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**Synthetic Neuroactive Steroids Targeting GABA-A Receptors as a Potential Therapy for
Contraceptive-Associated Mood Disorders**

A Thesis Presented

by

ALEXANDRIA DAVIS

To the Keck Science Department

of

Claremont McKenna, Scripps, and Pitzer Colleges

In Partial Fulfillment of

The Degree of Bachelor of Arts

Senior Thesis in Human Biology

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ABSTRACT

4-10% of women who use hormonal oral contraceptives (HOCs) discontinue use after experiencing symptoms of anxiety, irritability, and depression in the first 3 months of administration. Previous studies have acknowledged a relationship between HOCs and emotional dysregulation but have not identified mechanisms by which HOCs have this effect. As a result, effective treatment options for HOC-induced mood disorders are lacking. Current theories implicate combined-HOCs, particularly those containing ethinyl estradiol and levonorgestrel (EE-LNG), in reducing neuroactive steroid levels. Specifically, HOC users show decreases in allopregnanolone, a positive allosteric modulator associated with neuronal inhibition and emotional regulation. The objective of this study is to assess the effectiveness of integrating synthetic neuroactive steroid treatment with HOC use as a potential therapy for contraceptive-related mood alterations. Recent FDA approval of synthetic neuroactive steroids such as brexanolone and zuranolone for the treatment of postpartum disorder underscores their potential effectiveness and highlights their relevance for addressing hormonal-related mood changes. Additionally, recent interest in ganaxolone as a therapeutic option for major depressive disorder as well as severe anxiety disorders further establishes restoration of neuroactive steroid activity as a target for treatment. Evaluating the effectuality of synthetic neuroactive steroids allows for a greater understanding of the role of HOC-disrupted neuroactive steroid levels in emotional regulation, and offers a potential treatment avenue for the vulnerable subset of women struggling with the adverse effects of HOCs. Using a combination of self-report mood questionnaires, fMRI scans, and EEG recordings, this study will assess the efficacy of synthetic neuroactive steroids in alleviating mood symptoms and elucidate their underlying neurobiological mechanisms. This research contributes to women's health by advancing understanding and treatment options for mood disorders induced by hormonal contraceptives, advocating for comprehensive care and informed decision-making.

INTRODUCTION

The Evolution of Hormonal Contraceptives

In 1960, Enovid, the first hormonal oral contraceptive pill (HOC), liberated women from traditional constraints, paving a path for reproductive rights by offering women autonomy in deciding if and when they would have children. For decades, women have fought for equality, encompassing the right to self-agency and equitable access to healthcare. While the development of HOCs was a seminal event in this struggle, repercussions to women's health, specifically implications on brain function remain poorly understood (Concas et al., 2022; Porcu et al., 2019). This gap in understanding begs the question: At what price do women truly possess the right to make decisions about their health and have full agency over their bodies? This question serves as a reminder that the journey towards equality demands ongoing scrutiny and advocacy for women's health and well-being.

With Congress's declaration of the Comstock Act in 1873 came the prohibition of all contraceptive and abortion materials. Prior to this ban, family planning tools, including antiseptic spermicides, condoms, suppositories, and herbal concoctions, were accessible to women under certain limitations. Many of these methods were deemed ineffective and posed a danger to the health of both the woman and the fetus they carried ("Birth Control Throughout History," n.d.). Rather than fostering concern for women's health, the growing number of women utilizing preventative measures raised fears for some for the religious morality of society. Thus, Congress issued the Comstock Act, believing public morality could be restored if unethical behaviors encouraged by reproductive intervention came to a stop (Boomer, n.d.). The lack of reproductive rights women desperately needed left them with few options, compromising their safety as they resorted to dangerous procedures to avoid or terminate unwanted pregnancies (*Enovid: The First*

Hormonal Birth Control Pill | *Embryo Project Encyclopedia*, n.d.). Observing the negative impact of these laws on women's health, Margaret Sanger and Gregory Pincus developed Enovid as a safe and accessible option for fertility control. To combat laws prohibiting contraceptives, this pill's secondary effects was marketed as a treatment option for menstrual disorders (*Biography*, n.d.; Burrows, n.d.).

Enovid used high doses of synthetic estrogen and progestin, more specifically mestranol and norethisterone, in a combined formula to suppress ovulation, thicken the cervical mucus, and alter the uterine lining to ensure the egg could not be fertilized (Antoniou-Tsigkos et al., 2022; Kao, 2000; Petitti & Sidney, 2005). While the development of Enovid proved to be novel in preventing unwanted pregnancies in many women, it was not without its challenges. The high concentration of hormonal components in Enovid led to reports of side effects such as weight gain, bloating, nausea, internal bleeding, and conditions as extreme as venous thromboembolism which increased the user's risk for stroke or heart attack (Anderson & Johnston, 2023; Burkman et al., 2011). Researchers strove to limit adverse effects by reducing the amount of synthetic hormones in the pill as well as considering the use of other synthetic hormones to mimic the natural hormonal changes in the menstrual cycle (Liao & Dollin, 2012).

Today, HOCs remain one of the most commonly used forms of contraception in the United States, with 24% of women opting for the pill among various contraceptive choices (Diep et al., 2023). Following years of development, the dosage of synthetic estrogen and progesterone in HOCs has gradually decreased. Initially ranging from 100 - 175 µg of estrogen and 10 mg of progesterone in the first pill, modern-day HOCs now typically contain 30 - 50 µg of estrogen and as little as 0.3 mg of synthetic progesterone (Christin-Maitre, 2013; Kao, 2000; Sivasankaran & Jonnalagadda, 2021). Additionally, four generations of synthetic progesterone, otherwise known

as progestins, have been developed to act as components in combined and progestin-only contraceptives. Increased variation of synthetic hormones has allowed researchers to investigate which hormone combinations reduce side effects, making the pill more accessible to women with different health conditions, as well as more effective. Compared to the first HOC pill, more recent versions use various combinations of newer synthetic estrogen and progesterone combinations and ease associated side effects with increased effectiveness (Sivasankaran & Jonnalagadda, 2021). Enovid marked an initial stride, paving a path for further advancements and research. By laying the groundwork for expansion, its foundation facilitated progress in methods like contraceptive patches, intrauterine devices (IUDs), and contraceptive shots (Anderson & Johnston, 2023). Through the enhancement of birth control methods, hormonal contraceptives have become a source of hope for women, allowing them the right to choose what their bodies are used for.

While the formula of HOC pills has changed considerably since Enovid, side effects and health implications continue to pose an issue for HOC users. As a result, there are limitations on who can use them and an eventual discontinuation in women without contraindications. Most notably, estrogen's association with an elevated risk for thrombosis in women who smoke, have a history of hypertension, or are obese suggests that estrogen-containing hormonal contraceptives should be avoided for these groups of women (Anderson & Johnston, 2023). More commonly reported side effects in women taking combined oral contraceptives (COCs) consist of irregular menstruation, breast tenderness, weight gain, headaches, and acne, ultimately leading to the cessation of birth control pills within 3 months of starting (Chen et al., 2022; Creinin et al., 2021; Mukanga et al., 2023). Though non-hormonal contraceptives such as condoms, intrauterine devices without hormones, and sterilization present options for women seeking to prevent

pregnancy, HOCs offer benefits that non-hormonal contraceptives do not. In addition to family planning, due to their hormonal effects, they are prescribed for the treatment of health conditions like endometriosis, acne, and polycystic ovary syndrome. These uses make HOCs an appealing therapeutic solution with reversible effects and versatile use for women (Fiffick et al., 2023).

HOCs have evolved to become invaluable aids for a large population of women, serving as both a preventative measure and a treatment of other distressing health conditions. Their multifaceted function underscores their importance in women's reproductive health. As researchers work to increase their benefits, it should be a goal to decrease side effects as much as possible, ensuring confidence and effectiveness in its users. However, many researchers have focused on bettering physiological HOC side effects, overlooking adverse effects such as mood changes (Martell et al., 2023). A growing number of HOC users report an onset of symptoms with varying degrees of depression, irritability, or anxiety after starting the pill. These negative emotional effects are one of the most common reasons why HOCs are discontinued within the first three months of use. As a result, barriers are created as contraceptive options are limited for women (Mu & Kulkarni, 2022). This disparity is not only one of health but also of equity. Women have fought for a right to their bodies for generations, and part of what that means is having access to safe and well-tolerated contraceptive options. Equitable health care for women includes the development of a safe oral contraceptive that incorporates concern for how the brain and mental health of women are impacted. While hormonal contraceptives offer women authority over their own health management, we must consider their potential impact on the mental health and cognitive function of those who use them.

Mental Health

Mental health has often been overshadowed by physical well-being and the prioritization of physiological explanations. It encompasses emotional well-being and includes social and psychological aspects. The mental health of an individual impacts their day to day routines and interactions, and plays a role in how they handle challenges. While emotional well-being does not gain enough attention, at least 1 in 5 adults in the US struggle with mental health, meaning its prevalence is fairly high in society (*About Mental Health*, 2023). Similar to physical health, it affects the overall well-being and quality of life of an individual, however, this is achieved by influencing the thoughts, feelings, and actions of the individual. Struggles with mental health often lead to consequences such as impaired relationships, difficulty in work or school, substance abuse, and increased rates of suicide (*About Mental Illness*, n.d.; *WHO Highlights Urgent Need to Transform Mental Health and Mental Health Care*, n.d.). As the prevalence of mental illness becomes more apparent in society, there is a need to explore its causes and treatments to reduce its impact on both an individual and the public.

Compared to men, women have higher rates of depression, anxiety, and suicide attempts. Reasons for this disproportion include biological, social, and cultural influences (*The Gender Gap in Mental Health*, 2022). Men and women encounter distinct risk factors, particularly shaped by societal expectations and gender roles, leading to differing opportunities and challenges in their experiences (*Mental Health Disparities: Women's Mental Health*, 2017). Paired with increased risk factors and limited resources for treating mental illness, women exhibit heightened levels of stress, anxiety, and depression. Additionally, hormonal fluctuations increase susceptibility to developing mental disorders unique to women (*Can Menopause Cause Depression?*, 2024). Sex hormones such as estrogen and progesterone, which are responsible for

maintaining the reproductive cycle, additionally work to interact with neurotransmitters such as serotonin to promote feelings of happiness, and gamma-aminobutyric acid (GABA) to calm neuronal excitability involved in negative emotions. Disruption of such hormones and their functions leads to increased feelings of sadness, irritability, and anxiety (*Understanding How Hormonal Changes Impact Emotional Health for Teens* | *Relational Psych*, n.d.). The impact of hormonal imbalances in women can specifically be observed in thyroid conditions, premenstrual dysphoric disorder, postpartum depression, and menopause, where changes in hormone levels are linked to challenges with mood regulation (Algas-Sasaki, 2024).

Symptoms of depressive disorders in women are characterized by a decline in mood, loss of hope, decreased self-efficacy and self-esteem, irritability, anhedonia, appetite changes, decreased attention, reduced energy, and suicidal ideation. Additionally, excessive crying, withdrawal from everyday life, and excessive guilt are indicators of depression (*Depression Among Women* | *CDC*, 2023). Anxiety disorders, on the other hand, are distinguished by feelings of restlessness, heart palpitations, the inability to make decisions, and feelings of nervousness, panic, or impending doom (*Anxiety Disorders*, 2023). Depending on the severity of symptoms and the circumstances contributing to symptoms, individuals meeting the criteria for diagnosis are treated with the use of talk therapy, medications, or lifestyle changes (Algas-Sasaki, 2024; *Anxiety Disorders*, 2023). It is important to emphasize that mental health is multifaceted and incorporates biological, social, and cultural factors when assessing underlying reasons for symptoms (*Mental Illness - Symptoms and Causes*, n.d.). For this reason, treatment differs depending on the individual. Moreover, pathophysiological factors and molecular etiologies continue to be explored by researchers to obtain a firm understanding of the intricacies

contributing to mental illness as there remains more to learn (Fries et al., 2023; Kaltenboeck & Harmer, 2018).

Taking the time to understand the causes and biological elements of mental disorders is essential to address the challenges presented by mental illness. Furthermore, understanding the implications of gender differences on mood disorders and their causes is crucial for the consideration of targeted interventions and support systems for this vulnerable population. By acknowledging the unique challenges faced by women that expose them to an increased risk for mood disorders we can begin to understand the multi-layered nature of mental well-being. In doing so, we can develop more effective strategies for prevention and treatment, effectively reducing the burden of mental illness.

Hormonal Contraceptives and their Implications on Mental Health

Researchers have yet to come to a universal agreement regarding the correlation between HOCs and mood disorders (Masama et al., 2022). While many studies have supported an association between HOCs and emotional well-being, some studies yield no significant relationship (Concas et al., 2022). Factors attributing to this variability include differences in study methodologies and limitations, user demographics, and personal patient histories. Many studies use self-reporting to collect data, which is considered an unreliable method. Additionally, the methodology of experiments may have resulted in biased data as women who experienced mood effects stopped taking HOCs and were thus excluded from studies (Johansson et al., 2023). Due to the variance in study methodologies, there is no conclusive data regarding the relationship between HOCs and emotional symptoms. To address this gap in understanding using a more reliable method and reduced biases, researchers cross-referenced the diagnosis of

depression and the prescription of psychotropic drugs among women beginning HOC use before their diagnosis (Skovlund et al., 2016; Zettermark et al., 2018). Researchers evaluating the prevalence of mood deterioration upon oral contraceptive use generally observe that while the majority of women taking HOCs report no noticeable change in their emotional well-being, 4-10% describe an onset of psychological symptoms such as depression, irritability, and anxiety (Lundin et al., 2017). Moreover, a higher frequency of suicide attempts and suicides is reported in HOC users compared to nonusers (Skovlund et al., 2018). These studied correlations imply a subset of women may be more susceptible to emotional side effects, thus prompting the question, who is most vulnerable to these side effects?

Among the subset of women susceptible to the development of psychological side effects are women with a history of anxiety and depression, and adolescents beginning contraceptive use around the age of 16 (de Wit et al., 2020; Johansson et al., 2023; Lundin et al., 2017). Previous longitudinal studies have tracked the development of affective disorders in adolescent women being administered HOCs over time, revealing that 16-year-old girls were at high risk for depressive symptoms during both oral contraceptive use and also later in life (Anderl et al., 2020; de Wit et al., 2020). Furthermore, studies have also suggested women who have discontinued HOC use after reporting anxiety, mood swings, and feelings of depression were more likely to experience those same adverse effects if reintroduced to contraceptive pills. When comparing participants who received placebos to participants who received combined oral contraceptives, women using HOCs demonstrated higher scores of depression and stress, indicating a correlation between the use of HOCs and mood deterioration. Mood changes were also supported by decreased reactivity in the emotion circuits of the brain, particularly the left insula and frontal gyrus which correlated with difficulties in emotional regulation (Gingnell et

al., 2013; Lundin et al., 2017). This relationship between combined oral contraceptives and emotional side effects has been particularly observed in progestin-containing contraceptives using levonorgestrel (LNG) as a synthetic progesterone component (Gingnell et al., 2013; Lundin et al., 2017; Skovlund et al., 2016).

Neurological studies strive to illuminate the multifaceted relationship between HOC use and brain function. Resting state functional connectivity is the correlation of neuronal activity between functionally related brain regions while not engaging in any specific task (Biswal, 2015). In simpler terms, resting state functional connectivity refers to how various areas of the brain with the same neural network communicate and collaborate to coordinate activity even when there is no task that requires activity. In a study assessing the impact of combined HOC use on the development of adolescent brains, Sharma et al. (2020) reported observing increased resting state functional connectivity in fMRIs for pubertal HOC users compared to adult-onset HOC users. Furthermore, the researchers noted more resting state functional connectivity in adult-onset HOC users compared to nonusers. This increase in activity was distinguished in regions of the brain associated with emotional regulation, decision making, memory formation, and reward processing. Additionally, pubertal HOC users demonstrated heightened resting state functional connectivity in regions involved in empathy and emotional understanding (Sharma et al., 2020). Increased resting state functional connectivity suggests heightened activity in specific regions of the brain. This connectivity disruption can result in challenges with emotional regulation and reward processing, as implicated regions of the brain adapt to accommodate heightened connectivity (Chung et al., 2019). Consequently, difficulties in affective modulation and reward response may increase susceptibility to mental disorders such as depression, anxiety, and irritability, which rely on the control of these processes (Sharma et al., 2020).

Moreover, on an endocrine level, emerging evidence demonstrates lower endogenous estradiol levels in oral contraceptive users compared to non-users (Lundin et al., 2017). Endogenous estradiol hormone is a sex steroid hormone produced within the body. Sex steroid hormones are namely responsible for the development of secondary sex characteristics, reproductive processes, sexual behaviors, and sexual differentiation (Pillerová et al., 2021). Additionally, they are implicated in emotional and cognitive processes due to their influence on cortical and subcortical regions of the brain; recent findings have demonstrated a correlation between estrogen levels and activity in the dorsolateral prefrontal cortex (DLPFC) when experiencing emotional regulation. Higher levels of estrogen in adolescent women resulted in better emotional processing as there was more DLPFC activity (Chung et al., 2019; Gingnell et al., 2013; Skovlund et al., 2016). Additionally, researchers have theorized that low levels of estrogen correlate with a greater risk for depression in women as estrogen is involved in anti-inflammatory processes and neuroprotective mechanisms in the brain (Morssinkhof et al., 2021; Zhang et al., 2023).

Furthermore, Masama et. al (2022) reported elevated levels of stress and inflammatory markers in HOC users including cortisol and C-reactive protein. The higher levels of cortisol observed in HOC users suggest an increased vulnerability to stress-related mood disturbances. This is supported by the study's findings where higher cortisol levels in women using HOCs correlated with high scores of depression and stress (Masama et al., 2022).

Research appears to have elicited many findings about the association between hormonal oral contraceptives and their effects on cognitive and emotional processes that contribute to psychological symptoms. Though there is evidence of a relationship, underlying biological mechanisms and the prevalence of hormonal contraceptive side effects are not yet fully

understood. Based on the data described above, it becomes clear that mental health is complex and multifaceted, involving various processes in the brain rather than one simple explanation. While examining some of the known effects of hormonal contraceptives on individual processes, there is a notable indication of hormonal influence on brain function and possible psychological implications. In modifying hormone production using synthetic hormones, cognitive processes are ultimately affected, justifying the importance of understanding the role hormones play in brain function. More specifically, there is a need to explore the effects of hormones on neurobiological mechanisms that alter neural responses to emotional and cognitive stress, as these processes contribute to the development or prevention of disorders like depression and anxiety.

Uninterrupted Mechanism of Ovulation

Ovulation refers to the female reproductive process in which a mature egg is released from the follicle of the ovary and enters the fallopian tube where it is moved to the uterus to await fertilization (Holesh et al., 2024). This process is mitigated by hormones such as gonadotropin releasing-hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone. GnRH is a hypothalamic hormone that regulates the secretion of FSH and LH from the anterior pituitary gland where they are produced. Low levels of GnRH typically trigger release of FSH, while high levels trigger LH release. GnRH can be inhibited or promoted depending on the amount of estrogen or progesterone present. In addition, estrogen is responsible for establishing the endometrial lining of the uterus and promoting cervical mucus changes conducive for sperm survival and mobility. In a series of phases that include the follicular phase, ovulation, the luteal phase, and menstruation, hormone levels

fluctuate and work in a feedback mechanism with the hypothalamic-pituitary-gonadal (HPG) axis to facilitate the release of eggs from the ovaries in preparation for fertilization (**Fig. 1**) (Homburg, 2014).

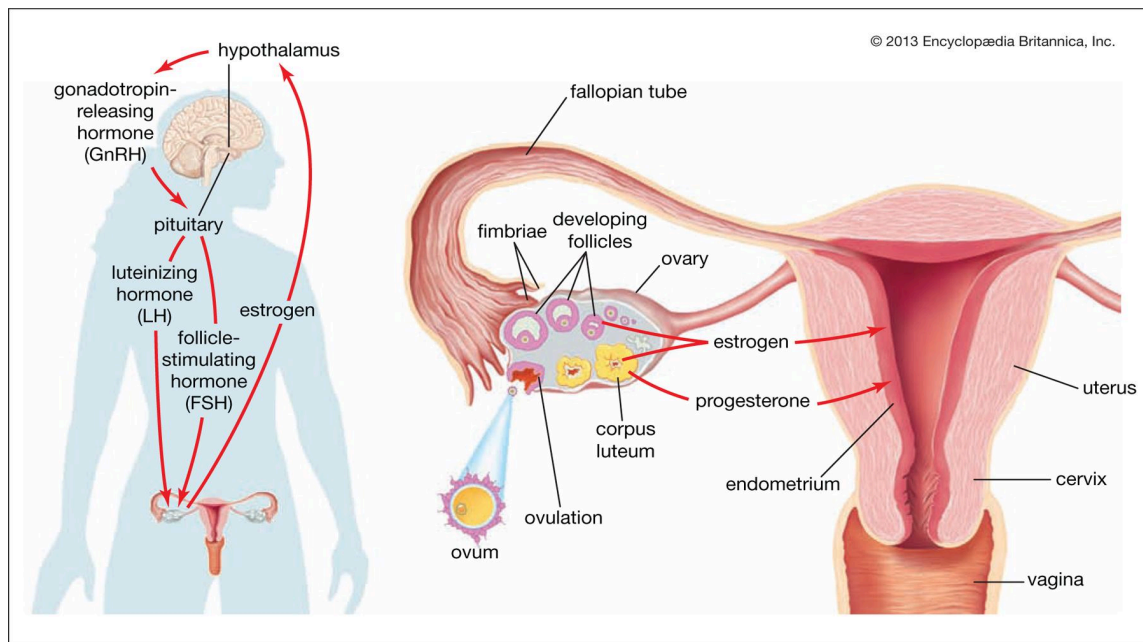


Figure 1: Hypothalamic-pituitary-gonadal (HPG) axis regulating the progression of ovulation through a feedback mechanism involving gonadotropin releasing-hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone (Britton et al., 2020; *Ovulation | Physiology, Hormones & Fertility | Britannica*, 2024).

Follicular Phase:

In the follicular phase, ovarian follicles carrying immature ova mature and develop into primary follicles. This growth is stimulated by FSH which allows the granulosa cells surrounding the ovarian follicles to multiply in layering and grow. At the start of this phase, 1,000 follicles exist and growth is initiated by FSH. As the follicular phase progresses however, only one follicle is considered viable as it grows larger and stronger than all others. This dominant follicle

will be the follicle to later release a mature egg into the ovary. As ovarian follicles grow, estrogen is produced (Holesh et al., 2024; Homburg, 2014).

Ovulation:

Estrogen levels continue to rise as follicles grow. As estrogen increases, the uterus undergoes changes to create an environment in which the egg can be easily fertilized by the sperm. Eventually high levels of estrogen will cross a threshold and turn off negative feedback in the hypothalamus. The hypothalamus, being responsible for the regulation of GnRH secretion, will thus begin to release increased levels of GnRH which in turn allows for the production and secretion of LH. Climbing levels of LH increase the activity of enzymes that break down proteins. As a result, the wall of the ovary deteriorates, making it easier for the matured egg to burst through, where it will then be taken up by the fallopian tube and brought to the uterus for fertilization (Holesh et al., 2024; Homburg, 2014).

Luteal Phase:

The now burst follicle is stimulated by FSH and LH, allowing the empty follicle to become the corpus luteum, which produces progesterone and small amounts of estrogen. Rising progesterone suppresses the hypothalamus and contributes to the upkeep of the endometrial lining in the uterus. The lining of the inner uterine wall will become thicker as it prepares for potential embryo implantation. The corpus luteum breaks down if the egg is not fertilized, leading to a decline in progesterone and estrogen levels. A decline in progesterone subsequently results in the menstrual phase as progesterone is no longer maintaining the endometrial lining and will thus slough off (Holesh et al., 2024; Homburg, 2014). The entire cycle will repeat to make up the menstrual cycle and control reproductive function (**Fig. 2**).

The menstrual cycle

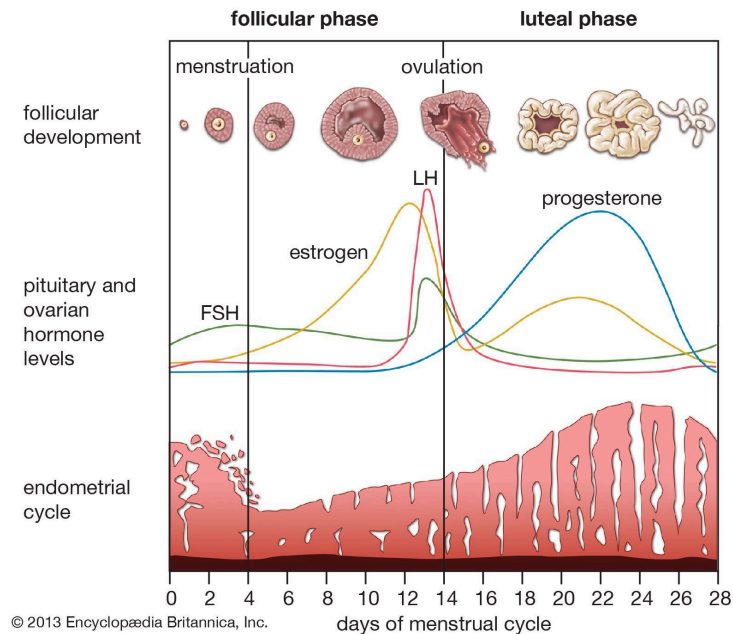


Figure 2: Hormonal fluctuations throughout the various phases of the menstrual cycle in preparation for ovulation where the matured egg is released into the uterus to await fertilization (*Ovulation | Physiology, Hormones & Fertility | Britannica, 2024*).

Hormonal Mechanism of Oral Contraceptives

The most common contraceptives used among women are combined oral contraceptives, composed of synthetic forms of estrogen and progesterone (Mu & Kulkarni, 2022). Synthetic hormones mimic natural hormones and bind to hormone receptors without exerting the same physiological effects as their natural counterparts, thereby inhibiting the HPG axis. By targeting the HPG axis, synthetic hormones disrupt normal hormonal fluctuations that facilitate ovulation like the production of GnRH and the release of FSH and LH (Anderson & Johnston, 2023). The most commonly used estrogen component found in combined oral contraceptive formulas is ethinyl estradiol (EE), however newer estrogen analogues, including estradiol and estradiol valerate, are also used to reduce side effects (Concas et al., 2022; Mu & Kulkarni, 2022; Porcu et al., 2019). These estrogen components are paired with progestins, typically nortestosterone

derivatives such as levonorgestrel (LNG), desogestrel, norethindrone, norgestimate, or gestodene (Concas et al., 2022; Mu & Kulkarni, 2022). Both estrogen and progestin possess distinct functions in HOC formulas to prevent pregnancy independent of each other, but together their combined role improves efficacy and mitigates side effects (Cooper et al., 2024).

Progestins block ovulatory processes by suppressing the release of high amounts of LH that enable ovulation (Anderson & Johnston, 2023; Britton et al., 2020; Cooper et al., 2024; Horvath et al., 2000). Through suppression of LH, ovulation is prevented as proteolytic enzymes never break down the ovarian wall to allow the egg to burst through the follicle and travel to the uterus. Additionally, progestins prevent pregnancy by thickening the cervical mucus, decreasing fallopian tube motility, and thinning the endometrium (Anderson & Johnston, 2023; Britton et al., 2020; Horvath et al., 2000). By thickening the cervical mucus, progestins make it more difficult for the sperm to penetrate and move through the cervical mucus, thus preventing the sperm from reaching the upper reproductive tract where fertilization will occur (Cooper et al., 2024).

While estrogen plays a role in regulating the reproductive cycle and preventing pregnancy, its influence is fairly limited compared to progestin. Estrogens are responsible for suppressing the production of FSH (Anderson & Johnston, 2023; Britton et al., 2020; Horvath et al., 2000). As a result, egg maturation and development of follicles is prevented, providing an alternate manner in which the egg cannot be fertilized as it never reaches the uterus. Additionally, estrogen functions to stimulate the endometrium and counteract progestin induced thinning of the endometrial lining during HOC use. Estrogen will stabilize the effects of progestin, regulating the menstrual cycle, and reducing side effects such as abnormal bleeding patterns (Archer et al., 2022; Hatcher & Kowal, 1990; Homminga et al., 2022). The combined

administration of both progestin and estrogen allows for greater efficacy and expands their ability to suppress the release of gonadotropic hormones from the pituitary gland to exert their intended effects (**Fig. 3**) (Rivera et al., 1999).

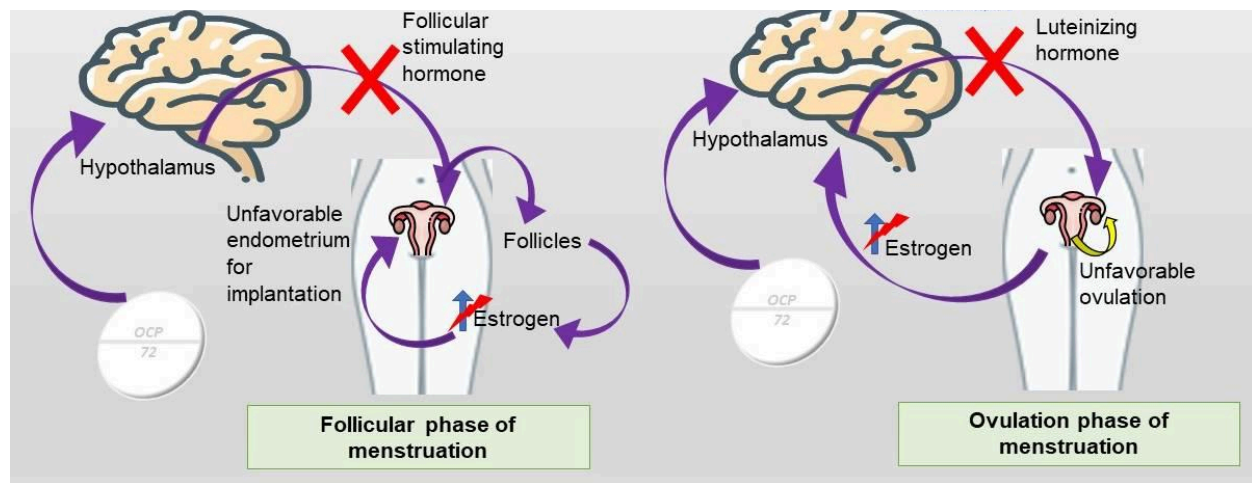


Figure 3: Combined oral contraceptives use estrogen derivatives and progestin to mimic natural hormones and create a block at hypothalamic and pituitary regions to hinder the release of FSH and LH and stop ovulation (Kumar, 2018).

Neuroactive Steroids

Neuroactive steroids, which can be synthesized in the central or peripheral nervous systems or peripherally before crossing the blood brain barrier, are a class of endogenous steroids that modulate rapid neuronal excitability (Del Río et al., 2018; Reddy, 2010). Examples of such steroids include allopregnanolone, pregnenolone, progesterone, and estradiol, which are implicated in regulation of cognition, behavior, mood, and neuroplasticity due to their ability to interact with neurotransmitters (Del Río et al., 2018). Studies on vehicle-treated rats revealed a decrease in neuroactive steroid levels in plasma, cerebrocortical, and hippocampal levels of long-term HOC users treated with ethinyl estradiol and levonorgestrel (EE-LNG) (**Fig. 4**). More specifically, serum levels of naturally occurring neuroactive steroids were reduced by 30-80% in

rats taking HOCs for 4 weeks or more compared to non-HOC users (**Fig. 5**). It was also observed that neuroactive steroid levels normalized within two weeks of HOC discontinuation (Concas et al., 2022; Porcu et al., 2019). Disruption of allopregnanolone levels among rats coincides with observations that individuals with depression and anxiety disorders often display dysregulated allopregnanolone levels at the cerebrocortical and plasma regions (Bäckström et al., 2014; Porcu & Morrow, 2014; Rapkin et al., 2006; Rasmusson et al., 2017).

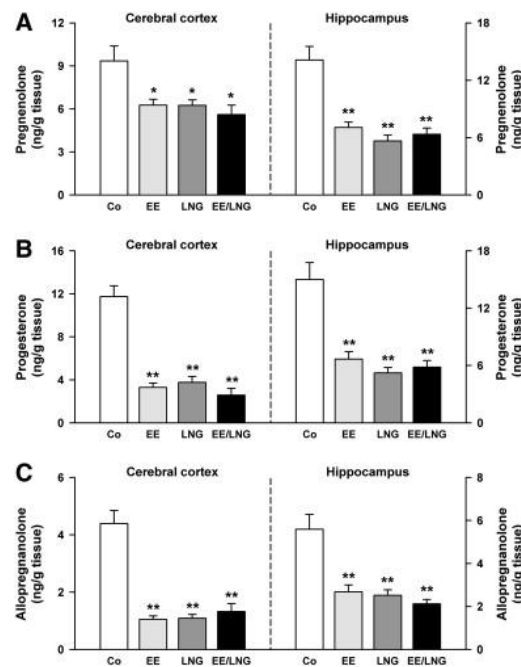


Figure 4: Neuroactive steroid concentrations decrease with long term treatment of hormonal oral contraceptives (Porcu et al., 2012).

Neuroactive Steroids	Female Rats			Women
	Cerebral Cortex	Hippocampus	Plasma	Plasma
Pregnenolone	↓	↓	↓	↓
Progesterone	↓	↓	↓	↓
Allopregnanolone	↓	↓	↓	↓
Estradiol			↓	↓
Testosterone	↓			↓

Figure 5: Long term use of combined hormonal contraceptives leads to the decrease of neuroactive steroid concentrations in the cerebral cortex, hippocampus, and plasma regions of the brain in both female rats and women (Porcu et al., 2019).

HOCS prevent ovulation by regulating hormone production and secretion to disrupt normal hormonal changes that lead to ovulation. This disruption prevents the increase in neuroactive steroids derived from naturally occurring hormones (Concas et al., 2022). In particular, allopregnanolone, a progesterone metabolite, cannot be derived from synthetic progestins found in HOCs (**Fig. 6**). Consequently, allopregnanolone concentrations decrease, limiting their effects on inhibitory processes (Pletzer et al., 2023).

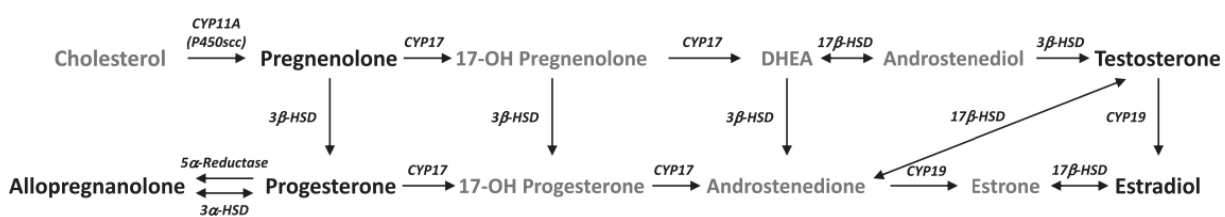


Figure 6: Biosynthetic pathway illustrating pregnenolone and progesterone acting as precursors to other naturally occurring neuroactive steroids (Porcu et al., 2019).

When regulated normally, allopregnanolone functions as a positive allosteric modulator to gamma-aminobutyric acid (GABA) receptors in order to exert its inhibitory effects. By binding to GABA-A receptors, allopregnanolone promotes receptor binding to the GABA neurotransmitter (Del Río et al., 2018). GABA acts as the main inhibitory transmitter in the central nervous system, meaning its activation will reduce neuronal excitability and regulate the overall activity of the brain. Increased inhibition and thus increased regulation of activity promotes anxiolytic, sedative, and antidepressant effects, as emotional processes are managed to a greater degree (Rupprecht & Holsboer, 1999). Given the role of allopregnanolone in increasing GABAergic neurotransmission and its function, which is key to these calming effects, researchers have considered allopregnanolone as a target treatment of psychiatric disorders such as depression, anxiety, and seizure disorders. More specifically, researchers have focused on

allopregnanolone's function as a positive allosteric modulator which enhances GABAergic activity and inhibition and thus neuronal inhibition (**Fig. 7**)(Zorumski et al., 2019). Disruption to neuroactive steroid levels has been implicated in various neuropsychiatric disorders, highlighting its importance of these molecules in maintaining brain homeostasis and mental health.

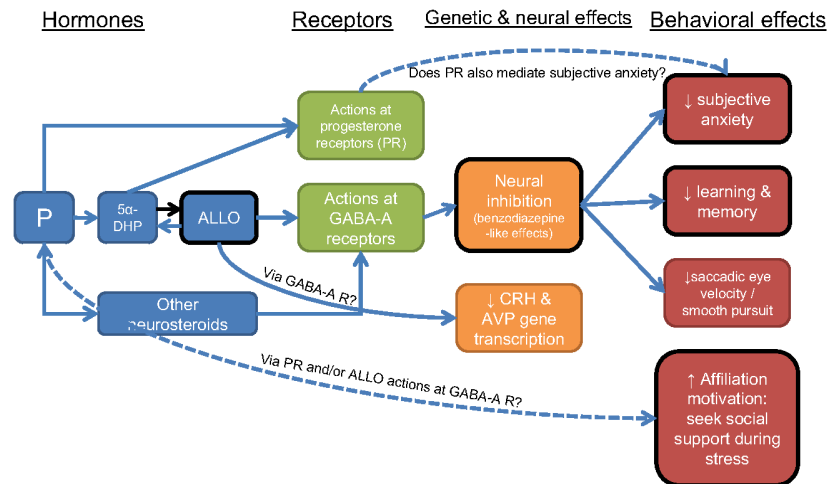


Figure 7: Summary of the relationship between naturally occurring neuroactive steroids and their behavioral implications due to biological processes. Pregnenolone acts as a precursor to allopregnanolone which will bind to GABA-A receptors to promote inhibitory and excitatory balance, thereby influencing emotional regulation and anxiety (Wirth, 2011).

While neuroactive steroid regulation is a subject of interest as a therapeutic intervention due to their role in modulating brain activity, their use poses limits therefore making it a less than ideal target. Limitations include poor bioavailability, concerns for safety and tolerability, and risk for addiction (Porcu et al., 2016). Current researchers are striving to understand its biological mechanism in pursuit of neurosteroidogenesis as a potential means of restoring neuroactive steroid concentrations and function (Zorumski et al., 2019). Additionally, as of recently, the FDA has approved the use of synthetic neuroactive steroids, brexanolone and zuranolone, as a treatment for severe postpartum disorder. Deficits in GABA-A receptor functions have been

linked to symptoms contributing to postpartum depression. Both zuranolone and brexanolone function to mimic allopregnanolone as positive allosteric modulators of the GABA-A receptor (Cornett et al. 2021). In doing so, these synthetic neuroactive steroids bind to GABA-A receptors to enhance GABAergic neurotransmission, and produce antidepressant effects (Belelli et al. 2019). Another synthetic neuroactive steroid with intended calming effects is ganaxolone, which has been used to treat seizures in severe seizure disorders and as of recently has gained attention for its possible anxiolytic effects and antidepressant effects. These treatments have not yet been perfected as adverse effects are still not yet fully understood, and their efficacy only lasts so long, but they show appeal due to their fast-acting effects, and efficacy (Reddy et al. 2023). Given all of the information from above, it is clear that neuroactive steroids play an important role in emotional regulation and therefore the development of mood disorders. Furthermore, targeting these steroids and their function is a potential avenue for treatment of emotional symptoms correlated with hormonal oral contraceptives.

RESEARCH PROPOSAL

Research Goals

The purpose of this study is to evaluate the effectiveness of synthetic neuroactive steroids and their interactions with GABA-A receptors, as a potential therapeutic intervention for alleviating symptoms of anxiety, depression, or irritability induced by the use of hormonal oral contraceptives (HOCs). This study will examine the effects of administering synthetic neuroactive steroids like brexanolone, ganaxolone, and zuranolone as an adjunctive therapy for combined oral contraceptives to evaluate their positive or negative impact on the mood levels of participants struggling with psychological side effects. Furthermore, this study aims to establish a stronger understanding of the physiological relationship between hormonal contraceptives and mood disorders by considering underlying neurobiological mechanisms and processes associated with emotional regulation. This will be achieved by comparing the findings of biological markers with behavioral and mood questionnaires to elucidate the neurobiological mechanism by which combined HOCs may lead to the development of psychosocial disorders. In acknowledging the neurobiological implications of hormonal contraceptives and their role in the possible development of negative psychological side effects, researchers can explore therapeutic interventions to address the emotional and physiological needs of HOC users struggling with mood symptoms. Ultimately, with this study we aim to fill a current gap in treatment options for women struggling with contraceptive-induced anxiety, depression and irritability which appear to have physiological foundations.

Hypothesis

We hypothesize that administering synthetic neuroactive steroids as a therapeutic intervention for combined oral contraceptive users experiencing negative psychological effects will alleviate feelings of anxiety and depression induced by ethinyl estradiol and levonorgestrel (EE-LNG) contraceptives. Brexanolone, ganaxolone, and zuranolone will act as positive allosteric modulators of GABA-A receptors, thereby carrying out allopregnanolone's normal role in binding to GABA and promoting GABAergic neurotransmission. We expect results to show increased GABA-A receptor activity in post-treatment electroencephalograms (EEGs). This will be reflected by decreased theta peak (4-8 Hz) frequency in EEGs after receiving treatment with synthetic neuroactive steroids, demonstrating inhibited neural responses as the receptor's response to GABA is improved, and thus resulting in calming effects. Increasing GABA's inhibitory effects on neural activity will alter the balance of connectivity between different brain regions, ultimately leading to less resting state functional connectivity in the salience network, central executive network, reward network, and subcortical limbic network in post-treatment functional magnetic resonance images (fMRIs). We anticipate decreased resting state functional connectivity will promote emotional regulation, reduced anxiety, and simulate the effect of antidepressants. We expect the results of this study will show that lower mood scores are associated with higher levels of GABA-A receptor activity and reduced resting state functional connectivity in the brain as a result of synthetic neuroactive steroid administration.

Materials and Methods

Study Design and Participants:

For this randomized, double-blind, controlled study, ≥ 300 participants will be recruited through patient referrals from OB/GYN clinics, medical centers, university campuses, and student health centers. Additionally, we will use participant registries, surveys, and media searches to expand the scope of participant searches and improve diversity. Subjects will be sourced from various locations throughout the United States to include diverse demographics representative of the population being studied. Women 18 and older meet eligibility criteria if they (1) currently use combined oral contraceptives with an ethinyl estradiol and levonorgestrel (EE-LNG) formula, (2) have been using these oral contraceptives for at least three months, and (3) have been experiencing mood-related side effects such as anxiety or depression while using contraceptives. Individuals with (1) a history of severe mood disorders that pre-date contraceptive use, (2) an allergy or intolerance to allopregnanolone, progesterone, or ganaxolone, (3) a positive pregnancy test, or (4) a current prescription for psychotropic medication will be excluded from this study (Deligiannidis et al., 2021; Dichtel et al., 2020; Gerbasi et al., 2021; Kanes et al., 2017; Marinus Pharmaceuticals, 2023; Meltzer-Brody et al., 2018; Qiu et al., 2024). To ensure participants meet the eligibility criteria to participate in the study, they will be pre-screened.

This study will be submitted for review and approval to the appropriate Institutional Review Board (IRB) and any independent ethics committees necessary to ensure this research is conducted ethically and considers the overall well-being of all participants.

Participants found eligible will be asked to sign an informed consent form which establishes that they understand the nature of the study, procedures, potential risks or benefits,

and their rights as a participant in the study (Meltzer-Brody et al., 2018). Additionally, all candidates will need to undergo a full physical examination with an electrocardiogram (ECG) to ensure that the individual is healthy enough to receive treatment given the possibility of serious side effects (Ali et al., 2021; Health, 2022; Meltzer-Brody et al., 2018). Before beginning any treatment, all participants will complete initial assessments to determine a baseline measure, allowing for the comparison of results before and after the treatment phase to determine significance. To establish baseline measures, we will administer self-assessment mood-questionnaires, quantify GABA-A receptor activity by conducting electroencephalography (EEG) testing, and examine of resting state functional connectivity using functional magnetic resonance imaging (fMRI) (Lambert et al., 2023; Meltzer-Brody et al., 2018, 2018; Sharma et al., 2020).

Randomization/Masking:

This study will use a randomized, double-blind, controlled trial design to explore the therapeutic potential of synthetic neuroactive steroids to reduce mood-related symptoms brought on by hormonal oral contraceptives. A computer-generated sequence will randomly and equally assign all participants to one of six treatment groups that will receive either an FDA-approved synthetic neuroactive steroid with an EE-LNG oral contraceptive, or a corresponding placebo. Thus, treatment groups will include (1) EE-LNG contraceptive + Brexanolone, (2) EE-LNG contraceptive + Brexanolone placebo, (3) EE-LNG contraceptive + Zuranolone, (4) EE-LNG contraceptive + Zuranolone placebo, (5) EE-LNG contraceptive + Ganaxolone, and (6) EE-LNG contraceptive + Ganaxolone placebo. Active treatment groups and placebo groups will have properties that render the two indistinguishable such as appearance, dosage, and route of

administration. Neither investigators nor participants will know which treatment is which, allowing a double-blind design and increasing the likelihood of reliable and unbiased results.

Administration Procedures:

We will administer synthetic neuroactive steroids and their corresponding placebos to treatment groups following basic procedures and protocols that have been established and tested in clinical trials for the respective drugs. These established protocols include dosage, administration schedule, delivery route, and cumulative treatment duration (Deligiannidis et al., 2021; Dichtel et al., 2020; Kanesh et al., 2017; Marinus Pharmaceuticals, 2023; Meltzer-Brody et al., 2018; Qiu et al., 2024). It is important to clarify that the route of administration, treatment duration, and dosage instructions vary for each synthetic neuroactive steroid being used, thus each treatment group will adhere to their own treatment schedule (See **Table 1** for summary of drug protocols for each treatment group)(Deligiannidis et al., 2021; Kanesh et al., 2017; Marinus Pharmaceuticals, 2023; Meltzer-Brody et al., 2018; Qiu et al., 2024). Scheduled follow-ups will remain uniform across all treatment groups relative to the completion of treatment. Every participant in each treatment group will take hormonal oral contraceptives (HOCs), specifically a combined EE-LNG contraceptive, regularly.

Table 1: Summary of the administration procedures recommended by preclinical trials for each synthetic neuroactive steroid and placebo treatment group

Synthetic Analogue of Allopregnanolone	Route of Administration	Dosage	Treatment Duration	Treatment Details
Brexanolone	Intravenous Infusion (IV)	*See administration details	72 hours	Hours 0-4: 30 µg/kg per hour Hours 4-24: 60 µg/kg per hour Hours 24-52: 90 µg/kg per hour Hours 52-56: 60 µg/kg per hour Hours 56-60: 30 µg/kg per hour COC taken as normal
Brexanolone Placebo	Intravenous Infusion (IV)	*See administration details	72 hours	Hours 0-4: 30 µg/kg per hour Hours 4-24: 60 µg/kg per hour Hours 24-52: 90 µg/kg per hour Hours 52-56: 60 µg/kg per hour Hours 56-60: 30 µg/kg per hour COC taken as normal
Zuranolone	Oral Capsule	20-30 mg	2 weeks	Zuranolone taken once daily in the evening with food. COC taken as normal
Zuranolone Placebo	Oral Capsule	20-30 mg	2 weeks	Placebo taken once daily in the evening with food. COC taken as normal
Ganaxolone	Intravenous Infusion (IV)	*See administration details	7-10 weeks	Begin infusion at 225 mg 2x a day Week 1, 2, 4, 6: 450 mg 2x a day Week 8 & 10: Drug is tapered COC taken as normal
Ganaxolone Placebo	Intravenous Infusion (IV)	*See administration details	7-10 weeks	Begin infusion at 225 mg 2x a day Week 1, 2, 4, 6: 450 mg 2x a day Week 8 & 10: Drug is tapered COC taken as normal

Active Brexanolone and Brexanolone Placebo Treatment Groups:

Brexanolone treatments will be administered under medical supervision to monitor and mitigate potentially serious side effects (Ali et al., 2021; Gerbasi et al., 2021; Health, 2018). Over the course of 72 hours, participants will receive treatment through continuous intravenous infusion (IV) beginning with a lower dose and continuously going through titration and increasing this dosage. The starting dose will be dependent on the weight of the patient, and the standard dosage used in clinical trials will need to be adjusted for each participant (Kanes et al., 2017; Meltzer-Brody et al., 2018). Starting doses will begin at 30 µg/kg per hour for hours 0-4. From this point, the following schedule is maintained during administration: 60 µg/kg per hour for hours 4-24; 90 µg/kg per hour during hours 24-52; 60 µg/kg per hour from hours 52-56; 30 µg/kg per hour for hours 56-60 (Ali et al., 2021; Kanes et al., 2017; Meltzer-Brody et al., 2018). After completing 60 hours of IV, participants will remain under observation for 12 hours as a safety precaution against adverse effects. Administration of brexanolone will be stopped immediately if participants observe any side effects or worsening in mood. The placebo administration of brexanolone involves the use of an IV solution that produces no effect as there is no active component. The procedure and dosage of administration for the placebo group will be identical to that of the active form of brexanolone. We will conduct follow up assessments on participants in both treatment groups three days after completing the regimen, followed by assessments two weeks, six weeks, and twelve weeks after finishing the treatment. Assessments will include the Brief Irritability Test (BITe), the Generalized Anxiety Disorder 7-item scale (GAD-7), and the Patient Health Questionnaire-9 (PHQ-9), as well as electroencephalography (EEG) monitoring, and functional magnetic resonance imaging (fMRI).

Active Zuranolone and Zuranolone Placebo Treatment Groups:

Zuranolone treatments will differ from brexanolone in that zuranolone will be administered orally in the form of a capsule over the course of two weeks (Health, 2018, 2022). Additionally, no extra medical attention is necessary for participants taking zuranolone. As a result, participants will take their daily dose of zuranolone from a more convenient location such as their homes (Deligiannidis et al., 2021). We will instruct participants to ingest a single capsule of zuranolone each night with food for two weeks straight (Deligiannidis et al., 2021; Health, 2022; Qiu et al., 2024). Based on standard measurements of earlier studies using zuranolone, each capsule will contain 30 mg of zuranolone (Deligiannidis et al., 2021). If 30 mg proves to have strong adverse effects on any patient, heeding the advice of clinical trials, we will instead administer 20 mg of zuranolone (Deligiannidis et al., 2021; Qiu et al., 2024). We will stop administration of zuranolone immediately if participants observe any side effects or worsening in mood.

Placebo administration of zuranolone will involve the use of capsules identical in size, shape, and packaging of zuranolone capsules. Participants in the zuranolone-placebo controlled treatment will take these capsules using the same procedure and schedule as the active form of zuranolone: every night with food for two weeks straight. Participants in both treatment groups, like the brexanolone treatment group, will complete follow-up assessments three days after completing the treatment plan, followed by assessments two weeks, six weeks, and twelve weeks after completing the treatment. Assessments will include the BITE, GAD-7, and PHQ-9 questionnaires, as well as EEG monitoring, and fMRI imaging.

Active Ganaxolone and Ganaxolone Placebo Treatment Groups:

Ganaxolone treatments will be administered by closely following procedures of pre-clinical and pilot studies that have already been established (Dichtel et al., 2020; Marinus Pharmaceuticals, 2023). For a period of 7-10 weeks, depending on how quickly results manifest due to administration of synthetic neuroactive steroids, participants will receive a treatment of intravenous ganaxolone (Dichtel et al., 2020). Similar to brexanolone treatments, the IV will begin with a lower dose of 225 mg which is administered twice a day. From this point, the following schedule is maintained during administration: 450 mg twice a day during week one, two, four, six; drug tapers (decreases) weeks 8 and 10 (Dichtel et al., 2020). We will adjust dosage of ganaxolone according to the participant's tolerance (Dichtel et al., 2020). Additionally, we will stop administration of ganaxolone immediately if participants report any side effects or worsening in mood. The placebo administration of ganaxolone, like brexanolone, will feature the use of an IV solution that is similar in size and packaging to the intravenous solution used in the treatment group for the active form of ganaxolone. Administration of the ganaxolone placebo will exactly mimic the procedure, schedule, and dosage of active ganaxolone. We will conduct follow-up assessments with participants three days after completion of the treatment plan, followed by assessments two weeks, six weeks, and twelve weeks after cessation of treatment. These assessments will include the BITE, GAD-7, and PHQ-9 questionnaires, as well as EEG monitoring, and fMRI imaging.

Measurements:

Self-Report Mood Assessments:

All participants in each study will undergo assessment using the Brief Irritability Test (BITe), Generalized Anxiety Disorder 7-item scale (GAD-7), and Patient Health Questionnaire-9 (PHQ-9) before receiving treatment with synthetic neuroactive steroids (Holtzman et al., 2015; Kroenke et al., 2001; Spitzer et al., 2006). We will conduct subsequent evaluations three days post-treatment and during follow-ups at two weeks, six weeks, and twelve weeks. These self-report questionnaires will determine the severity of irritability, anxiety, and depression among participants. BITe is a five-item assessment designed to gauge irritability by considering factors such as agitation, daily temperament, and feelings of aggression towards others. Participants will rate the frequency of anger-driven feelings or behaviors on a scale of 1 to 6, where 1 will indicate 'never' and 6 will indicate 'always' (**Fig. 8A**) (Holtzman et al., 2015). In comparison, GAD-7, another itemized scale, will measure the severity of anxiety symptoms over the past two weeks in participants. In this scale, participants will rate the prevalence of 7 anxious behaviors on a scale of 0-3, where 0 will indicate the behavior is not present and 3 will indicate the behavior is present almost everyday (**Fig. 8B**) (Spitzer et al., 2006). PHQ-9 will determine depression severity in participants by evaluating feelings of sadness and hopelessness, changes in appetite, energy, sleep quality, suicidal ideation, and changes in cognition. Participants will score how often within the last two weeks they have experienced the behaviors and thoughts associated with depression on a scale of 0 to 3, where 0 represents not at all and 3 represents almost everyday (**Fig. 8C**) (Kroenke et al., 2001). Threshold scores for GAD-7 and PHQ-9 representing mild, moderate, and severe levels of anxiety are 5, 10, and 15 (Spitzer et al., 2006).

Post-treatment scores will be compared to baseline scores.

8A

	Never	Rarely	Sometimes	Often	Very Often	Always
1. I have been grumpy.						
2. I have been feeling like I might snap.						
3. Other people have been getting on my nerves.						
4. Things have been bothering me more than they normally do.						
5. I have been feeling irritable.						

Scoring
 Never = 1, Rarely = 2, Sometimes = 3, Often = 4, Very Often = 5, Always = 6
 Total irritability score = Sum items 1-5

Figure 8A: The Brief Irritability Test (BITe) will assess the severity of irritability in each participant. Behaviors and feelings associated with irritability will be evaluated on their prevalence using a scale of 1 to 6, where 0 indicates the behavior never occurs. The higher the total score the more severe irritability (Holtzman et al., 2015).

8B

GAD-7

Over the last 2 weeks, how often have you been bothered by the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3

Total Score — = Add Columns — + — + —

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all <input type="checkbox"/>	Somewhat difficult <input type="checkbox"/>	Very difficult <input type="checkbox"/>	Extremely difficult <input type="checkbox"/>
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Figure 8B: The Generalized Anxiety Disorder 7-item scale (GAD-7) will examine the severity of anxiety in each participant. The prevalence of common symptoms of anxiety will be scored by participants using a scale of 0 to 3, where 3 will indicate the symptom has been a daily bother. Minimal anxiety: 0-5; mild anxiety: 5-10; moderate anxiety: 10-15; severe anxiety: 15-21 (Spitzer et al., 2006).

8C

The Patient Health Questionnaire (PHQ-9)

Patient Name _____ Date of Visit _____

Over the past 2 weeks, how often have you been bothered by any of the following problems?

	Not At all	Several Days	More Than Half the Days	Nearly Every Day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed or hopeless	0	1	2	3
3. Trouble falling asleep, staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself - or that you're a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or, the opposite - being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

Column Totals _____ + _____ + _____

Add Totals Together _____

10. If you checked off any problems, how difficult have those problems made it for you to Do your work, take care of things at home, or get along with other people?

☐ Not difficult at all ☐ Somewhat difficult ☐ Very difficult ☐ Extremely difficult

Figure 8C: The Patient Health Questionnaire-9 (PHQ-9) will evaluate the severity of depression in participants. Frequency of symptoms will be rated on a scale of 0 to 3, where 3 will indicate the symptom has been noted daily. Minimal depression: 0-5; mild depression: 5-10; moderate depression: 10-15; severe depression: 15-21 (Kroenke et al., 2001; Spitzer et al., 2006).

Electroencephalography (EEG):

GABA-A receptor activity will be quantified using EEG tests before and after the administration of synthetic neuroactive steroids. EEG recordings will measure electrical activity in the form of waves induced by inhibition or excitation of the GABAergic neurotransmitter in the brain (Rayi & Murr, 2024). During the EEG assessment, EEG electrodes will be applied to the scalp using the 10 to 20 international system for electrode placement which will ensure activity is captured from regions corresponding with emotional regulation and processing. These

regions will include the salience network, central executive network, reward network, and subcortical limbic network (Rayi & Murr, 2024; Sharma et al., 2020). Once electrodes are connected to the EEG amplifier and impedance levels are confirmed to be less than 5 kohms, a recording will be taken for 30 minutes as the participant lies relaxed with their eyes closed (*Electroencephalogram (EEG)*, 2017; “What Happens during an Electroencephalogram (EEG)?,” 2018; Rayi & Murr, 2024). The EEG recording will be filtered to isolate frequency bands associated with GABAergic neurotransmission and emotional regulation. Specifically, isolation of gamma (30-80 Hz) and theta (4-8 Hz) bands will allow abnormalities related to GABAergic neurotransmission and emotional regulation to be observed (Rayi & Murr, 2024; Wyss et al., 2017). Along with completing self-report questionnaires to determine mood levels during post-treatment follow-ups, participants will also undergo EEG testing on the same day. Following treatment, EEG testing will occur three days post-treatment and during follow-ups at two weeks, six weeks, and twelve weeks. Post-treatment EEG recordings will be compared to baseline EEG recordings.

Functional Magnetic Resonance Imaging (fMRI):

Use of fMRI scans will measure resting state functional connectivity in the brains of participants in response to supplementation of synthetic neuroactive steroids that target GABA-A receptors (Belelli et al., 2019; Nasrallah et al., 2017; Sharma et al., 2020). Participants will receive fMRI scans before beginning treatment, two weeks, six weeks, and twelve weeks after finishing their given treatment.

Statistical Analysis:

We will analyze self-questionnaire data using a repeated measures analysis of variance (ANOVA) to evaluate each treatment group's average mood scores and their changes over time. Follow-up times and treatment groups will act as independent variables. If changes in mood scores within each treatment group are found to be statistically significant we will conduct a post-hoc analysis to determine what is causing significant differences or at what point in time are differences arising. To analyze fMRI data we will use the General Linear Model (GLM) to analyze the relationship between conditions such as treatment groups and follow-up times and their effect on brain activity. We will follow standard protocol for fMRI statistical analyses and conduct whole-brain analysis of the GLM to determine significant changes in activation patterns when comparing treatment groups and times (Monti, 2011). For EEG data, we will use EEG analysis software to conduct EEG Power Spectral Density Analysis to observe peak frequency and power of theta and gamma bands (*5 Basics of EEG 101*, 2021). We will run ANOVA to compare statistical differences between treatment groups and follow-up points to assess peak frequencies and power spectra.

Results

Self-Report Mood Scores

In this section, we present our expected results for self-report mood scores for anxiety, depression, and irritability following treatment completion. To assess the efficacy of synthetic neuroactive steroids in treating contraceptive-induced mood disorder symptoms, we will administer quantitative measures including the Brief Irritability Test (BITe), the Generalized Anxiety Disorder 7-item scale (GAD-7), and the Patient Health Questionnaire-9 (PHQ-9). We will assess the mean scores of each treatment group and observe how they change over the course of 12 weeks, compared to each other. Our objective is to establish a correlation between treatment with synthetic neuroactive steroids and lower average mood scores immediately following treatment.

We expect after completion of the proposed treatment, average scores of anxiety, depression, and irritability for active treatment groups will fall significantly lower than the baseline measurement and gradually increase over the course of 12 weeks post-treatment. Upon initial assessment three days after concluding the treatment, statistically significant lower average mood scores will be observed across treatment groups for brexanolone, zuranolone, and ganaxolone compared to baseline scores taken at the start of the study. For at least two weeks post-treatment, we expect no significant change in average mood scores and they will remain fairly low before gradually beginning to increase again. Once participants approach the 6-week mark, average mood scores for all three assessments will have increased across all active treatment groups. Specifically, the brexanolone treatment group is expected to show a statistically significant increase in average mood score in each assessment group (BITe, GAD-7, and PHQ-9). In comparison, we anticipate the zuranolone treatment to show a statistically

significant increase in average mood score yet maintain a lower average than the brexanolone treatment group. We predict the ganaxolone treatment group will show a nonsignificant increase in average mood score across each assessment compared to brexanolone and zuranolone. By week 12, average mood scores are predicted to be relatively near baseline measurements for all treatment groups. Variation in mood scores among treatment groups may be attributed to differences in duration of each drug's effects, although detailed analysis of this aspect will be reserved for the discussion section. We anticipate that placebo groups will exhibit minimal decreases in average mood scores relative to baseline levels. While there may be fluctuations over time, there is no statistically significant data across all placebo treatment groups (**Fig 9**).

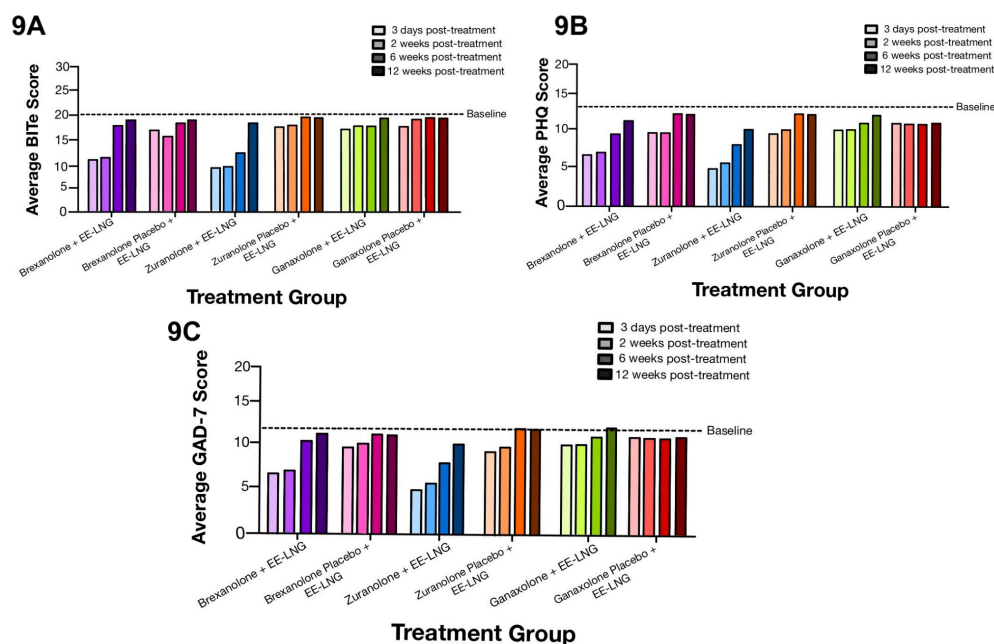


Figure 9: Expected changes in average mood scores following treatment with synthetic neuroactive steroids compared to corresponding synthetic neuroactive steroid placebos. 3 days immediately following treatment, average mood scores in active groups across all assessments will be significantly lower than baseline measures taken before treatment. Subsequent follow-ups at 2 weeks, 6 weeks, and 12 weeks will show increase in average mood scores measured using (9A) the Patient Health Questionnaire (PHQ), (9B) Generalized Anxiety Disorder 7-item scale (GAD-7), and (9C) Brief Irritability Test (BITE). Significant increases in average mood scores will be seen in week-6. Placebo groups exhibit minimal changes compared to active groups.

EEG Data

Within this segment, we outline our anticipated outcomes for the statistical analysis of the electroencephalogram (EEG) data collected. EEGs will be statistically analyzed to assess the significance of theta peak frequency changes before and after administration of synthetic neuroactive steroids over 12 weeks. We aim to observe how peak frequency changes compared to initial assessments before treatment, and how this frequency continues to change over time. EEG analysis allows us to make inferences about GABA-A receptor activity and its interaction with synthetic neuroactive steroids, thus allowing us to evaluate the efficacy of the proposed treatment. Our objective with this analysis is to delineate a relationship between synthetic neuroactive steroids and their GABAergic effects. Additionally, comparing these assessments to average mood scores establishes a correlation between mood and GABA-A receptor activity, furthering our understanding of the physiological relationship between HOC use and its impact on mood.

Frontal and parietal EEGs recording the effects of the administration of GABA-A receptor modulators such as pentobarbital and diazepam reveal acute effects on mid-frequency range oscillations and theta frequency and strength. Specifically, pentobarbital results in oscillations lower in the beta range with decreased theta rhythm strength while diazepam induces oscillations with a peak of 50 as well as reduces theta rhythm strength (**Fig. 10**)(Lambert et al., 2023).

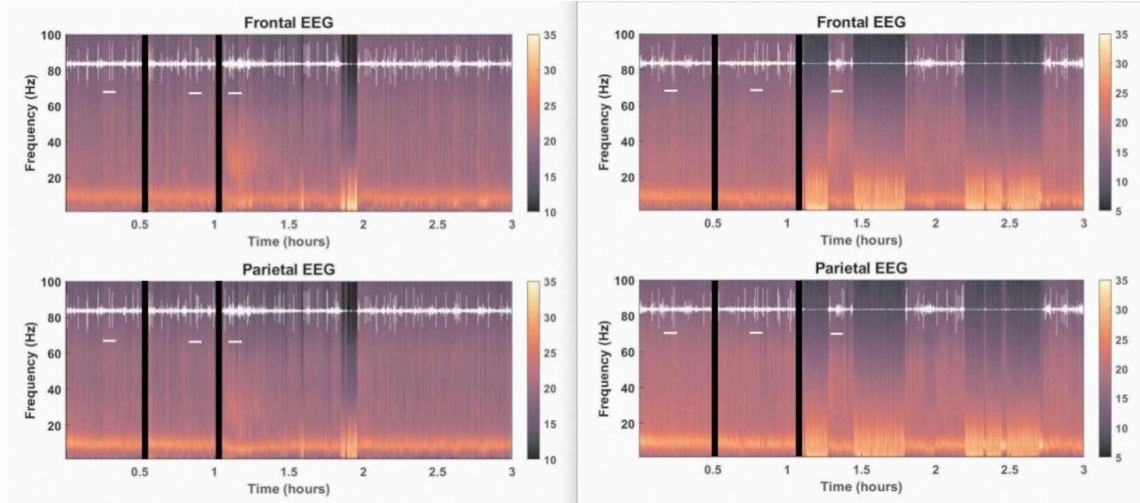


Figure 10: EEG spectrogram representing the expected results of EEG testing following treatment with synthetic neuroactive steroids. Frontal and parietal EEGs above illustrate an acute reduction in theta oscillations (4-8 Hz) and mid-frequency alterations after the administration of pentobarbital (right image) and diazepam (left image), positive allosteric modulators of GABA-A receptors. Black lines show vehicle and drug injection (Lambert et al., 2023).

We expect that treatment with active synthetic neuroactive steroids will result in post-treatment EEG spectrograms similar to those generated to measure the effects of administration of pentobarbital and diazepam. Anticipated effects include reductions in theta rhythm strength, reflected by a decrease in theta rhythm peak frequency, and changes to the mid-frequency oscillation peaks as seen in both diazepam and pentobarbital application. These effects will gradually decrease as follow-up sessions progress after treatment completion. By week-12, mid-frequency oscillation peaks and theta rhythm peak frequency implicating theta strength will return to baseline levels across all treatment groups. We predict that the changes in mid-frequency oscillation peaks and theta rhythm peak frequency seen in follow ups after three days and two weeks will be statistically significant when compared to baseline. Oscillation peaks and their frequencies during week-6 and week-12 will undergo no significant changes when compared to their initial data. We expect this trend to be seen throughout each synthetic

neuroactive steroid treatment group. In comparison, no significant changes will be observed across placebo groups.

fMRI Data

In the following section we describe our predicted findings for the statistical analysis of the fMRI data collected during each follow-up session for each treatment group. By conducting fMRIs we aim to observe functional connectivity patterns in the salience network, central executive network, reward network, and the subcortical limbic network, where processes such as pleasure, reward responses, and emotional regulation are understood to be most influenced by HOC use. Through a comparative analysis of fMRI connectivity patterns before and after treatment across all groups, we seek to determine the significance of brain activity changes resulting from synthetic neuroactive steroid administration. This statistical data provides insight on the specific brain regions impacted by HOC use and their potential relationship with neuroactive steroids, offering insights into the feasibility of replenishing these steroids to alleviate mood symptoms.

We predict fMRIs conducted before treatment will demonstrate higher functional connectivity in regions of the brain including the salience network, central executive network, reward network, and the subcortical limbic network (**Fig. 11A-11D**). Following the supplementation of synthetic neuroactive steroids in participants, fMRIs will demonstrate low levels of functional connectivity in these same areas. We anticipate that the relationship between synthetic neuroactive steroid administration and decreased functional connectivity will yield statistically significant results during the day-3 and week-2 follow-ups across all active treatment groups. In comparison, we predict no significant results when evaluating the effects of synthetic neuroactive steroids on functional connectivity during week-6 and week-12. Additionally, it is

anticipated that functional connectivity will be lowest three days after the treatment has concluded, and will gradually increase in two week, six week, and twelve week follow-ups. In week-12 fMRIs, functional connectivity will be similar to initial assessments completed before the start of the study.

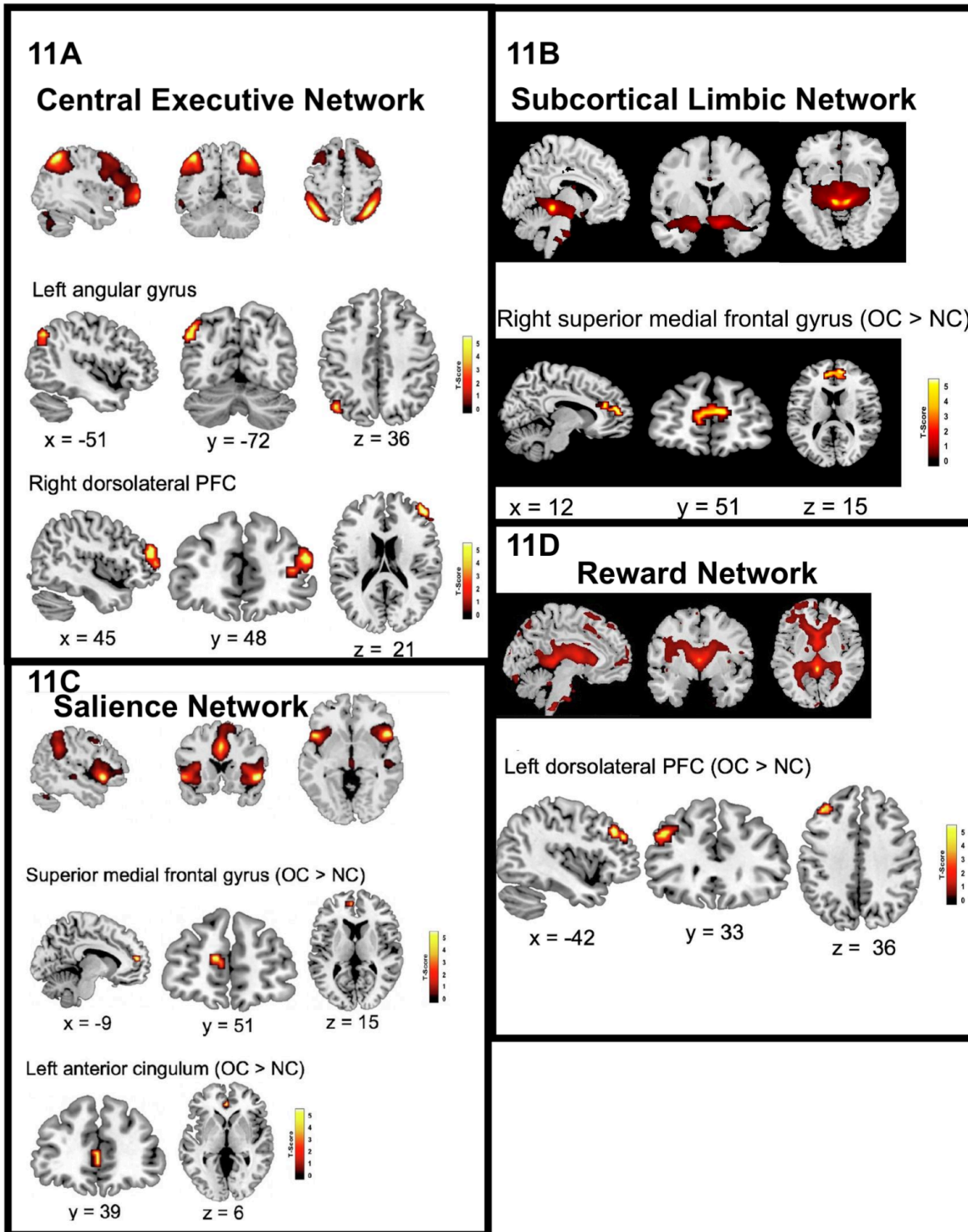


Figure 11: fMRI scans showing increased resting state functional connectivity expected before beginning treatment, particularly in regions of the brain including (11A) the central executive network, (11B) the subcortical limbic network, (11C) the salience network, and (11D) the reward network. Areas of strong activation (yellow coloring) correspond with regions responsible for emotional regulation, pleasure and reward systems, and decision making. XYZ represents MNI coordinates. OC = oral contraceptive users; NC = naturally cycling women (Sharma et al., 2020).

Discussion and Conclusion

This study is designed to test the use of synthetic neuroactive steroids to target GABA-A receptors, as a potential therapeutic avenue for the treatment of mood disorders induced by hormonal oral contraceptives. By using a comprehensive array of measures including self-report mood questionnaires, EEGs to monitor and quantify GABA-A receptor activity, and fMRI scans to observe functional connectivity, the overarching objective of the study is to further understand neurobiological mechanisms underlying the effects of hormonal contraceptives on mood disorders and develop a possible approach for treatment.

The integration of various measures allows for a deeper understanding of the relationship between hormonal contraceptives and emotional side-effects reported in 4-10% of users (Lundin et al., 2017). All of the measures are intended to serve as one piece to a greater puzzle in understanding underlying neurobiological mechanisms linking HOC use with adverse emotional effects. EEG recordings showing reduced theta rhythm peak frequency following administration of synthetic neuroactive steroids suggests increased inhibitory activity mediated by the GABAergic system as neuronal excitation has decreased compared to baseline assessments (Lambert et al., 2023; Wyss et al., 2017; Belevi et al. 2019). These EEG recordings exemplifying reduced activity suggests an increase in GABA-A receptor activity mediated by synthetic neuroactive steroids. This behavior aligns with the proposed mechanism of action of positive allosteric modulators targeting GABA-A receptors thus promoting inhibitory effects of GABA neurotransmission ultimately leading to neuronal hyperpolarization and suppression of activity (Lambert et al., 2023). Post-treatment EEG data, in which there will be an initial reduced theta rhythm peak frequency compared to initial EEGs recorded before treatment, will gradually show increase in theta strength in follow-up assessments. EEG data trends will support

the hypothesis that there is increased GABA-A receptor activity in post-treatment EEGs as synthetic neuroactive steroids bind to GABA-A receptors and improve the receptor's response to GABA (**Fig.12**).

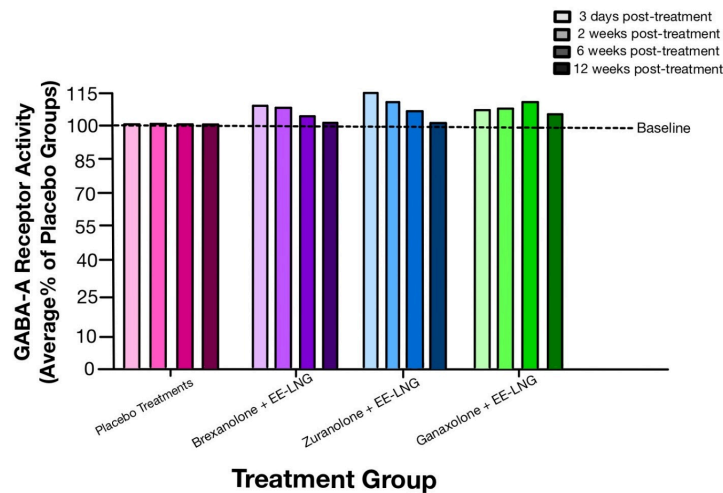


Figure 12: Post-treatment EEGs for brexanolone, zuranolone, and ganaxolone treatment groups will demonstrate an increase in GABA-A receptor activity compared to initial baseline measurements and placebo groups. Receptor activity will decrease over time.

Additionally, the complementary use of fMRI provides a greater perspective in understanding the broader neurobiological impact of synthetic neuroactive steroids on brain function and their implications for emotional processing and regulation. Decreased functional connectivity after synthetic neuroactive steroid treatment within neural networks implicated in emotional regulation such as the salience network, central executive network, reward network, and subcortical limbic network suggest a relationship between GABAergic neurotransmission and emotional processing (Sharma et al., 2020; Nasrallah et al. 2017). Furthermore, amplification of GABAergic neurotransmission, which promotes inhibition on neuronal activity, will cause imbalances across brain regions, prompting these regions to adjust in neural activity (Sharma et al., 2020). As a result, brain regions will alter their functional connectivity patterns, and less

activity across regions of the brain associated with emotional regulation will be seen in fMRI scans (Sharma et al., 2020; Nasrallah et al. 2017). Increased functional connectivity reflects higher neural activity, prompting the brain to shift neural networks, meaning processes could in turn be modified in response to this high activity. This will in turn influence how the brain regulates emotion, memory formation and retrieval, and responses to rewards (Sharma et al., 2020).

Study Limitations

Using neuroactive steroids as a therapeutic solution for psychological disorders is a topic still being heavily studied yet rising in popularity. Neuroactive steroids require careful management. Due to safety and tolerability considerations which can pose ethical dangers, only a select few synthetic neuroactive steroids have been approved with the intended purpose of treating depression or anxiety disorders, thereby limiting treatment groups.

Another potential limitation is that in recruiting participants for this study, we chose to exclude patients who have a history of mental illness or are under the age of 18 due to safety precautions, ethical reasons, and in order to isolate contraceptives induced mental disorders. However, individuals with a history of mental illness and who are around the pubertal age of 16 are most vulnerable to contraceptive induced psychological effects. Thus, in excluding these patients there is a possibility of excluding the target population.

Additionally, each drug that will be used has a different route of administration as well as treatment duration. This could imply that very different processes occur in each treatment group, impacting variation in efficacy and results.

Conclusion

Through a comparison of data obtained from self-report questionnaires, EEG recordings, and fMRI scans, the goal is to further develop the relationship and interconnectedness between high GABA-A receptor activity, altered functional connectivity patterns, and mood states. By uncovering these intricate relationships, the study provides a promising avenue for understanding the neurobiological underpinnings of mental disorders, particularly in the context of hormonal contraceptive use. These insights not only advance the understanding of the pathophysiology of mood disorders but also hold significant implications for the development of targeted therapeutic interventions tailored to individuals experiencing adverse mood effects associated with hormonal contraceptives.

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