of postsynaptic cell activates NMDAR, which promote Ca^{2+} influx and following activation of postsynaptic processes that enhance sensitivity of receptors to glutamate and release of transmitter substance (early LTP, red arrows); for LTP to last, the events must also lead to changes in protein synthesis and formation of new synapses (late LTP, blue arrows). (From Kandel ER, Schwartz JH and Jessell TM, 2000).

2.3.2 Extracellular field potential: an electrophysiological recording to measure LTP.

Field potentials are extracellular potentials recorded from groups of nerve cells in response to synaptic or antidromic stimulation. In laminated structures, field potentials provide surprisingly detailed information on cellular activity. In the hippocampal formation, the dense packing of the cell bodies, the roughly parallel position of the apical dendrites of hippocampal neurons, and the ease with which they can be synchronously activated are three histological features that make this brain structure high favorable for field potential studies.

A field potential is generated by extracellular current flowing across the tissue resistance between the recording electrode and, in general, the ground electrode. Although measurable extracellular voltages are generated by action potentials in a single neuron and form the basis of single-unit recording, synaptic currents generated by single neurons are generally too small to be detected. In laminated structures, the synchronous and localized currents generated by synaptic activation of population neurons gives rise to a characteristic and easily measured response called field extracellular postsynaptic potential (fEPSP). Synaptically generated current flows into the dendrites in the activated region; inside the cells, current flows proximally and distally away from the synaptic region, exiting where membrane area is greatest, notably in the region of the soma. The current loop is completed extracellularly, with current flowing radially from distal and proximal sources towards the sink in the synaptically active region. With a distal ground electrode, these synchronous extracellular currents give rise to fEPSPs that are negative in the region of current sinks and positive in regions of strong current sources [75]. In particular, in the hippocampus, the current sink generated by synaptic activation is in the molecular layer, while the passive source is in the cell body region and in distal dendrites. Such negative field potentials were taken as a measure of the intensity of excitatory synaptic activity and the efficacy of postsynaptic activation.

In electrophysiology experiments, a train of electrical pulses is generally used to depolarize the neuron with high stimulus frequency to induce a rapid Ca^{2+} influx through NMDA receptors and following postsynaptic modifications. LTP is dependent on NMDA receptors (NMDAR) in synapses between CA3 and CA1 hippocampal neurons. Specifically, for field electophysiological recordings, a stimulating electrode is placed in the stratum radiatum of Schaffer collateral terminals and a glass micropipette (0.5–1MΩ) filled with artificial cerebrospinal fluid (ACSF) is placed in the CA1 hippocampal region, at 200–600 μm from the stimulating electrode, in order to measure

fEPSP (Fig. 4a). Stimulus intensity was adjusted to evoke fEPSPs of amplitude about 50% of maximal amplitude with minimal contamination by a population spike. Evoked responses are recorded until reaching stability in the amplitude of fEPSP (at least 10 minute) and only once this is achieved, LTP is induced by an high-frequency stimulation such as trains of 2 x 100 Hz stimulation with a 200-ms interval between bursts. This strong presynaptic stimulation causes a rapid and substantial Ca^{2+} influx at the postsynaptic level which initiates LTP (Fig. 4b). This is believed to resemble the physiological activity that takes place in the brain during learning processes.

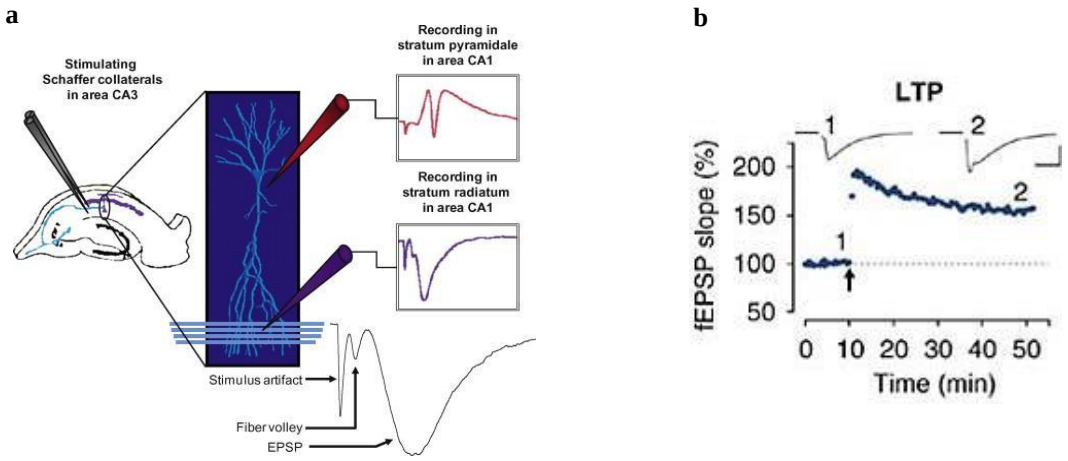


Figure 4 – Extracellular field recording at hippocampal CA1 synapses.
(a) Recording configuration and typical physiologic responses in a hippocampal slice recording experiment. Electrode placements and responses from stratum pyramidale (cell body layer) and stratum radiatum (dendritic regions) are shown. In addition, the typical waveform of a population EPSP is illustrated, showing the stimulus artifact, fiber volley, and population EPSP. (From Sweatt JD, 2010). (b) Inset, fEPSP waveforms recorded during basal synaptic trasmission (1) and after LTP induction (2). Bottom, sample experiments illustrating stratum radiatum CA1-LTP. Time course of fEPSP slope normalized to baseline is plotted as a function of time. Panel demonstrates LTP elicited by high-frequency stimulation (black arrow). (from Citri A and Malenca RC, 2008) .

3. NEUROINFLAMMATION

Although the CNS is an immune-privileged site, due to the presence of the blood-brain-barrier (BBB) which partially separates the CNS from the peripheral immune system, inflammatory reactions can occur within the CNS [76]. Indeed, neuroinflammation is recognized to be a prominent feature in the pathology of several acute and chronic brain diseases [77]. Neuroinflammation is a term used to describe the broad range of immune responses of the central nervous system, differing from peripheral inflammation in several ways, primarily concerning the principle cells involved. It consists of a protective response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is the protective attempt by the organism to remove the injurious stimuli and initiate the healing process for the tissue [78]. This process is orchestrated by the mobilization and interaction of several cell types and signaling molecules, producing a response that is both local and systemic (Fig. 5). The cell types that play a central role in the inflammatory response are microglia, considered the immune resident cells of the CNS, and the astrocytes, which perform many functions including maintenance of the extracellular ion balance and support of the BBB. These cells regulate both the induction and limitation of neuroinflammatory processes [79].

3.1 Molecular components of neuroinflammation

Cytokines and chemokines are neuromodulatory molecules involved in the regulation of CNS-immune system interactions and mediation of neuroinflammation. They are important for the coordination of immune responses throughout the body and for the brain's immune function that serve to maintain immune surveillance, facilitate leukocyte traffic, and recruit other inflammatory factors. These molecules are produced not only by white blood cells or leukocytes but also by a variety of other cells as a response to various stimuli under both pathological and physiological conditions. Cytokines cause the exacerbation or reduction of neuroinflammation: pro-inflammatory cytokines such as interleukin (IL)-1 β and tumor necrosis factor (TNF) α play an integral role in pathological inflammation and acceleration of disease; conversely, several cytokines, including IL-4, are largely anti-inflammatory. Chemokines are small chemotactic cytokines and although they have very low physiological concentrations within the CNS, levels of certain chemokines such as monocyte chemoattractant protein (MCP)-1 are strongly up-regulated in chronic neuroinflammation [80]. They are involved in the up-regulation and chemotaxis of astrocytes and microglia in response to an

inflammatory stimulus and may also disrupt neuronal function and adversely affect neurogenesis. Besides, cytokines and chemokines have adverse effects on the integrity of the BBB, allowing it to become more permeable and enabling the entry of leukocytes into the brain [81]. Upon stimulation by pathogens or abnormal cells, immune cells, as well as cells of the nervous system such as microglia, astrocytes, and even neurons, can release cytokines and chemokines that in turn interact with their receptors [82]. The binding of a cytokine or chemokine ligand to its receptor results in the trigger of a cascade of signaling events that regulate various cellular functions such as phagocytosis, cytokine secretion, cell activation, cell proliferation, etc.; for example, IL-1 β, when bound to the IL-1 receptor, starts a signal transduction cascades, namely mitogen-activated protein kinase pathways (MAPK) [83]. p38 MAPK is a stress-activated protein kinase and its activation results in many pro-inflammatory responses and the production of IL-8 and IL-6 [84].

The complement cascade, activated by the lectin-binding pathways, is an important feature of immunity and inflammation; it contributes to processes such as mast cell degranulation, chemotaxis, and cell lysis. Complement was not perceived to play a role within the CNS until relationships between complement proteins and glial cells were observed such as the roles of C3a, C3b, C5a in the chemotaxis and phagocytic functions of microglia in neuroinflammation [85]. As with many aspects of innate immunity, the complement cascade is generally considered a double-edged sword within the CNS exhibiting a protective effect at physiological and acute levels but causing damage if stimulated chronically.

The enzyme cyclooxygenase (COX) converts arachidonic acid to thromboxanes and eicosanoid groups such as prostaglandins (PGs), lipid metabolites that are involved in several physiological and pathological processes, including inflammation. Two distinct COX isoforms have been characterized, COX-1 and COX-2, that differ in terms of regulatory mechanisms, tissue distribution and preferential coupling to upstream and downstream enzymes in the CNS. The pathways of its two common isoforms COX-1 and COX-2 are becoming increasingly associated with neuroinflammation and neurodegeneration, as well as COX inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), therapeutic potentials [86]. Both these isoforms have different roles both in normal physiology and pathology. COX-1, which is constitutively expressed in most tissues, has been classically considered as the isoform primarily responsible for homeostatic PG synthesis; COX-1expression leading to prostaglandin synthesis is seen in microglia [87], and it is thought that activation of these cells could lead to an excessive

release of prostaglandins. Many aspects of the COX-1 pathway are pro-inflammatory, resulting in damaging neuroinflammation and cognitive impairment [88]. Pathological associations have also been observed in traumatic brain injury and neurodegenerative diseases such as Alzheimer's disease [89]. By contrast, COX-2 is mainly induced in response to inflammatory stimuli, which led to the concept that selective inhibition of COX-2 can reduce inflammation [90,91]. However, in the CNS, COX-2 is also constitutively expressed, mainly in hippocampal and cortical glutamatergic neurons where it has a pivotal role in synaptic activity and long-term synaptic plasticity [92] .

The strong involvement of COX-1 in neuroinflammation compared to COX-2 could be related to its expression in microglia, whereas COX-2 overexpression may only be exhibited in neuronal damage. Cytokine signaling also interacts with these pathways. IL-1 β induced MAPK activation promotes COX-2 gene expression and the cyclooxygenase pathway itself stimulates the production of IL-6, which shows one of many positive feedback loops in the systemic inflammatory response [93]. The complexities of eicosanoid signaling in neuroprotection and neurodegeneration highlights the difficulties in forming therapeutic strategies.

3.2 Microglia: a central player in neuroinflammation

Microglia plays a crucial role in the process of neuroinflammation because these innate immune cells perform the primary immune surveillance and macrophage-like activities of the CNS. Indeed, much of the innate immune capacity of the CNS is mediated by microglia. These cells are the resident macrophages of the CNS that populate all regions of the CNS and reside in both the white matter and gray matter of the brain and spinal cord. They are derived from the yolk sac, and takes up residence in the brain during development, representing up to 12% of the cells of the CNS [94]. In the mature CNS, microglia have an active role in immune surveillance of their microenvironment by acting as scavengers of debris and microbial pathogens using their processes. Other immune-related activities include the propagation of inflammatory signals that are initiated in the periphery; these responses are pivotal in the coordinated communication between the immune system and the brain. In response to cytokines and other signalling molecules from acute inflammation as well as in the case of pathological states within the nervous system including lesions, ischemic stroke and infection or in case of severe or chronic stressful conditions, microglia transform from a surveying, ramified state to an activated, phagocytic one; activated microglial cells rapidly alter their transcriptional

profile and produce proinflammatory factors like cytokines and chemokines, glutamate, and reactive oxygen species among others and help mobilize the adaptive immune response and cell chemotaxis, leading to trans-endothelial migration of immune cells across the BBB. Furthermore, these alterations allow microglia to migrate towards sites of injury or infection and potentially increase their phagocytic efficiency [95,96]. Active microglia also undergo cytoskeletal rearrangements that alter the pattern of receptor expression on the cell surface. Toll-like receptors (TLRs) are important signal transduction proteins in the innate immune system and inflammatory response. These are pattern recognition receptors that are activated on detection of a foreign microbe and initiate downstream signaling cascades. TLR-4 is of particular importance as it is induced by lipopolysaccharide (LPS), an endotoxin found in the outer membrane of gram-negative bacteria and commonly used to induce an inflammatory response in animal models [97]. TLR-4 activation induces TNFα and IL-1 β release and is a key receptor in proinflammatory signaling. Microglia express a range of TLRs that activate these cells and initiate a neuroinflammatory reaction. Besides, microglia express the two classes of major histocompatibility complex, MHC class 1and MHC class 2, and although these antigen presenters are mainly involved in the reaction to infectious disease, they are thought to play a role in the development of neuroinflammation [98].

In general, microglial activation and the increased expression of cytokines are intended to protect the CNS and benefit the host organism. Nonetheless, these cells can remain activated for extended periods, so that an amplified, exaggerated, or chronic microglial activation can establish a feedback loop that perpetuates inflammation and ultimately results in pathological changes and neurobehavioral complications such as depression and cognitive deficits [99]. Thus, a critical balance between repair and proinflammatory factors determines the rate of progression and outcome of a neurodegenerative process [100].

3.3 Neuroinflammation and synaptic plasticity: the role of immune system

Over the past two decades, it became evident that the immune system plays a central role in modulating learning, memory and neural plasticity [101]. Although the immune mechanism has an essential neuroprotective role in the brain, it is also physiologically implicated in the remodeling of synaptic circuits. The CNS and the immune systems are known to be engaged in intense bidirectional crosstalk, and glial cells are considered as a crucial third element of the synapse, contributing to neurotransmission and several

forms of synaptic plasticity like LTP. However, a status of neuroinflammation rapidly disrupts this system altering neuronal networks functioning and leading to cognitive impairments. The increased production of immune diffusible mediators, such as cytokines and prostaglandins, can disturb the delicate balance needed for the neurophysiological actions of immune processes and generate deleterious effects on synaptic transmission and brain plastic phenomena. Cognitive impairments are very common in inflammatory disorders, such as trauma, neurological diseases, severe or chronic stress and aging [102-105] but the mechanisms responsible of these deficits are still poorly understood. Immune responses are complex, being either neuroprotective or detrimental, and largely depending on the stimulus, the context, the duration of the inflammatory process and the type of activated inflammatory cells. Certainly, a greater understanding of the immune-mediated control mechanisms of synaptic plasticity could represent the basis for the development of a novel immune-centered therapeutic approach to neurological disorders. Moreover, the effects of these conditions are mediated by some immune diffusible mediators, as will be described below.

3.3.1 TNFα and IL1-β

Among others proinflammatory cytokines, IL-1β and TNFα are the best-studied molecular actors in both physiological and pathological conditions and have been associated with LTP.

TNFαis a proteolytically cleaved transmembrane protein whose activity is performed through TNF receptor type 1 (TNFR1) and type 2 (TNFR2) [106]. In a physiological state, the glial pathway that regulates TNF release is itself controlled by TNFa, but when the balanced system is strongly disturbed, the homeostatic mechanism fails [107]. This cytokine is an important regulator of synapse function and its involvement in neural plasticity processes has been much studied. It was shown that TNFα is involved in LTP maintenance by binding to both TNFR1 and TNFR2 [108]. The detrimental effect of TNFαon memory was first demonstrated by showing impaired learning in adult mice that over-express TNFαwithin the CNS [109]. Consistently, several data suggest that TNFαmassively released during neuroinflammation exerts different brain area-specific effects; it has been shown that microglia-released TNFα improves LTP at C-fiber synapses in spinal dorsal horn, whereas it impairs hippocampal LTP at CA3-CA1 synapses and the same effect was observed after intrahippocampal injection of TNF in healthy mice [110,111]. In contrast with these findings, most studies reported less

consistent effects of excess TNFα signaling on memory functioning and one study showed that chronic exposure to TNFα potentiates LTP in CA1 region [112-114]. These contradictory data do not provide definitive conclusions regarding the role of TNFα in memory processes; however, they can suggest that basal levels of TNFα are not required for memory, as TNFα deficient mice demonstrate no memory impairments, and the negative influence of TNFα appears to be both dose- and age-dependent.

IL-1β is the product of the proteolytic cleavage of its mature form pro-IL-1β. IL-1β exerts its biological action by binding to IL-1 receptor type 1 (IL-1RI), competing with IL-1 receptor antagonist (IL-1ra), the endogenous inhibitor of IL-1β. A bulk of data indicates the detrimental effect of IL-1 β in synaptic mechanisms, like LTP, underlying learning and memory. It was demonstrated that increased levels of IL-1β inhibit LTP in CA1, CA3, and DG of the hippocampus, either after in vitro application of the cytokine or in vivo intracerebroventricular administration [101]. To explore the effects of chronic exposure to IL-1 β, a recent study examined the effects of chronic transgenic over-expression of IL-1 β within the hippocampus, showing that sustained elevation of IL-1β levels, which resulted in neuroinflammation, produced marked memory deficit [115,116]. IL-1 β has also been shown to dose-dependently affect Ca^{2+} conductance through NMDARs, being able to improve or inhibit Ca^{2+} influx at low or high concentration, respectively [117]. Among others, these are the putative mechanisms by which IL-1β improves or impairs LTP induction. Studies conducted in animal models of disease associated with an inflammatory state show that LTP is impaired in correlation with IL-1 β levels and memory deficits are recovered by treatment with antagonist of IL1-β receptor [118]. Thus, these data clearly demonstrate a detrimental effect of elevated IL-1β levels on memory functions.

3.3.2. Prostaglandins

Prostaglandins (PGs) are important inflammatory mediators, synthesized from arachidonic acid by the enzyme COX 1 and 2. Within the brain, PGs are mainly produced following the induction of COX-2 by various stimuli, including inflammatory challenges, particularly IL-1 β, as well as synaptic activity [101]. Among the others, PGE_2 exert a variety of biological activities in the brain; however, it is only recently shown that PGs are involved in memory functioning and synaptic plasticity [119]. Basal levels of PGE_2 seem to have a role in neural plasticity; this has been demonstrated by several lines of research showing that COX-2 activity is upregulated during LTP, PGs

production by COX-2 can occur in postsynaptic dendritic spines, and inhibition of COX, but not COX-1, activity results in impaired hippocampal LTP, which can be completely rescued by exogenous application of PGE_2 [90,120-122]. Although basal levels of PGE_2 seem to be required for LTP, the increase of PGE_2 levels, which occurs in some pathological conditions, may be detrimental for LTP. Specifically, selective inhibition of COX-2 was found to reverse memory and synaptic plasticity impairments produced by several conditions associated with neuroinflammation, including Alzheimer's disease, acute and chronic LPS administration, stress and aging [123-126] .

Besides, the transgenic over-expression of COX-2 resulting in marked elevation of brain PGE_2 levels, as well as a direct administration of PGE_2 into the brain, was found to impair memory functioning but only in aged mice, suggesting that developmental compensatory mechanisms are sufficient to counteract the detrimental effects of elevated PGE_2 levels in young animals, and that memory deficits occur when these mechanisms are deteriorating during aging [127]. Finally, memory impairments induced by IL-1 β administration were found to be blocked by co-administration of the non-specific COX inhibitors suggesting that the detrimental effects of IL-1 β on LTP are at least partly mediated by elevated PGE_2 levels [128]. However, the mechanism that explains how PGE_2 affect synaptic plasticity is complex and not well understood because they act on different receptor subtypes, which have different functions in intracellular signal transduction based on the cell type involved, the action site (pre- or post-synaptic), and the amount of prostaglandins [119].

Four subtypes of G-protein coupled receptors for PGE2 have been identified: EP1, EP2, EP3, and EP4, each encoded by distinct genes. It was been shown that these EPs subtypes are heterogeneously expressed both in neurons and astroglial cells, whit EP2 and EP3 abundantly expressed in the hippocampus and cortex, while EP1 and EP4 only at detectable levels [129]. Interestingly, EP2 and EP4 are well merged with synaptophysin, but not PSD-95, indicating that these two subtypes of EPs are expressed in presynaptic terminals and providing important avenues for PGE2 signaling in synaptic activity. Binding of PGE_2 to these receptor subtypes initiates a signal transduction via secondary messengers, such as cAMP. Specifically, the activation of EP1 results in a release of intracellular Ca^{2+} from inositol trisphosphate (IP3) stores, whereas EP2 or EP4 activation stimulates adenylate cyclase, resulting in increased levels of intracellular cAMP; conversely, activation of EP3 inhibits adenylate cyclase [130]. These opposite effects of EP2 and EP3 on the production of cAMP could be involved in the modulation of synaptic plasticity. Another important aspect is that an high-frequency stimulation,

that induces LTP, causes a bidirectional trafficking of EP2 and EP3, which are mainly located in the cytosol and at the membrane, respectively, resulting in an increase in EP2 and a decrease in EP3 at the membrane; this predominant location of EP2 at the membrane, can easily induce the production of a large amount of cytosolic cAMP, wich in turn activates PKA, leading to the activation of CREB, a transcription factor involved in maintenance of LTP [131]. It is also possible that in the hippocampus, PGE2 works as a retrograde messenger, acting at the presynaptic site where EP2 is distributed [132]. Therefore, it is important to regulate the production of PGE_2 for the maintenance and successful accomplishment of ordinary synaptic function.

3.3.3. BDNF

Most research on the relations between immune mechanisms and neurotrophins concerning neurobehavioral plasticity focused on BDNF. This is understandable given that the production and signaling of BDNF via the TrkB receptor has been implicated in almost every aspect of neural and behavioral plasticity, including hippocampal-dependent memory and LTP [133,134]. BDNF and its receptors are expressed not only by neurons but also by astrocytes and microglia. For example, BDNF secreted by activated microglia can influence neuronal functioning, producing neuroprotective effects, but it can also act in an autocrine manner to promote proliferation and survival of microglia [135]. BDNF, acting via Trk-B receptors on microglia, can also induce sustained elevation of intracellular Ca^{2+} in these cells, which in turn can have anti-inflammatory effects [136]. Several lines of evidence suggest that decreased BDNF signaling seems to contribute to the detrimental effects of immune processes on learning, memory, and LTP. For instance, it was shown that, following learning and memory tasks, mice exhibit increased expression of BDNF as well as ERK1/2 activation, which underlies BDNF's effects on memory consolidation; whereas IL-4 deficient mice or IL-1ra transgenic mice, which display impaired performance, do not shows such an increase [137]. In addition, it seems that the detrimental effect of IL-1 β on memory is mediated by alteration in BDNF signaling cascades; this hypothesis is supported by studies in which intra-hippocampal IL-1 β or LPS administration reduces BDNF and TrkB expression, while the block of LPS effect increases the expression of this neurotrophin [138,139]. The inhibition of prostaglandin synthesis, which disturbs learning, memory, and LTP, is also accompanied by diminished production of PGE_2 and BDNF following spatial learning and LTP. Moreover, the endogenous elevation of BDNF levels is sufficient to reverse this detrimental effect and restore a parallel increase in LTP and learning-related BDNF

and PGE_2 [122]. Together, these converging lines of data suggest that a reduction in BDNF expression may underlie the detrimental effect of immune activation on memory. Moreover, not all findings are consistent with a role for BDNF because it has been shown that BDNF expression did not vary following LPS administration, which causes marked learning deficits [123].

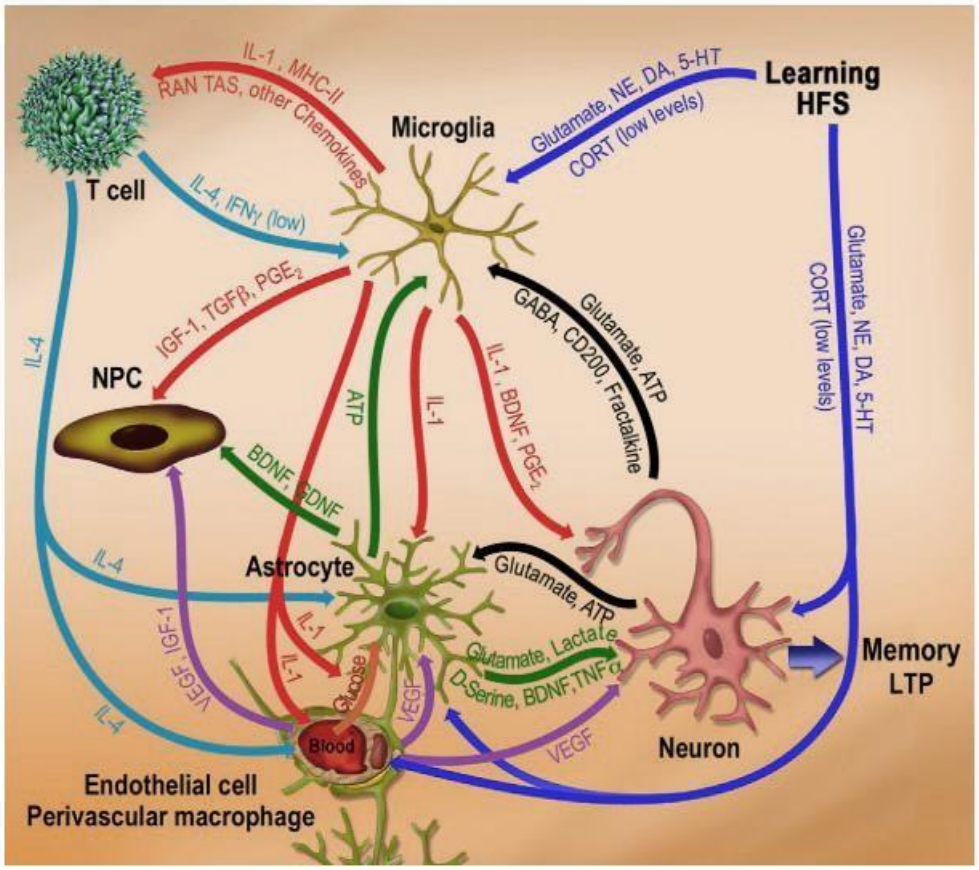


Figure 5 - Schematic description of the complex interplay between immune system and brain in regulating neural plasticity and behavioral processes at molecular and cellular level.
During learning or high frequency stimulation (HFS) that induces LTP, the external glutamatergic, monoaminergic and adrenocortical input can activate neurons, microglia and astrocytes (blue arrows). Signaling via specific receptors expressed on these glia cells induces the production of various mediators. For example microglial cells can release IL-1, as well as other inflammatory mediators, (red arrows) which can in turn further activate astrocytes, inducing the secretion of several compounds that are critical for memory formation and synaptic plasticity, such as D-serine, BDNF, TNFa and additional glutamate (green arrows). IL-1 has also been shown to facilitate glucose uptake and the production of lactate by astrocytes, which are important for long-term memory consolidation. Microglia and astrocytes also secrete various compounds that directly influence neuronal functioning and neural precursor cells (NPCs) (which underlie hippocampal neurogenesis), including BDNF, IGF-1, TGFb, and low levels of TNFa and PGE2. Microglial-derived IL-1 can also directly influence neurons by upregulating NMDA receptor functioning. Importantly, the production of IL-1 and other glial mediators is tightly regulated by neuronal-derived factors , including GABA, CD200 and fractalkine. (black arrows). Microglial expression of IL-1, MHC-II and various chemokines can influence T cells, which play an important role in learning and neurogenesis (light blue arrows). Finally, IL-1 can activate endothelial cells, which produce various trophic factors important for memory, neural plasticity and neurogenesis (purple arrows). (From Yirmiya R and Goshen I, 2011).

4. MAJOR DEPRESSIVE DISORDERS

The most common form of depression is the Major Depressive Disorder (MDD), a seriously disabling public health problem of very high prevalence affecting more than 300 million people across the globe, an increase of more than 18% between 2005 and 2015 [140]. This disorder is twice as common in women as in men, with an incidence that is more than 12% in men and 20% in women [141]. The age-of-onset is prevalent for the entire lifespan though the most common time of onset is between the ages of 20 and 30 years, with a later peak between 30 and 40 years [142]. Clinically, it is a mental disorder characterized by the prolonged presence of specific somatic and cognitive abnormalities in combination with sad, empty or irritable mood, low self-esteem, and loss of interest or pleasure in normally enjoyable activities, or anhedonia; in addition, there are several other symptoms causing marked functional impairment, such as poor concentration and memory, sleep disturbance, lack of energy and appetite disorders [143]. In severe cases, depressed people may have symptoms of psychosis including delusions or hallucinations, and recurrent morbid thoughts about death and suicidal ideation; this last one is a serious problem because about 60% of people who commit suicide had depression [144]. Furthermore, it is important to note that depression is often accompanied by other psychiatric disorders, especially substance use disorder, impulse control disorder, and anxiety disorders [141].everal aetiological and pathophysiological factors contribute to the development and maintenance of depressive symptoms; MDD can be described in terms of dysfunctions on neurochemical, neuroendocrine, immunological, functional and structural anatomical, and cognitive levels (Fig. 6). Although so far there is no clear understanding of the neurobiological basis underlying the pathology of depression, it's known that many brain regions, such as amygdala, thalamus, hippocampus, prefrontal and so on, are involved in this disorder. Human brain imaging studies on autopsy of depressive patients have demonstrated many abnormities in these regions and the most frequently reported findings obtained by this technology are diminished grey-matter volumes and reduced glial densities of hippocampus and prefrontal cortex (PFC) in depressive patients, while it is still inconclusive whether these alterations in hippocampus and PFC represent a precipitating factor or are just a result of major depression.

4.1 Neurobiology of depression

Depressive disorders are complex neurobiological conditions for which there is no simple explanation as to what causes it. It is clear now that MDD is associated with a

wide range of risk factors that, over the years, have stimulated the development of a whole array of hypothesis of depression concerning the development of clinical symptoms in terms of dysfunctions on different neurobiological levels. Only some hypothesis of depression will be described below.

4.1.1 Neurochemical hypothesis

The most influential neurobiological discoveries related to depression have probably been neurotransmitter-related abnormalities with the monoamines having received most attention. Monoamines are neurotransmitters and neuromodulators that include serotonin (5-HT), dopamine, norepinephrine, and epinephrine. The monoamine hypothesis of depression not only proposes that the biological basis for depression is directly related to decreased monoaminergic transmission, especially serotonergic, but also that many antidepressant drugs acutely increase synaptic levels of the monoamine neurotransmitters tending to restore normal function in depressed patients. Early observations of the ability of tricyclic antidepressants to relieve depressive symptoms and potentiate serotonin and noradrenaline activity triggered a wide range of neurochemical investigations in patients with depression. It has been shown, for example, that depression is associated with decreased serotonin transporter binding in the midbrain and amygdala, decreased 5-HT1A receptor binding in frontal, temporal and limbic regions and increased density of monoamine oxidase A (MAO-A), an enzyme which metabolizes monoamines [145-147]. All these findings are consistent with the idea of some sort of monoaminergic dysfunction. Furthermore, studies exploring the effects of experimental manipulations of monoaminergic pathway activity, for example, through restricting the availability of the serotonin precursor tryptophan, report that only those who had previously suffered from depressive episodes or who have a family history of depression develop depression-like symptoms in response to impaired monoamine function [148]. The inability to induce depression in those without vulnerability to the disorder thus suggests that impaired monoamine pathway activity alone is probably not sufficient to cause depressive episodes.

The monoamine hypothesis has enjoyed considerable support since it attempts to provide a pathophysiologic explanation of depression and it has been of great importance in the development of safe and effective pharmacologic agents for its treatment. However, it does not address key issues such as why antidepressants are also effective in other disorders such as panic disorder, obsessive-compulsive disorder, and

bulimia, or why all drugs that enhance serotonergic or noradrenergic transmission are not necessarily effective in depression.

Despite the main focus has been on monoamines in the last decades, there is also accumulating evidence for changes in other neurotransmitter systems associated with depression, specifically the gamma-aminobutyric acid (GABA) system and the glutamate system. For instance, it was shown that in depressed patient, GABA levels appears to be lowered in the plasma and cerebrospinal fluid and the density of specific GABAergic interneurons is reduced in prefrontal and occipital cortical regions [149,150]. Abnormalities of glutamate in plasma, serum, cerebrospinal fluid, and brain tissue have also been described in patients with depression and magnetic resonance spectroscopy imaging has yielded some evidence for decreased levels of glutamate especially in anterior brain regions [151,152]. Furthermore, ketamine, a glutamatergic NMDA receptor antagonist, has repeatedly been shown to exert rapid antidepressant effects [153].

4.1.2 Neurotrophic hypothesis

The brain-derived neurotrophic factor (BDNF) is a neurotrophin widely expressed in the brain where it is implicated in neuronal growth, synaptic plasticity, neuronal survival, and it has been suggested as a biomarker of depressive disorders [154]. Clinical findings have detected decreased BDNF expression in the hippocampus of depressed suicide patients, and increased levels in patients medicated with antidepressants before death [155,156]. Furthermore, serum BDNF in living depressed patients is abnormally low but can be restored following pharmacological antidepressant treatment [157]. These observations provided a basis for neurotrophin theory stating that depression is caused by decreased levels of this neurotrophic factor in both human depression and a range of rodent models of the disorder and that antidepressants neutralize this deficit [158]. Because BDNF is known to enhance synaptic plasticity in various brain regions, it is reasonable to postulate that improving BDNF function may be beneficial and, supporting this idea, direct injection of BDNF into the hippocampus of experimental animals induces behavioral changes similar to antidepressant treatment. Thus, BDNF and its tyrosine related kinase (Trk) B receptor, seems to be promising targets of novel antidepressant therapies.

Despite these observations, this hypothesis of depression has never received universal support, with a considerable number of studies generating evidences that directly contradict the existence of a simple relationship between total brain BDNF levels and mood. For instance, while the antidepressant efficacy is suppressed in

experiments using inducible BDNF knock-out mice, depression-related behaviors are only seen in females, showing significant gender differences [159]; moreover, forebrain-specific conditional TrkB receptor knockout mice do not exhibit depression-related behaviors such as increased behavioral despair in the forced swim test, whereas it has been demonstrated that activation of TrkB receptor is required for antidepressant-induced behavioral effects [160,161]. Furthermore, while a loss of BDNF may not generate spontaneous depressive traits, it may represent a predisposing factor, and manipulation such as chronic stress is necessary for its manifestation and subsequent detection.

One hypothesis that can explain this contradictory role of BDNF has been put forward by Castren et al. (2007), who suggests that BDNF may act as a 'critical tool' in modulating activity-dependent plasticity within emotional processing networks, the integrity of which may be compromised in depression [162]. The physiological function of such plasticity, as well as the extent to which it is modulated, may determine the magnitude and the direction of the impact BDNF levels have on mood.

4.1.3 Genetic factors

There is strong evidence that genetic factors may play a significant role in the onset of MDD. Depression is known to run in families, suggesting that genetic factors contribute to the development of this disease with about 40% risk if a biological parent has been diagnosed with the illness. However very little is known for certain about the genetic basis of the disease; it is clear from studies of families that major depression is not caused by any single gene but variations in many genes, each with a small effect, combine to increase the risk of developing depression and predispose some people to become ill.

Several studies have identified chromosomal regions with linkage to the disorder, and some of these loci have been replicated in more than one study, although no single chromosomal region has been replicated in every family study of genetic linkage in depression. Holmans et al. (2007) found evidence of linkage of recurrent, early-onset depression to chromosome 15q25-q26, but the population attributable risk was small [163]. No specific molecular risk factor has been reliably identified. One common polymorphic variant of the serotonin-transporter–linked polymorphic region (5-HTTLPR), which affects the promoter of the serotonin transporter gene, causes reduced uptake of the neurotransmitter serotonin into the presynaptic cells in the brain and confers a predisposition to depression; brain imaging reveals functional differences in emotion-related areas of the brain among carriers of the different common

polymorphisms of 5-HTTLPR although a direct relation to depression is unclear [164]. In a large epidemiologic study, Caspi et al. (2003) found that 5-HTTLPR predicted depression only in association with defined life stresses [165]. Some environmental factors could confer a predisposition to depression by affecting the genome epigenetically; for example, increased maternal care in rodents causes an epigenetic change in the promoter region of the glucocorticoid-receptor gene [166].

Other hypothesized genomic influences are BDNF polymorphisms; BDNF Val66Met (G196A, rs6265) polymorphism is located at an exon and changes an amino acid at codon 66 from Val to Met. Positive association studies have been published; however, whether the Met or Val allele is a risk allele for depression has not been confirmed. Frielingsdorf et al. (2010) reported that Met homozygotes may have a greater risk for major depressive disorder [167]. Another study found that Val homozygotes have an increased chance of depression [168]. However, negative association results have also been found and a meta-analysis involving 14 studies reported that BDNF Val66Met was not significantly associated with depression in a total sample and that a significant effect was found only in men [169]. Taken together, these results indicate that the genetic effect of BDNF Val66Met on depression is not fully understood.

The largest genome-wide study to date failed to identify variants with genome-wide significance in over 9000 cases [170]. Recently, the first genetics study has been published with positively identified two variants in intronic regions near SIRT1 and LHPP with genome-wide association with major depressive disorder [171].

4.2 Is major depression an inflammatory disorder?

Despite MDD cannot be considered a pure 'inflammatory' disease, evidence is accumulating to show a link between inflammation and depression and the fact that these two conditions are closely connected and may fuel each other in a bidirectional pathway. Despite the mechanisms by which inflammatory mediators modulate the pathways that are implicated in the etiology of depression is not completely understood, it is becoming increasingly clear that inflammation is able to affect cognition, increase the complexity and severity of illness presentation and contribute to the production of a pattern of symptoms, clustering in a syndrome named 'sickness behaviour' closely related to depression [172-174]. Several studies have pointed to the presence of increased expression of pro-inflammatory cytokines, whose main cellular source in the brain is microglia, and their receptors both in peripheral blood and cerebrospinal fluid (CSF) of some depressed subjects [175]; also, patients treated with cytokines are at increased risk of

developing this disease, while the blockade of cytokines or inflammatory signaling pathway components, such as COX-2, has been shown to reduce depressive symptoms [176,177]. Thus, these studies indicate an association between inflammatory markers and MDD but not the causal relationship and it has not been found yet a correlation between inflammatory levels and the severity of depressive symptoms. Interestingly, it was demonstrated that inflammatory cytokines can change brain function and structure through mechanisms that include an increase in glutamate-induced neurotoxicity, effects on monoamine neurotransmission and HPA axis. Besides, proinflammatory cytokines increase the activity of serotonin transporter (SERT) proteins, resulting in an increase of serotonin reuptake and in a reduction of extracellular serotonin, and they are able to up-regulate enzymes such as tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), with a resulting decrease in tryptophan (TRP) availability for serotonin synthesis [178].

Another interesting point that correlates neuroinflammatory processes and major depression is that patients suffering from this disorder display many cognitive dysfunctions. It's known that neuroinflammation plays a central role in modulating synaptic plasticity and other cellular mechanisms that underlie cognition through several mechanisms, including alteration of gene expression, modification in neuronal function, decreased neurogenesis, and impaired long-term potentiation. Despite inflammatory mediators influence synaptic plasticity processes through mechanisms described in the section 3.3, a growing body of evidence links inflammatory etiology, depression and cognitive impairment to a cytokine-induced imbalance in the kynurenine pathway, which is responsible for tryptophan degradation and hence plays a major role in serotonin synthesis [179].

4.3 The life environment: a key factor in the pathogenesis of major depression

Environmental factors also play critical roles in a person's risk of developing depression. A number of environmental factors have been found to contribute to depression vulnerability, including *in utero* exposure to infection, lack of nutrients, maternal stress, social disadvantage, childhood maltreatment, bullying, traumatic events, endocrine abnormalities, cancers and exposure to stress [180,181]. Among these environmental factors, stressful life experiences, especially those occurring early in life, have been suggested to exert a crucial impact on brain development leading to permanent functional changes that may contribute to lifelong risk for mental health outcomes. Indeed, the results of a large number of experimental and clinical studies as

well as many meta-analyses demonstrated that stressful life events have a potent relationship with the risk of depression [165,182]; during periods of heightened neural plasticity throughout development, brain regions involved in the regulation of emotion and the mediation of the stress response appear to be particularly sensitive to the effects of stressful events. Such experience-dependent plasticity may produce altered neural circuits and maladaptive responsiveness to the environment that, ultimately, lead to an enhanced risk for depression [183]. The mechanisms that mediate the impact of life environment on depression risk have been studied for decades.

4.3.1 Stress, Hypothalamic–Pituitary–Adrenal Axis and depression

The brain is a target of stressful experiences, and increasing attention has been focused on the influence imposed by stressful life events over depression. Stress response a wide range of physical responses that occur as a direct effect of a stressor causing an upset in the homeostasis of the body; upon immediate disruption of either psychological or physical equilibrium, the body responds by a series of changes including nervous, endocrine and immune systems, which provide the maintenance of homeostasis. Nevertheless, long-time activation of the stress system can cause harmful or even fatal consequences by elevating the risk of depression and other disorders.

Among the systems involved in stress response, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most consistent neurobiological findings in major depression psychiatry; excessive activation of the HPA axis is observed in about 70% of depressed people, and continuous administration of antidepressants helps to attenuate this activation [184]. The HPA axis is responsible for adaptation to changed environmental conditions and mobilization of the organism's reserves during exposure to stress of different etiologies. Briefly, in response to a stressor, neurons in the hypothalamic paraventricular nuclei secrete corticotropin-releasing hormone (CRH), which exerts its action on the hypophysis to initiate the release into the blood circulation of adrenocorticotropic hormone (ACTH), which stimulates the release of corticosteroids, particularly cortisol, from the adrenal cortex. The final hormonal product of this axis, cortisol, binds to mineralocorticoid and glucocorticoid receptors to form hormone–receptor complexes, which are then transported into the cell nucleus where they interact with specific DNA regions, the glucocorticoid-response elements, to activate the expression of hormone-dependent genes. Cortisol, in turn, induces feedback inhibition in hypothalamus and hypophysis, suppressing the production of CRH and corticotropin, respectively.

There is considerable evidence supporting the hypothesis according to which dysfunction of the HPA system is characteristic of a depressive state. Depressed patients frequently show elevated cortisol levels in plasma and urine, elevated CRH levels in cerebrospinal fluid, and increased levels of CRH messenger RNA and protein in limbic brain regions [185]; they also exhibit increased size of the hypophysis and suprarenal glands or decreased function of corticosteroid receptors [186,187]. Besides, in many patients with depression has been found a decreased size of the hippocampus due to elevated levels of cortisol that can reduce neurogenesis and a decreased numbers of neurons and glia, possibly reflecting reduced neurogenesis or due to reduced BDNF levels, which is known to be affected by stress and cortisol [188-190].

4.3.2 Microglia as mediator of the effects of the living enviroment in major depression

The nervous and the immune systems are engaged in an intense bidirectional interplay, along with the ongoing changes in the living environment [191]. In this perspective, microglia cells has recently attracted a lot of interest in the biological psychiatry field [192]. These cells can modify their features and function according to the inputs from the environment because equipped with receptors for a plethora of molecules through which they can sense environmental changes and respond performing either beneficial or harmful functions, accordingly to the context. For instance, these cells respond to sensory and behavioral experience by modulating their interactions with neuronal circuits, notably regulating adult hippocampal neurogenesis and elimination and formation of synapses [193].

The role of microglia in interfacing environmental stimuli and changes in brain function has suggested that these cells may underlay the interplay between environmental stimuli and vulnerability to major depression; however, its specific role is only beginning to be explicated. Preclinical study have provided an interesting demonstration of the role played by microglial cells in mediating the effects of stress on depression, showing that stress exposure produces dynamic bi-directional alterations in microglia status that, in turn, are causally involved in stress-induced depressive-like behavior [194]. Yirmiya et al. (2015) found that microglia modifications are dependent on the duration of the stress exposure [195]. In particular, a short-term exposure to stress, such as unpredictable stress (US) paradigm, results in microglial activation consisting in i) rapid and targeted movement of microglial processes, (ii) proliferation and resultant increase in the density of microglial cells, (iii) morphological alterations, including enlargement of the soma, increase in the diameter of primary processes, shortening of

distal processes, and, in fully activated microglia, complete retraction of all processes and assumption of an amoeboid morphology, (iv) enhanced phagocytic activity, and (v) production and secretion of inflammatory cytokines and other mediators. On the other hand, exposure to chronic stress regimens induces an intense and prolonged microglial activation leading to the microglial decline, senescence, and dysfunction. For example, using the chronic unpredictable stress (CUS) model of depression in mice, it has been found that some microglial cells, following an initial period of proliferation and activation, undergo apoptosis with a consequent reduction in their number and assumption of a dystrophic morphology characterized by smaller soma size and shorter and thinner processes [196]. In addition, it has been showed that counteracting the changes in microglial activity induced by stress, through pharmacological manipulations, can prevent or reverse the depressive-like phenotype. In particular, during the early phase of stress exposure, the administration of minocycline, a drug used to inhibit microglial activation, or imipramine, an antidepressant with anti-inflammatory properties prevents specific stress-induced effects on microglia and behavior [197]. Thus, microglia represent a promising therapeutic target for the treatment of depression; either microglia-suppressing or -stimulating drugs can serve as antidepressants, depending on the microglial status of the patient.

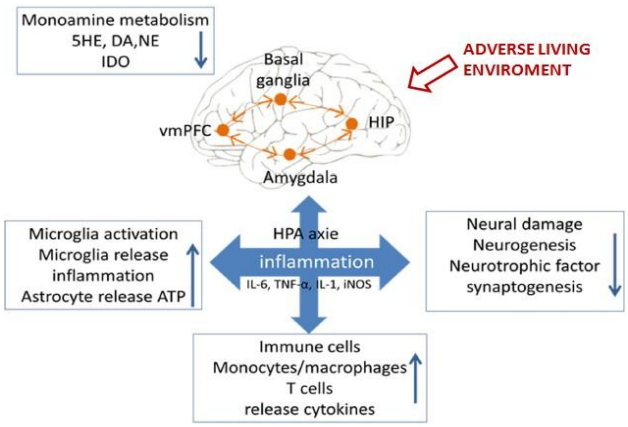


Figure 6 – Relationship between inflammation and major depressive disorders. Living environment is an important factor in the occurrence of depression symptoms. Adverse living enviroment can lead to i) disorderly release of neurotransmitters; ii) excessive activation of microglial cells, causing them to release toxic substances to disrupt the balance of inflammation and anti-inflammation, and (iii) release of inflammatory cytokines by immune macrophage, indirectly causing neurological circuit disorders. (Adapted from Zhang L, Zhang J and You Z, 2018).

4.4 Antidepressant treatment

Antidepressants are drugs used for the treatment of major depressive but have proven effective for a broad range of psychiatric illnesses, including several anxiety disorders, bulimia, and dysthymia. Among the various classes of antidepressants, such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and others, the advent of selective serotonin reuptake inhibitors (SSRIs) in the early 1990s represented a turning point in the treatment of depression [198].

4.4.1 How antidepressant work on neuroplasticity?

If depression entails a deficit in neuroplasticity, then antidepressant treatments may enhance neuroplasticity and even reverse cognitive deficits produced during the symptomatic period. A small literature does address the improvement of cognitive deficits, and even of structural damage, in depressed patients treated with a variety of antidepressant drugs. The possible mechanisms of how typical antidepressants could contribute to cellular survival and plasticity processes in the brain have been reviewed in several articles and could be explained by different pathways. First, the hypothesis that an up-regulation of CREB by antidepressants and activation of monoaminergic systems has been characterized in some studies indicating that CREB can be activated directly by the cAMP–PKA pathway or it can be directly induced by Ca^{2+}-dependent protein kinases and PKC via stimulation of 5-HT receptors and CREB can also be directly phosphorylated by activating the Ras–MAPK signaling pathway [199,200]. It has also been shed light on how changes in CREB function at the molecular level could produce persistent changes in behavior [201]. The transcription factor CREB is responsible for gene expression of many proteins involved in the neuroplasticity of hippocampus, such as BDNF, GluR1, etc [202]. Since the atrophy of hippocampus has been consistently found to play a key role in the vulnerability, chronicity, and treatment-resistance of MDD, improving the neurogenesis of hippocampus through activation of postsynaptic monoamine receptors may effectively promote depression recovery [203]. Thus, taken together, these data provide direct evidence that CREB can contribute to the therapeutic actions of antidepressants. Second, antidepressants would regulate neuroplasticity by reducing the release of presynaptic glutamate, especially the depolarization-evoked release of glutamate [204]. The reduced glutamate release may imply decreased neurotoxic efficacy and strengthened synaptogenesis, synaptic connections, and neurogenesis. Third, the antidepressants may work on neuroplasticity through enhancing AMPA to NMDA throughput; antidepressants can bind to the glycine-binding site of NMDA

receptor and inactivate this site [205]. The inactivation of NMDA receptor activity would result in inhibition of eukaryotic elongation factor 2 (eEF2) and enhance the expression of BDNF through subsequent signaling [206]. Besides, antidepressants would upregulate the expression of AMPA subunits GluR1 and potentiate the function of AMPA [207]. The depolarization of AMPA receptor would activate the voltage-dependent calcium channels (VDCCs) and induce the influx of Ca^{2+} into cytoplasm, which would further trigger the exocytosis of BDNF. Then the extracellular BDNF would further stimulate its membrane receptor TrkB and regulate gene expression and neuroplasticity through subsequent signaling [208]. Thus stimulation of AMPA and inactivation of NMDA would work synergistically to improve neuroplasticity in the brain. Fourth, antidepressants may improve neuroplasticity directly through LTP-like process. It has been repeatedly revealed that hippocampal synaptic plasticity was suppressed by stress through diminished amount of LTP, while antidepressant would reverse the negative efficacy of stress and potentiate synaptogenesis and synaptic connectivity through inducing LTP-like processes [209]. Last but not least, antidepressants may also improve neurogenesis in the hippocampus through activation of the 5-HT1A receptor [210].

4.5 SSRIs

SSRIs are the first-line treatments for major depression because of its specificity for the serotonin reuptake proteins, lack most of the adverse side effects of TCAs and MAOIs. SSRIs are no more efficacious or have rapid onset of action than the older antidepressants drugs but their improved safety and tolerability in clinical use have led to being the most widely used of all antidepressants. SSRIs are frequently prescribed also for anxiety disorders, occasionally for posttraumatic stress disorder (PTSD) and also to treat depersonalization disorder, although generally with poor results. The exact mechanism of SSRIs is unknown. SSRIs are believed to inhibit the reuptake of neurotransmitter serotonin into the presynaptic cell, increasing its extracellular level in the synaptic cleft available to bind postsynaptic receptors [211]. Specifically, in the brain, presynaptic cells that send information releasing neurotransmitters, including serotonin, into the synaptic cleft. The neurotransmitters are then recognized by surface receptors expressed by postsynaptic cells. About 10% of the neurotransmitters are lost in this process while the other 90% are released from the receptors and taken up again by monoamine transporters into the presynaptic cells, a process called reuptake. SSRIs inhibit the reuptake by allowing the serotonin to remain in the synaptic cleft longer than normal and thus to repeatedly stimulate its receptors at the postsynaptic level. The

observation that the increase of the synaptic concentrations of serotonin leads to the improvement of the depressive symptoms drove to the adoption of the monoamine hypothesis of depression (Fig. 7). However, there is no widely accepted theory on the beneficial effects of SSRIs on mood and anxiety, even because a large number of studies show conflicting results of pharmacotherapy with SSRIs and patient's success in achieving remission often depends on several factors. Clinical studies and meta-analyses have shown that 30-50% of patients treated with SSRI do not show a significant response and 60-70% do not achieve remission [212]. Partial remission is characterized by the presence of residual symptoms that are predictors of relapse. A second unexplained feature of antidepressant drug action is the temporal disconnection between the onset of the serotonin uptake inhibition in the CNS and the onset of the antidepressant activity; the former occurs within hours of the first administration while the latter takes at least three weeks. Thus, if the effect alone was sufficient to mediate an antidepressant response, SSRIs should work within hours rather than weeks. However, antidepressants tend to lose efficacy during treatment even if in clinical practice some strategies are used to try to overcome this limit, including switching medication, the increase in the dose and the combination with other drugs [213].

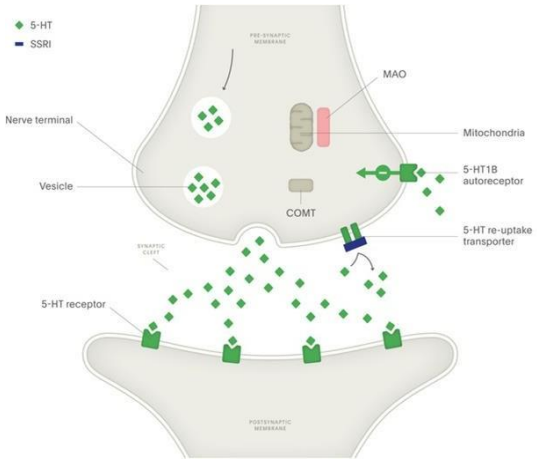


Figure 7 - The mechanism of SSRIs action

Depression is associated with reduced levels of the monoamines in the brain, such as serotonin (5-HT). The selective 5-HT re-uptake inhibitors (SSRIs) are thought to restore the levels of 5-HT in the synaptic cleft by binding at the 5-HT re-uptake transporter preventing the re-uptake and subsequent degradation of 5-HT. This re-uptake blockade leads to the accumulation of 5-HT in the synaptic cleft and the concentration of 5-HT returns to within the normal range. This action of SSRIs is thought to contribute to the alleviation of the symptoms of depression. In the presence of the SSRI, small amounts of 5-HT continue to be degraded in the synaptic cleft. (From Rang HP, Dale MM and Ritter JM, 2001).

4.5.1 "Undirected susceptibility to change" hypothesis

To explain the incomplete efficacy of SSRIs, a novel view of the role SSRI action named the "undirected susceptibility to change" hypothesis (USC) has been proposed [214]. According to such view, the relationship between SSRIs administration, the consequent change in serotonin levels, and the recovery from depression is not a direct

one. In particular, increasing serotonin levels *per se* does not univocally lead to recover from MDD, but may even worsen the symptomatology. The reason is that high serotonin levels increase brain plasticity and thus enhance biological sensitivity to context, that is, susceptibility to the quality of the environment. Consequently, an individual treated with SSRI is more affected by environmental stimuli, both favorable and adverse, compared to an untreated individual. SSRI treatment is effective because it enhances individual's reactivity to the environment, allowing, in the case of a favorable environment, to be beneficially affected. This hypothesis claims thus that increasing serotonin levels is not the direct cause of recovery from depression but a permissive factor (Fig. 8). The USC hypothesis has been recently demonstrated at preclinical and clinical levels. In particular, it has been showed in a mouse model of depression that the treatment with fluoxetine, one of the most commonly prescribed SSRIs, affects the molecular and behavioral depression-like phenotype according to the quality of the living environment: when administered in an enriched environment, it led to an improvement, while, when administered in a stressful environment, to a worsening; at clinical level, it has been confirmed that the effects of the SSRI citalopram are dependent on the environment [215,216].

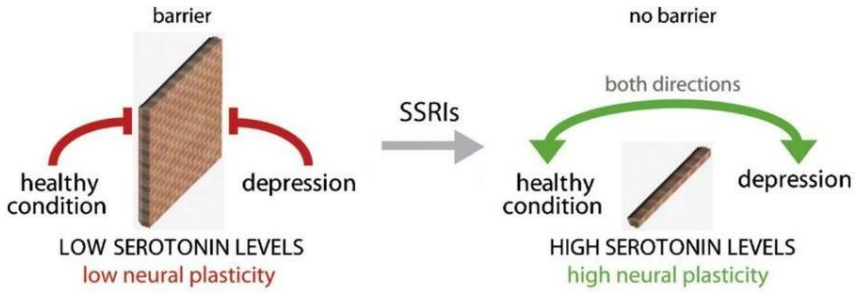


Figure 8 - Schematic description of the undirected change susceptibility model.
Serotonin levels determine vulnerability to MDD and capacity to recover from it. Low serotonin levels are associated to reduced neural plasticity. This condition does not allow to shift from an healthy condition to a depression condition (i.e., vulnerability) and vice versa (i.e., capacity to recover). By contrast, when serotonin levels are high, the increased levels of neural plasticity allow to shift from one condition to the other, increasing both vulnerability and capacity to recover. The change in neural plasticity levels can be induced by SSRI administration that increase the extracellular level of serotonin (modified from Branchi, 2011).

4.5.2 SSRIs induce neuroplasticity

Serotonin metabolism has been widely associated with brain plasticity and potentiality for modification. Indeed, it shapes neuronal networks during development and modulates a wide spectrum of essential brain functions ranging from perception and

cognitive appraisal to emotional responses in the mature brain [217]. For instance, modifications in mouse aggressive behavior associated with social status are been shown to be mediated by changes in the levels of this monoamine [218]. At the brain level, serotonin is involved in neural plasticity through a number of mechanisms, including neurite outgrowth, synaptogenesis, neurogenesis, and cell survival during brain development and at adulthood. Such effects seem to be mediated, among other factors, by BDNF pathway and this is supported by several studies showing that the BDNF pathway and the serotonergic system activity are strongly connected. In particular, it has been shown that serotonin modulates BDNF mRNA levels in the brain and in turn, an increase in BDNF levels leads to an enhanced brain serotoninergic activity [219-221]. These modifications that appear to mediate, at least partially, the therapeutic effects of antidepressants overlap with the molecular and cellular mechanisms of neural plasticity and more in particular synaptic plasticity. This suggests that an intimate relationship between these two phenomena is likely to exist. A direct evidence of the role of SSRIs in inducing neural plasticity has been provided by a study on an animal model of amblyopia, whose induction or recovery is possible only early in development, during a critical period in which the visual brain areas are highly plastic and thus can be wired according to the stimuli provided. The results obtained in this study showed that the neural plasticity can be restored after the end of the critical period, through a fluoxetine chronic administration, with a consequent full recovery of visual functions from amblyopia in adult rats [222]. These findings clearly show the strong effect exerted by SSRIs, in particular fluoxetine, on neural plasticity.

4.6 The Bidirectional Relationship between Diabetes and Major depression

The main challenge for medicine in the 21st century is the management of comorbidity of mental and metabolic disorders. Diabetes is one of the largest global health emergencies; it is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [223].

Over the last 20 years, several lines of evidence suggest comorbidity between diabetes and depression: diabetic patients are twice as likely to experience depression than nondiabetic individuals, and the majority of the cases remain under-diagnosed. On the other hand, depression occurrence is a well-established risk factor for the onset of diabetes. Diabetic patients with depression have poor glycemic control, reduced quality of life, and an increased risk of diabetes complications, consequently having an

increased mortality rate. Evidence exists on the biological mechanisms that may explain the link between these two disorders. Lifestyle factors are hypothesized to play a role in priming or reinforcing the comorbidity of depression and diabetes; for example, people with depression are more likely to be sedentary and eat diets rich in saturated fat and refined sugars, which may contribute to the increased risk of developing diabetes [224,225]. A meta-analysis study also found that depression was significantly associated with nonadherence to diabetes treatment recommendations including missed medical appointments, diet, exercise, medication use, glucose monitoring, and foot care [226]. This suggests the possibility that there may be a mutually-reinforcing phenomenon. In addition, there are a number of shared biological changes that occur in diabetes and depression and offer a novel perspective in considering this association. It is well known that both hypoglycemia and hyperglycemia can have major effects on brain function in areas of cognition and; in particular, it has been shown that diabetes negatively affects hippocampal integrity and neurogenesis, which may interact with other aspects of neuroplasticity and contribute to mood symptoms in diabetes [227]. Chronic inflammation, as well as dysfunction of HPA axis, may also underlie the comorbidity [228]. For instance, cytokines and other inflammatory markers, such as increased C-reactive protein, TNF-α, and proinflammatory cytokines, are increased in diabetes and the metabolic syndrome and are implicated in causing sickness behavior in animal models and depression in humans. These shared biological mechanisms provide a model by which environmental factors, ranging from the intrauterine environment to the surrounding environment, can influence the risk of comorbidity. Numerous environmental factors, including childhood adversity, the neighborhood environment and poverty influence the susceptibility to depression and diabetes [229]. Despite the complex nature of the available body of evidence, comorbidity of depression and diabetes is highly possible. Thus, a more precise understanding of the underlying mechanisms of this bidirectional relationship would ideally open the door for new and advanced therapeutic and preventive options for both conditions.

5. CURRENT STATE OF THE ART AND AIM OF THE STUDIES

5.1 Current state of the art

Major depression constitutes an enormous medical, individual, societal and economical challenge. SSRIs are the most commonly prescribed antidepressant; however, their efficacy is variable and incomplete and a new and effective pharmacological approach is warranted. The USC hypothesis, positing that SSRI treatment does not affect mood per se but, by increasing brain plasticity, makes the individual more susceptible to the influence of the environment, could explain the apparent inconsistency of the findings reported so far on the SSRI efficacy [214]. The finding currently available identifies the living conditions, such as socioeconomic status and lifestyle, as a key moderator of the outcome of SSRI treatment. Few clinical studies have attempted to investigate the influence of the environment on antidepressant action, showing that antidepressants are more effective in patients with a good quality of life, while having no or even detrimental consequences in patients experiencing stressful conditions. Thus, though the influence of the living environment in driving SSRI effects on depressive symptomatology starts to be unraveled, no information on its role in moderating SSRI effects on the inflammatory response is available. In the study published on Molecular Psychiatry in 2017, it was investigated whether treatment with fluoxetine (FLX), an SSRI, affects molecular, cellular and behavioral endophenotypes of depression according to the quality of the living environment [216]. In particular, it has been investigated the modifications in molecular and cellular processes previously described to be affected by SSRI administration including BDNF expression, HPA axis activity, LTP, neurogenesis, and behavior. The results obtained have shown that the administration of fluoxetine, in a mouse model of depression exposed to a favorable environment, promotes a significant recovery from the depression-like phenotype, whereas in stressful conditions this antidepressant treatment led to a worse prognosis. Other parameters, such as neurogenesis, and signaling pathways involving mitogen-activated protein kinase and CREB, were affected only when the drug was administered in the stressful environment. Furthermore, FLX treatment potentiates BDNF expression, as well as the reduction of corticosterone levels, in the enriched condition, but not in the stressful condition (Table 1). Finally, FLX affect CA1 synaptic plasticity in mice exposed to stress condition, while, in the enriched condition, both treated and untreated mice developed a robust LTP; such potentiation might have masked the effect of FLX because of a ceiling effect due to the saturation of LTP magnitude (Table 1). These

results represent a critical step in the development of a personalized medical approach and strategies aimed at improving the effectiveness of antidepressant treatment by controlling environmental conditions.

Table 1. Effects of fluoxetine, compared with vehicle, in the enriched and the stressful conditions

	Reduction in liking-type anhedonia	*Reduction in wanting-type anhedonia*	*Cognitive bias*	*Neurogenesis (Ki67)*	*ERK signaling*	*CREB signaling*	*BDNF levels*	*Reduction in CORT levels*	*LTP*
Enriched condition	–	↑	↑	–	–	–	↑	↑	–
Stressful condition	↓	↓	–	↓	↓	↓	–	–	↑

Table 1. The effects of the SSRI fluoxetine are highly dependent on the quality of the living environment. Fluoxetine administration in an enriched condition leads to a significant recovery from the depression-like phenotype compared with vehicle, while fluoxetine treatment under stress led to a worsening of the depression-like phenotype. Such environment-dependent effect is pervasive, as it concerns most of the features analyzed at the molecular, cellular and behavioral level. (From Alboni S et al., 2017).

Inflammation is one of the most recent and promising targets of therapeutic strategies for MDD [230]. It has been hypothesized that SSRIs affect mood through changes in immune function. Though some studies showed that antidepressants have anti-inflammatory effects, an increasing number of studies are reporting a pro-inflammatory action [231-234]. In addition, the bi-directional crosstalk between SSRI administration and inflammation has been described also by studies investigating the effects of anti-inflammatory drugs on antidepressant efficacy. Indeed, experimental and clinical studies have shown that anti-inflammatory drugs, such as celecoxib or aspirin, can either improve or impair treatment outcome [235,236]. Such opposite results suggest that additional factors may moderate the complex interplay between antidepressants and inflammation; the influence of the quality of the living environment may, at least partially, explain this apparent incongruence regarding the effects of SSRI treatment on the inflammatory response. Alboni et al. (2016) investigated whether fluoxetine treatment could affect the inflammatory response, which notably involves microglial cells, according to the quality of the living environment [237]. To investigate possible changes in microglial function, it has been measured the expression levels of pro- and anti-inflammatory markers in isolated hippocampal microglial cells and whole hippocampus, as well as microglial density, distribution, and morphology to investigate the surveillance state of these cells. The results obtained have shown that the increased neural plasticity, induced by fluoxetine, leads to opposite effects on inflammation, according to the starting inflammatory conditions of the animals. In particular, when the inflammatory levels were low, as in the enriched environment, SSRIs increase them,

while when the inflammatory levels were high, as in the stressful environment, SSRIs decrease them. In addition, neural plasticity requires inflammatory responses to be kept within a tightly controlled range; indeed, brain plasticity processes, such as learning and memory, neurogenesis, neuronal excitability, and synaptic scaling are dependent on the highly regulated interaction among microglia, cytokine production and neurons; consequently, any deviation from such range, caused either by immune activation or suppression, results in plasticity impairment [101] (Fig. 9). Therefore, it can be hypothesized that since fluoxetine enhances neural plasticity, fluoxetine keeps inflammation within a range that is permissive for plasticity. This dual effect of fluoxetine on inflammatory markers may explain the inter-individual differences in SSRI action and effects confirming the complexity of the crosstalk among antidepressant effects, inflammatory processes and microglial activity, which is moderated by the quality of the living environment.

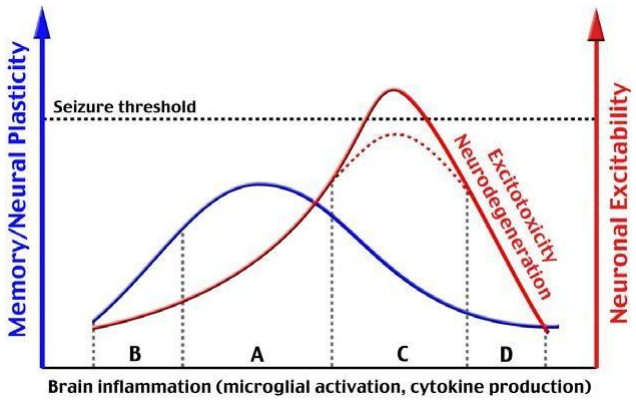


Figure 9 - Memory, neural plasticity and neuronal excitability as a function of brain inflammation.
Immune processes in the brain, including microglial activation and inflammatory cytokine production play a complex dual role in learning, memory, and neural plasticity (blue graph), as well as neuronal excitability (red graph). Locally controlled and properly timed activation of immune processes is involved in the increased neuronal excitability that underlies neural plasticity and memory consolidation (section A in graph). Any deviation from the physiological range, either by excessive immune activation or by immune suppression, results in memory and plasticity impairments: insufficient activation of immune parameters, exemplified by genetic models of immune/cytokine deficiency or treatment with immune-suppressive drugs, produces impairments in learning and memory, associated with reduced excitability, inability to mount LTP and suppressed neurogenesis (section B in graph). On the other hand, the intense brain immune activation and ‘‘cytokine storm’’ that characterizes infections, injury, and exposure to extreme stress, can induce hyper-excitability of neuronal circuits. The inflammation-induced pathological hyper-excitability is associated with disturbances in learning, memory, and neural plasticity (section C in graph). If the immune over-activation is even more severe and/or chronic, excitotoxicity, apoptosis and neurodegenearation may ensue, resulting in reduced neuronal excitability and further impairments in learning, memory and neural plasticity (section D in graph) (From Yirmiya R and Goshen I, 2011)

As mentioned above, the view offered by the USC hypothesis leads to new perspectives on how to improve SSRI efficacy by controlling the living conditions. However, it is not always possible to act on the quality of the patient’s living environment and his personal history is unchangeable. In these cases, a pharmacological

approach could be used to modulate the factors underlying the link between living environment and SSRI efficacy, in order to improve the antidepressant outcome even in patients who live in unfavorable conditions. Several publications report an association between the quality of the living condition and metabolism. For instance, it is well known that having an active physical and social life profoundly modulates the metabolic markers, including hormones and adipokine levels [238]. On the other hand, the neuroendocrine pathway, which consists in the brain-adipocyte axis, is reportedly involved in the vulnerability to MDD and antidepressants efficacy; indeed, it has been shown that metabolic syndrome and obesity are associated with MDD and are involved in the vulnerability of this disorder [229]. Therefore, modification of metabolism could represent a potential approach to modulate the interplay between environment and SSRIs to improve antidepressant treatment outcome.

Although the hippocampus has long been considered as a functionally unitary structure with properties preserved along its extension, recently a dichotomous view, under which the hippocampus has different physiological and functional properties along its dorsal-ventral axis, has emerged [16-19,239-244]. These variations could result from structural differences in hippocampal neurons, Different GABAergic control, as well as changes in glutamate release. However, increasing evidence highlights the role that microglial cells play in regulating neuronal networks and functions, neuronal transmission and synaptic plasticity [193]. For instance, it is well known that the disruption of CX_3CL1-CX_3CR1 signaling, which mediate the interaction between neuron and microglia, leads to impairments in the development and plasticity of neuronal circuits as well as electrophysiological properties of excitatory synapses in hippocampus [245-249]. Therefore, it is possible to hypothesize that microglial cells in the dorsal and ventral hippocampus could have different characteristics and that these could somehow explain the physiological and functional differences found along the dorsal-ventral hippocampal axis.

5.2 Aim of the studies

The general aim of the following studies is to explore the interplay between neural plasticity and inflammation which are involved in the vulnerability and recovery from psychiatric disorders such as MDD, and are widely reported act as key factors in determining SSRI outcome. Indeed, microglia and inflammatory system are increasingly emerging as effectors translating the influence of the living environment into changes in brain function. To this purpose, we modulate the inflammatory levels

with two different pharmacological agents, who modulate in opposite way molecules related to a pro- or anti-inflammatory action, and we evaluated their effects on neural plasticity at both molecular and cellular level.

As further step, we investigated the effects of the combined administration of fluoxetine and metformin, a drug able to improve metabolic profile, in a mouse model of depression. The aim of the this study was to verify whether the pharmacological modulation of metabolism, which is reported to mimic the quality of the living condition on mood, may improve the limited efficacy of FLX when administered in a stressful environment. In particular, we wanted identify the metabolic targets in the brain-adipocyte axis that lead to a beneficial effect on depressive symptoms, even when the patient is treated in non-supportive conditions.

Finally, we verified if the known electrophysiological differences along the hippocampal longitudinal axis could be due to different properties of microglia in dorsal and ventral hippocampus and, accordingly, if these cells may differently be involved in modulating synaptic plasticity in these two hippocampal regions, with the final goal of elucidating the mechanisms underlying the cross-talk between neurons and microglia.

5.3 Experimental approach and outline of the thesis

In *Study 1*, we investigated the interplay between neural plasticity and inflammation, assessing the level of plasticity following a decrease or increase levels of molecules related to inflammatory response. It is well known that neural plasticity is influenced by several factors including inflammation and we hypothesized that neural plasticity varies in function of inflammatory activity in an inverted U-shape relation. To investigate such interaction, we acutely treated adult male mice with different doses of the nonselective non-steroidal anti-inflammatory drug (NSAID), ibuprofen (IBU), or the endotoxin lipopolysaccharide (LPS), which respectively decrease and increase inflammatory levels, and we assessed the effects of treatments on hippocampal neural plasticity both at molecular and cellular levels, and the expression levels of inflammatory and plasticity markers. The results showed that LPS and IBU have different effects on behavior and inflammatory response. Specifically, LPS treatment induced a reduction of body temperature, a decrease of body weight and a reduced food and liquid intake. Besides, it led to increased expression of inflammatory markers, both in the total hippocampus and in isolated microglia cells, and enhanced concentration of PGE_2. On the other hand,

IBU increased the level of anti-inflammatory markers, decreased tryptophan 2,3-dioxygenase (TDO2), the first committed step in the kynurenine pathway, which is involved in inflammation, and PGE_2 levels. Though LPS and IBU administration differently affected mediators related to pro- or anti-inflammatory responses, they produced overlapping effects on neural plasticity. Indeed, higher doses of both LPS or IBU induced a statistically significant decrease in LTP, expression levels of BDNF, the neurotrophic factor most involved in plasticity process, and in the phosphorylation of the AMPA receptor subunit GluR1, compared to the control group. Such effect appears to be dose-dependent since only the higher, but not the lower, dose of both compounds led to plasticity impairment. Overall, these findings indicate that the two extreme inflammatory conditions lead to an impairment of the brain function, suggesting that the neural plasticity is possible only within a strict range of inflammatory levels and that any deviations from such range results in reduced plasticity.

With the *Study 2***,** we explored whether the combined administration of fluoxetine and metformin, a widely used drug to treat type II diabetes and other metabolic syndromes, could enhance the limited effectiveness of the treatment with fluoxetine alone when administered in stressful conditions. Indeed, metabolism could be an important target to modulate the influence of the environment on the individual; in particular, metformin affects the metabolic profile at both peripheral and central levels since it crosses the BBB, and, though its molecular mechanisms of action are yet to be fully determined, preclinical studies reported that MET affects brain plasticity and modulate neurotrophic factor levels, such as BDNF [250]. Thus, in our study, adult C57BL/6 male mice were exposed to stressful condition for 6 weeks, the first two aimed at inducing a depression-like phenotype. During the remaining 4 weeks, mice received vehicle, fluoxetine, metformin or a combination of fluoxetine and metformin. We measured behavioral phenotypes of depression and we assessed the expression levels of several genes involved in MDD and antidepressant response in the dorsal and ventral hippocampus. Our results showed that the combined treatment was more effective than FLX alone in ameliorating the depression-like phenotype in a stressful environment, and this was associated with an increase in IGF2 mRNA expression, enhanced LTP and BDNF expression, specifically in the dorsal hippocampus. Overall, these findings suggest that this polypharmacological approach could be more effective to treat MDD compared to the currently antidepressant therapy, especially when patients experience stressful living conditions.

It is well known that microglial cells, in addition to their function as resident immune cells, interacting directly with neurons, regulate neuronal networks and functions, neuronal transmission and synaptic plasticity [193]. In *Study 3*, we verified a possible contribution of microglial cells in regulating the different features observed in terms of synaptic plasticity and neurotrasmission along the longitudinal axis of hippocampus, as described previously [18]. In particular, we focused our attention on how these cells may differently modulate plasticity processes in the dorsal and ventral hippocampal region, with the ultimate goal to highlight the mechanisms underlying the interaction between neuronal and immune cells. First of all, we quantitatively and qualitatively characterized microglial cells in dorsal and ventral hippocampus of adult C57BL/6 male mice. Thus, to explore the role assumed by microglia in the two hippocampal poles, we interfered with these cells using both pharmacologic and genetic approaches. In particular, we used PLX5622, a microglial colony-stimulating factor 1 receptor (CSF-1R) inhibitor that causes a rapid and transient depletion of microglia, or minocycline, a tetracycline antibiotic which inhibits the physiological functioning of microglia. In addition, we used mice genetically deleted for CX3CR1 ($CX_3CR1^{KO/KO}$), the fractalkine receptor expressed exclusively on microglia that interacts with its unique nauronal ligand, the fractalkine (CX_3CL1). The results show that microglial cells differ in terms of distribution and morphology in the CA1 region of dorsal and ventral hippocampus. Specifically, the dorsal pole has an increase in the number of microglia which, however, appear to have a smaller soma area than those of ventral hippocampus; instead, the microglial arborization area is not different in these two regions. Electrophysiological analyses show that the pre-synaptic probability of glutamate release in CA1 hippocampal region differs in the two hippocampal poles and that the pharmacological and genetic strategies used to interfere with microglia cells do not affect this property. On the contrary, CA1-LTP, which is more potentiate in the dorsal region than the ventral, results inverted by PLX5622 and minocycline treatments as well as in $CX_3CR1^{KO/KO}$ mice. Based on our results, we can hypothesize that hippocampal plasticity processes are regulated by microglia- in a region-dependent manner along the dorsal-ventral axis.

EXPERIMENTAL SECTION

STUDY 1

Brain, Behavior, and Immunity 81 (2019) 484–494

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Interplay between inflammation and neural plasticity: Both immune activation and suppression impair LTP and BDNF expression

Maria Teresa Golia[a,1], Silvia Poggini[b,1], Silvia Alboni[c,1], Stefano Garofalo[a], Naomi Ciano Albanese[b], Aurelia Viglione[b,d], Maria Antonietta Ajmone-Cat[e], Abygaël St-Pierre[f], Nicoletta Brunello[c], Cristina Limatola[a,g], Igor Branchi[b,2], Laura Maggi[a,2,*]

[a] Department of Physiology and Pharmacology, Laboratory Affiliated to Istituto Pasteur-Italy, Sapienza University of Rome, Italy
[b] Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy
[c] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[d] PhD Program in Neuroscience, Scuola Superiore di Pisa, Pisa, Italy
[e] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
[f] Axe Neurosciences, Centre de recherche du CHU de Québec, Québec, Canada
[g] IRCCS Neuromed, Pozzilli, Isernia, Italy

ARTICLE INFO

Keywords:
Inflammatory level
Neuroplasticity
LTP
Neurotrophin
NSAIDs
LPS
Ibuprofen
Cytokines
IL-1β

ABSTRACT

An increasing number of studies show that both inflammation and neural plasticity act as key players in the vulnerability and recovery from psychiatric disorders and neurodegenerative diseases. However, the interplay between these two players has been limitedly explored. In fact, while a few studies reported an immune activation, others conveyed an immune suppression, associated with an impairment in neural plasticity. Therefore, we hypothesized that deviations in inflammatory levels in both directions may impair neural plasticity. We tested this hypothesis experimentally, by acute treatment of C57BL/6 adult male mice with different doses of two inflammatory modulators: lipopolysaccharide (LPS), an endotoxin, and ibuprofen (IBU), a nonselective cyclooxygenase inhibitor, which are respectively a pro- and an anti-inflammatory agent. The results showed that LPS and IBU have different effects on behavior and inflammatory response. LPS treatment induced a reduction of body temperature, a decrease of body weight and a reduced food and liquid intake. In addition, it led to increased levels of inflammatory markers expression, both in the total hippocampus and in isolated microglia cells, including Interleukin (IL)-1β, and enhanced the concentration of prostaglandin E_2 (PGE_2). On the other hand, IBU increased the level of anti-inflammatory markers, decreased tryptophan 2,3-dioxygenase (TDO2), the first step in the kynurenine pathway known to be activated during inflammatory conditions, and PGE_2 levels. Though LPS and IBU administration differently affected mediators related with pro- or anti-inflammatory responses, they produced overlapping effects on neural plasticity. Indeed, higher doses of both LPS and IBU induced a statistically significant decrease in the amplitude of long-term potentiation (LTP), in Brain-Derived Neurotrophic Factor (BDNF) expression levels and in the phosphorylation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor subunit GluR1, compared to the control group. Such effect appears to be dose-dependent since only the higher, but not the lower, dose of both compounds led to a plasticity impairment. Overall, the present findings indicate that acute treatment with pro- and anti-inflammatory agents impair neural plasticity in a dose dependent manner.

1. Introduction

Inflammation is key in the protective response to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani et al., 2007; Medzhitov, 2010). To orchestrate this process, the immune cells produce a wide range of soluble mediators such as cytokines, chemokines and free radicals (Chertov et al., 2000). A dysregulated or excessive response can however become detrimental for tissue integrity.

* Corresponding author at: Department of Physiology and Pharmacology, Sapienza University, Piazzale Aldo Moro 5, 00185 Rome, Italy.
E-mail address: laura.maggi@uniroma1.it (L. Maggi).
[1] These authors equally contributed as first author.
[2] These authors equally contributed as last author.

https://doi.org/10.1016/j.bbi.2019.07.003
Received 19 January 2019; Received in revised form 29 June 2019; Accepted 3 July 2019
Available online 04 July 2019

Some of these mediators are able to cross the blood–brain barrier (BBB) and modulate the function of central nervous system (CNS), since they can directly interact with both neurons and glia, including microglia, which are the tissue-resident macrophages of the brain (Branchi et al., 2014; Paolicelli et al., 2011; Tay et al., 2017; Tremblay et al., 2011).

The literature of last decades focused on the detrimental consequences of immune system activation in the brain, reporting that neuroinflammation is associated to a number of pathological conditions including acute and chronic neurodegenerative diseases (Chen et al., 2016; Hotamisligil and Erbay, 2008; Hurley and Tizabi, 2013; Libby, 2006; Wyss-Coray and Mucke, 2002). More recently, mediators involved in the promotion of inflammation, have been associated with the onset and progression of major depression in vulnerable individuals (Zunszain et al., 2013), and other psychiatric disorders (Najjar et al., 2013; Reus et al., 2015). The detrimental impact of inflammation on brain function has been hypothesized to be mediated by the neurotoxic potential of excessive levels of inflammatory mediators, as well as by their effects on neural plasticity, i.e. the capability of the CNS for structural and functional changes. Indeed, high levels of inflammatory molecules have been reported to decrease a wide number of neural plasticity markers such as long-term potentiation (LTP), Brain-Derived Neurotrophic Factor (BDNF) levels, and neurogenesis (Raison et al., 2006). On the other hand, it has been suggested that also a depression of the basal production of mediators formerly exclusively known as inflammatory signals, such as prostaglandins and cytokines, have a detrimental effect on synaptic plasticity and LTP (Shaw et al., 2003). As an example, endogenous basal levels of prostaglandin E_2 (PGE_2) regulate membrane excitability, synaptic transmission, and plasticity in hippocampal CA1 pyramidal neurons (Chen and Bazan, 2005; Chen et al., 2002) and are necessary for memory acquisition (Rall et al., 2003), consolidation (Teather et al., 2002), and retention (Cowley et al., 2008; Sharifzadeh et al., 2006; Shaw et al., 2003). Similarly, cytokines such as Interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA) and transforming growth factor (TGF)-β play functional roles in the mechanisms of synaptic plasticity and cognitive functions (Levin and Godukhin, 2017; Maingret et al., 2017; Ross et al., 2003).

According to this new emerging framework, inflammatory mediators and brain immune cells would be critical in regulating neural plasticity, including processes such as growing and pruning of dendrites and axons, shaping of synapses and associated structures, apoptosis and neurogenesis of neurons (Yirmiya and Goshen, 2011). These produce neuritic remains that are removed by the immune system to allow brain tissue remodeling, keeping neuronal homeostasis. Indeed, it is increasingly reported that the microglial cells maintain neural function, allowing key brain processes, including neural plasticity, to take place (Yirmiya and Goshen, 2011). However, such maintenance process is effective when microglial activation and production of immune mediators are in a physiological range, while too high or too low levels of these same molecules are both detrimental, hampering neuronal functions and microglia to exert its physiological role in brain function. Although some previous data are in line with this hypothesis, direct evidence supporting it is still limited.

Basing on this view, we tested whether basal brain immune processes should be in a range to be permissive for neural plasticity so that either an over- activated or suppressed state impairs such capability of the brain. To assess the potential inverted U-curve regulation of neural plasticity by inflammatory mediators, we administered in adult male mice either the endotoxin lipopolysaccharide (LPS) or the nonselective non-steroidal anti-inflammatory drug (NSAID) ibuprofen (IBU). LPS is commonly used to stimulate the innate immune system (Martin et al., 2013) and promotes pro-inflammatory gene expression, including cytokines such as IL-1β, Interleukin 6 (IL-6) or Tumor Necrosis Factor (TNF)-α (Kubera et al., 2013; Trotta et al., 2014). By contrast, IBU exerts dose-dependent anti-inflammatory, analgesic and antipyretic effects mainly by its inhibitory effect on cyclooxygenases (COX) enzymatic activity and, consequently, on the synthesis of prostaglandins (PG) and other prostanoids, key messenger molecules in the process of inflammation, pain, and fever (Rainsford, 2013; Shaw et al., 2005), and - as mentioned above - in physiological neural functioning. In addition, several COX- independent effects of NSAIDs have also been described, including the modulation of the activity and/or expression of crucial signaling molecules regulating in the expression of inflammatory genes, as well as genes involved in survival, plasticity, memory formation, cognition and behavior [for a review see (Ajmone-Cat et al., 2010)]. In non-inflamed subjects, NSAIDs central effects are however poorly explored.

We assessed the effects of LPS and IBU treatments on physiological indices, and both molecular and cellular endpoints in the hippocampus, an especially plastic area of the brain, including LTP at Schaffer collateral-CA1 synapses, the most commonly used paradigm of synaptic plasticity (Bliss and Collingridge, 2013; McEachern and Shaw, 1999), and the expression levels of a number of inflammatory and plasticity markers, including BDNF, which is deeply involved in neural plasticity (Lu et al., 2014).

2. Materials and methods

2.1. Animals

Fifty C57BL/6 male mice 12–15 week old were used and kept under 12-hour light-dark cycle at 22–25 °C. Mice were purchased at Envigo Italia (Udine, Italy). All procedures were carried out in accordance with the EC guidelines (EEC Council Directive 2010/63/UE86/609 1987), Italian legislation on animal experimentation (Decreto Legislativo 26/2014). In addition, animals were examined for signs of discomfort as indicated in the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research edited by The National Academies Press.

2.2. Housing condition

For the entire duration of the experiment, animals were housed in standard laboratory condition, four to five individuals per cage. Each cage was a Plexiglas box (48 × 37.5 × 21 cm) with metal tops and sawdust as bedding. Pellet food and tap water were provided ad libitum. The day before the treatment, mice were moved in a smaller cage (33.1 × 15.9 × 13.2 cm) in order to monitor them individually.

2.3. Treatment

Mice were acutely treated through intraperitoneal (i.p) injection of LPS (serotype 0127:B8; RBI/Sigma) diluted in saline at two different doses: 0.33 and 0.83 mg/kg, IBU (Ibuprofen sodium salt, Sigma Aldrich) diluted in saline at two different doses: 30 and 70 mg/kg or saline (0.9% NaCl) 100 mL/100 g. LPS and IBU solutions were freshly prepared before use. LPS doses were selected in accordance with previous studies which investigated classical acute sickness induced response and the pro-inflammatory response in the brain (Godbout et al., 2005; Medeiros et al., 2015; O'Connor et al., 2009). IBU doses were selected according to the study by O'Mara and colleagues reporting that, testing different doses between 30 up to 110 mg/kg, they obtained a reduced induction of LTP with a clear dose-response effect (Shaw et al., 2003, 2005). In addition, further studies on the effects of IBU on brain function used similar dosages (70 mg/kg in drinking water; Warner-Schmidt et al., 2011). All measurements were performed 3 h after the injection, since this experimental timing is reported to be enough to assess the short term effect of the treatments on physiological, inflammatory and plasticity endpoints (Fan et al., 2013; Shaw et al., 2005; Skelly et al., 2013; Teeling et al., 2010). The animals were assigned to various treatments in order to obtain balanced body temperature and weight across the experimental groups.

2.4. Physiological indexes

The day before the treatment, immediately before and three hours after the injection, rectal temperature (MicroTherma 2T Hand Held, Thermometer) and body weight were measured. At the same time points, we assessed the amount of food and liquid consumed by each animal.

2.5. RNA extraction and RT-qPCR on entire hippocampus

Three hours after treatments, the animals were sacrificed by decapitation, the brains removed, the hippocampi dissected, rapidly frozen and stored at −80 °C for further molecular analyses. Half of the experiments were conducted on hemispheres isolated from the same brain used for electrophysiology. Total RNA was prepared to combine extraction with TRI Reagent® and GenElute™ Mammalian Total RNA Miniprep Kit and (Sigma Aldrich, Milan, Italy) as previously described (Alboni et al., 2017). Two μg of total RNA were reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA USA) in a final reaction volume of 20 μL (Alboni et al., 2013). The cDNA was stored at −20 °C until Real-Time PCR was performed, in Roche LightCycler® 480 (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) using Power SYBR Green mix (Life Technologies Corporation, Carlsbad, CA, USA). Specific forward and reverse primers, at a final concentration of 150 nM, were used to measure mRNA levels were as follows: for Tryptophan 2,3-Dioxygenase (TDO2) F5′-TGTGGTCCTGAGACACTTC AGT-3′ and R5′-ACACCAGTTTGAGCTCTGTCTTC-3′; for prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX-2) F5′-TTCTAC GGAGAGAGTTCATC-3′ and R5′-CAGTTTATGTTGTCTGTCCA-3′; for prostaglandin-endoperoxide synthase 1 (PTGS1, also known as COX-1) F5′-TCGAAGGAGCTCCTCGCTCT-3′ and R5′-CTGGTTCTGGCACGGAT AGT-3′; for interleukin-1 beta (IL-1β) F5′- TGAAAGCTCTCCACCTCA ATG-3′ and R5′-CCAAGGCCACAGGTATTTTG-3′; for transforming growth factor beta (TGF-β) F5′-CCTTGCCCTCTACAACCAAC-3′ and R 5′-CTTGCGACCCACGTAGTAGAC-3′; for interleukin 1 receptor antagonist (IL-1RA) F5′-AAGCCTTCAGAATCTGGGATAC-3′ and R5′-TCA TCTCCAGACTTGGCACA-3′; for CCAAT Enhancer Binding Protein Delta (CEBPD) F5′-GCGGCCTTCTACGAG-3′ and R5′-GCCATGGAGTCAATG TAG-3′; for total Brain-Derived Neurotrophic Factor (BDNF) F5′-CCAT AAGGACGCGGACTTGTAC-3′ and R5′-AGACATGTTTGCGGCATCC AGG-3′ and for Glyceraldehyde 3-phosphate dehydrogenase (GADPH) F 5′-TTCGCAAAACAAGTTCACCA-3′ and R5′-TCGTTGTGGTTGTAAATG GAA-3′ as a house-keeping gene. The cycling parameters were: 95 °C 10 min and 95 °C 15 s, 60° 1 min for 40 cycles. Melt curve analysis and agarose gel separations were performed at the end of every RTqPCR to confirm formation of a single PCR product. No-template and no reverse transcriptase controls were also added for each target to exclude possible sample contamination (i.e. genomic DNA). Ct (cycle threshold) value was determined by the LightCycler® 480 Software (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) and mRNA expression was calculated with the ΔΔCt method with GAPDH as an endogenous control as previously described (Benatti et al., 2016). It was demonstrated that amplification efficiency of the target genes and endogenous control gene were approximately equal. Relative expression of the genes of interest was performed by using as calibrator (RQ value = 1) expression levels in the hippocampus of saline-treated animals.

2.6. PGE_2 extraction and measurement

A detailed procedure for PGE_2 extraction has been described elsewhere (Minghetti et al., 2000). In brief, 200 μL of ice-cold Tris-HCl buffer pH 7.5 containing 10 μg/ml of the COX inhibitor indomethacin (stock solution 100× in ethanol) to avoid ex vivo PGE_2 synthesis, and 10 μM of the radical scavenger Butylated hydroxytoluene (BHT stock solution 100× in ethanol) to avoid auto-oxidation, were added to each frozen sample, which was quickly thawed, homogenized with a Teflon pestle (Sigma) – 20 cycles in an ice bath – vigorously vortexed and centrifuged at 14000 rpm for 45 min at +4 °C. The supernatants were collected and stored at −80 °C until analysis. Pellets were resuspended in 200 μL of 0.1 M NaOH for protein determination (see below).

PGE_2 was measured in tissue extracts by an enzyme immunoassay kit (PGE_2 EIA kit, detection limit for PGE_2: 15.6 pg/mL, Arbor Assays). All measurements were run at least in duplicate for each sample. Results were expressed as pg PGE_2/mg of total proteins measured in the pellets obtained after the extraction procedure.

The BCA Protein Assay kit (Pierce, Rockford, IL), based on bicinchoninic acid for the colorimetric detection and quantification of total proteins, was used to determine the amount of proteins in the pellets obtained after extraction of PGE_2. Protein concentrations were reported with reference to standards curves of bovine serum albumin (working range: 5 μg/mL–400 μg/mL).

2.7. Isolation of CD11b-positive cells, total RNA extraction and real-time PCR

Three hours after treatment mice were anesthetized and decapitated. Brains were removed, isolated hippocampi were cut into small pieces and single-cell suspension was achieved by enzymatic digestion in trypsin (0.25 mg/ml) solution in Hank's balanced salt solution (HBSS). The tissue was further mechanically dissociated using a wide-tipped and the suspension was applied to a 30 μm cell strainer (Miltenyi Biotec). Cells were processed immediately for MASC MicroBead separation. CD11b-positive (+) cells were magnetically labelled with CD11b MicroBeads. The cell suspension was loaded onto a MACS Column (Miltenyi Biotec), placed in the magnetic field of a MACS Separator, and the negative fraction was collected. After removing the magnetic field, $CD11b^+$ cells were eluted as positive fraction. Vitality and purity of $CD11b^+$ cells were assessed by flow cytometry (FACS) as previously indicated (Garofalo et al., 2015). Upon sorting, total RNA was isolated using RNeasy Mini Kit and processed for Real-Time PCR (RT-PCR). The quality and yield of RNAs were verified using the NANODROP One system (Thermo Scientific). Sample were lysed in Trizol reagent for isolation of RNA. Reverse transcription reaction was performed in a thermocycler (MJ Mini Personal Thermal Cycler; Biorad) using IScript TM Reverse Transcription Supermix (Biorad) according to the manufacturer's protocol, under the following conditions: incubation at 25 °C for 5 min, reverse transcription at 42 °C for 30 min, inactivation at 85 °C for 5 min. RT-PCR was carried out in a I-Cycler IQ Multicolor RT-PCR Detection System using SsoFast EvaGreen Supermix (Biorad) according to the manufacturer's instructions. The PCR protocol consisted of 40 cycles of denaturation at 95 °C for 30 s and annealing/extension at 60 °C for 30 s. For quantification analysis, the comparative Threshold Cycle (C_t) method was used. The C_t values from each gene were normalized to the C_t value of GAPDH in the same RNA samples. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) and expressed as fold change in arbitrary values. The following primers were used: for IL-1β F5′-GCAACTGTTC CTGAACTCAACT-3′ and R3′-ATCTTTTGGGGTCCGTCAACT-5′; for tumor necrosis factor (TNF)-α F5′-GTGGAACTGGCAGAAGAG-3′ and R 3′-CCATAGAACTGATGAGAGG-5′; for interleukin (IL)-6 F5′-GATGGAT GCTACCAAACTGGA-3′ and R3′-TCTGAAGGACTCTGGCTTTG-5′; for arginase (Arg)-1 F5′-CTCCAAGCCAAAGTCCTTAGAG-3′ and R3′-AGGA GCTGTCATTAGGGACATC-5′; for chitinase like-3 (Ym1) F5′-CAGGTCT GGCAATTCTTCTGAA-3′ and R5′-GTCTTGCTCATGTGTGTAAGTGA-3′; for GADPH F5′-TCGTCCCGTAGACAAAATGG-3′ and R3′-TTGAGGTCA ATGAAGGGGTC-5′ as a house-keeping gene.

2.8. Western blot analyses

For protein extraction, the hippocampi were derived from mice

treated with high IBU and LPS doses. The same amount of proteins (50 µg/ sample) was loaded onto 8.75% SDS polyacrylamide gel and electrophoretically transferred to nitrocellulose paper at 4 °C for 2 h. Blots were incubated for 1 h with 5% nonfat dry milk or 3% BSA (bovine serum albumin) in Tris-buffered saline containing 0.2% Tween 20 to block nonspecific binding sites and then incubated overnight at 4 °C with specific primary Abs. After washing, membranes were incubated with HRP-conjugated secondary Abs and immunoreactivity was detected by ECL. Densitometric analysis of immunoreactive bands was performed using Chemi-Doc XRS and Quantity One software (Bio-Rad).

2.9. Electrophysiological analysis

In order to perform electrophysiological experiments, acute hippocampal slices were prepared from 3 months old male mice. Three hours after treatment, animals were anesthetized by inhalation of halothane (Sigma-Aldrich S.r.l., Milan, Italy) and decapitated. The brain was rapidly removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF) solution composed of (in mM): NaCl 125, KCl 4, CaCl2 2.5, MgSO4 1.5, NaH2PO4 1, NaHCO3 26 and glucose 10. ACSF was continuously oxygenated with 95% O2 + 5% CO2 to maintain the proper pH (7.4).

One of the two hemispheres isolated were conserved for molecular analysis. Following removal, one hemisphere was blocked on the stage of a vibrating microtome (Thermo Scientific, USA) and 350 µm thick slices were cut in ice-cold ACSF. The slices were then transferred to an incubation chamber containing oxygenated ACSF, where they were allowed to recover for 1 h at 30 °C prior to electrophysiological recording.

For field recordings, individual slices were transferred to the interface slice-recording chamber (BSC1, Scientific System Design Inc) to perform experiments within 1–6 h after slice preparation. Slices were maintained at 30 to 32 °C and constantly superfused with ACSF at the rate of 2 mL/min. Solutions were applied to the slices by a peristaltic pump. A concentric bipolar stimulating electrode (SNE-100 × 50 mm long, Elektronik–Harvard Apparatus GmbH) was placed in the stratum radiatum to stimulate Schaffer collateral fibers. Stimuli consisted of 100 µs constant current pulses of variable intensity, applied at 0.05 Hz. A glass micropipette (0.5–1 MΩ) filled with ACSF was placed in the CA1 hippocampal region, at 200–600 µm from the stimulating electrode, in order to measure orthodromically-evoked field extracellular postsynaptic potentials (fEPSP). Stimulus intensity was adjusted to evoke fEPSPs of amplitude about 50% of maximal amplitude with minimal contamination by a population spike. Evoked responses were monitored on-line and stable baseline responses were recorded for at least 10 min. Only the slices that showed stable fEPSP amplitudes were included in the experiments. LTP was induced by high-frequency stimulation (2 train of stimuli at 100 Hz, of 1 s duration and separated by 3 s). To analyze the time course of fEPSP slope, the recorded fEPSP was routinely averaged over 1 min (n = 3). fEPSP slope changes following the LTP induction protocol were calculated with respect to the baseline (30 min after vs 1 min before LTP induction).

The paired-pulse ratio (PPR) was measured from responses to two synaptic stimuli at 50 ms inter-stimulus interval. The PPR was calculated as the ratio between the fEPSP amplitude evoked by the second stimulus (A2) over the first (A1, A2/A1).

2.10. Statistical analyses

All data were analyzed with one-way ANOVA or Student's *t*-test with the Statistical software Statview II (Abacus Concepts, CA, USA), comparing LPS high and low dose, IBU high and low doses and saline-treated mice. When a significant main effect was found, selected pairwise comparisons were made using Tukey post hoc analysis following ANOVA and Bonferroni's post hoc analysis following Student's *t*-test.

3. Results

We examined the acute effect of different doses of the pro-inflammatory stimulus, LPS, and the anti-inflammatory drug, IBU, on neural plasticity. In particular, 3 h after the i.p. injection with these agents, we assessed the effects of the treatments by measuring (i) changes in body temperature and weight and the amount of food and liquid consumed, (ii) changes in expression levels of molecular targets related to inflammation and plasticity processes and (iii) CA1 hippocampal plasticity measured through field potential recordings in acute slices.

3.1. Assessment of behavioural and physiological responses

It has been largely demonstrated that LPS administration produces sickness behavior (Yirmiya, 1996; Yirmiya et al., 2001), whereas treatment with NSAIDs, such as IBU, under inflammatory conditions is effective in relieving inflammation symptoms (Pecchi et al., 2009). To verify the effectiveness of the treatment we assessed a number of endpoints, including body weight, temperature, food and liquid intake. We measured all these parameters the day before the treatment and no significant difference between the experimental groups has emerged (data not shown).

A difference of body temperature before and 3 h after the injection showed a main effect of the treatment [$F(4,45) = 9.304$, $p < 0.0001$]. Post hoc comparison revealed that both high and low doses of LPS significantly decreased the body temperature compared to the other groups ($p < 0.05$; Fig. 1A), as previously reported (Skelly et al., 2013). Also the body weight (Fig. 1B) was affected by the treatment [$F(4,45) = 18.925$, $p < 0.0001$], with post hoc analysis revealing that LPS treated mice displayed a significant reduction of weight compared to saline, high and low dose of IBU. Accordingly, we observed the effect of treatment on the food intake [$F(4,36) = 7.161$, $p = 0.0002$], which was decreased by the LPS treatment (Fig. 1C). Specifically, post hoc analysis revealed that mice treated with both doses of LPS consumed significant lower amount of food compared to IBU 70 mg/kg treated mice ($p < 0.05$). Finally, the amount of liquid drank was affected by the treatment [$F(4,44) = 2.483$, $p = 0.0574$], the mice treated with the higher dose of LPS displayed a significant reduced consumption of liquid compared to the control group (Fig. 1D). Taken globally, these results confirmed the effectiveness of the LPS treatment on behavioral parameteres at both doses.

3.2. Hippocampal expression levels of inflammatory markers are affected by treatments

We assessed the treatment effects at molecular level measuring the hippocampal expression levels of selected markers known to be involved in inflammatory processes as well as in brain plasticity. In particular, we analyzed the hippocampal levels of IL-1β, IL-1RA, TGF-β, CEBPD, and TDO2 mRNAs.

Regarding the pro-inflammatory cytokine IL-1β, the main effect of the treatment was shown, [$F(4,44) = 14.122$, $p = 0.0001$], with the post hoc analysis revealing that mice treated with both high and low dose of LPS had significant increased levels of IL-1β mRNA compared to the saline mice ($p < 0.01$). In addition, mice receiving the low or high dose of LPS displayed a significantly higher level of IL-1β expression compared to mice treated with the two doses of IBU ($p < 0.01$; Fig. 2A). When we measured the level of the IL-1RA mRNA, we observed a main effect of treatment [$F(4,22) = 4.333$, $p = 0.010$] and the post hoc analysis showed that the group treated with the lower dose of LPS had significant increased level of IL-1RA compared to the mice receiving IBU (high and low dose; $p < 0.05$ for both), higher dose of LPS ($p < 0.05$), and saline ($p < 0.01$; Fig. 2B). We found the main effect of treatment also on the levels of the CEBPD [$F(4,40) = 4.419$, $p = 0.005$], an inflammation-responsive transcription factor known to

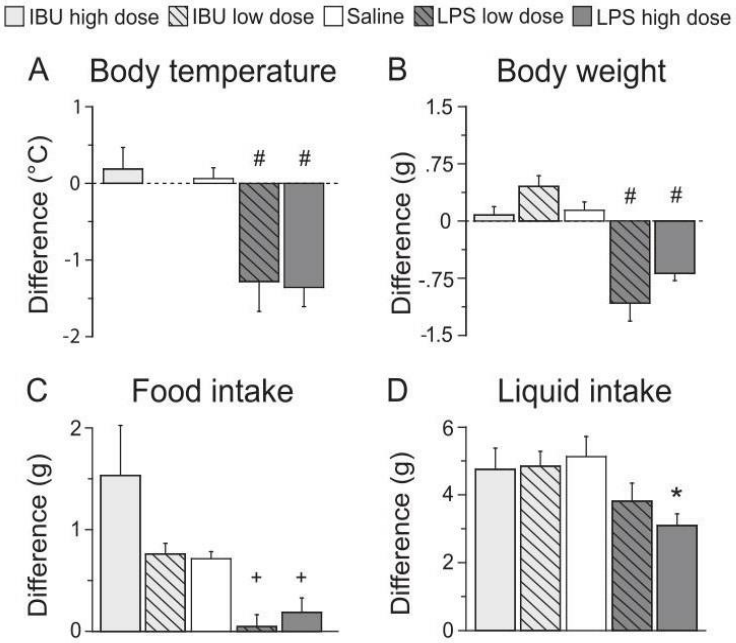


Fig. 1. Physiological and behavioral parameters are affected by LPS treatments. Histogram representing the variations in (A) body temperature and (B) weight, in (C) food and (D) liquid intake. Treatments as indicated in the legend, n = 7–15 mice per group. * $p < 0.05$ vs saline, + $p < 0.05$ vs IBU high dose and # $p < 0.05$ vs saline, IBU low dose and IBU high dose. Data are shown as mean ± s.e.m.

be upregulated by a pro-inflammatory challenges, including LPS (Chang et al., 2012). Post hoc analysis highlighted that the animals treated with the lower dose of LPS displayed significant increased expression levels compare to the saline mice ($p < 0.01$). In addition, they were significantly higher compared to both the high and low doses of IBU ($p < 0.05$ for both; Fig. 2C). mRNA expression of TGF-β, a cytokine with immunosuppressive and anti-inflammatory properties, was significantly affected by treatments [$F(4,44) = 3.226$, $p = 0.021$]. The high LPS dose significantly decreased its hippocampal levels compared to saline injected animals ($p < 0.01$; Fig. 2D). The level of TDO2,

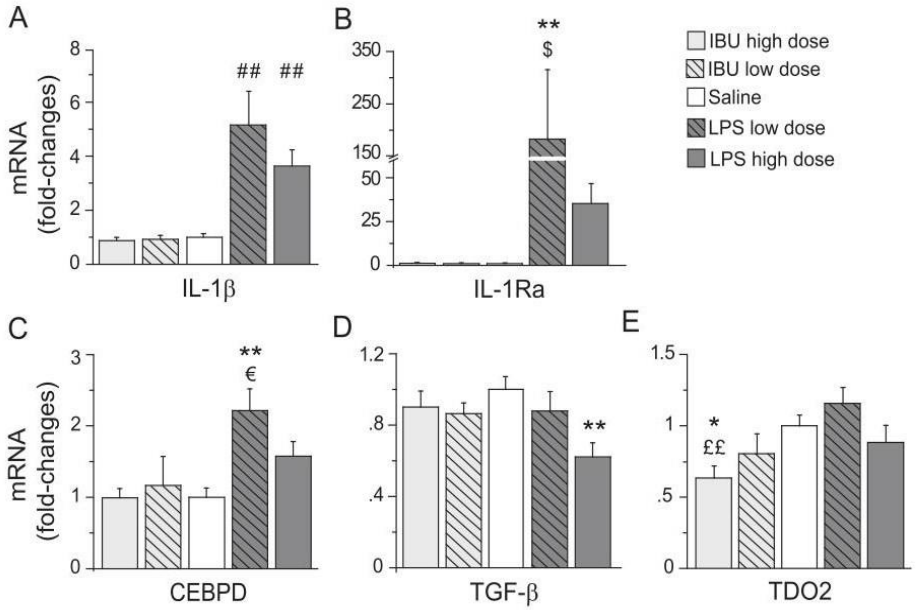


Fig. 2. Expression levels of pro- and anti-inflammatory markers are modulated by treatments. Bars graph representing the hippocampal mRNA level of (A) IL-1β, (B) IL-1RA, (C) CEBPD, (D) TGF-β and (E) TDO2. Treatments as indicated in the legend, n = 5–10 mice per group. ## $p < 0.01$ vs saline, high and low IBU doses, $ $p < 0.05$ vs the two doses of IBU and high dose of LPS, * $p < 0.05$ vs saline, ** $p < 0.01$ vs saline, € $p < 0.05$ vs IBU low and high doses and ££ $p < 0.01$ vs LPS low dose. Data are shown as mean ± s.e.m. Note that LPS increase or reduce inflammatory and anti-inflamamtory markers, respectively, and IBU reduce TDO2.

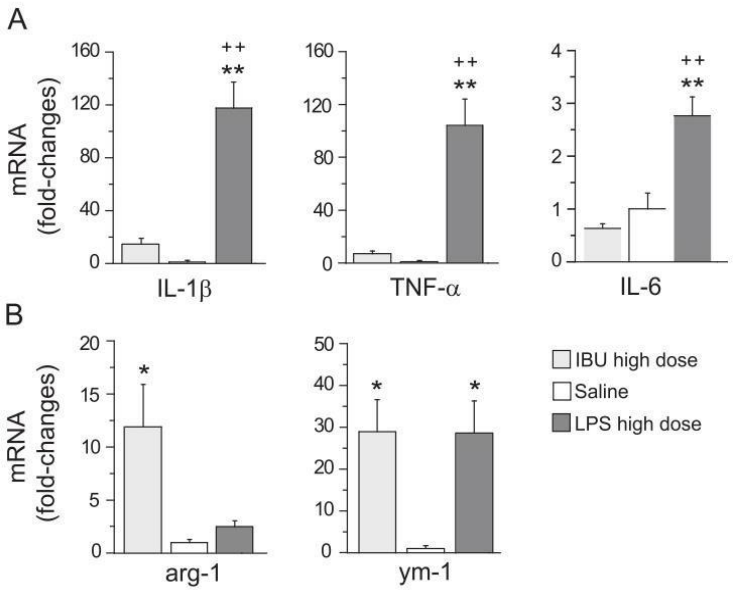


Fig. 3. mRNA expression levels in CD11b + cells isolated from the hippocampus are differently modulated by treatments. Results of RT-PCR analysis are shown as fold changes relative to saline treated mice. Bars graph representing the expression of selected (A) pro-inflammatory (IL-1β, TNF- α, IL-6) and (B) anti-inflammatory (arg-1, ym-1) genes. Treatments as indicated in the legend, n = 5 mice per group. * $p < 0.05$ vs saline, ** $p < 0.01$ vs saline and + + $p < 0.01$ vs IBU. Data are shown as mean ± s.e.m. Note that LPS and IBU increase the expression of pro- and anti-inflammatory markers, respectively.

which catalyzes the first and rate-limiting step of the tryptophan-kynurenine pathway, crucial for neuronal bioenergetics and neurotransmission and involved in the immune-inflammatory responses, was also affected by treatments [$F(4,39) = 3.290$, $p = 0.020$]. High IBU dose reduced TDO2 levels compared to both saline ($p < 0.05$) and low dose of LPS ($p < 0.01$; Fig. 2E).

3.3. Modulation of microglial activation state by IBU and LPS

Different activation states of microglia, the resident macrophage of the brain, are characterized by a wide range of expression of phenotypic markers, including cytokines, chemokines, and surface receptors associated with pro- or anti-inflammatory actions (Franco and Fernandez-Suarez, 2015). In order to investigate how high doses of both treatments affect microglial phenotype, we isolated and analyzed CD11b + cells that mainly comprise microglia. RT-PCR analysis in CD11b + cells revealed a main effect of treatments for both anti- and pro-inflammatory related markers. In particular, only LPS treatment augmented pro-inflammatory-related genes expression (Fig. 3A) such as IL-1β ([$F(2,13) = 17.525$], $p < 0.001$), TNF- α ([$F(2,13) = 17.525$], $p < 0.001$) and IL-6 mRNA ([$F(2,14) = 24.694$], $p < 0.001$) levels compared to both saline and IBU treated mice ($p < 0.01$ for each comparison). IBU treatment showed a different profile, increasing the expression of anti-inflammatory-related genes (Fig. 3B), such as Arg-1 ([$F(2,11) = 5.539$], $p = 0.027$) and Ym-1 ([$F(2,13) = 4.864$], $p = 0.031$), compared to saline ($p < 0.05$ for each comparison). Ym-1 expression was modulated positively also by LPS ($p < 0.05$ to saline).

3.4. The COX-2/PGE_2 pathway is affected by IBU and LPS

COX catalyzes the first committed step in the synthesis of PGs from arachidonic acid (AA). The isoforms COX-1 is a reference gene that is expressed constitutively in the majority of tissues, while COX-2 is an immediate, early-response gene that is highly involved in inflammatory responses, being highly inducible by LPS and inhibited by IBU. However, in mammalian brain, COX-2 is not only induced in microglia/macrophages in response to inflammatory stimuli, but also constitutively expressed in specific neuronal populations under normal physiological conditions, mainly in hippocampal dentate gyrus granule cells and pyramidal cell neurons, the piriform cortex, superficial cell layers of neocortex, and the amygdala (Minghetti, 2004). Its expression is “dynamically” regulated by normal synaptic activity, being rapidly increased during seizures or ischemia.

We observed that high dose of treatments modulates in an opposite manner the levels of PGE_2 produced by COX activity ([$F(2,9) = 4.824$], $p = 0.0377$). In particular, in the total hippocampal extract, LPS increased whereas IBU decreased the levels of this lipid ($p < 0.05$; Fig. 4A), being the vehicle in between. In the whole hippocampus the analyses of COX-2 expression levels did not reveal a significant effect of treatments (Fig. 4B). In contrast, COX-1 expression was affected by treatments ([$F(4,31) = 5.489$], $p = 0.002$) and significantly reduced by high doses of both LPS and IBU compared to saline ($p < 0.05$). In addition, LPS high dose significantly reduced COX-1 expression level compared to LPS low dose ($p < 0.005$; Fig. 4C).

Interestingly, in isolated microglia, COX-2 and prostaglandin E receptor subtype 2, EP2, expression was significantly affected by treatments ([$F(2,13) = 24.261$], $p < 0.001$ and [$F(2,11) = 19.01$], $p < 0.001$, respectively). In particular, post hoc analysis revealed that LPS treatment increased the expression of COX-2 compared to saline ($p < 0.01$) and IBU ($p < 0.05$, Fig. 4D) and of EP2 ($p < 0.01$ to saline and IBU, Fig. 4E). In contrast, prostaglandin E receptor subtype 3, EP3, was not affected by treatments (Fig. 4F).

3.5. Dose response effects of IBU and LPS treatments on neuronal plasticity

We then explored long-term plasticity in CA1 hippocampal region by recording extracellular fEPSP evoked by a robust Shaffer collaterals stimulation (two 100 Hz bursts, separated by a 3 s interval). We found a main effect of treatment [$F(4,56) = 4.757$, $p = 0.0022$]. Both LPS and IBU at the high dose significantly reduced the amplitude of LTP (Fig. 5A). Mice treated with saline showed a sustained LTP amplitude (1.323 ± 0.149), that was decreased of about 40% following IBU high dose (1.156 ± 0.079) or LPS high dose treatment (1.200 ± 0.136, $p < 0.05$ for each comparison). In contrast, low doses of LPS and IBU did not affect the amplitude of LTP (1.346 ± 0.088, and 1.327 ± 0.161, respectively). Overall, these results demonstrate that

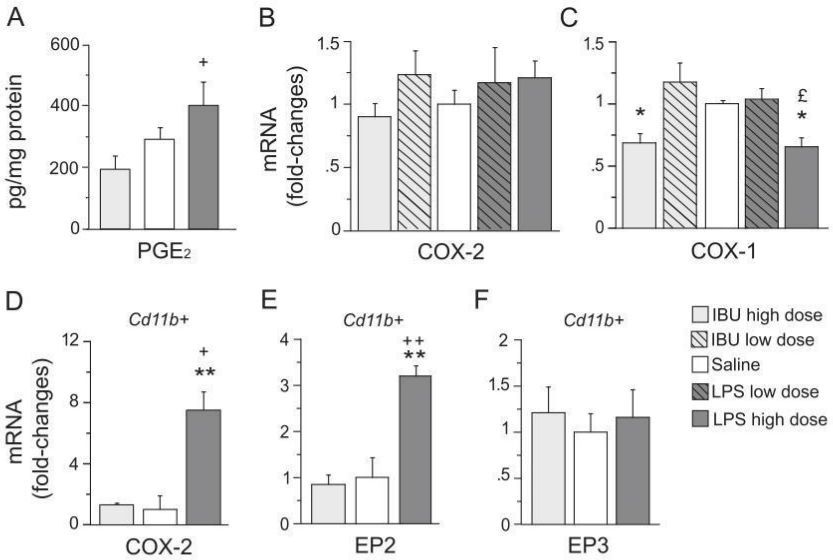


Fig. 4. LPS and IBU treatments modulate *COX-2/PGE2 pathway in total hippocampus and Cd11b + cells.* Bars graph representing levels of components of prostaglandins signaling in the total hippocampus: (A) prostaglandin E2 (PGE_2) lipid, (B) COX-2 and (C) COX-1 mRNA; or in microglia (Cd11b +) isolated cells: (D) COX-2, (E) EP3 and (F) EP2 mRNA. Treatments as indicated in the legend, n = 5–10 for all groups. Data are shown as mean ± s.e.m. ** $p < 0.01$ vs saline and + $p < 0.05$ vs IBU; * $p < 0.005$ vs saline and £ $p < 0.005$ vs LPS low dose (Bonferroni's post hoc).

IBU and LPS treatments have a detrimental effect on long-term plasticity mainly affecting the postsynaptic LTP expression.

We then explored whether the short-term plasticity (PPR) was affected by the treatments. PPR was estimated by stimulating Schaffer collateral inputs to the pyramidal neurons with two stimuli 50 ms apart.

A main effect of treatment emerged [$F(4,74) = 4.629$, $p = 0.0022$], with post hoc analysis revealing that the low LPS dose, but not the low IBU dose (1.302 ± 0.033 and 1.221 ± 0.033, respectively), increased PPR compared to saline (1.149 ± 0.022, $p < 0.01$), whereas both the high IBU (1.148 ± 0.116, $p < 0.01$) and LPS doses (1.165 ± 0.086, $p < 0.05$; Fig. S1) had no effect.

To assess the effect of activation and suppression of inflammatory mediators on neural plasticity at molecular level, we investigated whether the expression levels of BDNF, a neurotrophin that play a key role in hippocampal plasticity (Leal et al., 2017), were differently affected by the treatment with IBU and LPS. The measure of hippocampal level of BDNF mRNA showed the main effect of the treatment [$F(4,41) = 5.618$], $p = 0.001$). The post hoc analysis revealed that the higher doses of IBU and LPS were associated with significant reduced expression level of this neurotrophin compared to saline mice ($p < 0.01$ for both; Fig. 5B).

In addition, as AMPA receptor functionality is regulated by phosphorylation during activity-dependent plasticity, we evaluated the effects of high dose of treatments on GluR1 phosphorylation levels. We observed that Ser845 phosphorylation on GluR1 subunit was significantly affected by treatments ([$F(2,11) = 23.552$], $p < 0.001$). In particular, following both LPS and IBU administration, the level of phosphorylation was similarly decreased compared to saline (by 40%, $p < 0.01$ for LPS and IBU; Fig. 5C).

4. Discussion

The present results show that treatments with LPS and IBU, besides modulating in opposite direction molecules related with a pro- or anti-inflammatory action, converge in impairing neural plasticity measured at both molecular and cellular levels. Such effects appear to be dose-dependent since only the higher, but not the lower, dose of both compounds produced such effect.

According with the literature, LPS produced several pro-inflammatory effects. As expected, it induced a generalized defense response comprising physiological, and behavioral changes (Hart et al., 1988; Pecchi et al., 2009; Wieseler-Frank et al., 2005; Yirmiya, 1996; Yirmiya et al., 2001). In particular, LPS significantly reduced body temperature and weight compared to all the other treatment groups and decreased food intake compared to the high IBU dose group. This significant hypothermic response is in line with previous studies reporting a reduction of body temperature after few hours from LPS injection (Fan et al., 2013; Skelly et al., 2013).

In addition, LPS increased hippocampal expression of inflammatory transcripts. We found that both high and low LPS doses significant raised IL-1β expression compared to all the other groups, is in line with the literature describing IL-1β as a key mediator of the inflammatory response (Lopez-Castejon and Brough, 2011) and a main contributor to the pathogenesis of LPS-induced acute inflammation (Ulich et al., 1991). The lower dose of LPS significantly increased the level of both IL-1RA and CEPBD compared to both IBU doses and saline. IL-1RA is a natural inhibitor of the pro-inflammatory effect of IL-1β since it blocks further IL-1β activity and terminates the inflammatory response (Arend et al., 1998).

Previous studies have largely demonstrated that the IL-1RA expression can be induced in monocytes and macrophages through treatment with LPS (Jenkins and Arend, 1993). CEBPD is a transcription factor involved in the regulation of genes associated with activation and/or differentiation of macrophages, and consequently, with an important regulatory role of immune responses. CEBPD is expressed at a relatively low level under normal physiological conditions and it is upregulated by a variety of pro-inflammatory challenges, such as IL-6, IL-1β and LPS (Chang et al., 2012). In addition, while in neurons, CEBPD participates in memory formation and synaptic plasticity, in glial cells it regulates the pro-inflammatory program (Pulido-Salgado

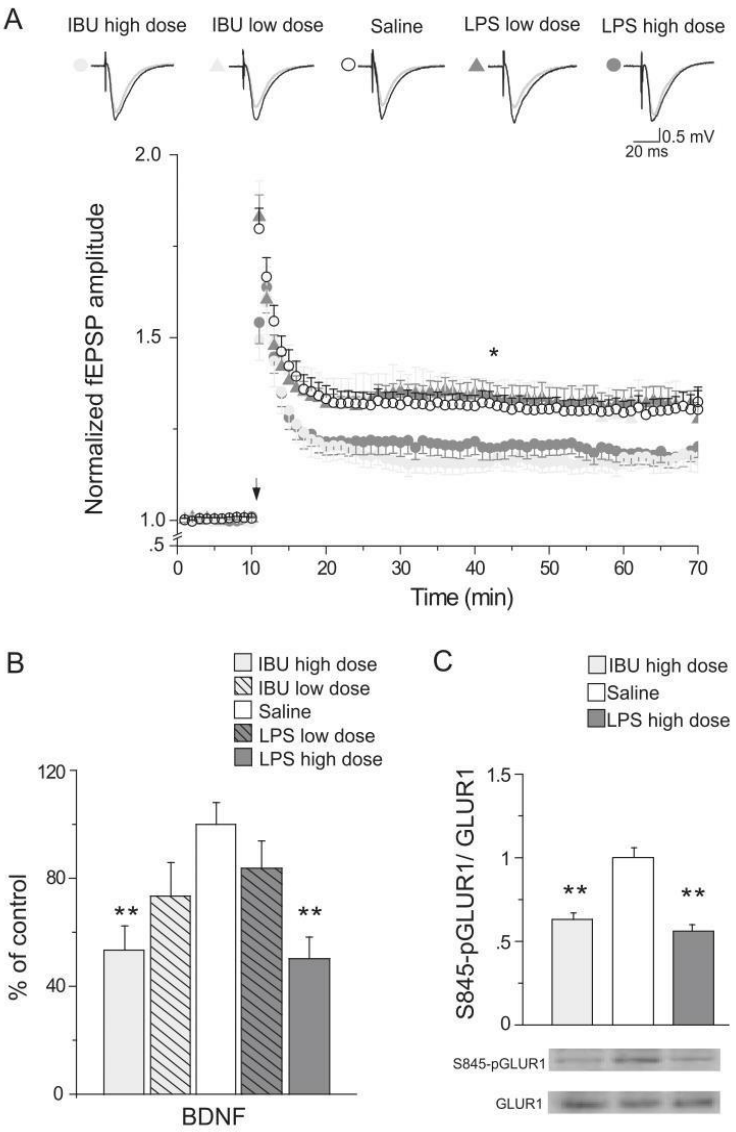


Fig. 5. High doses of treatments affect LTP, BDNF expression level and GluR1 subunit phosphorylation. (A) Inset, field potential waveforms (averages of three traces) before (black) and after (gray) 30 min of HFS induction, for each treatment as indicated. Bottom, Time course of normalized averaged amplitudes of fEPSPs. Arrows indicate LTP induction (HFS, two 100 Hz bursts, separated by a 3 s interval). Treatments as indicated in the inset. N/n refers to the number of slices on total number of mice analyzed (N = 8–25, n = 4–9). * $p < 0.05$ high dose of drugs vs saline. Note that only high dose of IBU and LPS reduce LTP amplitude 30 min after HFS. (B) Histogram represents BDNF hippocampal mRNA level. Higher doses of both IBU and LPS significantly decrease the levels of BDNF compared to saline mice. Treatments as indicated in the legend, n = 5–10 mice per group. ** $p < 0.01$ vs saline. Data are shown as mean ± s.e.m. (C) Histogram represents hippocampal levels of AMPA receptor subunits GluR1 phosphorylation at Ser 845 (S845-pGLUR1) normalized respect to total GluR1. Bottom, representative western blots analyses. Treatments as indicated in the legend, n = 5 for all groups. ** $p < 0.01$ vs saline. Data are shown as mean ± s.e.m. Note that both LPS and IBU treatments induce a decrease in GluR1 phosphorylation at Ser 845.

et al., 2015). The high LPS dose missed to reach a statistically significant increase of IL-1RA and CEBPD, likely because the pro-inflammatory pathways triggered by different LPS doses do not overlap, as previously reported (Deng et al., 2013b; Dudele et al., 2015; Morris et al., 2014). Moreover, increased expression of IL-1RA in the hippocampus of animals treated with the low dose of LPS, together with the increase in CEBPD expression, may counteract the detrimental effects induced by IL-1β on brain plasticity. The expression of TGF-β, a pleiotropic cytokine with immunosuppressive and anti-inflammatory properties was significantly reduced by the high LPS dose, in line with previous findings (Kim and Kim, 2011; Mitchell et al., 2014).

In order to ascertain the effect of treatments on the main resident macrophage population of the brain, (i.e. microglia) we freshly isolated microglial cells (CD11b-positive cells) and we analyzed the expression profile of a number of inflammatory-related markers, which play critical physiological roles in brain plasticity modulation as well. It is known that LPS treatments can trigger microglial activation and transcriptional induction of downstream inflammatory mediators, including the release of cytokines and prostanoids. Accordingly, we found that in CD11b-positive cells, LPS increased the expression of typical pro-inflammatory-related genes, such us IL-1β, TNF- α, IL-6, and COX-2 (Hein and O'Banion, 2009). EP2, known as a downstream target of PGE_2 involved in the inflammatory cascade, was also largely increased by LPS, supporting previously finding (Minghetti, 2004; Shie et al., 2005; Zhang et al., 2015) and confirming the efficacy of acute LPS treatment.

It is known that LPS promotes PGE_2 synthesis by a sustained upregulation of COX-2 (Font-Nieves et al., 2012). In accordance, in the total hippocampal extract, we observed that high dose of LPS enhanced

PGE_2 levels. Although COX-2 was clearly enhanced in microglia cells, in the hippocampus we observed only a tendency toward an increase of the transcript, indicating that microglia is the main target of LPS action. We also analyzed the expression of COX-1, the constitutive isoform that can be expressed by several cell types in the CNS (Minghetti and Pocchiari, 2007), and we observed that LPS down-modulated COX-1 mRNA in total hippocampal extract. The contribution of the enzymes COX-1 and COX-2 in PGE_2 formation varies depending on the stimuli and cell type. For example, astrocytes respond to LPS by a COX-2-dependent production of prostanoids, mainly PGE_2, and by reducing COX-1 (Font-Nieves et al., 2012). Similarly, on organotypic hippocampal cultures, LPS treatment promotes PGE_2 synthesis by a sustained up-regulation of cyclooxygenase-2 (COX-2) and down regulation of COX-1 mRNA (Ajmone-Cat et al., 2013).

Concerning IBU, we observed that in naïve mice, as expected, the acute exposure did not modify physiological parameters, such us body temperature, weight and food intake. However, in naïve conditions the drug affected constitutive components of the immune/plasticity-related machinery.

For example, the high IBU dose reduced TDO2, the first committed step in the kynurenine pathway, which is involved in inflammation and whose expression is highly restricted to the hippocampal circuitry, supporting the recent finding that TDO2 is a novel neuronal target of IBU (Campbell et al., 2014; Woodling et al., 2016). As mentioned above, the tryptophan-kynurenine pathway is also crucial for neuronal bioenergetics and neurotransmission, suggesting an important modulation of plasticity mechanisms by NSAIDs in naïve animals.

With regard to COX-2 expression levels in the total hippocampus, we did not observe any significant effect, though the high IBU dose showed a trend towards of a reduction of COX-2 levels, in line with previous studies (Hawkey, 1999; Mitchell et al., 1993). In addition, IBU reduced COX-1 expression, although the primary regulatory effect of NSAID, such as IBU, is ascribable to the modulation of the COX-enzymes activity. Notably, the main end-product of COX activity in the brain, PGE_2, was reduced by IBU, demonstrating the effectiveness of the treatment in naïve animals.

Interestingly, on freshly isolated microglia, IBU treatment increased two anti-inflammatory markers, such as Arg-1 and Ym-1, and did not affect the pro-inflammatory genes analyzed. This suggests an immunomodulatory effect of this NSAID on microglia in naïve animlas, able to shift the balance of the constitutive expression of immune mediators towards the anti-inflammatory/protective ones. Overall, these results reveal an immunomodulatory effect of IBU in naïve animals. It is worth noting that very limited studies investigating the effects of IBU on naïve animals are available (Ajmone-Cat et al., 2006; Shaw et al., 2005; Zaminelli et al., 2014).

Though LPS and IBU produced significantly different effects on physiological endpoints and expression of inflammatory markers, they led to overlapping effects on plasticity at a cellular level: both treatments, at high doses, significantly reduced LTP amplitude by around 40%.

LPS has been reported to affect LTP most likely through an increase in pro-inflammatory cytokines. Among these, IL-1β that has notably been demonstrated to have detrimental effects on LTP induction and maintenance (Bellinger et al., 1993; Ikegaya et al., 2003; Ross et al., 2003). The impairment of LTP induced by LPS could be also due to the reduction of TGF-β, a cytokine deeply involved in modulating synaptic transmission and physiological mechanism underlying LTP (Caraci et al., 2015).

Interestingly, both cytokines are involved in the regulation of BDNF expression and signaling (Ohja et al., 2018; Sometani et al., 2001; Tong et al., 2008), another key molecule in the regulation of LTP. Specifically, reduced BDNF levels have been found to be associated to a reduction of LTP (Leal et al., 2014), as we here observed.

We found that similarly to high LPS, also high IBU dose reduced BDNF expression, suggesting that the neurotrophin is a common target likely responsible for the reduction of LTP observed after both treatments.

With regard to other potential mechanisms linking high dose IBU with reduced BDNF expression levels in naïve animals, evidence indicates that this could be the broad-spectrum COX inhibition produced by IBU (Hein and O'Banion, 2009; Shaw et al., 2003). Notably, IBU and LPS affect in opposite directions COX-2 activity and/or expression, and hence PGE_2 production, as we expectedly found. Nevertheless, the effects of PGE_2 are dependent by (i) the cell type involved (astroglia vs pre and post-synaptic neurons), (ii) the amount of PGE_2 and (iii) the functioning of PGE_2 receptors (Hein and O'Banion, 2009; Sang et al., 2005; Shaw et al., 2003). Interestingly, it has been shown that PGE_2 generated by COX-2 regulates hippocampal long-term potentiation (LTP) under basal conditions (Chen et al., 2002), and pathological induction of COX-2 activity significantly disrupts hippocampal synaptic function (Yang and Chen, 2008). A recent hypothesis proposes a dominant role of EP3 receptor, an inhibitory G protein that has the highest abundance within the hippocampus and colocalizes with PSD95, in interfering with postsynaptic cascades involved in synaptic strengthening and in the regulation of cAMP response element binding protein (CREB) phosphorylation, that in turn regulate BDNF transcription (Hein and O'Banion, 2009). Accordingly, neuroinflammation-induced LTP reduction may be caused by elevated PGE_2 binding to increased number of EP3 receptors, reducing cAMP levels, and thereby BDNF mRNA expression (Hein and O'Banion, 2009). On the other hand, it is possible to speculate that following IBU treatment, the decreased level of PGE_2 could as well favor the activity of EP3 receptor that presents the higher affinity for the lipid compared to the other receptors (Kiriyama et al., 1997).

It is worth noting that both IBU and LPS converge in reducing COX-1 expression, which is constitutively present in the hippocampus, acting as a housekeeping enzyme by regulating normal physiological processes. Beside its role in the cross regulation of COX-2 expression, COX-1 activity could interfere at some levels of the aforementioned plasticity processes. Finally, several COX-independent effects of IBU could also contribute in the modification of plasticity described here, including the modulation of transcription factors involved in the expression of inflammatory/plasticity related molecules (Ajmone-Cat et al., 2010; Bernardo and Minghetti, 2008; Hein and O'Banion, 2009).

Interestingly, the phosphorylation of the GluR1 subunit of the AMPA receptor, known to be modulated at Ser845 during plasticity processes, is similarly reduced by both LPS and IBU treatments, in accordance with the reduction of LTP, further supporting the inhibitory action of the drugs. It is known that also the level of phosphorylation at this site is regulated by the cAMP levels that in turn affect the balance between kinase and phosphatase activity.

PPR, is a typical form of short-term plasticity associated with changes in transmitter release probability (Branco and Staras, 2009; Dunwiddie and Haas, 1985; Zucker and Regehr, 2002). We showed that low, but not high, dose of LPS increases PPR, an opposite behavior of what was observed for LTP modulation. Accordingly, the concentration of LPS previously shown to affect CA1 LTP, produced no effect on PPR (Iwai et al., 2014). As discussed, LPS produces specific dose-dependent effects, mainly due to the different triggering of pathway downstream Toll-like receptor 4 and to the level of expression of pro-inflammatory mediators (Deng et al., 2013b; Dudele et al., 2015; Morris et al., 2014). Indeed, many of these mediators, such as IL-1β, known to affect neurotransmission both at pre- and post-synaptic sites (O'Connor and Coogan, 1999; Yirmiya and Goshen, 2011), induce opposite effects by varying dosage (Deng et al., 2013a). Thus, it is possible that short- and long-term plasticity processes are differently affected by distinct LPS concentration.

Thus, the complex mechanisms underlying IBU an LPS mediated inhibition of plasticity needs further investigation. It is important to highlight that present molecular data comprise mainly mRNA transcript levels and the assessment of a number of protein levels/activation

profile will provide a more comprehensive picture of the interaction between inflammatory markers and hippocampal plasticity processes.

Overall, the present findings suggest that neural plasticity is impaired by acute treatment of both high doses of LPS and IBU that caused, respectively, an activation and a suppression of inflammatory mediators. The impact appears to be dose-dependent because only higher doses of either LPS or IBU, impair neuronal plasticity. This is in line with previous studies and in particular the seminal review paper by Yirmiya and Goshen (Yirmiya and Goshen, 2011) suggesting that both anti- and pro-inflammatory mediators are required for brain functioning since unbalances towards an immune suppression or activation have detrimental consequences for the healthy brain (Hewett et al., 2012; Santello et al., 2012; Yirmiya and Goshen, 2011). A relevant theoretical consequence of the present findings is that the ultimate effect of anti- and pro-inflammatory challenges are determined by the baseline inflammatory condition of the individual. Indeed, the beneficial or detrimental impact should be evaluated according to the capability to normalize or exacerbate the inflammatory balance. Finally, previous studies suggest that an increase in neural plasticity, induced through selective serotonin reuptake inhibitor administration, counterbalances both the activation and the suppression of the immune response keeping inflammation in a strict range (Alboni et al., 2016). Integrating these results with the present ones, a clear interplay between inflammatory mediators and neural plasticity emerges, indicating that these are mutually regulating processes. Therefore, therapeutic interventions aimed at targeting specifically neural plasticity may result in a more effective outcome if designed to simultaneously act on both the processes.

Acknowledgments

We thank Fatima Abdallah and Gloria Matte Bon who provided technical support and Barbara Cortese for critical reading and editing. The research was supported by the grant from the Italian Ministry of Health Ricerca Finalizzata - RF-2018-12367249 and ERANET neuron 2017 MicroSynDep, both to IB.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.07.003.

References

Ajmone-Cat, M.A., Bernardo, A., Greco, A., Minghetti, L., 2010. Non-steroidal anti-inflammatory drugs and brain inflammation: effects on microglial functions. Pharmaceuticals 3, 1949–1965.

Ajmone-Cat, M.A., Iosif, R.E., Ekdahl, C.T., Kokaia, Z., Minghetti, L., Lindvall, O., 2006. Prostaglandin E2 and BDNF levels in rat hippocampus are negatively correlated with status epilepticus severity: no impact on survival of seizure-generated neurons. Neurobiol. Dis. 23, 23–35.

Ajmone-Cat, M.A., Mancini, M., De Simone, R., Cilli, P., Minghetti, L., 2013. Microglial polarization and plasticity: evidence from organotypic hippocampal slice cultures. Glia 61, 1698–1711.

Alboni, S., Benatti, C., Montanari, C., Tascedda, F., Brunello, N., 2013. Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus. Eur. J. Pharmacol. 721, 158–167.

Alboni, S., Poggini, S., Garofalo, S., Milior, G., El Hajj, H., Lecours, C., Girard, I., Gagnon, S., Boisjoly-Villeneuve, S., Brunello, N., Wolfer, D.P., Limatola, C., Tremblay, M.E., Maggi, L., Branchi, I., 2016. Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment. Brain Behav. Immun. 58, 261–271.

Alboni, S., van Dijk, R.M., Poggini, S., Milior, G., Perrotta, M., Drenth, T., Brunello, N., Wolfer, D.P., Limatola, C., Amrein, I., Cirulli, F., Maggi, L., Branchi, I., 2017. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol. Psychiatry 22, 635.

Arend, W.P., Malyak, M., Guthridge, C.J., Gabay, C., 1998. Interleukin-1 receptor antagonist: role in biology. Annu. Rev. Immunol. 16, 27–55.

Bellinger, F.P., Madamba, S., Siggins, G.R., 1993. Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res. 628, 227–234.

Benatti, C., Blom, J.M., Rigillo, G., Alboni, S., Zizzi, F., Torta, R., Brunello, N., Tascedda, F., 2016. Disease-induced neuroinflammation and depression. CNS Neurol. Disord. Drug Targets 15, 414–433.

Bernardo, A., Minghetti, L., 2008. Regulation of glial cell functions by PPAR-gamma natural and synthetic agonists. PPAR Res. 2008, 864140.

Bliss, T.V., Collingridge, G.L., 2013. Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol. Brain 6, 5.

Branchi, I., Alboni, S., Maggi, L., 2014. The role of microglia in mediating the effect of the environment in brain plasticity and behavior. Front. Cell. Neurosci. 8, 390.

Branco, T., Staras, K., 2009. The probability of neurotransmitter release: variability and feedback control at single synapses. Nat. Rev. Neurosci. 10, 373–383.

Campbell, B.M., Charych, E., Lee, A.W., Moller, T., 2014. Kynurenines in CNS disease: regulation by inflammatory cytokines. Front. Neurosci. 8, 12.

Caraci, F., Gulisano, W., Guida, C.A., Impellizzeri, A.A., Drago, F., Puzzo, D., Palmeri, A., 2015. A key role for TGF-beta1 in hippocampal synaptic plasticity and memory. Sci. Rep. 5, 11252.

Chang, L.H., Huang, H.S., Wu, P.T., Jou, I.M., Pan, M.H., Chang, W.C., Wang, D.D., Wang, J.M., 2012. Role of macrophage CCAAT/enhancer binding protein delta in the pathogenesis of rheumatoid arthritis in collagen-induced arthritic mice. PLoS ONE 7, e45378.

Chen, C., Bazan, N.G., 2005. Lipid signaling: sleep, synaptic plasticity, and neuroprotection. Prostaglandins Other Lipid Mediat. 77, 65–76.

Chen, C., Magee, J.C., Bazan, N.G., 2002. Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. J. Neurophysiol. 87, 2851–2857.

Chen, W.W., Zhang, X., Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases (Review). Mol. Med. Rep. 13, 3391–3396.

Chertov, O., Yang, D., Howard, O.M., Oppenheim, J.J., 2000. Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. Immunol. Rev. 177, 68–78.

Cowley, T.R., Fahey, B., O'Mara, S.M., 2008. COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. Eur. J. Neurosci. 27, 2999–3008.

Deng, H., Maitra, U., Morris, M., Li, L., 2013a. Molecular mechanism responsible for the priming of macrophage activation. J. Biol. Chem. 288, 3897–3906.

Deng, M., Scott, M.J., Loughran, P., Gibson, G., Sodhi, C., Watkins, S., Hackam, D., Billiar, T.R., 2013b. Lipopolysaccharide clearance, bacterial clearance, and systemic inflammatory responses are regulated by cell type-specific functions of TLR4 during sepsis. J. Immunol. 190, 5152–5160.

Dudele, A., Fischer, C.W., Elfving, B., Wegener, G., Wang, T., Lund, S., 2015. Chronic exposure to low doses of lipopolysaccharide and high-fat feeding increases body mass without affecting glucose tolerance in female rats. Physiol. Rep. 3 (11), e12584. https://doi.org/10.14814/phy2.12584.

Dunwiddie, T.V., Haas, H.L., 1985. Adenosine increases synaptic facilitation in the in vitro rat hippocampus: evidence for a presynaptic site of action. J. Physiol. 369, 365–377.

Fan, L.W., Kaizaki, A., Tien, L.T., Pang, Y., Tanaka, S., Numazawa, S., Bhatt, A.J., Cai, Z., 2013. Celecoxib attenuates systemic lipopolysaccharide-induced brain inflammation and white matter injury in the neonatal rats. Neuroscience 240, 27–38.

Ferrero-Miliani, L., Nielsen, O.H., Andersen, P.S., Girardin, S.E., 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clin. Exp. Immunol. 147, 227–235.

Font-Nieves, M., Sans-Fons, M.G., Gorina, R., Bonfill-Teixidor, E., Salas-Perdomo, A., Marquez-Kisinousky, L., Santalucia, T., Planas, A.M., 2012. Induction of COX-2 enzyme and down-regulation of COX-1 expression by lipopolysaccharide (LPS) control prostaglandin E2 production in astrocytes. J. Biol. Chem. 287, 6454–6468.

Franco, R., Fernandez-Suarez, D., 2015. Alternatively activated microglia and macrophages in the central nervous system. Prog. Neurobiol. 131, 65–86.

Garofalo, S., D'Alessandro, G., Chece, G., Brau, F., Maggi, L., Rosa, A., Porzia, A., Mainiero, F., Esposito, V., Lauro, C., Benigni, G., Bernardini, G., Santoni, A., Limatola, C., 2015. Enriched environment reduces glioma growth through immune and non-immune mechanisms in mice. Nat. Commun. 6, 6623.

Godbout, J.P., Chen, J., Abraham, J., Richwine, A.F., Berg, B.M., Kelley, K.W., Johnson, R.W., 2005. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 19, 1329–1331.

Hart, P.H., Whitty, G.A., Piccoli, D.S., Hamilton, J.A., 1988. Synergistic activation of human monocytes by granulocyte-macrophage colony-stimulating factor and IFN-gamma. Increased TNF-alpha but not IL-1 activity. J. Immunol. 141, 1516–1521.

Hawkey, C.J., 1999. COX-2 inhibitors. Lancet 353, 307–314.

Hein, A.M., O'Banion, M.K., 2009. Neuroinflammation and memory: the role of prostaglandins. Mol. Neurobiol. 40, 15–32.

Hewett, S.J., Jackman, N.A., Claycomb, R.J., 2012. Interleukin-1beta in central nervous system injury and repair. Eur. J. Neurodegener. Dis. 1, 195–211.

Hotamisligil, G.S., Erbay, E., 2008. Nutrient sensing and inflammation in metabolic diseases. Nat. Rev. Immunol. 8, 923–934.

Hurley, L.L., Tizabi, Y., 2013. Neuroinflammation, neurodegeneration, and depression. Neurotox. Res. 23, 131–144.

Ikegaya, Y., Delcroix, I., Iwakura, Y., Matsuki, N., Nishiyama, N., 2003. Interleukin-1beta abrogates long-term depression of hippocampal CA1 synaptic transmission. Synapse 47, 54–57.

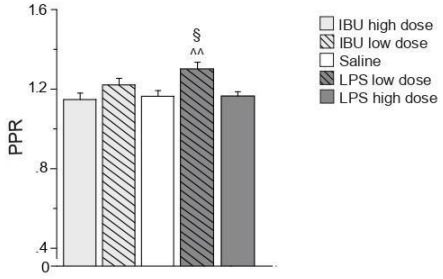
Iwai, T., Sawabe, T., Tanimitsu, K., Suzuki, M., Sasaki-Hamada, S., Oka, J., 2014. Glucagon-like peptide-1 protects synaptic and learning functions from neuroinflammation in rodents. J. Neurosci. Res. 92, 446–454.

Jenkins, J.K., Arend, W.P., 1993. Interleukin 1 receptor antagonist production in human monocytes is induced by IL-1 alpha, IL-3, IL-4 and GM-CSF. Cytokine 5, 407–415.

Kim, E.Y., Kim, B.C., 2011. Lipopolysaccharide inhibits transforming growth factor-beta1-

stimulated Smad6 expression by inducing phosphorylation of the linker region of Smad3 through a TLR4-IRAK1-ERK1/2 pathway. FEBS Lett. 585, 779–785.
Kiriyama, M., Ushikubi, F., Kobayashi, T., Hirata, M., Sugimoto, Y., Narumiya, S., 1997. Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 122, 217–224.
Kubera, M., Curzytek, K., Duda, W., Leskiewicz, M., Basta-Kaim, A., Budziszewska, B., Roman, A., Zajicova, A., Holan, V., Szczesny, E., Lason, W., Maes, M., 2013. A new animal model of (chronic) depression induced by repeated and intermittent lipopolysaccharide administration for 4 months. Brain Behav. Immun. 31, 96–104.
Leal, G., Bramham, C.R., Duarte, C.B., 2017. BDNF and hippocampal synaptic plasticity. Vitam. Horm. 104, 153–195.
Leal, G., Comprido, D., Duarte, C.B., 2014. BDNF-induced local protein synthesis and synaptic plasticity. Neuropharmacology 76, 639–656. https://doi.org/10.1016/j.neuropharm.2013.04.005.
Levin, S.G., Godukhin, O.V., 2017. Modulating effect of cytokines on mechanisms of synaptic plasticity in the brain. Biochem. Biokhimiia 82, 264–274.
Libby, P., 2006. Inflammation and cardiovascular disease mechanisms. Am. J. Clin. Nutr. 83, 456S–460S.
Lopez-Castejon, G., Brough, D., 2011. Understanding the mechanism of IL-1beta secretion. Cytokine Growth Factor Rev. 22, 189–195.
Lu, B., Nagappan, G., Lu, Y., 2014. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb. Exp. Pharmacol. 220, 223–250.
Maingret, V., Barthet, G., Deforges, S., Jiang, N., Mulle, C., Amedee, T., 2017. PGE2-EP3 signaling pathway impairs hippocampal presynaptic long-term plasticity in a mouse model of Alzheimer's disease. Neurobiol. Aging 50, 13–24.
Martin, S.A., Pence, B.D., Greene, R.M., Johnson, S.J., Dantzer, R., Kelley, K.W., Woods, J.A., 2013. Effects of voluntary wheel running on LPS-induced sickness behavior in aged mice. Brain Behav. Immun. 29, 113–123.
McEachern, J.C., Shaw, C.A., 1999. The plasticity-pathology continuum: defining a role for the LTP phenomenon. J. Neurosci. Res. 58, 42–61.
Medeiros, I.U., Ruzza, C., Asth, L., Guerrini, R., Romao, P.R., Gavioli, E.C., Calo, G., 2015. Blockade of nociceptin/orphanin FQ receptor signaling reverses LPS-induced depressive-like behavior in mice. Peptides 72, 95–103.
Medzhitov, R., 2010. Inflammation 2010: new adventures of an old flame. Cell 140, 771–776.
Minghetti, L., 2004. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J. Neuropathol. Exp. Neurol. 63, 901–910.
Minghetti, L., Greco, A., Cardone, F., Puopolo, M., Ladogana, A., Almonti, S., Cunningham, C., Perry, V.H., Pocchiari, M., Levi, G., 2000. Increased brain synthesis of prostaglandin E2 and F2-isoprostane in human and experimental transmissible spongiform encephalopathies. J. Neuropathol. Exp. Neurol. 59, 866–871.
Minghetti, L., Pocchiari, M., 2007. Cyclooxygenase-2, prostaglandin E2, and microglial activation in prion diseases. Int. Rev. Neurobiol. 82, 265–275.
Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J., Vane, J.R., 1993. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. PNAS 90, 11693–11697.
Mitchell, K., Shah, J.P., Tsytsikova, L.V., Campbell, A.M., Affram, K., Symes, A.J., 2014. LPS antagonism of TGF-beta signaling results in prolonged survival and activation of rat primary microglia. J. Neurochem. 129, 155–168.
Morris, M.C., Gilliam, E.A., Button, J., Li, L., 2014. Dynamic modulation of innate immune response by varying dosages of lipopolysaccharide (LPS) in human monocytic cells. J. Biol. Chem. 289, 21584–21590.
Najjar, S., Pearlman, D.M., Alper, K., Najjar, A., Devinsky, O., 2013. Neuroinflammation and psychiatric illness. J. Neuroinflammation 10, 43.
O'Connor, J.C., Lawson, M.A., Andre, C., Moreau, M., Lestage, J., Castanon, N., Kelley, K.W., Dantzer, R., 2009. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol. Psychiatry 14, 511–522.
O'Connor, J.J., Coogan, A.N., 1999. Actions of the pro-inflammatory cytokine IL-1 beta on central synaptic transmission. Exp. Physiol. 84, 601–614.
Ohja, K., Gozal, E., Fahnestock, M., Cai, L., Cai, J., Freedman, J.H., Switala, A., El-Baz, A., Barnes, G.N., 2018. Neuroimmunologic and neurotrophic interactions in autism spectrum disorders: relationship to neuroinflammation. Neuromolecular Med. 20, 161–173.
Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., Ragozzino, D., Gross, C.T., 2011. Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458.
Pecchi, E., Dallaporta, M., Jean, A., Thirion, S., Troadec, J.D., 2009. Prostaglandins and sickness behavior: old story, new insights. Physiol. Behav. 97, 279–292.
Pulido-Salgado, M., Vidal-Taboada, J.M., Saura, J., 2015. C/EBPbeta and C/EBPdelta transcription factors: basic biology and roles in the CNS. Prog. Neurobiol. 132, 1–33.
Rainsford, K.D., 2013. Ibuprofen: from invention to an OTC therapeutic mainstay. Int. J. Clin. Pract. Suppl. 9–20.
Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24–31.
Rall, J.M., Mach, S.A., Dash, P.K., 2003. Intrahippocampal infusion of a cyclooxygenase-2 inhibitor attenuates memory acquisition in rats. Brain Res. 968, 273–276.
Reus, G.Z., Fries, G.R., Stertz, L., Badawy, M., Passos, I.C., Barichello, T., Kapczinski, F., Quevedo, J., 2015. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience 300, 141–154.
Ross, F.M., Allan, S.M., Rothwell, N.J., Verkhratsky, A., 2003. A dual role for interleukin-1 in LTP in mouse hippocampal slices. J. Neuroimmunol. 144, 61–67.
Sang, N., Zhang, J., Marcheselli, V., Bazan, N.G., Chen, C., 2005. Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. J. Neurosci. 25, 9858–9870.
Santello, M., Cali, C., Bezzi, P., 2012. Gliotransmission and the tripartite synapse. Adv. Exp. Med. Biol. 970, 307–331.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3, 1101–1108.
Sharifzadeh, M., Tavasoli, M., Soodi, M., Mohammadi-Eraghi, S., Ghahremani, M.H., Roghani, A., 2006. A time course analysis of cyclooxygenase-2 suggests a role in spatial memory retrieval in rats. Neurosci. Res. 54, 171–179.
Shaw, K.N., Commins, S., O'Mara, S.M., 2003. Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor. Eur. J. Neurosci. 17, 2438–2446.
Shaw, K.N., Commins, S., O'Mara, S.M., 2005. Cyclooxygenase inhibition attenuates endotoxin-induced spatial learning deficits, but not an endotoxin-induced blockade of long-term potentiation. Brain Res. 1038, 231–237.
Shie, F.S., Breyer, R.M., Montine, T.J., 2005. Microglia lacking E prostanoid receptor subtype 2 have enhanced Abeta phagocytosis yet lack Abeta-activated neurotoxicity. Am. J. Pathol. 166, 1163–1172.
Skelly, D.T., Hennessy, E., Dansereau, M.A., Cunningham, C., 2013. A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1beta, [corrected] TNF-alpha and IL-6 challenges in C57BL/6 mice. PLoS ONE 8, e69123.
Sometani, A., Kataoka, H., Nitta, A., Fukumitsu, H., Nomoto, H., Furukawa, S., 2001. Transforming growth factor-beta1 enhances expression of brain-derived neurotrophic factor and its receptor, TrkB, in neurons cultured from rat cerebral cortex. J. Neurosci. Res. 66, 369–376.
Tay, T.L., Savage, J.C., Hui, C.W., Bisht, K., Tremblay, M.E., 2017. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J. Physiol. 595, 1929–1945.
Teather, L.A., Packard, M.G., Bazan, N.G., 2002. Post-training cyclooxygenase-2 (COX-2) inhibition impairs memory consolidation. Learn. Memory 9, 41–47.
Teeling, J.L., Cunningham, C., Newman, T.A., Perry, V.H., 2010. The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. Brain Behav. Immun. 24, 409–419.
Tong, L., Balazs, R., Soiampornkul, R., Thangnipon, W., Cotman, C.W., 2008. Interleukin-1 beta impairs brain derived neurotrophic factor-induced signal transduction. Neurobiol. Aging 29, 1380–1393.
Tremblay, M.E., Stevens, B., Sierra, A., Wake, H., Bessis, A., Nimmerjahn, A., 2011. The role of microglia in the healthy brain. J. Neurosci. 31, 16064–16069.
Trotta, T., Porro, C., Calvello, R., Panaro, M.A., 2014. Biological role of Toll-like receptor-4 in the brain. J. Neuroimmunol. 268, 1–12.
Ulich, T.R., Yin, S., Guo, K., Yi, E.S., Remick, D., del Castillo, J., 1991. Intratracheal injection of endotoxin and cytokines. II. Interleukin-6 and transforming growth factor beta inhibit acute inflammation. Am. J. Pathol. 138, 1097–1101.
Warner-Schmidt, J.L., Vanover, K.E., Chen, E.Y., Marshall, J.J., Greengard, P., 2011. Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans. PNAS 108, 9262–9267.
Wieseler-Frank, J., Maier, S.F., Watkins, L.R., 2005. Immune-to-brain communication dynamically modulates pain: physiological and pathological consequences. Brain Behav. Immun. 19, 104–111.
Woodling, N.S., Colas, D., Wang, Q., Minhas, P., Panchal, M., Liang, X., Mhatre, S.D., Brown, H., Ko, N., Zagol-Ikapitte, I., van der Hart, M., Khroyan, T.V., Chuluun, B., Priyam, P.G., Milne, G.L., Rassoulpour, A., Boutaud, O., Manning-Bog, A.B., Heller, H.C., Andreasson, K.I., 2016. Cyclooxygenase inhibition targets neurons to prevent early behavioural decline in Alzheimer's disease model mice. Brain 139, 2063–2081.
Wyss-Coray, T., Mucke, L., 2002. Inflammation in neurodegenerative disease–a double-edged sword. Neuron 35, 419–432.
Yang, H., Chen, C., 2008. Cyclooxygenase-2 in synaptic signaling. Curr. Pharm. Des. 14, 1443–1451.
Yirmiya, R., 1996. Endotoxin produces a depressive-like episode in rats. Brain Res. 711, 163–174.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Yirmiya, R., Pollak, Y., Barak, O., Avitsur, R., Ovadia, H., Bette, M., Weihe, E., Weidenfeld, J., 2001. Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. Neuropsychopharmacology 24, 531–544.
Zaminelli, T., Gradowski, R.W., Bassani, T.B., Barbiero, J.K., Santiago, R.M., Maria-Ferreira, D., Baggio, C.H., Vital, M.A., 2014. Antidepressant and antioxidative effect of Ibuprofen in the rotenone model of Parkinson's disease. Neurotox. Res. 26, 351–362.
Zhang, T., Gong, X., Hu, G., Wang, X., 2015. EP2-PKA signaling is suppressed by triptolide in lipopolysaccharide-induced microglia activation. J. Neuroinflammation 12, 50.
Zucker, R.S., Regehr, W.G., 2002. Short-term synaptic plasticity. Annu. Rev. Physiol. 64, 355–405.
Zunszain, P.A., Hepgul, N., Pariante, C.M., 2013. Inflammation and depression. Curr. Top. Behav. Neurosci. 14, 135–151.

Supplementary Fig.1 Golia et al. "*Interplay between inflammation and neural plasticity...*"



STUDY 2

Hindawi
Neural Plasticity
Volume 2019, Article ID 4651031, 12 pages
https://doi.org/10.1155/2019/4651031

Research Article

Combined Fluoxetine and Metformin Treatment Potentiates Antidepressant Efficacy Increasing IGF2 Expression in the Dorsal Hippocampus

Silvia Poggini,[1,2] Maria Teresa Golia,[3] Silvia Alboni,[4] Giampaolo Milior,[3,5] Livio Pepè Sciarria,[3] Aurelia Viglione,[1,6] Gloria Matte Bon,[1] Nicoletta Brunello,[4] Stefano Puglisi-Allegra,[7,8] Cristina Limatola,[3,9] Laura Maggi,[3] and Igor Branchi[1]

[1]*Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy*
[2]*PhD Program in Behavioral Neuroscience, Sapienza University of Rome, Rome, Italy*
[3]*Department of Physiology and Pharmacology, Laboratory Affiliated to Istituto Pasteur Italia, Sapienza University of Rome, Rome, Italy*
[4]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[5]*Inserm U1127, CNRS UMR7225, Sorbonne Universités, UPMC Univ Paris 6 UMR S1127, Institut du Cerveau et de la Moelle épinière, Paris 75013, France*
[6]*PhD Program in Neuroscience, Scuola Normale Superiore di Pisa, Pisa, Italy*
[7]*Department of Psychology, Sapienza University of Rome, Rome, Italy*
[8]*Santa Lucia Foundation (IRCCS Fondazione Santa Lucia), Rome, Italy*
[9]*IRCCS Neuromed, Pozzilli, Isernia, Italy*

Correspondence should be addressed to Igor Branchi; igor.branchi@iss.it

Received 30 July 2018; Revised 8 November 2018; Accepted 18 November 2018; Published 21 January 2019

Academic Editor: Clive R. Bramham

An increasing number of studies show that selective serotonin reuptake inhibitors (SSRIs) exert their therapeutic action, at least in part, by amplifying the influence of the living environment on mood. As a consequence, when administered in a favorable environment, SSRIs lead to a reduction of symptoms, but in stressful conditions, they show limited efficacy. Therefore, novel therapeutic approaches able to neutralize the influence of the stressful environment on treatment are needed. The aim of our study was to test whether, in a mouse model of depression, the combined administration of SSRI fluoxetine and metformin, a drug able to improve the metabolic profile, counteracts the limited efficacy of fluoxetine alone when administered in stressful conditions. Indeed, metabolic alterations are associated to both the onset of major depression and the antidepressant efficacy. To this goal, adult C57BL/6 male mice were exposed to stress for 6 weeks; the first two weeks was aimed at generating a mouse model of depression. During the remaining 4 weeks, mice received one of the following treatments: vehicle, fluoxetine, metformin, or a combination of fluoxetine and metformin. We measured liking- and wanting-type anhedonia as behavioral phenotypes of depression and assessed the expression levels of selected genes involved in major depressive disorder and antidepressant response in the dorsal and ventral hippocampus, which are differently involved in the depressive symptomatology. The combined treatment was more effective than fluoxetine alone in ameliorating the depressive phenotype after one week of treatment. This was associated to an increase in IGF2 mRNA expression and enhanced long-term potentiation, specifically in the dorsal hippocampus, at the end of treatment. Overall, the present results show that, when administered in stressful conditions, the combined fluoxetine and metformin treatment may represent a more effective approach than fluoxetine alone in a short term. Finally, our findings highlight the relevance of polypharmacological strategy as effective interventions to increase the efficacy of the antidepressant drugs currently available.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of major depressive disorder (MDD), which constitutes an enormous medical, individual, societal, and economical challenge and afflicts up to 10–15% of the population worldwide [1]. However, the efficacy of SSRIs is variable and incomplete: 60–70% of patients do not experience remission and 30–40% do not show a significant response [2]. To explain such incomplete efficacy, a novel hypothesis—named *undirected susceptibility to change*—posits that the increase in serotonin levels induced by SSRIs does not affect mood *per se* but enhances brain plasticity and thus amplifies the influence of the environment on the individual [3–6]. Therefore, SSRI treatment has not a univocal effect, but in a favorable environment, it would lead to a reduction of symptoms while in a stressful environment, it has limited efficacy and may even lead to a worse prognosis [7].

A number of evidence support a role for serotonin in increasing brain plasticity and enhancing susceptibility to the environment [3, 8, 9]. In addition, the *undirected susceptibility to change* hypothesis has been recently demonstrated at preclinical and clinical levels. In preclinical studies, it has been shown that fluoxetine (FLX), one of the most commonly prescribed SSRIs, affects the molecular and behavioral depression-like phenotype according to the quality of the living environment: when administered in an enriched environment, it led to an improvement while, when administered in a stressful environment, FLX treatment led to a worsening of depression-like endpoints such as an enhanced anhedonic behavior and a reduced neurogenesis [5, 10]. At a clinical level, it has been demonstrated that the commonly used SSRI citalopram amplifies the influence of the living conditions on mood, confirming that SSRI effects are affected by the environment [11].

The SSRI action on brain plasticity and susceptibility to the environment opens new perspectives on how to improve the efficacy of these antidepressants by improving the quality of the patients' living environment. However, often, it is not possible to act on the environment because of constraints due to patient's personal history and unchangeable life circumstances. In these cases, the pharmacological modulation of the factors underlying the link between the living environment and SSRI action represents a novel and desirable strategy to improve treatment outcome in patients living in adverse conditions, very common in depressed patients.

Metabolism is markedly affected by the quality of the living environment. For instance, having an active physical and social life profoundly modulates metabolic markers [12, 13]. In turn, the metabolic profile affects vulnerability to MDD and antidepressant efficacy. As an example, normalization of metabolic markers has been associated to remission following antidepressant treatment, while no change occurs in nonremitters [14]. Therefore, modification of metabolism represents a potential approach to modulate the interplay between the environment and SSRIs in order to improve treatment outcome.

The aim of the present study was to assess whether the pharmacological modulation of metabolism may improve the limited efficacy of FLX when administered in a stressful environment. To this purpose, we used metformin (MET), a widely used drug to treat type II diabetes and other metabolic syndromes [15]. It affects the metabolic profile at both peripheral and central levels since it crosses the blood-brain barrier [16, 17]. Though the underlying molecular mechanisms of MET action are yet to be fully determined, it has been reported in preclinical models that MET affects brain plasticity, increasing long-term potentiation (LTP) in the hippocampal CA1 region [18] and modulating neurotrophic factor levels, such as the brain-derived neurotrophic factor (BDNF) [19].

Our prediction was that the combined FLX and MET treatment is more effective than FLX alone in counteracting a depression-like phenotype in a stressful environment. According to our hypothesis, such enhanced efficacy is ascribable to the combined action of the two drugs: FLX increases brain plasticity, favoring a change in mood, while MET, which affects the metabolic profile, drives the change toward an improvement. To this aim, we measured BDNF expression and LTP as molecular and cellular markers of neural plasticity, in addition to liking- and wanting-type anhedonia as endpoints of depression-like response.

2. Materials and Methods

2.1. Animals. C57BL/6 male mice 12–15 weeks old were used and kept under a 12-hour light-dark cycle at 22–25°C. Mice were purchased at Envigo Italia (Udine, Italy). All procedures were carried out in accordance with the European law (EEC Council Directive 2010/63/UE86/609 1987), Italian legislation on animal experimentation (Decreto Legislativo 26/2014). In addition, animals were routinely examined for signs of discomfort as indicated by the animal care and use of the National Academy of Sciences of USA guidelines (National Research Council 2003).

2.2. Housing Condition. For the entire duration of the experiment, animals were housed in the IntelliCage system (TSE Systems, NewBehavior AG, Zürich, Switzerland), which is an apparatus for automatic monitoring of mouse behavior. It consists of a large acrylic cage (20.5 cm high, 58 cm × 40 cm at the top, and 55 cm × 37.5 cm at the base, Model 2000 Tecniplast, Buguggiate, VA, Italy) with 4 walls separating each corner from the center so that they form 4 identical triangular conditioning chambers (15 × 15 × 21 cm). Animals have access to the chamber by entering a front hole (chamber entrance). Only one mouse at a time can enter the chamber. Once entered, it is identified through a transponder antenna system. The system is able to collect data about the number and duration of visits and the number, duration, and side (right or left) of nosepokes and licks. The floor of the cage was covered with bedding and contains four sleeping shelters in the center while on the top, a food rack is present filled with standard mouse chow (food ad libitum). An additional cage (SocialBoxes) was used to expand the existing IntelliCage to a multiarea system; thus, we increased the

capacity of the system to test simultaneously more mice. One week before being moved to the IntelliCage, each animal was injected with a subcutaneous transponder (T-IS 8010 FDX-B; Datamars SA, Switzerland). Mice have been gradually habituated to the IntelliCage environment during a 14-day period (habituation period).

2.3. Treatment. After the first two weeks of stressful condition, aimed at inducing a depression-like phenotype, mice continued to be exposed to the unpredictable chronic mild stress (see below) for 4 weeks receiving one of the following treatments: VEH, FLX, MET, or FLX and MET together.

FLX (Fluoxetine HCl, Santa Cruz, USA) and MET (Metformin, Sigma-Aldrich, St Louis, MO, USA) were dissolved in water and in saccharin solution and delivered ad libitum in the drinking bottles for 4 weeks. Compared to injection, this administration method allows avoiding the stress due to the manipulation. The solutions were prepared according to the mouse average weight and daily water consumption in order to provide an average daily intake of 30 mg/kg of FLX [20] and 200 mg/kg of MET, respectively [21, 22]. Bottles with FLX and with FLX and MET were wrapped in tin foil to protect the substance from light. Metformin, fluoxetine, and their combination were dissolved in both water and saccharin solution to avoid that the saccharin preference could affect the amount of drug received. The average amount of fluoxetine or metformin administered did not differ among the experimental groups receiving the same compound. Though we did not perform a pharmacokinetic analysis, to our knowledge, no interaction between fluoxetine and metformin has been reported.

2.4. Environmental Conditions. All mice were exposed to the stressful condition for two weeks to induce the depression-like behavior. For the following 4 weeks, the subjects went on being exposed to the stressful condition or were exposed to the standard condition.

2.4.1. Stressful Condition. Mice were exposed to unpredictable chronic mild stress procedure to induce depression-like behavior (Figure S1). To prevent habituation to stress, mice were exposed each day to a different stressful procedure, randomly chosen among the procedures provided by the IntelliCage. The procedures were *short open door*: the door to access water or saccharin solution remains open for only 1.5 seconds; *delay*: the door opens with a delay of 1, 1.5, 2, and 2.5 seconds after the first nosepoke; *open door 25%*: the door opens only following 25% of nosepokes; and a*ir puff*: when the mouse performs a visit, it has a 20% chance to receive an air puff (2 bar) which lasts 1 sec or until the animal leaves the corner. In the latter case, the doors remain closed. Once each one of these procedures ended, in order to reopen the doors and drink again, the animals had to leave the corner and start a new visit. The duration of each stressful procedure was randomly chosen: 12, 18, or 24 hrs. In addition, during the stressful condition, no shelter or tissue paper was provided.

2.4.2. Standard Condition. Mice were socially housed in the IntelliCages and exposed to Plexiglas shelters of different colors and shapes (four red transparent Tecniplast plastic nest boxes and four white opaque boxes) and to tissue paper. New paper was provided every 5 days, and the plastic shelters were cleaned every week (Figure S2(a)).

2.5. Behavioral Tests. Behavioral endpoints investigated are liking- and wanting-type anhedonia. These were automatically assessed by the IntelliCage avoiding any bias or stress due to the experimenter.

2.5.1. Liking-Type Anhedonia: Saccharin Preference. To assess liking-type anhedonia, we measured the saccharin preference. Two bottles were present in each corner of the IntelliCage, one containing tap water and the other containing the 0.1% saccharin solution; both were freely available 24/24 h. Water and saccharin solution were substituted every day. The position of water and saccharin in each corner was counterbalanced across the four corners. The saccharin preference was determined as follows: (saccharin solution consumed/saccharin solution consumed + water consumed) × 100. We measured the baseline saccharin preference across a two-day period (i) at the end of the habituation period, (ii) at the end of the first two weeks of exposure to the stressful condition (aimed at inducing the depression-like phenotype), (iii) 1 week after the beginning of the treatment period, and (iv) at the end of the treatment period. Mice were exposed to the saccharin solution only (all bottles filled with saccharin solution) during the first two days of the IntelliCage habituation period in order to make them used to the saccharin flavor. In the remaining 12 days, mice could choose between water and saccharin solution.

2.5.2. Wanting-Type Anhedonia: Progressive Ratio Schedule. To assess wanting-type anhedonia, i.e., the drive for obtaining a reward, we used the progressive ratio reinforcement schedule that utilizes a multiplicative increase in the number of responses (nosepokes) required to dispense a unit of reinforce (i.e. access to saccharin). In particular, water was always accessible after one nosepoke while saccharin solution was accessible only after a specific number of nosepokes that increases progressively. After each series of 8 visits, the number of nosepokes required to access saccharin increases according to the following schedule: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, and 24. After reaching the 24 nosepoke level, mice had free access to saccharin following one nosepoke. The time for performing the nosepokes increased gradually according to the number of nosepokes requested from one to 24 sec. Mice were exposed to this test at the end of the habituation period, immediately before the treatment period and after both 1 and 4 weeks of treatment. To make the mice aware of the progressive ratio testing, the three LEDs on the top of each door were kept turned on throughout the test. Each test session lasted two days.

2.6. RNA Extraction and RT-RTqPCR. Following 4 weeks of treatment in the stressful condition, animals were sacrificed by decapitation, the brains were removed, and the dorsal and ventral parts of the hippocampus were dissected, rapidly frozen, and then stored at −80°C for further molecular

analyses. The same animals tested for behavior were analyzed for mRNA expression. Total RNA, from the ventral and dorsal hippocampi, was prepared combining extraction with TRI Reagent® and GenElute™ Mammalian Total RNA Miniprep Kit and (Sigma Aldrich ®, Milan, Italy) as previously described [4]. Two μg of total RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA USA) in a final reaction volume of 20 μL [23]. The cDNA was stored at −20°C until real-time PCR that was performed in Roche LightCycler® 480 (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) using Power SYBR Green Mix (Life Technologies Corporation, Carlsbad, CA, USA). The following forward and reverse sequences were used at the final concentration of 150 nM: for IGF1 F5′-TGCTCT TCAGTTCGTGTG-3′ and R5′-ACATCTCCAGTCTCCT CAG-3′; for IGF2 F5′-CGCTTCAGTTTGTCTGTTCG-3′ and R5′-GGAAGTACGGCCTGAGAGGTA-3′; for BDNF F5′-CCATAAGGACGCGGACTTGTAC-3′ and R5′-AGAC ATGTTTGCGGCATCCAGG-3′; for p11 (S100a10) F5′-CTTCAAAATGCCATC CCAAA-3′ and R5′-TATTTTGT CCACAGCCAGAGG-3′, for leptin F5′-AAGAAGATC CCAGGGAGGA and R5′-TGATGAGGGTTTTGGTGTCA, and for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) F5′-TTCGCAAAACAAGTTCACCA-3′ and 5′-TCGTTG TGGTTGTAAATGGAA-3′ as a house-keeping gene. Melt curve analyses and agarose gel separations were performed at the end of every RTqPCR to confirm formation of a single PCR product. The Ct (cycle threshold) value was determined by the LightCycler® 480 Software (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany), and mRNA expression was calculated with the ΔΔCt method with GAPDH as endogenous control as previously described [24]. Relative expression of the genes of interest was performed by using as calibrator (RQ value = 1) expression levels in the ventral hippocampi of vehicle-treated animals. All qPCR reactions based upon the same primer set were run in the same amplification plate to compare the levels of mRNA expression between the two parts of the hippocampus.

2.7. Electrophysiology

2.7.1. Hippocampal Slice Preparation. In order to perform electrophysiological experiments, acute hippocampal slices were collected. At the end of the treatment period in the stressful condition, animals were anesthetized by inhalation of halothane (Sigma-Aldrich S.r.l., Milan, Italy) and decapitated. The brain was rapidly removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF) solution composed of the following (in mM): NaCl 125, KCl 4, CaCl2 2.5, MgSO4 1.5, NaHPO4 1, NaHCO3 26, and glucose 10. ACSF was continuously bubbled with 95% O2 + 5% CO2 to maintain a pH close to 7.4.

Following removal, the brain was hemisected along the longitudinal fissure to separate the two hemispheres. Brain dissection was carried out according to the slicing plane chosen and the structure to be investigated. Specifically, for experiments on the ventral hippocampus, slices were cut perpendicular to the longitudinal axis from the temporal pole of the brain. For experiments on the dorsal hippocampus, coronal slices were cut from the frontal pole. Dorsal and ventral slices have been identified as the distance, in μm, from the frontal and temporal pole, respectively (approximately from 400 to 1750 μm). The brain tissues were blocked on the stage of a vibrating microtome (Thermo Scientific, USA), and 350 μm-thick slices were cut in ice-cold ACSF. The slices were then transferred to an incubation chamber containing oxygenated ACSF, where they were allowed to recover for 1 h at 30°C prior to electrophysiological recording. After this period, the slices were transferred to the interface slice-recording chamber (BSC1, Scientific System Design Inc.) to perform experiments within 1–6 h after slice preparation. Dorsal and ventral slices were prepared from separate hemispheres of the same brain and were obtained alternately from the right or left hemisphere.

2.7.2. Extracellular Field Recordings. For field recordings, individual slices were maintained at 30–32°C and superfused with ACSF at 2 mL/min by a peristaltic pump. A concentric bipolar stimulating electrode (SNE 100 × 50 mm long, Elektronik Harvard Apparatus GmbH) was placed in the stratum radiatum to stimulate Schaffer collateral fibers. Stimuli consisted of 100 μs constant current pulses of variable intensities, applied at 0.05 Hz. A glass micropipette (0.5–1 MΩ) filled with ACSF was placed in the CA1 hippocampal region, at 200–600 μm from the stimulating electrode, in order to measure orthodromically evoked field extracellular postsynaptic potentials (fEPSP). Stimulus intensity was adjusted to evoke fEPSP of amplitude about 50% of the maximal amplitude with minimal contamination by a population spike. Evoked responses were monitored online, and stable baseline responses were recorded for at least 10 min. Only the slices that showed stable fEPSP amplitudes were included in the experiments. LTP was induced by high-frequency stimulation (HFS) (1 train of stimuli at 100 Hz of 1 s duration), repeated after 30 min. To analyze the time course of the fEPSP slope, the recorded fEPSP was routinely averaged over 1 min ($n = 3$). The fEPSP slope changes following the LTP induction protocol at 31 and 61 min post tetanus were calculated with respect to those of the baseline (1 minute before induction). N/n refers to the number of slices on the total number of mice analyzed.

The paired-pulse ratio (PPR) was measured from responses to two synaptic stimuli at 50 ms interstimulus interval. PPR was calculated as the ratio between the fEPSP amplitude evoked by the second stimulus (A2) and that by the first (A1; A2/A1).

fEPSP were recorded and filtered (low pass at 1 kHz) with an Axopatch 200A amplifier (Axon Instruments, CA) and digitized at 10 kHz with an A/D converter (Digidata 1322A, Axon Instruments). Data acquisition was stored on a computer using pClamp 9 software (Axon Instruments) and analyzed offline with Clampfit 10 program (Axon Instruments).

2.8. Data and Statistical Analysis.

All data were analyzed with one-way ANOVA with the statistical software StatView II

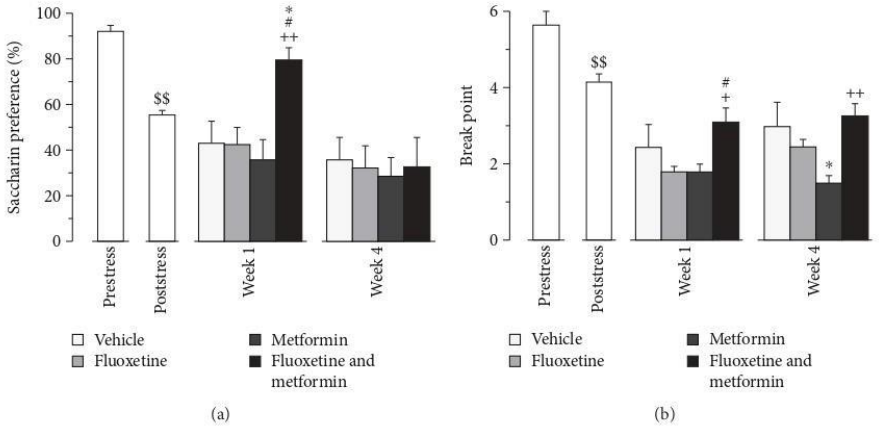


FIGURE 1: Effects of treatment with fluoxetine, metformin, or their combination on depression-like behavior. (a) Liking-type anhedonia. Saccharin preference significantly decreased following exposure to stressful procedure. After the first week of treatment, FLX-MET mice displayed an increased saccharin preference compared to both VEH, FLX, and MET mice. (b) Wanting-type anhedonia. The breakpoint level was significantly reduced after the unpredictable chronic mild stress. Following the first week of treatment, FLX-MET mice showed a significant increase of the breakpoint level compared to both FLX and MET mice. Treatments as indicated in the legend, $n = 9 - 10$ mice per group. $\$\$p < 0.0001$ pre- vs poststress, $^{*}p < 0.05$ and $^{**}p < 0.001$ vs VEH, $^{\#}p < 0.05$ and $^{\#\#}p < 0.0001$ vs FLX, and $^{+}p < 0.05$ and $^{++}p < 0.001$ vs MET. Data are presented as mean + SEM.

(Abacus Concepts, CA, USA), comparing VEH versus FLX-, MET-, and FLX plus MET-treated mice. When a significant main effect was found, selected pairwise comparisons were made using Tukey's post hoc analysis.

3. Results

3.1. FLX and MET Combination Is Effective in Alleviating Depression-Like Behavior. As behavioral phenotypes of depression, we assessed the liking- and wanting-type anhedonia, which have been previously shown to be susceptible to stress and SSRI treatment [5, 10, 25].

The two weeks of chronic stress before treatment was effective in inducing a depression-like profile. In particular, the saccharin preference (liking-type anhedonia) dropped from around 90 to 55 percent ($F(1, 37) = 87.870$, $p < 0.0001$, Figure 1(a)) and the breakpoint level (wanting-type anhedonia) was significantly reduced ($F(1, 39) = 17.874$, $p < 0.0001$, Figure 1(b)). No difference in weight between treated and control groups was found (data not shown), indicating that the stress procedure did not differentially affect the experimental groups. Following the induction of a depression-like profile, mice receiving the combination of FLX and MET showed an improvement of their behavioral phenotype when compared to those of the other experimental groups. Specifically, following 1 week of treatment, liking-type anhedonia was significantly affected by treatment ($F(3, 34) = 6.126$, $p = 0.0019$); post hoc analysis revealed that FLX-MET mice displayed an increased saccharin preference compared to VEH, FLX, and MET mice ($p < 0.05$, $p < 0.05$, and $p < 0.001$, respectively; Figure 1(a)). Wanting-type anhedonia was significantly affected by treatment as well ($F(3, 35) = 3.047$, $p = 0.0414$). FLX-MET mice showed a significant increase of the breakpoint level compared to both FLX and MET mice ($p < 0.05$ and $p < 0.05$, respectively; Figure 1(b)). At the end of the treatment, the prolonged exposure to stress (6 weeks) led to a marked anhedonic profile in all groups, flattening the potential differences in liking-type anhedonia. As for wanting-type anhedonia, a significant main effect of treatment was found ($F(3, 35) = 4.329$, $p = 0.0107$). In particular, FLX-MET mice reached a higher breakpoint level compared to MET mice ($p < 0.001$, Figure 1(b)).

All mice to be treated in the standard condition showed a significant increase of the depression-like phenotype following the two weeks of exposure to the stressful condition (Figures S2(b) and (c)). In particular, both liking-type anhedonia ($F(1, 41) = 43.721$, $p < 0.0001$) and wanting-type anhedonia ($F(1, 40) = 10.681$, $p = 0.0022$) were significantly reduced. Afterwards, when receiving VEH, FLX, MET, or FLX-MET in a standard condition, they showed no difference in depression-like behavior. In particular, all experimental groups showed a full recovery, displaying no anhedonic response, both at 1 and 4 weeks of treatment.

3.2. IGF2 mRNA Levels Are Increased in the Dorsal Hippocampus of Mice Receiving the Combined Treatment. To explore the molecular bases of treatment effect, we analyzed gene expression of selected targets reported to be involved in MDD and metabolism. In particular, we focused on IGF2 and IGF1, p11, BDNF, and leptin mRNA expression in the dorsal and ventral hippocampus. These hippocampal areas have been reported to be differently involved in MDD and antidepressant efficacy [26–30].

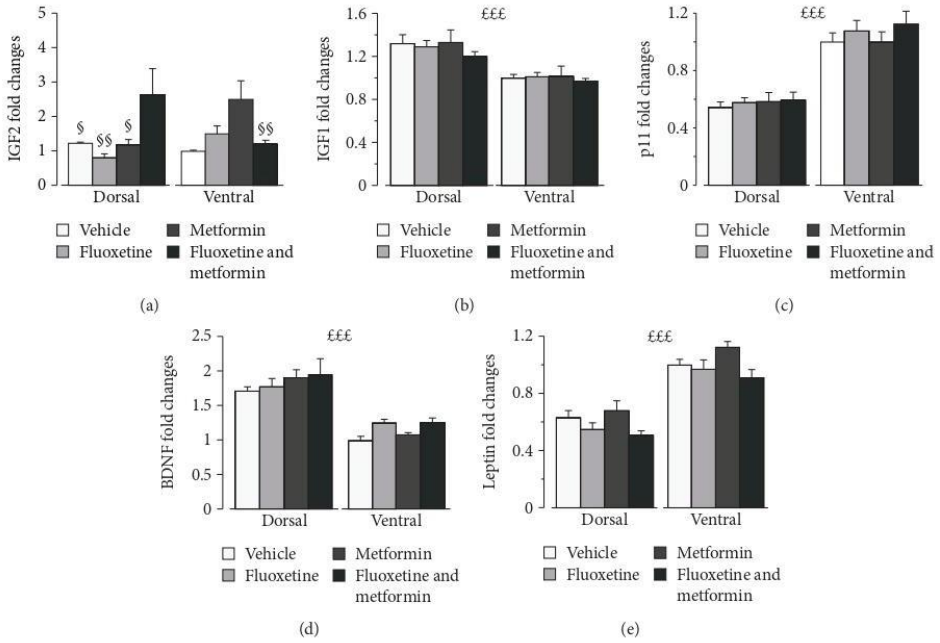


FIGURE 2: Effects of fluoxetine, metformin, or their combination on expression of genes involved in brain plasticity in the dorsal and ventral hippocampi. (a) IGF2 is significantly increased by the combined treatment compared to VEH and FLX alone, suggesting that this growth factor is involved in the antidepressant action of the FLX-MET treatment. Such effect concerned the dorsal hippocampus, where FLX-MET treatment increased IGF2 expression compared to all the other groups. (b) IGF1, (c) p11, (d) BDNF, and (e) leptin expression was not affected by treatment, but IGF1 and BDNF levels were overall higher in the dorsal hippocampus, while p11 and leptin were higher in the ventral hippocampus. Treatments as indicated in the legend, $n = 6 - 8$ mice per group. $^{£££}p < 0.001$, the main effect of the hippocampal region; $^{\$}p < 0.05$ and $^{\$\$}p < 0.01$ vs FLX-MET in the dorsal region. Data are presented as mean + SEM.

IGF2 analysis revealed a significant main effect of treatment ($F(3, 50) = 3.370$, $p = 0.0256$) and a significant interaction treatment × hippocampal region ($F(3, 50) = 5.912$, $p = 0.0015$; Figure 2(a)). Post hoc analysis revealed that, overall, mice receiving the combined treatment showed higher IGF2 expression compared to those of the VEH and FLX groups. With regard to the dorsal hippocampus, they displayed higher IGF2 levels compared to VEH ($p < 0.05$), MET ($p < 0.05$), and FLX ($p < 0.01$). FLX-MET mice showed also significantly higher IGF2 levels in the dorsal region compared to the ventral region ($p < 0.01$). As for the other genetic markers investigated, IGF1, BDNF, p11, and leptin, we found no effect of treatment but a significant main effect of the hippocampal region ($Fs(1, 50) = 29.161, 96.221, 114.972, 126.865$, $ps < 0.001$, Figures 2(b)–2(e)). In particular, IGF1 and BDNF levels were higher in the dorsal hippocampus, while p11 and leptin were higher in the ventral hippocampus.

3.3. LTP in the Dorsal and Ventral Hippocampal Regions Is Differentially Affected by Treatment. We explored plasticity processes in the CA1 hippocampal region by recording LTP evoked by two spaced (30 minutes apart) Schaffer collateral stimulations in both the dorsal and ventral hippocampus. Interestingly, during the second stimulation, the main effect of treatment emerged ($F(3, 60) = 3.321$, $p = 0.026$). In addition, the main effect on the hippocampal area and tr eatment × hippocampal region interaction were very close to reach statistical significance ($F(1, 60) = 3.473$, $p = 0.067$ and $F(3, 60) = 2.523$, $p = 0.066$, respectively). Post hoc analysis revealed that, in the dorsal hippocampus, FLX-MET-treated mice show an increased LTP amplitude (1.495 ± 0.065) compared to MET- (1.226 ± 0.061, $p < 0.05$) and VEH- (1.260 ± 0.058, $p < 0.05$, Figure 3(a), left) but not to FLX- (1.406 ± 0.069) treated mice. By contrast, in the ventral region, the combined treatment showed a trend toward a reduction of LTP amplitude compared to FLX and VEH alone (1.387 ± 0.058 vs 1.525 ± 0.065 and 1.479 ± 0.061, Figure 3(a), right) but was similar to MET alone (1.323 ± 0.0759). Finally, in the VEH group, the magnitude of LTP was higher in the ventral compared the dorsal hippocampus ($p = 0.012$).

With regard to PPR, a main effect on the hippocampal region was observed following treatment ($F(1,139) = 157.357$, $p < 0.001$), being its value higher on the dorsal hippocampus for all treatments ($p < 0.001$, Figure 3(b)). In the VEH group, PPR was 1.402 ± 0.034 and 1.081 ± 0.041, in the dorsal and ventral hippocampi, respectively. No treatment effect was observed.

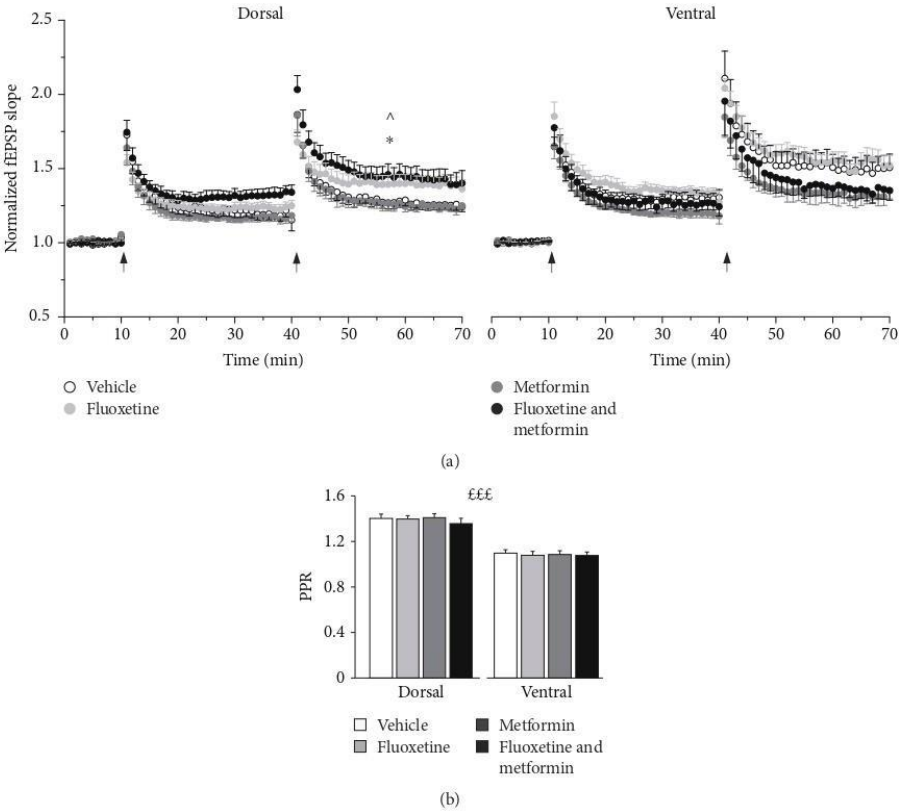


FIGURE 3: LTP in the dorsal and ventral hippocampus is differentially affected by fluoxetine, metformin, or their combination. (a) LTP from extracellular records in the dorsal and ventral hippocampi. The time course of fEPSP slope responses evoked at 0.05 Hz and normalized as detailed in Materials and Methods. Arrows indicate repeated spaced HFS (100 Hz trains of 1 sec duration, 30 minutes apart). Treatments as indicated in the legend (in the dorsal hippocampus, VEH: $n = 12/8$, FLX: $n = 10/9$, MET: $n = 12/9$, and FLX-MET: $n = 10/7$ and in the ventral hippocampus, VEH: $n = 10/10$, FLX: $n = 8/8$, MET: $n = 9/8$, and FLX-MET: $n = 11/9$). Note that in the dorsal hippocampus, FLX-MET mice show an increased LTP compared to MET or VEH mice and that in VEH, LTP is higher in the ventral compared to the dorsal hippocampus. Tukey's t-test post hoc analysis, 20 minutes after the second HFS. $^{*}p < 0.05$ FLX-MET vs VEH; $^{\wedge}p < 0.05$ FLX-MET vs MET. (b) PPR. Bar histogram indicates averaged PPR values for the dorsal hippocampus (VEH: $n = 20/12$, FLX: $n = 20/12$, MET: $n = 19/11$, and FLX-MET: $n = 21/12$) and ventral hippocampus (VEH: $n = 14/11$, FLX: $n = 16/11$, MET: $n = 21/12$, and FLX-MET: $n = 16/10$). Note that PPR is not affected by treatments and it is higher in the dorsal compared to the ventral hippocampus. Treatments as indicated in the legend, $^{£££}p < 0.001$, the main effect of the hippocampal region. Data are presented as mean + SEM.

4. Discussion

The present results show that the combination of FLX and MET administered in the stressful condition ameliorates the depression-like phenotype compared to FLX and VEH alone after one week but not after four weeks of treatment. The combination of FLX and MET led also to increased IGF2 expression and enhanced LTP, specifically in the dorsal hippocampus, at the end of treatment.

Previous findings by us [5, 10] indicate that FLX alone administered in a chronic stress condition has limited beneficial effects or leads to a worsening of depression-like behavior. This is in line with previous studies [31–34]. However, other studies found that mice treated with SSRI in a stressful environment show an improvement of the depression-like profile [35, 36]. Here, we confirm that, compared to VEH, FLX has limited beneficial effects when administered in adverse conditions. However, the cotreatment of MET and FLX counteracts the detrimental effects induced by the exposure to stress following 1 week of treatment. In particular, FLX and MET combination increases the saccharin preference to the level that the mice had before chronic stress compared to both VEH and FLX alone. Similar results have been found for wanting-type anhedonia, mice

treated with the combined treatment showing a higher motivation to obtain the reward compared to those receiving FLX only. The results collected at four weeks show no difference among the experimental groups, indicating that the combined treatment has not long-lasting beneficial effects on depression-like behavior and should be used for subacute interventions. It is worth noting that, when administered in standard condition, treatments did not produce different effects, all experimental groups showing a recovery of the anhedonic profile at both weeks 1 and 4 (Figure S2). These results confirm that the MET-FLX combination is an effective therapeutic approach when administered to subjects living in stressful conditions and support our hypothesis that FLX treatment outcome depends on the quality of the environment [7].

To explore the molecular mechanisms associated to the therapeutic action of the combined treatment, we analyzed the expression of selected genes reportedly involved in MDD and modulated by FLX and MET [37, 38]. In particular, we focused on IGF2 that is a key molecule in vulnerability to stress and a potential molecular target able to trigger antidepressant action [39–41]. A decrease in IGF2 hippocampal expression is significantly associated to depression-like behavior induced through chronic restraint stress [40–42]. Accordingly, IGF2 overexpression was found to rescue the neurobehavioral effects of stress exposure [40]. In addition, recent evidence indicates that IGF2 administration enhances adult neurogenesis in the hippocampal dentate gyrus [43], considered a marker of recovery from MDD [44, 45], indicating IGF2 as involved in switching from depressive-like to healthy phenotype. IGF2 has been also reported to be a key target of ketamine [39], a novel antidepressant drug, which has a rapid but not long-lasting action [46], similarly to the effect of the combined FLX-MET treatment reported here. This suggests that IGF2 might be involved mainly in the first-phase recovery from MDD. Here, we found that IGF2 is significantly increased by the combined treatment compared to VEH and FLX alone, suggesting that this growth factor might be involved in the antidepressant action of the FLX-MET treatment. However, since IGF2 expression has been associated to enhanced learning and memory [47], the differences in wanting-type anhedonia, assessed through a progressive ratio learning paradigm, could be ascribed also to the differences in learning abilities associated to IGF2 levels. The differences in the IGF2 expression levels concern mainly the dorsal hippocampus. Despite that the classic view on anatomical segregation of the hippocampal function considers the dorsal part to be involved in learning and memory while the ventral part in emotional and stress responses [48, 49], an increasing number of studies are challenging this dichotomy view [50, 51]. Indeed, novel evidence indicates that the dorsal hippocampus is implicated in MDD [52–54] and is an important target for antidepressants [29, 30, 53–55]. For instance, though the ventral region shows the highest expression levels of most markers of antidepressant action, such as the 5HT1A receptor in the dentate gyrus [56], the dorsal region expresses at high levels specific markers, including the 5-HT6 receptor, emerging as relevant regulators of depression-like behavior as well [57, 58].

We also analyzed the expression levels of other metabolic markers related to MDD such as IGF1, p11, BDNF, and leptin, but these were not affected by treatments. Nevertheless, all of them showed a significantly different expression in the two hippocampal regions. The adipose-derived hormone leptin is well known for its function in controlling energy homeostasis and has been recently involved in regulating mood and emotion [59, 60]. Low levels of leptin are associated to depression in humans, and preclinical models as well as pharmacological studies indicate leptin as a potential antidepressant drug [61]. Here, we observe an higher leptin expression in the ventral compared to the dorsal hippocampus. Such specificity is in line with previous data showing that leptin differently affects memory and food intake when administered in the dorsal or the ventral hippocampus [62]. Similar to leptin, the expression of p11 (also known as S100A10), involved in the regulation of depression-like behavior and response to antidepressants [63–65], was not modified by treatments but its expression levels were higher in the ventral compared to the dorsal hippocampus.

BDNF is a neurotrophic factor particularly abundant in hippocampal neurons [66] that has been indicated as a key player in the pathophysiology of MDD. Indeed, according to the "neurotrophic hypothesis of depression," the psychopathology is associated with the reduction of brain BDNF levels and antidepressant treatments alleviate depressive symptoms increasing its levels [6, 67]. BDNF is reported to be expressed at higher levels in the dorsal compared to the ventral hippocampus [68]. We here confirm this finding. In addition, we replicate data from our and other research groups showing that FLX treatment does not increase the levels of this neurotrophin in a stressful environment [4]. In line with previous data, BDNF levels were not affected also by MET [69]. Akin to IGF1, BDNF levels were higher in the dorsal compared to the ventral hippocampus.

Similar to gene expression, physiological properties differ along the longitudinal axis of the hippocampus. For instance, in the CA1 region [70–72], the LTP magnitude is smaller in the ventral than in the dorsal hippocampus [73–75] and is differentially modulated by stress in the two regions [76–81], being reduced or not affected in the dorsal but increased in the ventral hippocampus following both acute stress [82, 83] and chronic stress [27]. In line with these findings, we show here that the LTP magnitude was smaller in dorsal compared to ventral hippocampus. This might be due to the different distribution and effects exerted by the corticosteroid receptors, mineralocorticoids (MRs) and glucocorticoids (GRs), on LTP after exposure to stress. In particular, it has been reported that MRs, more expressed in the ventral part [84], facilitate LTP [85, 86], while GRs, more abundant in the dorsal part of the hippocampus [84], impair LTP [87].

Interestingly, the treatments differentially affected LTP in the two hippocampal regions. In particular, the FLX-MET cotreatment produced a significant increase in LTP amplitude in the dorsal hippocampus, which parallels the significant IGF2 expression increase observed in this region. Given the role of IGF2 in modulating biological processes involved in neuronal plasticity [88], such as promoting dendritic spine formation [89] and enhancing pERK1/2 and

GluR1 [47], the IGF2 increase might be involved in the plasticity enhancement that we observed. By contrast, no significant difference in LTP was found in the ventral part, suggesting that FLX and MET, alone or in combination, do not regulate plasticity in this area. This evidence suggests that the FLX and MET combination affects the electrophysiological activity specifically in the dorsal hippocampus which has been reported as a potential target for antidepressant treatments [29, 30, 53–55]. Such LTP amplitude enhancement, in addition to the increased IGF2 expression, in a brain region reportedly involved in learning processes further supports that these changes might contribute to the differences in the progressive ratio paradigm used to assess wanting-type anhedonia.

The major limitations of the present study include the lack of the analysis of the molecular and cellular endpoints after 1 week of treatment in order to better investigate the association between behavioral changes and modifications in neurophysiological substrates. In addition, a pharmacokinetic analysis of the possible interaction between metformin and fluoxetine would have better illustrated whether the coadministration affects their bioavailability. Finally, given that MDD affects mostly female with a female : male ratio of approximately 2 : 1, the assessment of the effect of the treatments not only in males but also in female individuals will be extremely relevant.

5. Conclusions

In conclusion, previous works by us and others have found that FLX administration has beneficial effects in an enriched environment but has no effects or even leads to detrimental outcome when administered in a stressful environment [4, 5, 90]. Here, we show that the combined FLX and MET treatment is more effective than FLX and VEH alone in a short term when administered in individuals exposed to a stressful condition. Therefore, this polypharmacological strategy appears effective to counteract the potential limited efficacy of FLX in individuals living in adverse conditions. This might be highly relevant in the clinic because, with very few exceptions, people cannot rapidly and effectively change their life circumstance and adverse conditions are very common in depressed patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The Italian Ministry of Health and the ERANET agency had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. An earlier version of the present study has been presented as conference abstract at the 31st ECNP Congress—Barcelona 2018.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Silvia Poggini and Maria Teresa Golia equally contributed as first authors; Laura Maggi and Igor Branchi equally contributed as last authors.

Acknowledgments

Nadia Francia and Stella Falsini (Istituto Superiore di Sanità) provided editorial support. The research was supported by the grant from the Italian Ministry of Health Ricerca Finalizzata (RF-2011-02349921) and ERANET Neuron 2017 MicroSynDep (both to IB).

Supplementary Materials

Figure S1: experimental design of the stressful condition. Mice were exposed to stress for six weeks. The first two weeks induced the depression-like phenotype. During the following four weeks of stress, the subjects were treated with fluoxetine, metformin, or their combination. Figure S2: experimental design and effects of fluoxetine, metformin, or their combination on depression-like behavior in standard condition: (a) first, mice were exposed for two weeks to the stressful condition to induce a depression-like phenotype. Afterwards, they were treated for four weeks in the standard condition. (b) Liking-type anhedonia. Saccharin preference significantly decreased following exposure to stress. Treatments did not produce different effects. (c) Wanting-type anhedonia. The breakpoint level was significantly reduced after the unpredictable chronic mild stress. Treatments did not produce different effects. *(Supplementary Materials)*

References

[1] World Health Organization, *Depression and Other Common Mental Disorders: Globalization and Health Estimates*, 2017.

[2] M. H. Trivedi, A. J. Rush, S. R. Wisniewski et al., "Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice," *The American Journal of Psychiatry*, vol. 163, no. 1, pp. 28–40, 2006.

[3] J. F. M. Vetencourt, A. Sale, A. Viegi et al., "The antidepressant fluoxetine restores plasticity in the adult visual cortex," *Science*, vol. 320, no. 5874, pp. 385–388, 2008.

[4] S. Alboni, R. M. van Dijk, S. Poggini et al., "Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment," *Molecular Psychiatry*, vol. 22, no. 4, pp. 552–561, 2017.

[5] I. Branchi, S. Santarelli, S. Capoccia et al., "Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice," *PLoS One*, vol. 8, no. 4, article e62226, 2013.

[6] E. Castren and M. Kojima, "Brain-derived neurotrophic factor in mood disorders and antidepressant treatments," *Neurobiology of Disease*, vol. 97, Part B, pp. 119–126, 2017.

[7] I. Branchi, "The double edged sword of neural plasticity: increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover," *Psychoneuroendocrinology*, vol. 36, no. 3, pp. 339–351, 2011.

[8] J. Belsky, C. Jonassaint, M. Pluess, M. Stanton, B. Brummett, and R. Williams, "Vulnerability genes or plasticity genes?," *Molecular Psychiatry*, vol. 14, no. 8, pp. 746–754, 2009.

[9] M. J. Bakermans-Kranenburg and M. H. van IJzendoorn, "The hidden efficacy of interventions: gene×environment experiments from a differential susceptibility perspective," *Annual Review of Psychology*, vol. 66, no. 1, pp. 381–409, 2015.

[10] S. Alboni, M. van Dijk, S. Poggini et al., "Hippocampus-related effects of fluoxetine treatment under stressful vs enriched conditions," *Molecular Psychiatry*, vol. 22, no. 4, p. 483, 2017.

[11] F. Chiarotti, A. Viglione, A. Giuliani, and I. Branchi, "Citalopram amplifies the influence of living conditions on mood in depressed patients enrolled in the STAR*D study," *Translational Psychiatry*, vol. 7, no. 3, article e1066, 2017.

[12] S. Häfner, A. Zierer, R. T. Emeny et al., "Social isolation and depressed mood are associated with elevated serum leptin levels in men but not in women," *Psychoneuroendocrinology*, vol. 36, no. 2, pp. 200–209, 2011.

[13] E. Corpeleijn, E. J. M. Feskens, E. H. J. M. Jansen, M. Mensink, W. H. M. Saris, and E. E. Blaak, "Lifestyle intervention and adipokine levels in subjects at high risk for type 2 diabetes: the study on lifestyle intervention and impaired glucose tolerance Maastricht (SLIM)," *Diabetes Care*, vol. 30, no. 12, pp. 3125–3127, 2007.

[14] B. Weber-Hamann, J. Kratzsch, D. Kopf et al., "Resistin and adiponectin in major depression: the association with free cortisol and effects of antidepressant treatment," *Journal of Psychiatric Research*, vol. 41, no. 3-4, pp. 344–350, 2007.

[15] I. Pernicova and M. Korbonits, "Metformin–mode of action and clinical implications for diabetes and cancer," *Nature Reviews Endocrinology*, vol. 10, no. 3, pp. 143–156, 2014.

[16] Y. Wang, G. Li, J. Goode et al., "Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes," *Nature*, vol. 485, no. 7396, pp. 128–132, 2012.

[17] C. Grommes, J. C. Karlo, A. Caprariello, D. Blankenship, A. DeChant, and G. E. Landreth, "The PPARγ agonist pioglitazone crosses the blood-brain barrier and reduces tumor growth in a human xenograft model," *Cancer Chemotherapy and Pharmacology*, vol. 71, no. 4, pp. 929–936, 2013.

[18] R. Lennox, D. W. Porter, P. R. Flatt, C. Holscher, N. Irwin, and V. A. Gault, "Comparison of the independent and combined effects of sub-chronic therapy with metformin and a stable GLP-1 receptor agonist on cognitive function, hippocampal synaptic plasticity and metabolic control in high-fat fed mice," *Neuropharmacology*, vol. 86, pp. 22–30, 2014.

[19] N. Ghadernezhad, L. Khalaj, H. Pazoki-Toroudi, M. Mirmasoumi, and G. Ashabi, "Metformin pretreatment enhanced learning and memory in cerebral forebrain ischaemia: the role of the AMPK/BDNF/P70SK signalling pathway," *Pharmaceutical Biology*, vol. 54, no. 10, pp. 2211–2219, 2016.

[20] S. C. Dulawa, K. A. Holick, B. Gundersen, and R. Hen, "Effects of chronic fluoxetine in animal models of anxiety and depression," *Neuropsychopharmacology*, vol. 29, no. 7, pp. 1321–1330, 2004.

[21] D. M. Cheng, N. Pogrebnyak, P. Kuhn et al., "Polyphenol-rich Rutgers Scarlet Lettuce improves glucose metabolism and liver lipid accumulation in diet-induced obese C57BL/6 mice," *Nutrition*, vol. 30, no. 7-8, pp. S52–S58, 2014.

[22] B. D. Green, N. Irwin, N. A. Duffy, V. A. Gault, F. P. M. O'Harte, and P. R. Flatt, "Inhibition of dipeptidyl peptidase-IV activity by metformin enhances the antidiabetic effects of glucagon-like peptide-1," *European Journal of Pharmacology*, vol. 547, no. 1–3, pp. 192–199, 2006.

[23] S. Alboni, C. Benatti, C. Montanari, F. Tascedda, and N. Brunello, "Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus," *European Journal of Pharmacology*, vol. 721, no. 1–3, pp. 158–167, 2013.

[24] S. Alboni, C. Benatti, G. Capone et al., "Time-dependent effects of escitalopram on brain derived neurotrophic factor (BDNF) and neuroplasticity related targets in the central nervous system of rats," *European Journal of Pharmacology*, vol. 643, no. 2-3, pp. 180–187, 2010.

[25] S. Alboni, S. Poggini, S. Garofalo et al., "Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment," *Brain, Behavior, and Immunity*, vol. 58, pp. 261–271, 2016.

[26] A. Tanti and C. Belzung, "Neurogenesis along the septo-temporal axis of the hippocampus: are depression and the action of antidepressants region-specific?," *Neuroscience*, vol. 252, pp. 234–252, 2013.

[27] V. Pinto, J. C. Costa, P. Morgado et al., "Differential impact of chronic stress along the hippocampal dorsal-ventral axis," *Brain Structure & Function*, vol. 220, no. 2, pp. 1205–1212, 2015.

[28] D. F. Hawley and J. L. Leasure, "Region-specific response of the hippocampus to chronic unpredictable stress," *Hippocampus*, vol. 22, no. 6, pp. 1338–1349, 2012.

[29] R. J. McLaughlin, M. N. Hill, A. C. Morrish, and B. B. Gorzalka, "Local enhancement of cannabinoid CB1 receptor signalling in the dorsal hippocampus elicits an antidepressant-like effect," *Behavioural Pharmacology*, vol. 18, no. 5-6, pp. 431–438, 2007.

[30] C. M. Padovan and F. S. Guimaraes, "Antidepressant-like effects of NMDA-receptor antagonist injected into the dorsal hippocampus of rats," *Pharmacology, Biochemistry, and Behavior*, vol. 77, no. 1, pp. 15–19, 2004.

[31] J. C. Brenes and J. Fornaguera, "The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum," *Behavioural Brain Research*, vol. 198, no. 1, pp. 199–205, 2009.

[32] M. A. Prendergast, D. P. Yells, S. E. Balogh, S. R. Paige, and S. E. Hendricks, "Fluoxetine differentially suppresses sucrose solution consumption in free-fed and food-deprived rats–reversal by amantadine," *Medical Science Monitor*, vol. 8, no. 10, pp. BR385–BR390, 2002.

[33] S. Sammut, I. Bethus, G. Goodall, and R. Muscat, "Antidepressant reversal of interferon-alpha-induced anhedonia," *Physiology & Behavior*, vol. 75, no. 5, pp. 765–772, 2002.

[34] M. Tonissaar, T. Mallo, M. Eller, R. Haidkind, K. Koiv, and J. Harro, "Rat behavior after chronic variable stress and partial lesioning of 5-HT-ergic neurotransmission: effects of citalopram," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 32, no. 1, pp. 164–177, 2008.

[35] J. M. Bessa, A. R. Mesquita, M. Oliveira et al., "A trans-dimensional approach to the behavioral aspects of depression," *Frontiers in Behavioral Neuroscience*, vol. 3, 2009.

[36] R. Rygula, N. Abumaria, G. Flügge et al., "Citalopram counteracts depressive-like symptoms evoked by chronic social stress in rats," *Behavioural Pharmacology*, vol. 17, no. 1, pp. 19–29, 2006.

[37] E. Tunc-Ozcan, S. L. Wert, P. H. Lim, A. Ferreira, and E. E. Redei, "Hippocampus-dependent memory and allele-specific gene expression in adult offspring of alcohol-consuming dams after neonatal treatment with thyroxin or metformin," *Molecular Psychiatry*, vol. 23, no. 7, pp. 1643–1651, 2017.

[38] A. Śmieszek, A. Czyrek, K. Basinska et al., "Effect of metformin on viability, morphology, and ultrastructure of mouse bone marrow-derived multipotent mesenchymal stromal cells and Balb/3T3 embryonic fibroblast cell line," *BioMed Research International*, vol. 2015, Article ID 769402, 14 pages, 2015.

[39] S. F. Grieco, Y. Cheng, H. Eldar-Finkelman, R. S. Jope, and E. Beurel, "Up-regulation of insulin-like growth factor 2 by ketamine requires glycogen synthase kinase-3 inhibition," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 72, pp. 49–54, 2017.

[40] Y. W. Luo, Y. Xu, W. Y. Cao et al., "Insulin-like growth factor 2 mitigates depressive behavior in a rat model of chronic stress," *Neuropharmacology*, vol. 89, pp. 318–324, 2015.

[41] B. M. Andrus, K. Blizinsky, P. T. Vedell et al., "Gene expression patterns in the hippocampus and amygdala of endogenous depression and chronic stress models," *Molecular Psychiatry*, vol. 17, no. 1, pp. 49–61, 2012.

[42] S. Jung, Y. Lee, G. Kim et al., "Decreased expression of extracellular matrix proteins and trophic factors in the amygdala complex of depressed mice after chronic immobilization stress," *BMC Neuroscience*, vol. 13, no. 1, p. 58, 2012.

[43] O. Bracko, T. Singer, S. Aigner et al., "Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis," *The Journal of Neuroscience*, vol. 32, no. 10, pp. 3376–3387, 2012.

[44] S. R. Ferrón, E. J. Radford, A. Domingo-Muelas et al., "Differential genomic imprinting regulates paracrine and autocrine roles of IGF2 in mouse adult neurogenesis," *Nature Communications*, vol. 6, no. 1, p. 8265, 2015.

[45] Y. Kita, Y. Ago, K. Higashino et al., "Galantamine promotes adult hippocampal neurogenesis via M1 muscarinic and α7 nicotinic receptors in mice," *The International Journal of Neuropsychopharmacology*, vol. 17, no. 12, pp. 1957–1968, 2014.

[46] D. C. Mathews and C. A. Zarate Jr, "Current status of ketamine and related compounds for depression," *The Journal of Clinical Psychiatry*, vol. 74, no. 5, pp. 516-517, 2013.

[47] D. Y. Chen, S. A. Stern, A. Garcia-Osta et al., "A critical role for IGF-II in memory consolidation and enhancement," *Nature*, vol. 469, no. 7331, pp. 491–497, 2011.

[48] D. M. Bannerman, J. N. P. Rawlins, S. B. McHugh et al., "Regional dissociations within the hippocampus–memory and anxiety," *Neuroscience and Biobehavioral Reviews*, vol. 28, no. 3, pp. 273–283, 2004.

[49] O. F. O'Leary and J. F. Cryan, "A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis," *Trends in Pharmacological Sciences*, vol. 35, no. 12, pp. 675–687, 2014.

[50] B. A. Strange, M. P. Witter, E. S. Lein, and E. I. Moser, "Functional organization of the hippocampal longitudinal axis," *Nature Reviews Neuroscience*, vol. 15, no. 10, pp. 655–669, 2014.

[51] M. S. Fanselow and H. W. Dong, "Are the dorsal and ventral hippocampus functionally distinct structures?," *Neuron*, vol. 65, no. 1, pp. 7–19, 2010.

[52] J. F. Rodriguez-Landa, C. M. Contreras, and R. I. Garcia-Rios, "Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor," *Behavioural Pharmacology*, vol. 20, no. 7, pp. 614–622, 2009.

[53] Y. C. Ho and S. Wang, "Adult neurogenesis is reduced in the dorsal hippocampus of rats displaying learned helplessness behavior," *Neuroscience*, vol. 171, no. 1, pp. 153–161, 2010.

[54] C. Goeldner, D. Reiss, B. L. Kieffer, and A. M. Ouagazzal, "Endogenous nociceptin/orphanin-FQ in the dorsal hippocampus facilitates despair-related behavior," *Hippocampus*, vol. 20, no. 8, pp. 911–916, 2010.

[55] R. Liu, W. Dang, M. Jianting et al., "Citalopram alleviates chronic stress induced depression-like behaviors in rats by activating GSK3β signaling in dorsal hippocampus," *Brain Research*, vol. 1467, pp. 10–17, 2012.

[56] K. F. Tanaka, B. A. Samuels, and R. Hen, "Serotonin receptor expression along the dorsal–ventral axis of mouse hippocampus," *Philosophical Transactions of the Royal Society B*, vol. 367, no. 1601, pp. 2395–2401, 2012.

[57] G. V. Carr, L. E. Schechter, and I. Lucki, "Antidepressant and anxiolytic effects of selective 5-HT6 receptor agonists in rats," *Psychopharmacology*, vol. 213, no. 2-3, pp. 499–507, 2011.

[58] K. C. Liu, J. Y. Li, H. H. Tan et al., "Serotonin$_6$ receptors in the dorsal hippocampus regulate depressive-like behaviors in unilateral 6-hydroxydopamine-lesioned Parkinson's rats," *Neuropharmacology*, vol. 95, pp. 290–298, 2015.

[59] Y. Milaneschi, W. K. Simmons, E. F. C. van Rossum, and B. W. J. H. Penninx, "Depression and obesity: evidence of shared biological mechanisms," *Molecular Psychiatry*, 2018.

[60] D. J. Bond, A. C. Andreazza, J. Hughes et al., "A longitudinal study of the relationships between mood symptoms, body mass index, and serum adipokines in bipolar disorder," *The Journal of Clinical Psychiatry*, vol. 78, no. 4, pp. 441–448, 2017.

[61] X. Y. Lu, C. S. Kim, A. Frazer, and W. Zhang, "Leptin: a potential novel antidepressant," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 5, pp. 1593–1598, 2006.

[62] S. E. Kanoski, M. R. Hayes, H. S. Greenwald et al., "Hippocampal leptin signaling reduces food intake and modulates food-related memory processing," *Neuropsychopharmacology*, vol. 36, no. 9, pp. 1859–1870, 2011.

[63] P. Svenningsson, Y. Kim, J. Warner-Schmidt, Y. S. Oh, and P. Greengard, "p11 and its role in depression and therapeutic responses to antidepressants," *Nature Reviews Neuroscience*, vol. 14, no. 10, pp. 673–680, 2013.

[64] J. L. Warner-Schmidt, E. Y. Chen, X. Zhang et al., "A role for p11 in the antidepressant action of brain-derived neurotrophic factor," *Biological Psychiatry*, vol. 68, no. 6, pp. 528–535, 2010.

[65] B. Lu, G. Nagappan, and Y. Lu, "BDNF and synaptic plasticity, cognitive function, and dysfunction," *Handbook of Experimental Pharmacology*, vol. 220, pp. 223–250, 2014.

[66] J. M. Conner, J. C. Lauterborn, Q. Yan, C. M. Gall, and S. Varon, "Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS:

evidence for anterograde axonal transport," *The Journal of Neuroscience*, vol. 17, no. 7, pp. 2295–2313, 1997.

[67] R. S. Duman, "Pathophysiology of depression: the concept of synaptic plasticity," *European Psychiatry*, vol. 17, Supplement 3, pp. 306–310, 2002.

[68] J. Faure, J. D. K. Uys, L. Marais, D. J. Stein, and W. M. U. Daniels, "Early maternal separation alters the response to traumatization: resulting in increased levels of hippocampal neurotrophic factors," *Metabolic Brain Disease*, vol. 22, no. 2, pp. 183–195, 2007.

[69] J. S. Allard, E. J. Perez, K. Fukui, P. Carpenter, D. K. Ingram, and R. Cabo, "Prolonged metformin treatment leads to reduced transcription of Nrf2 and neurotrophic factors without cognitive impairment in older C57BL/6J mice," *Behavioural Brain Research*, vol. 301, pp. 1–9, 2016.

[70] C. Papatheodoropoulos and G. Kostopoulos, "Dorsal-ventral differentiation of short-term synaptic plasticity in rat CA1 hippocampal region," *Neuroscience Letters*, vol. 286, no. 1, pp. 57–60, 2000.

[71] K. Maruki, Y. Izaki, M. Nomura, and T. Yamauchi, "Differences in paired-pulse facilitation and long-term potentiation between dorsal and ventral CA1 regions in anesthetized rats," *Hippocampus*, vol. 11, no. 6, pp. 655–661, 2001.

[72] L. L. Colgin, D. Kubota, Y. Jia, C. S. Rex, and G. Lynch, "Long-term potentiation is impaired in rat hippocampal slices that produce spontaneous sharp waves," *The Journal of Physiology*, vol. 558, no. 3, pp. 953–961, 2004.

[73] N. Maggio and M. Segal, "Unique regulation of long term potentiation in the rat ventral hippocampus," *Hippocampus*, vol. 17, no. 1, pp. 10–25, 2007.

[74] C. Papatheodoropoulos and G. Kostopoulos, "Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation," *Neuroscience Letters*, vol. 279, no. 3, pp. 177–180, 2000.

[75] G. Milior, M. A. di Castro, L. P.'. Sciarria et al., "Electrophysiological properties of CA1 pyramidal neurons along the longitudinal axis of the mouse hippocampus," *Scientific Reports*, vol. 6, no. 1, article 38242, 2016.

[76] D. N. Alfarez, M. Joels, and H. J. Krugers, "Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro," *The European Journal of Neuroscience*, vol. 17, no. 9, pp. 1928–1934, 2003.

[77] N. Maggio and M. Segal, "Striking variations in corticosteroid modulation of long-term potentiation along the septotemporal axis of the hippocampus," *The Journal of Neuroscience*, vol. 27, no. 21, pp. 5757–5765, 2007.

[78] R. Garcia, "Stress, metaplasticity, and antidepressants," *Current Molecular Medicine*, vol. 2, no. 7, pp. 629–638, 2002.

[79] J. J. Kim and K. S. Yoon, "Stress: metaplastic effects in the hippocampus," *Trends in Neurosciences*, vol. 21, no. 12, pp. 505–509, 1998.

[80] J. J. Kim, E. Y. Song, J. J. Kim, E. Y. Song, and T. A. Kosten, "Stress effects in the hippocampus: synaptic plasticity and memory," *Stress*, vol. 9, no. 1, pp. 1–11, 2006.

[81] P. M. Maras, J. Molet, Y. Chen et al., "Preferential loss of dorsal-hippocampus synapses underlies memory impairments provoked by short, multimodal stress," *Molecular Psychiatry*, vol. 19, no. 7, pp. 811–822, 2014.

[82] A. Vlachos, N. Maggio, and M. Segal, "Lack of correlation between synaptopodin expression and the ability to induce LTP in the rat dorsal and ventral hippocampus," *Hippocampus*, vol. 18, no. 1, pp. 1–4, 2008.

[83] N. Maggio and M. Segal, "Differential modulation of long-term depression by acute stress in the rat dorsal and ventral hippocampus," *The Journal of Neuroscience*, vol. 29, no. 27, pp. 8633–8638, 2009.

[84] D. A. F. Robertson, J. E. Beattie, I. C. Reid, and D. J. K. Balfour, "Regulation of corticosteroid receptors in the rat brain: the role of serotonin and stress," *The European Journal of Neuroscience*, vol. 21, no. 6, pp. 1511–1520, 2005.

[85] A. Avital, M. Segal, and G. Richter-Levin, "Contrasting roles of corticosteroid receptors in hippocampal plasticity," *The Journal of Neuroscience*, vol. 26, no. 36, pp. 9130–9134, 2006.

[86] V. Korz and J. U. Frey, "Stress-related modulation of hippocampal long-term potentiation in rats: involvement of adrenal steroid receptors," *The Journal of Neuroscience*, vol. 23, no. 19, pp. 7281–7287, 2003.

[87] O. Wiegert, Z. Pu, S. Shor, M. Joels, and H. Krugers, "Glucocorticoid receptor activation selectively hampers N-methyl-D-aspartate receptor dependent hippocampal synaptic plasticity in vitro," *Neuroscience*, vol. 135, no. 2, pp. 403–411, 2005.

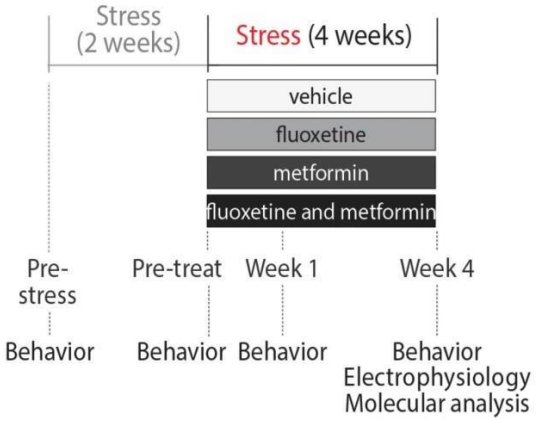
[88] M. J. Schmeisser, B. Baumann, S. Johannsen et al., "IκB kinase/nuclear factor κB-dependent insulin-like growth factor 2 (Igf2) expression regulates synapse formation and spine maturation via Igf2 receptor signaling," *The Journal of Neuroscience*, vol. 32, no. 16, pp. 5688–5703, 2012.

[89] M. Pascual-Lucas, S. Viana da Silva, M. di Scala et al., "Insulin-like growth factor 2 reverses memory and synaptic deficits in APP transgenic mice," *EMBO Molecular Medicine*, vol. 6, no. 10, pp. 1246–1262, 2014.

[90] A. Cohen, P. R. Houck, K. Szanto, M. A. Dew, S. E. Gilman, and C. F. Reynolds, "Social inequalities in response to antidepressant treatment in older adults," *Archives of General Psychiatry*, vol. 63, no. 1, pp. 50–56, 2006.

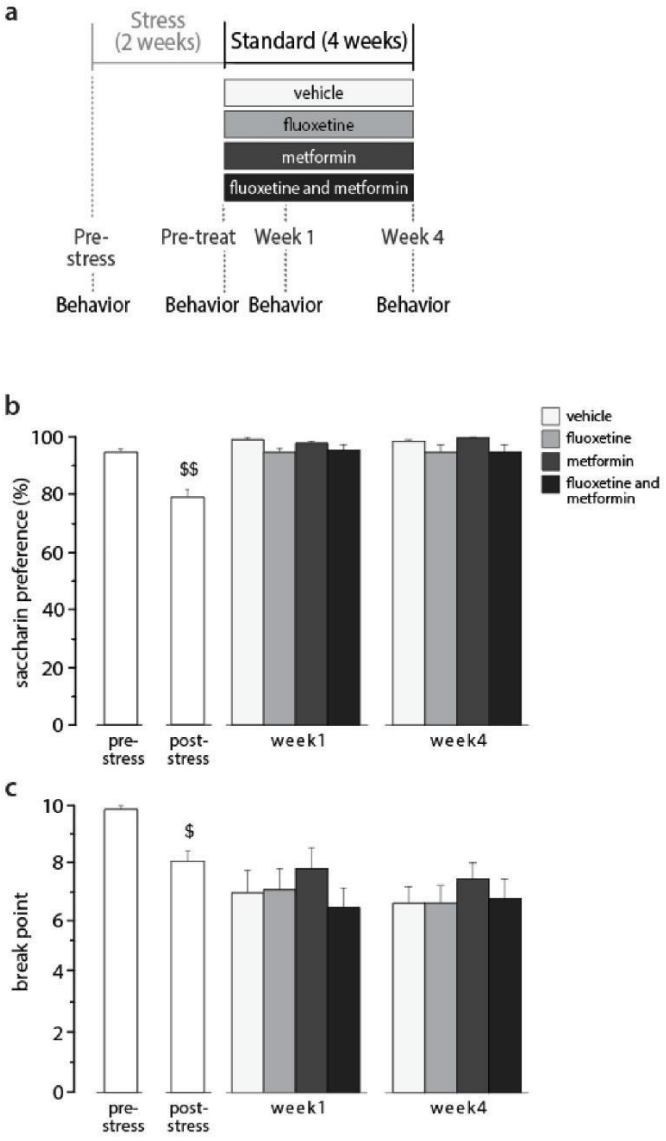
SUPPLEMENTARY MATERIALS – Poggini et al.

FIGURE S1



Experimental design of the stressful condition. Mice were exposed to stress for six weeks. The first two induced the depression-like phenotype. During the following four weeks of stress, the subjects were treated for with fluoxetine, metformin or their combination.

FIGURE S2



Experimental design and effects of fluoxetine, metformin or their combination on depression-like behavior in standard condition: (a) First mice were exposed for two weeks to the stressful condition to induce a depression-like phenotype. Afterwards, they were treated for four weeks in the standard condition. (b) Liking-type anhedonia. Saccharin preference significantly decreased following exposure to stress. Treatments did not produce different effects. (c) Wanting-type anhedonia. The breakpoint level was significantly reduced after the unpredictable chronic mild stress. Treatments did not produce different effects. Treatments as indicated in the legend, n=9-10 mice per group. $$p<0.0001 and $p=0.0022 pre- vs post-stress. Data are presented as mean + S.E.M.

STUDY 3 - Microglia modulation of synaptic plasticity in dorsal and ventral hippocampus *(unpublished data)*.

Abstract

Although it was initially believed that the hippocampus could act in a unitary manner with properties preserved along its extension, recent evidence highlighted differences concerning physiological and functional properties along the longitudinal axis of hippocampus (Fanselow and Dong, 2010; Dougherty et al., 2012; Malik et al., 2015; Maruki et al., 2001; Maggio and Segal, 2007; Papatheodoropoulos and Kostopoulos, 2000a,b; Milior et al., 2016). In parallel, increasing evidence are emerging about the role that microglial cells play in regulating neuronal networks and functions, neuronal transmission and synaptic plasticity, supporting the existence of a close relationship between the central nervous system and the immune system (Tremblay, 2011). Here, for the first time, we examined the possible contribution of microglial cells in modulating short- and long-term plasticity in the CA1 region of dorsal and ventral hippocampus. To this purpose, we interfered with physiological functioning of microglial cells, using different approaches: i) a pharmacological depletion with PLX5622 *in vivo*, ii) a pharmacological inhibition with the tetracycline antibiotic minocycline *in vitro*, and iii) a mice model of genetic microglial deletion for CX3CR1 (fractalkine receptor) in which the microglia-neuron communication, mediated by CX3CR1 and its unique neuronal ligand CX3CL1 (fractalkine), is interrupted. In addition to having confirmed that short- and long-term plasticity are considerably different in dorsal and ventral hippocampus, we found that both minocycline and PLX5622 treatment, inverts the LTP amplitude compared to the control group in both hippocampal regions, without affecting the pre-synaptic probability of glutamate release; the same results were obtained in CX_3CR1 knockout mice. Analysis of microglial distribution and morphology in the two hippocampal poles also shows significant differences, supporting our hypothesis that microglia could differently modulate neuronal functions in the dorsal and ventral hippocampus.

Introduction

The hippocampus is a medial temporal lobe structure extending in a C-shaped fashion from dorsal (septal)-to-ventral (temporal), corresponding to a posterior-to-anterior axis

in human (Strange et al., 2014). While intrinsic circuitry is conserved along the longitudinal axis, dorsal and ventral regions have different connectivity with cortical and subcortical areas; specifically, dorsal hippocampus receives visual and spatial information from sensory cortices via the medial entorhinal cortex, while, ventral hippocampus is connected to the amygdala, prefrontal cortex (PFC) and hypothalamus (Amaral and Witter, 1989; Pitkanen et al., 2000); these differences lead the dorsal hippocampus to be involved mainly in cognitive functions, while the ventral in regulating emotional responses (Fanselow and Dong, 2010). Several studies based on gene expression, anatomical, behavioral and electrophysiological measurements have suggested that hippocampus has different functional organizations along its longitudinal axis. In particular, it has been proposed that either this brain region is organized in multiple functional domains or it is structured according to a spatial gradient (Cembrowski et al. 2016; Thompson et al. 2008; Kjelstrup et al. 2008). Longitudinal variations in the electrophysiological properties of hippocampal neurons showed that CA1 pyramidal cells differ in excitability, short- and long-term plasticity (Dougherty et al., 2012; Malik et al., 2015; Maruki et al., 2001; Maggio and Segal, 2007; Papatheodoropoulos and Kostopoulos, 2000a,b; Milior et al., 2016). In parallel, that is emerging that microglia, in addition to their function as resident immune cells in the brain parenchyma, are also involved in regulating neuronal networks and functions, neuronal transmission, and synaptic plasticity (Tremblay, 2011), supporting the existence of a close relationship between the central nervous system and the immune system. Among the candidate pathways which could mediate the communication between microglial cells and neurons, fractalkine signaling is critical in various contexts of health and disease (Sheridan and Murphy, 2013). This chemokine is expressed by neurons, either as membrane-bound or soluble form, while its unique receptor CX3CR1 is considered to be exclusively present on microglia in the healthy brain (Jung et al., 2000). Fractalkine signaling was recently found to control key microglial functions, such as the regulation of maturation, function and modification of synapses, adult neurogenesis and synaptic activity (Paolicelli et al., 2011; Tremblay et al., 2010a; Maggi et al., 2011; Bachstetter et al., 2011).

The aim of the present study was to assess whether the known electrophysiological differences present along the hippocampal longitudinal axis could be due to different properties of microglia in dorsal and ventral hippocampus and, accordingly, if these cells may differently be involved in modulating short- and long term synaptic plasticity

in these two hippocampal regions, with the final goal of elucidating the mechanisms underlying the cross-talk between neurons and microglia.

In particular, first we analyzed the microglial distribution and morphology in dorsal and ventral hippocampus; then, we investigated the consequences of interfering with microglial cells, using pharmacological approaches (minocycline or PLX) or through fractalkine signaling deficiency, on short- and long-term plasticity in dorsal and ventral hippocampus.

Materials and Methods

Animals

All experiments were conducted in conformity with European Directive 2010/63/EU and the Italian D.lg. 4.05.2014 and all methods were carried out in accordance with relevant guidelines and regulations. Adult (8–12 weeks old) male mice were used: C57BL/6J wild-types and $CX3CR1^{GFP/GFP}$, on a C57BL/6J background (from the Jackson Laboratory, Charles River, where the Cx3cr1 gene was replaced by a green fluorescent protein (GFP) reporter) (Jung et al., 2000). In this work we refer to these mice as $CX_3CR1^{KO/KO}$ mice. Mice were housed under a12-h light–dark cycle at 22–25 °C with food and water provided ad libitum. All efforts were made to minimize the number of animals used and their suffering.

Electrophysiology

Hippocampal slice preparation - In order to perform electrophysiological experiments, acute hippocampal slices were collected. Animals were anesthetized by inhalation of halothane (Sigma-Aldrich S.r.l., Milan, Italy) and decapitated. The brain was rapidly removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF) solution composed of the following (in mM): NaCl 125, KCl 4, CaCl2 2.5, MgSO4 1.5, NaHPO4 1, NaHCO3 26, and glucose 10. ACSF was continuously bubbled with 95% O2 + 5% CO2 to maintain a pH close to 7.4. Following removal, the brain was hemisected along the longitudinal fissure to separate the two hemispheres. Brain dissection was carried out according to the slicing plane chosen and the structure to be investigated. Specifically, for experiments on the ventral hippocampus, slices were cut perpendicular to the longitudinal axis from the temporal pole of the brain. For experiments on the dorsal hippocampus, coronal slices were cut from the frontal pole. Dorsal and ventral slices have been identified as the distance, in μm, from the frontal

and temporal pole, respectively (approximately from 400 to 1750 μm). The brain tissues were blocked on the stage of a vibrating microtome (Thermo Scientific, USA), and 350 μm-thick slices were cut in ice-cold ACSF. The slices were then transferred to an incubation chamber containing oxygenated ACSF, where they were allowed to recover for 1 h at 30°C prior to electrophysiological recording. Dorsal and ventral slices were prepared from separate hemispheres of the same brain and were obtained alternately from the right or left hemisphere.

Extracellular field recordings - For field recordings, individual slices were transferred to an interface slice-recording chamber (BSC1, Scientific System Design Inc) with a total fluid dead space of about 3 ml where they were visualized with a Wild M3B stereomicroscope (Heerbrugg, Switzerland). They were maintained at 30–32 °C and superfused with ACSF at 2 ml/min by a peristaltic pump. A concentric bipolar stimulating electrode (SNE-100×50 mm long, Elektronik–Harvard Apparatus GmbH) was placed at a constant distance in the stratum radiatum to stimulate Schaffer collateral fibres in the CA1 region. Stimuli consisted of 100 μs constant current pulses of variable intensity, applied at 0.05 Hz. An ACSF-filled glass micropipette (0.5–1 MΩ) was placed at 200–600 μ m from the stimulating electrode to measure orthodromically-evoked field extracellular postsynaptic potentials (fEPSP). They were recorded and filtered (low pass at 1 kHz) with an Axopatch 200 A amplifier (Axon Instruments, CA) and digitized at 10 kHz with an A/D converter (Digidata 1322 A, Axon Instruments). Stimulus intensity was adjusted to evoke fEPSPs of amplitude about 50% of maximal amplitude with minimal contamination by a population spike. Evoked responses were monitored online, and stable baseline responses were recorded for at least 10 min. Only the slices that showed stable fEPSP amplitudes were included in the experiments. LTP was induced by high-frequency stimulation (HFS) (2 train of stimuli at 100Hz, of 1 s duration and separated by 3s) and recorded for 1 hour. To analyze the time course of the fEPSP amplitude, the recorded fEPSP was routinely averaged over 1 min. fEPSP slope changes following the LTP induction protocol were calculated with respect to the base line (30 min after vs 1 min before LTP induction). Averaged fEPSPs at 35 min after these trains were normalized to baseline values before high-frequency stimulation. The paired-pulse ratio (PPR) was measured from responses to two synaptic stimuli at 50 ms inter-stimulus interval. The PPR was calculated as the ratio between the fEPSP amplitude evoked by the second stimulus (A2) divided by that induced by the first (A1; A2/A1). fEPSP were recorded and filtered (low pass at 1 kHz) with an Axopatch 200A

amplifier (Axon Instruments, CA) and digitized at 10 kHz with an A/D converter (Digidata 1322A, Axon Instruments). Data were stored on a computer using pClamp 9 software (Axon Instruments) and analyzed off-line with Clamp-fit 10.4 program (Axon Instruments).

Treatments

Only wilt-types mice were subjected to pharmacological treatments. Specifically, to inhibit microglial functions, dorsal and ventral hippocampal slices has been treated with the tetracycline antibiotic minocycline at 1 µM for 1 hour prior of experiments and for all the duration of electrophysiological recordings. To transiently deplete microglia, mice were treated for seven consecutive days with a selective inhibitor of CSF1 receptor, essential for microglia proliferation, differentiation and survival (Elmore et al., 2014). The inhibitor, PLX5622, was gently provided by Plexxikon inc. (Berkeley, USA) and formulated in standard chow at 1200 mg/kg by Research Diets.

Light microscopy

Immunoperoxidase staining – Transverse sections of the brain (50 lm thick) were cut in sodium phosphate buffer (PBS; 50 mM at pH 7.4) using a vibratome and stored at _20 _C in cryoprotectant (30% glycerol and 30% ethylene glycol in PBS) until further processing (Tremblay et al., 2010b). For light microscopy, sections were washed in PBS to remove the cryoprotectant, quenched with 2% H2O2 in 70% methanol for 10 min at room temperature (RT), washed in Tris–buffered saline (TBS; 50 mM at pH 7.4) containing 1% Triton X100, and processed freely-floating for immunoperoxidase staining. Briefly, sections were pre-incubated for 1 h at RT in a blocking solution of TBS containing 10% fetal bovine serum, 3% bovine serum albumin, and 1% Triton X100. They were incubated overnight at 4 °C with primary antibody against IBA1 (rabbit-anti-IBA1; Wako Pure Chemical Industries) and rinsed in TBS. Incubation in secondary antibody (goat anti-rabbit) was followed by incubation with ABC solution (Vectastain). Finally, sections were stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and mounted onto glass slides, dehydrated in ascending concentrations of ethanol, cleared in citrisol, and coverslipped with DPX (Electron Microscopy Sciences; EMS).

Light microscopy imaging and analysis - Color pictures were acquired in CA1 stratum radiatum of dorsal and ventral hippocampus using an Infinity 2 camera (5 MP; Lumenera), at 10X for the cellular density and spacing analysis, and at 40X for the

morphology analysis. All the analysis was performed with the ImageJ software (National Institutes of Health). To determine cellular densities and spacing, the CA1 stratum radiatum was delineated in 10X pictures by using the freehand selection tool, based on the stereotaxic atlas of Paxinos and Franklin (2013), and its area measured in pixels and converted into mm2. The center of each microglial cell body was marked with a dot using the paintbrush tool. The analyze particles function was then used to automatically record cell numbers as well as spatial coordinates, enabling to determine the nearest neighbor distance for each cell by using the nearest neighbor distance plugin. Total cell number was divided by the total area to determine cellular density on a per animal basis. A spacing index was calculated as the square of the average nearest neighbor distance multiplied by microglial density on a per animal basis (Tremblay et al., 2012). To analyze morphology only cells whose cell body and proximal processes were perfectly in focus were included in the analysis. Every IBA1-immunopositive microglia in a particular picture was analyzed before moving on to the next picture as to not introduce selection bias (Tremblay et al., 2012). For each microglia, the soma area was determined by drawing a line around the cell body by using the freehand selection tool. The arborization area was determined with the polygon selection tool to connect the most distal extremities of every process. The soma and arborization areas were calculated in pixels and converted into micrometers. A morphological index was determined by using the formula: soma area/arborization area. The analyzes were performed with the GraphPad Prism software (v. 6.01, GraphPad Software, San Diego, CA, USA).

Statistical analysis

Tests of significance were made with with one-way ANOVA or Student's t-test. When a significant main effect was found, selected pairwise comparisons were made using Tukey post hoc analysis. Levels of significance were set as $*p < 0.05$; $**p < 0.01$. All parameters are reported as mean±SEM and n/N refers to numbers of slices/number of animals analysed.

Results

Dorsal and ventral hippocampus differs in terms of microglial distribution and morphology.

Based on the differences concerning the physiological and functional properties along the longitudinal axis of hippocampus (Milior et al., 2016) and the emerging involvement of microglial cells in regulating neuronal networks and functions,

neuronal transmission and synaptic plasticity (Tremblay, 2011), we first investigate whether, in C57BL/6J mice, the dorsal and ventral hippocampus can differ in microglial distribution and morphology. By IBA1 immunostaining analysis, we found that microglial density is different in the two hippocampal regions; in particular, in dorsal hippocampus microglial cells are significantly more dense (0,29 ± 0,01 cells/mm^2) than in the ventral (0,22 ± 0,01 cells/mm^2; $p<0.01$). By contrast, for what concerns microglial spacing index, the dorsal pole (0,46 ± 0,01 a.u.) doesn't differ from the ventral one (0,43 ± 0,01 a.u.) (Fig. 1a,b). Besides, microglial cell body area is increased in ventral hippocampus (24,16 ± 0,99 μm^2) compared to the other region (20,37 ± 0,67 μm^2; $p<0.05$), but any difference was observed in the arborization area(1105,14 ± 42,15 μm^2 and 1152,82 ± 88,23 μm^2, respectively) (Fig. 1c). These quantitative and morphological results could be related to differences in microglial basal properties along the longitudinal axis of hippocampus.

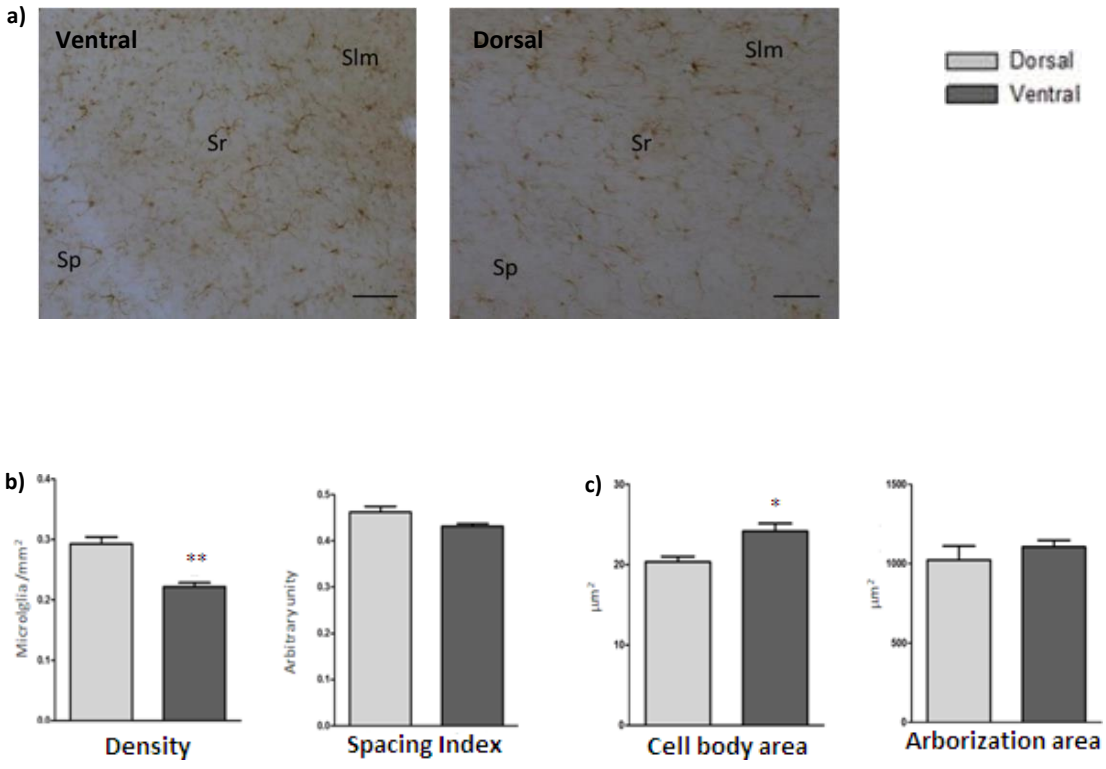


Figure 1. Quantitative and qualitative analysis of microglial cells in dorsal and ventral hippocampus.
a) Representative images of IBA1-stained microglia captured at low (10X) magnification from dorsal and ventral hippocampal sections of wild-types mice. Histograms represent b) microglial density and spacing index and c) microglial cell body area and arborization area. *p < 0.05 and **p < 0.01 One Way ANOVA, Tukey post hoc test. n/N refers to the number of microglial cells on total number of mice analyzed (n=5-6, N=4). Arbitrary units (a.u.). (Data obtained by Tremblay ME)

Microglial inhibition or depletion doesn't affect the pre-synaptic probability of glutamate release.

To investigate the possible repercussion of changes in microglial distribution and morphology on the synaptic and neuronal properties, we performed electrophysiological recordings on acute hippocampal slices. Specifically, in order to explore if microglia cells may differently control short-term synaptic plasticity of excitatory synapses made by Schaffer collaterals onto CA1 neurons in dorsal and ventral hippocampus, we measure changes in the paired-pulse ratio (PPR), a form of short-term plasticity generally associated with changes in transmitter release probability 70. As reported in previous publications (Milior et al., 2016; Maggio and Segal, 2007, Maruki et al., 2001), we found that PPR values changes along the hippocampal longitudinal axis; in particular, post hoc comparison shows that PPR is significantly increased in dorsal hippocampus (1.64 ± 0.03) compared to the ventral one (1.21 ± 0.03; $p<0.01$) (Fig. 2). Since PPR depends on a transient increase of transmitter release due to residual presynaptic calcium, this difference means that the ventral region has a higher probability of glutamate release compared to the dorsal [12,15]. Following minocycline treatment, we found that PPR remains significantly higher in dorsal (1.50 ± 0.04) than in ventral region (1.23 ± 0.05; $p<0.01$); similarly, PLX5622 treated-mice show an increased PPR in dorsal region (1.47 ± 0.04) compared to the ventral (1.19 ± 0.04; $p<0.01$) (Fig. 2). These results suggest that the pharmacological inhibition or depletion of microglial cells doesn't affect the pre-synaptic probability of glutamate release.

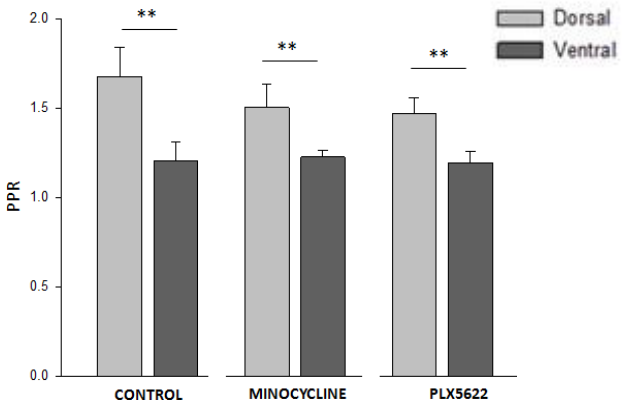


Figure 2. Short-term plasticity at Schaffer collateral synapses in dorsal and ventral hippocampus.
Averaged PPR values in control condition and in groups treated with minocycline and PLX5633. Data are shown as mean ± s.e.m.
** $p<0.01$ One Way ANOVA, Tukey post hoc test. n/N refers to the number of slices on total number of mice analyzed (n=5-11, N=4-8).

Microglial inhibition or depletion affects long-term potentiation.

To investigate the role of microglial cells in modulating synaptic/neuronal properties, we evaluated long-term plasticity (LTP) at the CA1 region of dorsal and ventral

hippocampus by measuring changes in fEPSP amplitude with time after a high-frequency stimulation (HFS). In the control group, we found that the amplitude of LTP, measured at 35 min after HFS, is increased in slices prepared from the dorsal hippocampus (1.56 ± 0.03) compared to the ventral (1.26 ± 0.03; $p<0.01$) (Fig. 3a), confirming what previously published (Milior G. et al., 2016). Then, we performed electrophysiological recordings in the experimental groups in which microglial cells activity was blocked through the pharmacological strategies; we found that microglial inhibition *in vitro*, using minocycline (at 1μM), modifies LTP amplitude, which results to be significantly higher in ventral (1.47 ± 0.04) than in dorsal hippocampus (1.36 ± 0.03; $p<0.05$) (Fig. 3b). Similarly to minocycline, we observed that in mice treated with PLX5622, LTP is more potentiated in ventral hippocampus (1.47 ± 0.04) than in the dorsal (1.32 ± 0.04; $p<0.05$) (Fig. 3c). In addition, the statistical comparison of LTP amplitude (35 minutes after LTP induction) showed that LTP recorded in dorsal hippocampal slices is significantly reduced by both pharmacological treatments compared to the control group ($p<0.001$ for each comparison); by contrast, in the ventral hippocampus, both treatments significantly increase the amplitude of LTP compared to the control group ($p<0.001$ for each comparison) (Fig. 3d).

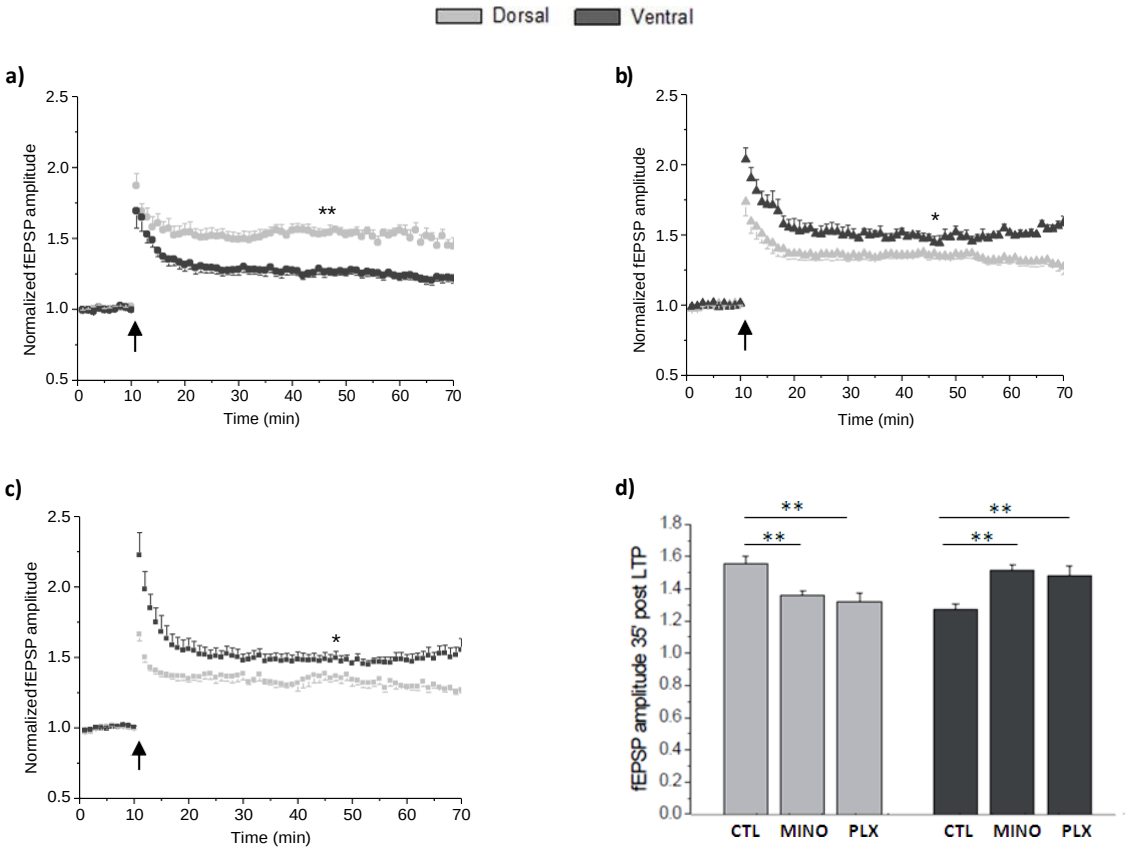


Figure 2. Long-term plasticity at Schaffer collateral synapses in dorsal and ventral hippocampus.
Time course of normalized averaged amplitudes of fEPSPs made from dorsal (light gray) and ventral (dark gray) hippocampal slices in a) control, b) minocycline, and c) PLX5622 groups. Arrows indicate LTP induction by HFS (2 trains at 100 Hz of 1 s duration with 3 s inter-train interval). * $p < 0.05$, One Way ANOVA, Tukey post hoc test.
d) LTP of fEPSP amplitude measured 35 minutes after HFS from dorsal (light gray) and ventral (dark gray) hippocampal slices of three experimental groups. **$p<0.01$, Two Way ANOVA, Tukey post hoc test. Data are shown as mean ± s.e.m. n/N refers to the number of slices on total number of mice analyzed (n=5-8, n=4-8).

Genetic deletion of the CX_3CR1 microglial receptor modifies the LTP amplitude.

In order to further validate the results obtained we repeat the same experiment in animals with a genetic deletion of CX_3CR1, a microglial receptor that guarantees the communication between neurons and microglia and which is known to influence synaptic plasticity processes. We evaluated the short-term plasticity and we found that, similarly to control, the PPR is significantly higher in dorsal (1.58 ± 0.07) than in ventral region (1.25 ± 0.05; $p<0.01$) (Fig. 3a). Concerning long-term plasticity we observed that the amplitude of LTP was higher in the ventral (1.36 ± 0.05) compared to the dorsal hippocampus (1.19 ± 0.03; $p<0.05$) (Fig. 3b), resembling results obtained with minocycline and PLX5622 treatments. These results showed that an impairment of a neuron-microglia signaling is sufficient to affect LTP amplitude in the two hippocampal regions.

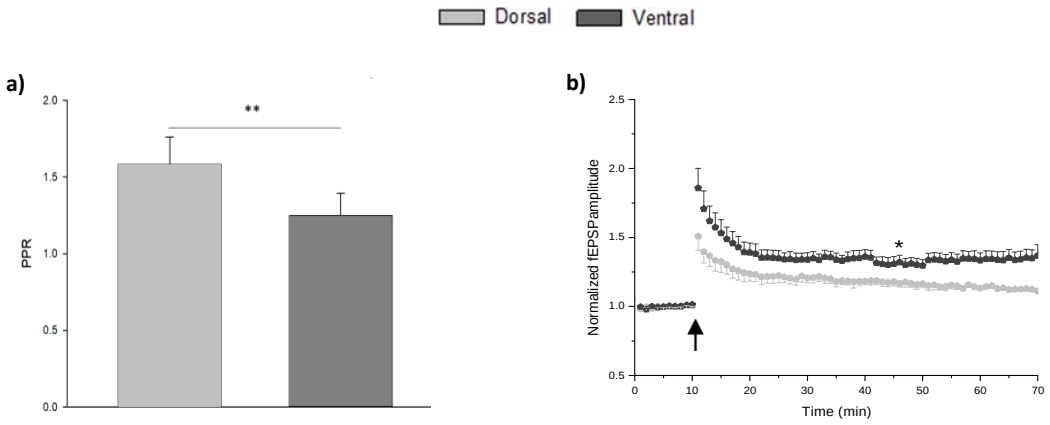


Figure 3. Short- and long-term plasticity recorded in dorsal and ventral hippocampus of $CX_3CR_1^{KO/KO}$ mice
a) Histogram represents averaged PPR values. ** $p<0.01$ One Way ANOVA, Tukey post hoc test. Data are shown as mean ± s.e.m. n/N refers to the number of slices on total number of mice analyzed (n=7-8, N=5). b) Time course of normalized averaged amplitudes of fEPSPs made from dorsal (light gray) and ventral (dark gray) hippocampal slices Arrows indicate LTP induction by HFS (2 trains at 100 Hz of 1 s duration with 3 s inter-train interval). * $p<0.05$, One Way ANOVA, Tukey post hoc test. Data are shown as mean ± s.e.m. n/N refers to the number of slices on total number of mice analyzed (n=5-8, N=5).

Discussion

Although for a long time it was thought that the hippocampus acted as a unitary structure, recent evidence demonstrated different physiological and functional properties along the hippocampal longitudinal axis (Fanselow and Dong, 2010). In parallel, it is emerging that microglia, in addition to their function as resident immune cells in the brain parenchyma, is also involved in regulating neuronal networks and functions, neuronal transmission and synaptic plasticity (Tremblay, 2011) supporting the existence of a close interaction between the central nervous system and the immune system. In this study we focused our attention on understanding whether microglial cells act in a region-specific manner in modulating short- and long-term hippocampal synaptic plasticity, which are reported to vary considerably along the longitudinal axis of hippocampus (Maruki et al., 2001; Maggio and Segal, 2007; Milior et al., 2016). For the first time, our results demonstrated that microglial cells have a different distribution and morphology along the longitudinal axis of hippocampus, being more dense and with a smaller cell body area in the dorsal hippocampus compared to the ventral. Morphological remodeling is an important requisite for all microglial activities, from monitoring synaptic information flow to phagocytosis, and it is associated with changes in transcriptional activity and functional state of microglia (Walker et al., 2014). Therefore, the morphological variation that we observed in the two hippocampal regions could be related to transcriptional and functional differences of microglial cells. For a better characterization, additional analysis on microglial cells isolated from dorsal and ventral hippocampus, is underway; in particular, the evaluation of expression levels of phenotypic and functional microglial markers (e.g. cytokines), as well as the gene expression of microglial targets involved in the interactions with the neuronal counterpart (e.g. CX3CR1) will clarify the functional status of these cells in the two hippocampal regions. Future studies of electron microscopy, aimed at evaluating the microglial phagocytosis of synaptic elements and the physical interaction between microglia and synaptic terminals in the two hippocampal regions, will help to understand if microglial cells differently act on the remodeling and activity of neuronal circuits in dorsal and ventral hippocampus.

To investigate the possible microglia-mediated effects on neural plasticity in the two hippocampal regions, we have pharmacologically depleted or inhibited microglial cells, using PLX5622, an inhibitor of colony-stimulating factor 1 receptor (CSF-1) which is essential for microglial proliferation, differentiation and survival (Elmore et al., 2014),

or the tetracycline antibiotic minocycline, respectively. Exploiting extracellular recordings at the Schaffer collateral synapse, we demonstrated that microglial functionality loss doesn't influence short-term plasticity which was significantly higher in the dorsal hippocampal region than in the ventral one, similarly to the control group; this evidence indicates that microglial cells don't play a relevant role in regulating the presynaptic probability of glutamate release in the two hippocampal poles. By contrast, microglia seems to take action in modulating long-term potentiation (LTP), one of the most striking examples of strengthening synapses efficiency. In the control group, we confirmed the reported decrease in LTP amplitude along the dorsal-ventral hippocampal axis (Milior et al., 2016); interestingly, we showed that following pharmacological microglial inhibition or depletion, LTP was more potentiated in the ventral region than in the dorsal, suggesting that an impaired microglial functionality inverted the amplitude of LTP compared to the control condition. These findings, besides confirming the involvement of microglia in modulating neuronal transmission and plasticity, highlight that microglial cells act in a region-specific manner on the regulation of hippocampal LTP.

In addition to these results, we analyzed mice with a genetic deletion of fractalkine receptor CX_3CR1 ($CX_3CR^{KO/KO}$), which is expressed specifically on the surface of microglia and binds its unique neuronal ligand, fractalkine CX3CL1 (Jung et al., 2000). CX3CR1-CX3CL1 signaling was found to crucially control key microglial functions such as regulation of synaptic activity and remodeling and/or maturation of synaptic circuits (Paolicelli et al., 2011; Tremblay et al., 2010a; Maggi et al., 2011; Ragozzino et al., 2006; Bertollini et al., 2006), suggesting that it is critical for mediating neuron-microglia crosstalk. The results that we obtained in $CX_3CR^{KO/KO}$ mice suggest that fractalkine signaling strongly influence neuroplasticity in these two hippocampal regions. In particular, in mice lacking CX3CR1, LTP amplitude was significantly augmented in ventral hippocampus compared to the dorsal, while the presynaptic probability of glutamate release remains higher in the dorsal hippocampal region compared to the ventral one, summarizing the effects obtained following pharmacological blockade of microglial functions. Taken together, these evidences support the idea that differences in synaptic plasticity reported along the hippocampal longitudinal axis could be strongly influenced by microglial activities in a region-specific manner.

These preliminary evidences need further investigations to implement and clarify differences in microglial cells activity and neuronal interactions in the two hippocampal

regions. For example, it will be interesting to analyze factors released by microglial cells involved in neuronal plasticity modulation such as the brain-derived neurotrophic factor (BDNF), that modulates inhibitory neurotransmission and NMDA receptors (Ferrini and Koninck, 2013; Crozier et al., 2008). Indeed, it has been demonstrated that selective deletion of microglial or genetic removal of BDNF reduces the synaptic expression of two specific glutamate receptor subtypes, GluN2B and GluA2, without affecting the density of neurons or synapses in the cortex and hippocampus. These results suggest that microglial BDNF may alter synaptic levels of NMDA receptor subunit (Parkhurst et al., 2013). A role in LTP modulation could be also played by IL-1 β, a soluble factor produced by microglia, which crucially influences AMPA receptors (Lai et al., 2006) and at higher doses it significantly inhibit LTP induction (Di Filippo et al., 2008). Therefore, it could be possible that microglial cells differ in the amount of BDNF and/or IL1-b released in dorsal versus ventral hippocampus, which in turn could specifically affect LTP induction and expression in the two regions. Interestingly, it has been reported that BDNF is differently expressed along the hippocampal axis, with higher levels in the dorsal region (Faure, et al., 2007). BDNF binds to tropomyosin-related kinase (Trk) B receptor, highly expressed also on microglia; TRkB activation increases increases synaptic strength synaptic protein expression, induces LTP, modulates dendritic arborization and spine morphology, and increases synaptic strength (Chen et al., 2010). Therefore, the differences observed in LTP amplitude along the hippocampal axis could be due to a different activation of BDNF-TrkB signaling in dorsal and ventral hippocampus.

Additionally, it would be important to evaluate the expression levels of microglial neurotransmitter receptors in dorsal versus ventral hippocampus. Microglial cells are well known to express receptors for glutamate, GABA, as well as other neurotransmitters, which induce microglia to increase or decrease the release of some neuroactive molecules, known to exert profound effects on neurons (Liu et al., 2016). For example, the microglial response to glutamate mediated by NMDA and kainate receptors enhance the release of Tumor Necrosis Factor (TNF)α, while the activation of AMPA receptors inhibit it (Hagino et al., 2004; Acarin et al., 1996); indeed, the GABAergic microglial receptors attenuates the release of proinflammatory microglial cytokines such as ILs and TNF-a (Kuhn et al., 2004; Lee et al., 2011), in response to GABAergic tone. It has been well established that microglial activation and consequent overproduction of proinflammatory cytokines have detrimental effects on hippocampal LTP induction and maintenance (Liu et al., 20117; Bellinger et al., 1993; Ikegaya et al.,

2003). Based on this evidence, the region-specific modulation of hippocampal LTP exerted by microglia could also arise from different expression levels of microglial neurotransmitter receptors.

Interestingly, our results highlighted a relevant role of CX_3CR1-CX_3CL1 signaling in modulating neuronal plasticity along the longitudinal axis of hippocampus. It has already been shown that neuronal CXCL1 significantly inhibits LTP in wild-type mice but not in $CX_3CR1^{KO/KO}$ mice, and this impairment requires the activation of AR3 receptors (Maggi et al., 2009). Besides, it has been demonstrated that mice lacking microglial CX3CR1 show enhanced plasticity compared to wild-type mice indicating that microglial cells drive alterations of plasticity phenomena (Maggi et al. 2011). Moreover, since CX_3CL1 reduces spontaneous glutamate release and postsynaptic glutamate currents (Limatola et al., 2005) linked to dephosphorylation of the GluR1 AMPA receptor subunit (Ragozzino et al., 2006), we can imagine that the disruption of CX_3CL1-CX_3CR1 signaling results in different phosphorylation state of GluR1 AMPA receptors in dorsal versus ventral hippocampus, producing an inversion of LTP amplitude. In view of this consideration, we are evaluating the expression levels of CX3CR1 in microglia isolated cells, as mentioned above, and CX3CL1 in the neuronal counterpart, as well as the AMPA receptor functionality by measuring phosphorylation of the GluR1, known to be modulated at Ser845 during plasticity.

All these investigations will implement and clarify the role of microglial cells in regulating the physiological properties of CA1 pyramidal neurons along the longitudinal axis of the hippocampus.

References

Acarin L, González B, et al. (1996) Microglial response to N-methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. J. Comp. Neurol. 367:361–74.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31, 571–591.

Bachstetter AD, Morganti JM, Jernberg J, Schlunk A, et al. (2011) Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. Neurobiol. Aging 32, 2030–2044.

Bellinger FP, Madamba S, Siggins GR (1993) Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res. 628, 227–234.

Bertollini C, Ragozzino D, Gross C, et al. (2006) Fractalkine/CX3CL1 depresses central synaptic transmission in mouse hippocampal slices. Neuropharmacology 51, 816–821.

Cembrowski MS, Bachman JL, Wang L, Sugino K, Shields BC, Spruston N (2016) Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. Neuron 89, 351–368.

Chen LY, Rex CS, Sanaiha Y, Lynch G, Gall CM (2010) Learning induces neurotrophin signaling at hippocampal synapses. Proc Natl Acad Sci U S A. 107:7030–7035.

Crozier RA, Bi C, Han YR, Plummer MR (2008) BDNF modulation of NMDA receptors is activity dependent. J Neurophysiol.;100:3264–3274.

Di Filippo M, Sarchielli P, Picconi B, Calabresi P (2008) Neuroinflammation and synaptic plasticity: theoretical basis for a novel, immune-centred, therapeutic approach to neurological disorders. Trends Pharmacol Sci.;29(8):402–12.

Dougherty KA, Islam T, Johnston D (2012) Intrinsic excitability of CA1 pyramidal neurones from the rat dorsal and ventral hippocampus. The Journal of physiology 590, 5707–5722.

Elmore MRP, et al. (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neur. 82 380–397.

Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65, 7–19.

Ferrini F, De Koninck Y (2013) Microglia control neuronal network excitability via BDNF signalling. Neural Plast. 2013:429815.

Faure J, Uys JDK, Marais L, Stein DJ, Daniels WMU (2007) Early maternal separation alters the response to traumatization: resulting in increased levels of hippocampal neurotrophic factors. Metabolic Brain Disease 22(2), 183–195.

Hagino Y, Kariura Y, et al. (2004) Heterogeneity and potentiation of AMPA type of glutamate receptors in rat cultured microglia. Glia;47:68–77.

Ikegaya Y, Delcroix I, Iwakura Y, Matsuki N, Nishiyama N (2003) Interleukin-1beta abrogates long-term depression of hippocampal CA1 synaptic transmission. Synapse 47, 54–57.

Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, et al. (2000) Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol. Cell. Biol. 20, 4106–4114.

Kjelstrup KB, Solstad T, Brun VH, Hafting T, Leutgeb S, Witter MP, Moser EI, Moser MB (2008) Finite scale of spatial representation in the hippocampus. Science 321, 140–143.

Kuhn SA, van Landeghem FK, Zacharias R, et al. (2004) Microglia express GABA(B) receptors to modulate interleukin release. Mol. Cell. Neurosci. 25:312–22.

Lai AY, Swayze RD, El-Husseini A, Song C (2006) Interleukin-1 beta modulates AMPA receptor expression and phosphorylation in hippocampal neurons. J. Neuroimm. 175, 97–106.

Lee M, Schwab C, McGeer PL. (2011) Astrocytes are GABAergic cells that modulate microglial activity. Glia 59:152–65.

Limatola C, Lauro C, Catalano M, Ciotti MT, et al. (2005) Chemokine CX3CL1 protects rat hippocampal neurons against glutamate-mediated excitotoxicity. J. Neuroimm. 166,19–28.

Liu H, Leak RK, Hu X (2016) Neurotransmitter receptors on microglia. Trends in. Neuroscience, 1(2), 52–58.

Liu Y, Zou L-J et al. (2017) TNF-α Differentially Regulates Synaptic Plasticity in the Hippocampus and Spinal Cord by Microglia-Dependent Mechanisms after Peripheral Nerve Injury.J. Neurosci. 25; 37(4): 871–881.

Limatola C, Lauro C, Catalano M, Ciotti MT, Bertollini C, Di Angelantonio S, et al. (2005) Chemokine CX3CL1 protects rat hippocampal neurons against glutamate-mediated excitotoxicity. J. Neuroimmunol. 166 19–28 C.

Maggi L, Scianni M, Branchi I, D'Andrea I, Lauro C, Limatola C (2011) CX(3)CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. Front. Cell. Neurosci. 5, 22.

Maggi L, Trettel F, Scianni M, Bertollini C, Eusebi F, Fredholm BB, et al. (2009) LTP impairment by fractalkine/CX3CL1 in mouse hippocampus is mediated through the activity of adenosine receptor type 3 (A3R). J. Neuroimm. 215, 36–42.

Maggio N, Segal M (2007) Unique regulation of long term potentiation in the rat ventral hippocampus. Hippocampus 17, 10–25.

Malik R, Dougherty KA, Parikh K, Byrne C, Johnston D (2015) Mapping the electrophysiological and morphological properties of CA1 pyramidal neurons along the longitudinal hippocampal axis. Hippocampus.

Maruki K, Izaki Y, Nomura M, Yamauchi T (2001) Differences in paired-pulse facilitation and long-term potentiation between dorsal and ventral CA1 regions in anesthetized rats. Hippocampus 11, 655–661.

Milior G, Di Castro MA, Sciarria LP, Garofalo S, et al. (2016) Electrophysiological Properties of CA1 Pyramidal Neurons along the Longitudinal Axis of the Mouse Hippocampus. Sci Rep. 6;6:38242.

Paolicelli RC, Bolasco G, Pagani F, Maggi L, et al. (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458.

Papatheodoropoulos C, Kostopoulos G (2000a) Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. Neurosci Lett 279, 177–180.

Papatheodoropoulos C, Kostopoulos G (2000b) Dorsal-ventral differentiation of short-term synaptic plasticity in rat CA1 hippocampal region. Neurosci Lett 286, 57–60.

Parkhurst CN, Yang G, Ninan I, Savas JN, et al. (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 9;155(7):1596-609.

Paxinos G, Franklin KB (2013) The Mouse Brain in Stereotaxic Coordinates. Academic Press, New York.

Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Annals of the New York Academy of Sciences 911, 369–391.

Ragozzino D, Di Angelantonio S, Trettel F, Bertollini C, Maggi L, Gross C, et al. (2006) Chemokine fractalkine/CX3CL1 negatively modulates active glutamatergic synapses in rat hippocampal neurons. J. Neurosci. 26, 10488–10498.

Rogers JT, Morganti JM, Bachstetter AD, et al. (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J. Neurosci. 31, 16241–16250.

Sheridan GK, Murphy KJ (2013) Neuron-glia crosstalk in health and disease: fractalkine and CX3CR1 take centre stage. Open Biol. 3, 130181.

Strange BA, Witter MP, Lein ES, Moser EI (2014) Functional organization of the hippocampal longitudinal axis. Nat Rev Neurosci 15, 655–669.

Thompson CL, Sayan D, et al. (2008) Genomic anatomy of the hippocampus. Neuron 60, 1010–1021.

Tremblay ME, Lowery RL, Majewska AK (2010a) Microglial interactions with synapses are modulated by visual experience. PLoS Biol. 8, e1000527.

Tremblay E, Riad M, Majewska A (2010b) Preparation of mouse brain tissue for immunoelectron microscopy. J. Vis. Exp. – JoVE.

Tremblay ME (2011) The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. Neuron Glia Biol. 7, 67–76.

Tremblay ME, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60, 541–558.

Walker FR, Beynon SB, et al. (2014) Dynamic structural remodelling of microglia in health and disease: a review of the models, the signals and the mechanisms. Brain Behav. Immun. 37,1–14.

GENERAL DISCUSSION AND CONCLUSION

MDD represents a relevant global public health burden at clinical, social and economic levels. One of the most relevant causes of this disorder is the incomplete efficacy of antidepressant treatments. In the present studies, we endeavored to identify factors involved in determining the SSRI outcome with the potential translational aim of developing a more effective antidepressant treatment.

Previous studies report that the administration of antidepressant fluoxetine, an SSRI, leads to an opposite effect on inflammation according to the starting inflammatory conditions, maintaining inflammatory levels in a strict range which appears permissive for brain plasticity processes [101,237].

Here, we demonstrated that, following LPS or Ibuprofen administration, the increased production of molecules related to both pro- or anti-inflammatory action determines plasticity impairment at molecular and cellular levels, reducing LTP amplitude, BDNF expression levels, as well as the GluR1 subunit phosphorylation at Ser845. Therefore, these and other reported evidences suggest a clear interplay between neural plasticity and inflammation, indicating that these are mutually regulating processes and that inflammatory levels should be kept within a strictly controlled range to establish the neural plasticity needed for the SSRIs beneficial action. In a translational perspective, this concept implies that to improve the efficacy of the antidepressant treatment in clinical settings, patients' inflammatory levels should be controlled before and during pharmacological therapy. In addition, by combining the antidepressant treatment with pro- or anti-inflammatory compounds aimed at counterbalancing any deviation in inflammatory levels, a more effective antidepressant strategy could be achieved. Although the beneficial effects of a co-treatment involving an SSRI and an anti-inflammatory compound have already reported in both pre-clinical and clinical studies [251,252], new studies should be devoted to evaluating whether, as suggested by the theoretical framework proposed here, a pro- or anti-inflammatory add-on could improve the outcome of antidepressant treatment.

An important emerging factor involved in the antidepressant outcome is the living environment; the treatment with SSRIs in a stressful environment leads to a worse depressive prognosis, enhancing brain plasticity and thus amplifying the influence of the environment on the individual. Since, in most cases, it is not possible to act on the living environment because of constraints due to patients' personal history, we proposed the pharmacological regulation of metabolism as a potential new approach to improve

antidepressant treatment outcome. The combined treatment with fluoxetine, used to enhance neural plasticity, and metformin, a drug able produce metabolic effects overlapping with those induced by a healthy lifestyle, ameliorates depressive-like behavior compared to just SSRI treatment in adverse living conditions; however, it has not long-lasting beneficial effects and should be used for subacute interventions. Interestingly, the therapeutic action of this polypharmacological approach potentiates LTP and increases the expression of IGF2, a key molecule involved in the vulnerability to stress [253] and in modulating biological processes related to neuronal plasticity, specifically in the dorsal hippocampus which was recently indicated as an important target for antidepressants treatments [254-258]. The expression of IGF2, which rescues the neurobehavioral effects of stress exposure and improves learning and memory, suggests that it could be a potential molecular target capable of triggering the antidepressant action and therefore the recovery from MDD. To support this, IGF2 has been reported to be a key target of ketamine, an antidepressant drug that has a rapid but not long-lasting action [259,260], similar to the effect concerning our polypharmacological treatment; this suggests that IGF2 could be mainly involved in the first-phase recovery from MDD. Other markers related to MDD, such as IGF1, leptin, p11, and BDNF are not affected by co-treatment in adverse environmental conditions, but they result more expressed in hippocampal dorsal region compared to the ventral one. Among the other, the result concerning BDNF supports the neurotrophic hypothesis of depression, according to which the psychopathology is associated with the reduction of brain BDNF levels and antidepressant treatments alleviate depressive symptoms increasing its levels [261], and the involvement of dorsal hippocampus in the antidepressant outcome.

Similar to gene expression, also hippocampal LTP is differentially modulated by stress in dorsal and ventral hippocampus. It is largely proven that several types of stress affect neuronal plasticity, generally inhibiting it, but mechanisms underlying this modulation are still under explorations.

Based on the described interplay between inflammation and hippocampal plasticity processes, we decided to further investigate whether the dorsal and ventral hippocampal regions, show differences in the function of cells highly involved in inflammatory responses, i.e. microglia cells, that could underlie a selective sensitivity to antidepressant treatment. From our preliminary study, it emerged for the first time that in standard environmental conditions, microglial cells show specific distribution and cell body morphology in the dorsal versus ventral hippocampus and that they regulate

long-term plasticity in a region-specific manner, further strengthening the relevant role of these cells in modulating plasticity process.

It is reported that the expression of neurotransmitter receptors (such as AMPA and NMDA receptors) varies gradually along the hippocampal axis [15], as well as BDNF, a neurotrophic factor involved in modulating inhibitory neurotransmission, NMDA receptors and LTP [262,263]. We can speculate that differences in microglia number, functionality or activation state along the hippocampal axis, could specifically modulate the amount of factors known to affect plasticity, producing the observed differences in LTP levels. To investigate this possibility, it would be necessary to measure microglial and neuronal expression of neurotransmitter receptors and their phosphorylation levels (e.g. GABAergic and glutamatergic receptors) [264], transcriptional activity and functional status of microglia as well as microglial phagocytosis of synaptic elements and physical interaction between microglia and synaptic terminals. This would greatly implement and clarify the role of these cells in regulating the physiological properties of CA1 pyramidal neurons along the longitudinal axis of the hippocampus in a standard living environment as well as in adverse conditions, i.e. following stress.

Overall, this study indicates that neuroinflammatory processes, as well as microglia cells which are highly involved in inflammatory responses, must be strictly regulated to instate the neural plasticity needed for the SSRI beneficial action, and that, in adverse environmental conditions, the co-administration of compounds affecting the metabolic profile can improve the antidepressant efficacy, affecting hippocampal synaptic plasticity. In a translational view, our evidences support the idea that, to improve the efficacy of antidepressant drugs, information concerning not only the mood of the patients but also their living environment and selected physiological endpoints (e.g. inflammatory levels) should be considered, in order to develop a more effective antidepressant therapy. This implies the development of personalized therapeutic interventions based on a global assessment of the biological and psychosocial characteristics of patients. This is relevant for MDD, a disorder whose onset and progression are multifactorial rather than triggered by a single chemical imbalance.

REFERENCES

1. Bartsch T, Wulff P (2015) The hippocampus in aging and disease: from plasticity to vulnerability. Neuroscience 19(309):1-16.

2. Andersen P (2007) The hippocampus book. Oxford, New York Oxford University Press.

3. Deshmukh SS, Knierim JJ (2012) Hippocampus. Wiley Interdisc. Rev. Cogn. Sci. 3(2):231-251.

4. Knierim JJ (2015) Hippocampus. Curr. biology 25(23):R1116-R1121.

5. Soudry Y, Lemogne C, Malinvaud D, Consoli SM, Bonfils P (2011) Olfactory system and emotion: common substrates. Eur. Ann. Otorhinolaryngol Head Neck Dis. 128(1):18-23.

6. Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J. Neurol Neurosurg. Psychiatry 20(1):11-21.

7. Squire LR (2009) The Legacy of Patient H.M. for Neuroscience. Neuron 15, 61(1):6–9.

8. O'Keefe J, Nadel L (1978) The Hippocampus as a Cognitive Map. Oxford University Press.

9. Moser EI, Kropf E, Moser M-B (2008) Place Cells, Grid Cells, and the Brain's Spatial Representation System. Ann. Rev. Neurosci. 31:69.

10. Kötter R, Stephan KE (1997) Useless or helpful? The "limbic system" concept. Rev. Neurosc. 8(2): 139–45.

11. Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. Nature Reviews Neuroscience 10:397–409.

12. Campbell S, MacQueen G (2004) The role of the hippocampus in the pathophysiology of major depression. Journal of Psychiatry Neuroscience 29:417–426.

13. Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31:571–591.

14. Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Annals of the New York Academy of Sciences 911:369–391.

15. Strange BA, Witter MP, Lein ES, Moser EI (2014) Functional organization of the hippocampal longitudinal axis. Nat. Rev. Neurosci. 15:655–669.

16. Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures?. Neuron 65:7–19.

17. Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J. Neurosci. 13:3916–3925.

18. Milior G, Di Castro MA, et al. (2016) Electrophysiological properties of CA1 pyramidal neurons along the longitudinal axis of the mouse hippocampus. Sci. Rep. 6(6):38242.

19. Cembrowski MS, Bachman JL, Wang L, Sugino K, Shields BC, Spruston N (2016) Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. Neuron 89:351–368.

20. Kjelstrup KB, Solstad T, Brun VH, Hafting T, Leutgeb S, Witter MP, Moser EI, Moser M-B (2008). Finite scale of spatial representation in the hippocampus. Science 321:140–143.

21. Small SA, Schobel SA, Buxton RB, Witter M P, Barnes CA (2011) A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat. Rev. Neurosci. 7, 12(10): 585–601.

22. Joels M (2008) Functional actions of corticosteroids in the hippocampus. Eur. J. Pharmacol. 583(2-3): 312–321.

23. Hughes JR (1958) Post-tetanic potentiation. Physiological Reviews, 38(1):91–113.

24. Citri A, Malenka RC (2008) Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. Neuropsychopharmacology Rewiev 33:18–41.

25. Sheng M, Sabatini BL, Südhof TC (eds.) (2012).The Synapse. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press 251-289, 331-348.

26. Byrne JH, LaBar KS, LeDoux JE, Schafe GE, Sweatt JD, Thompson RF (2009) Learning and memory: basic mechanisms. In: From Molecules to Networks, eds. Byrne JH, Roberts JL 2nd, Oxford, UK: Academic Press 539-608.

27. Bourne JN, Harris KM (2008) Balancing structure and function at hippocampal dendritic spines. Annu. Rev. Neurosci. 31:47-67.

28. Miladinovic T, Nashed MG, Singh G (2015) Overview of Glutamatergic Dysregulation in Central Pathologies. Biomolecules 5(4):3112–3141.

29. Henley JM, Wilkinson KA (2016) Synaptic AMPA receptor composition in development, plasticity and disease. Nat. Rev. Neurosci. 17(6):337-50.

30. Rosenmund C, Stern-Bach Y, Stevens CF (1998) The Tetrameric Structure of a Glutamate Receptor Channel. Science, 280:1596–1599.

31. Henley JM, Barker EA, Glebov OO (2011) Routes, destinations and delays: recent advances in AMPA receptor trafficking. Trends Neurosci. 34:258–268.

32. Seeburg PH, Hartner J (2003) Regulation of ion channel/neurotransmitter receptor function by RNA editing. Curr. Opinion in Neurobiology.

33. Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15(1):193–204.

34. Isa T, Itazawa S, Iino M, Tsuzuki K, Ozawa S (1996) Distribution of neurones expressing inwardly rectifying and Ca(2+)-permeable AMPA receptors in rat hippocampal slices. The Journal of Physiology 15,491(Pt 3):719-33.

35. Derkach V, Barria A, Soderling TR (1999) Ca2_/calmodulin-kinase II enhances channel conductance of alpha-amino-3- hydroxy-5- methyl-4-isoxazolepropionate type glutamate receptors. Proc. Natl. Acad. Sci. USA 96:3269–3274.

36. Banke TG, Bowie D, Lee H, Huganir RL, Schousboe A, Traynelis SF (2000) Control of GluR1 AMPA receptor function by cAMPdependent protein kinase. J. Neurosci. 20:89–102.

37. Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. Neuron 30,80(3), 704–717.

38. Hansen KB, Yi F, Perszyk RE, Furukawa H, et al. (2018) Structure, function, and allosteric modulation of NMDA receptors. J. Gen. Physiol. 6,150(8):1081-1105.

39. McBain CJ, Mayer ML (1994) N-Methyl-D-aspartic acid receptor structure and function. Physiol. Rev. 74:723–760.

40. Laube B, Hirai H, Sturgess M, Betz H, Kuhse J (1997) Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. Neuron 18:493–503.

41. Benveniste M, Mayer ML (1991) Kinetic analysis of antagonist action at N-methyl-D-aspartic acid receptors. Two binding sites each for glutamate and glycine. Biophys. J. 59:560–573.

42. Wolosker H (2007) NMDA receptor regulation by D-serine: new findings and perspectives. Mol. Neurobiol. 36:152–164.

43. Mothet JP, Le Bail M, Billard JM (2015) Time and space profiling of NMDA receptor co-agonist functions. J. Neurochem. 135:210–225.

44. Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465.

45. Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Ann. Rev. Phys. 64:355–405.

46. Katz B, Miledi R (1968) The role of calcium in neuromuscular facilitation. J. of Phys. 195:481-492.

47. Hebb DO (1949) Classic text pointing out the theoretical importance of 'LTP'-like synaptic modifications. The Organization of Behavior. John Wiley: New York.

48. Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. 23:649–711.

49. Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097.

50. Kauer JA, Malenka RC, Nicoll RA (1988) A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. Neuron 1:911–917.

51. Hvalby O, Lacaille JC, Hu GY, Andersen P (1987) Postsynaptic long-term potentiation follows coupling of dendritic glutamate application and synaptic activation. Experientia 43:599–601.

52. Tao HW, Poo M-M (2001) Retrograde signaling at central synapses. Proc. Natl. Acad. Sci. USA 25,98(20):11009–11015.

53. Zakharenko SS, Patterson SL, Dragatsis I, Zeitlin SO, Siegelbaum SA, Kandel ER, Morozov A (2003) Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1-CA3 synapses. Neuron 39(6):975–990.

54. Südhof TC (2001) The synaptic cleft and synaptic cell adhesion. Synapses, Johns Hopkins University Press, Baltimore, MD 275-313

55. Nicoll RA (2003) Expression mechanisms underlying long-term potentiation: a postsynaptic view. Philos. Trans. R. Soc. Lond. 358:721–726.

56. Isaac JT, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: implications for the expression of LTP. Neuron 15: 427–434.

57. Bredt DS, Nicoll RA (2003) AMPA receptor trafficking at excitatory synapses. Neuron 40:361–379.

58. Derkach VA, Oh MC, Guire ES, Soderling TR (2007) Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat. Rev. Neurosci. 8:101–113.

59. Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD (2004) Recycling endosomes supply AMPA receptors for LTP. Science 305:1972–1975.

60. Ehrlich I, Malinow R (2004) Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. J. Neurosci 24:916–927.

61. Stein V, House DR, Bredt DS, Nicoll RA (2003) Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. J. Neurosci. 23:5503–5506.

62. Ehrlich I, Klein M, Rumpel S, Malinow R (2007) PSD-95 is required for activity-driven synapse stabilization. Proc. Natl. Acad. Sci. USA 104:4176–4181.

63. Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CAMKII blocks induction but not expression of LTP. Science 245:862-866.

64. Barria A, Muller D, Derkach V, et al. (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CAM-KII during long-term potentiation. Science 276:2042-2045.

65. Soderling TR, Derkach VA (2000) Postsynaptic protein phosphorylation and LTP. Trends Neurosc. 23:75–80.

66. Lee H-K, Takamiya K, He K, Song L, Huganir RL (2010) Specific roles of AMPA receptor subunit GLUR1 (GLUA1) phosphorylation sites in regulating synaptic plasticity in the Ca1 region of hippocampus. J. Neurophysiol.103:479-489.

67. Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GLUR1 subunit. Neuron. 16:1179-1188.

68. Oh MC, Derkach VA, Guire ES, Soderling TR (2006) Extrasynaptic membrane trafficking regulated by GLUR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. J. Biol. Chem. 281:752-758.

69. Opazo P, Labrecque S, Tigaret CM, Frouin A, et al. (2010) CAMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron 67:239-252.

70. Correia SS, Bassani S, Brown TC, Lisé MF, et al. (2008) Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. Nat Neurosci.11:457-466.

71. Sutton MA, Schuman EM (2006) Dendritic protein synthesis, synaptic plasticity, and memory. Cell 127: 49–58.

72. Davis S, Vanhoutte P, Pages C, Caboche J, Laroche S (2000) The mapk/erk cascade targets both ELK-1 and CAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J Neurosci. 20:4563-4572.

73. Schafe GE, Swank MW, Rodrigues SM, Debiec J, Doyere V (2008) Phosphorylation of ERK/MAP kinase is required for long-term potentiation in anatomically restricted regions of the lateral amygdala in vivo. Learn. Mem.15:55-62.

74. Steward O, Schuman EM (2001) Protein synthesis at synaptic sites on dendrites. Annu Rev Neurosci 24: 299–325.

75. Richardson TL, Turner RW, Miller JJ (1987) Action-potential discharge in hippocampal CA1 pyramidal neuron: current sourcedensity analysis. J. Neurophysiol. 58:981–996.

76. Bechmann I (2005) Failed central nervous system regeneration: a downside of immune privilege? Neuromol Med. 7(3):217–28.

77. DiSabato DJ, Quan N, Godbout JP (2016) Neuroinflammation: the devil is in the details. J. Neurochem. 139(2):136-153.

78. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, et al. (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9:7204-7218.

79. Quan N, Banks WA (2007) Brain-immune communication pathways. B.B.I. 21(6):727-35.

80. Sokolova A, Hill MD, Rahimi F, Warden LA, Halliday GM, Shepherd CE (2009) Monocyte chemoattractant protein-1 plays a dominant role in the chronic inflammation observed in Alzheimer's disease. Brain Pathology 19:392–398.

81. Takeshita Y, Ransohoff RM (2012) Inflammatory cell trafficking across the blood-brain barrier: chemokine regulation and in vitro models. Immunological Reviews 248:228–239.

82. Benveniste EN (1992) Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. American Journal of Physiology 263(1):C1–C16.

83. Ridley SH, Sarsfield SJ, Lee JC, Bigg HF, Cawston TE, et al. (1997) Actions of IL-1 are selectively controlled by p38 mitogen-activated protein kinase: regulation of prostaglandin H synthase-2, metalloproteinases, and IL-6 at different levels. J. of Imm. 158:3165–3173.

84. Jung YD, Fan F, McConkey DJ, Jean ME, Liu W, et al. (2002) Role of P38 MAPK, AP-1,and NF-kappaB in interleukin-1beta-induced IL-8 expression in human vascular smooth muscle cells. Cytokine 18:206–213.

85. Eikelenboom P, Veerhuis R (1996) The role of complement and activated microglia inthe pathogenesis of Alzheimer's disease. Neurobiology of Aging 17:673–680.

86. Phillis JW, Horrocks LA, Farooqui AA (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. Brain Res. Rev. 52:201–243.

87. Hoozemans JJ, Rozemuller AJ, et al. (2001) Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. Acta Neuropathologic. 101:2–8.

88. Matousek SB, Hein AM, Shaftel SS, et al. (2010) Cyclooxygenase-1 mediates prostaglandin E(2) elevation and contextual memory impairment in a model of sustained hippocampal interleukin-1beta expression. Journal of Neurochemistry 114:247–258.

89. Schwab JM, Beschorner R, Meyermann R, Gozalan F, Schluesener HJ (2002) Persistent accumulation of cyclooxygenase-1-expressing microglial cells and macrophages and transient up regulation by endothelium in human brain injury. J. of Neurosurg. 96:892–899.

90. Aid S, Langenbach R, Bosetti F (2008) Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2. J. of Neuroinflamm. 5:17.

91. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA (1999) Inducible cyclooxygenase may have anti-inflammatory properties. Nat. Med. 5:698–701.

92. Cowley TR, Fahey B, O'Mara SM (2008) COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. The European Journal of Neuroscience 27:2999–3008.

93. Anderson GD, Hauser SD, McGarity KL, Bremer ME, et al. (1996) Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. The Journal of Clinical Investigation 97:2672–2679.

94. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, et al. (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845.

95. Russo MV, McGavern DB (2015) Immune Surveillance of the CNS following Infection and Injury. Trends Immunol. 36:637–650.

96. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 8:752–758.

97. Hoshino K, Takeuchi O, Kawai T, Sanjo H, et al. (1999) Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J. of Imm. 162:3749–3752.

98. Al Nimer F, Beyeen AD, Lindblom R, et al. (2011) Both MHC and non-MHC genes regulate inflammation and T-cell response after traumatic brain injury. B.B.I. 25:981–990.

99. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9:46–56.

100. Gendelman HE (2002) Neural immunity: friend or foe? J. of NeuroVirology 8(6):474–479.

101. Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behavior and Immunity 25(2):181–213.

102. Hauss-Wegrzyniak B, Lynch MA, Vraniak PD, Wenk GL (2002) Chronic brain inflammation results in cell loss in the entorhinal cortex and impaired LTP in perforant path-granule cell synapses. Exp. Neurol. 176:336–341.

103. Jacobsen JS, Wu CC, et al. (2006) Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 103:5161–5166.

104. Li ST, Matsushita M, Moriwaki A, Saheki Y, Lu YF, Tomizawa K, et al. (2004) HIV-1 Tat inhibits long-term potentiation and attenuates spatial learning. Ann. Neurol. 55:362–371.

105. O'Donnell E, Vereker E, Lynch MA (2000) Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: analysis of underlying mechanisms. Eur. J. Neu. 12:345–352.

106. MacEwan D J (2002) TNF receptor subtype signalling: differences and cellular consequences. Cellular Signalling 14 (6):477–492.

107. Santello M, Bezzi P, Volterra A (2011) TNFα controls glutamatergic gliotransmission in the hippocampal dentate gyrus. Neuron 69(5):988–1001.

108. Becker D, Deller T, Vlachos A (2015) Tumor necrosis factor (TNF)-receptor 1 and 2 mediate homeostatic synaptic plasticity of denervated mouse dentate granule cells. Sci. Rep. 5:12726.

109. Fiore M, Probert L, Kollias G, Akassoglou K, Alleva E, Aloe L (1996) Neurobehavioral alterations in developing transgenic mice expressing TNFalpha in the brain. Brain Behav. Immun. 10:126–138.

110. Liu Y, Zhou L-J, Wang J, Li D, Ren WJ, et al. (2017) TNF-α differentially regulates synaptic plasticity in the hippocampus and spinal cord by microglia-dependent mechanisms after peripheral nerve injury. J. of Neuroscience 37(4):871–881.

111. Ren W-J, Liu Y, Zhou L-J, Li W, Zhong Y, et al. (2011) Peripheral nerve injury leads to working memory deficits and dysfunction of the hippocampus by upregulation of TNF-α in rodents. Neuropsychophar. 36(5):979–992.

112. Golan H, Levav T, Mendelsohn A, Huleihel M (2004) Involvement of tumor necrosis factor alpha in hippocampal development and function. Cereb. Cortex 14:97–105.

113. Scherbel U, Raghupathi R, Nakamura M, Saatman KE, Trojanowski JQ, Neugebauer E, et al. (1999) Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. Proc. Natl. Acad. Sci. USA 96:8721–8726.

114. Yamada K, Iida R, Miyamoto Y, Saito K, Sekikawa K, Seishima M, Nabeshima T (2000) Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-alpha gene: implications for emotional behavior. J. Neuroimmunol. 111:131–138.

115. Moore AH, Wu M, Shaftel SS, Graham KA, O'Banion MK (2009) Sustained expression of interleukin-1beta in mouse hippocampus impairs spatial memory. Neurosc. 164:1484–1495.

116. Hein AM, Stasko MR, Matousek SB, Scott-McKean JJ, Maier SF, et al. (2010) Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice. Brain Behav. Immun. 24:243–253.

117. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, et al. (2003) Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J. of Neuroscience 23(25):8692–8700.

118. Han T, Qin Y, Mou C, Wang M, Jiang M, Liu B (2016) Seizure induced synaptic plasticity alteration in hippocampus is mediated by IL-1β receptor through PI3K/Akt pathway. American Journal of Translational Research 8(10):4499–4509.

119. Hein AM, O'Banion MK (2009) Neuroinflammation and memory: the role of prostaglandins. Mol. Neurobiol. 40:15–32.

120. Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P (1996) COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. Proc. Natl. Acad. Sci. USA 93:2317–2321.

121. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron 11:371–386.

122. Shaw KN, Commins S, O'Mara SM (2003) Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor. Eur. J. Neur. 17:2438–2446.

123. Kotilinek LA, Westerman MA, et al. (2008) Cyclooxygenase-2 inhibition improves amyloid-beta-mediated suppression of memory and synaptic plasticity. Brain 131:651–664.

124. Casolini P, Catalani A, Zuena AR, Angelucci L (2002) Inhibition of COX-2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat. J. Neurosci. Res. 68:337–343.

125. Shaw KN, Commins S, O'Mara SM (2005) Cyclooxygenase inhibition attenuates endotoxin-induced spatial learning deficits, but not an endotoxin-induced blockade of long-term potentiation. Brain Res. 1038:231–237.

126. Dhir A, Padi SS, Naidu PS, Kulkarni SK (2006) Protective effect of naproxen (non-selective COX-inhibitor) or rofecoxib (selective COX-2 inhibitor) on immobilization stress-induced behavioral and biochemical alterations in mice. Eur. J. Pharmacol. 535:192–198.

127. Andreasson KI, Savonenko A, Vidensky S, et al. (2001) Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. J. Neurosci. 21:8198–8209

128. Matsumoto Y, Yamaguchi T, Watanabe S, Yamamoto T (2004) Involvement of arachidonic acid cascade in working memory impairment induced by interleukin-1 beta. Neuropharmacology 46:1195–1200.

129. Zhu PM, Genc Ai, Zhang X, Zhang J, Bazan NG, Chen C (2005) Heterogeneous expression and regulation of hippocampal prostaglandin E2 receptors. J Neurosci Res 81:817–26.

130. Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structure, properties, and functions. Physiol Rev 79:1193–226.

131. Akaneya Y (2007) The Remarkable Mechanism of Prostaglandin E_2 on Synaptic Plasticity Gene. Regul. Syst. Bio. 1:83–89.

132. Sang N, Zhang J, Marcheselli V, et al. (2005) Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. J. Neurosci. 25:9858–70.

133. Barnes P, Thomas KL (2008) Proteolysis of proBDNF is a key regulator in the formation of memory. PLoS One 3:e3248.

134. Lu Y, Christian K, Lu B (2008) BDNF: a key regulator for protein synthesis dependent LTP and long-term memory? Neurobiol. Learn. Mem. 89:312–323.

135. Elkabes S, DiCicco-Bloom EM, Black IB (1996) Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. J. Neurosci. 16:2508–2521.

136. Mizoguchi Y, Monji A, Kato T, et al. (2009) Brain-derived neurotrophic factor induces sustained elevation of intracellular Ca2+ in rodent microglia. J. Immunol. 183:7778–7786.

137. Derecki NC, Cardani AN, Yang CH, Quinnies KM, et al. (2010) Regulation of learning and memory by meningeal immunity: a key role for IL-4. J. Exp. Med. 207:1067–1080.

138. Barrientos RM, Sprunger DB, Campeau S, Watkins LR, Rudy JW, Maier SF (2004) BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. J. Neuroimmunol. 155:119–126.

139. Shaw KN, Commins S, O'Mara SM (2001) Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus. Behav. Brain Res. 124:47–54.

140. World Health Organization, W.H.O. (2017) Depression: let's talk" says WHO, as depression tops list of causes of ill health.

141. Kessler RC, Berglund P, et al. (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). Jama 289:3095-3105.

142. Kessler RC, Angermeyer M, Anthony JC et al. (2007) Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. World Psychiatry 6(3):168–176.

143. American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: American Psychiatric Association.

144. Marquet RL, Bartelds AI, Kerkhof AJ, Schellevis FG, Van der Zee J (2005) The epidemiology of suicide and attempted suicide in Dutch General Practice 1983-2003. BMC family practice 6:45.

145. Gryglewski G, Lanzenberger R, Kranz GS, Cumming P (2014) Meta-analysis of molecular imaging of serotonin transporters in major depression. J. of Cerebral Blood Flow and Metabolism 34(7):1096–1103.

146. Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, et al. (2000) Brain serotonin1A receptor binding measured by positron emission tomography with [11C] WAY-100635: Effects of depression and antidepressant treatment. Arch. Gen. Psych. 57(2):174–180.

147. Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, et al. (2006) Elevated monoamine oxidase A levels in the brain: An explanation for the monoamine imbalance of major depression. Arch. General Psychiatry 63(11):1209–1216.

148. Ruhé HG, Mason NS, Schene AH (2007) Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: A metaanalysis of monoamine depletion studies. Mol. Psy. 12(4):331–359.

149. Croarkin PE, Levinson AJ, Daskalakis ZJ (2011) Evidence for GABAergic inhibitory deficits in major depressive disorder. Neurosc. and Biobehavioral Reviews 35(3):818–825.

150. Sanacora G (2010) Cortical inhibition, gamma-aminobutyric acid, and major depression: There is plenty of smoke but is there fire? Biological Psychiatry 67(5):397–398.

151. Sanacora G, Zarate CA, Krystal JH, Manji HK (2008) Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nature Reviews Drug Discovery 7(5):426–437.

152. Yüksel C, Öngür D (2010) Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. Biological Psychiatry 68(9):785–794.

153. Kishimoto T, Chawla JM, Hagi K, et al. (2016) Single-dose infusion ketamine and non-ketamine N-methyl-D-aspartate receptor antagonists for unipolar and bipolar depression: A meta-analysis of efficacy, safety and time trajectories. Psychol. Med. 46(7):1459–1472.

154. Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev. 64:238–258.

155. Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50:260–265.

156. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R (2005) Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res. Mol. Brain Res. 136:29–37.

157. Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S (2005) Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. Eur Arch Psychiatry Clin. Neur. 255:381–386.

158. Groves JO (2007) Is it time to reassess the BDNF hypothesis of depression? Mol. Psych. 12:1079–1088.

159. Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, Parada LF, Nestler EJ (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol. Psychiatry 61:187-197.

160. Zorner B, Wolfer DP, Brandis D, Kretz O, et al. (2003) Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than “depressive”. Biol. Psych. 54:972-982.

161. Saarelainen T, Hendolin P, Lucas G, Koponen E, et al. (2003) Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J. Neurosci, 23:349-357.

162. Castren E, Voikar V, Rantamaki T (2007) Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7:18–21.

163. Holmans P, Weissman MM, Zubenko GS, et al. (2007) Genetics of recurrent early-onset major depression (GenRED): final genome scan report. Am. J. Psychiatry 164:248- 58.

164. Pezawas L, Meyer-Lindenberg A et al. (2005) 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat. Neurosci. 8:828-34.

165. Caspi A, Sugden K, Moffitt TE, Taylor A et al. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386—389.

166. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat. Neurosci. 7:847-54.

167. Frielingsdorf H, Bath KG, Soliman F, Difede J, Casey BJ, Lee FS (2010) Variant brain-derived neurotrophic factor val66met endophenotypes: implications for posttraumatic stress disorder. Ann. N Y Acad. Sci. 1208:150–157.

168. Ribeiro L, Busnello JV, Cantor RM, Whelan F, Whittaker P, Deloukas P, Wong ML, Licinio J (2007) The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and depression in Mexican-Americans. Neuroreport 6(18):1291–1293.

169. Verhagen M, Van der Meij A, Van Deurzen PA, Janzing JG, Arias-Vasquez A, Buitelaar JK, Franke B (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol. Psychiatry 15:260–271.

170. Ripke S, Wray NR , Lewis CM , Hamilton SP , et al. (2013) A mega-analysis of genome-wide association studies for major depressive disorder. Mol. Psychiatry 18:497-511.

171. Converge consortium (2015) Sparse whole-genome sequencing identifies two loci for major depressive disorder. Nature 523(7562):588–91.

172. Raison CL, Miller AH (2011) Is depression an inflammatory disorder? Curr. Psych. Rep. 13(6):467-475.

173. Kiecolt-Glaser JK, Derry HM, Fagundes CP (2015) Inflammation: Depression Fans the Flames and Feasts on the Heat. Am. J. Psych. 172(11):1075-1091.

174. Haroon E, Raison CL, Miller AH (2012) Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychophar. 37(1):137-162.

175. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010) A meta- analysis of cytokines in major depression. Biol. Psychiatry 67(5):446-457.

176. Miller AH, Maletic V, Raison CL (2009) Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65(9):732-741.

177. Kohler O, Benros ME, Nordentoft M, et al. (2014) Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. JAMA Psychiatry. 71:1381–1391.

178. Allison DJ, Ditor DS (2014) The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. J. Neuroinflammation 11(1):151.

179. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B (2007) Kynurenine pathway in major depression: evidence of impaired neuroprotection. J. Affect. Disord. 98:143–151.

180. Lin,YL, Wang S (2014) Prenatal lipopolysaccharide exposure increases depression-like behaviors and reduces hippocampal neurogenesis in adult rats. Behav. Brain. Res. 1(259):24–34.

181. Gullander M, Hogh A, Hansen ÅM, Persson R, Rugulies R, et al. (2014) Exposure to work place bullying and risk of depression. J. Occup. Environ. Med. 56(12):1258–65.

182. Eaton WW (1978) Life events, social supports, and psychiatric symptoms: a re-analysis of the New Haven data. J. Health Soc. Behav. 19:230—234.

183. Hornung OP, Heim CM (2014) Gene-environment interactions and intermediate phenotypes: early trauma and depression. Front. Endocrinol. 5:14.

184. Pariante CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31(9):464-468.

185. Burke HM, Davis MC, Otte C, Mohr DC (2005) Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 30:846-56.

186. MacMaster FP, Russell A, Mirza Y, et al. (2006) Pituitary volume in treatment-naïve pediatric major depressive disorder. Biol. Psychiatry 60:862-6.

187. Modell S, Yassouridis A, Huber J, Holsboer F (1997) Corticosteroid receptor function is decreased in depressed patients. Neuroendocrinology 65:216–22.

188. MacQueen GM, Campbell S, et al. (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. Proc. Natl. Acad. Sci. USA 100:1387-92.

189. Rajkowska G (2000) Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. Biol. Psychiatry 48:766-77.

190. Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H (2007) Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int. J. Neuropsychopharmacol. 10:741-58.

191. Branchi I, Alboni S, Maggi L (2014) The role of microglia in mediating the effect of the environment in brain plasticity and behavior. Front. Cell. Neurosci. 8:390.

192. Muller N (2014) Immunology of major depression. Neuroimmunomodulation 21:123–130.

193. Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A (2011) The role of microglia in the healthy brain. J. Neurosci. 31:16064–16069.

194. Beumer W, Gibney SM, Drexhage RC, et al. (2012) The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. J. Leukoc. Biol. 92:959–975.

195. Yirmiya R, Rimmerman N, Reshef R. (2015) Depression as a microglial disease. Trends Neurosci. 38(10):637-658.

196. Kreisel, T. et al. (2014) Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neuro-genesis. Mol. Psychiatry 19, 699–709

197. Alboni S, Benatti C, Montanari C, Tascedda F, Brunello N (2013) Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus. Eur. J. Pharmacol. 721:158–167.

198. Whooley MA, Simon GE (2000) Managing depression in medical outpatients. The New England journal of medicine 343:1942-1950.

199. Nestler EJ, Terwilliger RZ, Duman RS (1989) Chronic antidepressant administration alters the subcellular distribution of cAMP-dependent protein kinase in rat frontal cortex. J. Neuro. 53:1644–1647.

200. Thome J, Sakai N, Shin K et al. (2000) cAMP response elementmediated gene transcription is upregulated by chronic antidepressant treatment. J. Neurosci. 20:4030–4036.

201. Chen AC, Shirayama Y, Shin K, Neve RL, Duman RS (2001) Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect. Biol. Psych. 49:753–762.

202. Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33:88–109.

203. MacQueen G, Frodl T (2011) The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol. Psychiatry 16:252-264.

204. Sanacora G, Treccani G, Popoli M (2012) Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuroph. 62:63–77.

205. Du J, Machado-Vieira R, et al. (2006) Enhancing AMPA to NMDA throughput as a convergent mechanism for antidepressant action. Drug Discov. Tod. Ther. Strat. 3:519–526.

206. Monteggia LM, Gideons E, Kavalali ET (2013) The role of eukaryotic elongation factor 2 kinase in rapid antidepressant action of ketamine. Biol. Psychiatry 73:1199–1203.

207. Martinez-Turrillas R, Frechilla D, Del Río J (2002) Chronic antidepressant treatment increases the membrane expression of AMPA receptors in rat hippocampus. Neuropharmacology 43:1230–1237.

208. Yoshii A, Constantine-Paton M (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev. Neurobiol. 70:304–322.

209. Popoli M, Gennarelli M, Racagni G (2002) Modulation of synaptic plasticity by stress and antidepressants. Bipolar Disord. 4:166–182.

210. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809.

211. Hiemke C, Hartter S, (2000) Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacology & therapeutics 85:11-28.

212. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. The American journal of psychiatry 163:28-40.

213. Fava GA (2002) Long-term treatment with antidepressant drugs: the spectacular achievements of propaganda. Psychotherapy and psychosomatics 71:127-132.

214. Branchi I (2011) The double edged sword of neural plasticity: Increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover. Psychoneuroendocrin. 36:339-351.

215. Branchi I, Santarelli S, Capoccia S, Poggini S, D'Andrea I, Cirulli F, Alleva E (2013) Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice. PLoS One 8:e62226.

216. Alboni S, van Dijk RM, Poggini S, et al. (2017) Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol Psych. 22(4):552-561.

217. Lesch KP, Waider J (2012) Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. Neuron 76:175-191.

218. Larson ET, Summers CH (2001) Serotonin reverses dominant social status. Behavioural Brain Research 121(1-2):95–102.

219. Mattson MP, Maudsley S, Martin B (2004) BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends in Neurosc. 27(10):589-94.

220. Zetterström TSC, Pei Q, Madhav TR, Coppell AL, et al. (1999) Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain. Neuropharmacol. 38(7):1063–1073.

221. Mössner R, Daniel S, Albert D, Heils A, et al. (2000) Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). Neurochem. Int. 36:197–202.

222. Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, et al. (2008) The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320(5874): 385–388.

223. World Health Organization, W.H.O (2014) "About diabetes".

224. Payne ME, Steck SE, George RR, Steffens DC (2012) Fruit, vegetable, and antioxidant intakes are lower in older adults with depression. J. Acad. Nutr. Diet. 112:2022–7.

225. Weyerer S (1992) Physical inactivity and depression in the community. Evidence from the Upper Bavarian Field Study. Int. J. Sports Med. 13:492–6.

226. Gonzalez JS, Safren SA, Cagliero E, Wexler DJ, Delahanty L, Wittenberg E, et al. (2007) Depression, self-care, and medication adherence in type 2 diabetes: relationships across the full range of symptom severity. Diabetes Care 30:2222–7.

227. Ho N, Sommers MS, Lucki I (2013) Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. Neurosci. Biobehav. Rev. 37:1346–62.

228. Champaneri S, Wand GS, Malhotra SS, Casagrande SS, Golden SH (2010) Biological basis of depression in adults with diabetes. Curr. Diab. Rep. 10:396–405.

229. Holt RI, De Groot M, Golden SH (2014) Diabetes and Depression. C. Diab. Rep. 14(6):491.

230. Miller AH, Raison CL (2016) The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature reviews. Immunology 16:22-34.

231. Basterzi AD, Aydemir C, Kisa C, Aksaray S, et al. (2005) IL-6 levels decrease with SSRI treatment in patients with major depression. Hum. Psychopharm. 20:473–476.

232. Lanquillon S, Krieg JC, Bening-Abu-Shach U, Vedder H (2000) Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology 22:370–379.

233. Hannestad J, Della Gioia N, Bloch M (2011) The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a metaanalysis. Neuropsychopharm. 36:2452–2459.

234. Song C, Halbreich U, Han C, Leonard BE, Luo H (2009) Imbalance between pro- and anti-inflammatory cytokines, and between Th1 and Th2 cytokines in depressed patients: the effect of electroacupuncture or fluoxetine treatment. Pharmacopsychiatry 42:182–188.

235. Akhondzadeh S, Jafari S, et al. (2009) Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. Depr. Anx. 26:607–611.

236. Brunello N, Alboni S, Capone G, et al. (2006) Acetylsalicylic acid accelerates the antidepressant effect of fluoxetine in the chronic escape deficit model of depression. Int. Clin. Psychopharm. 21:219–225.

237. Alboni S, Poggini S, Garofalo S, Milior G, et al. (2016) Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment Brain, Behavior, and Immunity 58:261–271.

238. Corpeleijn E, Feskens EJ, Jansen EH, et al. (2007) Lifestyle intervention and adipokine levels in subjects at high risk for type 2 diabetes: the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM). Diabetes care 30:3125-3127.

239. Dougherty KA, Islam T, Johnston D (2012) Intrinsic excitability of CA1 pyramidal neurones from the rat dorsal and ventral hippocampus. J. of physiology 590:5707–5722.

240. Malik R, et al. (2015) Mapping the electrophysiological and morphological properties of CA1 pyramidal neurons along the longitudinal hippocampal axis. Hippocampus.

241. Maruki K, Izaki Y, Nomura M, Yamauchi T (2001) Differences in paired-pulse facilitation and long-term potentiation between dorsal and ventral CA1 regions in anesthetized rats. Hippocampus 11:655–661.

242. Maggio N, Segal M (2007) Unique regulation of long term potentiation in the rat ventral hippocampus. Hippocampus 17:10–25.

243. Papatheodoropoulos C, Kostopoulos G (2000a) Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. Neurosc. Lett. 279:177–180.

244. Papatheodoropoulos C, Kostopoulos G (2000b) Dorsal-ventral differentiation of short-term synaptic plasticity in rat CA1 hippocampal region. Neurosc. Lett. 286:57–60.

245. Paolicelli RC, Bolasco G, Pagani F, Maggi L, et al. (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456–1458.

246. Tremblay ME, Lowery RL, Majewska AK (2010a) Microglial interactions with synapses are modulated by visual experience. PLoS Biol. 8:e1000527.

247. Maggi L, Scianni M, et al. (2011) CX(3)CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. Front. Cell. Neur. 5:22.

248. Ragozzino D, et al. (2006) Chemokine fractalkine/CX3CL1 negatively modulates active glutamatergic synapses in rat hippocampal neurons. J. Neurosc. 26:10488–10498.

249. Bertollini C, Ragozzino D, et al. (2006) Fractalkine/CX3CL1 depresses central synaptic transmission in mouse hippocampal slices. Neuropharmacology 51:816–821.

250. Ghadernezhad N, Khalaj L, Pazoki-Toroudi H, Mirmasoumi M, Ashabi G (2016) Metformin pretreatment enhanced learning and memory in cerebral forebrain ischaemia: the role of the AMPK/BDNF/P70SK signalling pathway. Pharmaceut. Biol. 54(10):2211–2219.

251. Kohler O, Petersen L, Mors O, Gasse C (2015) Inflammation and depression: combined use of selective serotonin reuptake inhibitors and NSAIDs or paracetamol and psychiatric outcomes. Brain and behavior 5:e00338.

252. Kopschina Feltes P, Doorduin J, et al. (2017) Anti-inflammatory treatment for major depressive disorder: implications for patients with an elevated immune profile and non-responders to standard antidepressant therapy. J. of psychopharmacology 31:1149-1165.

253. Luo YW, Xu Y, et al. (2015) Insulin-like growth factor 2 mitigates depressive behavior in a rat model of chronic stress. Neuropharmacology 89:318–324.

254. McLaughlin RJ, Hill MN, Morrish AC, Gorzalka BB (2007) Local enhancement of cannabinoid CB1 receptor signalling in the dorsal hippocampus elicits an antidepressantlike effect. Behavioural Pharmacology 18(5-6):431–438.

255. Padovan CM, Guimaraes FS (2004) Antidepressant-like effects of NMDA-receptor antagonist injected into the dorsal hippocampus of rats. Pharmacology, Biochemistry, and Beh. 77(1):15–19.

256. Rodriguez-Landa JF, Contreras CM, Garcia-Rios RI (2009) Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor. Beh. Phar. 20:614–622.

257. Liu R, Dang W, Jianting M et al. (2012) Citalopram alleviates chronic stress induced depression-like behaviors in rats by activating GSK3β signaling in dorsal hippocampus. Brain Research 1467:10–17.

258. Liu KC, Li JY, Tan HH et al. (2015) Serotonin6 receptors in the dorsal hippocampus regulate depressive-like behaviors in unilateral 6-hydroxydopamine-lesioned Parkinson's rats. Neuropharmacology 95:290–298.

259. Mathews DC, Zarate Jr CA (2013) Current status of ketamine and related compounds for depression. The Journal of Clinical Psychiatry 74(5):516-517.

260. Grieco SF, Cheng Y, Eldar-Finkelman H, Jope RS, Beurel E (2017) Up-regulation of insulin-like growth factor 2 by ketamine requires glycogen synthase kinase-3 inhibition. Progress in Neuro-Psychopharmac. & Biolog. Psych. 72:49–54.

261. Castren E, Kojima M (2017) Brain-derived neurotrophic factor in mood disorders and antidepressant treatments.Neurobiology of Disease 97 B:119–126.

262. Ferrini F, De Koninck Y (2013) Microglia control neuronal network excitability via BDNF signalling. Neural Plast. 2013:429815.

263. Crozier RA, Bi C, Han YR, Plummer MR (2008) BDNF modulation of NMDA receptors is activity dependent. J Neurophysiol.;100:3264–3274.

264. Liu H, Leak RK, Hu X (2016) Neurotransmitter receptors on microglia. Trends in. Neuroscience, 1(2), 52–58.