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FGFR1-5HT1AR heteroreceptor complexes differently modulate GIRK currents in the hippocampus and the raphe nucleus of control rats and of a genetic rat model of depression

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ABSTRACT

The midbrain raphe serotonin neurons provide the main ascending serotonergic projection to the forebrain, including hippocampus that has a recognized role in the pathophysiology of depressive disorder. The activation of G protein-coupled inwardly-rectifying potassium (GIRK) channels by serotonin 5HT1A receptors at the soma-dendritic level of serotonergic raphe neurons and glutamatergic hippocampal pyramidal neurons reduces neuronal activity. The presence of FGFR1-5HT1A heteroreceptor complexes in this raphe-hippocampal serotonin neuron system has been demonstrated, but functional receptor-receptor interactions in the heterocomplexes have only been studied in CA1 pyramidal neurons of control Sprague Dawley (SD) rats. In the present research, the short-term effects of FGFR1-5HT1A complex activation were studied in hippocampal pyramidal neurons, both in CA1 and CA2 areas, and midbrain dorsal raphe serotonergic neurons of SD rats and a genetic rat model of depression, the Flinders sensitive line (FSL) rats selected from SD strain, using an electrophysiological technique. The results obtained demonstrate that FGFR1-5HT1A heteroreceptor activation by specific agonists reduced the ability of the 5HT1AR protomer to open the GIRK channels via the allosteric inhibitory interplay produced by agonist activation of the FGFR1 protomer, resulting in increased neuronal firing in the raphe-hippocampal 5HT system of SD rats. In contrast, apart from CA2 neurons, the inhibitory allosteric effects of FGFR1 agonist on the 5HT1AR protomer were unable to have this influence on GIRK channels in FSL rats. According to these data, 5HT1AR activation impaired hippocampal plasticity in both SD and FSL rats, as determined by long-term potentiation induction capability in the CA1 field, but not in SD rats following simultaneous FGFR1-5HT1A heterocomplex activation. While, due to the impairment in heterocomplex activation, long-term potentiation was precluded in FSL rats. It is thus hypothesized that in the genetic FSL model of depression, there is a considerable decrease of the allosteric inhibition mediated by the FGFR1 protomer on the 5HT1AR protomer, resulting in a reduced opening of the GIRK channels in the raphe-hippocampal serotonin pathway. The consequent increase in inhibition in dorsal raphe 5HT nerve cell and glutamatergic hippocampal CA1 pyramidal nerve cell firing may contribute to the onset of major depression.

ABBREVIATION LIST

Dorsal raphe nucleus (DRN)
Dentate gyrus (DG)
Serotonin (5HT)
Serotonin reuptake transporter (SERT)
G protein-coupled receptors (GPCRs)
Adenylyl cyclase (AC)
Phospholipase-C (PLC)
Inositol 1,4,5-triphosphate (IP3)
Diacylglycerols (DAGs)
Protein kinase A (PKA)
GIRKs (G protein coupled inward rectifier K channels)
Phosphorylated ERK1/2 (pERK)
Nuclear factor-light chain-enhancer of activated B cell (NF-κB)
Calmodulin (CaM)
CaM-dependent protein kinase II (CaMKII)
Fibroblast growth factor receptor 1 (FGFR1)
Sprague Dawley (SD)
Flinders sensitive line (FSL)
Forced swim test (FST)
Serotonin reuptake inhibitors (SSRIs)
Brain derived neurotrophic factor (BDNF)
Vascular endothelial growth factor (VEGF)
Granule cells (GCs)
Neuronal progenitor cells (NPCs)
Long-term potentiation (LTP)
Long-term depression (LTD)
N-methyl-D-aspartate receptors (NMDA)
Major depression disease (MDD)
Prefrontal cortex (PFC)
Hippocampus (HPC)
(2R, 6R)-Hydroxynorketamine (HNK)
Subventricular zone (SVZ)
Subgranular zone (SGZ)
Muscarinic acetylcholine receptors (mAChRs)
Bioluminescence-Resonance Energy Transfer (BRET)
Fluorescence-Resonance Energy Transfer (FRET)
Genome-wide association studies (GWAS)
Diisopropylfluorophosphate (DFP)
Field excitatory postsynaptic potentials (fEPSPs)

INTRODUCTION

The serotonin (5HT) system regulates several brain processes, including mood, cognition, and emotions [1, 2], and its dysregulation has been linked to the neuropathology of various mental diseases, including depression and anxiety [2]. The raphe nuclei of the lower brainstem are the principal sources of 5HT in the brain [3]. Indeed, serotonergic neurons are found in these areas of the midbrain, where they send ascending projections to the telencephalon and diencephalon, where they release serotonin from large numbers of 5HT nerve terminals to activate high-affinity synaptic and extra-synaptic receptors and extracellular channels through volume transmission [4, 5]. The hippocampus is one of several brain areas that receives intense serotonergic innervation from both the median and dorsal raphe nuclei [6]. Serotonin influences hippocampal activities in this area by activating various 5HT receptor subtypes expressed in excitatory and inhibitory neurons, as well as glial cells.

At least 15 primary 5HT receptor subtypes have been found [7] which are typically connected to G protein, with the exception of 5HT3 being coupled to ion channels. Among these serotonin receptors, the 5HT1A has been widely studied for its function in mood, anxiety, and cognitive regulation [8]. 5HT1A receptors are divided into two categories based on where they are located: 5HT1A autoreceptors, which are found on the soma and dendrites of serotonergic neurons in the midbrain raphe nuclei, and 5HT1A post-junctional receptors, which are found on non-5HT neurons such as glutamatergic hippocampal pyramidal neurons and astrocytes [9]. The activation of 5HT1A autoreceptors leads to a reduction in the firing rate of serotonergic neurons [10], resulting in a decrease in activity-dependent serotonin release [11]. The activation of the 5HT1A post-junctional receptor on pyramidal neurons reduces neuronal firing activity [12]. The inhibitory effect of 5HT1A receptors on the activity of both serotonergic nerve cells and hippocampal pyramidal neurons has been demonstrated to be due to the activation of G protein-coupled inwardly-rectifying potassium channels (GIRK), which deeply hyperpolarize neurons by increasing K^+ conductance [13-15].

It should be mentioned that 5HT1A receptors may create a significant number of heterocomplexes in the plasma membrane with other receptors [16]. Borroto-Escuela and colleagues demonstrated the presence of FGFR1-5HT1A heteroreceptor complexes in the dorsal hippocampus, as well as the dorsal raphe and median raphe of the midbrain of

Sprague Dawley (SD) control rats, using an *in situ* Proximity Ligation Assay [17, 18]. This heteroreceptor complex, in particular, has been demonstrated to be involved in neuroplasticity in the rat hippocampus and midbrain raphe serotonergic neurons, mostly via allosteric 5HT1A protomer increase of FGFR1 protomer signaling, resulting to antidepressant-like effects [18]. Borroto-Escuela and colleagues discovered that intracerebroventricular infusions of FGF-2 with the 5HT1A agonist 8-OH-DPAT resulted in an enhanced antidepressant effect, as measured by the forced swim test (FST), when compared to 5HT1A agonist alone. It showed that the co-treatment had a larger and perhaps faster antidepressant effect than selective serotonin reuptake inhibitors (SSRIs), which particularly boost extracellular 5HT levels and are widely used to treat mood disorders pharmacologically [19]. The hypothesized mechanism for the antidepressant-like effects produced by co-activating the two protomers of the FGFR1-5HT1A heterocomplex involves the uncoupling of the 5HT1A Gi/o driven opening of the GIRK channels. It is most likely the result of an antagonistic allosteric interaction in the FGFR1-5HT1A heterocomplex, in which the agonist-activated FGFR1 protomer promotes a conformational shift in the 5HT1A protomer, limiting its capacity to open GIRK channels. In keeping with these findings, our previous research found that activating FGFR1 with a specific agonist significantly diminish the opening of GIRK channels produced by the 5HT1A receptor in pyramidal neurons of the CA1 area of the dorsal hippocampus in SD rats [19]. Thus, the allosteric interaction between 5HT1A receptor and FGFR1 protomer would prevent 5HT1A receptor activation from hyperpolarizing glutamatergic pyramidal neurons. This may explain the possible faster antidepressant benefits of co-activation of the two receptor protomers. However, the i.c.v. combined treatment with FGF2 and 8-OH-DPAT over a 48-hour period in a genetic rat model of depression, Flinders sensitive line (FSL) rats selected from SD strain, failed to produce any antidepressant effects in FST, indicating that the synergistic effect of the co-treatment was missing in FSL rats [19].

AIM OF THE STUDY

Based on the findings described above, and considering the impact of the allosteric receptor protomer interplay in possibly developing new more rapidly acting antidepressant drugs targeting these heteroreceptor complexes, through an electrophysiological approach, the current thesis work aimed to verify the allosteric interaction between the 5HT1A serotonin receptor and the FGFR1 receptor at the plasma membrane of neurons in the dorsal raphe nucleus and the hippocampal CA1 and CA2 areas of control rats and the animal genetic model of depression known as the Flinders sensitive line rat. Because 5HT1A receptors and GIRK potassium channels have a functional link, it was possible to investigate how the 5HT1A-FGFR1 heteroreceptor activation influences the hyperpolarization mechanism that governs the activity of serotonergic raphe neurons and hippocampal glutamatergic neurons. The electrophysiological technique of patch clamp, both in loose patch and in whole cell configuration, was used to examine first the contribution of heteroreceptor activation in the autonomous firing activity of serotonergic neurons followed by the GIRK-mediated membrane currents of both serotonergic neurons and hippocampal neurons. These experiments allowed us to unveil how heteroreceptor complex stimulation by specific agonists affected the GIRK current in both depressed (FSL) and control (SD) rats. Furthermore, as it has been found that the heteroreceptor plays a critical function in controlling membrane potential and neuronal activity, we investigated the involvement of heteroreceptors in the induction of long-term synaptic potentiation in CA1 of both control and depressed rats.

CHAPTER 1: ROLE OF SEROTONIN IN HEALTH AND DISEASE

1.1 Morpho-functional organization of raphe nuclei

The raphe nuclei are an aggregation of neuronal bodies, with poorly defined cyto-architectural limits, originally named and divided into nuclei from B1 to B9. The organization of this neuronal population foresees the presence of cell bodies grouped around the midline of the brain stem along its entire rostrum-caudal extension, both in animals [20, 21] and in humans [22], with two types of terminations: axons with large varicosities or small varicosities. Through diffuse projections, involving different brain areas, the raphe neurons use various neurotransmitters to control numerous physiological functions.

The serotonergic neurons that compose the majority of the raphe nuclei are heterogeneous populations that are found throughout the brain stem, but we can distinguish two major groups based on their distribution and main projections: the rostral group (nuclei B6-B9), which is close to the upper part of the brain stem and borders the midbrain and rostral pons, and the caudal group (nuclei B1-B5), which is close to the lower region of the brain stem, extending from the caudal pons to the caudal portion of the medulla oblongata (Fig.1).

Numerous nuclei with parallel and complementary projections are present in each group. The caudal group includes many nuclei: the raphe magnus, the dark nucleus, and the pale nucleus, with greater projections to the caudal brainstem and the spinal cord. On the other hand, the rostral group, that accounts around 85% of total serotonergic neurons in the brain, includes the linear caudal nucleus, the nucleus of the dorsal raphe and the median raphe nucleus, with greater projections to the caudal brainstem.

Several immunohistochemical studies have revealed that raphe nuclei include not only the cell bodies and dendrites of serotonergic neurons, but also a dense network of serotonergic fibers that, as evidenced by the presence of a specific enzyme, the tryptophan hydroxylase, can produce serotonin [23].

The dorsal raphe nucleus (DRN), a heterogeneous and bilateral brainstem nucleus, comprises the majority of serotonin-expressing neurons in the brain. These serotonergic neurons are located throughout the dorsal nucleus, including the dorsal, ventral, interfascicular, and ventrolateral subdivisions [24].

Less than half of DRN neurons is serotonergic, with the others releasing neurotransmitters such as GABA, dopamine, glutamate, nitric oxide, and a multitude of peptides [25],

resulting in clusters of serotonergic and non-serotonergic neurons. In addition to serotonin, other neurotransmitters and neuropeptides contribute to the connection and control of its serotonergic neurons [26].

The DRN is of enormous interest because it regulates several behavioral and physiological processes such as anxiety, depression, sleep, memory, and reward via its extensive network of connections and projections [27]. As a result, this nucleus is frequently connected with brain dysfunctions, including diseases such as Alzheimer's disease and depression.

1.2 Projections of the dorsal nucleus of the raphe

The dorsal raphe nucleus projections are crucial in modulating physiological functions in various areas of the brain. The axons of the DRN neurons, in fact, contribute numerically to the majority of serotonergic innervation and are characterized by small varicosities, which do not form authentic chemical synapses, but contribute to the communication system known as volume transmission [28-30]. Serotonergic neurons in the rostral region of the DRN, than those in the more caudally, project into the rostral areas of the brain [31]. The majority of the ascending and descending circuits used by the neurons in the dorsal raphe nucleus are also used by other regions of the raphe. The medial, the dorsal, and the ventral path are the three main ascending connection to the other brain region.

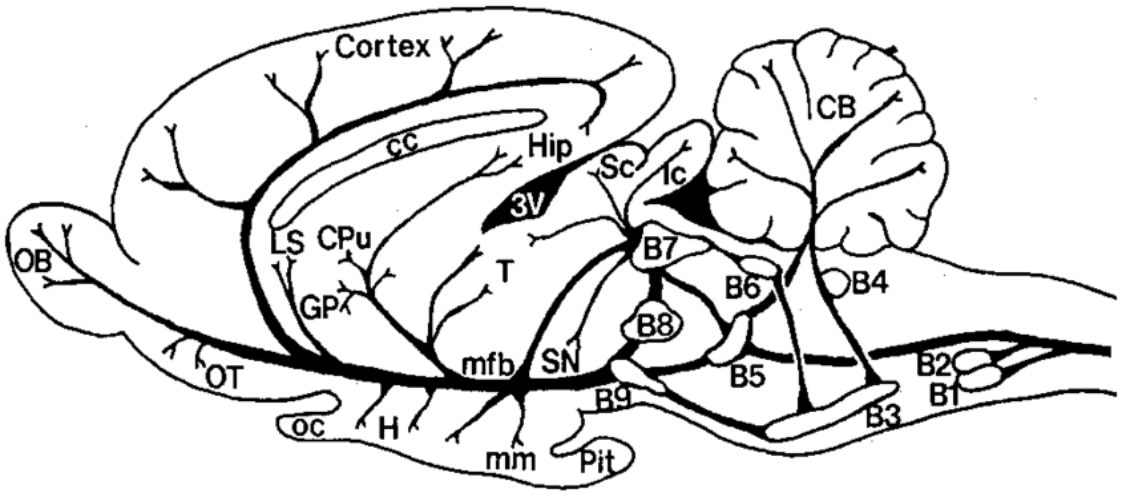

Figure 1 Schematic drawing of the location of the serotonergic nuclei (B1-B9) and their major projections in the brain. B7: dorsal raphe nucleus, B8: median raphe nucleus [32].

The dorsal and ventral pathway are the most significant efferent projections for the DRN since they can connect to various forebrain targets. The five descending paths that emerge

from the DRN are the bulbospinal pathway, the cerebellar pathway, the propriobulbar pathway, and the dorsal integumental nucleus, a tract that innervates the locus coeruleus, and the nucleus of the pontine raphe. Despite the fact that the individual neurons project onto separate destinations, these targets are really functionally related to one another by branching fibers [33].

The hippocampus, a crucial component of the limbic system found in the temporal lobe of each cerebral cortex, is reached by one of the ventral ascending projections of the dorsal nucleus of the raphe, in particular the majority of the neurons that reach the hippocampus dwell in the caudal regions of the nucleus, close to the midline [34, 35].

In the hippocampus, immunohistochemical staining revealed the presence of thin serotonergic axons, whose morphology suggests that they originate from the dorsal nucleus [36]. Consequently, serotoninergic projections from the raphe nuclei, which constitute the raphe-hippocampal pathway, modulate the hippocampus. Particularly, the serotonin released by the diffuse networks of serotonergic nerve terminals arising from the dorsal and median raphe nuclei modifies the hippocampus internal circuits by the 5HT1A receptor subtype activation [3, 37, 38].

As a result, different abnormalities of the physiological activities controlled by the dorsal raphe nucleus, the hippocampus, and other projection sites of this nucleus are implicated in dysfunctions at the level of the serotonergic system.

1.3 Hippocampal connectivity

The so-called "tri-synaptic" ring, the traditional literary representation of the hippocampal anatomical connectivity, is seen in a cross section of its sagittal axis (Fig.2). The dentate gyrus (DG) region receives the strongest projections from the entorhinal cortex, which has the largest cortical input to the hippocampus. Through the mossy fibers path, the DG area projects to the CA3 region and the CA3 region projects to the CA1 region via the Schaffer collaterals path. In order to complete the cycle, CA1 reaches back toward the entorhinal cortex.

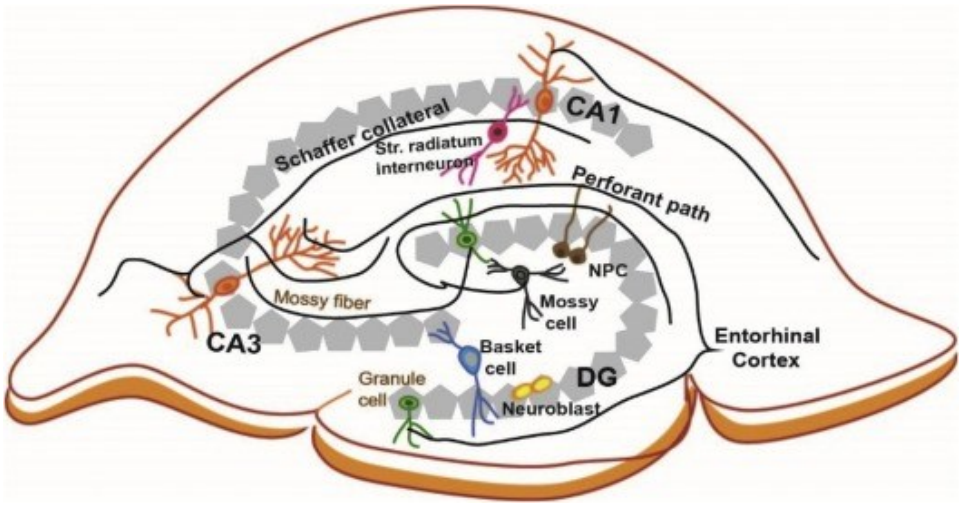

Figure 2 Hippocampal neural circuit diagrams [39].

The observation that CA3 axons also project into CA1 and transmit collaterals that form synapses on adjacent CA3 neurons is an essential addition to the traditional tri-synaptic circuitry. Many significant theories on CA3 have been influenced by this recurrent collateral way, including the self-associative memory system, which exhibits appealing dynamics essential to maintaining dispersed memory.

The hippocampus also receives direct inputs from the perirhinal and postrhinal cortex in addition to its primary inputs from the entorhinal cortex. Further, it receives significant subcortical inputs from the amygdala, locus coeruleus, raphe, nucleus reuniens, and medial septum, justifying the hippocampus strong theta rhythm. Likewise, CA1 sends signals to the amygdala, prefrontal cortex, and nucleus accumbens [40].

It is worth noting that the raphe nuclei send fibers to the interface of the radiatum layer and to the lacunosum-molecular in the region of CA1, sending a dense serotonergic input to the hippocampus, a brain region of mammals necessary for the development of declarative memory. The perforating pathway, which connects CA1 directly to the entorhinal cortex, as well as the Schaffer collaterals, which connect CA3 to CA1, can both be modulated by serotonin release at this innervation site. Direct investigation has proven that the serotonergic neurons in the raphe can be stimulated by local optogenetic activation of the serotonergic axons in the hippocampus [41].

The dorsal nucleus of the raphe also sends axons straight to the hippocampus. As previously stated, the DRN efferences in the hippocampus originate from both serotonergic and non-serotonergic sources, and they primarily come from the more caudal regions of the nucleus, close to the midline. In fact, the serotonergic axons that reach the hippocampus contain

minor varicosities and are quite thin, according to the immunohistochemistry stains. Injury experiments have demonstrated that the dorsal nucleus is not the primary source of hippocampus serotonergic innervation, but rather the median raphe nucleus [42].

1.4 Serotonin and serotonergic transmission in the dorsal raphe nucleus and hippocampus

Tryptophan hydroxylase and L-aromatic amino acid decarboxylase are the enzymes that catalyze a two-step process in which serotonin is produced from L-tryptophan. After release, serotonin binds post-synaptically to a 5HT receptor and is taken up by the serotonin transporter (SERT) located on the pre-synaptic axonal membrane. Finally, serotonin is metabolized by monoamine oxidase in the pre-synaptic compartment (Fig.3).

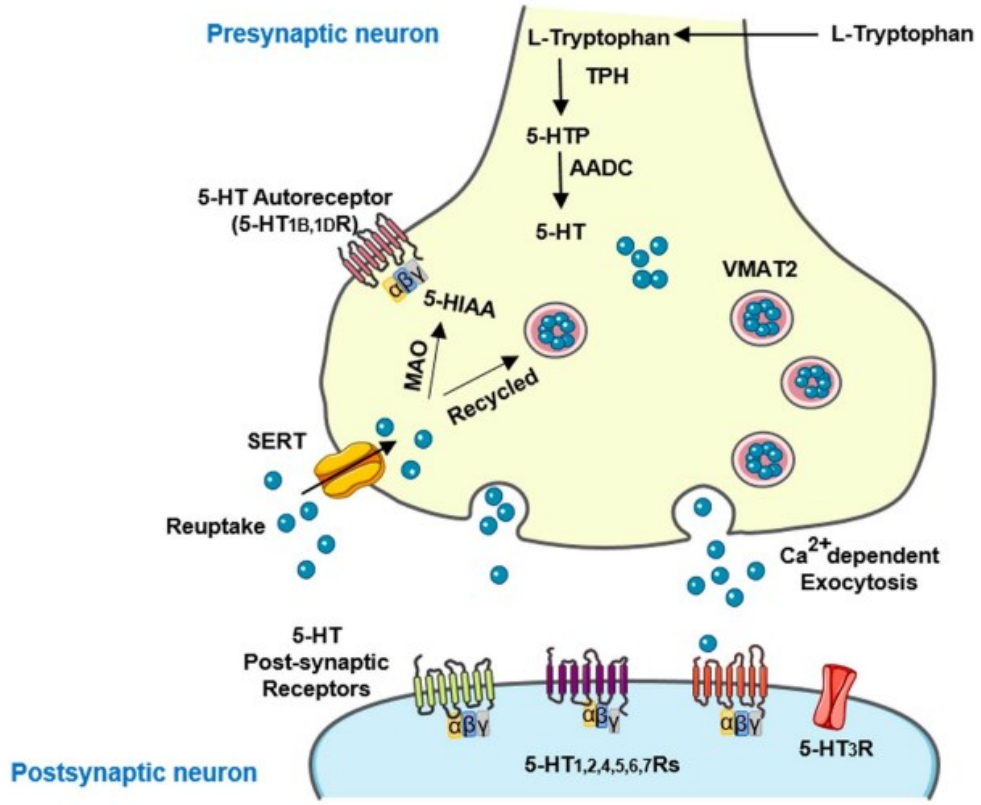

Figure 3 5HT Synthesis and Metabolism [43].

On the basis of both structural and functional properties, 5HT receptors are currently classified into seven classes (from 5HT1 to 5HT7). Apart from the 5HT3 receptor, which is an ionotropic receptor-channel that allows the passage of sodium and potassium, they are

all metabotropic receptors, or G protein-coupled receptors (GPCRs). The receptors have been further categorized into receptor subtypes, each of which has a unique function.

G protein-coupled receptors include the 5HT1A, B, D, E, F, 5HT2A, B, C, 5HT4, 5HT5A, B, 5HT6, and 5HT7 receptors, whereas ligand-gated ion channels include the 5HT3A, B, C, D, and E receptors [44]. The 5HT1 family of receptors is the most frequently expressed (5HT1A, 5HT1B, 5HT1D, 5HT1E and 5HT1F). Seven transmembrane alpha-helices, coupled by three extracellular and three intracellular loops, make up the common structure of the serotonergic GPCRs. The intracellular loop and C-terminal tail of specific G protein families, such as Gs, Gi/o, and Gq/11, interact upon ligand binding, resulting in the production of second messengers. G-i/o proteins are the targets of 5HT1Rs, G-q/11 proteins are the targets of 5HT2Rs, and G-s proteins are the targets of 5HT4, 5HT6, and 5HT7 receptors. Despite the fact that 5HT5Rs key mechanistic pathway has not been identified, there are reports indicating that it connects to Gi/o. These GPCRs function via a number of intracellular functional proteins, such as voltage-gated N-type Ca^{2+} channels, hyperpolarizing K^{+} channels, adenylyl cyclase (AC), and phospholipase-C (PLC) (Fig.4).

While 5HT2Rs releases second messengers such inositol 1,4,5-triphosphate (IP3) and diacylglycerols (DAGs) by hydrolyzing membrane phospholipids through its coupling with PLC, 5HT1Rs block neuronal activity by inhibiting adenylyl cyclase activity, lowering cAMP levels, and activating the MAPK pathway. The MAPK pathway is completed by the activation of the Erk1 and Erk2 proteins, which travel to the nucleus and activate transcription factors like Elk1 and CREB through phosphorylation [45-47] (Fig.4).

The activation of 5HT5ARs causes Ca^{2+} release, which triggers the production of cyclic ADP ribose, while 5HT4, 5HT6, and 5HT7 receptors stimulate AC, causing a rise in cAMP, the activation of protein kinase A (PKA), and a number of signaling molecules, including CREB (Fig.4).

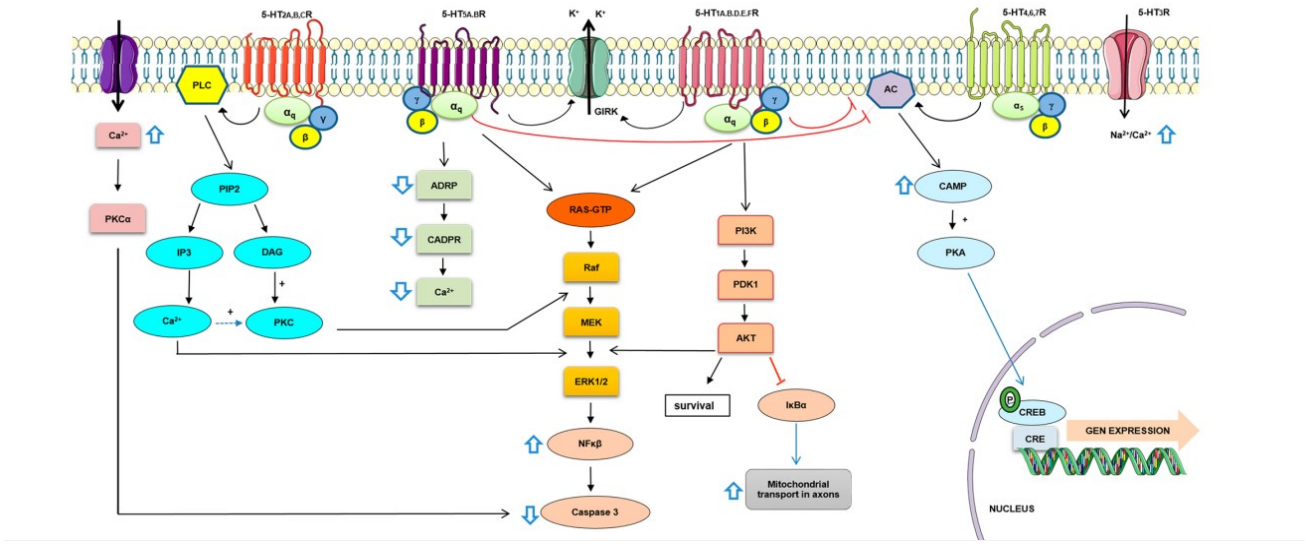

Figure 4 Summary of proposed signaling mediated by 5HT receptor subtypes [43].

The inhibitory receptor subtypes all have high serotonin affinity. Among which, the 5HT1A receptors are widely dispersed throughout the central nervous system; in particular, they are found on the same 5HT neurons at the body and dendritic levels in the mesencephalic and medullary nuclei of the raphe, as well as at the postsynaptic level. The endogenous transmitter or agonists that activate 5HT1A receptors also activate G protein-coupled inward rectifying potassium channels, also known as GIRKs, which are highly expressed in pyramidal neurons of the hippocampus and in the nerve cells of the dorsal raphe [15] The 5HT1A receptors in the dorsal raphe nucleus function as autoreceptors, receptors that are responsive to the neurotransmitter released by the neuron on which they are located. They do this by controlling the functionality of serotonergic transmission. According to Adell and Artigas [31], their activation causes the hyperpolarization of neuronal membrane, which inhibits 5HT cells and reduces serotonin release in the proximity of the cell body and in the projection regions [32]. Therefore, controlling the firing pattern of serotonergic neurons and the subsequent release of serotonin is the fundamental function of these autoreceptors. As a result, manipulating these autoreceptors is crucial for modulating some of the many behaviors regulated by serotonin.

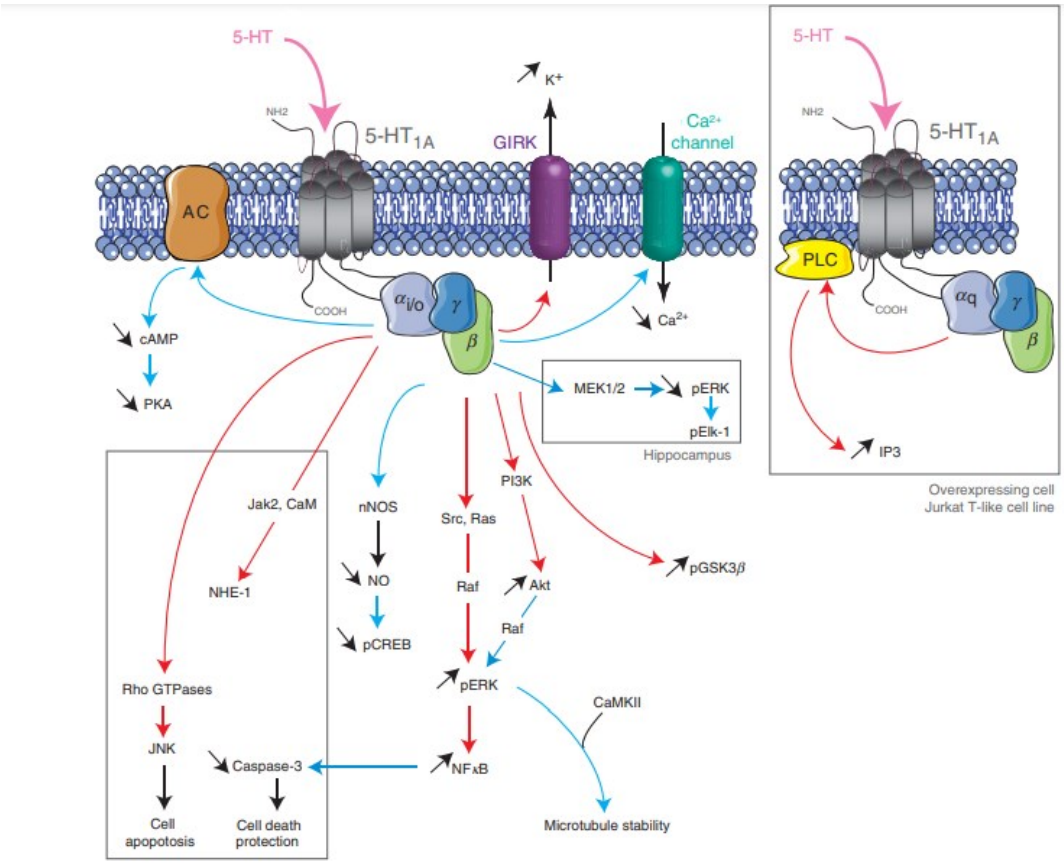

Figure 5 5HT1AR signaling pathway through Gi/o proteins: the 5HT1AR is negatively linked to AC/PKA/cAMP signaling. The simultaneous alterations in K^+ (raise) and Ca^{2+} (reduction) conductance that result from its activation reduce neurotransmitter release in neurons. Additionally, 5HT1AR activation controls ERK phosphorylation. Blue arrow, inhibition; red arrow, stimulation [48].

1.5 The role of 5HT1A receptors

Autoreceptors and heteroreceptors are the two populations of 5HT1A receptors found in the mammalian brain. Autoreceptors are found on the soma and dendrites of serotonergic neurons in the raphe nuclei; when engaged, they block neuronal activity and reduce serotonin release. Studies on animals have shown that the antidepressant-like effect is induced by the activation of postsynaptic 5HT1A receptors or the inhibition of presynaptic 5HT1A receptors. The lack of functional 5HT1A receptors favors a less depressive phenotype, as evidenced by the fact that mice lacking the 5HT1A receptor were less immobile during the forced swim test compared to non-genetically modified controls [49]. The 5HT1A receptor is the most remarkable member of the serotonin receptor family 1, being a presynaptic autoreceptor and playing a part in several physiological processes, such

as the regulation of neuronal growth and plasticity. The studies also demonstrate how the mechanisms behind schizophrenia, depression, and anxiety include the 5HT1A receptor. Depending on the tissues under investigation, 5HT1AR activation affects the ERK (or MAPK) signaling pathway differently (Fig.5). A family of serine/threonine kinases called MAPK is known to mediate long-lasting structural alterations in cells and control physiological reactions such as cell division, proliferation, death, long-term potentiation, and other types of synaptic plasticity [50].

When phosphorylated on both tyrosine and threonine residues, these kinases become active. The phosphorylation of transcription factors and gene transcription is the final outcome of MAPK pathway activation. The nuclear transcription factor phospho-Elk-1, basal levels of phosphorylated ERK1/2 (pERK), and phosphorylated extracellular signal-regulated kinase (MEK) were all lowered in the rat hippocampus in response to 5HT1AR activation [51], whereas no changes in pERK levels were observable in the cortex. In contrast, treatment of a systemic 5HT1AR agonist causes a rapid rise in phosphorylated ERK levels in the paraventricular nucleus and dorsal raphe nucleus of adult rats [52].

Stimulation of endogenous 5HT1AR in hippocampus primary cultures activates Akt via a G protein-mediated and PI3K-dependent mechanism. Src tyrosine kinase, small GTPases Ras, and Raf activation have all been demonstrated to be necessary for ERK activation by 5HT1AR stimulation in transfected CHO cells. Stimulation of endogenous 5HT1AR in hippocampus primary cultures activates Akt via a G protein-mediated and PI3K-dependent mechanism [53, 54]. Due to negative feedback that inhibits Raf activity through an Akt PI3K-dependent cascade, this ERK activation is quick but transient [55]. Transient ERK phosphorylation has been demonstrated to activate the nuclear factor-light chain-enhancer of activated B cell (NF-κB) pathway in transfected CHO cell lines, which in turn inhibits caspase-3 and prevents cell death [55].

Unexpectedly in another investigation, 5HT1AR were shown to induce Jun N-terminal kinase (JNK)-dependent pro-apoptotic pathways via Pertussis toxin-sensitive and Rho family low molecular weight receptors, also in transfected CHO cell line, processes reliant on GTPases [56].

These findings collectively imply that the 5HT1AR can simultaneously stimulate both ERK-dependent anti-apoptotic pathways and JNK-dependent pro-apoptotic pathways, and that the outcome of 5HT1AR stimulation is likely reliant on the respective contributions of these pathways in a tissue-specific context. In transfected CHO cells, it has also been shown that short-term regulation by 5HT1A agonist of Na^+/H^+ exchanger (NHE) is G protein-

dependent via Gi2 and Gi3. NHE is involved in the regulation of intracellular pH as well as other processes, such as cell differentiation, by mediating the exchange of extracellular Na^+ for intracellular H^+. The activation of Gi2 and/or Gi3 by the 5HT1AR results in the activation of Janus kinase 2 (Jak2) and tyrosine phosphorylation of calmodulin (CaM), which in turn increases CaM affinity for NHE-1 and causes NHE-1 activation [57].

Additionally, Ca^{2+}/CaM-dependent protein kinase II (CaMKII) and MEK-dependent loss of microtubule stability are intracellular consequences induced by 5HT1AR activation. This has been seen, particularly, in cultured cortical neurons. By reducing the number of surface NR2B subunits in dendrites through a mechanism dependent on microtubule/kinesin-based dendritic transport of N-methyl-D-aspartate receptors (NMDA) receptors, and regulated by CaMKII and ERK signaling pathways, activation of 5HT1AR exerted a strong reduction of currents through the NMDA-type glutamate receptor channels in these cells [58].

Gi/o proteins are known to activate a number of effectors through the 5HT1A receptor as the G βγ -mediated activation of a K^+ current, suppression of Ca^{2+} current, stimulation of phospholipase C, and activation of the mitogen-activated protein kinase Erk2 are additional functions of the 5HT1A receptor. The principal 5HT1A receptor inhibitory effectors are GIRK channels that can represent an alternative research subject for the investigation of 5HT1A-mediated signaling in depressive disorders and antidepressant treatments.

Given that there is only limited expression of GIRK 4 subunits in the brain, GIRK neuronal channels are tetramers mostly made up of GIRK 1-3 subunits. Since the predominant form of GIRK channels is a heterotetramer containing GIRK1 and GIRK2 subunits, the GIRK2 subunit specifically plays a significant role in the function of the GIRK channel. It is responsible for the generation of GIRK-mediated currents in a number of brain regions, including the locus coeruleus and the hippocampus [59].

Desensitization and internalization of the 5HT1A receptor are crucial processes that control the 5HT1A ability of receptor to function. It is interesting to note that 5HT1A receptor activation (caused by increased amounts of the endogenous mediator) can lead to 5HT1A autoreceptors internalization in the dorsal raphe nucleus but not postsynaptic receptors found in hippocampal neurons, suggesting the different ways that 5HT1A receptors regulate their pre- and postsynaptic activity [60].

According to a novel theory put forth by several studies, 5HTR heterocomplexes may cause depression by altering their plasma membrane densities and/or allosteric receptor-receptor interactions. They may therefore be functionally compromised, and novel depression

treatment targets. Changes in heterocomplex density and/or allosteric receptor-receptor interactions can lead to malfunction and be referred as vulnerabilities.

Beyond the serotonergic hypothesis of depression, the theory expands into the molecular integration processes carried out by a number of 5HT1A heterocomplexes and additional serotonergic receptors with protomers like fibroblast growth factor receptor 1 (FGFR1) and oxytocin receptor [16].

1.6 Serotonin and the neural circuits of the hippocampus

Samuels and colleagues' findings indicate that 5HT1ARs play a critical role in mature DG granule cells (GCs) mediating the effects of serotonin selective reuptake inhibitors on behavior, neurotrophic factors, and neurogenesis [61].

Chronic 5HT1AR activation on granule cells triggers signaling pathways that ultimately lead to the release of neurotrophic factors including brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), which in turn encourage the growth of neuronal progenitor cells (NPCs) and the differentiation and maturation of newly formed GCs (Fig.6).

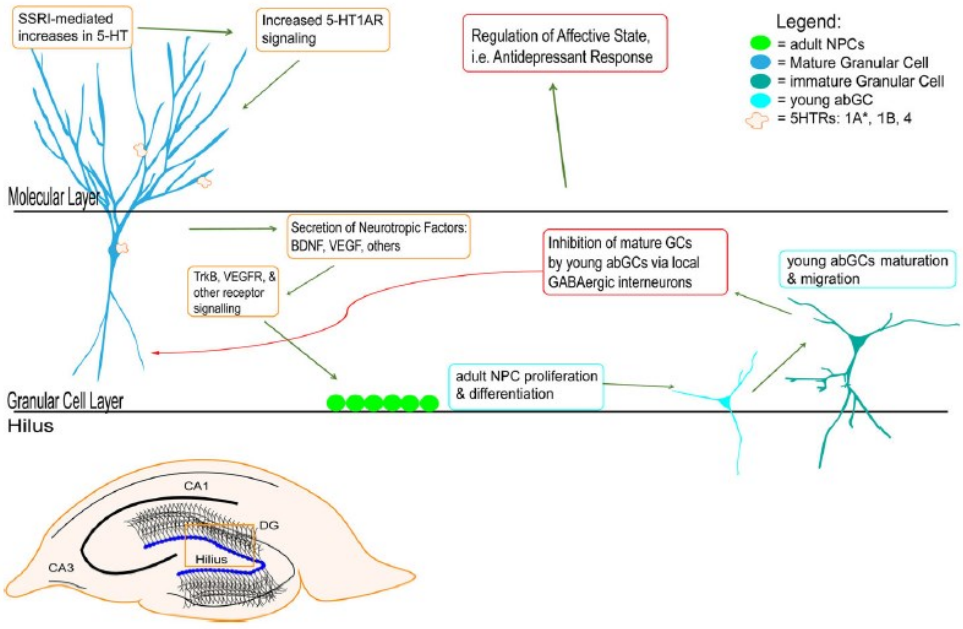

Figure 6 Serotonin influence on dentate gyrus [62].

Persistent SSRI use raises 5HT levels, which causes 5HTR activation on dentate gyrus granule cells. The release of downstream growth factors like BDNF, VEGF, and others

occurs as a result of the activation of 5HT1ARs on mature granule cells. These growth factors then bind to receptors on NPCs in the sub-granular zone that begin to multiply and differentiate. Then, the adult born granule cells start to move, mature, and eventually integrate into the granule cell layer.

Some authors suggest that GABAergic interneurons are important in the etiology of depression. The immature GCs, which have different plasticity characteristics than the mature DG GCs, can activate nearby GABAergic interneurons, resulting in significant inhibitory input to the elderly GCs [63-66]. According to this concept, inhibition of mature GCs, by direct activation of 5HT1ARs or via local microcircuitry, is crucial for the antidepressant response.

It is interesting to note that 5HT1ARs exhibit a distinct expression pattern in the mouse DG, where expression levels sharply rise along the dorsoventral axis until the majority of DG 5HT1ARs are expressed in the ventral pole [67]. The ventral and dorsal hippocampus may play different roles, with the ventral being essential for regulating emotional affective states and the dorsal being more involved in cognitive processes, according to additional studies [68], for instance in the Morris water maze, dorsal injuries impair spatial memory but ventral lesions do not [69, 70]. Modern research shows that precise optical stimulation of the ventral hippocampal projections to the nucleus accumbens or the basolateral amygdala (through channel rhodopsin 2, ChR2) promotes anxiety-related behaviors [71]. On the other hand, suppression of the ventral hippocampus projections to the medial prefrontal cortex reduces anxiety-related behavior [72]. In the contextual fear conditioning model, freezing behavior is decreased when granule neurons in the dorsal DG are directly activated with acute ChR2 stimulation; however, this effect is not present when stimulating the ventral hippocampus [70]. On either side, anxiolytic-like behavioral effects are produced when ventral DG is acutely optogenetically inhibited but not dorsal DG.

Dorsal and ventral DG presumably play different roles in mediating various behaviors as a result of their distinctive connections. The medial septal nucleus, which relays information from the V1, S1, and talamus nuclei, as well as the dorsolateral and caudomedial entorhinal cortex, all provide input to the dorsal DG. The mammillary complex, dorsal lateral septum, lateral entorhinal cortex, and anterior cingulate cortex receive efferent outputs from the dorsal DG [68]. The fact that several of these areas are essential for memory, movement, and exploration shows how crucial the dorsal hippocampus is for cognitive rather than mood-related processes. In contrast, the medial septal nucleus and rostral medial entorhinal cortex send information from the auditory and piriform cortices to the ventral DG. The

medial entorhinal cortex, prefrontal cortex, nucleus accumbens, hypothalamus, amygdala, and rostral and ventral lateral septal nuclei are among the regions that ventral DG projects to, in contrast, dorsal DG [68]. The dorsal and ventral hippocampus differ from one another in terms of electrophysiology, chemistry, and anatomical structure in addition to circuit connectivity [68]. The relevance of 5HTR signaling in possibly influencing emotional affect and the antidepressant response is illustrated by the ventral having larger amounts of 5HT and 5HT innervation compared to the dorsal [73].

In the ventral DG and dorsal CA1, two different hippocampal subfields, 5HT1ARs are strongly expressed [61]. Given that dentate gyrus 5HT1ARs are required and sufficient for mediating the behavioral effects of SSRIs, the position of these receptors in the ventral pole enables them to directly affect limbic circuitry to control mood-related behavior. It will need more investigation to ascertain if certain pharmacological or electrical manipulations of the ventral DG might represent a cutting-edge therapeutic approach for the treatment of anxiety and depression.

CHAPTER 2: SEROTONIN AND SYNAPTIC PLASTICITY IN HEALTH AND DISEASE

2.1 Synaptic plasticity in depression

The monoaminergic theory of depression, despite being widely accepted, is unable to fully explain the pathophysiology of the disease or the precise pharmacodynamics of antipsychotic drugs. Given these drawbacks, focus has shifted to other viable targets. In particular, new research suggests that the pathophysiology of depression is caused by dysfunctions in glutamatergic systems and a dysregulation of synaptic plasticity [74-76].

Long-term potentiation (LTP) and long-term depression (LTD), the two main subtypes of synaptic plasticity at glutamatergic synapse, are assumed to be the biological foundations of learning and memory in the brain. LTP and LTD involve activity-dependent strengthening or weakening of synaptic connections, respectively [77, 78]. When glutamatergic NMDAR are activated, LTP and LTD are stimulated. This causes intracellular cascades that change the surface expression levels and/or functionality of AMPARs, which can then increase or decrease AMPAR-mediated synaptic transmission [77, 78].

There have been reports of anomalies in the cycling of glutamate and glutamine in depressed patients, as well as higher glutamate levels in plasma, CSF, and important brain regions such the prefrontal cortex (PFC) and hippocampus (HPC) [76, 79]. Additionally, depression is associated with abnormal NMDAR and AMPAR gene expression, density, and function [80, 81]. In areas implicated in major depression disease (MDD), specifically the HPC and PFC, chronic stress, which in turn raises glucocorticoid levels, is linked to pathological glutamate excitotoxicity and synaptic dysfunction, which eventually results in reductions of dendritic branching and spine density of pyramidal neurons [74, 82, 83].

Chronic stress in rodents reduces the expression of fundamental receptors and proteins involved in synaptic plasticity, such as AMPAR (GluR1, 2 and 3), NMDAR (GluN1 and 2B) subunits and other synaptic proteins (such as synapsin 1 and postsynaptic density 95, PSD95) in these regions. Acute and chronic stress, as well as exogenous glucocorticoids, might disturb the usual balance between LTP and LTD in pyramidal neurons, favoring the latter, especially in the mouse hippocampus, according to important preclinical research [83-86]. Stress weakens synapses and causes neuronal atrophy in the HPC and PFC by blocking LTP and/or promoting LTD, paralleling or possibly contributing to the structural

and functional changes observed in MDD [86-88]. Human postmortem and imaging studies, in particular, revealed significant regional reductions in the number and size of neurons and glia, as well as reduced grey matter volume, in the hippocampus, orbital, dorsolateral and medial PFC, and ventral striatum [83, 87]. Along with the primary symptoms of emotional dysregulation and anhedonia, MDD is also linked to a variety of cognitive deficits, including reduced attention, episodic memory, and executive function. All of these deficits could be mediated by impaired synaptic plasticity processes and loss of connectivity between these important brain regions, which are particularly vulnerable to stress [83, 87, 89].

It is significant to highlight that, in addition to the glutamatergic system, hippocampal and prefrontal GABAergic networks have also shown functional and structural abnormalities in both depressed individuals and animal models of depression [76, 90, 91]. Thus, increasing data suggests that chronic stress and depression may reduce inhibitory control in the PFC and HPC, which may contribute to the emotional and cognitive symptoms of depression [76, 90, 91]. Importantly, chronic stress increases dendritic spine density, dendrite length, and branching of neurons in the amygdala, leading to hypertrophy in this part of the brain in contrast to the substantial atrophy found in the HPC and PFC [83]. These regional variations in stress-induced synaptic and structural plasticity may decrease inhibitory control and increase amygdala activity, which would exacerbate depression related abnormal stress response, depressive symptoms, and anxiety [83]. In the past ten years, neurotrophic factors have received significant attention as key mediators of the antidepressant response because they are now understood to control adult synaptic plasticity and neuronal survival in addition to brain growth and differentiation during development [86, 88].

Preclinical studies, that demonstrate that chronic stress causes significant reductions in BDNF levels, which, at least in part, mediate synaptic dysfunction and neuronal atrophy in vulnerable brain regions like the HPC and PFC, support the relationship between neurotrophins and stress/depression [92, 93]. In line with this finding, depressive patients have lower expression or function of BDNF and/or its high-affinity receptor TrkB, which is linked to plasticity-related alterations such hippocampus shrinkage [92]. Importantly, preclinical research has shown that practically all kinds of antidepressants boost BDNF expression in the mouse HPC when administered chronically (but not acutely); BDNF signaling, in turn, encourages synaptic plasticity, synaptogenesis, and hippocampal/cortical function [92, 94]. The delay in the onset of therapeutic effects with traditional

antidepressants may be explained by the fact that increases in neurotrophin levels happen after modifications to monoamine signaling [95].

The revelation that ketamine, a non-selective NMDA receptor antagonist, provides strong, immediate (within hours), fast (within days), and persistent (within one week) antidepressant effects in people with treatment resistant depression [96], has sparked a lot of interest in figuring out the molecular processes that control the drug's clinical effectiveness. Ketamine prolonged antidepressant effectiveness can be solely attributable to the acute receptor actions due to its short half-life [80, 97]. Instead, the activation of essential downstream signaling cascades, which leads to long-lasting modifications in neural circuits, appears to be its mode of action [97]. Ketamine may enhance the ratio of AMPA to NMDA receptor throughput by directly blocking NMDARs and indirectly improving AMPAR activity, which in turn appears to activate critical signaling pathways, most notably BDNF and mammalian target of rapamycin (mTOR) [86, 88, 98]. Ketamine, specifically, increases the surface expression of AMPAR subunits GluR1 and/or GluR2, glutamate transporters, and other synaptic proteins, enhances excitatory synaptic transmission, and increases mushroom spines, dendritic branching, vascularization, and glial coverage in the HPC and/or PFC [86, 97, 99-101]. The rodent HPC and medial PFC show synaptogenesis after receiving a single systemic injection of ketamine, which reverses stress-induced synaptic destabilization and neuronal atrophy [100-102].

(2R, 6R)-Hydroxynorketamine (HNK), an active ketamine metabolite, demonstrated comparable antidepressant efficacy without the NMDA receptor binding capabilities or significant adverse effects of its parent molecule [103]. HNK significantly increased AMPAR-mediated synaptic transmission and upregulated surface GluR1 and GluR2 in the HPC, similar to ketamine, though its precise mode of action is unclear [103]. Based on their findings, the authors proposed that the HNK metabolite, which seems to act in an NMDAR-independent manner, is necessary and sufficient for ketamine therapeutic actions [103]. But others have since challenged this theory, and more importantly, a number of subsequent studies have failed to replicate the antidepressant effects of HNK in three different depression model systems [104], provoking some controversy. Given these contradictory findings, it is critical to ascertain how this metabolite contributes to ketamine's clinical effectiveness and to continue researching this novel, potential candidate antidepressant drug capacity to alter AMPAR and synaptic activity. Despite the fact that the key biochemical and structural effects of ketamine discussed, such as on AMPAR/NMDAR throughput, BDNF and mTOR signaling, synaptogenesis, are now well established, the

mechanisms behind the drug's antidepressant effects on a "systems level" are still unknown. One hypothesis holds that ketamine can "reset the system" by triggering synaptic plasticity and synaptogenesis to restore normal connectivity between the hippocampus, prefrontal cortex, and related regions [83, 86, 105].

Antidepressants may have impacts on stress-induced amygdala enlargement, but ketamine may restore proper top-down control of amygdala functioning by repairing prefrontal connection abnormalities [83, 105]. Although it is likely that ketamine and traditional antidepressants work in a similar way, ketamine has the unique capacity to cause these changes that take place within hours, which accounts for its quick antidepressant effect. In contrast, ketamine more directly targets these important downstream signaling pathways that are relevant to the antidepressant response than do standard antidepressants [101]. Given the mounting evidence, it is remarkable how little is known about synaptic plasticity mechanisms in important brain circuits linked to depressive disorders or the antidepressant effects of ketamine and other medications, particularly in vivo.

2.2 Serotonin and neurogenesis

It has been well acknowledged that animals develop new neurons throughout lifespan in two distinct areas: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the DG in the hippocampus [106]. While neurons born in the SGZ migrate into the granular layer of the DG and mature into granule neurons, those born in the SVZ travel down the rostral migratory stream into the olfactory bulb and become interneurons.

Adult neurogenesis is a multi-step process that includes neural progenitor proliferation and fate specification, neuronal migration and maturation, and the synaptic integration of newborn neurons into mature neuronal circuitry. Throughout the neurogenesis process, various well-established molecular markers are employed to identify cells at specific times, with electrophysiological cell membrane characteristics being well defined [106, 107].

Chronic, but not acute, antidepressant treatment accelerates the rate of maturation and integration of young granule cells into the DG circuitry, differentiation of precursor cells into young granule cells and proliferation of dividing neuronal progenitor cells in the SGZ [108, 109]. Additionally, given that they do not boost neurogenesis in the SVZ, chronic antidepressants appear to have effects that are unique to the SGZ [108].

Importantly, loss of the behavioral antidepressant response is observed following localized ablation of the adult hippocampus neurogenic niche via cranial irradiation, indicating a

requirement for adult neurogenesis in mediating the behavioral benefits of persistent antidepressant treatment [110, 111]. However, it is significant to highlight that in rodents, ablating adult hippocampal neurogenesis does not result in an increase in anxiety- and depression-related behaviors [110, 112]**.** Similar to this, while postmortem samples of untreated depressed patients showed declines in the number of DG GCs, progenitor cell numbers did not seem to be affected [113]. Moreover, under normal circumstances, directly increasing neurogenesis through a genetic method does not provide an antidepressant-like phenotype [114]. Thus, while boosting adult hippocampal neurogenesis is essential for the antidepressant response, it is insufficient to mediate an antidepressant response, and there is little evidence to imply that deficits in adult hippocampal neurogenesis may be involved in the pathophysiology of depression.

Multiple 5HT receptors are presumably connected to the mechanisms by which SSRIs boost adult hippocampus neurogenesis. 8-OH-DPAT, a 5HT1AR agonist, boosts neurogenesis in both the SGZ and SVZ [110, 115]. Furthermore, SSRIs do not increase neurogenesis in mice that are germline deficient for 5HT1ARs [110]. It is interesting to note that a recent work by Samuels and colleagues found that targeted deletion of the 5HT1AR gene from mature DG GCs, but not from immature GCs, eliminated the behavioral response to SSRI therapy and decreased the neurogenic response [61]. These findings suggest that SSRI-induced increases in adult hippocampus neurogenesis are likely mostly mediated through 5HT1ARs. As a result, increases in neurogenesis may contribute to the antidepressant response. Finding out whether either increase in neurogenesis may be brought on by signaling via various serotonin receptors would be of special interest. This theory needs more investigation utilizing highly localized 5HT1AR-deficient mice.

Additionally, although not being as well known as SGZ and SVZ adult neurogenesis, numerous studies have revealed that adult neurogenesis can take place in other parts of the brain, including the cortex and hypothalamus [116]. It will be fascinating to find out whether cortical neurogenesis is involved in mediating how antidepressants affect behavior positively, as some authors have hypothesized that GABAergic interneurons are important in the etiology of depression [90].

2.3 Molecular connections between serotonin and neuroplasticity

It is well known that 5HT and other forms of neuroplasticity have a biochemical connection [117, 118]. The majority of prior research on the 5HT1A receptor was conducted based on relationships between serotonergic receptors and neurotrophins, tyrosine kinases involved

in cytoskeletal rearrangement, astroglial interactions, or cell adhesion molecules. The relationship between serotonin and neurotrophins is mostly based on mouse research showing strong reciprocal interactions between BDNF and 5HT. 5HT raises BDNF mRNA in raphe neuronal cultures, and BDNF injection to cell cultures enhances genesis of serotonergic neurons and dendritic lengths, demonstrating that BDNF promotes the morphology and function of serotonergic neurons [119]. Additionally, research on both animal [120-123] and human [113, 124, 125] subjects points to an improvement in neural plasticity as a result of raised 5HT levels following SSRI therapy. Electroconvulsive therapy and SSRI therapy, both short- and long-term, are linked to modifications in BDNF expression [126, 127] .

According to studies on the molecular relationships, changes in monoamine levels due to antidepressant administration cause elevation of BDNF levels through interaction with CREB and TrkB. [126, 128]. Antidepressants have no effect on mice with a common genetic polymorphism in the BDNF gene (Val66Met), although this has not been observed in people who carry the Met allele [129]. Contradictory results have been discovered in individual antidepressant research, and individuals who are Val/Met heterozygous and Met-allele carriers have shown a somewhat greater response in meta-analyses [130]. However, neurotrophins might be simply one neuroplastic process that SSRIs promote. The activity of signaling pathways involved in neural plasticity, such as ERK or MAPK, is modulated by some serotonergic receptors in addition to their conventional relationship with G proteins [8]. Although genetic variants in SERT or MAO and their knockdown have an impact on neuronal structure [131-133], the effects appear to be less pronounced than those of serotonergic receptor modification [134]. Numerous serotonergic receptors are connected to ERK or other protein kinases that control the cytoskeleton in neuronal cells, suggesting that intracellular serotonergic signals have a role in long-term cell protective mechanisms. The ability of the 5HT1A receptor to promote neurogenesis and dentritic development in the hippocampus serves as evidence for this [135].

5HT may engage in interactions with synaptic adhesion molecules, according to additional data [136]. The polysialylated version of the neural cell adhesion molecule (PSA-NCAM), which is involved in synaptogenesis and neurite remodeling, is increased by serotonin. Additionally, PSA-NCAM is regarded as a marker of growing neurons, with expression levels dropping as the neurons mature. It has been shown that fluoxetine increases PSA-NCAM expression in the prefrontal cortex and changes the connection and plasticity of cortical interneurons [137, 138]. The ability of NMDA-antagonists like ketamine to

promote synaptic plasticity may depend on the metabolites of ketamine activating the AMPA receptors [139]. It is known that SSRIs and other antidepressant classes can affect the transcription of NMDA or AMPA receptors, which in turn has an effect on the glutamate system [140]. In a mouse model of depression-like phenotype, recent excellent work revealed an environmental effect of neuroplasticity mediated by SSRIs [141]. Notably, this study showed that persistent stress and an enriched environment have opposite effects, indicating that SSRIs may operate as catalysts for environmental vulnerability [142]. The study of environment-treatment interactions (or gene-environment-treatment interactions) in the field of human psychopharmacology, where prospective studies are currently rare, is made possible by this paradigm.

In conclusion, 5HT is a neurotransmitter with unique neuroplastic effects. During embryonic development and the early stages of postnatal neuronal maturation, serotonergic neurons are highly active in shaping them. This neuroplastic role is largely preserved into adulthood in particular brain regions. A large number of these effects are mediated by synaptic adhesion molecules, serotonergic receptors with direct linkages to neuromodulatory signaling pathways, or connections to BDNF. Negative environmental experiences during antidepressant therapy may possibly have negative effects. Environmental stimuli may be ignored contributors to serotonergic neuroplasticity. All of the aforementioned pathways were discovered in animals; however, research on the relationship between 5HT and neuroplasticity in humans is still in its infancy. As a result, there is a high demand for human translational studies.

CHAPTER 3: GPCR-RTK RECEPTOR INTERACTION AND ITS ROLE IN DISEASE

3.1 Receptor-receptor interaction: discovery and their role in disease

Receptor-receptor (R-R) interactions, which are direct interactions between membrane receptors, have been investigated using a variety of technologies, ranging from traditional biochemical methods to biophysical techniques [143] .

Membrane receptors are involved in cell signaling when they recognize particular extracellular chemicals and translate the signal inside the cells by triggering complex intracellular pathways. Transmembrane proteins are used to classify and split a variety of cell surface receptors into families based on their topologies. G Protein-Coupled Receptors and Tyrosine Kinase Receptors (RTK) are two prominent families of membrane receptors. Since they cross through the cell membrane seven times, GPCRs are also known as seven transmembrane domain receptors. Their mechanism involves the activation of many heterotrimeric G proteins and has both genomic and non-genomic effects. Gs, Gi/o, Gq, and G12/13 are the four primary G proteins. In contrast, RTKs are receptors recognized for their participation in growth factor, cytokine, and hormone pathways. With some exceptions, these receptors are typically single subunit receptors that dimerize once bound to their ligand. This conformational state results in the transphosphorylation of their cytoplasmic domains and the activation of intracellular pathways, which have a variety of effects on the genome and non-genome.

Using the information that neuropeptides and monoamines frequently coexist in the same synaptic vesicles, Kjell Fuxe's team initially proposed the possibility of molecular integration of various transmitter signals via transmembrane receptor-receptor integration [144]**.**

In order to provide evidence for the existence of direct R-R contacts in the plasma membrane between various kinds of GPCRs, it was claimed that their interactions in the plasma membrane take place in putative heteroreceptor complexes of GPCRs. In 1993, the word "heteromerization" was coined to refer to a particular direct interaction between many kinds of GPCRs [145]. A 1999 research showing that the non-functional GABAβ1 and GABAβ2 receptors form a signaling heterodimer at the cell surface supported the idea of GPCR heteromerization [146]. Monomers, homo- and heteroreceptor complexes with receptor assemblies of uncertain stoichiometry and shape, as well as adapter proteins, are

now included in the R-R interaction field in the central nervous system. Moreover, the original idea of R-R interaction between GPCR receptors has been expanded to include additional receptor types, including RTKs. It is evident that RTKs and GPCRs can be transactivated not only through the release of neurotrophic factors caused by GPCRs but also through the sharing of signaling pathways or through direct allosteric R-R contact [5, 147].

About fifteen years ago, Fuxe's group first proposed the idea that RTKs and GPCRs directly interact [5], and the first report of such an interaction involved the adenosine A2A and FGF receptors using the yeast two-hybrid system. The same group also provided evidence for the enhancement of synaptic plasticity following the combined activation of the two receptors [148]. Bioluminescence-RET (BRET) was used to confirm this complex existence after that. Two empirically quantifiable requirements must be met in order to classify an interaction as R-R interaction: in particular, the binding of a ligand to one receptor must result in a discernible change in the biochemical properties of the other receptor, including the recognition, decoding, and trafficking of ligands. In addition, the mean distance assessed using an atomic force microscope or RET-based method, such as BRET or fluorescence-RET (FRET), must be less than 10 nm [144].

The orthosteric and allosteric binding sites of the adjacent protomer, its G protein activation and selectivity, signaling cascade, and the appearance of new allosteric sites are all modulated by the allosteric mechanisms in receptor heteromers, enabling a noticeably increased range of GPCR recognition and signaling. Thus, because to conformational changes in distinct domains that modify receptor protomer function and pharmacology, allosteric R-R interactions in heteroreceptor complexes confer variety, specificity, and bias to the receptor protomers. It follows that R-R interaction has garnered a lot of attention in the realm of scientific study due to its intriguing potential as a new target for the treatment of neurological and mental illnesses. GPCRs and neuropsychiatric diseases are in fact closely connected. It has long been recognized that a wide variety of GPCRs have a role in several types of illnesses.

The development of a molecule that inhibits the R-R interaction, such as a protein or an oligopeptide that prevents the formation of the heteroreceptor complex, is a potential method for treating diseases involving heteroreceptor complexes [143]. Successful introduction of transmembrane interface interfering peptides has resulted in a considerable reduction of these heteroreceptor complexes [18]. The FGFR1-5HT1A heteroreceptor complex is one instance where the R-R interaction should be promoted rather than

destroyed since doing so can boost excitability and plasticity while also preventing depression, a condition in which this complex is active. In addition, it is also likely that positive or negative modulators of one protomer might have a significant role in modifying the function of the other protomer engaged in the heteroreceptor complex through comparable mechanism [143]. Understanding these modulatory effects, however, would likely result in new prospects for the introduction of novel therapeutic medications as it is presently mostly unclear how allosteric modulators at one protomer impact the function of the other in an R-R contact.

3.2 Heteroreceptor complexes: overview

In the 1980s, Agnati and Fuxe proposed the idea that direct interactions between receptors might result in heteroreceptor complexes with allosteric receptor-receptor interactions based on neuropeptides capacity to alter the binding properties of monoamine receptor subtypes in membrane preparations [149-151]. According to Kenakin [152] and Tsai [153], the term "allostery" refers to a long-distance cross-talk involving various distal sites in proteins, in which activation of one site results in conformational or dynamic changes, releasing energy that, by traveling along particular pathways of the protein structure, can change the conformational or dynamic properties of other sites. When this idea is used to allosteric receptor-receptor interaction, it denotes modifications to a protomer pharmacology and signaling that are brought on by certain events that can cross the receptor interface and include another protomer of the heteromer. The sentence "receptor-receptor interactions" actually refers to the molecular process in which "the binding of a ligand to the orthosteric or allosteric sites of one receptor causes, via direct allosteric interactions, a change in the ligand recognition, decoding, and trafficking processes of another receptor" [154].

To look at it another way, the phenomenon of allosteric receptor-receptor interaction encompasses intramembrane protein-protein physical interactions operating through the receptor interface, resulting in transient protein complexes or mosaics capable of emerging functions, in which integration of the incoming extracellular signals is realized already at the plasma membrane level [155]. In fact, GPCRs can exhibit a variety of temporary conformations in addition to their unbound and bound states. These transient conformations are regulated by dynamic association and allosteric interactions with ligands, additional receptors, and proteins including adaptors and scaffolding proteins. The conformational

flexibility of receptors impacts their capacity to activate various cell signaling pathways [156-158].

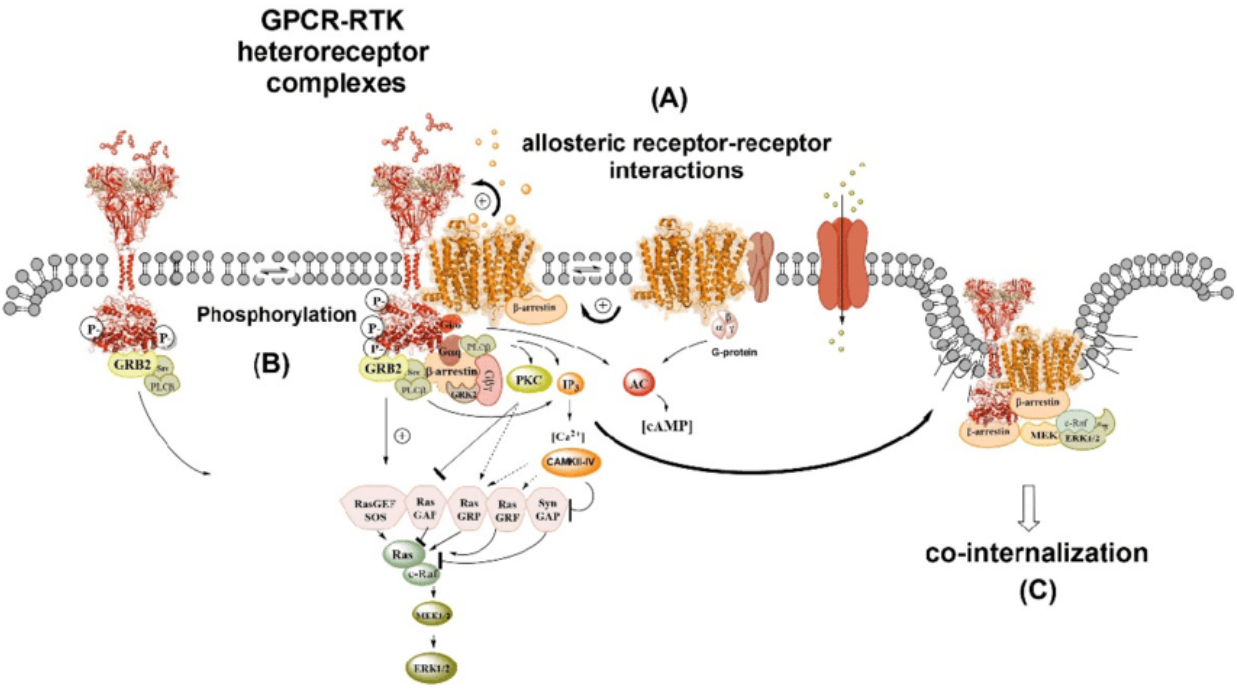

Figure 7 GPCR-RTK heteroreceptor complexes [159].

An increasing number of studies has revealed that GPCRs can form highly dynamic homodimers, heterodimers, and oligomers with other receptors, including GPCRs themselves and RTKs, in addition to signaling as monomers (Fig.7). These highly dynamic oligomers play an important role in the regulation of GPCR ligand binding, function, and cross-talk, as well as in cell pathology [160-163]. Accordingly, heteromers are linked to novel receptor dynamics in recognition, pharmacology, signaling, and trafficking, and may result in differential signaling depending on the heteromer context of the targeted receptor. This means that while individual receptors activated by a given neurotransmitter can typically trigger canonical responses associated with the recruitment of intracellular mediators, heteromers are related to novel receptor dynamics in recognition, pharmacology, signaling, and trafficking, and may lead to differential signaling. To accomplish several functional results for a single neurotransmitter input, the CNS cells may, more broadly, employ the method of heteromer synthesis. Serotonin receptors serve as a model because they take part in various transactivation mechanisms and heteroreceptor complexes involving RTKs and other GPCRs, which modulate the activity of hippocampal circuits and related outputs like mood-regulating limbic areas [164, 165].

There are two primary methods that receptor monomers might assemble into oligomers in relation to the receptor biophysical interaction. The first is a "domain contact" dimerization, which is the primary mechanism of GPCR association. It involves a connection between the receptor protomers, that can be either covalent (disulfide bond) or non-covalent (electrostatic and hydrophobic interaction) [143], that occurs at particular binding surfaces, resulting in minimal to no change in the conformation of the monomer. A group of triplet amino acid homologies that may be implicated in the receptor interface of heteromers, generating "adhesive guides," have been discovered using a mathematical technique [166]. For instance, numerous receptor heteromers, including 5HT1A receptor-fibroblast growth factor receptor 1 and FGFR1-platelet-derived growth factor receptor alpha (PDGFR), include the triplet of amino-acid residues LLG (Leucine-Leucine-Glycine) at their interface. Intriguingly, electrostatic interactions between arginine and phosphorylated serine residues are characterized by "covalent-like" stability, thus representing another significant mechanism in protein heteromerization [167, 168]. Other important mechanisms in protein heteromerization include small-XXX-small motifs, in which two residues are typically glycine, but also alanine or serine [169]. When a monomer domain is swapped with the matching domain of another monomer, a dimerization process known as "domain swapping" dimerization occurs, leading to a significant conformational shift [154, 170].

Direct interactions between receptors in heterocomplexes have been identified using a variety of techniques, depending on how close the involved proteins are to one another [143, 154]. These techniques range from binding and functional studies, the use of bivalent ligands [171], and biophysical proximity assays, like resonance energy transfer methods [147, 172] and line-scan fluorescence cross-correlation spectroscopy [173], to biochemical methods, like co-immunoprecipitation [174] and proximity ligation assay (PLA) [175]. These cutting-edge approaches have allowed for the characterization of numerous interactions between GPCRs and various types of receptors and proteins [154, 160, 162, 176-179].

3.3 Transactivation mechanisms: overview

Numerous studies have demonstrated that GPCRs and RTKs, which activate comparable signaling molecules and regulate related physiological activities, may interact rather than work in isolation. This cross-communication integrates and combines information from various sources and offers a complicated level of control over a number of regulatory

cellular mechanisms. It can take place in both healthy and pathological circumstances [180, 181]. In fact, it is now generally accepted that GPCRs perform a growth-promoting action via RTKs and their downstream signaling cascades in addition to traditional second messenger-regulated processes [5, 181, 182]. In other words, by combining the diverse array of GPCRs with the potent signaling capabilities of RTKs, GPCR activation can boost RTK signaling activity. The process by which GPCR agonists were able to quickly activate the epidermal growth factor receptor (EGFR) in fibroblasts, known as transactivation, was first reported [183]. Importantly, ligands of heterologous RTKs can also transactivate RTKs; for example, EGF governs the migration of developing cortical neurons by transactivating tropomyosin-related kinase receptors [184]. Basically, depending on whether the RTKs ligands are involved or not, transactivation of RTKs by GPCRs can be mediated by two alternative ways. In accordance with the ligand-dependent triple-membrane-passing-signal mechanism [183, 185, 186], RTK transactivation is dependent on the activation of membrane-bound matrix metalloproteases (MMPs) by GPCR signaling, which in turn causes the proteolytic cleavage of a pro-ligand for a given RTK. In other words, ligand-dependent RTKs transactivation by GPCRs is characterized by three transmembrane signal transmission events. Three processes are involved in the extracellular activation of the RTK: extracellular ligand-mediated GPCR activation, subsequent processing of the RTK ligand precursor and release of the mature form, and activation of RTK intracellular signaling [186].

3.4 GPCR-FGF2 receptors complexes and functional transactivation

A crucial multifunctional neurotrophic factor known as fibroblast growth factor 2 (FGF2) encourages neuronal growth and morphogenesis, neurogenesis, survival, migration, and differentiation to regulate critical CNS activities, including brain development [187-191], adult neurogenesis, neuro-regeneration following brain injury [192], synaptic plasticity and axonal branching both *in vitro* and *in vivo* [193]. According to Johnson and Williams [194], FGF2 signals through four RTKs in rodents and five in humans [195]. Three primary pathways are then activated as a result of ligand binding to the FGFR: PLC/PKC, Ras/MAPK (including ERK, JNK, and p38), and PI3K/AKT [196].

In recent years, a number of examples of GPCR-FGFR heterocomplexes and associated transactivation, producing significant neurotrophic effects, have been documented. The two-hybrid system has demonstrated that there is a direct physical contact between the A2A and FGFRs [148]. These receptors are simultaneously activated, but not individually, by

the co-transmitter FGF2, which results in differentiation and neurite elongation in PC12 cells, spine morphogenesis in primary neuronal cultures, and cortico-striatal plasticity [148].

Bioluminescence resonance energy transfer methods have further confirmed the existence of this heteroreceptor complex [147]. Recently, has been identified a complicated interaction between FGFR1 and 5HT receptors. In fact, FGFR1-5HT1A heterocomplexes have been identified and studied in the rat hippocampus and primary hippocampal neurons using a variety of approaches. While co-activation of the heterocomplex with both FGF2 and 8-OH-DPAT agonist results in synergistic increases in heterocomplex signal, in extensions of PC12 cells, in neurite densities and axon growth in primary hippocampal neurons, and in significant antidepressant effects [18].

The development of antidepressant medications may target the hippocampus and midbrain raphe FGFR1-5HT1A heteroreceptor complexes, according to these findings. The hippocampus and ascending midbrain raphe 5HT neurons trophic actions and neuroplasticity are in fact connected to the activation of this target, which can promote 5HT nerve terminal networks expansion and prevent atrophy, both of which have antidepressant effects [164, 165].

Recently has been identified a relationship between FGFR1 and muscarinic acetylcholine receptors (mAChRs). In fact, heteroreceptor complexes between M1 receptors and FGFR1 have been found in the rat hippocampus, cerebral cortex, and basic hippocampal neuronal cultures, as well as Src-mediated transactivation of FGFR1 by M1 agonists that improves neurite outgrowth in primary hippocampal neurons [175, 197, 198]. In addition to explaining the mechanisms of action of cholinergic drugs and the acetilcholine trophic effects, FGFR activation by mAChRs may also aid in the development of novel treatment approaches for pathologies involving hippocampus cell atrophy, such as Alzheimer disease and depression. Interestingly, the possibility of D2-FGFR1 and A2A FGFR1 autoreceptor complexes at the soma-dendritic level of central catecholamine neurons has been hypothesized based on evidence that the FGF2-FGFR1 system is involved in the ventral tegmental dopamine neurons and the locus coeruleus noradrenaline neuron system, and that these two systems strongly contribute to antidepressant effects [164].

Overall, these data provide a new perspective on how the brain network, including both neuronal and glial cells, mediates the therapeutic action of antidepressants that is 5HT dependent.

3.5 FGFR1-5HT1A heteroreceptor complex as target for the treatment of major depression

The discovery of GPCR-containing heteroreceptor complexes can cause transactivation of RTKs with an effect on neuronal plasticity, has the potential to open a new research area for the treatment of many diseases, including Major Depressive Disorder [5, 148, 199]. It is strongly hypothesized that MDD may be significantly impacted by the interaction between the FGFR1 and the serotonin receptor 5HT1A [17, 18, 164, 200].

Using the in-situ Proximity Ligation Assay and co-immunoprecipitation methods in the rat dorsal hippocampus, the FGFR1-5HT1A heteroreceptor complex was initially identified (Fig.8). These complexes were discovered in the pyramidal cell layers from CA1 to CA3 fields, in the dorsal leaflet of the DG, in the dorsal raphe but not in the cerebral cortex. The specificity of this contact has also been validated using a short transmembrane peptide (TM V) that prevents the formation of complexes, and the existence of this interaction has also been confirmed using the BRET technique in cellular cultures [18]. Rat medullary raphe RN33B cells, HEK293T cells, and midbrain 5HT neurons have all shown evidence of heteroreceptor complexes between 5HT1A and FGFR1, where combined FGF-2/8-OH-DPAT treatment catches more FGFR1-5HT1A heteroreceptor complexes with increased affinity and raises FGFR1 and ERK1/2 phosphorylation as well as the number of cell processes [17, 200].

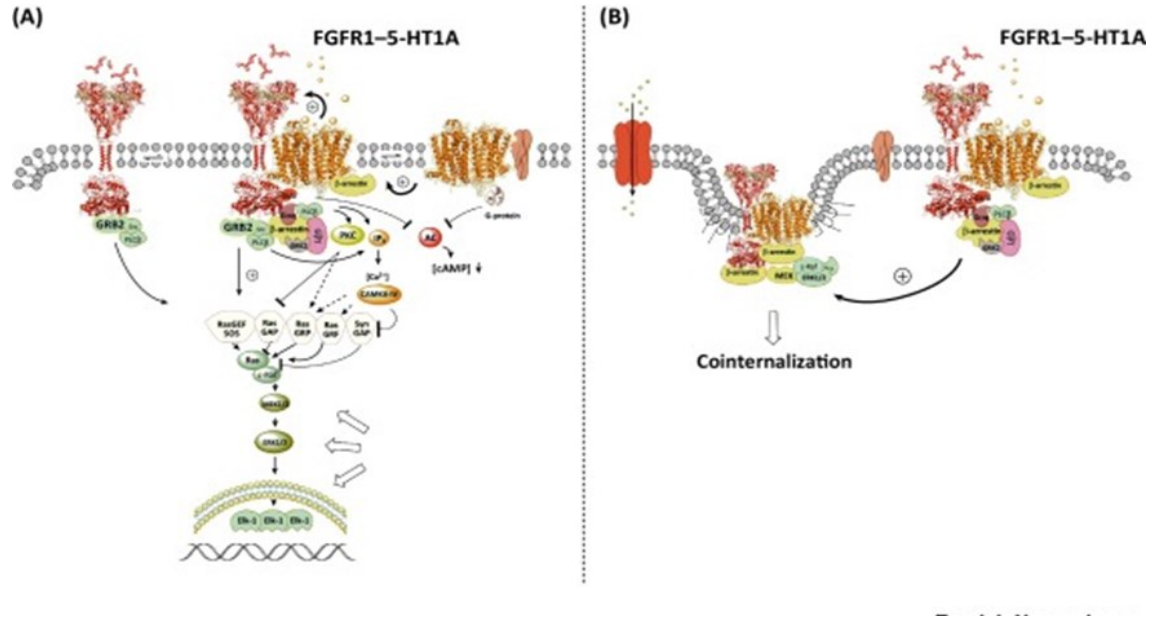

Figure 8 Illustration of the FGFR1-5HT1A heteroreceptor complex, as well as the signaling and balance that the 5HT1A and FGFR1 homodimers contribute in the plasma membrane [164].

As evidenced by the elevated phosphorylation of FGFR1 and ERK 1/2, especially after combined agonist treatment, 5HT1A agonist-induced FGFR1 transactivation and pathways activation were identified as able of allosteric R-R interactions in hippocampus cultures [18]. This coactivation promotes the heterodimerization of FGFR1 and 5HT1A, which strengthens the recruitment of β-arrestins to the heterocomplex and increases the internalization of the complex. β-arrestins are known to be crucial for several cellular functions, including the facilitation of signal transduction pathways and the desensitization of membrane receptors. The observation that receptor endocytosis is necessary for 5HT1A receptor-mediated activation of the ERK cascade [201] suggests another aspect of 5HT receptor signaling. G protein-coupled receptor desensitization, or the uncoupling of the receptor from the G protein as a result of arrestin binding and G protein-coupled receptor kinase phosphorylation, had historically been thought to be the source of endocytosis of G protein-coupled receptors [202]. However, the mechanisms that simultaneously stop the 5HT1A receptor coupling to G proteins can contribute to the transduction of Ras-dependent signals. As scaffolding components, β-arrestins have been demonstrated to mediate receptor signaling in addition to their roles in the desensitization of GPCRs. Thus, GPCRs and signaling proteins can be coupled by β-arrestins without the need for G protein-mediated activation [203].

Studies in cell culture have demonstrated that β-arrestins can support a variety of signaling pathways, including as the MAP kinase family of serine/threonine kinases, notably ERK1/2, p38 kinase, and c-Jun N-terminal kinases, the Src family of tyrosine kinases, phosphatidylinositol 3 kinase (PI3-K). Many GPCRs signaling pathway, including the neurokinin-1, angiotensin, parathyroid hormone, vasopressin, β-adrenergic, chemokine, and dopamine receptors, have been shown to be triggered by β-arrestins [204]. The desensitization of G protein coupling and the activation of ERK1/2 for the protease activated receptor-2 (PAR2) in vitro were both shown to be mediated by β-arrestins [205]. The number of receptor complexes generated and the affinities of the interactions between the two receptor protomers increase as a result of the agonist regulation of this heteroreceptor complex, particularly with a combined agonist therapy. Additionally, it has been discovered that FGF2 and 5HT1A agonists increase neuroplasticity in vitro through this pathway providing evidence for a possible benefit of a therapy based on this heteroreceptor complex in preventing and reversing the hippocampal shrinkage seen in MDD. However, there are currently no in vivo evidence to support such a notion, limiting its usefulness.

During the administration of antidepressant drugs, FGFR1-5HT1A heteroreceptor complexes are interacting. The steady increase in FGFR1 and ERK1/2 phosphorylation shows that co-activation of the FGFR1 and 5HT1A protomers by FGF2 and 5HT1A enhances FGFR1 signaling [164]. However, it is important to point out that the FGFR1-5HT1A heteroreceptor complex, which is activated by the joint activation of 5HT1A and FGFR1 protomers, may also play a role in the antidepressant-like effects observed in the hippocampus [164]. The hippocampus formation is linked to important parts of the brain emotional and mood circuits, placing it in a position to effectively affect how these emotional networks function, which is known to be tied to the pathophysiology of MDD. The discovery of FGFR1-5HT1A heteroreceptor complex in several midbrain 5HT neurons, in the dorsal and median raphe nuclei of Sprague Dawley rats, and rat medullary raphe cell cultures is a nice starting point for trying to rationalize the hunt for heteroreceptors in the hippocampus [17].

A combination of FGFR1 and 5HT1A agonists has been shown to significantly increase the number of processes formed by each medullary raphe neuron as well as the amounts of serotonin immunoreactivity per cell in raphe cells, suggesting a potential impact on increasing plasticity and serotonin production in 5HT cells [200]. In fact, the FGFR1-5HT1A heteroreceptor complex is one of the allosteric R-R interactions that may eventually be disrupted or dysfunctional in depression, leading to dysregulation of the ascending midbrain 5HT neurons and diminished trophic support.

Together, the researches on the hippocampus and midbrain raphe suggest that during agonist co-activation, synergistic allosteric R-R interaction arises within the FGFR1-5HT1A heteroreceptor complex. By enlisting 5HT1A autoreceptors into FGFR1-5HT1A complexes, the development of these complexes is thought to contribute to antidepressant effects by causing their decoupling from GIRK channels. As a result, the midbrain 5HT neurons may exhibit both increased trophism and decreased 5HT1A autoreceptor function; additionally, better trophism may occur in other brain regions, such as the hippocampus area. Therefore, combined agonist therapy has the ability to boost neuronal activity and reverse the atrophy occurring in several CNS regions, including the hippocampus and raphe, thus constituting a crucial event for long-lasting antidepressant effects. For this reason, assessing potential changes to this heteroreceptor interaction is a crucial step in the creation of an antidepressant strategy that targets the FGFR1-5HT1A complexes.

CHAPTER 4: PATOPHYSIOLOGY OF DEPRESSION

4.1 Epidemiology of depression

Major Depressive disorder (MDD) is one of the leading causes of disability in the world [206]. In the United States, close to 10% of adults struggle with depression. According to the National Institutes for Mental Health, in 2015, about 16 million people had at least one major depressive episode [207].

Emotions are a critical part of the human experience, but sometimes our emotions get out of hand. Depression is actually a neurological condition that can really affect everyday life, it is a medical disorder that lingers for at least two consecutive weeks, and significantly interferes with one's ability. Depression can have a lot of different symptoms: a low mood, changes in appetite, feeling worthless or excessively guilty, sleeping either too much or too little, poor concentration, restlessness or slowness, loss of energy or recurrent thoughts of suicide. Subjects showing at least five of those symptoms, according to psychiatric guidelines, are qualified for a diagnosis of depression [208].

Depression has physical manifestations inside the brain. First of all, there are morphological changes inside the brain as smaller frontal lobe and hippocampal volumes [209]. On a more microscale, depression is associated with a few things: the abnormal transmission or depletion of certain neurotransmitters, especially serotonin, norepinephrine, and dopamine [210], blunted circadian rhythms, or specific changes in the REM and slow-wave parts of your sleep cycle [211], and hormone abnormalities, such as high cortisol and deregulation of thyroid hormones [212, 213].

Neuroscientists still do not have a complete picture of what causes depression, it seems to be related with a complex interaction between genes and environment [214], but we do not have a diagnostic method that can accurately predict when it will show up. The progression of the disease is variable: some patients rarely experience a remission while others go through years with no symptoms between isolated depressive episodes. However, some patients show chronicity of symptoms that decreases the possibility of full remission following the treatment, also because lower recovery rates are associated with symptom severity. Despite the different outcomes, an observational study showed that 17% of participants with MDD remained in a chronic state of depression after 39 months, and another 40% had a fluctuating course of depression, while 43% were in remission from baseline [215].

4.2 Pathophysiology of depression

The rate of major depressive disorder has and continues to increase among the population, without difference between high and low-income countries in the rates of depression. Both high- and low-income countries experience a high prevalence of depression at varying rates in the U.S. this is about eight percent of the population over 12-month [207].

4.2.1 Pathology

The comprehension of the pathophysiology of major depressive disorder advanced quickly in the last few years, but there is not a unique hypothesis or mechanism that clarify exhaustively all aspects of the disease. Psychosocial and biological stressors can trigger different depressive episodes in different patients, suggesting the multifactorial etiology of the disease. Animal research has been a useful tool for the neurobiological investigation of depressive disorder, but the translation of findings to clinical practice in often complicated [216].

4.2.2 The monoamine hypothesis

Until recently, scientists were pretty sure that depression was caused by an imbalance of certain chemicals within the brain. Named monoamine hypothesis of depression, this theory said that a lack of neurotransmitters like serotonin and dopamine, two chemicals associated with feelings of pleasure and reward, would lead to depression. This theory is based on the evidence, obtained in mid-20th century, that the antihypertensive reserpine had the side-effect to cause depression through the depletion of monoamines. On the other hand, the monoamine approach was supported by the indication that tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs) enhanced monoamine neurotransmission by different mechanisms with a significative improvement in depressed patient symptoms [217] and new generation selective medications, such as autoreceptor antagonists and serotonin agonists are clinically effective [218]. This model has had good luck in previous years, also in light of the results obtained following the analysis of neurotransmitter levels in the brains of depressed patients. In fact, the alteration of dopaminergic signaling emerged mainly from the brains of patients obtained post-mortem [219]. Moreover, some researchers found that, in patients with a family history of depression, people had a particular genetic variation in the gene that codes for the serotonin receptor 5HT are more susceptible to depression after stressful life events [220]. However, the dilemma with this hypothesis of depression is that even though antidepressant drugs

increase monoamine neurotransmitters immediately it often takes several weeks for a detectable effect, so clearly there must be something else to explain this long delay [221].

4.2.3 Hypothalamic–pituitary–adrenal axis changes

Alterations in the balance of the hypothalamus-pituitary-adrenal axis have been the focus of the debate regarding depression for several years [222, 223]. From the research carried out, it emerged that in the most severe cases with melancholic characteristics, plasma cortisol levels are increased. This increment seems to be the result of the combination between a reduction in excessive stress-mediated cortisol release and a decreased negative feedback regulation at glucocorticoid receptors level [224]. Furthermore, it should be noted that the deregulation of the HPA axis is often associated with a reduction in brain volumes, including the hippocampus [225], and that a failure in the normalization of cortisol activity after treatment is often associated with poor clinical response [226]. Despite these evidences, clinical studies on HPA axis functionality regulation have not proved effective [227, 228].

4.2.4 Inflammation

Cytokines are involved in sickness behavior and specialized role of immune signaling molecules in the brain is to affect neural activity and cognition [229]. Moreover, peripheral cytokines can have an effect on neurons, astrocytes and microglia, directly crossing the blood-brain barrier or indirectly via the afferent pathways [230]. It is interesting to note that a link between long-term psychological and emotional stress and the release of proinflammatory cytokines has also been suggested. One of the first discoveries revealing this connection was that long-term caregiver stress was shown to be linked to higher production of transcripts containing NF-kappa B response elements, a transcription factor that promotes inflammation, in circulating monocytes [231]. Numerous studies have since examined circulating markers and found that, in comparison to respective control groups, the plasma levels of IL-6 increased in the elderly caring for a partner with a chronic illness, people with low socio-economic status, people who experienced abuse or maltreatment during childhood, and patients with depression [232]. Additionally, research on animals showed that modest chronic stress raises levels of IL-6, IL-1β, and TNF-α in the blood [233], while frequent social defeat stress raises the level of IL-6 in the blood [234]. These observations have motivated researchers to investigate the use of non-steroidal anti-inflammatory pharmaceuticals in the management of major depressive disorder [235].

4.2.5 Neuroplasticity and neurogenesis

According to some researchers, depression may be correlated with alterations in brain plasticity. This process enables neurons to modify and alter their signaling in response to new inputs. Although it is rare in the adult brain, there are some areas of the brain that continue to divide and connect new cells over the course of our live. In line with this concept, persistent stress has a detrimental effect on neuroplasticity. Additionally, people with depression have a comparable alteration in neuroplasticity.

The discovery of pluripotent stem cells in the adult brain, which have the potential to create new neurons through a process known as neurogenesis, is one of the most significant scientific discoveries of the 20th century. Neuroplasticity, a word used more widely to describe the development and adaptability at the neuronal level, may be affected by inflammation and HPA axis dysfunction, both of which are brought on by environmental stress [236].

Brain-derived neurotrophic factor, a neurotrophic factor that promotes neurogenesis and typically induces dendritic sprouting and the creation of new neurons, is decreased in people with severe depressive illness but it is found in greater concentrations in depressed individuals taking antidepressants if compared to non-treated patients [237].

The activity of neurotransmitter receptors and transporters is hypothesized to affect gene transcription, which in turn affects the presynaptic feedback mechanisms for norepinephrine and serotonin, that significantly influence neuroplasticity. Through this pathway, antidepressants can reverse or diminish the symptoms, supporting the neurotrophic model of depression development. Perhaps even more significant is the fact that antidepressant therapies, including either medication or psychosocial interventions, can increase BDNF levels in persons with depression [238].

It has been demonstrated in animal studies that restricting neurogenesis causes depression-like symptoms, especially under stressful conditions, and prevents antidepressant activity. Thus, it has been proposed that neurogenesis promotes resistance to stress, which may be the basis for the therapeutic effects of antidepressants [239].

When compared to non-depressed and treated groups, post-mortem examinations of persons with depression reveal a deficiency of granule neurons in the dentate gyrus of untreated individuals. When compared to those with untreated depression and even non-depressed individuals, patients who are receiving treatment for depression have a significant increase in dividing neural progenitor cells [240]. These results are in line with research on mice that demonstrate how antidepressants can function by boosting

neurogenesis in the adult brain. Numerous studies suggest that the capacity of antidepressants to stimulate hippocampus neurogenesis in rats and non-human primates determines their effectiveness [110, 241-243].

The hippocampus inherent structure favors a partition of memory input from the entorhinal cortex, which enhances the mouse's contextual and spatial learning processes [244] as well as in human [245] and although this ability is native to the dentate gyrus circuitry, it is significantly improved by the integration of additional neurons into the current circuits [114, 246-248]. A neurotoxin-induced lack of neurogenesis in rats results in symptoms resembling depression, as evidenced by the animals' decreased performance in the forced swimming or sucrose consumption tests, according to research. As long as neurogenesis is not suppressed, fluoxetine can reverse these symptoms. In addition, depressive-like symptoms start to show up 4 weeks later, which is how long it takes for new neurons to be recruited into memory circuits [242].

The existence of extra-neurogenic actions of antidepressants exerted on neural plasticity may help to partially reconcile the fact that not all antidepressant actions require neurogenesis and that the reduction of neurogenesis is not directly causing anxiety/depression-like behavior [112, 249]. Given that serotonin is a key regulator of cell division, neuronal migration and differentiation, as well as processes like apoptosis, axon branching, and dendritogenesis, SSRI antidepressants like fluoxetine appear to have a dual action, being able to enhance the generation of new neurons while also facilitating their plastic functions [118]; all of this would favor the recovery of dysfunctional conditions like depression, stress, or anxiety.

And at last, it is evident that people with major depressive disorder have disturbed FGF signaling. While FGF9 and FGF12 are elevated in the anterior cingulate and dorsolateral prefrontal cortex of depressive patients, levels of FGF1, FGF2, FGFR2, and FGFR3 decrease in cortical regions [250] and it has been discovered that during prolonged stress, precursors for myelinating oligodendrocytes secrete FGF2, which may stop glutamate abnormalities and counterproductive depressive characteristics [251]. So, since intracerebroventricular injection of FGF2 produced antidepressant effects, which were also seen after FGF2 infusion into the prefrontal cortex in chronic unpredictable stress models of depression, direct relationships were hypothesized [252]. Particularly remarkable is the possibility that FGF2 indirectly increased neuronal activity by stimulating astrocyte proliferation, which is inhibited in rodent depression models. The therapeutic effects of tricyclic antidepressants and selective serotonin reuptake inhibitors may also be influenced

by increased FGF2 levels [253]. When monoamine oxidase inhibitors are used to treat cortical astrocytes, both the low and high molecular weight variants of FGF2 increase [254], and antidepressant treatments increased FGF2 expression in the cerebral cortex and hippocampus in rodents [255].

4.2.6 Structural and functional brain changes

Scientists are no longer certain that depression is caused by a simple chemical imbalance. Some researches and treatments have been in conflict with that assumption, and the condition is extremely complex. As a result, researchers are looking into a variety of other explanations: according to brain imaging studies, patients with depression have less grey matter in several brain regions, including the cingulate cortex, the hippocampus and the amygdala [256]. As a result, some experts believe that depression is caused by anatomical variations rather than neurochemical ones. Depression, in essence, reduces the regions of the brain that are necessary for controlling our emotions and making decisions to take care of ourselves, making it difficult for patients to recover from feelings of despair. Advances in technology and computing have had a huge impact on our understanding of brain structure and function over the last quarter-century, but meaningful insights have only begun to emerge in the last decade, as it became possible to scan larger numbers of patients and reliably combine neuroimaging data.

Structural investigations in depressed individuals have consistently demonstrated that hippocampus volume is reduced in serious depression compared to persons who are not depressed [257], and some investigations have linked the degree of volume loss to the length of untreated lifelong depression [258, 259]. Thus, according post-mortem analyses, the volume of the dentate gyrus in untreated individuals with depression is around half that of a non-depressed comparison group and a group of patients with depression who underwent therapy [113, 260]. Whether the smaller hippocampus can be reversed with treatment, and whether it is required for an antidepressant response, is yet to be shown in clinical studies.

Functional neuroimaging reveals the brain networks responsible for important functions including self-awareness, mood regulation and altered reward pathways linked to anhedonia. Studies looking at these networks in depressive conditions have discovered that while the insula and dorsal lateral prefrontal cortex are hypoactive in people with depression, the amygdala and other structures, such the subgenual anterior cingulate, are typically more active and connected [261, 262]. Nevertheless, the brain alterations that have

been linked to severe depression are associated with a very diverse range of clinical presentations and are consequently quite variable, making it challenging to repeat findings from one research to another [263]. Ongoing research links pre-existing brain abnormalities to the selection of the best therapy. Varying methods of treatment, such as medicine, psychotherapies, and stimulation treatments have different impacts.

4.2.7 Genes

Major depressive illness is somewhat heritable, according to twin and adoption studies [264]. When compared to people who do not have first degree relatives who have been diagnosed with major depression, first degree relatives of patients with major depression have a three times higher chance of acquiring major depressive disorder. Unfortunately, it has been challenging to accurately identify the related genes. Up until 2018, few gene hits have been reproduced from genome-wide association studies (GWAS), which have so far discovered a number of genes, each with a tiny impact [265]. Current GWAS have, however, started to reliably identify risk alleles and have demonstrated repeatable findings that could start to shed light on the pathophysiology of major depressive disease [266]. Studies that looked at more uniform patients with severe disease are also encouraging since they have found loci that increase the likelihood of major depressive disorder [267]. Given the wide range of results, studies of epigenetic variables are currently being conducted in addition to genomic ones.

4.2.8 Environmental milieu

Major depressive illness may be precipitated by, or perhaps be caused by, certain life experiences, which has long been acknowledged [268]. Early research, for instance, looked at the effects of stressful life events that occurred close together with severe depressive episodes, such as up to a year before the onset of the episode [269, 270]. Adults might become stressed out by life-threatening or persistent illnesses, money problems, losing their jobs, being divorced, losing a loved one, and becoming the target of violence. Even though a portion of patients is susceptible to the consequences of stressful life events and another group appears relatively resilient [271, 272], potentially reflecting biological predispositions, the connections between stressful life events and depression have been proven to be substantial. A second strategy has looked at childhood problems, including as abuse and trauma, which seem to be linked to a propensity for significant depression in adulthood when faced with stressful life events.

Such studies have stratified adversity and found at least two types of molecular variations that predispose people to major depressive disorder: molecules whose effects are dependent on adversity and molecules whose effects are always present, regardless of adversity [273]. Both purely epigenetic processes and gene-environment interactions have been revealed in these experiments. Early childhood trauma is connected to depression in later life by alterations in the HPA axis, specifically glucocorticoid receptor hypofunction, in both animal and clinical research [227]. In particular, early exposure to adversity throughout childhood causes DNA methylation at important locations in the glucocorticoid receptor gene, lowering the expression of this gene [274]. Therefore, exposure to sexual and physical abuse or emotional neglect affects the risk, severity, and recurrence of severe depression [275].

4.2.9 Epigenetics

The thrilling finding that some genes are activated by environmental conditions during the past ten years has shown that the environment may have a direct influence on how genetic information is interpreted. This process has been termed as the gene-environment interaction and it is governed by epigenetic processes [276]. The study of this phenomenon has revealed perhaps novel paths and processes through which environmental variables may influence the neurobiology of the brain affecting, for instance, neural plasticity [277, 278].

The possibility for clinical treatments to be based on these findings must first be explored in studies establishing therapeutic techniques that can modulate harmful epigenetic effects, despite the fact that these results are fascinating and have inspired future genetics research.

4.3 Pharmacotherapy

The first aim of pharmacotherapy for major depressive disorder is the enhancement of monoaminergic neurotransmission [279] even if newer drugs have different target as NMDA receptor, melatonin or GABA receptor. Most of the antidepressant that have monoaminergic pathway as target produce effects on the synapse, which impacts on intracellular signaling, albeit the precise mechanism is still unknown [221]. The alteration of second messenger pathways has repercussions on gene expression, neurogenesis and synaptic plasticity, that ultimately lead to therapeutic benefits [280]. The pharmacological effects of antidepressants are diverse and complicated, and the grouping of antidepressants into classes based on their principal pharmacological action is overly simplistic, but it

remains useful in practice, when the clinical effects of antidepressants are broad and overlapping.

Trials examining the potency of antidepressant drugs have traditionally focused on efficacy, and in clinical contexts have usually assessed this potency somewhat crudely, seeking a 50% reduction in symptoms [281]. Some of the earliest developed antidepressants, such as the tricyclics and MAOIs, remain among the most efficacious drugs available, but are in minimal use today [282]. In most settings, and in particular at the beginning of treatment, these medications have been displaced by newer drugs with more pharmacologically selective actions and, consequently, fewer side-effects [283].

Therefore, over the last quarter of a century, the selective serotonin reuptake inhibitors have become the first-line antidepressant medication class, despite only moderate efficacy that can take weeks to produce a measurable benefit. Furthermore, SSRIs can also produce significant side-effects that patients do not tolerate, including sexual dysfunction, weight gain, nausea, and headaches [284].

In a network meta-analysis that compared efficacy and acceptability of antidepressant medications in the acute treatment of major depressive disorder [285], all pharmaceuticals, including the two WHO-recommended essential antidepressants amitriptyline and clomipramine, showed more effectiveness than placebo, with amitriptyline and other dual-acting medications (such as mirtazapine, duloxetine, and venlafaxine) ranking highest. Only agomelatine and fluoxetine were more palatable than placebo in terms of acceptability, but other antidepressants were comparable, with the exception of clomipramine, which was accepted more badly than placebo. The research looked at head-to-head comparisons as well, and many of the same medications performed better than other antidepressants (such as amitriptyline, mirtazapine, venlafaxine, paroxetine, and vortioxetine); nevertheless, analyses using aggregated data cannot detect effects at the individual level, thus in reality, prescribing antidepressants is still a clinical decision [285].

Despite the high rate of placebo reactions, the discovery that antidepressants are a successful therapy for severe depressive illness is comforting. Additionally, certain drugs may be designed to treat specific forms of depression and are likely effective and tolerable for those conditions. Giving sedative antidepressants for depression along with anxiety or sleeplessness and turning on antidepressants for depression combined with psychomotor retardation are two instances. Although it is not yet practical to choose the optimal antidepressant merely based on depression symptoms, this knowledge combined with clinical expertise helps educate and enhance care [285].

4.4 Flinders Sensitive Line as a genetic model of depression

Flinders Sensitive Line rats are a genetically verified model of depression. The main purpose of developing FSL rats was to establish a breed that was genetically resistant to the effects of the anticholinesterase drug diisopropylfluorophosphate (DFP), in order to understand the processes involved in DFP tolerance. In contrast, the generated strain was revealed to be genetically more vulnerable to DFP. The observation of higher sensitivity to cholinergic agonists in depressed persons than in normal controls [286] strengthened the potential that the FSL rat may be a model for depression. FSL rats resemble depressed people in that they exhibit symptoms such as reduced appetite, poor psychomotor performance, sleep and immune system problems, all of which are indicators of depressive disorder. Immune abnormalities, the development of certain forms of anxiety, a greater prevalence of irritable bowel syndrome and asthma are all related with this illness. Instead, they exhibit normal cognitive processes and hedonic responses: anhedonia and cognitive deficits, in addition to normal reward-related behavior and food-motivated learning, are the most prominent instances of the disparity with respect to symptoms of depression in humans.

Since known antidepressants reduced rat immobility time in the Forced Swim Test, the FSL rat depression model is commonly used to test suspected or proven antidepressants [287]. The FST is a validated behavioral test used to evaluate antidepressant medicines, experimental treatments aimed at inducing or preventing depressed states, and the actual depressive condition of animals representing depression models, such as the FSL. It is a commonly utilized test because it exposes the animal to stress, which is linked to a higher risk of serious depression. During the FST, the animal is placed in a container filled with water from which it cannot escape; initially, it will try to escape but will soon exhibit immobility (indicated as floating without carrying out any movement other than those necessary to keep the nose out of the water). The duration of the test varies depending on the species being tested: for rats, two separate sessions are performed 24 hours apart (handling phase or pre-test and test phase). A proportion of rat immobility more than 50% of the time during the test phase indicates the existence of depressed moods.

CHAPTER 5: MATERIAL AND METHODS

5.1 Animals

The animals used - Sprague Dawely rats and Flinders Sensitive Line (FSL) rats - have been reared and housed in standard cages with water and food ad libitum. The environment temperature has been maintained at 21 ± 1 °C, the humidity was 50 ± 5 % and the light/dark cycle was 12-12 h. Adult male rats (2-3 month-old) were killed to perform brain explant for electrophysiological experiment execution. The study was carried out in accordance with the current Italian legislation on animal experimentation (D.lgs 26/2014; protocol codes: BEF09.N.4CO and BEF09.N.O0C, approved by the Institutional Review Board of Italian Health Ministry), which is in strict accordance with the European Council Directives on animal use in research (n. 2010/63/EU).

5.2 Forced Swim test

FSL animals were individually placed in a vertical glass cylinder (58 cm height, 20 cm diameter) containing water (25 ºC) to a height of 50 cm. Two swimming sessions were conducted: a 10 min pre-test followed 24 h later by a 5 min test [288]. The total duration of immobility behaviour was recorded during the second session of 5 min.

Immobility was defined as floating passively in an upright position in water with only small movements necessary to keep the head above the water surface. FSL rats were considered as depressed and thus used for electrophysiology experiments only if the percentage of immobility time was higher than 50% (FSL average of % immobility time 55.66% ± 1.35 *vs* SD average of % immobility time 36.89% ± 3.46, Student t test *** $p< 0.001$).

5.3 Slice preparation

After anesthetization with isoflurane and killing by decapitation, brains were quickly removed from SD and FSL rats (SD: n = 26 from different litters; FSL: n = 32 from different litters) and incubated in chilled oxygenated solution containing in mM: 110.0 choline Cl^-, 2.5 KCl, 1.3 NaH_2PO_4, 25.0 $NaHCO_3$, 0.5 $CaCl_2$, 7.0 $MgCl_2$, 20.0 dextrose, 1.3 Na^+ ascorbate, 0.6 Na^+ pyruvate, 5.5 kinurenic acid (pH: 7.4; 320 mOsm). Hippocampal transversal slices (400 μm thick) or brainstem coronal slices (300 μm thick) were obtained by vibrating microtome (Campden Instruments) and allowed to recover in oxygenated artificial Cerebrospinal Fluid (aCSF) containing in millimolar: 125.0 NaCl, 2.5 KCl, 1.3

NaH_2PO_4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 1.3 $MgCl_2$, 1.3 Na^+ ascorbate, 0.6 Na^+ pyruvate, 10.0 dextrose (pH: 7.4; 320 mOsm).

The slices were kept in steadily oxygenated aCSF for at least 1 h at room temperature (experiments in CA2) or 3 h (experiments in dorsal raphe nucleus - DRN) to allow cell recovery before electrophysiological recordings. Slices were then transferred into a recording chamber where they were continuously superfused throughout the electrophysiological recordings with oxygenated aCSF at a rate of 3 mL/min.

5.4 Analysis of GIRK currents

Several scientific works have already proven that serotonin 5HT1A receptors are able to open GIRK channels, generating a hyperpolarizing outward potassium current [289, 290]. Thus, 5HT1A receptor activation and its modulation related to FGFR1-5HT1A heteroreceptor complex formation can be analyzed using whole cell patch clamp technique by monitoring the holding current (Ih) trend and input resistance (IR) deviations, as previously described [19]. Indeed, changes in K^+ conductance across the plasma membrane lead to input resistance decrease and Ih – required to keep membrane potential constant at the prefixed value of -70mV – increase.

Whole cell electrophysiological recordings were performed under visual guidance using a Zeiss Axioskop microscope equipped with an infrared camera connected to a monitor. Furthermore, recordings were carried out using an Axopatch-200B amplifier and WinWCP software for data acquisition and analyses. As whole cell recording technique consists of the connection between intracellular environment and recording pipette solution, the recordings pipettes were filled with an internal solution containing in mM: 126 potassium gluconate, 8 NaCl, 0.2 EGTA, 10 HEPES, 3 Mg_2ATP, 0.3 GTP (pH = 7.2; 290 mOsm).

No correction was made for junction potential between internal and external solutions.

5.5 CA2 pyramidal neurons whole cell analysis

Somata of neurons to be registered were identified in CA2 pyramidal cell layer based on their typical shape, localization and high membrane capacitance and low resistance (C = 229.8 ± 28.6 pF; IR = 242.8 ± 27.5 MΩ). After whole cell configuration establishment, we measured resting membrane potential (RMP) and in voltage clamp mode input resistance and membrane capacitance (C) by recording holding current in response to 300 ms, 5 mV hyperpolarizing step. The Ih variation was recorded in voltage clamp mode keeping the membrane potential at -70 mV. The current necessary to induce action potential firing was

measured in current clamp mode by injecting increasing 50 pA depolarizing steps. If the visually identified neuron showed typical electrophysiological features of CA2 neurons [291], we started to record GIRK currents as described below.

An important assumption that it can be done approaching a heteroreceptor complex between an RTK and a GPCR is that the activation of the protomers establishing this interaction could alter the capability of GPCR to interact and affect GIRK channel-mediated current. Therefore, using GIRK current as electrophysiological readout it is possible to study R–R interactions through its modulation in presence of different receptor agonists. We quantified the opening of GIRK channels, evaluating IR decrease, a positive increase of the Ih necessary to maintain the membrane potential at -70 mV, and inward current increase at hyperpolarized potentials (about −110/120 mV) by applying hyperpolarizing voltage ramps.

To evaluate if FGFR1-5HT1A interaction might result in a modulation of 5HT1A activity, GIRK current dynamics were analysed in presence of single or combined treatment with the agonists for the receptors under investigation. In particular, after break into whole cell configuration, the cytoplasm goes through a dialysis with the pipette recording solution that induce an Ih modification and stabilization. Therefore, in each cell a baseline of stable Ih was obtained before agonist application. Once Ih reached a stable value – for at least 10 minutes – a single or combined bath application of the 5HT1A agonist 8-OH-DPAT (5 μM; BioTechne s.r.l., Italy) and/or selective FGFR1 agonist SUN11602 (10 μM; BioTechne s.r.l., Italy), were performed.

5.6 Loose Seal Cell Attached Recordings in DRN serotonergic neurons

Neurons within DRN were visualized using a Zeiss Axioskop microscope equipped with an infrared camera connected to a monitor. Recordings were carried out using an Axopatch-200B amplifier and WinWCP software for data acquisition and analyses. Neurons selected for recordings were localized based on the rat brain atlas of Paxinos and Watson [292], and identified by means their typical firing properties [4]. Neurons showing an overall healthy appearance and clearly visible intact primary neurites were considered and recorded to evaluate firing frequency [293]. Electrodes were pulled from thick-walled borosilicate and each pipette was used for several recordings and was replaced if tissue debris attached to the tip. After positioning the pipette in gentle contact with the cell membrane, development of loose seal was monitored recording the firing activity of the cell. When was possible to record a spontaneous firing activity, gentle suction was slowly applied until detected spikes

increased their amplitude on the background noise. When there was the doubt that the mechanical stretches may have interfered with spiking activity or when the firing frequency was higher or lower than 2-4 Hz, the cell was discarded. After the recording of a 5 minutes long steady baseline, we performed a single or combined bath application for 2 minutes of the 5HT1A agonist 8-OH-DPAT (1 μM) and/or selective FGFR1 agonist SUN11602 (10 μM).

5.7 DRN serotonergic neuron whole cell analysis

DRN neuron whole cell analysis was carried out based on the protocols used for CA2 whole cell analysis and loose patch analysis. In fact, after the recording of a 5 minutes baseline using loose patch technique to identify the serotonergic neurons showing 2-4 Hz firing frequency, we moved to the whole cell recording configuration. At this point, the cells were recorded in voltage clamp mode and in each cell RMP and IR were determined. Subsequently, when we obtained the recording of a baseline with a stable Ih, we applied for 2 minutes in the recording bath 5HT1A agonist (1 μM 8-OH-DPAT), FGFR1 agonist (10 μM SUN11602) or a mixture of them. GIRK currents were quantified, as described in CA2 experiments.

5.8 Field Recordings

The synaptic plasticity was evaluated by measuring the ability to elicit long-term potentiation in SD and FSL rats. Field excitatory postsynaptic potentials (fEPSPs) were elicited in the CA1 stratum radiatum by stimulating the Schaffer collateral pathway. The stimulation intensity that produced a half-maximal response was chosen for test pulse and tetanic stimulation. Only those slices which produced the field excitatory postsynaptic potentials of 1 mV or higher in amplitude were accepted for experiments. Low-frequency test pulses (at 30-seconds intervals) were applied to elicit baseline responses. Once obtained a stable baseline of approximately 20 minutes, the 5HT1A agonist 8-OH-DPAT (5 μM) and/or selective FGFR1 agonist SUN11602 (10 μM) was applied in the perfusion bath for 10 minutes. After the agonist application, the agonist wash-out was performed and once obtained a stable baseline, the Schaffer collateral pathway was simulated applying the LTP protocol consisting in a stimulus pattern including 3 trains of 100 Hz applied for 1 sec separated by an interval of 20 sec [294]. The fEPSP was then monitored by recordings for 40 min. Slope (between 10% and 80% of max) of the fEPSP was analyzed and taken as

measures of synaptic strength; values were normalized to the mean value obtained over the last 20 min of the baseline period and expressed as a percent of this baseline value.

5.9 Data Analysis

Data are expressed as mean ± SEM. The number of samples (n) in each experimental condition is indicated in figure legends. All data were analysed using the commercial program GraphPad PRISM 6.0 (GraphPad Software, USA). Statistical analysis was performed by one-way or two-way analysis of variance (ANOVA) followed by Tukey's post-test. The significance threshold was established at $p=0.05$.

CHAPETR 6: RESULTS

6.1 Electrophysiological analysis of 5HT1A-activated GIRK currents and their modulation by FGFR1 agonist

6.1.1 Hippocampal neurons in SD and FSL rats

GIRK channels represent the main effectors of 5HT1A receptors in different types of neurons, such as serotonergic raphe neurons and pyramidal CA1 neurons [15, 19]. Based on our previous findings [19], we verified the existence of 5HT1A-activated GIRK-mediated current in CA1 and CA2 pyramidal neurons and its modulation by 5HT1AR-FGFR1 heteroreceptor complex activation in our experimental groups. Voltage-clamp experiments performed in SD rats showed an outward GIRK current induced by a ten-minute-long bath application of 5μM 8-OH-DPAT both in CA1 neurons, confirming our previous results [19], and CA2 nerve cells, which partially reversed upon agonist washout (Fig.9A). The 8-OH-DPAT-induced outward GIRK current was associated with a decreased input resistance, indicating channel opening (Fig.9B). Very similar results were obtained by recording CA1 and CA2 neurons in FSL rats, even though the amplitude of GIRK channel-mediated current elicited by 5HT1A receptor activation was basically smaller than that recorded in SD rats (Fig.9D,F). In both experimental groups, the 8-OH-DPAT-induced GIRK outward current matched the potassium reversal potential of –100 mV estimated considering the free potassium concentration inside (126 mM) the cell and outside (2.5 mM). The current-voltage relationship slope was steeper at hyperpolarized potentials in the voltage ramp protocol due to GIRK channel-induced inward rectifying response (Fig.9C). These results support the presence of Gi/o mediated GIRK current in CA1 and CA2 hippocampal neurons of SD and FSL rats. FGFR1 activation in CA1 and CA2 neurons of SD and FSL rats, following the application of the specific agonist SUN11602 (10 μM), did not affect the holding current (Fig.9D-F), pointing out its failure in GIRK channel opening. Co-application of 5 μM 8-OH-DPAT and 10 μM SUN11602 to activate FGFR1-5HT1A hetereceptors significantly reduced 5HT1A-induced GIRK currents (Fig.9D-F) and counteracted the IR decrease elicited by 5HT1A-induced GIRK activation (Fig.9E-G) in CA1 and CA2 neurons of SD rats, but only in CA2 neurons of FSL rats. Indeed, the co-application of the agonists mentioned above in CA1 neurons of FSL rats induced a GIRK current similar to that obtained with 5 μM 8-OH-DPAT alone (Fig.

9D). Following the washing out of the drugs from the bath, all the observed effects were reversed.

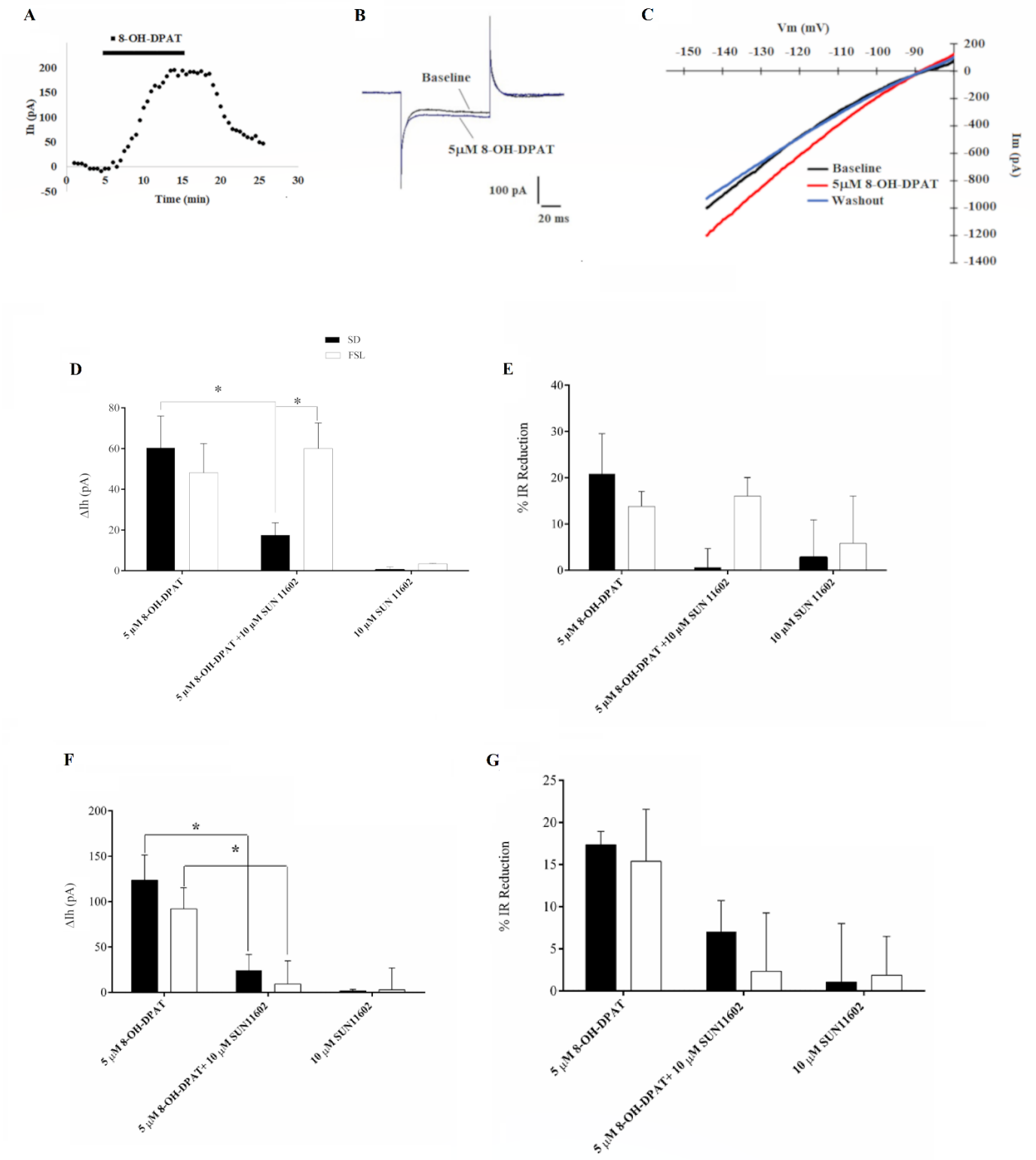

Figure 9 8-OH-DPAT-activated inwardly rectifying K^+ conductance in CA1 and CA2 neurons of Sprague Dawley and Flinders sensitive line rats. A) Time-course of a

representative experiment showing the effect on holding current (Ih) at -70mV of 5HT1A receptor agonist 8-OH-DPAT bath application mediated by inwardly rectifying K^+ conductance in CA2 hippocampal neurons. B) Representative trace showing that 8-OH-DPAT application induced an input resistance (IR) decrease, suggesting a membrane channel opening in CA2 hippocampal neurons. C) Representative current-voltage (Im-Vm) correlation plot. Traces are recorded before (Baseline; black line), during agonist application (5 μM 8-OH-DPAT; red line) and after washout (Washout; blue line). D) Summary graph including agonists tested on CA1 neurons. Combined application of 5 μM 8 OH-DPAT together with FGFR1 agonist 10 μM SUN 11602 significantly reduced the amplitude of the GIRK current, measured at -120mV, induced by 5HT1A activation in SD CA1 pyramidal neurons, but not in FSL CA1 pyramidal neurons. Two-way analysis of variance (ANOVA) F (2, 44) = 8,473, p= 0.0008; Tukey's post hoc: *p <0.05. Number of recorded cells (n) SD: 8-OH-DPAT (8), 8-OH-DPAT + SUN 11602 (10), SUN 11602(4); FSL: 8-OH-DPAT (12), 8-OH-DPAT + SUN 11602 (9), SUN 11602(7). E) GIRK channel opening decreased IR in SD CA1 neurons. As expected, while combined agonist treatment tended to reduce the IR drop elicited by 5HT1A induced GIRK activation in SD rats, in FSL rats there were no changes in IR. Number of recorded cells (n) SD: 8-OH-DPAT (8), 8-OH-DPAT + SUN 11602 (10), SUN 11602(4); FSL: 8-OH-DPAT (12), 8-OH-DPAT + SUN 11602 (9), SUN 11602(7). F) Combined application of 5 μM 8 OH-DPAT together with FGFR1 agonist 10 μM SUN 11602 in CA2 hippocampal neurons significantly reduced the amplitude of the GIRK current induced by 5HT1A activation in SD and FSL neurons. Two-way analysis of variance (ANOVA) F (2, 43) = 10,12, p= 0.0002; Tukey's post hoc: *p <0.05. G) GIRK channel opening decreased input resistance in CA2 neurons. As expected, combined agonist treatment tended to reduce the IR drop elicited by 5HT1A induced GIRK activation both in SD rats and in FSL rats. Two-way analysis of variance (ANOVA) F (2, 44) = 3,686, p= 0.0331. Number of recorded cells (n) SD: 8-OH-DPAT (8), 8-OH-DPAT + SUN 11602 (6), SUN 11602(5); FSL: 8-OH-DPAT (10), 8-OH-DPAT + SUN 11602 (10), SUN 11602(10). All data are expressed as mean ± SEM.

6.1.2 Dorsal raphe serotonergic neurons

5HT1A receptor activation in dorsal raphe neurons induces a hyperpolarizing current by activating GIRK channels [15]. This hyperpolarization can modify the serotonergic neuron autorhythmic firing activity, thus controlling the release of serotonin on target neurons. For this reason, the effects of 5HT1A and FGFR1 agonists on the spontaneous firing activity

of dorsal raphe serotonergic neurons were assessed in SD and FSL rats using the loose patch approach. Neurons with a stable firing activity showing a frequency ranging from 2 to 4 Hz (Fig.10A) were considered. A two-minute-long bath application of 1µM 8-OH-DPAT induced a drastic decrease in spontaneous firing in both groups of rats, which was maintained for at least 20 minutes, and then partially reversed after the end of the application (Fig.10C). No difference was found between SD and FSL rats. On the contrary, in SD rats only, co-application of 1 µM 8-OH-DPAT and 10 µM SUN11602 counteracted neuronal firing decrease (Fig.10B-C). However, the FGFR1 activation by specific agonist SUN116052 (10 µM) application did not affect spontaneous neuron firing in both animal groups (Fig.10B). These data led us to hypothesize that 5HT1A-induced GIRK current in dorsal raphe serotonergic neurons could be modulated by co-application of 5HT1A and FGFR1 agonists in SD rats, and that this modulation does not occur in FSL serotonergic neurons.

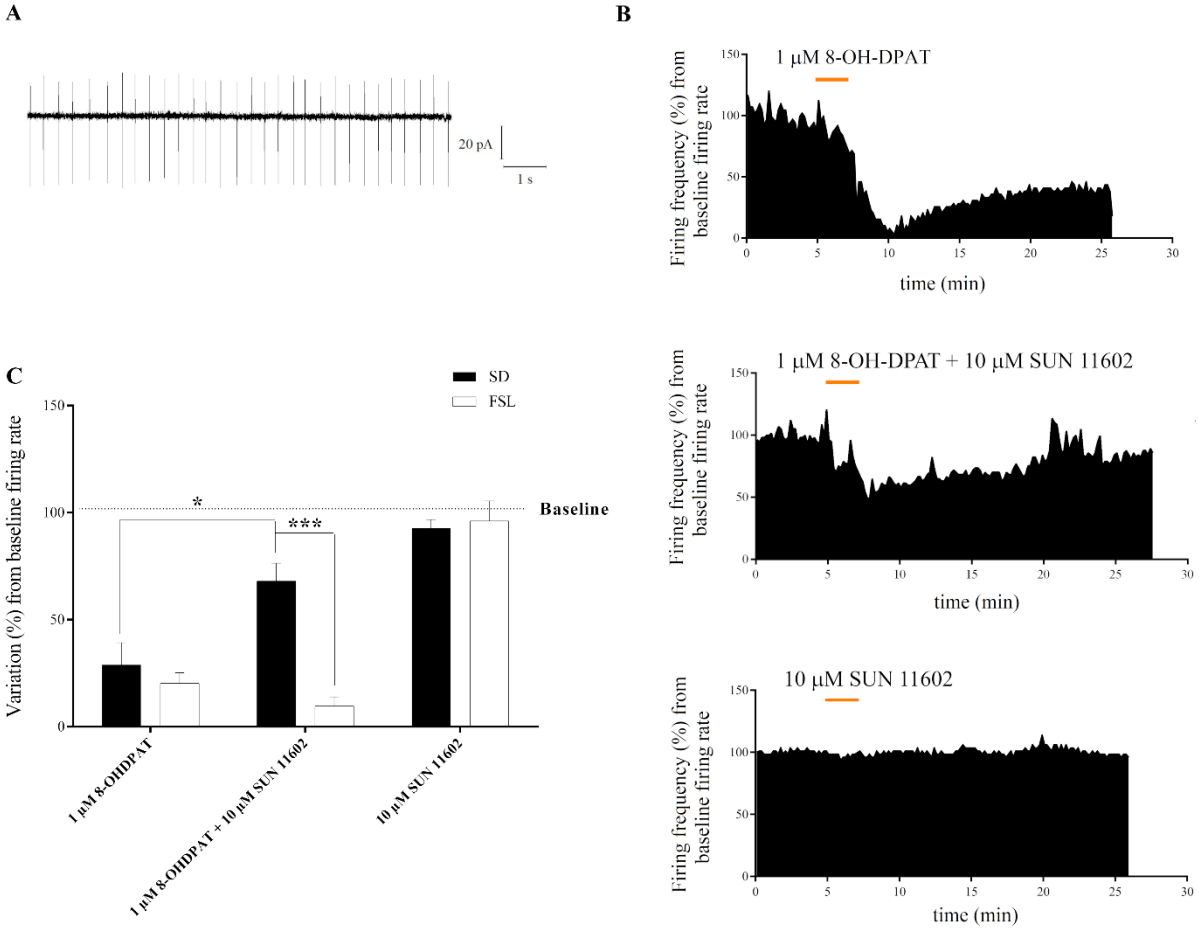

Figure 10 Analysis of dorsal raphe nucleus (DRN) neuron firing properties after 5-HT1A and/or FGFR1 agonist bath application. A) Trace shows a segment of the loose-patch

recording explaining the firing characteristics of the recorded DNR serotonergic neurons. B) Representative time – course variation of DRN neuron firing activity after 2-minutes-long bath application of 1μM 8-OH-DPAT, 10 μM SUN11602 or a combination of them. C) Variation of DRN neuron firing frequency induced by bath application of 1 μM 8-OH-DPAT and/or 10 μM SUN 11602 in Sprague-Dawley (SD) and Flinders Sensitive Line (FSL) rats. All data are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) $F(2, 38) = 51{,}48$, $p < 0.0001$; Tukey's post hoc: * $p < 0.05$; *** $p < 0.001$. Number of recorded cells (n) SD: 8-OH-DPAT (6), 8-OH-DPAT + SUN 11602 (6), SUN11602 (6); FSL: 8-OH-DPAT (11), 8-OH-DPAT + SUN 11602 (7), SUN11602 (8).

To verify this hypothesis, we measured, using the whole cell patch clamp technique, the GIRK current and its modulation by the aforementioned agonists in dorsal raphe serotonergic neurons of SD and FSL rats. In our experimental conditions, a two-minute-long bath application of 1μM 8-OH-DPAT elicited a small outward current at V-holding of -70 mV that reversed upon agonist washout (Fig.11A) in both groups of rats. As described for CA1 and CA2 neurons, input resistance tended to decrease also in serotonergic neurons during 8-OH-DPAT application, indicating GIRK channel opening (Fig.11B). Furthermore, voltage ramp protocols showed a potassium-like reversal potential and an inward rectifier nature of the 8-OH-DPAT-induced current (Fig.11C). Bath application of the specific FGFR1 agonist, SUN11602 (10 μM), did not affect holding current in SD and FSL rats, suggesting that FGFR1 activation could not open ion channels. The combined application of 5 μM 8-OH-DPAT and 10 μM SUN11602 failed to significantly vary the holding current (Fig.11D) in SD rats, indicating that 5HT1A-FGFR1 heterocomplex activation was able to reduce GIRK current amplitude in dorsal raphe serotonergic neurons of this group of rats, as occurs in CA1 and CA2 hippocampal neurons. This mechanism possibly underlies the effect exerted by agonist co-application on the spontaneous firing activity of serotonergic neurons in SD rats. On the contrary, in FSL serotonergic neurons, the agonist co-application did not reduce the activity of the 5HT1A receptor on the GIRK channels, suggesting a disturbance in FGFR1-5HT1A heteroreceptor complex operation. This result is consistent with the lack of effect of heterocomplex activation in counteracting 5HT1A-induced serotonergic neuron firing activity decrease. The measurement of membrane input resistance supported the difference found in the GIRK current elicited by agonist co-application between SD and FSL rats (Fig.11E).

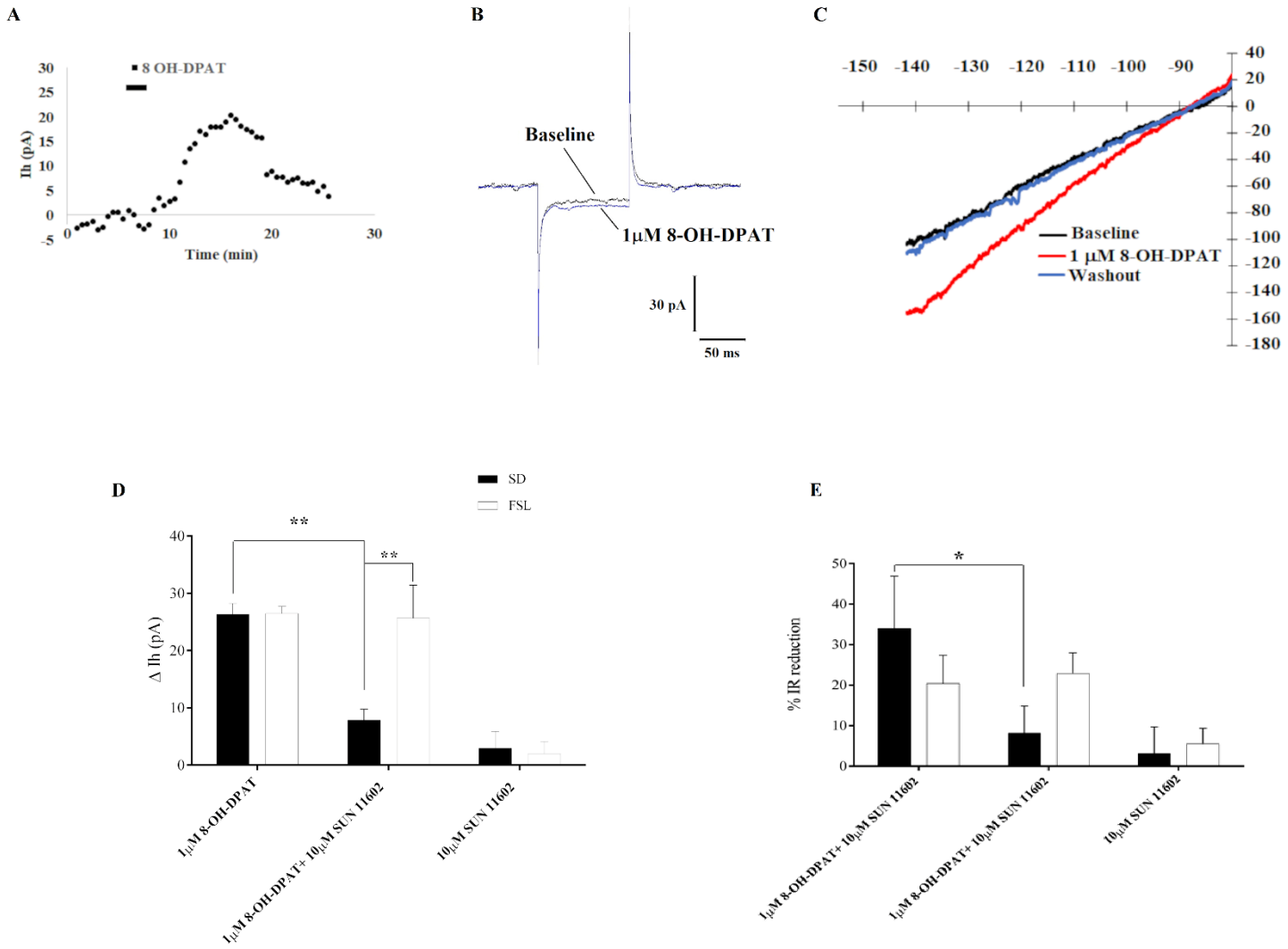

Figure 11 8-OH-DPAT-activated inwardly rectifying K+ conductance in dorsal raphe nucleus serotonergic neurons. A) Time-course of a representative experiment showing the effect on holding current (Ih) at -70 mV of 5HT1A receptor agonist 8-OH-DPAT bath application mediated by inwardly rectifying K+ conductance. B) 8-OH-DPAT application induces an input resistance (IR) decrease, suggesting a membrane channel opening. C) Representative current-voltage (Im-Vm) correlation plot. Traces are recorded before (Baseline; black line), during agonist application (1 mM 8-OH-DPAT; red line) and after washout (Washout; blue line). D) Whole cell patch clamp analysis of DNR neurons showed that the activation of 5HT1A induced an outward Gi/o-mediated current in DRN serotonergic neurons due to GIRK channel opening. Two-minutes-long combined application of 1 µM 8-OH-DPAT together with 10 µM SUN 11602 significantly reduced the amplitude of the 5HT1A-induced GIRK current at –120 mV in SD but not in FSL rats.

Two-way analysis of variance (ANOVA) F (2, 31) = 26,82, p < 0,0001; Tukey's post hoc: ** p <0.01. Number of recorded cells (n) SD: 8-OH-DPAT (5), 8-OH-DPAT + SUN 11602 (7), SUN 11602 (4); FSL: 8-OH-DPAT (7), 8-OH-DPAT + SUN 11602 (7), SUN 11602 (7); E) GIRK channel opening decreased IR of DRN neurons. Combined agonist treatment tended to reduce the IR drop elicited by 5HT1A-induced GIRK activation in SD rats but not in FSL. Two-way analysis of variance (ANOVA) F (2, 32) = 5,213 p = 0.0110; Tukey's post hoc: * p <0.05. Number of recorded cells (n) SD: 8-OH-DPAT (5), 8-OH-DPAT + SUN 11602 (7), SUN 11602 (4); FSL: 8-OH-DPAT (6), 8-OH-DPAT + SUN 11602 (8), SUN 11602 (8). All data are expressed as Mean ± SEM.

6.1.3 Synaptic plasticity evaluation

High-frequency stimulation (HFS) of stratum radiatum induced a comparable LTP in SD and FSL rats (Fig.12-13) that reached a stable value about 30 min after HFS. In SD rats, LTP induction was fully prevented by 8-OH-DPAT application. On the contrary, the co-application of 5HT1AR and FGFR1 agonists did not block LTP induction (Fig.12). In FSL rats, bath application of 8-OH-DPAT alone or co-application of 8-OH-DPAT and SUN 1602 induced complete inhibition of LTP (Fig.13).

As expected, the activation of 5HT1A receptors induces LTP inhibition both in SD and FSL rats, likely due to GIRK channel-mediated hyperpolarization of CA1 neurons. Consistently with whole cell experiment results in SD rats, FGFR1-5HT1AR co-activation allows LTP induction as not eliciting GIRK currents. Differently, due to the possible absence of functional 5HT1A-FGFR1 heterocomplexes in FSL rats, the co-application of agonists makes impossible LTP induction by inducing GIRK currents.

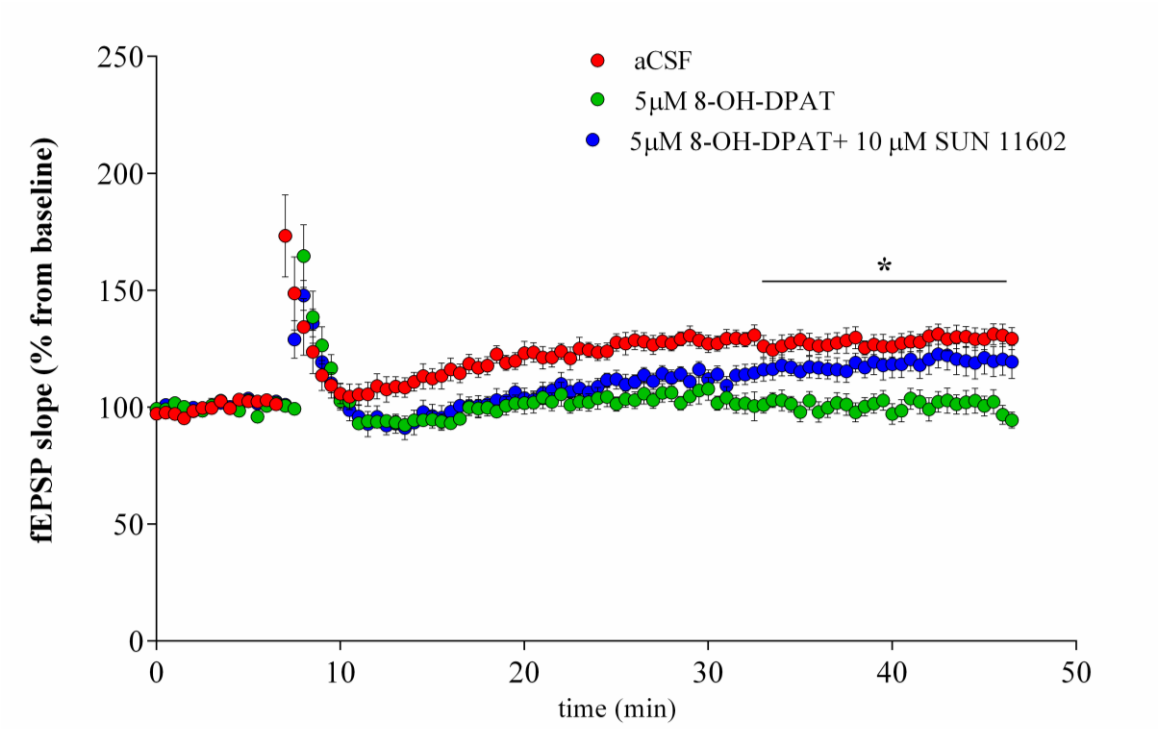

Figure 12 fEPSP slope (10% and 80% of max) recorded before and after the Schaffer Collateral high-frequency stimulation in SD rats. Values were normalized to the mean value obtained over the last 20 min of the baseline period and expressed as a percentage of this baseline value (n = 9 slices for aCSF, n= 7 for 5 µm 8-OH-DPAT, n = 10 slices for 5 µm 8-OH-DPAT + 10 µm SUN11602).

Two-way ANOVA test: $F(2, 782) = 245{,}3$, $p < 0{,}0001$; Tukey's multiple comparisons test: aCSF vs 5µm 8-OH-DPAT ** $p < 0{,}01$ from 20 to 40 min of recoding post HFS, 5µm 8-OH-DPAT vs 8-OH-DPAT + 10 µm SUN11602 * $p < 0{,}05$ from 23 to 40 min of recording post HFS

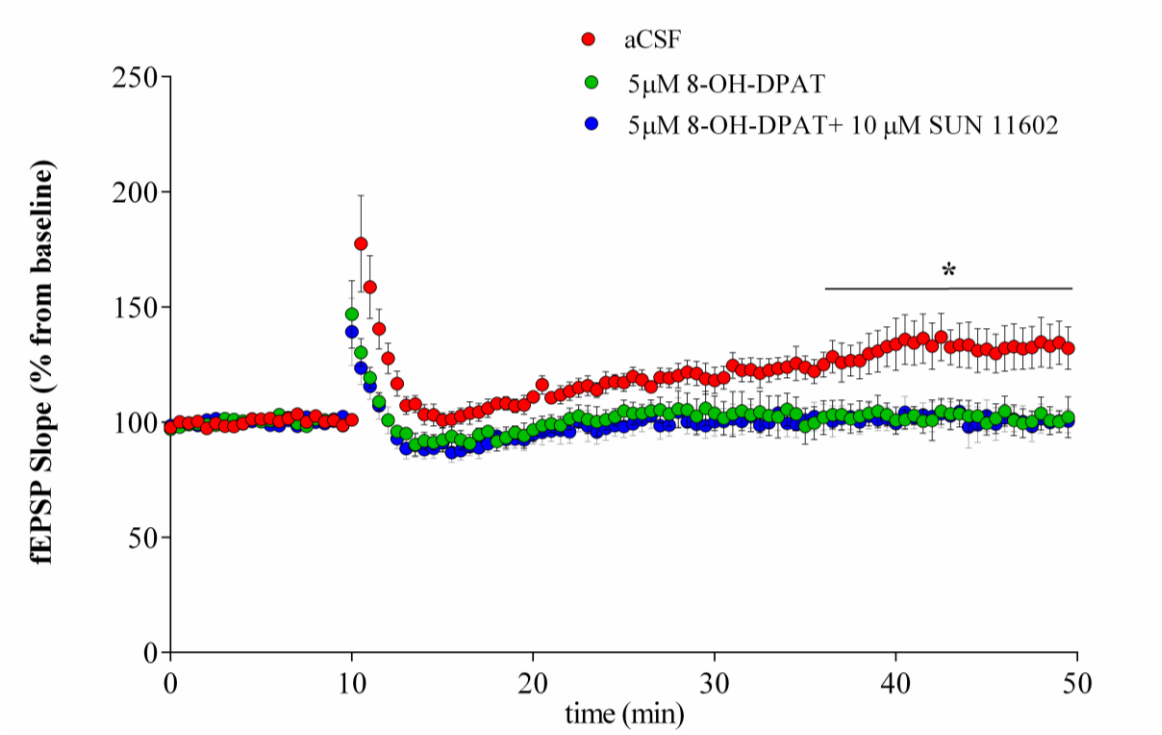

Figure 13 fEPSP slope (10% and 80% of max) recorded before and after the Schaffer Collateral high-frequency stimulation in FSL rats. Values were normalized to the mean value obtained over the last 20 min of the baseline period and expressed as a percentage of this baseline value (n = 6 slices for aCSF, n= 7 for 5µm 8-OH-DPAT, n = 7 slices for 5µm 8-OH-DPAT + 10 µm SUN11602).

Two-way ANOVA test F (2, 701) = 196,7; $p < 0,0001$; Tukey's multiple comparisons test: aCSF vs 5µm 8-OH-DPAT ** $p < 0,01$ from 23 to 40 min of recoding post HFS, aCSF vs 8-OH-DPAT + 10 µm SUN11602 ** $p < 0,01$ from 23 to 40 min of recording post HFS

CHAPETR 7: DISCUSSION

Allosteric receptor-receptor interactions can occur among the receptors present on the plasma membrane, leading to the formation of macromolecular complexes called receptor heteromers. These receptor complexes can determine an alteration of the biochemical characteristics of the receptors involved: the allosteric interactions in the heteroreceptor complexes, in fact, confer diversity and specificity to the receptor protomers due to allosterically induced conformational changes, which can lead to alterations in the function and pharmacology of the receptors involved. For this reason, they can represent an important pharmacological target for the treatment of numerous pathologies, including major depression [159].

Numerous studies have demonstrated the significance of the FGFR1-5HT1A heteroreceptor complex in the onset of major depression [17, 18, 200]. Based on earlier research by Professor Kjell Fuxe's team [18, 19], a number of electrophysiological analyses have been carried out in the current research project to confirm the functionality of the FGFR1-5HT1A heteroreceptor complex in a genetic model of depression.

As known, the activation of the 5HT1A receptor determines the opening of GIRK channels with subsequent hyperpolarization of the plasma membrane. Otherwise, the activation of the FGFR1 protomer, when involved in the formation of the FGFR1-5HT1A receptor heteromer, appears to be able to determine a reduction of the ability of the 5HT1A receptor to open GIRK channels [15]. As a result, the FGFR1 agonist may cause a conformational change in the 5HT1A protomer that decreases cell membrane hyperpolarization.

In DRN neurons the FGFR1-5HT1A heterocomplexes, positioned at the somatodendritic level, act as autoreceptors, and, when activated, they are able to reduce the inhibition mediated by the 5-HT1A activation on the firing activity of serotonergic neurons, and consequently raise the levels of serotonin that reach the different projection areas. With the use of a combination of 5HT1A and FGFR1 receptor agonists, we observed this modulation in DRN neurons of Sprague Dawley rats; the non-decrease in neuronal firing frequency, the lower whole cell recorded GIRK current, and the non-reduction in membrane resistance found in this group of rats lead to the confirmation of the existence of the heteroreceptor in DRN neurons and indicated its adequate functionality. Conversely, failure of the same combination to alter the 5HT1A receptor capacity to activate GIRK channels observed in Flinders Sensitive Line rats - which represent a genetic model of depression - indicates

either the absence of the heteroceptor in raphe nuclei or its ineffectiveness. In this view, a hyperfunctioning of GIRK channel in FSL rats, due to the lack of a functioning FGFR1-5HT1A heteromer able to modulate potassium current, might be the basis for the depressed-like behavior of FSL rats. In line with this suggestion, blockers of the GIRK channels are considered as antidepressants [295, 296].

In keeping with this evidence, the differences between SD and FSL rats might be explained by one of two theories. According to the first, depression may develop as a result of the FGFR1-5HT1A receptor heteromer being inactive or dysfunctional. In fact, these changes may be linked to the onset of serious depression because they may result in lower levels of serotonin reaching the raphe's projection regions. According to the monoaminergic theory of depression, there would be a deficiency in the serotonergic system's operation as well as the emergence of the classic signs of the depressive syndrome [297].

The second theory postulates that the dysfunction of the heteroreceptor complex is caused by depression itself and the ensuing changes to various circuits that frequently go along with this pathology; as a result, the absence or dysfunction of the FGFR1-5HT1A receptor heteromer would not be a cause of depression but rather an effect of the start of the pathological process. According to published research, depressed animals exhibit lower serotonin levels [298], which may be the cause of cellular adaptation events that lead to lower numbers of pre- and post-synaptic heteroreceptors [250]. Consistently to the hypothesis that the existence of the functioning heteroreceptor serves to avoid excessive hyperpolarization of pre- and postsynaptic neurons, the uncoupling of the two protomers in FSL rats might be related to lesser serotonin production and therefore a reduced requirement to minimize hyperpolarization. In addition, previous studies has shown that FGF2 can act as an anxiolytic and anti-depressive agent in rodents and that decreased levels of hippocampal FGF2 and FGF2 receptors have been found in post-mortem brains of individuals with mood disorders [299]. Thus, the existence of sufficient levels of serotonin and FGF2, as well, might be considered a prerequisite for the development of the receptor heteromerization.

The midbrain raphe serotonergic neurons provide the primary ascending serotonergic projections to the forebrain. In particular, serotonergic neurons of dorsal and medial raphe nuclei reach pyramidal neurons of the hippocampus making up the raphe-hippocampal pathway [3], which is thought to have a role in the pathophysiology of depressive disorder. In this scenario, the modulation of GIRK currents by FGFR1-5HT1A heteromer activation may represent an antidepressant effect.

In hippocampus pyramidal neurons the FGFR1-5HT1A heterocomplexes, located at the somatodendritic level, act as post-junctional (or post-synaptic) receptors, thus influencing the effects of serotonin on hippocampal neuronal firing. The FGFR1-5HT1A heterocomplex activity was demonstrated by electrophysiological tests to exist and operate in CA1 and CA2 neurons of the hippocampus of control animals, but only in the pyramidal neurons of CA2 in FSL rats. In fact, in the current study, we found that FGFR1 receptor activation decreases GIRK-mediated currents brought on by the administration of a 5HT1A receptor agonist in the CA1 and CA2 pyramidal neurons of control rats and CA2 neurons of FSL rats. The 5HT1A protomer capacity to activate GIRK channels may be reduced in hippocampal neurons, as in DRN nerve cells, as a result of the FGFR1 agonist binding to the receptor, which is thought to reduce hyperpolarization and increase neuronal activity.

On the other side, the analysis performed on the CA1 neurons of FSL rats reveals a different situation, where the co-application of the 5HT1A and FGFR1 receptor specific agonists does not determine a reduction of the GIRK hyperpolarizing current, indicating a change in the heteroreceptor's functioning in the depressed phenotype. This is further supported by data from the analysis of membrane resistances in the CA1 of FSL rats. These findings are consistent with behavioral studies done on FSL rats, which do not appear to benefit from the antidepressant effect owing to activation of the FGFR1-5HT1A heteromer generated by intracerebroventricular injection of the combination of receptor protomer agonists, as occurs in controls [300].

Since the FGFR1 protomer was unable to prevent 5HT1A receptors from opening GIRK channels, the results of the current thesis work suggest that the mechanism underlying the lack of antidepressant effect of receptor agonist treatment seen in vivo in the genetic model of depression may involve a disruption in allosteric receptor-receptor interactions in the FGFR1-5HT1A heteromer in hippocampal neurons of CA1. While taking into account the function that CA2 plays in the regulation of mood and social relationships, the 5HT1A-FGFR1 receptor heteromer appears to retain its function in hippocampal CA2 neurons in depressed rats, suggesting a different implication of these neurons in the raphe-hippocampal pathway involved in depression (Fig.14) [301].

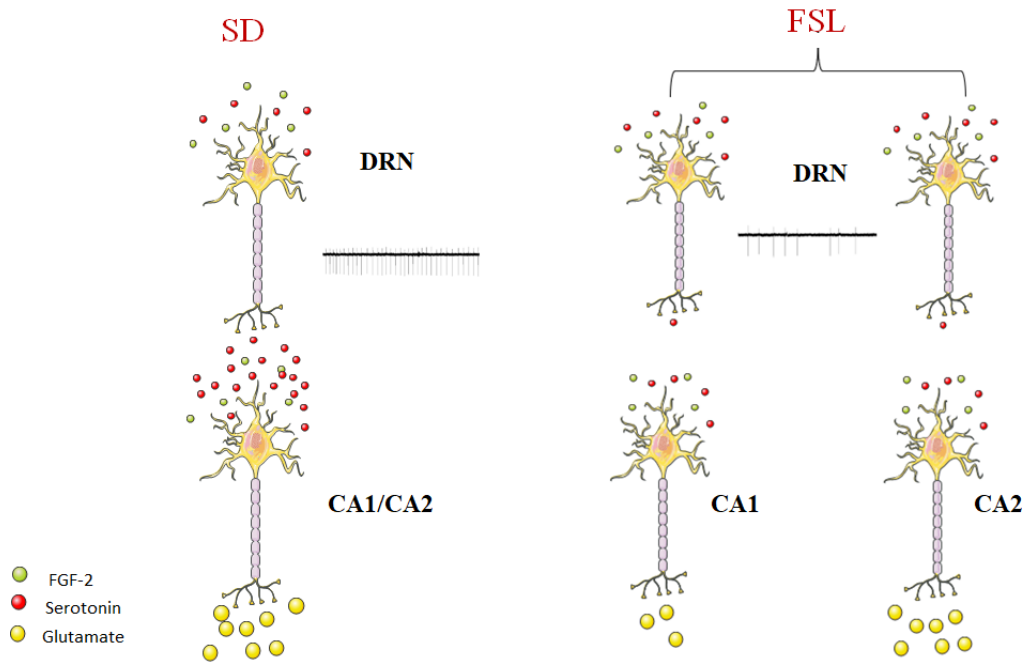

Figure 14 The FGFR1-5HT1AR heteroreceptor plays a fundamental role in the regulation of dorsal raphe nucleus neuron activity as it regulates the membrane potential of serotonergic neurons and their autonomous firing activity, controlling the release of serotonin on downstream targets. In the genetic model of depression this mechanism is disrupted in the DRN, resulting in less release of serotonin on the hippocampal CA neurons. In CA1 neurons of depressed rats the increase of GIRK-mediated hyperpolarization, due to the absence of functioning FGFR1-5HT1AR heteroreceptors, causes the inhibition of these glutamatergic neurons impairing long-term potentiation.

As a result of an altered FGFR1-5HT1A heteroreceptor activity in FSL rats, the CA1 pyramidal neurons would become susceptible to stronger GIRK currents in the presence of serotonin, which causes a greater hyperpolarization of the neurons themselves. Due to this, even if neurons are adequately stimulated, they could be unable to generate post-synaptic excitatory potentials necessary to trigger the LTP process. The capacity to transfer information from short-term memory into long-term memory is one of the major functions of the LTP event in the hippocampus as well as to retrieve information stored in long-term memory [302]. In order to assess and remember complicated information as well as to respond appropriately and adaptively to a given situation, long-term synaptic plasticity is a fundamental brain characteristic. Important preclinical evidence suggests that depression

may disrupt the normal balance between LTP and LTD in pyramidal neurons, favoring the latter, particularly in the murine hippocampus [83-86].

It is generally recognized that there is a biological link between 5HT and some types of neuroplasticity, as far as concern the regulation of neuronal morphology and circuitry development [117, 118]. The majority of previous studies on the 5HT1A receptor were based on the interactions of serotonergic receptors with neurotrophins, cytoskeletal rearrangement-related tyrosine kinases, astroglial connections, or cell adhesion molecules. It is possible that intracellular serotonergic signals have a role in long-term cell protective mechanisms since several serotonergic receptors are linked to ERK or other protein kinases that regulate the cytoskeleton in neuronal cells. This is supported by the fact that the 5HT1A receptor can encourage neurogenesis and dendritic growth in the hippocampus [135].

The FGFR1 and 5HT1A coactivation promotes their heterodimerization, enhancing the recruitment of β-arrestins to the heterocomplex and boosting the internalization of the complex [303]. It is well recognized that β-arrestins are essential for a number of cellular processes, such as facilitating signal transduction pathways and desensitizing membrane receptors. Another feature of 5HT receptor signaling is suggested by the finding that receptor endocytosis is required for 5HT1A receptor-mediated activation of the ERK cascade [201]. The ERK pathway is the MAPK signaling pathway that has received the most attention in research about depression. In post-mortem investigations, it was discovered that MDD patients had reduced Raf-ERK1/2 signaling in the PFC and hippocampus, as well as decreased hippocampal MEK5-ERK5 signaling [304, 305]. The Ras-dependent signals can be transmitted by the same processes that prevent the 5HT1A receptor from binding to G proteins. In addition to their involvement in the desensitization of GPCRs, β-arrestins have been shown to function as scaffolding elements that modulate receptor signaling. As a result, β-arrestins can connect GPCRs and signaling proteins without the necessity for G protein-mediated activation [203].

In light of this, the inability to trigger LTP in depressed rats could be due to the convergence of effects caused by heteroreceptor malfunction in the CA1 pyramidal neurons: lower neuronal activity, due to increased activation of the GIRK channels, and, on the other hand, decreased internalization of the complex mediated by β-arrestin recruitment, resulting in reduced activation of the pERK 1/2 pathway, which is required for synaptic plasticity processes. As a consequence, memory and learning deficits may result from this mechanism modifications in depressed patients.

Altogether the studies on the hippocampus and midbrain raphe indicate that synergistic allosteric R-R interaction develops within the FGFR1-5HT1A heteroreceptor complex during coactivation by agonists. The formation of these complexes is hypothesized to contribute to the antidepressant effects by recruiting 5HT1A receptors and triggering their dissociation from GIRK channels. As a result, the neurons could be more depolarized due to the reduction of 5HT1A autoreceptor activity in the midbrain 5HT neurons, as well as improved neurotransmitter release in other areas of the brain, such the hippocampal area. Because of this, the heteroreceptor activation would have the potential to increase neuronal activity and reverse the atrophy of various CNS areas, including the hippocampus and raphe, which is a necessary condition for the long-lasting antidepressant benefits.

The modification in the heteroreceptor activity seen in FSL may be a contributing factor to depression or may be the result of adaptive mechanisms put in place in brain as a response to a depressive disease.

CHAPTER 8: CONCLUSION AND FUTURE PERSPECTIVES

To fully understand this scenario and validate the assumptions generated by the findings of this analysis, further research will be required. Future possibilities include replicating the work under identical experimental settings using an alternative depression model, such as the olfactory bulbectomy animal model. In contrast to FSL rats, this represents a model of induced depression as the animal exhibits neurochemical, neuroendocrine, and behavioral traits after olfactory bulbectomy that are comparable to those seen in human depression. By more accurately simulating what occurs at the level of the dorsal raphe nucleus in the depressed person, the use of these animals will enable evaluation of the existence and functionality of the FGFR1-5HT1A heteromer both before and after the development of depressive disease. Furthermore, future goals will however include a more in-depth investigation that will allow the characterization of the biochemical processes involved in the allosteric interaction of the two receptors under investigation.

The outcomes of these future investigations, along with the conclusions drawn from the current electrophysiological analysis, may provide novel targets and pharmaceutical options for the prevention and treatment of depressive disorders that take into account the involvement of the FGFR1-5HT1A heteromers.

The data obtained so far have resulted in a submitted publication, which will be the basis for future studies that will allow a thorough understanding of one of the numerous aspects involved in the pathophysiology of depression.

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