TRYP
THERAPEUTICS

# BIOLOGIC AND CLINICAL RATIONALE FOR THE USE OF PSILOCYBIN FOR NOCIPLASTIC PAIN INDICATIONS

## BACKGROUND

Psychedelics have been used in many cultures for thousands of years, and recently there has been a resurgence of interest in using them to treat psychiatric conditions. These substances have a generally favorable safety profile, especially when compared with opioid analgesics. Clinical evidence to date for their use for chronic pain is limited; however, several studies and reports over the past 50 years have shown potential analgesic benefit in cancer pain, phantom limb pain and cluster headaches. While the mechanisms by which the classic psychedelics may provide analgesia are unclear, several possibilities exist given the similarity between 5-HT2A activation pathways of psychedelics and the nociceptive modulation pathways in humans.

Additionally, the alterations in functional connectivity (FC) seen with psychedelic use suggest a way that these agents could help reverse the pathologic changes in neural connections seen in chronic pain states. Given the current state of the opioid epidemic and limited efficacy of non-opioid analgesics, it is time to consider further research on psychedelics as analgesics in order to improve the lives of patients with chronic pain conditions.

## MOLECULAR MECHANISM OF ACTION FOR PSYCHEDELICS IN NOCIPLASTIC PAIN

The primary mechanism of action of psychedelics is via activation of the serotonin 5-HT2A receptor, which is a G protein-coupled receptor encoded by the HTR2A gene (1). Phenethylamine hallucinogens are typically selective for 5-HT2 subtypes, including 5-HT2A, 5-HT2B and 5-HT2C sites. Tryptamine hallucinogens, including psilocybin, bind non-selectively to most 5-HT receptors and may also bind to the σ1 receptor, the trace amine receptor and the 5-HT transporter (SERT). Ergolines, by contrast, display high affinity for most 5-HT, dopaminergic and adrenergic receptors (1).

## DOWNSTREAM EFFECTS OF PSYCHEDELICS

### *Modulation of gene expression and inflammation*

Several studies have looked at the downstream effects of psychedelics on gene expression. Nichols, *et. al* in a series of studies demonstrated that a single dose of LSD upregulates several transcripts in the prefrontal cortex via 5-HT2A activation, including neuron-derived orphan receptor 1 (nor1), ania3, krox-20 (egr-2), map kinase phosphatase 1 (mkp1), core/enhancer binding protein β (C/EBP-β) and arrestin domain containing 2-arrdc2 (2,3,4). Many of these genes appear to be involved in synaptic plasticity. 5-HT2A receptor activation by psychedelics increases the expression of immediate early genes (IEGs), including c-fos, period1, egr-1 and egr-2 in mouse somatosensory cortex (5,6). By contrast, although the non-hallucinogenic 5-HT2A agonist lisuride increased the expression of c-fos in mouse SSC, it had no effect on period-1, egr-1 or egr-2 expression. Administration of 2,5-dimethoxy-4-iodoamphetamine (DOI) to rats increases brain-derived neurotrophic factor (BDNF) mRNA levels in frontal, temporal and parietal cortices (7). The ability of 5-HT2A receptor activation to increase Arc expression is reportedly linked to effects on BDNF and glutamatergic signaling (8).

Studies have also examined the effect of psychedelics on inflammatory responses. The hallucinogen R–4-iodo-2,5-dimethoxyamphetamine (R-DOI) is a potent inhibitor of tumor necrosis factor α (TNF-α)-mediated inflammatory pathways in primary rat aortic smooth muscle cells in vitro (9) as well as in the vasculature and small intestine when administered in vivo (10). TNF inhibitors have been developed and approved in a number of inflammatory diseases including rheumatoid arthritis, psoriasis, inflammatory bowel disease and ankylosing spondylitis (a form of complex regional pain syndrome). At the present time, it is not clear whether other 5-HT2A agonists have similar effects on TNF-α pathways.

Normally, descending inhibitory 5-HT pathways help to modulate the transmission of pain signals in the spinal cord and decrease the sensitivity of dorsal horn neurons by inhibiting the c-fiber responses of wide dynamic range neurons (11,12). It has been suggested that malfunction of these descending inhibitory pathways plays a role in the development of hyperalgesia and allodynia (13,14,15).

In rat models, the descending inhibitory effects of 5-HT are mediated by activation of 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, 5-HT3 and 5-HT4 receptors; but after nerve ligation, only activation of the 5-HT2A subtype resulted in persisting 5-HT descending inhibition, suggesting a role for the latter receptor in pain caused by nerve injury. The 5-HT2A receptor may play a role in hyperalgesia and neuropathic pain. 5-HT2A mRNA are expressed by dorsal root ganglia (DRG) neurons (16). DRG neurons are depolarized by 5-

HT2A, and 5-HT2A receptors in the DRG have been shown to potentiate inflammatory pain.

Consistent with those findings, 5-HT2A antagonists reduce pain responses to inflammatory stimuli (17). Rats and mice treated with 2′,3′-dideoxycytidine (ddC), a reverse transcriptase inhibitor used to treat patients with HIV, or with vincristine, a chemotherapeutic agent, show evidence of thermal allodynia and mechanical hypersensitivity. The allodynia and hypersensitivity can be reversed by the selective 5-HT2A antagonist, glemanserin, and do not occur in 5-HT2A receptor knockout mice (18). These effects likely occur because ddC and vincristine increase 5-HT2A receptor expression in dorsal horn, which sensitizes spinal nociceptive responses. 5-HT2A receptors in the spinal cord have also been shown to undergo upregulation in models of inflammatory pain.

*Neural Imaging Advances for Understanding Chronic Pain*

Recent advances in brain imaging technology have allowed the mapping of neural connectivity within the brain. The identification of connections referred to as resting-state networks and the temporal connection between anatomically separate areas designated as functional connectivity (FC) has helped show how brain regions are connected as well as the patterns of connection that are associated with neuropathology or psychological phenomena. These brain network dynamics are revealed through fMRI resting state FC analysis. This work has identified brain connectivity networks that are essential for integration of information for complex cognitive function. Healthy brain networks have a characteristically efficient organization. There is growing evidence that disruption of these efficient networks is associated with several neurological conditions including but not limited to depression, anxiety, trauma, addiction, as well as many chronic pain states such as somatoform pain disorder, fibromyalgia, rheumatoid arthritis, centralized pain, chronic pelvic pain, lumbar back pain and phantom limb pain (19,20,21).

In evaluating the FC changes between healthy volunteers and subjects with chronic pain conditions, the common reorganization was in the extent of the association between prefrontal cortex and the insula. The extent of this reorganization was a function of the intensity of the chronic pain and its duration (22). Several imaging studies have demonstrated that psychedelics alter established patterns of connectivity within the brain by reducing the stability and integrity of established brain networks and by increasing the global integration between established brain networks. In patients with phantom limb pain, mental imagery exercises have been shown to decrease pain as well as reduce cortical reorganization, as seen on fMRI (23).

An fMRI study of mirror visual feedback (MVF) therapy in patients with phantom limb pain found that reduction in phantom pain after MVF therapy was associated with increased

activity in the prefrontal cortex. In a recent case report, a patient who combined MVF therapy and psilocybin had a profound and beneficial pain response compared with the use of MVF alone, suggesting possible synergistic effects. A possible explanation for this response was provided in studies performed by in the Carhart-Harris labs. In analyzing a series of fMRI studies involving psilocybin, Carhart-Harris, *et. al* proposed that psychedelics "disintegrate" brain networks while increasing the "repertoire of connectivity motifs" that form and fragment within a network. They suggest that psychedelics may have therapeutic potential in psychiatric conditions by disrupting spatiotemporal patterns of brain activity but that these drug-induced changes may need to be mediated by other therapeutic processes such as co-administered psychotherapy (24).

Psychedelics extend local functional connections to become more global with many additional brain regions. After the normal organization is disrupted, there is emergence of strong, topologically long-range functional connections that are not present in the normal state. Nichols, *et. al* hypothesized that after the psychedelic-induced brain network disruption, the formation of long-range functional connections may be solidified through local anti-inflammatory effects to allow "healthy" reconnections as the acute effects of the drug wear off (28).

## THE RATIONALE FOR USING PSYCHEDELICS TO ADDRESS NOCIPLASTIC PAIN

The literature on classic psychedelics and chronic pain is limited but does include several indicators that these agents have analgesic potential. There are several articles including case reports, case series, retrospective surveys and prospective non-randomized trials of either psilocybin or LSD in chronic pain conditions. The earliest published studies on psychedelics and analgesia are works from Dr. Eric Kast in the mid-1960s on analgesic response to LSD for cancer pain. In these studies, LSD not only acutely outperformed 2mg of PO hydromorphone or 100mg of PO meperidine but also produced analgesia that persisted for an average of 3 weeks after LSD administration (12).

More recently, two retrospective cross-sectional surveys of patients with cluster headaches showed that the use of hallucinogens such as LSD and psilocybin was associated with a reduction in headache severity and an extension of remission periods (25). Psilocybin was reported to be roughly as effective as high flow oxygen in aborting cluster headaches and was more effective than oral or intranasal triptan administration (but less effective than triptan administration by the subcutaneous route). Psilocybin was also used to prevent cluster headaches and was determined by users to be more effective than conventional pharmaceutical agents including verapamil, prednisone, topiramate and melatonin. In contrast to psychedelics, the authors found highly variable reports of efficacy when cannabis was self-administered as a treatment, worsening symptoms in some patients and improving symptoms in others.

The mechanisms by which chronic pain develops are not completely understood but likely involve a complex interplay between somatic and visceral afferent input, peripheral and central sensitization, emotional state, behavior and cognition. Distraction and changes in mood can have a powerful effect on the perception of pain (26). Recent randomized, double-blind trials demonstrated that psilocybin can relieve anxiety and depression in patients with life-threatening cancer (27). There is also evidence that specific 5-HT2A gene polymorphisms are associated with fibromyalgia, chronic widespread pain and pelvic pain, further demonstrating a significant role of the 5-HT2A receptor in pain perception (28).

There are several mechanisms whereby psychedelic drugs could potentially produce antinociceptive effects in chronic pain states. First, 5-HT2A receptor activation causes upregulation of genes associated with neuroplasticity and suppresses TNF-α-induced inflammation. Moreover, treatment with psychedelic drugs causes downregulation of 5-HT2A receptor binding sites (29). Agonist-induced downregulation of the 5-HT2A receptor is not linked to changes in the level of 5-HT2A mRNA, but rather likely occurs due to redistribution of the receptor from the cell surface to intracellular compartments. To date, studies have not examined whether psychedelic drugs induce 5-HT2A internalization in dorsal horn neurons. Such an effect could potentially counteract the sensitization of spinal nociceptive responses in neuropathic pain states.

The transition from acute pain to chronic pain, especially in patients with nociplastic pain, has been shown to involve neuroplasticity or other changes in nervous system structure and function. These neuroplastic changes have been detected at multiple levels of the central nervous system, ranging from the spinal cord to the cortex (30). Given the accumulating evidence of altered brain FC in chronic pain states, the ability of psychedelics to disrupt established brain connection patterns is the most intriguing potential analgesic mechanism for psychedelics. Should this prove to be the case, combining psychedelics with more traditional therapeutic modalities could result in synergistic therapeutic benefits. Potential psychedelic co-therapeutic modalities include MVF therapy, physical therapy, nerve blocks, neuromodulation techniques, psychotherapy or others with the goal of reversing some of the neuroplastic changes that resulted in the chronic pain state.

## FIBROMYALGIA CLINICAL STUDY RATIONALE

Fibromyalgia is a chronic syndrome that affects 2% to 4% of the population and is characterized by widespread musculoskeletal pain that often manifests with a cluster of co-occurring symptoms including sleep disturbances, fatigue, cognitive dysfunction, and mood disorders (e.g., anxiety and depression). The administration of TRP-8802, an oral

formulation of synthetic psilocybin, in concert with psychotherapy may be a potentially safe and effective treatment for symptoms associated with fibromyalgia. The pressing need for effective fibromyalgia treatments, known safety of psilocybin therapy, and mechanistic plausibility for potential benefit provide a backdrop for investigating TRP-8802 as a treatment for fibromyalgia.

Current treatments for fibromyalgia are inadequate, and there is an unmet need for treatment. Fibromyalgia treatment is challenging: many nonpharmacological therapies are inaccessible due to cost and lack of treatment providers, and most pharmacological therapies are only modestly effective while causing a significant side-effect burden. Some researchers have proposed that psychedelic drugs such as psilocybin may have potential therapeutic value for treating fibromyalgia and chronic pain. Much of this interest has been stoked by the results from small clinical trials among individuals with psychiatric disorders such as anxiety, depression and addiction (27,32,33,34,35), which show remarkable improvements in otherwise seemingly intractable conditions.

Studies with modern neuroimaging techniques (e.g., functional connectivity magnetic resonance imaging) have shown that psilocybin dramatically alters maladaptive patterns of connectivity between different brain regions, which in combination with psychotherapy may promote improved clinical symptoms. Basic science research has identified the serotonin 5-HT2A receptor as the primary target for psychedelics – a finding of interest given that polymorphisms in the encoding 5-HT2A gene are associated with fibromyalgia and chronic widespread pain (28).

While case series and retrospective surveys suggest that psychedelic use may improve chronic pain due to cluster headaches/migraine (36), phantom limb pain (38), and neuropathic pain (37), no studies have examined the potential role of psychedelics in fibromyalgia.

## PLANNED STUDY IN FIBROMYALGIA USING TRP-8802

To better understand the potential benefits of psilocybin in chronic pain management, Tryp Therapeutics is planning to perform an open-label, Phase 2a clinical trial at the University of Michigan to assess the clinical utility of TRP-8802 in concert with psychotherapy to treat chronic pain symptoms in patients with fibromyalgia. Participants will undergo an initial pain phenotyping assessment and will complete a daily electronic pain diary until the end of active treatment. TRP-8802-psychotherapy intervention, the structure of which has been used in previous psilocybin-based clinical trials, will begin with initial meetings between the patient and the therapist followed by two drug dosing sessions and then integration sessions following drug dosing.

Prior to dosing, baseline resting state fMRI, EEG and additional neural imaging procedures will be performed. During the two drug administration sessions, participants will receive TRP-8802 in a comfortable setting under the guidance of two therapists/facilitators. Participants will have an in-person study visit the day after each TRP-8802 administration visit to ensure safety and comfort. Psychotherapy with the same two therapists/facilitators will occur between and after TRP-8802 sessions with a focus on integration, helping participants gain insight and understanding from the experience. After the final session, participants will undergo three weekly integration sessions and then complete a post-treatment deep phenotyping session. Following the final dosing session, the fMRI, EEG and additional neural imaging procedures will be repeated. Participants will complete electronic patient reported outcomes questionnaires at 12 and 24 weeks after the final TRP-8802 dose to examine persistence of changes.

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