CONTROL OF CHRONIC PAIN BY METABOTROPIC GLUTAMATE RECEPTORS

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CERTIFICATE

This is to certify that **CHAUDHARI PRIYANKA PRITHVIPAL GUNINDER** submitted the Project report entitled "**Control of Chronic Pain by Metabotropic Glutamate Receptors**" towards the compulsory internal projects in the semester - VI of T.Y.B.Sc. and in the partial fulfillment of the award of the degree of Bachelor's of Science in Biotechnology of the University of Mumbai. The work included in the project is based on the literature survey and analysis of published research carried out by the student under my supervision, and no part of project has been submitted for any other degree or diploma.

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ABSTRACT

The sensation of pain is our body's innate mechanism to warn us that something is wrong. The pain usually fades away once the body has completely recovered from what was hurting and fixed the root cause of the pain. But when the pain persists for more than 3 to 6 months even after the body has healed completely, then that type of pain is generally referred to as Chronic Pain Syndrome. It also involves chronic depression and general anxiety disorder. Arthritis, back pain, cancer pain in the vicinity of a tumor, migraines or other forms of headaches, lasting scar tissue pain, fibromyalgia, repetitive stress injuries, Lyme disease, broken bones, acid reflux, or ulcers, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), endometriosis, surgery and neurogenic pain that is caused by damage to the nerves or other parts of the nervous system are associated with chronic pain syndrome. A few medications, lifestyle changes, and alternative medications have proven to help patients who suffer from chronic pain. Further research in this field is actively being conducted so that there are more efficient and safer options available to alleviate chronic pain with minimal side effects. Researchers and healthcare professionals need to understand how all four sections of the pain pathways—transduction, transmission, regulation, and perception interact such that modalities to avoid and manage different pain disorders can be developed and implemented. The mammalian nervous system contains all types of Metabotropic Glutamate Receptors (mGluRs) and they are found both presynaptically and postsynaptically in neurons. Because of their ability to modulate rather than mediate excitatory synaptic activity, mGluRs are considered promising valid targets for chronic pain management as they can inhibit nociceptive, recurrent, inflammatory, and neuropathic pain. Metabotropic glutamate receptors (mGluRs) are divided into three groups- Group I, Group II, and Group III based on agonist pharmacology, primary sequence, and G protein coupling to the effector. Group I mGluRs are mGluR1 and mGluR5. Group II mGluRs are mGluR2 and mGluR3. Group III mGluRs are mGluR4, mGluR6, mGluR7 and mGluR8. All of these mGluRs and their subtypes have various applications in chronic pain control and understanding the function, expression, their roles in pain modulation, and their involvement in the induction and maintenance of central sensitization may aid in the development of a feasible alternative for reducing hypersensitivity in chronic pain conditions. Metabotropic glutamate receptors could have a bright future in the treatment of chronic pain and related nervous system disorders as further research, assessment, and clinical trials are actively being conducted.

1. INTRODUCTION

1.1 CHRONIC PAIN

Pain that lasts for months or years is referred to as chronic pain. It may occur in any part of the body. It's possible that the pain will be constant or that it will come and go. Chronic pain can make it difficult to go about your everyday activities, such as working, socializing, and caring for yourself or others. It can cause depression, anxiety, and sleeping problems, all of which can exacerbate pain (refer to Figure no. 1.1). Chronic pain is distinct from acute pain, which is a different form of pain. When something hurts you, you experience acute pain. It doesn't last long and disappears until the body has recovered from whatever caused the pain. Chronic pain, on the other hand, lasts even after you've recovered from an accident or illness. It can happen for no apparent cause (Chronic Pain: Symptoms, Treatments, 2021).

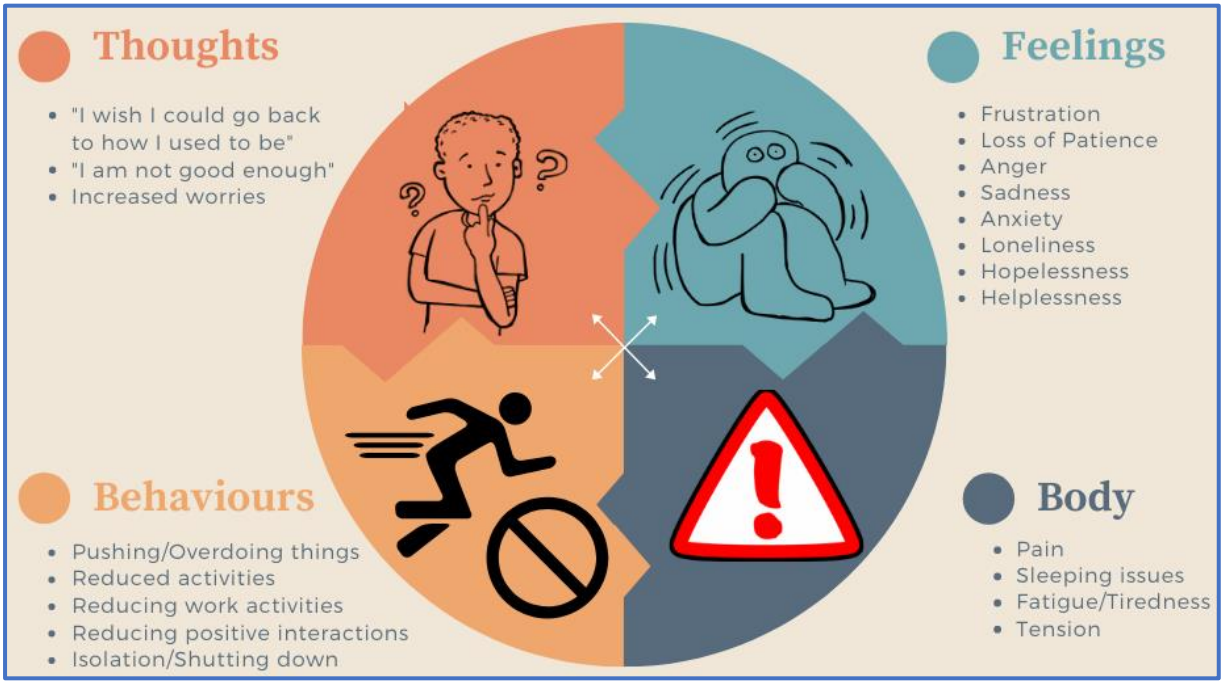

Figure no. 1.1: Chronic Pain Syndrome

Source: *Chronic Pain Treatment in London & Online | Therapy Central*. Therapy Central. (2021). Retrieved 15 April 2021, from https://therapy-central.com/what-we-do/chronic-pain-treatment/

Chronic pain may often be traced back to a specific cause. One may have a long-term illness, such as arthritis or cancer, that causes them to be in constant pain. However, accidents and diseases can alter the body, making one more susceptible to pain. Even after one has recovered from the original accident or illness, these changes will persist. A sprain, a broken bone, or a brief infection, for example, may result in chronic pain (Chronic Pain: Symptoms, Treatments, 2021).

Some people suffer from chronic pain that isn't caused by an accident or disease. This type of pain is referred to as psychogenic pain or psychosomatic pain by doctors. Psychological factors such as stress, anxiety, and depression can be the cause. Low levels of endorphins in the blood, according to many scientists, are the cause of this connection. Endorphins are natural chemicals that produce euphoria. Many sources of pain likely coexist. One may, have two separate diseases, for example, experiencing migraines and psychogenic suffering at the same time (Chronic Pain: Symptoms, Treatments, 2021).

Chronic pain can take several different forms and manifest itself all over the body. Some commonly associated illnesses with chronic pain are arthritis (joint pain), back pain, cancer pain in the vicinity of a tumor, migraines or other forms of headaches, lasting scar tissue pain, all-over muscle pain (such as with fibromyalgia), repetitive stress injuries, lyme disease, broken bones, acid reflux or ulcers, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), endometriosis, surgery and neurogenic pain that is caused by damage to the nerves or other parts of the nervous system (refer to Figure no. 1.3). Aching, burning, shooting, squeezing, stiffness, stinging, and throbbing are only a few of the ways people with chronic pain describe it. Anxiety, depression, fatigue (feeling excessively tired all of the time), insomnia, and mood swings are all common symptoms and issues associated with chronic pain. Blood checks, electromyography to assess muscle function, imaging tests, such as X-rays and MRI, nerve conduction studies to see whether the nerves are responding properly, reflex and balance tests, cerebrospinal fluid tests, and urine tests can be ordered by a healthcare practitioner or medical professional to determine the source of the pain (Chronic Pain: Symptoms, Treatments, 2021).

Medications to alleviate chronic pain can be prescribed by a healthcare provider based on the severity, type, or source of the pain. Anticonvulsants (seizure medications), antidepressants, corticosteroids, muscle relaxants, opioids (narcotics), nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen, topical medications, and sedatives to help with anxiety and insomnia. Chronic pain has also been shown to be relieved over time by some lifestyle changes and forms of alternative medicine like cognitive-behavioral therapy (CBT), counselling, occupational therapy, physical therapy, acupuncture, aromatherapy, biofeedback, exercises such as walking, swimming, yoga, and tai chi, hypnotherapy, mindfulness training, music, art, or pet therapy, relaxation techniques such as massage, meditation, and guided imagery, stress reduction, transcutaneous electrical nerve stimulation (TENS), nerve blocks, etc. (Chronic Pain: Symptoms, Treatments, 2021).

1.2 CLASSIFICATION OF CHRONIC PAIN

Chronic pain is classified into two types: Nociceptive chronic pain, which is induced by inflamed or broken tissue activating advanced pain receptors referred to as nociceptors, and Neuropathic chronic pain, which is caused by nervous system harm or malfunction (Yam, et al., 2018) (refer to Figure no. 1.2).

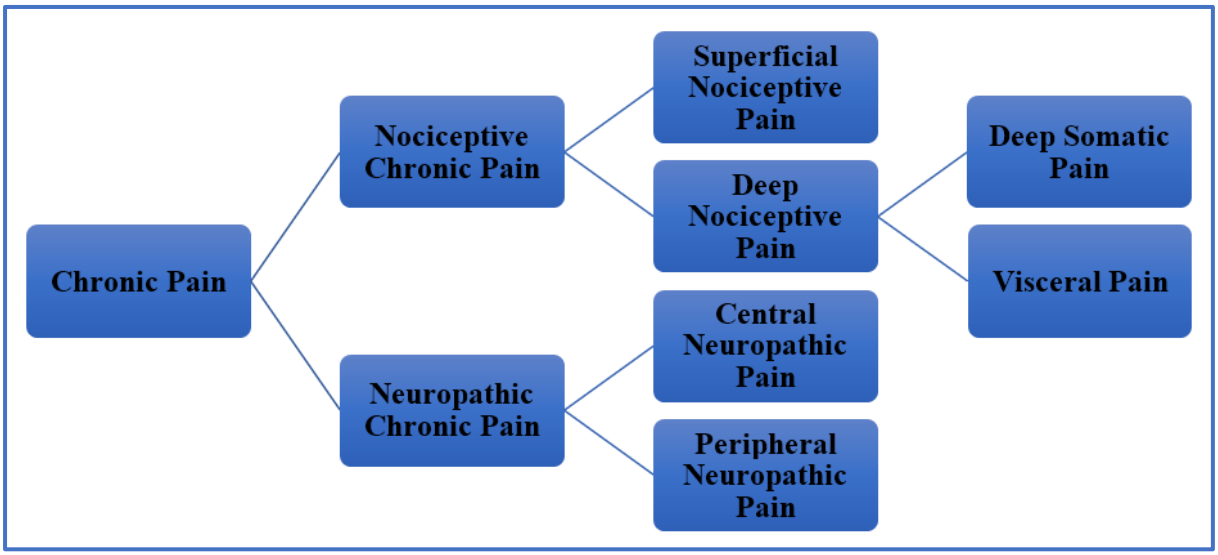

Figure no. 1.2: General classification of Chronic Pain

Neuropathic Pain	Mixed Pain	Nociceptive Pain
• Peripheral neuropathies (diabetes, HIV)	• Migraine and chronic daily headache	• Mechanical low back pain
• Postherpetic neuralgia	• Fibromyalgia	• Rheumatoid arthritis
• Trigeminal neuralgia	• Phantom limb pain	• Osteoarthritis
• Central post-stroke pain	• Complex regional pain syndrome	• Chronic inflammatory conditions
• Spinal cord injury	• Multiple sclerosis	• Somatoform pain disorder
• Neuropathic low back pain	• Low back pain	• Postoperative pain
	• Myofascial pain syndrome	• Sickle cell crisis
	• Skeletal muscle pain	• Sports/exercise injury

Figure no. 1.3: Various Chronic Pain Disorders

Source: *Guide to Chronic Pain Assessment Tools*. Practical Pain Management. (2021). Retrieved 15 April 2021, from https://www.practicalpainmanagement.com/resources/diagnostic-tests/guide-chronic-pain-assessment-tools

Superficial nociceptive pain and deep nociceptive pain are two forms of nociceptive chronic pain. The activation of nociceptors within the skin or superficial tissues causes superficial nociceptive pain. Deep nociceptive pain is divided into two categories: deep somatic pain and visceral pain. Deep somatic pain may be a dull, aching, poorly localized pain caused by the stimulation of nociceptors in ligaments, tendons, bones, blood vessels, fasciae, and muscles. The viscera are the origin of visceral pain (organs), when broken or inflamed, produces pain, where the sensation is located in an area distant from the location of pathology or injury (Yam, et al., 2018).

Central neuropathic pain, which originates within the brain or the spinal cord, and peripheral neuropathic pain, which originates within the peripheral nervous system, are the two kinds of neuropathic pain. Burning, tingling, electrical, stabbing, or pins and needles are common descriptions of peripheral neuropathic pain (Yam, et al., 2018).

Chronic pain is classified into seven groups by the International Classification of Diseases, Eleventh Revision (ICD-11) (Treede, et al., 2019) (refer to Table no. 1.1).

1. Chronic primary pain	identified as a three-month period of persistent pain in one or more body regions that is unrelated to another pain disorder.
2. Chronic cancer pain	referred to as Cancer or treatment-related visceral (inside the internal organs), musculoskeletal, or bony pain.
3. Chronic post-traumatic pain	described as pain that persists for more than three months after an injury or surgery, excluding contagious or pre-existing conditions.
4. Chronic neuropathic pain	caused by damage to the somatosensory nervous system.
5. Chronic headache and orofacial pain	described as pains that start in the head or face and last for 50 percent or more of the time over three months.
6. Chronic visceral pain	referred to as pain that originates in an internal organ.
7. Chronic musculoskeletal pain	identified as pain that originates in the bones, muscles, joints, or connective tissue.

Table no. 1.1: Classification of Chronic Pain by ICD-11

Source: Treede, R., Rief, W., Barke, A., Aziz, Q., Bennett, M., & Benoliel, R. et al. (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain*, *160*(1), 19-27. https://doi.org/10.1097/j.pain.0000000000001384

Pain can be thought of as a disorder in chronic primary pain syndromes (refer to figure no. 1.4) (left), but it first appears as a symptom of another disease in chronic secondary pain syndromes (right), such as breast cancer, a work injury, diabetic neuropathy, chronic caries, inflammatory bowel disease, or rheumatoid arthritis. It can be difficult to distinguish between primary and secondary pain conditions (arrows), but when pain is mild to extreme, the patient requires special attention. Chronic pain may persist after spontaneous healing or effective management of the underlying condition, and so chronic secondary pain diagnoses may persist and continue to guide treatment as well as health care statistics (Treede, et al., 2019). (IASP: The International Association for the Study of Pain)

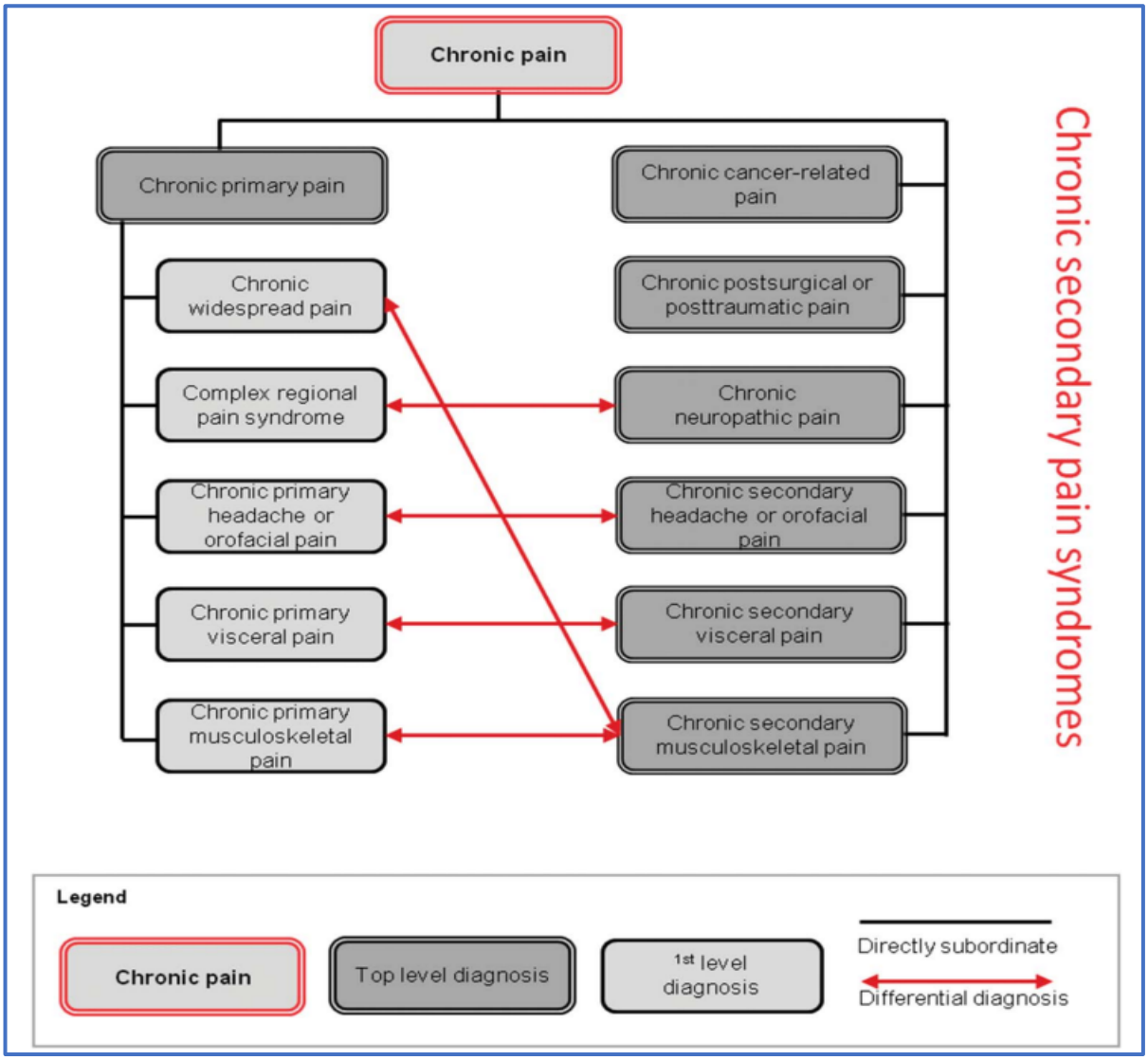

Figure no. 1.4: Structure of the IASP Classification of Chronic Pain

Source: Treede, R., Rief, W., Barke, A., Aziz, Q., Bennett, M., & Benoliel, R. et al. (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain*, *160*(1), 19-27. https://doi.org/10.1097/j.pain.0000000000001384

Unlike previous versions of ICD, which had a strictly linear structure, ICD-11 allows any given disease (“child”) to belong to more than one section (“parent”). This is referred to as "multiple parenting." (refer to figure no. 1.5) As an example, “chronic painful chemotherapy-induced polyneuropathy” is depicted (Treede, et al., 2019). (WHO: World Health Organization) (ICD: The International Classification of Diseases)

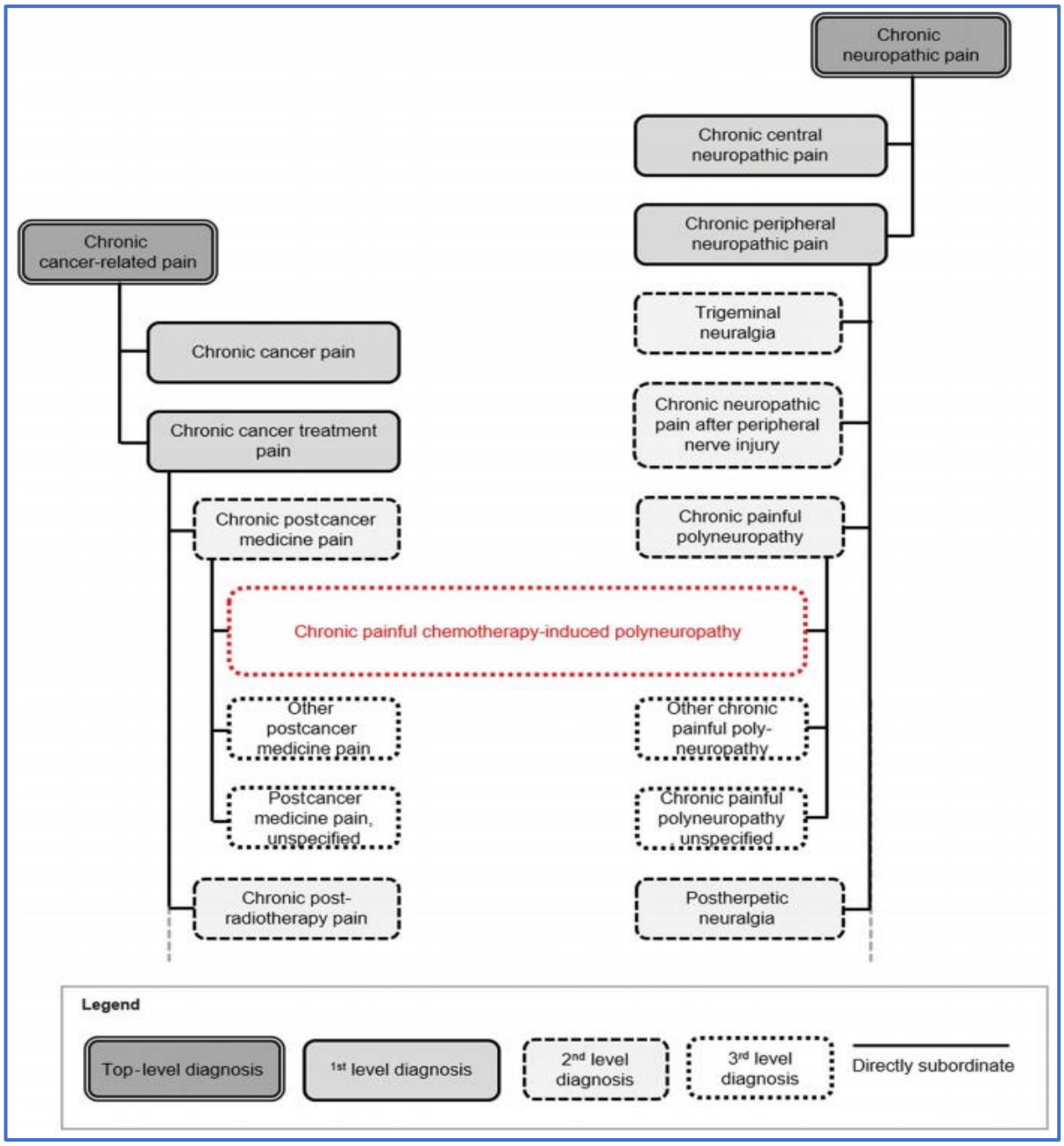

Figure no. 1.5: Multiple Parenting concept of WHO for ICD-11

Source: Treede, R., Rief, W., Barke, A., Aziz, Q., Bennett, M., & Benoliel, R. et al. (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain*, *160*(1), 19-27. https://doi.org/10.1097/j.pain.0000000000001384

1.3 MECHANISMS OF CHRONIC PAIN IN OUR NERVOUS SYSTEM

The core elements involved in the processes of pain physiology are transduction, transmission, modulation, and perception, and each phase is characterized by specific cellular mechanisms that allow the nociceptive signal to continue or eventually stop. The pathway from the induction of pain at the time of initial tissue injury to its final processing in the brain is made up of these components. Not all pain pathways, however, have all four components. Since the stimulation causes direct nerve damage, neuropathic pain bypasses the first step in translating a stimulus into an electrical impulse. Nociceptive and neuropathic pain are the two extremes of a "pain continuum," along which each process is still poorly understood (Table no. 1.2). For clinicians, however, identifying the mechanisms of pain is crucial for successful management because it informs medical decisions at any level (Christiansen & Cohen, 2018).

Clinical characteristics	Neuropathic pain	Nociceptive pain
Etiology	Direct injury to the nervous system, often with consequent maladaptive changes	Actual or potential tissue damage
Quality	Sharp, shooting, stabbing, electrical-like	Aching, throbbing, dull, pressure
Sensory complaints	Numbness, tingling, “pins and needles”	Uncommon
Motor complaints	Weakness if motor nerve affected; spasticity or dystonia with CNS lesions	Pain-induced weakness
Hypersensitivity	Allodynia and hyperalgesia	May have hypersensitivity in immediate vicinity of tissue injury (i.e., primary hyperalgesia)
Radiation	Distal, often in dermatomal distribution	Proximal, correlates with stimulus magnitude
Exacerbations	Common and unpredictable	Often associated with activity
Autonomic changes	Color or temperature changes, swelling, or sudomotor activity	Uncommon

Table no. 1.2: Comparison between neuropathic and nociceptive pains

Source: Christiansen, S., & Cohen, S. (2018). Chronic Pain: Pathophysiology and Mechanisms. *Essentials Of Interventional Techniques In Managing Chronic Pain*, 15-25. https://doi.org/10.1007/978-3-319-60361-2_2

The mammalian brain has acquired the highest degree of complexity as well as functioning ability. The brain does not only receive but also transmit information that is vital for our day-to-day activities. The exchange of this vital information is carried out by specialized cell systems that comprise the entire nervous system in vertebrate beings. The act of receiving, processing, and responding to an external electrochemical stimulus occurs within few seconds in our nervous system. Our nervous system is broadly classified into the central nervous system (CNS) and peripheral nervous system (PNS). The main components of the CNS are the brain and the spinal cord whereas, the PNS consists of the autonomic nervous system and somatic nervous system. The brain is further divided into forebrain, midbrain, and hindbrain. The somatic nervous system comprises 12 pairs of cranial nerves and 31 pairs of spinal nerves. The autonomic nervous system further comprises the sympathetic and parasympathetic nervous system (Christiansen & Cohen, 2018).

While it is commonly accepted that mechanism-based pain management is technically preferable to disease or etiologic-based treatment, it is difficult to implement in clinical practice. Since pain affects the entire nervous system, it's crucial to comprehend the various parts through which the signals pass. In general, transduction and transmission take place in the peripheral nervous system (PNS), while regulation (i.e., transformation) and perception take place in the central nervous system (CNS). The process of converting noxious stimuli from tissue damage to a nociceptive signal is known as transduction. Transformation is the biological modulation of the signal until it reaches the CNS. Finally, perception is the interpretation of a signal by the brain's cognitive and emotional responses, which take into account meaning, previous experiences, and expectations. This sequence was designed to defend against further injury from an evolutionary standpoint; however, given the long chain of biological events that occur, maladaptation at any point along the chain may result in pathological pain (Christiansen & Cohen, 2018).

Scientists are better able to develop treatments that concentrate on a particular pathway that has become maladaptive, contributing to pathological pain, through recognizing these fundamental mechanisms and continuing to research the cellular processes that are still unknown. Despite the discovery of multiple possible therapeutic targets, animal models and clinical trials have consistently shown that changing only one phase in the maladaptive process is often insufficient to eliminate pain (Christiansen & Cohen, 2018).

1.4 NEURONS AND ACTION POTENTIAL

When noxious stimuli are present, the basic pain system goes through three steps: transduction, transmission, and modulation. For example, in the nociceptive pathway, transduction occurs in the following order: (1) stimulus events are converted to chemical tissue events; (2) chemical tissue and synaptic cleft events are then converted to electrical events in neurons; and (3) electrical events in neurons are transduced as chemical events at synapses. The transmission will be the next mechanism after transduction was completed. It occurs as electrical events are transmitted along neuronal pathways and neurotransmitters in the synaptic cleft relay information from one cell's post-synaptic terminal to another cell's pre-synaptic terminal. Meanwhile, modulation occurs at all levels of nociceptive pathways through up-or down-regulation of the primary afferent neuron, dorsal horn (DH), and higher brain core. Everything of this leads to the same conclusion: the pain process has been activated and completed, allowing us to experience the unpleasant feeling elicited by the stimulus (Yam, et al., 2018).

Neurons are a primary component that links, receives, and processes all nociceptive information provided by the three events described above in the CNS and PNS. Sensory neurons (afferent neurons), interneurons (which transmit signals between afferent and efferent neurons), and motor neurons are the three types of neurons that reside in our bodies (efferent neurons). All neurons are electrically excitable and are divided into three parts: soma, axon (myelinated or unmyelinated), and dendrites (Yam, et al., 2018).

In our bodies, there are currently 86 billion neurons and other specialized cells present in the brain. These neurons are linked to form complex neural networks in which chemical and electrical signals are transmitted via specialized connections called synapses. This synapse is barely 20 nm or 0.02 micron in size. A neuron's synaptic signals are processed by the dendrites and soma of another neuron, and these signals may be inhibitory or excitatory, depending on the pharmacological effects of the signal. This process of exchange of chemicals at the synapse is called neurotransmission (synaptic transmission). The signals are transmitted inside the neurons by axons after they are received via dendrites or soma. This causes brief pulses, known as action potentials, to be produced within the neuron, which spread from the soma, move along the axons to trigger the synapses, and then be transmitted to other neurons, serving as a pathway to bring signals from their source to either the spinal cord or the brain, where a response is eventually interpreted and implemented (Yam, et al., 2018).

Sensory neurons and motor neurons are the two main types of specialized neurons. Sensory neurons, which are found in the dermis and epidermis and respond to stimuli such as contact, pass these signals along when the stimulus is present, while motor neurons' main job is to receive signals from the brain and spinal cord, then produce responses that cause muscle contractions and affect glandular outputs. Our bodies cannot respond to harmful stimuli from the environment without the involvement of neurons within the nervous system to relay signals (Yam, et al., 2018).

The entry of Ca^{2+} triggers the release of neurotransmitters at the axon terminal, and the nociceptive signals are then carried and transmitted across various neurons by an action potential. There are two major potentials that play distinct roles in the generation of action potentials, which enable signals to be transmitted through neurons. The resting potential and threshold potential of neurons are these. The resting and threshold potentials of a typical neuron's axon are approximately 70 and 55 mV, respectively. Since more Na^+ accumulates outside the cell than K^+ accumulates within the cell, the cells' resting potential is negatively charged. The activation of various ion channels controls the passage of these ions through the lipid bilayer membrane of neurons. Ion channels may be activated or inactivated by changing their conformation, allowing for the influx or efflux of particular ions (Yam, et al., 2018).

When nociceptors are stimulated by a noxious stimulus, two types of potentials, inhibitory postsynaptic potentials (IPSP) and excitatory postsynaptic potentials (EPSP) are produced and summed in the axon hillock (EPSP). The action potential is propagated through the axon along the neurons until the triggering threshold is reached. In general, the action potential begins when Na^+ enters through a voltage-activated Na^+ channel (Na_v), causing the membrane potential to depolarize (Yam, et al., 2018).

When the threshold potential is reached, all of the Na_v channels in the axon hillock are stimulated to open, resulting in a complete depolarization of the neurons until they reach peak potential (+40 mV). The Na_v channels return to their resting state at this stage, and the voltage-activated K^+ channels (Kv) are activated and opened to allow K^+ efflux, allowing the neurons to repolarize. The action potential has a stereotypical form, which means that the amplitude and time course of all action potentials in the cell are identical. During the supposedly refractory cycle of the neurons, persistent efflux of K^+ through the Kv and K^+ leakage channels causes the membrane potential to hyperpolarize (Yam, et al., 2018).

The K^+ channels eventually close, and the Na^+/K^+ transporters restore the resting potential by allowing three Na^+ to enter and two K^+ to exit. Ca^{2+} reaches the presynaptic terminal via the voltage-operated Ca^{2+} channels (VOCC) as the action potential passes to the axon terminal, triggering synaptic transmission (Yam, et al., 2018) (refer to Figure no. 1.6).

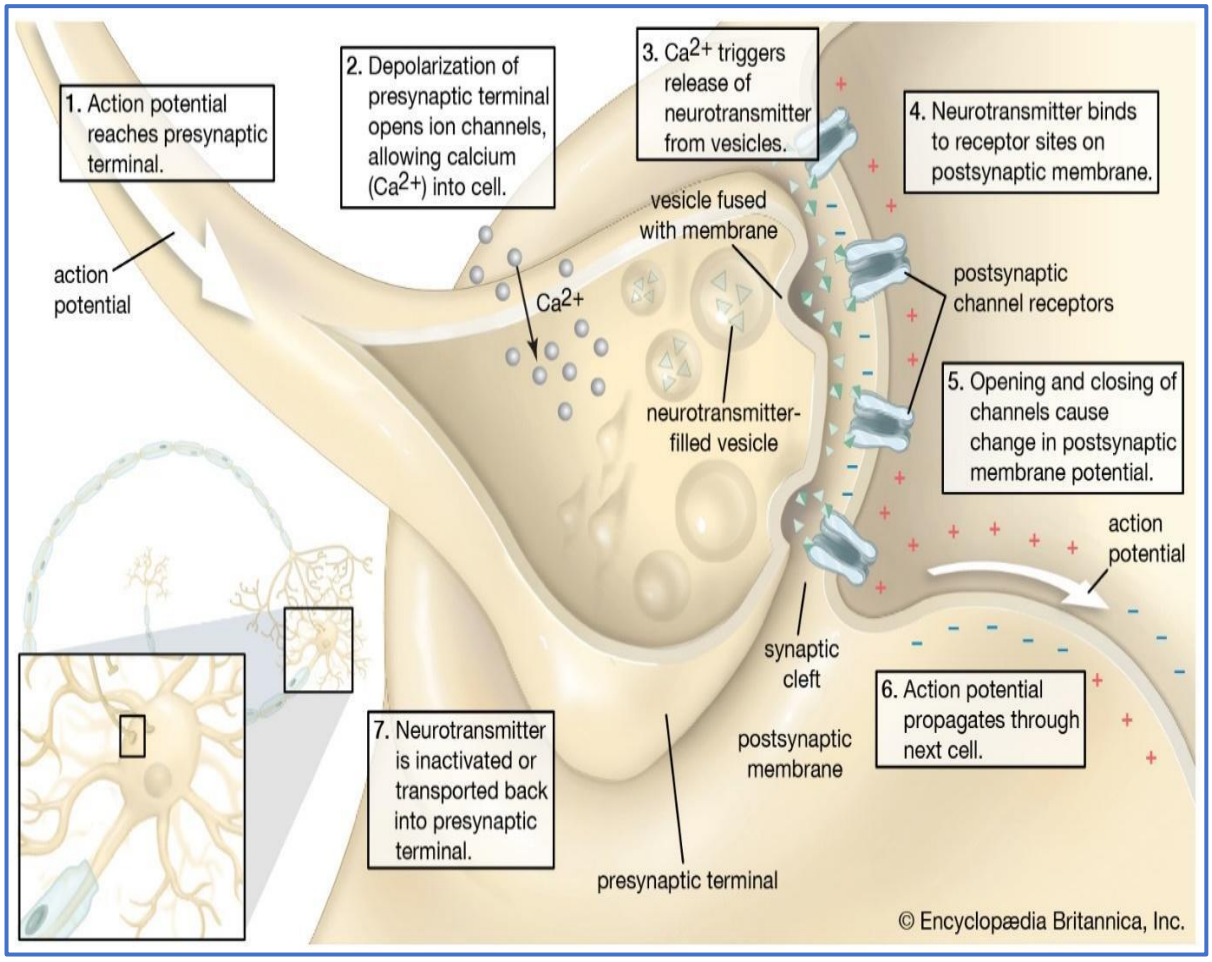

Figure no. 1.6: Neurotransmission at the Synaptic Cleft

Source: Das, D. (2011). *Synapse | anatomy*. Encyclopedia Britannica. Retrieved 15 April 2021, from https://www.britannica.com/science/synapse.

The chemicals exchanged during neurotransmission are neurotransmitters. They are chemicals that are produced within the neuron and are used to increase or decrease the intake of signal that is being passed along to the neighboring cells. Thousands of neurotransmitters are present in specialized sac-like structures called synaptic vesicles and are released into the synapse when the neuron cell is activated within a span of 0.5 to 4.0 milliseconds. The released neurochemicals are then received by specific receptors on the neighboring cells. The neurotransmitters then bind to the receptor proteins which causes an excitatory, inhibitory, or modulatory effect on the target cell or tissue (Das, 2011).

1.5 NEUROTRANSMITTERS

In the process of synaptic transmission, also known as neurotransmission, neurotransmitters are substances that neurons use to interact with one another and with their target tissues. Nerve endings synthesize neurotransmitters, which are then released into the synaptic cleft. Neurotransmitters then bind to receptor proteins in the target tissue's cellular membrane. The target tissue is stimulated, inhibited, or otherwise functionally altered. Each central neuron has several synapses with other neurons on the dendrites, soma, and initial segment of the axon, among other places. The final integrated responses can include many neurotransmitters, some excitatory, some inhibitory, and others modulatory (Erulkar & Lentz, 2020).

An excitatory neurotransmitter acts in an excitatory synapse as it causes the target cell to an action. If it inhibits the target cell, on the other side, it is an inhibitory neurotransmitter acting in an inhibitory synapse. As a result, the type of neurotransmitter determines the type of synapse and the reaction of the target tissue. Inhibitory synapses induce hyperpolarization of the target cells, leading them farther from the action potential threshold, thereby inhibiting their action. Excitatory neurotransmitters cause depolarization of the postsynaptic cells and produce an action potential. The neurotransmitter released into the synaptic cleft has just a few minutes or even seconds of action. It is either destroyed by enzymes such as acetylcholine esterase or recycled by reuptake mechanisms into the terminal button of the presynaptic neuron (Vasković, 2021).

Other synapse-associated chemical compounds known as neuromodulators exist alongside neurotransmitters. Neuromodulators are compounds that do not specifically stimulate ion-channel receptors but improve the excitatory or inhibitory responses of the receptors when combined with neurotransmitters. The duration of the substance's action on the synapse distinguishes neuromodulation from neurotransmission. Neuromodulators aren't broken down or reabsorbed as easily by presynaptic neurons. Instead, they spend a lot of time in the cerebrospinal fluid, where they influence (modulate) the behavior of a lot of other neurons in the brain. Neurohormones are other chemical substances that are associated. They're synthesized in neurons and then secreted into the bloodstream, where they're carried to distant tissues (Vasković, 2021).

Neurotransmitters are categorized as excitatory or inhibitory depending on their role. Excitatory neurotransmitters stimulate receptors on the postsynaptic membrane and intensify the action potential's effects, while inhibitory neurotransmitters prevent an action potential from occurring (Vasković, 2021). The chemical nature and molecular properties of neurotransmitters are also used to classify them. Amino acids, cholinergic, biogenic amines such as catecholamines and indolamines, neuropeptides, soluble gases, purines, and trace amines are the most common types of neurotransmitters . (refer to table no. 1.3).

NEUROTRANSMITTERS	
(A) Based on Chemical Nature	
1. Amino acids	Glutamate, Glycine, Gamma-aminobutyric acid, Aspartate, Arginine, D-serine
2. Cholinergic	Acetylcholine
3. Biogenic amines	**Catecholamines:** Dopamine**,** Epinephrine**,** Norepinephrine **Indolamines:** Serotonin**,** Histamine
4. Peptides	Vasopressin, Neurotensin, Oxytocin, Somatostatin, Endorphins, Enkephalins, Opioids, Substance P
5. Soluble gases	Nitric oxide, Carbon monoxide, Hydrogen sulphide
6. Purines	Adenosine triphosphate, Adenosine
7. Trace amines	Phenethylamine, N-methylphenethylamine, Tyramine Tryptamine, Octopamine, Synephrine
(B) Based on Function	
1. Excitatory Neurotransmitters	Cause depolarization of postsynaptic cells.
2. Inhibitory Neurotransmitters	Cause hyperpolarization of target cells

Table no. 1.3: The classification of common neurotransmitters based on their chemical nature and function

Source: Vasković, J. (2021). *Neurotransmitters*. Kenhub. Retrieved 15 April 2021, from https://www.kenhub.com/en/library/anatomy/neurotransmitters

1.6 TYPES OF NEUROTRANSMITTERS

Acetylcholine (ACh) is secreted by motor neurons that innervate muscle cells, basal ganglia, preganglionic neurons of the autonomic nervous system, and postganglionic neurons of the parasympathetic and sympathetic nervous systems. Acetylcholine receptors, also known as cholinergic receptors, are found in clusters on muscle cell membranes opposite presynaptic terminal active zones. About 7,000-30,000 sites per square micrometer are found in these receptor regions. These can also be found on the presynaptic terminals of neurons that release acetylcholine, as well as other neurotransmitter terminals. These are known as autoreceptors, and they are thought to control neurotransmitter release at the terminal. Its primary purpose is to promote muscle contraction. The parasympathetic endings of the vagus nerve are the only exception, where acetylcholine is an inhibitory neurotransmitter. The cardiac plexus is used to suppress the cardiac muscle. It's also found in sensory neurons and the autonomic nervous system, and it helps schedule a person's "dream state" while they're fast asleep. Acetylcholine is important for muscle function (Erulkar & Lentz, 2020) (Vasković, 2021).

Epinephrine (Epi) and **Norepinephrine** (NE), also known as adrenaline (Ad) and noradrenaline (NAd), are neurotransmitters and hormones secreted by the adrenal glands in response to stress. Axon terminals in the central nervous system and sympathetic fibers in the autonomic nervous system synthesize and activate them as neurotransmitters. Epinephrine is an excitatory neurotransmitter released by the adrenal glands chromaffin cells that helps the body prepare for the fight-or-flight response. Extra amounts of epinephrine are released into the bloodstream when a person is afraid or angry, which increases the heart rate, blood pressure, and increases the glucose release from the liver. Thus, preparing the body for risky and circumstances by raising nutritional supply to key tissues. Norepinephrine is an excitatory neurotransmitter that is produced by the brainstem, hypothalamus, and adrenal glands and released into the bloodstream. It raises the level of alertness and wakefulness in the brain. Most postganglionic sympathetic nerves in the body secrete it, which serves to activate the body's processes. Norepinephrine has been linked to mood disorders including depression and anxiety, where the body's concentration is abnormally low. An abnormally high concentration, on the other hand, can result in a disrupted sleep cycle. Receptors sensitive to epinephrine and norepinephrine are called adrenergic receptors and are divided into two types, α and β which are further classified into subtypes α1, α2, β1, and β2 (Erulkar & Lentz, 2020) (Vasković, 2021).

Dopamine (DA) is secreted by the neurons of the substantia nigra. It's a unique form of neurotransmitter because it has both excitatory and inhibitory effects. It serves as a precursor to norepinephrine at some synapses throughout the brain. Dopamine, which is part of the basal ganglia's extrapyramidal motor system, is essential for movement coordination by inhibiting excessive movements. It prevents the release of prolactin and promotes the secretion of growth hormone in the pituitary gland. Parkinson's disease is caused by a lack of dopamine, which is related to the degradation of the substantia nigra. The pathophysiology of psychotic disorders and schizophrenia are associated with the increase in dopaminergic neuron function. Drug and alcohol addiction can raise dopamine levels in the blood temporarily, causing confusion and inability to concentrate. Dopamine secretion in the bloodstream, plays a role in motivation and the ability to complete a mission. The D_1 and D_2 dopaminergic receptors are the two forms of dopaminergic receptors. The first catalyzes the synthesis of cAMP, while the second inhibit its synthesis. Calcium and potassium channels in the postsynaptic membrane are then regulated by these reactions. On the presynaptic membrane, dopaminergic receptors can also be found that control the calcium and potassium channels in the postsynaptic membrane. Dopaminergic receptors can also be found on the presynaptic membrane. The neurotransmitter is terminated until it is absorbed into the presynaptic terminal (Erulkar & Lentz, 2020) (Vasković, 2021).

Serotonin (5-hydroxytryptamine or 5-HT) is an inhibitory neurotransmitter that has been involved with the regulation of emotion and mood. Neurons in the brainstem and those that innervate the gastrointestinal tract secrete it. Serotonin is also present in platelets (thrombocytes), which release it during the coagulation process. By linking proteins and the cAMP second-messenger systems, serotonin receptors, stimulate calcium and potassium channels. The neurotransmitter is taken up by the presynaptic terminal and enzymatically destroyed after acting on postsynaptic receptors. Serotonin appears to prime muscle cells for an excitatory response to other neurotransmitters at synapses in the peripheral nervous system. Even though the brain contains just a small amount of serotonin present in the human body, there appears to be a clear link between serotonin levels in certain brain regions and certain behavioral patterns, such as sleep, sexual desire, and mood. It helps to regulate body temperature, pain perception, emotions, and the sleep cycle. Reduced immune system activity, mental disorders such as depression, anger management issues, obsessive-compulsive disorder, and even suicidal impulses, may all be caused by inadequate serotonin secretion (Erulkar & Lentz, 2020) (Vasković, 2021).

Gamma-Aminobutyric acid (GABA) is the most effective inhibitory neurotransmitter developed by neurons in the spinal cord, cerebellum, basal ganglia, and many areas of the cerebral cortex and is derived from glutamate. GABA's functions are closely linked to mood and emotions. It is an inhibitory neurotransmitter that acts as a brake on excitatory neurotransmitters, so it can cause anxiety when levels are abnormally low. It's found all over the brain and is responsible for lowering neuronal excitability in the nervous system. GABA is found in high concentrations in the central nervous system and is widely distributed in the brain. The enzyme glutamic acid decarboxylase (GAD) produces GABA from glutamate. As a result, the amounts of GABA and GAD in the nervous system are almost similar (Erulkar & Lentz, 2020) (Vasković, 2021).

Glutamate (Glu) is the most abundant and strong excitatory neurotransmitter in the central nervous system, and it works in tandem with GABA to maintain homeostasis. It's generated by neurons in many of the central nervous system's sensory pathways, as well as the cerebral cortex. The concentration of glutamate does not differ significantly from one region to the next. Glutamate concentrations are high in the dorsal grey matter of the spinal cord, which includes terminals of incoming dorsal roots. It helps to regulate the central nervous system's general excitability, as well as learning and memory. As a result, abnormal glutamate neurotransmission plays a role in the development of epilepsy, as well as cognitive and affective disorders. Problems with glutamate production or use have been linked to a variety of mental illnesses, including autism, obsessive-compulsive disorder (OCD), schizophrenia, and depression. Excess glutamate has been related to neurological disorders including Parkinson's, Alzheimer's, multiple sclerosis, stroke, and amyotrophic lateral sclerosis (ALS) (Erulkar & Lentz, 2020) (Vasković, 2021).

1.7 NEUROTRANSMITTER RECEPTORS

The two kinds of neurotransmitter receptors are ionotropic receptors and metabotropic receptors. Ionotropic receptors are ligand-gated receptors whereas, Metabotropic receptors are G-protein coupled receptors (Neurotransmitter Receptors Research - Life Science Products | StressMarq Biosciences Inc., n.d.).

The channel present on the ionotropic receptor can be modulated by the binding of a specific neurotransmitter as the ligand-binding causes the opening and closing of this channel, thus controlling the flow of ions such as Na^+, K^+, Ca^{2+}, Cl^-, etc. into the cell. Major types of ionotropic receptors include: GABA receptors, Glutamate NMDA receptors, Glutamate Kainate receptors, Glutamate AMPA receptors, Glycine receptors, Nicotinic Acetylcholine receptors (nAChR), and Serotonin 5-HT3 receptors (Neurotransmitter Receptors Research - Life Science Products | StressMarq Biosciences Inc., n.d.).

There are no channels present on the metabotropic receptor as the ligand-binding does not take place. But when the receptors are activated, they can modulate the pathways that control the action of neurotransmitters and the ion channels through secondary messengers. Major types of metabotropic receptors include: Adrenergic receptors, Dopamine receptors, GABAB receptors, Glutamate receptors (mGluRs), Histamine receptors, Muscarinic acetylcholine receptors (mAChR), Opioid receptors and Serotonin (5-HT) receptors (Neurotransmitter Receptors Research - Life Science Products | StressMarq Biosciences Inc., n.d.).

1.8 GLUTAMATE AND PAIN MECHANISMS

Glutamate is the central nervous system's (CNS) key excitatory amino acid, and it plays a role in both brain activity and pathology. Glutamate acts as a neurotransmitter by activating two forms of receptors: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). Increased synaptic efficacy occurs in the dorsal horn of the spinal cord at the synapse between primary afferent terminals and second-order neurons after damage or an injury, and glutamate-induced plasticity is a crucial phase in this process. This process, known as activity-dependent central sensitization, plays a role in the development of post-injury pain hypersensitivity and is similar to hippocampal long-term potentiation of synaptic strength, which is an effective memory-consolidation mechanism (Chiechio & Nicoletti, 2012).

In inflammatory and neuropathic pain models, both NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate), and many mGlu receptor subtypes have been implicated in the induction and maintenance of chronic pain by inducing long-lasting sensitization of the nociceptive pathways. Because of their ability to modulate rather than mediate excitatory synaptic activity, mGlu receptors can indeed be considered valid targets for chronic pain control. Understanding the function, expression, and role of mGluRs in pain modulation, and their involvement in the induction and maintenance of central sensitization, may aid in the development of a feasible alternative for reducing hypersensitivity in chronic pain conditions (Chiechio & Nicoletti, 2012).

1.9 METABOTROPIC GLUTAMATE RECEPTORS

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors (GPCRs) of class C that modulate synaptic transmission and neuronal excitability in the central nervous system. They are activated by the Glutamate neurotransmitter. Metabotropic glutamate receptors are a second messenger signaling pathway that allows glutamate to modulate cell excitability and synaptic transmission. These receptors lack ion channels and instead influence other channels by activating G-proteins, which are intermediate molecules. The eight members of the mGluR family (named mGluR1 to mGluR8) are divided into three classes based on agonist pharmacology, primary sequence, and G protein coupling to effector: Group I (mGluR1 and mGluRR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) (Metabotropic glutamate receptors | Introduction | BPS/IUPHAR Guide to PHARMACOLOGY, 2019) (refer to figure no. 1.8 & 1.9).

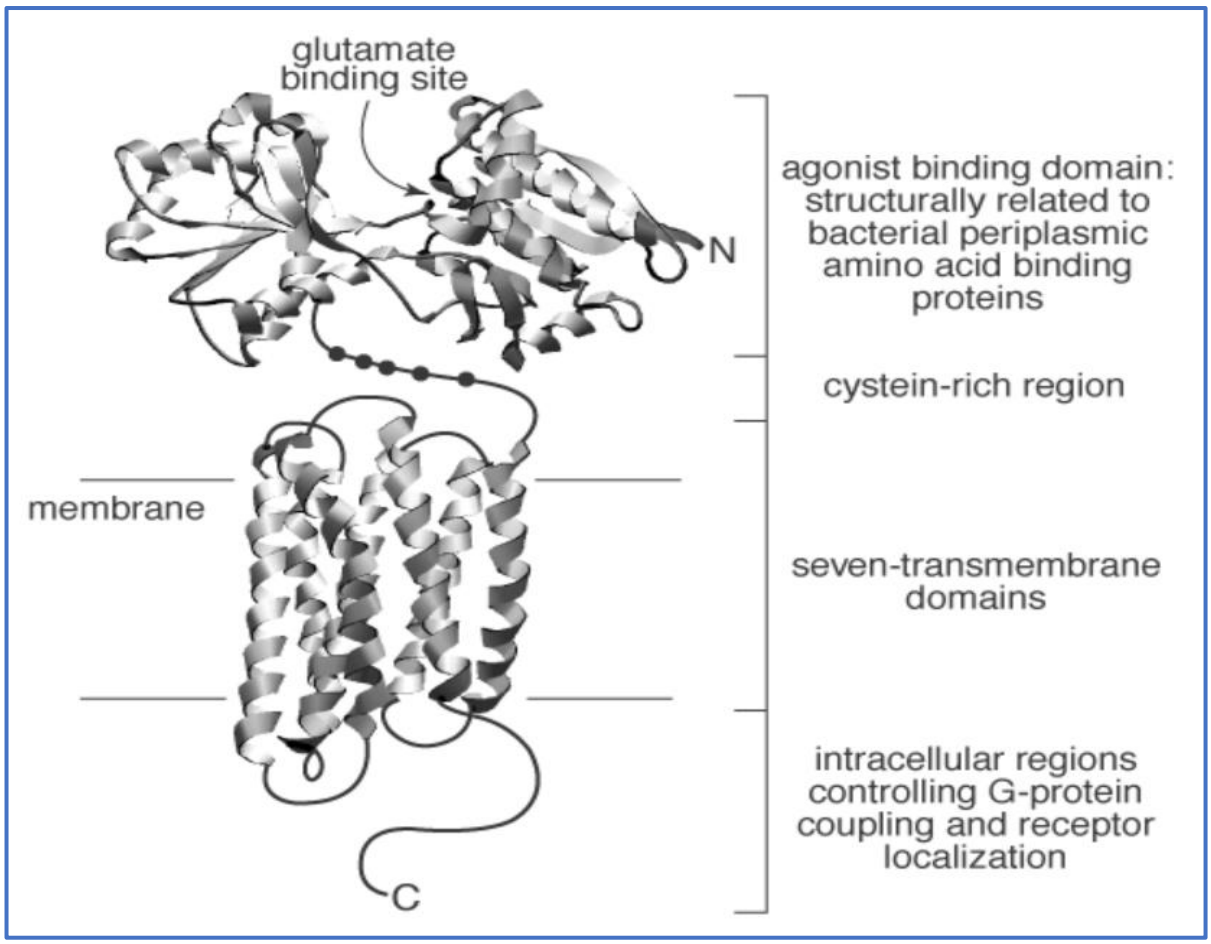

Figure no. 1.7: General structure of Metabotropic Glutamate Receptor

Source: *Metabotropic glutamate receptors | Introduction | BPS/IUPHAR Guide to PHARMACOLOGY*. Guidetoimmunopharmacology.org. (2019). Retrieved 15 April 2021, from https://www.guidetoimmunopharmacology.org/GRAC/FamilyIntroductionForward?familyId=40

A broad N-terminal extracellular domain of 560 amino acids, which contains the glutamate binding domain and confers agonist selectivity, distinguishes metabotropic glutamate receptors. The structure of the ligand-binding domain is identical to that of bacterial periplasmic binding proteins. The Venus flytrap domain (VFTD), a large bi-lobed extracellular domain where glutamate binds, is linked to the core G protein-activating seven-transmembrane domain (TM), which is common to all GPCRs, via a rigid cysteine-rich domain (CRD). A disulphide bridge connects mGluRs to form constitutive dimers (Metabotropic glutamate receptors | Introduction | BPS/IUPHAR Guide to PHARMACOLOGY, 2019) (refer to Figure no. 1.7 & 1.8)

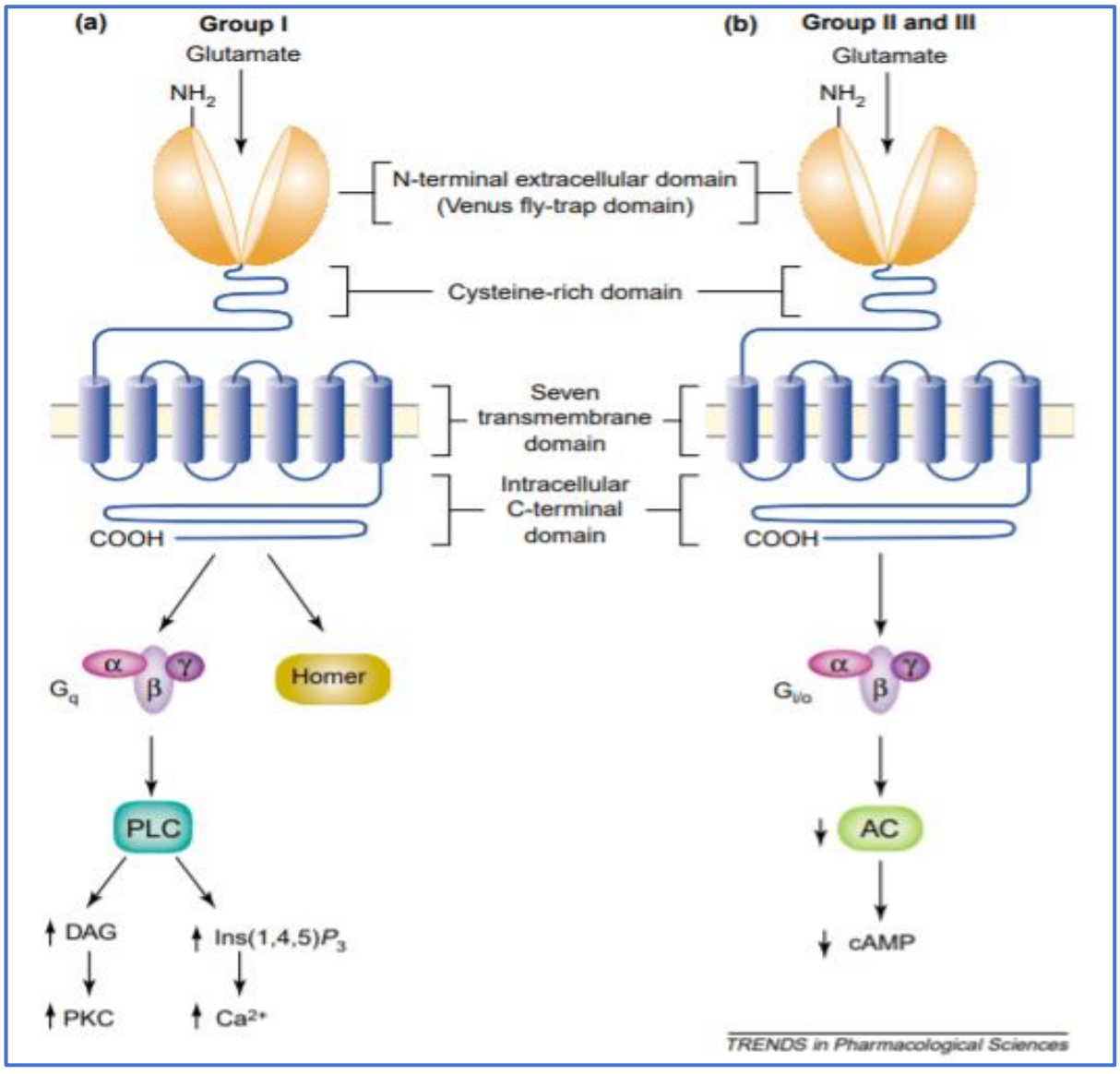

Figure no. 1.8: Schematic representation of Group I, Group II, and Group III mGluRs

Source: Kenny, P., & Markou, A. (2004). The ups and downs of addiction: role of metabotropic glutamate receptors. *Trends In Pharmacological Sciences*, *25*(5), 265-272. https://doi.org/10.1016/j.tips.2004.03.009

1.10 GENERAL FUNCTION OF mGluRs

mGluRs are neuromodulators that regulate neuronal excitability and the release of neurotransmitters such as GABA, purines, dopamine, 5-HT, and neuropeptides. Neuronal excitability is enhanced by Group I mGluRs, while neuronal excitability is suppressed by Groups II and III mGluRs. G_q proteins bind to Group I mGluRs, which activate phospholipase C. Adenylyl cyclase inhibition and cAMP formation are caused by Groups II and III receptors interacting with $G_{i/o}$ proteins which limits downstream protein kinase A (PKA) activation. Activation of group I mGluRs triggers phospholipase C (PLC) and causes phosphoinositide hydrolysis, resulting in the formation of IP3 and DAG, while activation of group II and group III mGluRs causes a decrease in intracellular levels of cyclic adenosine monophosphate (cAMP) through a negative coupling to adenylyl cyclase (AC) (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016) (refer to Figure no. 1.9 & 1.10).

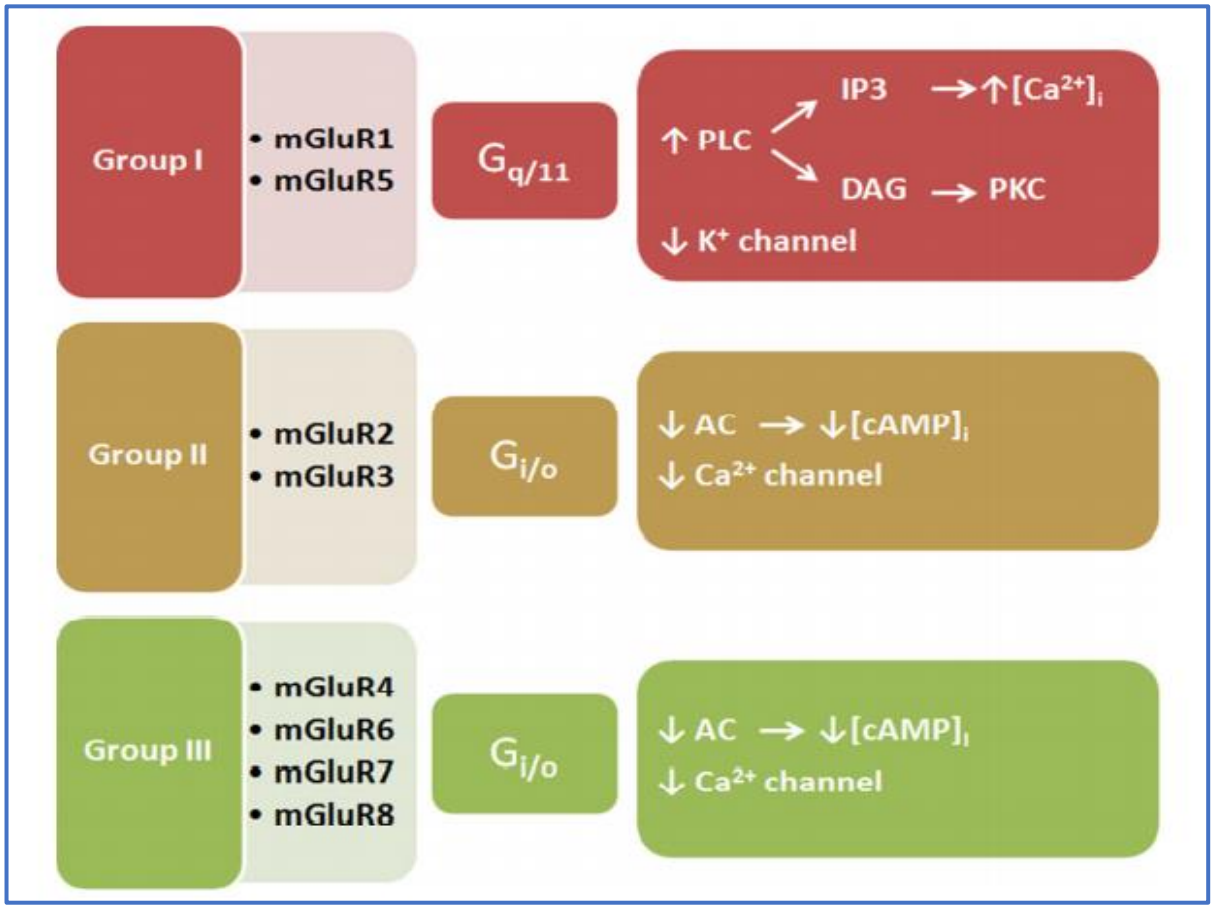

Figure no. 1.9: mGluRs groups and transduction pathways

Source: chiechio, S. (2016). Modulation of Chronic Pain by Metabotropic Glutamate Receptors. *Pharmacological Mechanisms And The Modulation Of Pain*, 63-89. https://doi.org/10.1016/bs.apha.2015.11.001

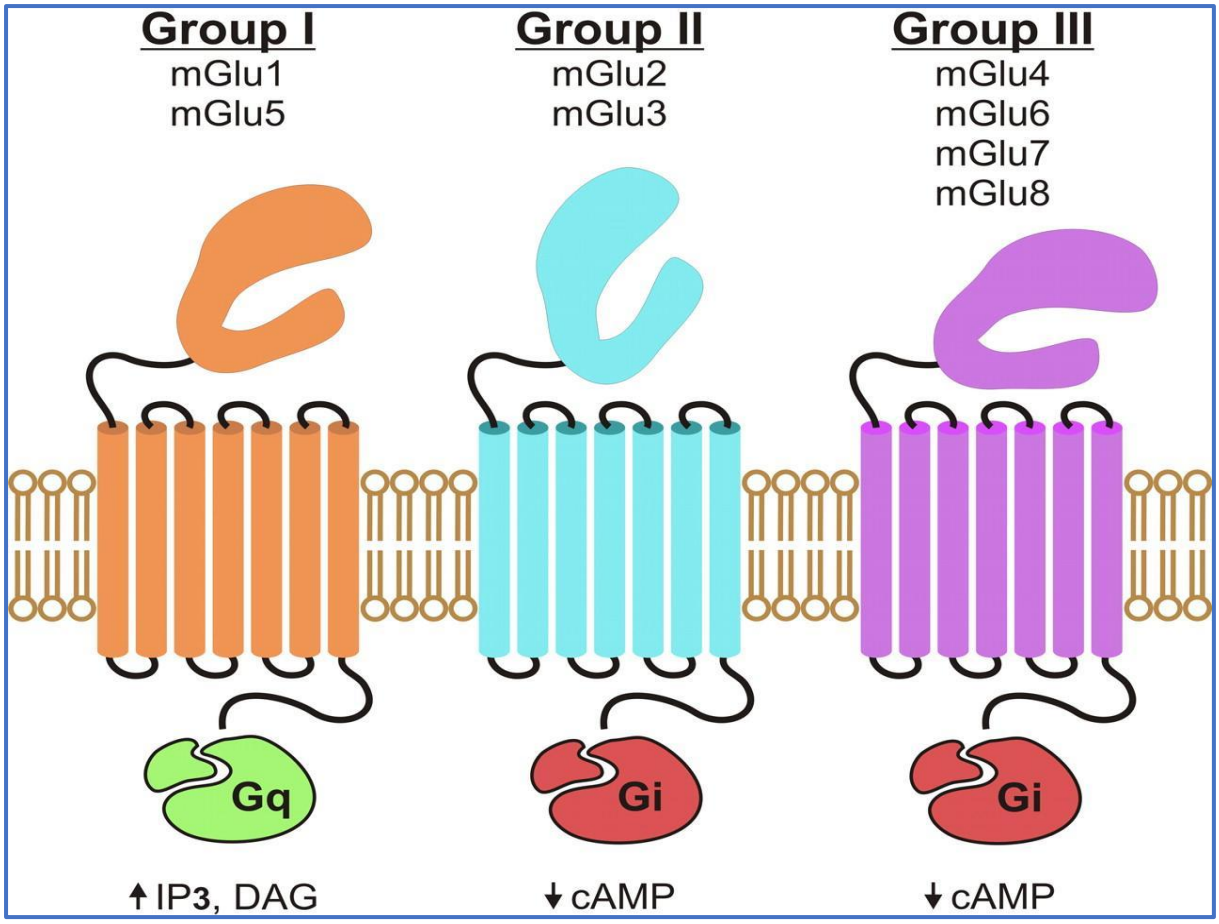

Figure no. 1.10: mGlu receptor families

Source: Julio-Pieper, M., Flor, P., Dinan, T., & Cryan, J. (2011). Exciting Times beyond the Brain: Metabotropic Glutamate Receptors in Peripheral and Non-Neural Tissues. *Pharmacological Reviews*, *63*(1), 35-58. https://doi.org/10.1124/pr.110.004036

Group I mGluRs are often found postsynaptically and stimulate phospholipase C (PLC) to increase diacylglycerol and inositol triphosphate levels. Protein kinase C (PKC) is activated, resulting in the release of intracellular Ca^{2+}, which inhibits presynaptic K^{+} channels and delays nerve terminal repolarization (Metabotropic glutamate receptors | Abcam, n.d.).

Group I mGluRs may also stimulate a variety of downstream effectors, which may play a role in synaptic plasticity regulation. mGluR5 receptors can be used to treat depression, fragile X syndrome, anxiety, obsessive-compulsive disorders, and levodopa-induced dyskinesia in Parkinson's disease by targeting negative allosteric modulators (Metabotropic glutamate receptors | Abcam, n.d.).

Group II mGluRs can inhibit adenylate cyclase and suppress neuronal excitability presynaptically. The mGluR2 and mGluR3 subtypes are strongly expressed in the hippocampus, cortex, nucleus accumbens, striatum, and amygdala and share a strong degree of sequence similarity. These receptors may be used to treat anxiety disorders and schizophrenia in the future (Metabotropic glutamate receptors | Abcam, n.d.).

Group III mGluRs function in the same way as Group II mGluRs, being expressed presynaptically and inhibiting adenylate cyclase to suppress neuronal excitability. mGluR4 and mGluR7 receptors are present in the brain, while mGluR6 receptors are found only throughout the retina and mGluR8 receptors are mostly found in the hippocampus, hypothalamus, and olfactory bulb at low levels. Group III mGluRs also play a role in synaptic remodeling as well as recurrent substance seeking and addiction, acting via a presynaptic process that can extend to postsynaptic modulation (Metabotropic glutamate receptors | Abcam, n.d.) (refer to Table no. 1.4).

Family and Receptors	Coupling	Characteristics
Group I	Excitatory	
mGluR1	G_q coupled	- Primarily postsynaptic - Expressed in neurons
mGluR5	G_q coupled	- Primarily postsynaptic - Expressed in neurons and astrocytes
Group II	Inhibitory	
mGluR2	G_i coupled	- Primarily presynaptic - Expressed in neurons and astrocytes
mGluR3	G_i coupled	- Pre- and postsynaptic - Expressed in neurons and astrocytes
Group III	Inhibitory	
mGluR4	G_i coupled	- Pre- and postsynaptic - Expressed in neurons and reactive astrocytes
mGluR6	G_i coupled	- Exclusively in postsynapses of retinal bipolar metabotropic (ON-center) cells
mGluR7	G_i coupled	- Pre- and postsynapses - Expressed in neurons
mGluR8	G_i coupled	- Primarily presynaptic - Expressed in neurons and reactive astrocytes

Table no. 1.4: Classification and features of mGluRs

Source: Rubio, M., Drummond, J., & Meador-Woodruff, J. (2012). Glutamate Receptor Abnormalities in Schizophrenia: Implications for Innovative Treatments. *Biomolecules And Therapeutics*, *20*(1), 1-18. https://doi.org/10.4062/biomolther.2012.20.1.001

2. METHODS & RESULTS

2.1 ORTHOSTERIC LIGANDS AND ALLOSTERIC MODULATORS OF mGluRs

Several synthetic compounds with the ability to modulate glutamatergic transmission by activating or inhibiting specific mGluR groups or subtypes have been developed. Orthosteric ligands and allosteric modulators have been used to investigate the specific role of mGluRs in pain modulation. A variety of subtype-selective positive allosteric modulators (PAM) and negative allosteric modulators (NAM) have been developed to potentiate or minimize the physiological activation of specific mGluRs by endogenous glutamate, and several have been used to understand the specific role of mGluR subtypes function in pain conditions (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016) (refer to Table no. 2.1).

Group/ Subtype	Activity	Other Effects	References
Group I ligands			
Quisqualic acid	Agonist	Selectivity for mGluR1 and mGlur5	Ohashi et al. (2002)
		Activity at AMPA receptors	Watkins, Krogsgaard-Larsen, and Honoré (1990)
(*S*)-3,5-DHPG	Agonist	More selective for mGluR5 over mGluR1	Schoepp et al. (1999)
(*S*)-4CPG	Antagonist	Activity at mGluR2	Schoepp et al. (1999)
(*S*)-4C3HPG	Antagonist	Activity at mGluR2	Schoepp et al. (1999)
(*S*)-MCPG	Antagonist	Activity at mGluR2	Schoepp et al. (1999)
mGluR1 selective			
AIDA	Antagonist		Lavreysen et al. (2003)
LY367385	Antagonist		Kingston, Burnett, Mayne, and Lodge (1995)
Ro67-4853	PAM		Knoflach et al. (2001)
CPCCOEt	NAM		Litschig et al. (1999)
JNJ16259685	NAM		Lavreysen et al. (2004)
YM-298198	NAM		Kohara et al. (2005)
FTIDC	NAM		Suzuki, Kimura, et al. (2007) and Suzuki, Tsukamoto, et al. (2007)
mGluR5 selective			
CHPG	Agonist	High selectivity at mGluR5 over mGluR1	Doherty, Palmer, Henley, Collingridge, and Jane (1997)
SIB-1757	NAM		Varney et al. (1999)
Fenobam	NAM		Porter et al. (2005)
MPEP	NAM	mGluR4 PAM	Gasparini et al. (1999)
MTEP	NAM		Cosford et al. (2003)

Group/ Subtype	Activity	Other Effects	References
Group II ligands			
DCG IV	Agonists	Potent agonist at mGluR2 and mGluR3	Schweitzer et al. (2000)
		NMDA receptor agonist activity	Ishida, Saitoh, Shimamoto, Ohfune, and Shinozaki (1993)
		Antagonist activity at group I and group III	
		mGluRs at high concentrations	Brabet et al. (1998)
2*R*,4*R*-APDC	Agonist		Schoepp et al. (1999)
LY379268	Agonist	Activate mGluR4, mGluR6 and mGluR8 at high concentrations	
			Monn et al. (1999)
LY341495	Antagonist	Little antagonism on group I and III mGluRs	Johnson et al. (1999)
			Jane, Thomas, Tse, and Watkins (1996)
LY354740	Agonist		Bond, Monn, and Lodge (1997)
EGLU	Antagonist	Little antagonism on group I and III mGluRs	Jane et al. (1996)
mGluR2 selective			
LY487379	PAM		Schaffhauser et al. (2003)
mGluR3 selective			
NAAG	Agonist		Schweitzer et al. (2000)
Group III ligands			
L-AP4	Agonist	Activate mGluR4, mGluR6 and mGluR8 with similar potencies	Schoepp et al. (1999)

Table no. 2.1: Orthosteric and Allosteric mGlur receptor ligands employed in pain studies

Source: Chiechio, S. (2016). Modulation of Chronic Pain by Metabotropic Glutamate Receptors. *Pharmacological Mechanisms And The Modulation Of Pain*, 63-89. https://doi.org/10.1016/bs.apha.2015.11.001

This table lists the most commonly used ligands in chronic pain research.

Group/ Subtype	Activity	Other Effects	References
L-SOP	Agonist	Activate mGluR7 at higher concentrations	Wright, Arnold, Wheeler, Ornstein, and Schoepp (2000)
CPPG	Antagonist		Jane et al. (1996)
mGluR4 selective			
LSP4-2022	Agonist	300-fold selectivity for mGluR4 over mGluR8	Goudet et al. (2012)
PHCCC	PAM	Activity on mGluR6	Beqollari and Kammermeier (2008)
VU0155041	PAM		Niswender et al. (2008)
mGluR7 selective			
AMN082	PAM		Mitsukawa et al. (2005)
MMPIP	NAM		Suzuki, Kimura, et al. (2007) and Suzuki, Tsukamoto, et al. (2007)
XAP044	NAM		Gee et al. (2014)
mGluR8 selective			
(*S*)-3,4-DCPG	Agonist	100-fold selectivity for mGluR8 over mGluR4	Thomas et al. (2001)
AZ12216052	PAM		Duvoisin et al. (2010)

2.2 mGluR SUBTYPES IN CHRONIC PAIN MODULATION

Depending on the particular receptor subtype activated as well as the anatomical and cellular localization, activation of mGlu receptors may either increase or decrease cell excitability. The mammalian nervous system contains all mGlu receptors and they are found both presynaptically and postsynaptically in neurons. mGluRs are promising candidates for chronic pain management because they inhibit both nociceptive and recurrent pain. Several mGluR subtypes are also expressed in glial cells, such as astrocytes, where they play an important role in neuronal excitability and pain hypersensitivity modulation. Pain hypersensitivity can be reduced by mGluRs working at various stages, from the periphery to brain regions involved in pain perception and regulation. mGluRs are ubiquitously expressed in the nervous system in both neurons and glial cells, with some selectivity for each subtype in particular brain regions, except for the mGluR6 subtype, which is restricted to dendrites of ON bipolar cells in the retina (Chiechio & Nicoletti, Metabotropic glutamate receptors and the control of chronic pain, 2012) (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016) (refer to Table no. 2.2).

mGlu receptor subtype	Target	Effects and consequences on pain threshold [Ref]	
			Peripheral terminals
mGlu5	TRPV1	Increased function	Hyperalgesia [29-31]
mGlu2/3		Decreased function	Analgesia [50-53]
			Dorsal Horn
mGlu1/5	ERK1/2	Increased phosphorylation	Hyperalgesia [34]
mGlu5	$K_{v4.2}$	Decreased A-type K* currents	Hyperalgesia [35]
mGlu2/3 mGlu4/7	Glutamate release	Decreased	Analgesia [48]
			Amygdala
mGlu1	CeA neurons	Increased activation following prolonged nociception	Hyperalgesia [36]
	CeLCneurons	Increased excitatory transmission	Hyperalgesia [38]
mGlu5	ERK1/2	Increased phosphorylation	Hyperalgesia [39]
	CeA neurons	Increased activation in brief and prolonged nociception	Hyperalgesia [36]
mGlu7	CeLC neurons	Inhibition of inhibitory synaptic transmission in normal condition	Hyperalgesia [70]
mGlu8		Inhibition of pain-related enhanced excitatory transmission	Analgesia [69,70]
			Periaqueductal gray
mGlu1/5 mGlu2/3	Descending antinociceptive pathway	Activation	Analgesia [42]
mGlu7	Glutamae and	Decreased	Hyperalgesia [68]
mGlu4/8	GABA release	Increased	Analgesia [68]

TABLE NO. 2.2: mGlu receptor subtypes modulate nociceptive processing through several mechanisms

Source: Chiechio, S., & Nicoletti, F. (2012). Metabotropic glutamate receptors and the control of chronic pain. *Current Opinion In Pharmacology*, *12*(1), 28-34. https://doi.org/10.1016/j.coph.2011.10.010

mGluRs modulate the experience of physiological pain within the pain neuraxis and play a role in the development of peripheral and central sensitization in chronic pain conditions. Group I mGluRs, are found at the postsynaptic level, where they positively modulate neuronal excitability, while group II and III mGluRs are found at the presynaptic level, where they negatively regulate neurotransmitter release. mGluR subtypes can have both pronociceptive and antinociceptive effects depending on the anatomical site of activation and it is generally accepted that although group I mGluRs mediate hyperalgesia, and group II mGluRs and III mGluRs can minimize hyperalgesia in inflammatory and neuropathic pain in animal models (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

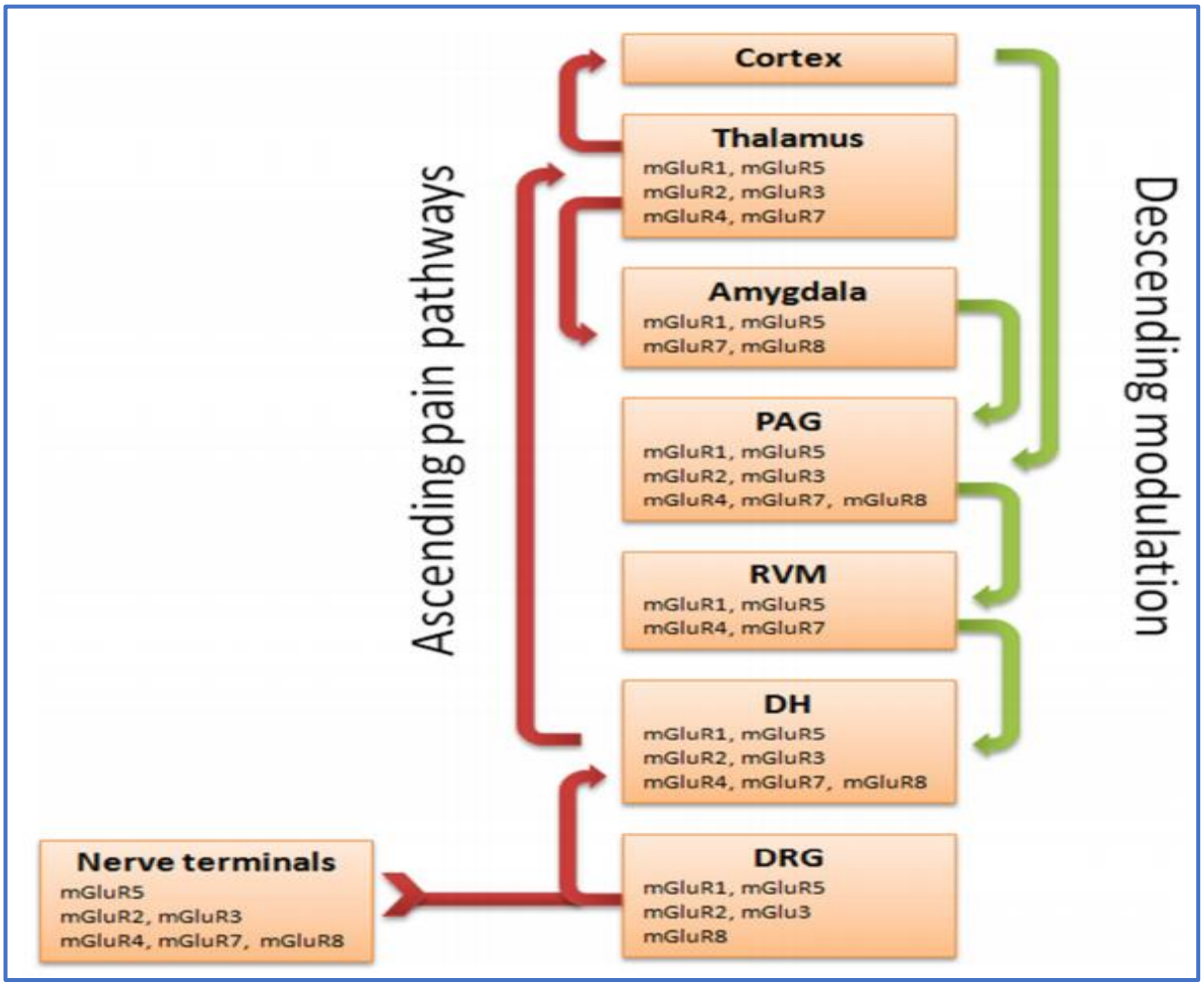

Figure no. 2.1: mGluRs subtypes in the pain pathways

Source: Chiechio, S. (2016). Modulation of Chronic Pain by Metabotropic Glutamate Receptors. *Pharmacological Mechanisms And The Modulation Of Pain*, 63-89. https://doi.org/10.1016/bs.apha.2015.11.001

mGluR expression in peripheral, spinal, and supraspinal structures involved in pain transmission and modulation. There are pain receptors that ascend and descend (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016). (PAG: periaqueductal grey; RVM: rostral ventromedial medulla; and DRG: dorsal root ganglion) (refer to Figure no. 2.1).

2.3 ROLE OF GROUP I mGluRs IN CHRONIC PAIN MODULATION

Inhibiting the Group I mGluRs and/or potentiating Group II and III mGluR signaling are the most common pharmacological interventions for pain relief. Glutamate released from primary afferent neurons is an important event in chronic pain states that leads to recurrent activation of spinal neurons and hypersensitivity. The mGluR5 subtype of group I mGluRs, along with NMDA receptors, plays an important role in nociceptive processing and central sensitization production by activating a variety of intracellular pathways (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Both mGluR1 and mGluR5 have been implicated in the physiological regulation of pain and the production of chronic pain states in pharmacological studies. Antibodies raised against the C-terminals of mGluR1 and mGluR5 will block the effects of group I mGluRs agonists, which cause spontaneous pain and increase inflammatory and neuropathic pain. mGluR5 antagonists are involved in animal models of inflammatory, neuropathic, and visceral pain in recent studies. Drugs that inhibit or negatively modulate mGluR5 are not only effective in chronic pain but also have positive reinforcing effects in neuropathic pain. This intriguing discovery makes the mGluR5 a promising candidate for chronic pain treatment (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

While both mGluR1 and mGluR5 antagonists or NAMs are successful in chronic pain models, it appears that the mGluR5 is a better target for chronic pain care. While the group I mGluR-mediated signaling is a key event in peripheral and central sensitization of pain, depending on the site of activation, the group I mGluRs may mediate both pronociceptive and antinociceptive effects. Although activation of group I mGluRs in peripheral sensory afferents in the dorsal horn (DH) of the spinal cord has been shown to help with nociception, the group I mGluRs can modulate pain sensitivity at supraspinal levels through different mechanisms (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Group I mGluR-mediated hyperalgesia is elicited in the periphery through a variety of mechanisms that often entail the cooperation of multiple receptors, ion channels, and intracellular pathways. Group I mGluRs, for example, enhance the activity of the transient receptor potential vanilloid 1 (TRPV1) in the peripheral terminal of sensory neurons, increasing pain sensitivity (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

In peripheral sensory neurons, mGluR5 has also been shown to modulate TRPV1 activity in a biphasic manner. Surprisingly, mGluR5-mediated TRPV1 role sensitization appears to necessitate the involvement of the scaffolding protein A-kinase anchoring protein 79/150. (AKAP150). In trigeminal sensory neurons responsible for mechanical hyperalgesia in muscle tissues, a functional association between group I mGluRs and TRPV1 receptors through AKAP150 has also been discovered. mGluR1 and mGluR5 play a pronociceptive function in the dorsal horn of the spinal cord by triggering ERK signaling, which inhibits Kv4.2-containing potassium channels, resulting in increased dorsal horn neuronal excitability (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Stimulation of group I mGluRs has both pronociceptive and antinociceptive effects at the supraspinal level. In inflammatory models of chronic pain, the group I mGluRs mediate hyperalgesic effects in the amygdala, and mGluR5 is involved in the regulation of visceral pain. Furthermore, in rats with inflammatory pain, mGluR1 appears to be involved in the crosstalk between the basolateral amygdala and the prefrontal cortex. Activation of all groups of mGluRs, including group I, mediates antinociceptive effects in the periaqueductal grey matter (PAG), presumably by activating the descending antinociceptive pathways through inhibition of GABAergic transmission. Moreover, mGluR5 seems to be involved in the analgesic effects of cannabinoids in the PAG (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

The prefrontal cortex, which is involved in decision-making and behavioral regulation is affected in patients with chronic pain and animal models of inflammatory and neuropathic pain, is also high in Group I mGluRs. In arthritis pain models, the prefrontal cortex's mGluR5 regulates amygdala neuronal hyperactivity and pain behavior. The thalamus, which is an essential center for the integration of pain sensations coming from the spinal cord, via the spinothalamic tract, and from the descending projection from the somatosensory cortex through the corticothalamic projections, also plays an important role in nociceptive processing by the Group I mGluRs. The regulation of nociceptive processing in the thalamic circuitry is largely mediated by glutamate via both NMDA receptors and the group I mGluRs, with the postsynaptic mGluR1 subtype playing a prominent role (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Pain and depression have a bidirectional effect on one another, implying the presence of shared or overlapping neural pathways. The medial prefrontal cortex (mPFC) is considered to be involved in both pain and depression, making it one of the neural circuits important to negative affection. Physical pain and mental distress can cause maladaptive changes in mPFC circuits, which can lead to depression. An uncomfortable mood alters mPFC circuits, distorting aversion appraisal and making people more prone to pain (Chung, Kim, & Kim, 2018).

The role of mGluR5 in the mPFC has been extensively studied in both pain and depressive disorders but there are still concerns about changes in mPFC activity during chronic pain and depression, and the functional roles of mGluR5 in altered mPFC activity. Unavoidable discomfort, such as somatic pain or psychological distress, affects mPFC circuitry and distorts perceptions of self-state and subsequent coping strategy decisions. The mGluR5 receptor in the mPFC may be a popular mediator of pain and depression and its levels will monitor the benefit for internal state evaluation, guide external state appraisal, and be active in updating behavioral outcome expectancies in the mPFC. Increased mPFC-mGluR5 levels during the pathological state will inhibit pyramidal neuron activity and reduce system flexibility, resulting in a loss of control over adequate information processing (Chung, Kim, & Kim, 2018).

Glutamate tends to play a key role among the multiple neurotransmitter systems causally related to the expression of social conduct. The altered function of mGlu5 receptors has been identified in many mouse models of autism spectrum disorders (ASD) and mental retardation, so it has gotten a lot of attention. In some of these animal models, inhibiting the activity of mGluR5 receptors through genetic or pharmacological manipulations enhanced social deficits. This influence, however, was followed by a decrease in exploratory activity and an increase in anxiety-like behavior during the test (Ramos-Prats, et al., 2019).

The mGlu5 receptor negative allosteric modulator 3-((2-methyl-1,4-thiazolyl)ethynyl)pyridine (MTEP) induced anxiolytic effects in the wild-type mice without influencing the social selection, in contrast to mGlu5 receptor ablation. Following social interaction, specific activation of the prefrontal cortex and dorsolateral septum in the Grm5/ mice were observed by mapping c-Fos expression in 21 distinct brain regions reported to be involved in social interaction. The absence of mGlu5 receptors altered the functional influence of prefrontal and hippocampal regions in the social interaction network dramatically. The significance of mGlu5 receptors in controlling brain functional connectivity during social interaction suggests that mGlu5 receptors play a complex role in sociability and anxiety (Ramos-Prats, et al., 2019).

2.4 ROLE OF GROUP II mGluRs IN CHRONIC PAIN MODULATION

The capacity of group II mGluRs to alleviate hyperalgesia in animal models of chronic pain has been extensively studied. Presynaptically on sensory nerve terminals, mGluR2 and mGluR3 are found. These receptors function as presynaptic autoreceptors or heteroreceptors in the synapse, regulating neurotransmitter release and responding primarily to glutamate released by astrocytes. Group II mGluR agonists suppress pain transmission by functioning at various levels of the pain neuraxis, including nociceptors, DH neurons, and supraspinal regions involved in pain control, such as the amygdala and PAG, when administered systemically. Group II mGluRs in the periphery reduce hyperalgesia after inflammation. This was demonstrated in a model of inflammatory pain called carrageenan-induced arthritis after local administration of a group II agonist, APDC, in the knee joint (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

TRPV1 channels and TTX-resistant sodium channels are regulated by group II mGluRs in sensory neuron peripheral terminals. Group II mGluRs play an important role in pain control by inhibiting synaptic transmission between primary afferent fibers and dorsal horn neurons in the dorsal horn of the spinal cord. At the supraspinal stage, including the amygdala, the PAG, and the medial prefrontal cortex, group II mGluRs mediate analgesia. Group II mGluRs are strongly expressed in the prefrontal cortex, where they inhibit glutamatergic excitatory transmission and modulate arthritis pain-related synaptic transmission, similar to group I mGluRs (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

mGluR2 appears to play a more prominent role in the regulation of pain transmission among group II mGluRs, at least in inflammatory pain. Several epigenetic drugs, such as L-acetylcarnitine, or histone deacetylase, or acetyltransferase inhibitors, have been shown to control mGluR2 expression in various regions such as the dorsal root ganglion (DRG) and dorsal horn (DH). Changes in the expression level of mGluR2 in the DRG and spinal cord have been shown to alter the analgesic efficacy of group II mGluR agonists. As a result, drug-induced mGluR2 overexpression can be a useful method for treating inflammatory and neuropathic pain. As a result, N-acetylcysteine, a medication that induces analgesia in models of inflammatory and neuropathic pain by increasing endogenous activation of mGluR2 and mGluR3 through the glutamate/cystine antiporter, has been shown to inhibit nociceptive transmission in humans, implying that group II mGluRs are promising targets for new analgesic drugs (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Group II mGluR2 and mGluR3 are primarily expressed presynaptically and inhibit the release of neurotransmitters such as glutamate and GABA. They couple negatively to adenylyl cyclase via Gi/Go proteins. The expression of Group II mGluRs in peripheral, spinal, and supraspinal elements of pain-related neural processing has been consistently linked to pain regulation. In preclinical models of acute and chronic pain, pharmacological studies have demonstrated anti-nociceptive/analgesic effects of group II mGluR agonists, but much less is known about the mechanisms and sites of action for mGluR2 and mGluR3 than for other mGluRs. The availability of orthosteric and new selective allosteric modulators acting on mGluR2 and mGluR3 has provided useful resources for elucidating (subtype) specific contributions of these receptors to the pathophysiology of pain and other disorders, as well as their therapeutic potential (Mazzitelli, Palazzo, Maione, & Neugebauer, 2018).

Preclinical research suggests that group II mGluRs play an important role in nociception and pain control. There is some evidence that the mGluR2 and mGluR3 subtypes play different roles in different neural circuits and areas, but with the availability of more selective compounds including allosteric modulators, this will be determined more thoroughly. These new methods can also be used to investigate the pathophysiological mechanisms of pain. The therapeutic potential of mGluR2/3 compounds for the treatment of pain can be supported by their efficacy in clinical trials on conditions other than pain (Mazzitelli, Palazzo, Maione, & Neugebauer, 2018).

The limbic area of the anterior cingulate cortex is involved in the emotional perception of pain. A GRM2Cre:tdtomato reporter mouse line was used to classify a population of pyramidal neurons in the murine anterior cingulate cortex's layer II/III. Although its role in the anterior cingulate cortex is unknown, GRM2 encodes the Group II mGluR2, which has analgesic properties in mouse and human models. Physiological properties of GRM2-tdtomato anterior cingulate cortex neurons were examined in slices from animals with neuropathic or inflammatory pain, as well as controls, using whole-cell patch-clamp techniques. GRM2-tdtomato anterior cingulate cortex neurons showed increased excitability after a hind-paw injection of Complete Freund's Adjuvant or chronic constriction injury, as measured by a rise in the amount of evoked action potentials and a decrease in rheobase (Chen, Kadakia, & Davidson, 2020).

In both inflammatory and neuropathic models, bath application of the mGluR2 subtype agonist (2R, 4R)-4-Aminopyrrolidine-2,4-dicarboxylate APDC (1 mM) reversed hyperexcitability. Under inflammatory and neuropathic pain, the layer II/III pyramidal GRM2-tdtomato anterior cingulate cortex neurons express functional Group II metabotropic glutamate receptors and experience changes in membrane biophysical properties (Chen, Kadakia, & Davidson, 2020).

Inflammatory and neuropathic damage sensitize GRM2-tdtomato pyramidal neurons in layer II/III of the mouse ACC. These neurons have functional group II mGluR receptors, which when activated suppressed the increased action potential discharge seen after injury but had no effect on membrane properties or discharge in the absence of injury. Future research aimed at identifying the GRM2-tdtomato ACC neurons' input pathways as well as their downstream connections will help us better understand how these neurons are incorporated into nociceptive and emotional circuitry. Drug development for mGluRs is a hot topic, and clinical trials of mGluR2/3 agonists have already been approved, indicating a favorable safety profile (Chen, Kadakia, & Davidson, 2020).

2.5 ROLE OF GROUP III mGluRs IN CHRONIC PAIN MODULATION

Group III mGluR subtypes are found in the pain neuraxis, from peripheral nerves to the CNS. The expression of mGluR8 has been found in unmyelinated fibers of digital nerves, where it inhibits adenylyl cyclase and thus negatively modulates the activity of TRPV1 receptors on nociceptors. The intraplantar injection of L-AP4, a group III mGluRs agonist, consistently reduces the hyperalgesia caused by capsaicin, a TRPV1 agonist. Peripheral group III mGluRs, including group II mGluRs, are involved in the regulation of hyperalgesia after inflammation. In the carrageenan-induced arthritic pain model, local administration of the group III mGluRs agonist L-AP4 in the knee joint reduces hyperalgesia. Activation of spinal group III mGluRs in the dorsal horn of the spinal cord has been shown to reduce the firing of projection second-order neurons by regulating glutamatergic transmission excess in inflammatory and neuropathic pain (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

The mGluR4 subtype among group III mGluRs has been demonstrated on both presynaptic terminals of C-fibers and spinal neuron terminals in the inner laminae II of the dorsal horn. By reducing glutamatergic transmission in the dorsal horn, mGluR4 stimulation prevents the production of inflammatory and neuropathic pain. Intrathecal injection of selective mGluR4 agonists or PAMs can minimize hypersensitivity in animal models of inflammatory and neuropathic pain without affecting normal nociception in naive animals, making the regulation of pain hypersensitivity mediated by mGluR4 especially intriguing. The mGluR7 subtype of group III mGluR has also been discovered in presynaptic terminals of sensory neurons in the dorsal horn's laminae I and II (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

The supraspinal region S, which is involved in pain, has a high expression of Group III mGluRs. In areas like the amygdala, PAG, and the rostral ventromedial medulla (RVM), however, mGluR7 and mGluR8 also have an opposing impact. While systemic mGluR7 and mGluR8 activation reduce inflammatory and neuropathic pain, local mGluR7 stimulation in the PAG and amygdala increases pain, while mGluR7 blockade reduces inflammatory and neuropathic pain (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

mGluR8 activation in the PAG, dorsal striatum, amygdala, and RVM, on the other hand, decreases inflammatory and neuropathic pain. Overall, the findings with group III mGluR ligands indicate that these receptors may be useful therapeutic targets for treating chronic pain hypersensitivity. This is particularly true for the mGluR4 subtype since its activation in the spinal cord does not interfere with physiological pain perception while alleviating chronic pain (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Recently, new ligands that target group III mGluR subtypes have been created. They primarily serve as positive or negative allosteric modulators (PAMs or NAMs) of glutamate transmission, and they help to explain the functional functions of group III mGluRs in a variety of pathological conditions, including epilepsy, anxiety, neurodegenerative diseases, and chronic pain. The existence of group III mGluRs in the pain neuraxis, particularly in the descending system, indicates that these analgesic endogenous substrates that run from the cortex to the first spinal synapse could be used to regulate pain. Group III mGluRs ligands will help with chronic pain relief as pathological pain is one of the most difficult conditions to treat due to a lack of effective treatments (Palazzo, et al., 2017).

The selectivity of the compounds on the specific mGluR subtype determines the multidirectional effects of group III mGluR stimulation on pain responses. Indeed, the positions of mGluR7 and mGluR8 in pain modulation are opposed. The results vary depending on the type of neuron (glutamate or GABA) on which the receptor is primarily located within the brain site. The total effects of group III mGluR ligands when given systemically are determined by the compound hydrophilicity, which affects receptor modulation at the peripheral, spinal, or supraspinal level. The blood-brain barrier can be penetrated by new subtype-selective allosteric modulators of group III mGluRs. Since mGluRs are commonly expressed in the pain pathway, understanding the mechanism and site of action by which mGluR modulation occurs is difficult (Palazzo, et al., 2017).

Local microinjections and/or perfusions of subtype-selective group III mGlu ligands were used in studies on the function of supraspinal group III mGluRs, and it was discovered that mGluR7 antagonists and mGluR8 agonists have analgesic activities in the PAG, dorsal striatum, and CeA, but have the opposite effect in the NTS. Furthermore, these allosteric modulators modulate pain responses in physiological and pathological states in various ways (Palazzo, et al., 2017).

mGluR8 agonists, in particular, inhibit pain only when the CeA and dorsal striatum are pathologically activated, not in the PAG, where DCPG is also effective in normal conditions. Local administration of mGluR antagonists into the PAG, such as MMPIP, reduces pain responses in chronic pain but does not affect healthy people. As a result, these antagonists behave similarly to opiates and group II mGluRs (Palazzo, et al., 2017).

While we still don't fully understand the function of each subtype of group III mGluR in the processing of noxious information inside supraspinal sites, the potential for silencing pain through targeting mGluRs seems limitless. Selective mGluR-subtype allosteric modulators are promising analgesic candidates. When glutamate transmission is disrupted, such as in pathological pain states, mGluR7 NAMs and mGluR8 PAMs may modulate it (Palazzo, et al., 2017).

The amygdala is a brain region that connects pain and negative emotions. We demonstrate here that activating a particular intrinsic neuromodulatory mechanism in the amygdala associated with type 4 metabotropic glutamate receptors (mGlu4) eliminates sensory and affective symptoms of chronic pain, including hypersensitivity to pain, anxiety and depression-related behaviors, and fear extinction impairment. The effects of mGlu4 activation appear to occur outside the central nucleus through modulation of multisensory thalamic inputs to lateral amygdala principal neurons and dorsomedial intercalated cells, according to the neuroanatomical and synaptic analysis of the amygdala circuitry (Zussy, et al., 2016).

Optogluram, a small diffusible photo switchable positive allosteric modulator of mGlu4, was also grown. Light can be used to regulate endogenous mGlu4 activity with this ligand. Via visual stimulation of optogluram in the amygdala of freely behaving animals, we were able to quickly and reversibly inhibit behavioral symptoms correlated with chronic pain using this picture pharmacological approach. By acting on unique amygdala networks, acute pharmacological or optopharmacological stimulation of mGlu4 may quickly reverse emotional and sensory symptoms of chronic pain despite central sensitization (Zussy, et al., 2016).

This research contributes to a deeper understanding of the processes underlying negative emotions associated with chronic pain, as well as new avenues for developing novel therapeutic strategies for chronic pain syndromes that combine specific pharmacological treatments with extinction-based behavioral therapies (Zussy, et al., 2016).

The mGluR7 gene may be used to treat Neurodevelopmental Disorders (NNDs). mGluR7 is found in areas of the brain that relate to the above-mentioned symptom domains, and it plays a role in synaptic physiology and conduct. Patients with idiopathic autism and other NDDs have been linked to single nucleotide polymorphisms and mutations in the GRM7 gene. Reduced mGlu7 expression and/or function has been shown in rodent models to cause symptoms that are similar to those seen in NDDs. Potentiation of mGlu7 activity has also been shown to be effective in a mouse model of Rett syndrome (Fisher, Seto, Lindsley, & Niswender, 2018).

The clinical basis for this strategy is based on GRM7 gene disruptions seen in patients with NDDs. Preclinical studies in rodent models indicate that decreased mGlu7 function is sufficient to mimic phenotypes associated with NDD symptom domains and that positive modulation of mGlu7 activity may improve some deficits, especially in a mouse model of RTT. NDDs, on the other hand, are extremely heterogeneous and are most likely the product of distinct molecular pathologies that converge to generate phenotypic circuits and behaviors. As a result, further research is required to determine which subpopulations could benefit from a mGlu7-mediated therapy (Fisher, Seto, Lindsley, & Niswender, 2018).

Apart from regulating voluntary movement, the dorsal striatum has recently been shown to inhibit pain. The medullary dorsal reticular nucleus connects it to the descending pain modulatory system, specifically the rostral ventromedial medulla. In addition to motor disorders, diseases of the basal ganglia, such as Parkinson's disease, are associated with pain and hyperactivation of the excitatory transmission. The activation of group III metabotropic glutamate receptors (mGluRs), which are found on presynaptic terminals and inhibit neurotransmitter release, is one way to counteract glutamatergic hyperactivation. Selective ligands for each mGluR subtype of group III, including positive and negative allosteric modulators, have recently been established, and the function of each subtype is beginning to emerge. In pathological conditions marked by elevated glutamate, the neuroprotective capacity of group III mGluRs has recently been demonstrated (Boccella, et al., 2019).

mGluR7 and mGluR8 are glutamatergic corticostriatal terminals in the dorsal striatum, and their activation inhibits pain in pathological conditions like neuropathic pain. In normal conditions, the two receptors in the dorsal striatum play different roles in pain regulation. Several experiments were performed to determine the function of each receptor in pain regulation after the discovery of more selective compounds for mGluR4, mGluR7, and mGluR8, in particular, positive and negative allosteric modulators. When looking for pain response, it's logical to administer each selective ligand locally as well as systemically, including in pain-controlling areas; among these, the dorsal striatum showed antinociceptive activity (Boccella, et al., 2019).

The limitations of the only existing selective agonist, the AMN082, which, in addition to showing off-target effects in vivo, quickly produces internalization, can explain the variability of the effects produced by mGluR7 stimulation, which are site and condition-dependent (healthy versus pathological) (and behaves as an antagonist). The effects of mGluR7 negative allosteric modulation, which has been shown to suppress pain and its comorbidities in chronic pain conditions, are different. Although there is some heterogeneity in responses for the mGluR8 that is dependent on the site of administration, there is strong evidence that stimulation of this receptor inhibits pain only in chronic pain conditions (Boccella, et al., 2019).

Although its role in inhibiting pain processing at the spinal level appears well established, mGluR4 remains the least studied in terms of pain regulation, perhaps because all research has concentrated on its striking impact on Parkinson's symptoms. Finally, stimulation of mGluR7 and mGluR8 in the dorsal striatum inhibits pain in neuropathic pain conditions, while the two receptors act differently in normal conditions: mGluR7 facilitates pain responses while mGluR8 does not have any effect. In all cases, however, mGluR7, mGluR8, and mGluR4, which are expressed in the CNS sites that regulate pain transmission, have pain-inhibiting effects in pathological pain (Boccella, et al., 2019).

More than 70% of patients who undergo paclitaxel develop paclitaxel-induced acute pain syndrome (P-APS), which is characterized by deep muscle aches and arthralgia. P-APS may be debilitating for patients, causing them to reduce or stop taking potentially curable medications. Despite being fairly common in clinical practice, P-APS has no specific cure and the underlying mechanisms are unknown. Concerning its role in neuropathic pain, the regulation of glutamatergic transmission by mGluRs has gotten a lot of attention (Wang, et al., 2020).

AMN082, a positive allosteric modulator of mGluR7, inhibited the production of paclitaxel-induced acute mechanical and thermal hypersensitivity in rats without affecting their normal pain conduct. AMN082 inhibited glial reactivity and reduced pro-inflammatory cytokine release during P-APS by activating mGluR7. The paclitaxel-induced acute mechanical and thermal hypersensitivity was alleviated by stopping the spinal glial reaction to the drug. Selective activation of mGluR7 by its positive allosteric modulator, AMN082, reduces spinal glial reactivity and the neuroinflammatory mechanism, which helps to block P-APS (Wang, et al., 2020).

3. DISCUSSION

The Introduction section of this literature review explains what chronic pain is and how it affects the daily lives of people who suffer from it. Chronic pain lasts for months or years and may occur in any part of the body. It makes it difficult to go about everyday activities, such as working, socializing, and caring for oneself or others. It can cause depression, anxiety, and sleeping problems, all of which can exacerbate pain. Current treatments and medications available to alleviate chronic pain can be prescribed by a healthcare provider based on the severity, type, or source of the pain. Anticonvulsants, antidepressants, corticosteroids, muscle relaxants, opioids, nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen, topical medications, sedatives to help with anxiety and insomnia, etc. are prescribed to chronic pain sufferers. Some lifestyle changes and forms of alternative medicine have also shown to relieve chronic pain (Chronic Pain: Symptoms, Treatments, 2021).

Chronic pain is classified into nociceptive chronic pain and neuropathic chronic pain. Nociceptive chronic pain is further divided into superficial nociceptive pain and deep nociceptive pain. Deep nociceptive pain is divided into deep somatic pain and visceral pain. Neuropathic pain is categorized into central neuropathic pain and peripheral neuropathic pain (Yam, et al., 2018). The International Classification of Diseases, Eleventh Revision (ICD-11) divides chronic pain into seven categories which include Chronic primary pain, Chronic cancer pain, Chronic post-traumatic pain, Chronic neuropathic pain, Chronic headache and orofacial pain, Chronic visceral pain and, Chronic musculoskeletal pain. Chronic pain is also classified by The International Association for the Study of Pain (IASP) for better understanding (Treede, et al., 2019). The core elements involved in the processes of pain physiology are transduction, transmission, modulation, and perception. Chronic pain affects the entire nervous system and that's why it's crucial to comprehend the various parts through which the signals pass (Christiansen & Cohen, 2018). The in-depth research conducted on neurons, neurotransmission, the role of neurotransmitters and neurotransmitter receptors leads to a better understanding of how chronic pain affects the nervous system and thereby affects the human body. Due to such research conducted, it was discovered that glutamate or glutamic acid which is the most abundant and strong excitatory neurotransmitter in the central nervous system plays a vital role in inflammatory and neuropathic pain models (Chiechio & Nicoletti, Metabotropic glutamate receptors and the control of chronic pain, 2012).

NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate), and many metabotropic glutamate receptors and their subtypes have been implicated in the induction and maintenance of chronic pain by inducing long-lasting sensitization of the nociceptive pathways (Chiechio & Nicoletti, Metabotropic glutamate receptors and the control of chronic pain, 2012). Metabotropic glutamate receptors (mGluRs) are divided into three groups- Group I, Group II, and Group III based on agonist pharmacology, primary sequence, and G protein coupling to the effector. Group I mGluRs are mGluR1 and mGluR5. Group II mGluRs are mGluR2 and mGluR3. Group III mGluRs are mGluR4, mGluR6, mGluR7 and mGluR8 (Metabotropic glutamate receptors | Introduction | BPS/IUPHAR Guide to PHARMACOLOGY, 2019). Because of their ability to modulate rather than mediate excitatory synaptic activity, metabotropic glutamate receptors and their subtypes can indeed be considered valid targets for chronic pain control. Understanding the function, expression, and role of mGluRs in pain modulation, and their involvement in the induction and maintenance of central sensitization, may aid in the development of a feasible alternative for reducing hypersensitivity in chronic pain conditions (Chiechio & Nicoletti, Metabotropic glutamate receptors and the control of chronic pain, 2012).

The Methods & Results section of this literature review explains how chronic pain can be modulated by metabotropic glutamate receptors and their specific roles to alleviate chronic pain. The orthosteric ligands and allosteric modulators have been used to investigate the specific role of mGluRs in pain modulation. A variety of subtype-selective positive allosteric modulators (PAM) and negative allosteric modulators (NAM) have been developed to potentiate or minimize the physiological activation of specific mGluRs by endogenous glutamate, and several have been used to understand the specific role of mGluR subtypes function in pain conditions. Depending on the particular receptor subtype activated as well as the anatomical and cellular localization, activation of mGlu receptors may either increase or decrease cell excitability. The mammalian nervous system contains all mGlu receptors and they are found both presynaptically and postsynaptically in neurons and that's why mGluRs are promising candidates for chronic pain management as they can inhibit both nociceptive and recurrent pain (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Several mGluR subtypes are also expressed in glial cells, such as astrocytes, where they play an important role in neuronal excitability and pain hypersensitivity modulation. Pain hypersensitivity can be reduced by mGluRs working at various stages, from the periphery to brain regions involved in pain perception and regulation. Group I mGluRs are mostly found at the postsynaptic level where they positively modulate neuronal excitability. Group II and III mGluRs are mostly found at the presynaptic level where they negatively regulate neurotransmitter release (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016). The role of Group I mGluRs, Group II mGluRs and, Group III mGluRs in chronic pain modulation are explained thoroughly in the Methods & Results section while also highlighting the major research discoveries and contributions in this field (refer to Figure no. 3.1).

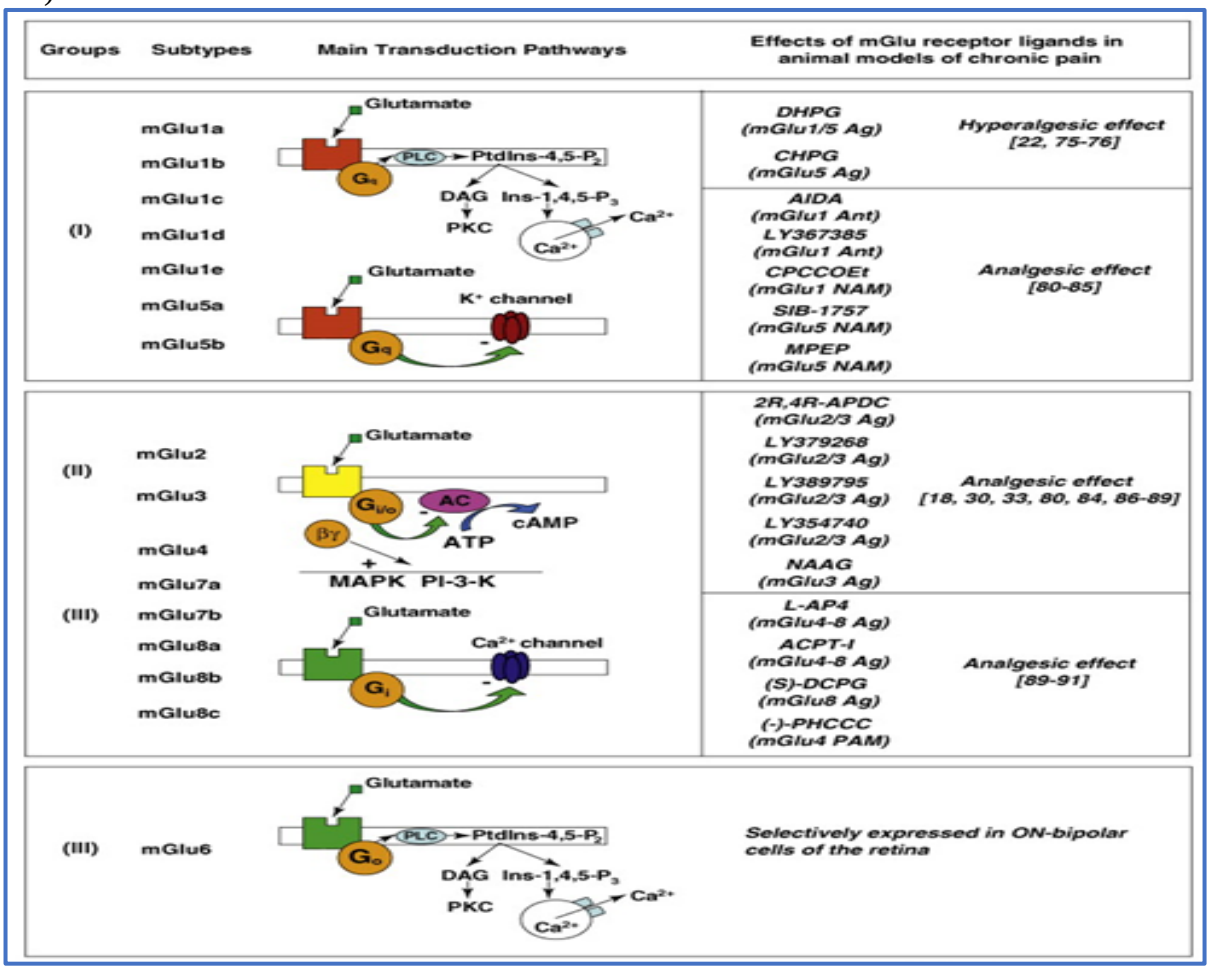

Figure no. 3.1: The transduction pathways, pharmacological profile and influence of mGlu receptor subtypes and their subtype-selective ligands in models of chronic pain

Source: Chiechio, S., Copani, A., Zammataro, M., Battaglia, G., IV, R., & Nicoletti, F. (2010). Transcriptional regulation of type-2 metabotropic glutamate receptors: an epigenetic path to novel treatments for chronic pain. *Trends In Pharmacological Sciences*, *31*(4), 153-160. https://doi.org/10.1016/j.tips.2009.12.003

4. CONCLUSION

The function of mGluRs in various types of pain has been studied over the last few decades. Due to the coexistence of mixed pain types that often occur in patients, treating chronic pain can be exceedingly difficult. The increasing availability of novel subtype-selective pharmacological ligands acting on mGluRs has aided in elucidating the precise function of each mGluR subtype in pain processing and nociceptive sensitization processes, indicating that these receptors may be promising targets for chronic pain treatment. In general, pain hypersensitivity can be effectively regulated by either blocking group I mGluRs or stimulating group II and group III mGluRs. This can be accomplished by using orthosteric ligands that directly activate or block particular mGluR subtypes, or allosteric ligands that modulate mGluR function positively (PAM) or negatively (NAM). Furthermore, in chronic pain models, an epigenetic approach resulting in increased mGluR2 expression has shown promising results (Chiechio, Modulation of Chronic Pain by Metabotropic Glutamate Receptors, 2016).

Despite the discovery of multiple possible therapeutic targets, animal models and clinical trials have consistently shown that changing only one phase in the maladaptive process is often insufficient to eliminate pain. Clinical therapies are still constrained by their side effect profiles, further complicating the therapeutic options accessible. Although it remains a daunting challenge, it is important for researchers and healthcare professionals to understand how all four sections of the pain pathways—transduction, transmission, regulation, and perception—interact such that modalities to avoid and manage different pain disorders can be developed and implemented (Christiansen & Cohen, 2018).

The function of specific mGluRs in various types of synaptic plasticity indicates that mGluR modulation could help treat cognitive impairments caused by a variety of neurodevelopmental/psychiatric disorders and neurodegenerative diseases. mGluRs can modulate presynaptic neurotransmission in the CNS by fine-tuning neuronal firing and neurotransmitter release in a complex, activity-dependent manner, according to preclinical and clinical evidence. Drugs that target mGluRs have been described as promising, novel pharmacological tools for the treatment of neurodegenerative and neuropsychiatric disorders, such as chronic pain, in recent studies (Crupi, Impellizzeri, & Cuzzocrea, 2019).

Furthermore, the diversity and heterogeneous distribution of mGluR subtypes in the brain may allow for the targeting of specific mGluR subtypes implicated in different CNS functions, which could aid in the development of novel treatment strategies for psychiatric and neurological disorders such as depression, anxiety, chronic pain, Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), among others (MS). In general, targeting glutamatergic neurotransmission through mGluR modulation holds a lot of promise for treating a variety of CNS diseases with fewer side effects. Multiple mGluRs could be targeted selectively in new therapeutic approaches. More clinical trials are needed to see whether targeting multiple mGluRs in human patients with neuropsychiatric and neurological disorders is successful (Crupi, Impellizzeri, & Cuzzocrea, 2019).

At doses that have been shown to be analgesic, mGluR1 antagonists have been linked to motor and cognitive impairment. Similarly, mGluR1 conditional knockouts in the cerebellum have been shown to have motor coordination deficits. While mGluR5 antagonists may have psychoactive properties, they appear to have less side effects than mGluR1, meaning that targeting mGluR5 for the production of new analgesics may be more promising. The production of tolerance after repeated systematic injections of Group II mGluRs agonists, which have proven antinociceptive effects, is a major concern for the treatment of chronic pain. Nonetheless, epigenetic upregulation of endogenous mGluR2 receptor expression may be able to overcome tolerance's disadvantage. Targeting Group III mGluRs can also reduce affective and cognitive disorders associated with chronic pain, such as anxiety, depression, or fear, which are of particular interest in drug development. Given the analgesic effects seen after targeting peripheral mGluRs, peripherally restricted molecules may provide adequate analgesia while reducing central-related side effects. Furthermore, new pharmacological tools such as photoswitchable or caged ligands, which allow for spatiotemporal tuning of mGluRs, could minimise off-target effects associated with glutamatergic system modulation outside the pain neuraxis (Pereira & Goudet, 2019).

The global metabotropic glutamate receptor market report 2021-2027 by QY Research suggests that major pharmaceutical companies are actively working towards the production of metabotropic glutamate receptors therapeutics for chronic pain, major depressive disorder, alcohol addiction, and neurological disorders. Metabotropic glutamate receptors could have a bright future in the treatment of chronic pain and related nervous system disorders as further research, assessment, and clinical trials are actively being conducted (Global Metabotropic Glutamate Receptor MRG Industry Growth And Competitive Analysis 2021-2027, 2021).

5. REFERENCES

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