4-13-2021

Effect of Social Isolation on Seizure Susceptibility through GABA-ergic Mechanisms

Will Chesner

Kaitlyn Twadell

Elise Pham

Sydney Wright

Effect of Social Isolation on Seizure Susceptibility through GABA-ergic Mechanisms

Will Chesner, Kaitlyn Twadell, Elise Pham, Sydney Wright

Santa Clara University, Library Research Award

April 13th 2021

PROJECT SUMMARY

The ongoing Covid-19 pandemic has incited an array of social, psychological, and emotional burdens among adolescents and adults. The lockdowns, social distancing measures, and general anxiety surrounding the virus have produced an unprecedented and pervasive degree of social isolation, particularly amongst adolescents. Social isolation has deleterious effects on physical and mental well-being and is a critical risk factor for morbidity among adults. However, the neural and physiological underpinnings and consequences of social isolation have yet to be fully explored. Social isolation has been understood as a source of chronic and early stress, with various developmental consequences.

In the past decade, researchers have found the stress associated with social isolation to worsen the development of epilepsy, among other neuropsychological disorders. There are various mechanisms and regions involved such as the GABAergic system, which is the neural system regulating GABA neurotransmission. There is a growing consensus in the scientific community that changes in the GABAergic system stemming from social isolation can lead to a lower seizure threshold.

This leads us to not only ask whether the GABAergic system, and other systems involved with neurotransmitters, could be affected by social isolation, but also whether this corresponding change in neurophysiology leads to a lowered seizure threshold. Our objective is to better understand the effects of social isolation on epilepsy development. The broad, long-term objective of this work is to provide clinicians with a framework for how to properly intervene and target therapies for neuropsychological disorders that might arise from early life stress.

Research Plan

SIGNIFICANCE

During early development, the brain tends to be much more neuroplastic. Childhood and adolescence are critical periods in which stressors and environmental alterations can disrupt the maturation of biological circuits that regulate homeostasis (Spear, 2000; Spear, 2004). Social isolation stress (SIS) has been associated with neurobiological alterations in the Central Nervous System (CNS), changes in stress reactivity, social behavior, the function of neurochemical and neuroendocrine systems (Mumtaz et al., 2018). Exposure to chronic SIS in early life has been

proposed to induce altered levels of dopamine, serotonin, GABA, and glutamate, as well as changes to the nitrergic (regulating nitric oxide levels) and adrenaline systems (Lapiz et al., 2003; Amiri et al., 2014; Chen et al., 2015; Mumtaz et al., 2018). As GABA is the principal inhibitory neurotransmitter in the brain regulating cellular communication, imbalances in GABA can intensify and trigger seizures (Treiman, 2001). Alterations in the GABAergic system are correlated with social isolation (Matsumoto et al., 2003; Mumtaz et al., 2018). Such neurochemical changes can induce a heightened response to new stimuli in socially isolated mice (Lapiz et al., 2003). The research proposed here will investigate the neurobiological consequences of social isolation in the GABAergic system. This will be done by measuring seizure threshold. Seizures are generally associated with GABA deficiency, though the extent to which GABA plays a role is disputed. By investigating social isolation and the neurobiological mechanisms underlying seizure susceptibility through the GABAergic system, the research proposed here may lead to targeted interventions for treating seizures and a better understanding of the adverse effects of SIS on neural function.

BACKGROUND

Social Isolation Stress (SIS)

SIS and Development

Adolescence is the period of transition from childhood to adulthood and involving many biological changes. Childhood and adolescence is a period when the brain is still in development, and thus stressors can negatively impact proper development, leading to immediate and long-term detrimental effects (Godoy et al., 2018).

Social isolation of rodents during adolescence and development is a commonly used method to study how adverse events during early life induce long-lasting neural and behavioral dysfunction. Rodents socially isolated around early adolescence exhibit altered behaviors and show cognitive impairments when later tested as adults (Orben et al., 2020).

SIS and Nervous System Disorders

Evidence suggests that social isolation can be a major source of stress that is correlated with a higher prevalence of neurological diseases (Friedler et al., 2015). Social isolation stress is a debilitating condition that can contribute to a predisposition to disease, prolonged fatigue, and varying responses to psychiatric drugs (Jaremka et al., 2014).

Social isolation can alter behavioral responses to other stress-inducing events, social behavior, neurochemistry, neuroendocrine and anatomical systems, in both humans and animals (Ferdman et al., 2007; Weiss et al., 2004). SIS in mice models has been shown to result in similar patterns of behavior seen in anxiety, depression, and schizophrenia in humans (Amiri et al., 2014; Nestler & Hyman, 2010).

Mechanistically, chronic social isolation has been shown to alter behavior in adverse ways through GABA receptors (Matsumoto et al., 2003). Taken together, social isolation as early life stress induces drastic developmental changes, nervous system, and mood disorders, which raise questions for further research on its underpinning biological mechanisms.

Social Isolation and Neurotransmitters

Mechanisms underlying GABA antagonization

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammal nervous system. GABA can bind with two receptors: $GABA_A$ and $GABA_B$. $GABA_A$ is a ligand-gated ion channel. When GABA binds with $GABA_A$ receptors, it causes an influx of chloride ions which hyperpolarizes the neuron and gives a rapid inhibitory effect (Herbison 2011). The $GABA_A$ receptor mediates an increase in membrane conductance, which is usually accompanied by membrane hyperpolarization. Hyperpolarization results in an increase in the firing threshold, which causes neuronal inhibition. Reduction in membrane resistance is caused by the facilitation of Cl^- ions influx (Olsen, 1986).

Social isolation and GABA

Looking at the effects on neurobiology, social isolation during the early stages of life can result in alterations of levels of neurotransmitters such as dopamine, serotonin, GABA, and glutamate (Mumtaz et al., 2018). Furthermore, Mumtaz et al., found that SIS also reduced $GABA_A$ receptor function in male mice. Studies have shown reduced responsiveness of $GABA_A$ receptors to GABA mimetic drugs, alterations in the expression of $GABA_A$-R subunits, and reduced $GABA_A$ receptor function in socially isolated male mice (Pinna et al., 2004). GABA expression and receptor function are disturbed following SIS, thus providing functional consequences for seizure threshold and the development of epilepsy.

Epilepsy as a Model to Study SIS

Neural correlates between GABA and epilepsy

Epilepsy is a complex disease characterized by many symptoms. Seizures are defined as a period of abnormal excitation of a neuronal population, usually lasting seconds to minutes (Jefferys, 2010; Scharfman, 2007). Epilepsy and seizures are not interchangeable for each other as epilepsy is defined as recurrent, spontaneous seizures. Many mechanisms have been linked with seizures. Research has turned towards mechanisms associated with the synaptic transmission that balance excitatory and inhibitory messages in the nervous system (Scharfman, 2007). Glutamate and GABA are the major excitatory and inhibitory neurotransmitters in the nervous system, respectively, where too much GABA decreases seizures and the overproduction of Glutamate increases seizures (Barker-Haliski & White, 2015; Matsumoto et al., 2003).

There is a great deal of experimental and clinical evidence showing that GABA plays a role in

epilepsy (Çavuş et al., 2016; Chowdhury et al., 2015; Miles et al., 2012; Treiman, 2001). Abnormalities of the GABAergic system have been observed in genetic and acquired models of epilepsy (Akyuz et al., 2021). Reduction of GABA concentrations and binding to $GABA_A$ has been detected in human studies of epileptic brain tissue (Kang & Barnes, 2013; Staley, 2015). GABA agonists have been shown to suppress seizures while GABA antagonists have been shown to produce seizures (Treiman, 2001).

Other mechanistic correlates with epilepsy

Glutamate is the principal excitatory neurotransmitter in the adult mammal brain. Past research has shown that a GABA(A)-mediated mechanism inhibits excitatory inputs caused by glutamate in the paraventricular nucleus (Li 2015). Glutamate binds to three different types of receptors: amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-d-aspartate (NMDA) receptors, and kainic acid receptors (KAR). When glutamate binds to AMPA and NMDA, the ionotropic receptors depolarize the postsynaptic neuron by allowing cations to pass into the cell. Aberrant neuronal activity such as that involved in seizures has been shown to regulate the number and location of AMPA receptors. This plasticity may contribute to weakening the neural network of the brain, contributing to heightened neuron excitability. Metabotropic glutamate receptors such as mGluR5 also play a role in mediating epileptic activity by increasing neuron excitability. Finally, group 1 mGluRs are regulated by the *Homer 1a* gene, which has been observed to downregulate AMPARs to cause changes to neural plasticity involved in seizures. Aberrant glutamate signaling and expression exacerbate neural plasticity issues that are common in epilepsy (Barker-Haliski & White, 2015).

The dopaminergic system is also thought to play a role in seizures. D1-like receptor agonists have been demonstrated to induce seizures as well as phosphate cAMP-regulated phosphoprotein of 32 kDa (DARPP-32). Also, seizure activity is absent or greatly reduced in mice that have a knocked-out D1R and DARPP-32. D5R activation has also been shown to activate cAMP and Plc signaling. The cAMP signaling pathway is thought to be involved in seizure control. Finally, D2R has been demonstrated across multiple studies to be the major DA receptor subtype involved in antiepileptogenic mechanisms in the limbic system (Bozzi & Borrelli, 2013).

Social Isolation, seizure susceptibility, and GABAergic systems

Seizure susceptibility is enhanced through social isolation by a reduction of $GABA_A$ receptors, which decreases the efficacy of GABA (Matsumoto et al., 2003). They administered a pharmacological modulator of $GABA_A$ receptor, known as allopregnanolone (ALLO), which suppressed seizure susceptibility. As ALLO synthesis diminished seizure vulnerability by upregulating $GABA_A$, long-term social isolation-induced functional alterations in the GABAergic system and increased seizure susceptibility.

Despite the growing body of research on the neural correlates of SIS and seizure susceptibility, there is a need to address the potential causal link between how SIS mediated GABA induce seizures. From contributing to the understanding of neurochemical modulators and mechanisms involved in SIS we hope this research can step into providing clinicians with underlying causes and tools in identifying disorders associated with chronic stress. With further research, illuminating chronic social isolation stress in childhood or adolescence can be used as a biomarker for disorders such as anxiety, epilepsy, among other neural and psychiatric disorders. These questions and areas for growth in the field guided our pursuit to look at the effects of SIS on seizure susceptibility.

APPROACH

Social Isolation Methods

Male C57BL/6 mice will be obtained 21 days postnatal, housed in a room with constant temperature and a reverse light schedule 12h light, 12h dark. Food and water are available ad libitum to both groups. On postnatal day 21, half of the mice will be randomly assigned to the group-reared and the other half will be randomly assigned to isolation-reared conditions. Isolation-reared mice will be housed individually for 3 weeks and then rehoused in groups of 3-4 mice for 2 weeks. Group-reared mice will be housed in groups of 3-4 for 3 weeks and randomly rehoused to a new group of 3-4. The new group of 3-4 mice will include both control-reared and isolation-reared mice to ensure that any differences seen between the groups will be due to the isolation period and not due to the resocialization period (Lukkes et al., 2009). All experimental protocols and procedures will be reviewed and obtain approval from the Animal Care and Use Committee at Santa Clara University. These social isolation methods are used for all three proposed studies.

Study-specific hypothesis #1: Socially isolated mice will have higher levels of Glutamate and lower levels of Dopamine and GABA relative to non-socially isolated mice. In this experiment, we seek to answer the question of whether the alterations in neurotransmitter concentrations in the hippocampus are brought about by social isolation.

Design:

After the 3-4 week housing period has been completed, all mice will go through microdialysis of the hippocampus to determine the levels of the following neurotransmitters, Gaba, Glutamate, dopamine, serotonin, acetylcholine, and norepinephrine. Microdialysis is a method widely used for sampling and quantitating neurotransmitters, neuropeptides, and hormones in the brain (Shippenberg & Thompson, 2008). Microdialysis is a localized sampling method, so we chose the hippocampus as the area to sample from because epileptic children generally experience seizures in the temporal and frontal lobes (“Childhood epilepsy”). After the neurotransmitter levels for each mouse are measured, they are placed in the same conditions as the rehousing

condition described in the social isolation methods section. The mice are housed like this for 10 weeks. Each week the microdialysis test is repeated for the same neurotransmitters.

Study-specific hypothesis #2: GABA antagonization is necessary for seizures in socially isolated mice. Experiments will be conducted with two groups, with varying social isolation housing and group housing (IV). Control mice will be group-housed. The experimental group will be reared in socially isolated housing. A synthetic ligand will be injected into all mice to inactive and silence $GABA_A$ receptor cells to determine if they are necessary to drive seizure susceptibility.

Design:

After 5 weeks of housing under group or social isolation conditions, all mice will be injected with pentylenetetrazole(PTZ) which is a $GABA_A$ receptor negative allosteric modulator. Prior research shows elevated nitric oxide levels in the brain are associated with increased seizure susceptibility in a pentylenetetrazole model (Miriski et al., 1994). They showed that PTZ-induced seizure is sensitive to small changes of nitric oxide (NO) levels in the brain; therefore, it is a valid animal model to evaluate epileptic activity.

Animals are administered ligand pentylenetetrazol (PTZ) (20 mg/ml in 0.9% saline; Sigma Chemical, St. Louis, MO). PTZ will be injected at a rate of 5.5mg.kg-1.min-1, which in a previous study elicited seizures within 10-18 minutes (Miriski et al., 1994). The total dose of PTZ given to elicit electroencephalographic/ behavioral seizure activity will be assessed. Blood samples will be taken after 15 min of infusion to determine the serum PTZ concentrations by gas chromatography. The concentrations should not be different between the two groups confirming that differences based on the alteration of PTZ kinetics did not play a role in the seizure threshold. An EEG is recorded and the PTZ dosage required to initiate a seizure is noted.

The dependent variable of seizures will be assessed based on a progression of motor activity and the score will be assigned on a scale of 0 to 6 (Miriski et al., 1994). An R score of 0 is indicative of no motor seizure activity; 1, only oral-facial movements; 2, head nodding; 3, myoclonic jerk movements; 4, forelimb clonus; 5, rearing; 6, rearing and falling.

Study-specific hypothesis #3: GABA receptor agonization does not induce seizures in socially isolated mice. To further investigate the neural correlates of seizure susceptibility as mediated by social isolation, we will look at the effects of GABA excitation through the use of a synthetic ligand GABA agonist diprofol (Zhang et al., 2018).

Design:

Experiments will be conducted with four experimental groups. The experimental group will be socially isolated-reared mice, while the control will be group-housed. Within each group, half of the mice will be randomly assigned to receive the GABA agonist and the other half will not.

In our two GABA agonist experimental groups, mice will be injected with 10 mg/ml of the synthetic ligand dipropofol (LMR-101; mol wt 354.53, purify 99.0%). Dipropofol is an established GABA receptor agonist in mice (Zhang et al., 2018). The dipropofol powder will be dissolved before injection. All experimental groups of mice will be injected with 30 mg/kg of the glutamate antagonist kainic acid monohydrate to induce seizures through another mechanism. Mice will be observed for two hours and the seizure threshold will be measured by recording the time of onset of seizures and recording seizure intensity on a scale of 0 to 6. An R score of 0 is indicative of no motor seizure activity; 1, only oral-facial movements; 2, head nodding; 3, myoclonic jerk movements; 4, forelimb clonus; 5, rearing; 6, rearing and falling.

OUTCOMES

Study 1 Predicted Outcomes:

It is well known that seizures induce increases in extracellular glutamate, which is why much research in the past has been done on its relationship to seizures, so we would expect a positive correlation between extracellular glutamate and social isolation of mice (Barker-Haliski & White, 2015). Changes in the GABAergic system brought about by social isolation have also been studied, and have been associated with decreased seizure threshold in mice (Matsumoto et al., 2003). So we would predict that increased social isolation would have a negative correlation with GABA levels. The dopaminergic system has been known to play a major role in the control of seizures arising from the limbic system, but not in the hippocampus (Bozzi & Borrelli, 2013). This mediating effect of dopamine leads us to predict a slight correlation (no directionality) between dopamine in the hippocampus. There is also a possibility of the serotonin system playing a role in lowering the seizure threshold in epileptic patients, so we also predict that there will be a slight correlation between serotonin and social isolation (Richerson & Buchanan, 2012). The other two neurotransmitters have very little research concerning their mechanisms underlying seizure threshold or epilepsy, so we would predict no correlation.

Study 1 Alternative Outcomes:

The predicted outcomes assume that the changes associated with social isolation will make the brain more closely reflect the neurobiology of an epileptic. There is a possibility that this is not true and the neurobiological mechanisms that underlie the lowering of seizure threshold in social isolation are different from that of epilepsy. Also, many of the studies referenced explore the entirety of the system associated with a neurotransmitter rather than just the neurotransmitter levels themselves. It's possible even if the levels of neurotransmitters do not change in the way we expected, there might still be a change in the neurotransmitter system. For example, the expression of certain GABA receptors might be an underlying mechanism and alteration within this system.

If the levels of neurotransmitters do not match our predicted outcomes, further analysis is still needed to determine if the systems associated with these neurotransmitters have been altered. For

example, rather than causing lower levels of GABA, SIS could cause a deleterious mutation to occur at the GABA receptor, decreasing the efficacy of GABA.

Study 2 Predicted Outcomes:

In socially isolated-reared mice, the infusion of PTZ at 5.5 mg.kg-1.min-1 will result in a progression of seizures. We expect the social isolation stress to induce a significant decrease in seizure threshold in the experimental group (social isolation reared group), compared to the group reared mice. A lower seizure threshold in the isolation-reared group, compared to the group-housed group, would allow us to make conclusions about the role of GABA in this system. Specifically, we expect to conclude GABA antagonization will induce a lower seizure threshold in socially isolated mice. We can then conclude that GABA is necessary for the seizure threshold.

Study 2 Alternative Outcomes:

The predicted outcomes assume GABA antagonization will decrease seizures in socially isolated mice relative to non-socially isolated mice. However, we may see similar results in both the socially isolated and group reared mice. If the mice exhibit similar (non-significant) seizure susceptibility through the behavioral range of 0-6 in both the non-socially isolated system and socially isolated system, we can hypothesize another mechanism is involved in seizure susceptibility solely in the GABAergic system.

Study 3 Predicted Outcomes:

Isolation-reared mice not treated with dipropofol will have the shortest onset time of seizures and with higher intensity on the behavioral scale of 0-6. It is expected that isolated mice without the GABA agonist will have the lowest seizure threshold and highest seizure susceptibility. Group-housed mice treated with dipropofol will have the longest onset time of seizures with lower intensity, indicating that nonisolated mice with the GABA agonist will have the highest seizure threshold and lowest seizure susceptibility. If isolation-reared mice and dipropofol-treated mice are less susceptible to seizures, we can conclude that agonizing GABA increases seizure threshold.

Study 3 Alternative Outcomes:

The predicted outcomes assume that GABA agonization increases seizure threshold in socially isolated mice. It is possible that the outcomes for our experimental groups will be opposite of what we expect, or that all groups will have the same seizure threshold. This would lead us to conclude that GABA agonization is not sufficient to increase the seizure threshold. Alternatively, it could lead us to ask questions about receptor expression rather than simply GABA release or agonization. For example, an unchanged seizure threshold can be attributed to the decreased expression of the $GABA_A$ receptor caused by social isolation, which means that even the GABA

agonziation of the limited amounts of receptors will not increase the seizure threshold significantly. Further research would be required to understand the regulation and expression of GABA in response to SIS. Regardless of the outcome, the insights gained from this test would still be valuable in understanding how social isolation affects the GABAergic system.

References

Akyuz, E., Polat, A. K., Eroglu, E., Kullu, I., Angelopoulou, E., & Paudel, Y. N. (2020). Revisiting the role of neurotransmitters in epilepsy: An updated review. Life Sciences, 118826.

Amiri, S., Shirzadian, A., Haj-Mirzaian, A., Imran-Khan, M., Balaei, M. R., Kordjazy, N., ... & Mehr, S. E. (2014). Involvement of the nitrergic system in the proconvulsant effect of social isolation stress in male mice. Epilepsy & Behavior, 41, 158-163.

Barker-Haliski, M., & Steve White, H. (2015). Glutamatergic mechanisms associated with seizures and epilepsy. Cold Spring Harb. Perspect. Med. p. a022863.

Bozzi, Y., & Borrelli, E. (2013). The role of dopamine signaling in epileptogenesis. Frontiers in cellular neuroscience, 7, 157.

Çavuş, I., Romanyshyn, J. C., Kennard, J. T., Farooque, P., Williamson, A., Eid, T., ... & Spencer, D. D. (2016). Elevated basal glutamate and unchanged glutamine and GABA in refractory epilepsy: Microdialysis study of 79 patients at the yale epilepsy surgery program. Annals of neurology, 80(1), 35-45.

Chadda, R., & Devaud, L. L. (2004). Sex differences in effects of mild chronic stress on seizure risk and GABAA receptors in rats. Pharmacology Biochemistry and Behavior, 78(3), 495-504.

Chen, H. J. C., Spiers, J. G., Sernia, C., & Lavidis, N. A. (2015). Response of the nitrergic system to activation of the neuroendocrine stress axis. Frontiers in neuroscience, 9, 3.

Childhood epilepsy: The brain. (n.d.). Retrieved March 17, 2021, from https://www.massgeneral.org/children/epilepsy/education/the-brain

Chowdhury, F. A., O'Gorman, R. L., Nashef, L., Elwes, R. D., Edden, R. A., Murdoch, J. B., ... & Richardson, M. P. (2015). Investigation of glutamine and GABA levels in patients with idiopathic generalized epilepsy using MEGAPRESS. Journal of Magnetic Resonance Imaging, 41(3), 694-699.

DeWit, D. J., MacDonald, K., & Offord, D. R. (1999). Childhood stress and symptoms of drug dependence in adolescence and early adulthood. American Journal of Orthopsychiatry, 69(1), 61-72.

Ferdman, N., Murmu, R. P., Bock, J., Braun, K., & Leshem, M. (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. Behavioural brain research, 180(2), 174-182.

Friedler, B., Crapser, J., & McCullough, L. (2015). One is the deadliest number: the detrimental effects of social isolation on cerebrovascular diseases and cognition. Acta neuropathologica, 129(4), 493-509.

Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & de Lima Umeoka, E. H. (2018). A comprehensive overview on stress neurobiology: basic concepts and clinical implications. Frontiers in behavioral neuroscience, 12, 127.

Herbison, A. E., & Moenter, S. M. (2011). Depolarising and hyperpolarising actions of GABAA receptor activation on GnRH neurons: towards an emerging consensus. Journal of neuroendocrinology, 23(7), 557.

Horton, R. W., Prestwich, S. A., & Meldrum, B. S. (1982). γ‐Aminobutyric acid and benzodiazepine binding sites in audiogenic seizure‐susceptible mice. Journal of neurochemistry, 39(3), 864-870.

Jaremka, L. M., Andridge, R. R., Fagundes, C. P., Alfano, C. M., Povoski, S. P., Lipari, A. M., ... & Kiecolt-Glaser, J. K. (2014). Pain, depression, and fatigue: loneliness as a longitudinal risk factor. Health Psychology, 33(9), 948.

Jefferys, J. G. (2010). Advances in understanding basic mechanisms of epilepsy and seizures. Seizure, 19(10), 638-646.

Johnson, E. W., De Lanerolle, N. C., Kim, J. H., Sundaresan, S., Spencer, D. D., Mattson, R. H., ... & Innis, R. B. (1992). “Central” and “peripheral” benzodiazepine receptors: opposite changes in human epileptogenic tissue. Neurology, 42(4), 811-811.

Juárez, J., & Vázquez‐Cortés, C. (2003). Alcohol intake in social housing and in isolation before puberty and its effects on voluntary alcohol consumption in adulthood. Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology, 43(3), 200-207.

Kang, J. Q., & Barnes, G. (2013). A common susceptibility factor of both autism and epilepsy: functional deficiency of GABA A receptors. Journal of autism and developmental disorders, 43(1), 68-79.

Lapiz, M. D. S., Fulford, A., Muchimapura, S., Mason, R., Parker, T., & Marsden, C. A. (2003). Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neuroscience and behavioral physiology, 33(1), 13-29.

Li, Yi-Fan, et al. "Interaction between glutamate and GABA systems in the integration of sympathetic outflow by the paraventricular nucleus of the hypothalamus." American Journal of Physiology-Heart and Circulatory Physiology 291.6 (2006): H2847-H2856.

Lukkes, Jodi L., et al. "Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses." Hormones and behavior 55.1 (2009): 248-256.

Maguire, J., & Salpekar, J. A. (2013). Stress, seizures, and hypothalamic–pituitary–adrenal axis targets for the treatment of epilepsy. Epilepsy & Behavior, 26(3), 352-362.

Miles, R., Blaesse, P., Huberfeld, G., Wittner, L., & Kaila, K. (2012). Chloride homeostasis and GABA signaling in temporal lobe epilepsy.

McCutcheon, J. E., & Marinelli, M. (2009). Age matters. European Journal of Neuroscience, 29(5), 997-1014.

McDonald, J. W., Garofalo, E. A., Hood, T., Sackellares, J. C., Gilman, S., McKeever, P. E., ... & Johnston, M. V. (1991). Altered excitatory and inhibitory amino acid receptor binding in hippocampus of patients with temporal lobe epilepsy. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 29(5), 529-541.

Mirski, M. A., Rossell, L. A., McPherson, R. W., & Traystman, R. J. (1994, December). Dexmedetomidine decreases seizure threshold in a rat model of experimental generalized epilepsy. In The Journal of the American Society of Anesthesiologists (Vol. 81, No. 6, pp. 1422-1428). The American Society of Anesthesiologists.

Mumtaz, F., Khan, M. I., Zubair, M., & Dehpour, A. R. (2018). Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. Biomedicine & Pharmacotherapy, 105, 1205-1222.

Matsumoto, K., Puia, G., Dong, E., & Pinna, G. (2007). GABAA receptor neurotransmission dysfunction in a mouse model of social isolation-induced stress: possible insights into a non-serotonergic mechanism of action of SSRIs in mood and anxiety disorders. Stress, 10(1), 3-12.

Matsumoto, K., Nomura, H., Murakami, Y., Taki, K., Takahata, H., & Watanabe, H. (2003). Long-term social isolation enhances picrotoxin seizure susceptibility in mice: up-regulatory role of endogenous brain allopregnanolone in GABAergic systems. Pharmacology Biochemistry and Behavior, 75(4), 831-835.

Nestler, E. J., & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. Nature neuroscience, 13(10), 1161.

Olsen, R. W. (1986). Midbrain GABA receptor deficit in genetic animal models of epilepsy. Neurotransmitters, seizures and epilepsy III, 279-291.

Orben, A., Tomova, L., & Blakemore, S. J. (2020). The effects of social deprivation on adolescent development and mental health. The Lancet Child & Adolescent Health.

Pinna, G., Costa, E., & Guidotti, A. (2004). Fluoxetine and norfluoxetine stereospecifically facilitate pentobarbital sedation by increasing neurosteroids. Proceedings of the National Academy of Sciences, 101(16), 6222-6225.

Richerson, G. B., & Buchanan, G. F. (2011). The serotonin axis: shared mechanisms in seizures, depression, and SUDEP. Epilepsia, 52, 28-38.

Scharfman, Helen E. (2007). The Neurobiology of Epilepsy. Current Neurology and Neuroscience Reports, U.S. National Library of Medicine, www.ncbi.nlm.nih.gov/pmc/articles/PMC2492886/.

Shippenberg, T. S., & Thompson, A. C. (1997). Overview of microdialysis. Current protocols in neuroscience, (1), 7-1.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. Neuroscience & biobehavioral reviews, 24(4), 417-463.

Spear, L. P. (2004). Adolescent brain development and animal models. Annals of the New York Academy of Sciences, 1021(1), 23-26.

Staley, K. (2015). Molecular mechanisms of epilepsy. Nature neuroscience, 18(3), 367-372.

Treiman, D. M. (2001). GABAergic mechanisms in epilepsy. Epilepsia, 42, 8-12.

Weiss, I. C., Pryce, C. R., Jongen-Rêlo, A. L., Nanz-Bahr, N. I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behavioural brain research, 152(2), 279-295.

Vlachos, I. I., Papageorgiou, C., & Margariti, M. (2020). Neurobiological Trajectories Involving Social Isolation in PTSD: A Systematic Review. Brain sciences, 10(3), 173.

Zhang, J., Chen, X., Kårbø, M., Zhao, Y., An, L., Wang, R., ... & Huang, Z. (2018). Anticonvulsant effect of dipropofol by enhancing native GABA currents in cortical neurons in mice. Journal of neurophysiology, 120(3), 1404-1414.