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A HOLISTIC APPROACH TO POST-TRAUMATIC STRESS DISORDER: ALPHA-2 ADRENERGIC RECEPTOR-SINOPHILIN COFILIN AXIS BIOLOGIC ANTIBODY TREATMENT PROPOSAL

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A HOLISTIC APPROACH TO POST-TRAUMATIC STRESS DISORDER: ALPHA-2 ADRENERGIC RECEPTOR-SINOPHILIN COFILIN AXIS BIOLOGIC ANTIBODY TREATMENT PROPOSAL

by

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DEDICATION

I want to dedicate this paper to all of the patients of which I have been a part of their medical journey, and to all that I have yet to meet.

ACKNOWLEDGEMENTS

I would like to thank my parents for their constant support of all of the dreams I have and my grandma for being one of my first patients and my inspiration.

I would like to thank all of my Southeastern Professors for their hand in my education and providing me with more support than I could have wished for.

I will never be able to express how much their encouragement, knowledge, and wisdom has shaped my life.

I would like to thank the School of Honors for being like another family for me.

I have developed relationships that will last a lifetime, and habits that have strengthened both my academic and spiritual maturity.

I would lastly like to thank Dr. Schraw for his persistent encouragement and being able to so encouragingly hold me up throughout this process,

Thank you.

ABBREVIATIONS LIST

Post-Traumatic Stress Disorder	PTSD
Tricyclic Antidepressant	TCA
Monoamine Oxidase Inhibitor	MAOI
Post-Traumatic Event	PTE
Blood Brain Barrier	BBB
Selective Serotonin Reuptake Inhibitors	SSRI
Adrenergic Receptor	AR
Adrenocorticotropic Hormone	ACTH
Corticotropin Releasing Hormones	CRH
Hypothalamic Pituitary Axis	HPA
Brain Derived Neurotrophic Factor	BDNF
Long-Term Potentiation	LTP
People for the Ethical Treatment of Animals	PETA
Contextual Fear Conditioning	CFC
Electric Foot Shock	EFS
Elevated Pulse Maze	EPM
Cognitive Behavioral Therapy	CBT

Abstract

PTSD affects about 5% of adults in the United States every year.[1] This thesis investigates the common biologic therapies for PTSD and the specificity factor of the catecholamine binding to related norepinephrine alpha-2A adrenergic receptors. The binding of these catecholamines to adrenaline receptors in the spinophilin cofilin axis of the dorsal hippocampus causes fear-memory modulation and storage by altering dendritic morphology and thus manipulating neuronal plasticity. Targeting the inhibition of these respective receptors and increasing the activity of cofilin, a protein responsible for breaking down actin utilized for dendrite reaction to stimulus appears promising. By preventing the binding of catecholamines in this axis which would depress cofilin, dendritic changes of elongation would be small rather than dramatized and prolonged as seen in PTSD, eliminating a change in plasticity and future responsiveness to recurring stimuli. The use of a polyclonal antibody rather than an SSRI or beta blocker – which only treats symptoms without changing the modulation of fear itself– was proposed due to the more bodily 'holistic' approach to PTSD rather than using an artificially synthesized pharmaceutical. Coupling the proposed antibody treatment with cognitive behavioral therapy can aid in making long term PTSD symptoms a thing of the past.

KEY WORDS: PTSD, Biologics, Adrenergic Receptors, Polyclonal Antibody

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CHAPTER 1 INTRODUCTION

Post-traumatic stress disorder (PTSD) is a mental illness that impacts individuals that have been faced with past intense trauma. As a result of this condition, patients tend to relive or be easily triggered by stimuli related to or neurologically associated with said traumatic event.[1] However, just because an individual goes through a traumatic event does not directly mean that they will develop PTSD, about 1 in 3 people who are exposed to a severe traumatic stimuli will be faced with the residual symptoms and may be diagnosed with PTSD.[1] PTSD has been studied to be a survival response to near death and/or stressful situations, while beneficial in adapting to external changes, the long-term wear on both the physical and mental state of an individual can be greatly depressed due to continual unnecessary induced stress.[1,2] Feelings associated with PTSD can include an array of associated diagnoses and symptoms such as flashbacks, anxiety, dissociation, self-isolating, paranoia, etc. all of which depending on the severity of the trauma experienced can cause longer-term mental and physical health effects.[2] In general though, for an individual to be diagnosed with PTSD after a traumatic event, symptoms usually have to have been occurring for at least a month for an accurate diagnosis.[2,3]

The Problem and Significance

Working in healthcare I have seen mental health struggles in patients from all walks of life. I have heard their stories, each unique and equally devastating. Whether it be a story of PTSD from a previously fought war, or family abuse, hurt and trauma is a common occurrence. No matter the age, background or socioeconomic standing, everyone experiences trauma, sometimes so life threateningly stressful it ends up doing just that. By better understanding the way the PTSD affects an individual, the goal would

be to stop the pain and provide a way that even the most selfless and innocent of our world could find relief after traumatic effects of the trauma they have experienced.

As of 2022, it has been observed that about fifteen percent of military personnel over the last 20 years have reported having been diagnosed with PTSD as a result of wartime and general deployment traumas, this statistic includes any sexual harassment trauma that may have been experienced while serving.[4] When left untreated PTSD can cause an array of issues including further depression and anxiety as well as self-isolation and suicidal thoughts.[5,6] One study by the veterans affairs PTSD research and information center calculated that about 20 percent of veterans with PTSD left untreated turn to substance and or alcohol abuse to self-medicate, and that of the 117 suicides that occur every day in the United States 18 percent of them are veterans, an occurrence thought to be due to traumatic combat exposures.[5] As of 2019, 20,000 members of the military were faced with instances of sexual harassment, keeping this in mind, it is understandable that some trauma could be birthed from these situations, especially among females in the Air Force, where these numbers have increased in the last year by 9%.[4-6] With the mental well-being of veterans being scarred by the events of wartimes, a more holistic approach to the health of the men and women who have sacrificed so much needs to be taken into consideration.

A holistic approach would approach the patient’s whole body, all of its systems as well as the environmental factors of their conditions.[4] A holistic approach would include in this case the examination of one's social and psychological extensions of health.[4] This entails not pushing pills to solve problems from past traumas but taking into account the effect such stressors already have had on the physical, social, and psychological workings of the person and finding the

healthiest option for both the patient's mind and body, treating both the physical and mental traumas.[4,7]

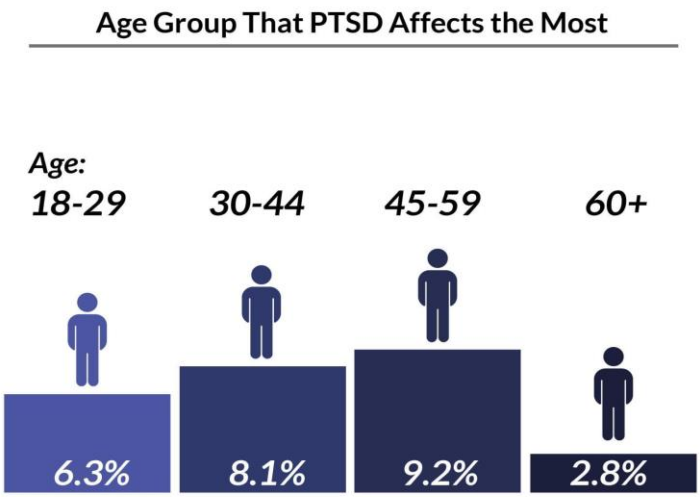


Figure 1: Types of traumas by percent of PTSD cases observed in the United States[8]

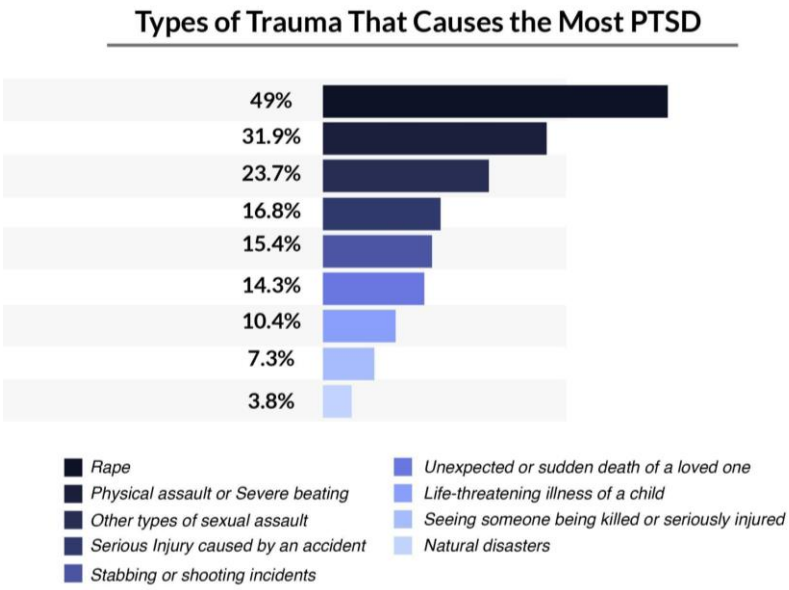


Figure 2: Percent by age of Americans affected by PTSD[8]

As a chemical response to trauma, individuals with PTSD commonly have an increase in their stress hormones i.e. catecholamines such as epinephrine, norepinephrine, and dopamine, all

of which are components of the adrenaline-mediated response to stress.[9] A study from 2018 analyzed the concentration of these catecholamines and found that the concentration of epinephrine and dopamine were not affected on patients observed to have PTSD, while the concentration of nor-epinephrine was significantly increased in the same individuals when compared to control patients that did not suffer from PTSD.[9] At this point in time there are not many pharmaceutical options available to treat PTSD and its related symptoms specifically and directly, and those that are need to be pre-administered prior to or just after the time of a traumatic experience.[10,11] Though ideally a treatment would be delivered as soon as a traumatic event causes the symptoms associated with PTSD long enough to be deemed PTSD that is not always the case. For numerous reasons an individual that needs help will not seek it whether it be for social, economic, or generational reasons.[1]

Current treatment includes the use of beta-adrenergic receptor blockers, commonly known as beta blockers of which are most notably used for the regulation of heart rate and blood pressure.[12] Beta blockers function by blocking the ability of catecholamines from binding to beta-adrenergic receptors, preventing memory consolidation altogether.[12] Though effective immediately after experiencing trauma, the longer the time between interaction to traumatizing stimuli and treatment delivery can decrease the effectiveness of beta blockers for PTSD memory modulation.[12,13] Other prescribed treatments include multiple other anxiolytics known as tricyclic antidepressants (TCA) and or along with monoamine oxidase inhibitors (MAOI) that may ease the anxious feelings associated with PTSD.[10,13–15] Prazosin, Propranolol, and Doxosin in particular are now more commonly being used along with the more popular Zolaf, Paxil, Prozac and Effexor.[13,16] These common SSRIs work in the regulation of the neurotransmitter serotonin, and by causing an inhibition of its respective uptake, in return the activity of the serotonin that is

being properly taken up by various channels is of greater significance.[6,17] These SSRI options however do not address the catecholamines norepinephrine or epinephrine which usually cause the more severe responses to a traumatic stimulus.[17] The use of MAOIs and TCAs though do target the catecholamines of interests proving to be associated with negatively correlated side effects such as arrhythmias, digestive and other endocrine related health complications.[6,16,17] Even along with their inefficient maintenance of the symptoms of PTSD, studies have indicated that when prescribed for combat related PTSD in veterans only 20 percent of individuals receiving SSRIs actually see a relief in symptoms.[6,17] Further exemplifying the need for a change in treatment, that of which is not only specifically tailored for the mind of the individual but a treatment of which aids in the rehabilitation of the individual as a whole.[6]

Research Question

With this study I hope to be able to propose a biologic approach, that is, a polyclonal antibody treatment that targets the alpha two nor-adrenergic receptors (AR) –one of the specific receptors that are found within the adrenaline receptor complexes that targets and alters neurotransmitter release in response to a stressful triggering stimuli– specifically the ARs that are part of the limbic system spinophilin cofilin axis, directed towards the hippocampal memory modulation region of the brain– pairing this proposed treatment with cognitive behavioral therapies for a more holistic treatment option of which rather than constantly taking medication, a patient could have as once a month injection and weekly therapy.[18] With this proposal I hope to also decrease the need for pharmaceutical interventions and thus, decrease the need of adding a chemical to the body, rather helping to manage what is already in the body and reshape the way the body reacts to past trauma by changing the intensity of the modulation of fear.

CHAPTER 2 PTSD AND NORADRENALINE RESPONSES

Biochemical Effects of PTSD

To best understand PTSD, the neurobiological groundwork of which fear and stress act on must first be addressed.[19] The major regions of the brain that are associated with the stress rendered response is the amygdala, hippocampus, prefrontal cortex and the nucleus accumbens of which mediate the various neurochemical releases and uptakes needed for the body to properly and in normal scenarios, provide a healthy response to a multitude of stressors.[20]

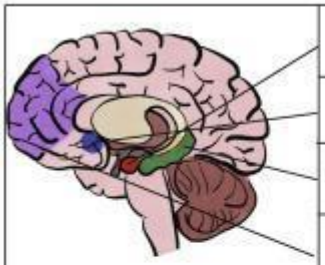
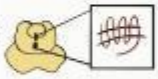
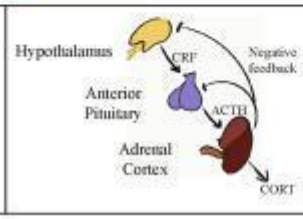
		PTSD	Depression
	Amygdala	Increase in size	Increase in size
	Nucleus Accumbens	Reduced activity in response to rewards	Reduced activity in response to rewards
	Hippocampus	Decrease in size	Decrease in size
	PFC	Dysfunctional activity Reduction in size	Dysfunctional activity Reduction in size
Raphe Nuclei 	$5\text{-HT}_{1A}R$	Enhanced $5\text{-HT}_{1A}R$ binding in Raphe Nuclei	Enhanced $5\text{-HT}_{1A}R$ binding in Raphe Nuclei
HPA axis 		CRF overactivity Hyper responsive to glucocorticoid negative feedback Low cort levels, or not different Disease associated SNPs present in FKBP5 gene	CRF overactivity Hyper active HPA axis Increased cort levels Disease associated SNPs present in FKBP5 gene
First line of treatment		SSRIs	SSRIs

Figure 3: Trademark Changes in the Brain in PTSD and Depression Comparatively.[21]

With the physical changes shown above, such structural changes are in turn destined to be linked to a shift in functional quality and efficiency of neurochemical processing.[19,20] Though not the focus of this study, the comparison to the changes in the brain during depressive episodes is also pictured to emphasize the difference between chronic PTSD and diagnosed depression as it so similarly alters one's physical brain as well as the related interactions within associated

pathways.[21,22] Most notable is the change in the reactivity and size of the amygdala and hippocampus.[23] The increase in volume and activity of the amygdala is linked to conditioned fear and the large flux of nor-epinephrine and cortisol release at a new, still chemically stressed 'resting' state.[23] The hippocampus, effected by a decrease in volume with depressed activity in early traumatic experiences, exemplifies the incomparable plasticity of hippocampal input.[23,24]

As pictured in figure 4 below the sympathetic nervous system displays neuroendocrine changes, such as neurotransmitter and associated chemical changes in response to the overactivity.[20] By activation of the pituitary glands and increasing acetylcholine concentrations within neurons, the adrenal glands are stimulated to decrease the cortisol concentrations and in turn associated macrophage and T cells increase their inflammation sympathetic response.[20] This increased response activates cytokines which alert the prefrontal cortex, hippocampus, and insula, more specifically the basolateral amygdala is much involved in this cycle as the emotional response mediator.[20] The inflammation of these regions of the brain cause the emotional responses to a stimulus which triggers this cycle to occur.[20] The various cytokines include various classes of immune biomarkers, specifically: interleukins, tumor necrosis factors, and C reactive protein –these factors are also associated with sleep loss as it is directly proportional to increased inflammation.[20] When the term inflammation is used in these scenarios it is describing the neural increase in the immune responsive biomolecules that activate during an episode of immunological depression and in turn react with a period of over reactivity, i.e. inflammation.[20] The proposed method will focus on the adrenal gland's role in this inflammation process, where the adrenal glands produce decreased levels of cortisol of which is directly linked to the activity and use of available nor-epinephrine.[20,25] The decrease in the amount of cortisol produced in turn leads to the inability to regulate all of the carbohydrate i.e. steroid production

that occurs during traumatic stimuli training.[20,25] The Glucocorticoids that are typically observed as to effect memory modulation is also linked to the norepinephrine concentrations within the basolateral amygdala, with many studies linking the role of norepinephrine as the primary memory modulation hormone.[20,25] The changes in the sympathetic neural plasticity changes the hormone concentrations and in turn also affect the adrenergic receptors (AR) response to such hormone increase, where an increase in norepinephrine directly increases the activity of AR.[24]

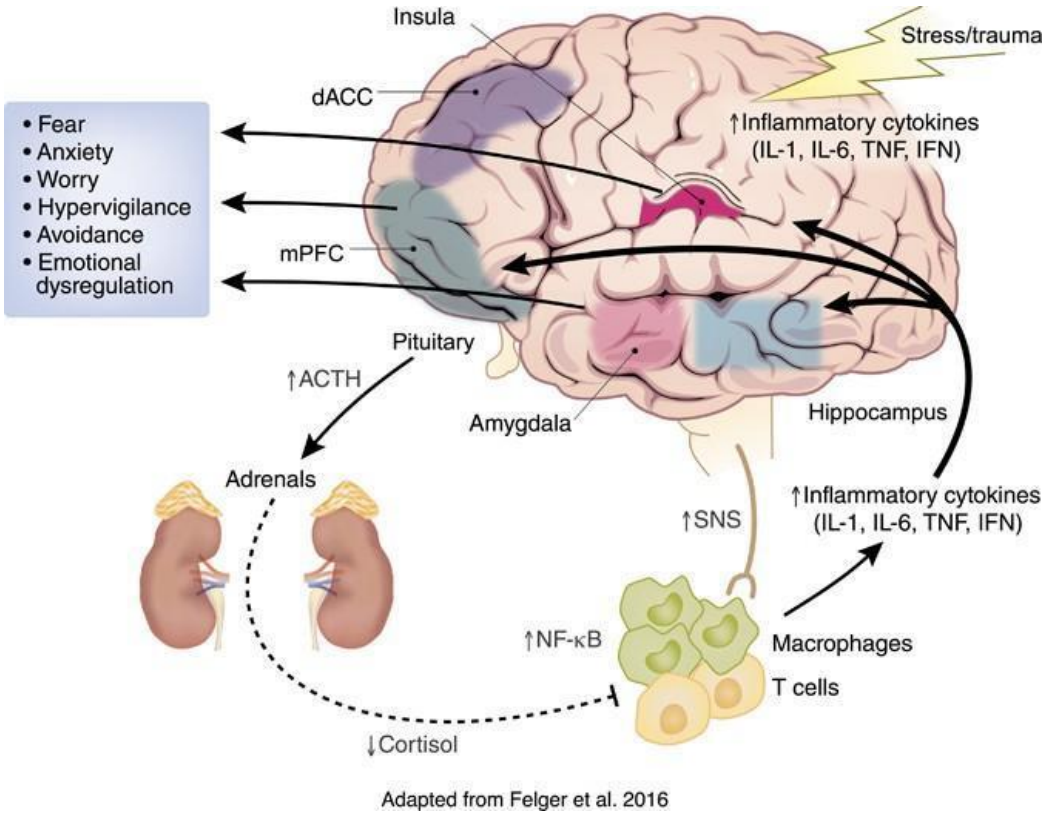


Figure 4: Adrenal and Neurochemical Changes and Stress Inflammation Response to PTSD[20]

As with the size and regional alterations that are associated with stress inputs, various glands and immunological cells also have a vicious response to effect these changes in PTSD neurochemical concentrations.[20] As previously mentioned, and increase in acetylcholine (ACTH) is associated with the adrenal associated reduction of cortisol, this results in the increase in norepinephrine of which accounts for the stress induced response by the cerebral cortex and

other neural regions.[22] Similarly shown below in figure 5, the specific up and down regulation of various hormones and biomolecules that interact within the brain also exemplify chain reactions of the slightest concentration changes.[19,21]

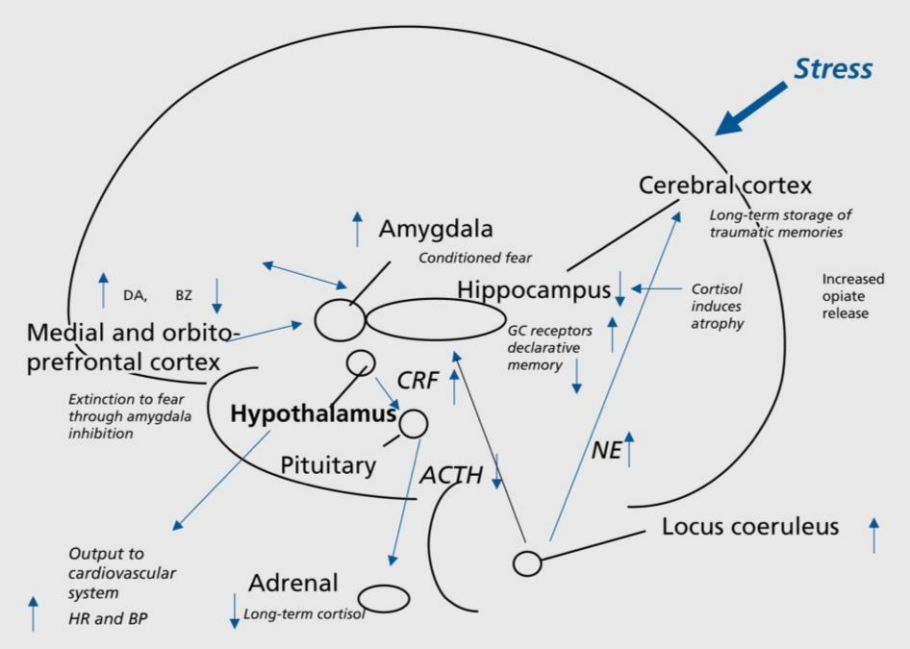


Figure 5: PTSD Specific Changes Due to Cortisol and Norepinephrine Changes [22]

As discussed, one of the biggest changes occurring in PTSD neurobiological workings is associated with a constant increased concentration of norepinephrine and cortisol.[23] As the primary chemical response to stress, the interactions of the various neuronal binding factors are what cause the central nervous system to react to stressful stimuli, which is then translated into peripheral –visible– stress responses.[22]

Adrenergic Receptor Complexes

It is known that PTSD works by the manipulation and alteration of various of the naturally occurring chemicals within the body. The most common to be affected by this phenomena is the adrenergic receptor complexes found on numerous organs that are capable of

receiving hormonal input from the adrenal glands which secrete epinephrine and nor-epinephrine associated with many processes for stress control, including those associated with PTSD.[26] With such an increase in norepinephrine in particular, negative associations in response to such large influxes of norepinephrine has been found to be associated with the formation of memories that trigger similar such a large amount of norepinephrine production.[26] The fear conditioning associated with PTSD is a result of increase norepinephrine release to the amygdala and hippocampal regions specifically.[26] By being able to alter stimuli processing of the specific regions of the brain containing alpha-2 adrenergic receptors responsible for this fear modulation and triggering an expected decrease in the storage or reliving of fearful memories should occur.[26,27] The idea of essentially distracting these receptors has always proved to be an extremely specific and daunting task, because of this most studies have been focused on the role of amygdalar co-factors and how they react with the adrenergic receptors, not as often norepinephrine which is not specific to the amygdalar or hippocampal regions.[26]

The other receptor complex components include the alpha 1 and 2 as well as beta 1,2 and 3 receptors.[28] Each of the subtypes of these receptors correlate to specific responses in the body of which results from the binding of the associated adrenal catecholamines.[28] Figure 6 below quickly overviews the various reactions in accordance to the specific bindings of norepinephrine.[28] The alpha receptor groups are G coupled receptors that focus more on the vascular and neurochemical processing of stimuli while the beta receptors mainly associate with the cardiological and gastrointestinal response to receptor activation.[19] Beta 1 and 2 receptors have been closely studied as a potential target for the treatment of cardiomyopathy.[12] Specifically the development of a polyclonal antibody to target the contractive nature of the various nodes of the lower cardiac space, altering the binding of epinephrine and norepinephrine to the hearts

associated smooth muscle surface receptors.[12] The classification of the adrenergic receptors is separated by inhibitory or excitatory.[27] With each of the sub classes, being separated by alpha or beta, which indicate a specific location of the receptor.[27] Commonly used as an inhibitor of the alpha AR, prazosin is a common pharmacologic, prescribed to decrease anxiety like vascular and inflammatory symptoms.[27] These alpha-2 inhibitors are primarily centered near the Locus Coeruleus –specifically, there are sub categories within the alpha, AR receptors as well: 2A,2B,and 2C, of which are chemically inhibited by endogenous biological components. In this study the chemical for inhibition is norepinephrine.[27]

When studying the fear memories that are associated with PTSD and the leading traumatic event, the utilization of pharmacologic agents to solve these issues is not as understood as scientists and physicians may make them out to be in terms of the actual path the drug takes to provide the relieving effects that most prescribed drugs provide.[29] The gray space that is present within these common pharmaceuticals is part of the drive to develop a more understood, and to an extent, natural way to ease the developed symptoms, and in turn decrease the noticeable, somatic effects that a trained traumatic experience has on individuals. One study in particular looked at an alpha-2 adrenergic receptor at the spinophilin cofilin axis located in the hippocampus, a region of which monitors processing of memories and the context in which a memory was first formed.[29] As shown in Figure 6 below, the various other types of alpha and beta receptors can be visualized with their effects listed.[29] For further investigation, the focus on the alpha-2 complex will be discussed in terms of the decreased sympathetic flow pattern that is being targeted for the duration of this study to better understand the specific finding of endogenous molecules and said molecules effect on the neural plasticity of fear modulation components.[29]

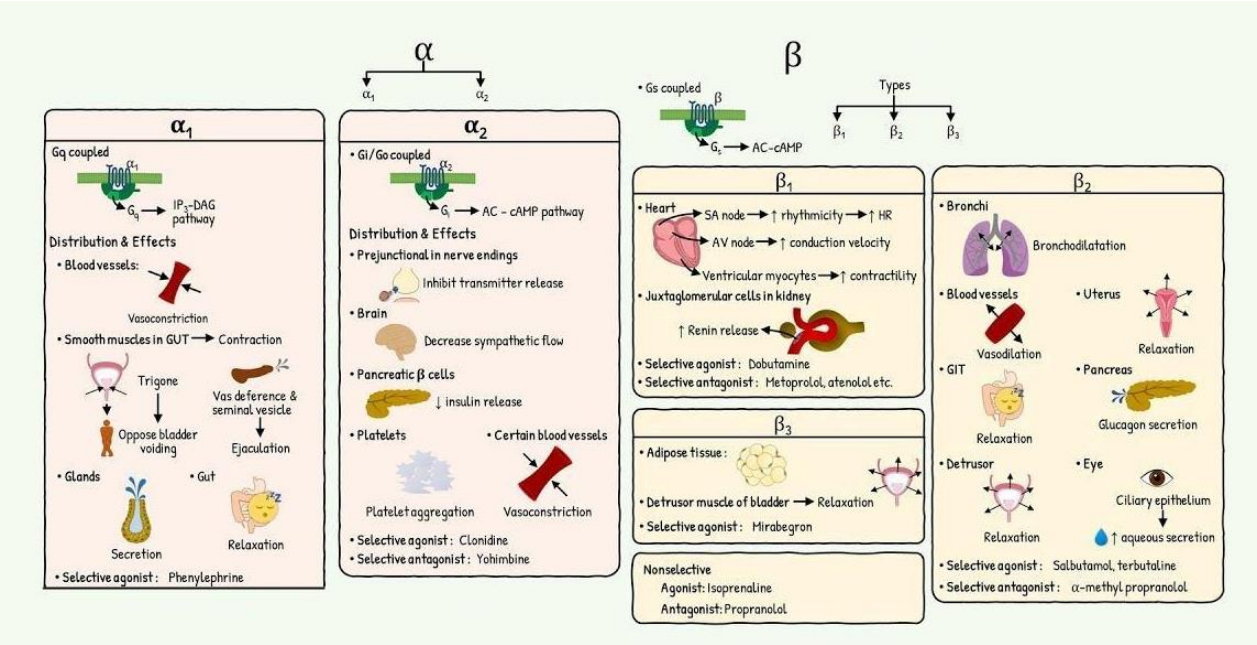


Figure 6: Adrenergic receptor types and their physiological role.[28]

Fear Modulation Components

Fear in general is a result of the increase in stress stimuli that activates the hypothalamus pituitary, adrenal axis (HPA) and the locus coeruleus along with the previously discussed noradrenergic receptors.[30] Though a natural and healthy process, the over consolidation of such traumatic exposure can cause an individual an inability to remove such event from their subconscious.[30] The HPA access is what controls hormone release, as previously mentioned, cortisol is able to aid in regulation of the AR complexes.[30] Corticotropin releasing hormones (CRH) along with its respective receptors also play a large role, as well as glucocorticoid receptors of which are genetic regulators of the fear consolidation process in individuals.[30] The hyper-suppression of cortisol of which associates with noradrenergic dysregulation is responsible for the overabundance of norepinephrine that alters the formation of memories when left too long, without regulation via cortisol.[30]

Besides making these chemical changes that alter and rewire the brains response to external stimulation, genetic alterations and regulation by FKPB5 has also been studied as it relates to childhood sexual abuse victims.[30] These individuals prematurely developed a chaotic response to various hormone changes as a result of childhood trauma. In these cases the hypothalamus, hippocampus and locus coeruleus have to compensate hormonal uptake irregularities, later causing developmental setbacks and or realization of previous adolescent trauma that may not have been able to be appropriately processed.[30] Being an endocannabinoid, norepinephrine is directly related to an increase in emotional responsiveness.[30] By targeting the mechanism by which this norepinephrine is up taken, the amount of norepinephrine present in the specific brain regions can hopefully be reduced to prevent further memory consolidation and plasticity adaptations during triggering events, and aid in the slow reduction of the response to the traumatic memory initially formed.[30]

An Alpha-2C adrenergic receptor variation can be associated with norepinephrine feedback complications post-traumatic event (PTE).[30] Colocalized with the increased norepinephrine released that is unable to be processed by the AR receptors, neuropeptide Y is found in decreased concentrations in individuals with PTSD, inversely proportional to the norepinephrine increase from the release of their respective neurons.[30] These abnormal changes in any of the alpha-2C or beta-1 or 2 receptors along with neuropeptide Y have been closely associated with PTSD.[30]

The gene GRP and STMN1 in humans have similarly neurologically been observed in the fear cycle associated with memory development.[30] The discussion of dopamine and serotonin within the cycle is also mentioned.[30] Consequently, the role of dopamine within PTSD systems is greatly understood where recently the plasticity of dopamine receptor to a region within the brain

is altered in war veterans with PTSD, where transmission complications associated with developed dopamine depression is positively correlated to chronic PTSD.[30] Similarly, serotonin levels in the amygdala also present a promoter region mutation of genomes, which have the likelihood of developing into multiple cases of PTSD.[30] Not as well understood, the inhibition of serotonin interaction with the amygdala can cause many similar psychiatric issues, having a large impact on the actual genotype of individuals.[30] The largest, most physically notable effect that fear and PTSD can have on the brain is the reduction of the volume of the hippocampus.[30] Brain derived neurotrophic factors (BDNF) account for this volume.[30] Despite the correlation between decreased hippocampal volume in individuals with PTSD, the actual genetic workings of variance of BDNF variations, have not been genetically determined.[30]

Alpha-2 Spinophilin Cofilin Adrenergic Receptors

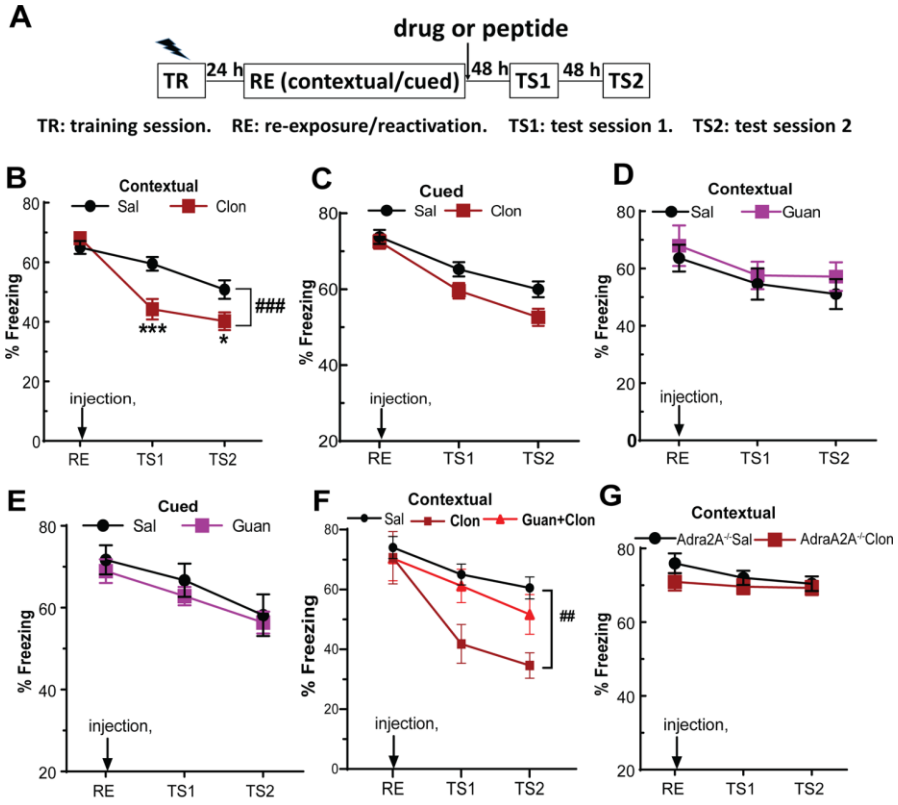


Figure 7: The effects of Alpha AR receptor agonists on freezing in response to traumatic stimulus.[29]

The alpha AR protein complex can be targeted by its respective agonist, two most notable pharmaceuticals being Clonidine and Guanfacine which both proved to have molecular influence on fear modulation, but the exact mechanism is still unknown.[29] Both of these target cofilin, a protein responsible for dendrite morphology.[29,31] Cofilin causes changes in the plasticity of dendrites via altering actin.[29] By changing the activity of cofilin, the dendrites are unable to elongate physiologically to partake in a long-term potentiation event, some of which could be recurring during a traumatic stimulative event.[29] As shown is figure 6 above, the use of an agonist of the alpha-2a AR receptors proved that this specific region of manipulation provided a good response to a stimuli, decreasing by around 25 percent the debilitating reactions to a stressing stimuli.[29] Commonly found in various psychiatric disorders, the plasticity of normal functioning neurons change, and the dendrites develop mutated morphologies and elongate, where they would usually contain shorter, flattened terminals on their communicating dendritic ends.[29] In the long-term potentiation seen in PTSD individuals, by being able to regulate and alter the activity of cofilin, the mediation of the downstream processes of the alpha-2A adrenergic receptors can be utilized as a prime area of where to target an antibody treatment with markers specific to the targeting of the spinophilin cofilin receptor site for inhibition.[29]

As shown in figure 8 below, cofilin is used as a lysing protein responsible for breaking down actin.[31] During long-term potentiation this protein is depressed, and sometimes uncontrolled actin growth is allowed where dendritic spines grow due to the inactivation of cofilin.[31] By bypassing the depression of the actin mediating protein cofilin, during stressful events of which trigger long-term potentiation (LTP) –or a related triggering stimuli can elicit

LTP– actin, will still be able to be broken down to prevent the dendritic mutations and as a result decrease stress reactions associated with fear modulation within the spinophilin cofilin axis in the hippocampus.[31]

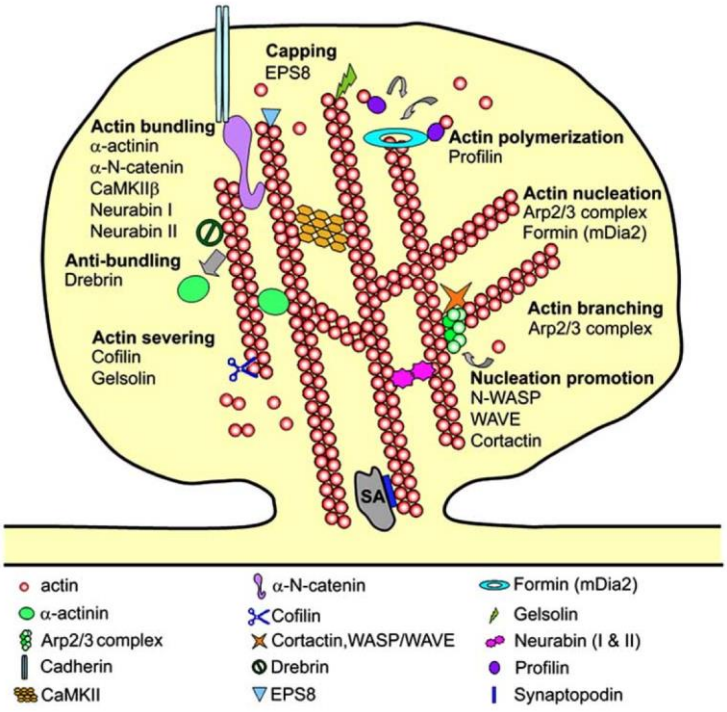


Figure 8: The role of cofilin in actin cutting and dendrite morphology[31]

CHAPTER 3 BIOLOGICS AND ANTIBODIES

Pharmaceuticals for PTSD and Their Mechanism

Some of the most common pharmaceuticals utilized for the treatment of PTSD can be seen below in figure 9. This table outlines both drug class and the mechanism by which it targets and alters neurochemical occurrences in individuals with PTSD.

Table 2

Treatment Options for PTSD

Drug	Place in Therapy	Dosing Range (mg)	Common Side Effects
SSRIs	1st line/(++) for avoidance and hyperarousal/(+) for reexperiencing	Fluoxetine: 20–80 Sertraline*: 50–200 Paroxetine*: 20–60 Citalopram: 20–40 Escitalopram: 10–20	Sexual dysfunction, sedation, appetite changes, headache, increased nervousness if dose started too high
TCAs	2nd, 3rd line/(+) for avoidance and hyperarousal	Imipramine: 25–250; Amitriptyline: 25–250	Sexual dysfunction, anticholinergic, sedation, cardiovascular
MAOIs	3rd line/(+) for avoidance and hyperarousal	Phenelzine: 30–90	Dietary restrictions, hypertension, sexual dysfunction
SNRIs	2nd line	Venlafaxine XR: 75–300	Gastrointestinal, sexual dysfunction, mild increase in heart rate/blood pressure
Nefazodone	2nd line/(+) for nightmares/sleep	200–600	Black box warning for hepatotoxicity
Atypical antipsychotics	2nd-line addition to antidepressant/ (++) for nightmares and reexperiencing	Risperidone: 0.25–2 Olanzapine: 2.5–10 Quetiapine: 25–100 Ziprasidone: 40–160 Aripiprazole: 2.5–20	Weight gain, metabolic syndrome, akathisia, dystonic reactions
Alpha-1 receptor antagonists	2nd-line addition to antidepressant/ (++) for nightmares/sleep/ (+) for overall symptoms	Prazosin: 1–10	Dizziness, orthostatic hypotension, drowsiness
Propranolol	Prevention of future symptoms if given within hours of event; not widely used	40 mg 3–4x/day x 7–10 days	Bradycardia, hypotension, dizziness
Anticonvulsants	2nd, 3rd line, best with comorbid bipolar disorder	Doses not well studied	Vary by drug

** FDA approved for PTSD. SSRIs: selective serotonin reuptake inhibitors; (++): evidence is positive; (+): good evidence but not as strong; TCAs: tricyclic antidepressants; MAOIs: monoamine oxidase inhibitors; SNRIs: serotonin/norepinephrine reuptake inhibitors.*

Figure 9: Current pharmaceuticals for the treatment of PTSD with dosages, references, and side effects.[32]

Unlike the anticipated use of biological agents for the treatment of PTSD by way of targeting the adrenergic receptor (AR) complex, the pharmaceuticals exemplified above are chemically, synthetically developed.[6,16] Surely enough, of the listed medication above, Sertraline and Paroxetine are the only FDA drugs approved for the treatment of PTSD, other medications help

only to ease the symptoms associated with this disorder but their primary purpose is not the treatment of PTSD as a whole, rather it outlying symptoms.[32,33] The actual synthesis of these treatments entails the complete synthesis from independent molecular structures to synthesize a new molecule tasked with either chemically enhancing or depressing a present homeostatic regulatory system.[33] The use of these pharmaceuticals potentially introduces sometimes foreign and or harmful i.e. new molecules to the body to induce a reaction and shift ones hormonal and in turn behavioral changes.[33,32]

Biological Agents

Modern medicine tends to lean more towards the use of pharmaceuticals which have the ability to become addictive and or change the natural chemical wiring of both your physical and cognitive functions.[34] Biologics are a large category of which derive any product that is made of naturally occurring biological components, such as proteins, amino acids, or any other biologically active, naturally occurring components within the body.[34] Biologics include monoclonal, and polyclonal antibodies as well as naturally occurring vaccines.[34] These products are hard to chemically characterize, and due to their biological variance, have differences between mass productions of the molecules of the developed biological product.[34] With the issue of mass production of biological agents, the idea of having slight inconsistencies of their development, is coined the term bio similar, which means there is no clinical difference in the actual composition of the product in terms of its purity or safety.[34] Biologic drugs have numerous advantages over the typical small molecule pharmaceuticals. Most pharmaceuticals prescribed although smaller in nature are considered less invasive when ingested orally but are consumer dependent; just because someone is prescribed a medication, for numerous reasons whether physically, mental or just concisely, they may not take it correctly, if at all.[35] With this

understood, biologics are more specific in that they are targeted to one specific region, and or complex process, and in many cases have less severe side effects in healthy individuals[35] Biologics, however, tends to be much larger molecules when compared to pharmaceuticals, that is single monoclonal antibody is about 100 times larger than a larger molecule pharmaceutical.[35] Though some biologics are more complex, and therefore unstable, they are able to utilize immunological circulation and regularly occurring processes of the immune system, and therefore do not require continuous administration. Removing the need for consumer dependency, increasing effectivity with increased treatment consistency, removing the variables that come along with self-administration of oral pharmaceuticals.[35] This idea is extremely important when dealing with mental health patients that may go through emotional cycles and at lows be hesitant to continue taking prescribed medications.[35] By utilizing biologics, the administration may be a more invasive injection but the consistency of the action of the treatment itself would be more effective overtime.[35] With all of this in mind the cost of a biologic antibody treatment can sum to a great expense. Unfortunately some of the most expensive but also promising treatment options for chronic conditions, biologics can cost between 30,000 to 50,000 dollars before insurance, which is surprising considering the production of these biologics is very similar to the cost to produce small molecule pharmaceuticals.[33,35]

Monoclonal and Polyclonal Antibodies

The biologics of interest for this proposal is the idea of the development of a therapeutic antibody for the treatment of post-traumatic stress disorder.[33] The first monoclonal antibody developed and approved in 1986 set the stage for what is now one of the largest worldwide utilized biologics.[33] Antibody technology is part of the manipulation of the B lymphocyte also known as B cells, in this processes B cells over-generate themselves when the infecting antigen,

and or species of interest interacts with their complex, causing them to multiply and spread throughout the body.[33] B cells are responsible for the release of antibodies targeting and in a sense, 'fighting' the initial, triggering antigens and or components.[33] Monoclonal and polyclonal antibodies are different in that monoclonal antibodies are developed using the same if not identical immune cells, were polyclonal antibodies use many different immune cells to target a specific antigen.[33] Polyclonal antibodies, by utilizing multiple different immune cells also known as immunoglobulin are therefore able to interact with multiple different types of the same antigen.[33] Different types of an antigen can arise due to different cell surface components on the targeted antigen where monoclonal antibodies are very specific, and are limited to only a single type of B cell of which can be utilized to produce correlating antibodies.[33] Polyclonal antibodies are cheaper to develop and have faster production time, also capable of multiplying via numerous hosts; monoclonal antibodies on the other hand are much more tedious to tend to in that they are much more sensitive to experimental conditions, such as reactivity, production time, and binding conditions.[33]

Antibodies that are directed against receptors specifically have had questionable selectivity but when tested in the past have proven to demonstrate acceptable selectivity for receptor targets, of interest in this study the anti-alpha 2A-AR.[36] The delivery mechanism of these antibodies must be extremely specific based on the cell tissue target for the desired increased specificity to be maintained.[36] The idea of having an antibody coordinate to a receptor implies that the mechanism of action is that mimicking the connectivity of the antibody to the corresponding hormone or molecule that triggers activity among the receptor of interest.[37] For the alpha-2A G coupled protein receptor complex that will be targeted in this study, the binding of an antibody to the receptors in the specific spinophilin cofilin axis will implement the

depression of the receptor.[37] By competitively binding to the receptors in this region, regularly stimulating hormones such as nor-epinephrine and dopamine will have no if very little effect on the chemical alteration of these specific receptors and therefore will decrease the memory consolidation of events of which can cause the increased dendritic mutations in response to abnormally high levels of such stimulating hormones, i.e. norepinephrine and dopamine.[37] In a sense the goal is to develop an antibody that works in the same mechanism as the pharmaceutical Clonidine, a medication used to treat hypertension and general stress disorders.[37] By utilizing the same pathway of binding to the AR receptor at the spinophilin cofilin axis, adding a factor that increases the specificity only for the activation of this specific region's receptors, the depression of the receptors would change the morphology and plasticity of dendritic spines.[37] If the designed antibody were allowed to react with other AR regions, then the main regulatory pathway to other AR receptors would be to modulate hypertension, showing the importance of the specificity of the antibody to target one area rather than all alpha-2A AR receptors, per region or axis their primary role differs and can cause adverse effects to the inhibition or depression of the protein complexes.[37]

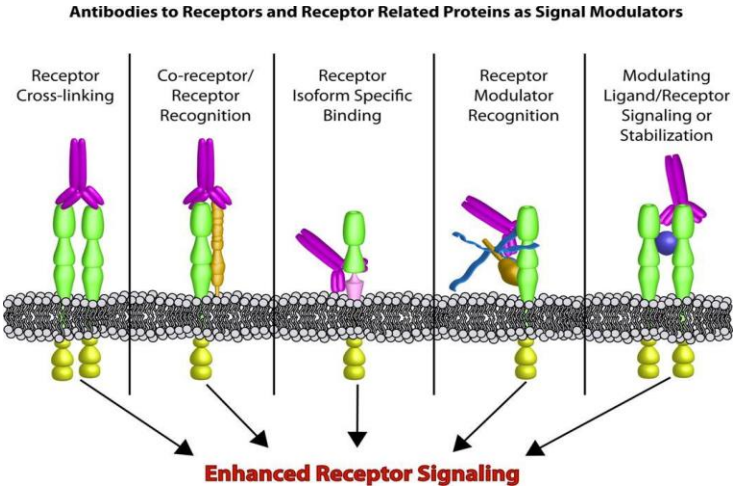


Figure 10: Receptor antibody methods of general signal modulation.[37]

A new study on the use of clonidine for the treatment of PTSD has proven to work by chemically binding to the alpha-2A receptors and reducing the amount of dendritic growth allowed to occur.[38] Cofilin acts as scissors for the actin within dendritic spines, inhibiting the growth of these spines. When functioning in PTSD, dendritic spines have already had their plasticity altered by an initial traumatic synaptic stimuli that results in the initial consolidation of a traumatic memory.[38] When hormones like norepinephrine bind to these receptors in the spinophilin cofilin axis, such established responses to a traumatic stimuli can be physically seen due to the established proximity of spines to a recurring, or triggering stimulus acting on the same synapse as the established reaction.[38] By providing an inhibitor of this hormonal interaction, though the removal of present dendritic mutations cannot be entirely erased, chemically mediated cofilin reactions and physical reactions to them can be depressed as to not be as debilitating as the usual behavioral symptoms related to PTSD.[38]

Why Biologics for PTSD: α2A-AR Antibody at the Spinophilin Cofilin Axis

The use of a polyclonal antibody for the treatment of PTSD would be targeted to inhibit the alpha-2A AR at the spinophilin cofilin axis where this antibody would then inhibit norepinephrine binding so cofilin is not inhibited in response to a large flux of nor-epinephrine into a synaptic connection.[38,39] This prevention would allow actin that usually would accumulate and modify dendritic spine structure to be cut by the active, uninhibited cofilin, preventing over modulation of traumatic memories when faced with a triggering stimulus.[38,39] Dendritic elongation would then be degraded and the plasticity of a signal no longer able to have as intense of an effect from a PTE stimuli.[38,39] Norepinephrine inhibits proteins associated with the activation of cofilin.[39] With this understood, cofilin inhibition due to the norepinephrine chain of events allows for dendritic growth of which increases plasticity and reactivity to developed

dendritic morphology, inhibiting the effects of such norepinephrine would cause the desired effect of dendritic stasis.[38,39]

As mentioned, the use of a chemically altering pharmaceutical rather than a component mediating biologic have many different long and short-term effects on an individual's overall health. The use of biologics prevents both the addition of unknown, man-made synthesized drugs to the body and also prevents issues associated with patient compliance. This idea is extremely important when dealing with mental health patients that may go through emotional cycles and at lows be hesitant to continue taking prescribed medications.[35] By utilizing biologics, the administration may be a more invasive injection but the consistency of the action of the treatment itself would be more effective overtime since the treatment would be administered less frequently and by the prescribing provider.[35]

CHAPTER 4: ANIMAL TESTING METHODS AND EXPERIMENTAL NOTES

Overview of Groups and Experimental

It is expected that non-modified rat species will be used for the macro-scale antibody testing. However, microscale the preparation of the polyclonal antibody will be conducted externally in a petri dish and administered into the rat's thigh and the rat monitored for 5 days before any data or observations are collected. The antibody will be prepared to include a specificity factor of which promotes the targeting of only the spinophilin cofilin AR receptors and consequently the intramuscular injection of it once allowed time to respond by the creation of B cells, will eventually be able to actively affect the axis of interest. When utilizing these animal species, it's understood that specific PETA regulations and breeding standards must be followed. For this reason, as a starting point only five rats, for each of the six proposed experimental group trials will be utilized.

For the modulation of PTSD in these rats, the electric foot shock experiment will be utilized to create a contextual fear response as part of this modulation, a corresponding bell ring, followed by the shocking of the rat will develop trained responses in the rat which mimic that of traumatic event stimulation in individuals with environmentally acquired PTSD. This procedure will work as follows: each rat will be placed in an electric shock chamber and at the beginning every hour for the first 12 hours of the day they will experience a bell ringing, accompanied by a shock to the foot. This training will occur for 2 days before injections and observations are recorded for the actual antibody effectiveness itself. Then, once the two days are up, the rats are left alone for five days, with no trauma being inflicted on them. Being left alone for five days resembles the action of waiting for treatment post a traumatic event, rather than being treated right after being exposed to a traumatic stimulus.

Of the experimental treatment groups, the first will be a group consisting of rats of which were trained by the electric foot shock and receive no treatment intervention; this will be the untreated, first control group. The second group will also be traumatized rats that are treated with the developed polyclonal antibody, this group would observe the effectiveness of the polyclonal antibody itself and monitor the physical and neurochemical effects of the rat during a re-implemented foot shock procedure. The third group would be rats of which went through the electric foot shock, received the polyclonal antibody injection, as well as undergo a fear extinction procedure, where they are trained that a once stressing stimuli is cognitively marked as safe. This third group will then be exposed to the bell ringing without the associated foot shock, resembling the individual that is treated with both the antibody and cognitive behavioral therapy.

The fourth group would consist of rats not treated with the polyclonal antibody, but of which are traumatized, undergoing the same fear extinction procedures, providing an experimental understanding of a behavioral therapy to a traumatic anticipated shock associated with the bell ringing. The fifth group would be the monitoring of five normal mice. This group will aid in having a baseline for non-traumatized rat activity and act as the second control. This group will be also injected with the polyclonal antibodies after the seven days of preparation that the other rats will go through just to keep the timeline of data collection consistent. The sixth and final group will contain rats treated with the SSRI Zoloft, which is commonly used to treat the symptoms of PTSD, not as often the source of PTSD itself. This group will act to compare the effectiveness of biologic vs pharmaceutical interventions. The variability of patient's compliance has to be acknowledged in that experimentally these rats are forced to take whatever we give them. However, in the real world a mentally struggling individual commonly refuses or misses

taking medication. This is a large issue when prescribing consumer dependent treatments, where a biologic injection is largely dependent on provider administration over longer intervals of time.

Rat Electric Foot Shock Contextual Fear Conditioning (CFC)

The use of an electric foot shock model is common to artificially traumatize smaller rodents and rats, providing similar modulations of fear such as freezing and anxiousness. The electric foot shock model is the use of a device of which a rodent is placed into and while standing on a metal gridded platform of which when unstimulated acts as a regular surface.[40,41] Whenever the researcher desires to stimulate the shock chamber, the rat is shocked with a 1 to 3.mA current, which predeceases an audio stimulus, in this case, a bell ringing.[40]

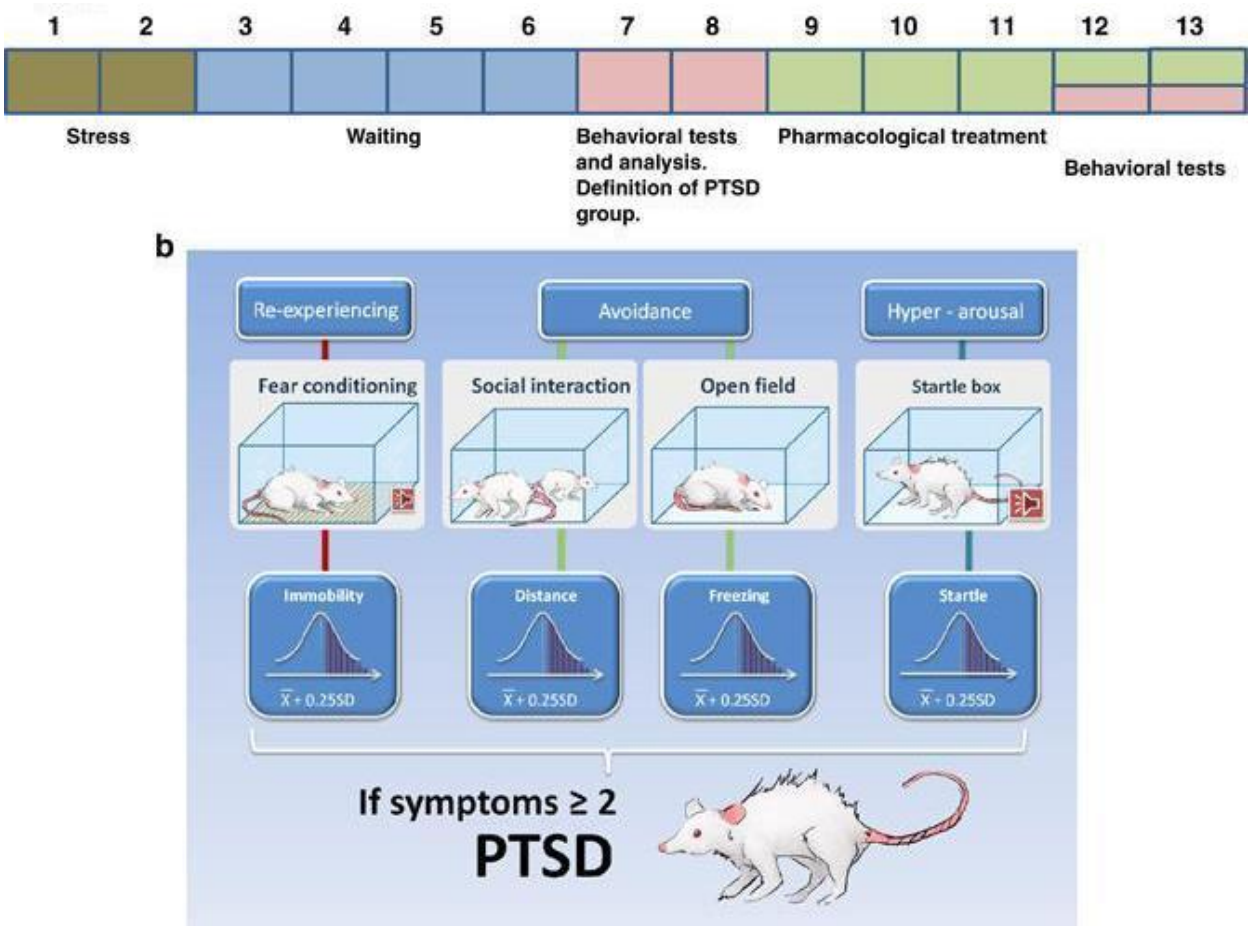


Figure 11: Electric foot shock model and timeline.[40]

By utilizing this model by the last day of fear training the rats are expected to have a predisposed reaction to the shock before the shock is even delivered, mimicking the reaction of PTSD victims to a stimulus, even if unharmful. For each of the experimental groups, this model will be utilized for two days from 7 AM to 7 PM and each rat will be shocked once an hour for two days. Though all of the rats will be exposed to the same stimulus, not all of them may develop symptoms of PTSD. This is where an extra day or two may be needed to distinguish which rats actually modulated the shock as trauma for future responses, or if the rat was able to naturally dissociate and consequently not react negatively to the stimulus once re-created. During this time of distinction for two extra days, the addition of ringing the bell followed by an absence of shocking the foot can be used as a way to see if the rodent still freezes or is hyperactive in response to the bell without the painful stimulus.

Injection and Monitoring

As briefly outlined in figure 11 above the fear conditioning of the utilized model takes about two days. It was previously discussed however, that a few additional days will be needed to determine at the end of the five day waiting period to determine which mice actually modulated PTSD.[40] Around day nine or ten the treatment of choice should be administered; for the proposed experiment, the administration route will be an intramuscular injection of the synthesized anti-spinophilin cofilin alpha 2A-AR receptor polyclonal antibody. Since rats are fairly small and fragile, it is important to make sure the handling is gentle enough to not disturb the sciatic nerve close to the thigh near the site of injection.[43] When injecting into the thigh, it's important to point the needle away from the sciatic/ femur region and that no blood is drawn once the needle is removed and injection fluid is slowly injected into the rat rather than rapidly

all at once.[43] Once injected, the re-initiation of the behavioral test will begin 2 to 4 days after treatment.

Data Collection and Analysis

The behavioral test that will be conducted to determine the effectiveness of the biologic intervention treatment, and the various groups include two different models of anxiety and stress analysis. The use of a trial known as the startle box, which is the presence of just the audio stimulus, rather than the associated electrical stimulus present during fear conditioning.[40,42]

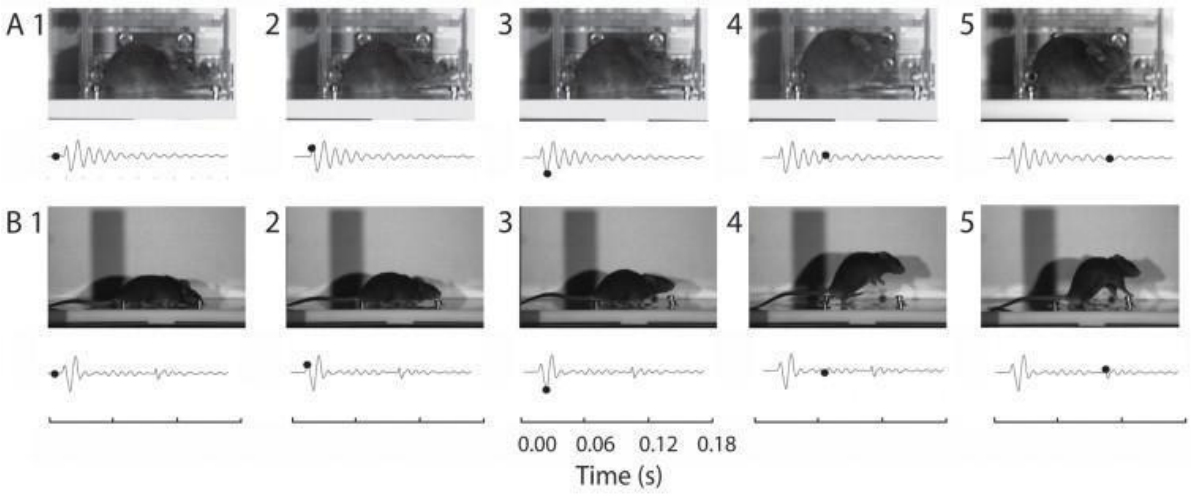


Figure 12: Startle box model testing rat response to a previously painful paired stimulus.[44]

In the image shown above in figure 12, the initial fear conditioning response from the rat can be seen side by side to the startle testing of the rats reflexes to the triggering stimuli.[44] In line A the training of the rat appears to have no physiological response to the paired stimuli, while once just the audio stimuli is present shown in line B the rat shows a hyperresponsiveness to re-living the non-painful portion of a modulated painful experience.[44]

Another way of behavioral analysis known as the open field model includes observing the behaviors of the rats when left alone in the box without any stimuli for 1 to 2 days and

comparing the rats of each trial to both the untreated rats and the normal, non-traumatized rats.[40,42] After the startle box and open field test are performed the use of the more complex elevated pulse maze (EPM) model can be used to see if trauma cognitively affects the modulation of new memories and or skills.[42] Though not utilized for this initial treatment proposal, EPM is a great expansion on the further effects that PTSD can have on the cognition development of individuals.[42]

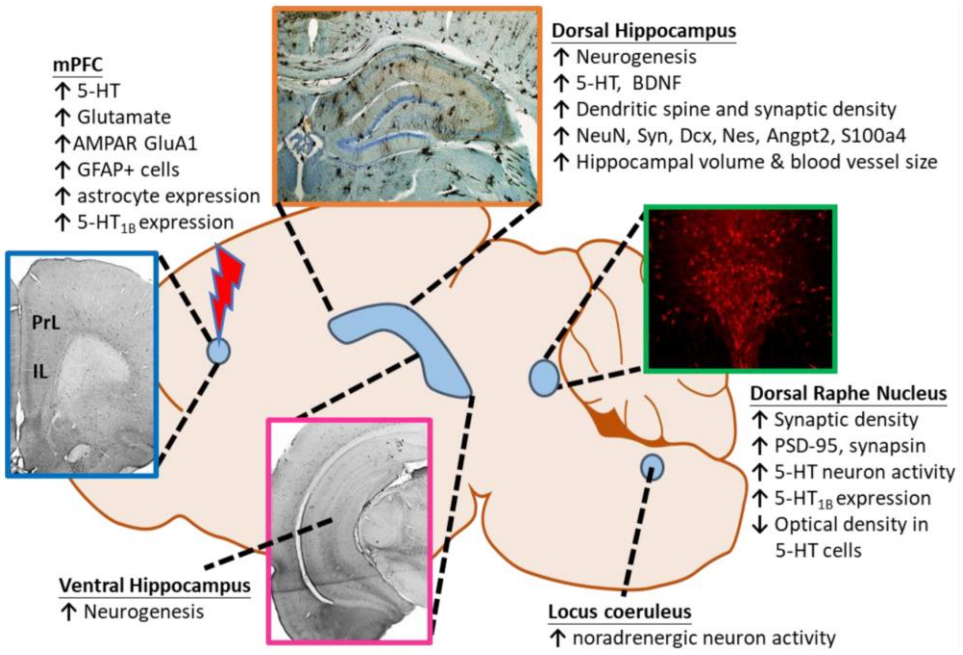


Figure 13: Neurochemical changes in rat response to fear inducing electric foot shock.[45]

As previously discussed and understood, rats as well as humans in response to a traumatic stimuli have alterations in dendritic spine and synaptic density as a result of increased norepinephrine, and dopamine surges in the dorsal hippocampus.[45] By injecting the proposed antibody it is hoped that this antibody will be confined to the inhibition of the spinophilin cofilin AR region, and aid in the decrease of dendritic spine growth.[45] Though the increased flux of norepinephrine, epinephrine and dopamine will still occur but the bonding of these

catecholamines to the targeted receptor will not affect the dendritic plasticity nor react to cause an overreaction to a triggering stimuli.[45] These fear conditioning procedures act as a way to artificially develop these catecholamines surges of which can be recorded initially by blood tests before and after treatment injection and before and after analyses of behavioral test effectiveness.[45]

CHAPTER 5: SPECIFIC AIMS, METHODS, AND EXPECTED OUTCOMES

Treatment Goal

The Alpha-2A adrenergic receptor at spinophilin cofilin axis where a respective antibody would inhibit norepinephrine binding so cofilin is not affected, and allowed to cut actin that usually would accumulate and modify dendritic spines, prevents over modulation of traumatic memories when faced with a triggering stimulus. Norepinephrine inhibits proteins associated with the activation of cofilin. With this understood, cofilin inhibition due to the norepinephrine flux chain of events post-stimulation from traumatic recurrence allows for dendritic growth of which increases plasticity and reactivity to developed dendritic morphology. Pairing the use of the Anti-Alpha-2A Spinophilin Cofilin Adrenergic Receptor Polyclonal Antibody to a behavioral therapy, the goal is to develop a more holistic approach to PTSD without the use of harsh, synthetic pharmacological agents.

Specific Aim 1: Observing the Interventional Neurochemical Effects of an Anti-Alpha-2A Spinophilin Cofilin Adrenergic Receptor Polyclonal Antibody on Fear Memory Modulation in Long-Term PTSD

As previously addressed the neurochemical shift of present catecholamines observed in traumatized rodents is responsible for the increased dendritic plasticity and is a response to fear modulation in the spinophilin cofilin axis. By utilizing the axis specific antibody proposed, the binding of nor-epinephrine will be inhibited and prevent the binding and depression of the cofilin protein allowing it to break down actin responsible for dendrite elongation.

Specific Aim 2: Observing the Physical and Cognitive Effects of an Anti-Alpha-2A Spinophilin Cofilin Adrenergic Receptor Polyclonal Antibody on Fear Memory Modulation in Long-Term PTSD

Understanding the chemical and physical, behavioral effects that the proposed treatment produces will allow a better understanding of the alternative therapies for fear consolidation monitoring and depress the hyper arousal physical responses to a traumatic event. This part of the study will focus on the observable changes correlated to the presence or absence of each treatment group.

Experimental Procedure for Aim 1 and 2:

The experimental procedures for specific aim one and two combined were inspired and mainly resemble that of the molecular psychiatry journal article by Saggu et al. and Goswami et al. outlining rodent fear modulation and protein injection.[29,46] Starting with initially six groups of five rodents with each separated and placed into their respective groups as depicted in the table below with their treatment courses outlined in Table 1.

Groups	Treatment	Modulation and Analysis Tests*
Group 1 (Control 1) n=5	Untreated PTSD.	Electric Foot Shock and Startle Box Analysis and Open Field Analysis
Group 2 n=5	Treated with 10 µL antibody infusion.	Electric Foot Shock and Startle Box Analysis and Open Field Analysis
Group 3 n=5	Treated with 10 µL antibody infusion and fear extinction.	Electric Foot Shock Followed by Fear Extinction Procedure and Startle Box Analysis and Open Field Analysis
Group 4 n=5	Traumatized and untreated but undergo fear extinction.	Electric Foot Shock Followed by Fear Extinction Procedure and Startle Box Analysis and Open Field Analysis
Group 5 (Control 2)	Normal mice, no traumatizing or administration of	Startle Box Analysis and Open Field Analysis

n=5	treatment.	
Group 6 n=5	Treated with 25mg of Zoloft in 20 µL of water (drug comparison daily).	Electric Foot Shock and Startle Box Analysis and Open Field Analysis

*All groups will have blood tests done at experimentally mentioned frequency regardless of group treatment.

Table 1: Experimental group treatment assignments of rats.

Following the National Institute of Health Guide for the Care and Use of Laboratory Animals, 30 male Lewis rats were split into six groups each weighing about 250 g. The rats were acclimated to a 12 hour light to dark cycle and their initial behavioral and chemical starting points were recorded and cannulation of the dorsal hippocampus was performed, with the cannula anchored to the top of the head and connected to a syringe of which would deliver the treatment once fear modulation was completed.[29,46]

During the previously outlined electric foot shock and bell fear modulation model for the preparation of traumatized mice, the polyclonal antibody preparation was prepared and purified with the addition of a specifying 16 amino acid cofilin sequence connected to a TAT resembling sequence that promotes permeability to the membrane of interest in the dorsal hippocampus, as well as controls the antibodies reactiveness with the receptor target.[29]

Once fear modulation is complete over the course of two days with the rats being shocked every one hour for the 12-hour light timeframe, the rats are to be left for five days where no shock or bell ring will occur. After the initial five days corresponding to whichever treatment group, they are in, their treatment will be administered. For the groups being treated with the antibody treatment, they will receive a 10 µL infusion of the developed antibody solution over a period of 50 minutes. During this time the group of which will be treated with the Zoloft will be given 25 mg dissolved in 10µL of deionized water.[46] Only for the group receiving the Zoloft

dose will the administration via cannula be continued every day on the first hour of the light cycle.[46]

After treatment administration via the injection through the cannula, the rats will be left for seven days and monitored for chemical and behavioral changes. During this time, they will not be shocked, but their catecholamine levels and physical and behavioral changes will be recorded and cataloged via blood test and video recording of each group over the 7 day rest period after treatment. Blood analysis will occur as follows: at the beginning of fear modulation, at the end of fear modulation, at the end of the first rest period, at the end of biologic or pharmaceutical injection, after the second seven day rest period, and after each behavioral analysis test is performed to have a better understanding of the starting and benchmark points of norepinephrine control throughout trauma modulation and intervention. For the groups that undergo the mimicking of cognitive behavioral therapy (CBT) i.e. fear extinction methods, in these rats an additional step will be added prior to the startle test series. The way by which this model works can be seen in figure 14.

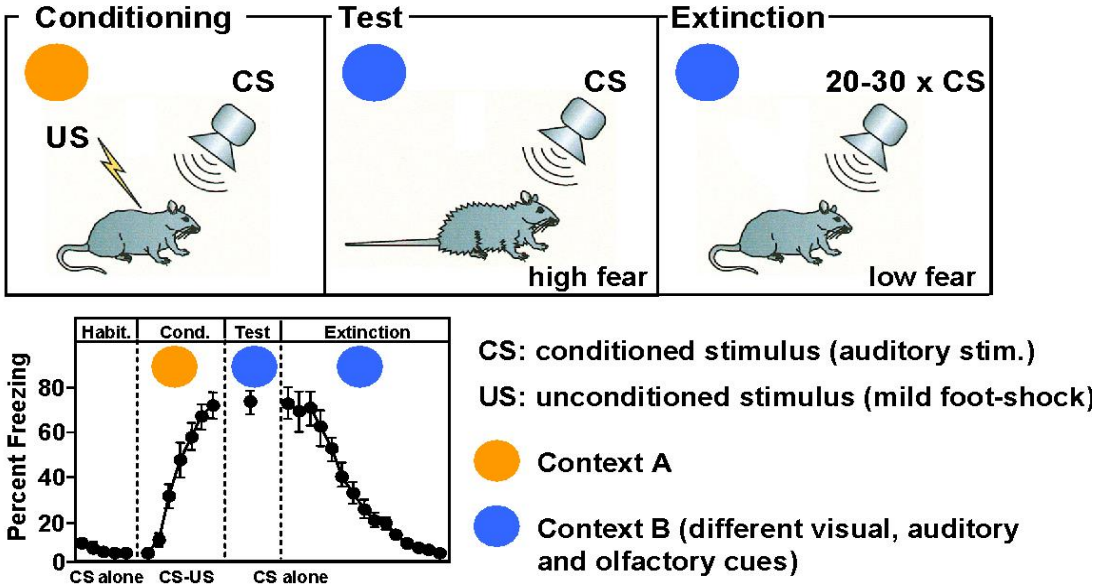


Figure 14: Fear extinction model in rats mimic use of CBT.[47]

When the seven days has passed with each of the groups, various catecholamine levels can be tested by tail IV blood analysis after the startle box procedures will be performed by placing the rat in a chamber similar to its initial fear conditioning box, and each of the 30 rats will be exposed to the same bell ring that had been previously accompanied by a foot shock. However, the electrical pulse will not occur, and the rat is expected to either respond to the bell, as if it had been shocked, or not, depending on the treatment group it was in. The catecholamine levels after each of these trials can be completed with a blood test as well to see the effect of a triggering episode on the various treatment groups.

The specific aims one and two can be analyzed during the same experimental procedures. That is, the physical and behavioral observations and data can be recorded by observing the change in mannerisms and developed tics of which could best be observed after the startle box behavioral analysis. The coupling of these analyses produces more coherent results rather than taking a whole other group of thirty rats and only looking at the physical changes and vice versa for the catecholamine chemical analyses.

Expected Outcomes

While the experimental for both of the aims are coupled, the outcomes for each part of the analysis of the varying treatments expect to remain independent and unaffected by the longitudinal nature of the experimental performed.

Anticipated Outcomes for Aim 1: Neurochemical Evaluation

The chemical concentrations of the dorsal hippocampus and HPA for each of the groups will be increased since all of the groups will be introduced to a stressing stimulus. For each group the expected outcomes are shown in table 2 below.

Treatment Group	Expected Observations
Untreated PTSD.	The concentration of all of the catecholamines are expected to be the highest in this group due to the uncharging flux that occurs during the initial modulation of the chock stimulus.
Treated with 10 µL antibody infusion.	The concentration of catecholamines is expected to be increased by a small amount due to the lack of allowance to bind to the AR receptor target.
Treated with 10 µL antibody infusion and fear extinction.	The concentration of catecholamines is expected to lower due to the decreased stress response as result of the fear extinction even despite the inability of uptake from the antibody inhibit AR complex in the spinophilin cofilin axis.
Traumatized and untreated but undergo fear extinction.	Concentrations of catecholamines would be expected to be slightly lower than if treated with the polyclonal antibody treatment. This is due to the presence of a stress response coupled with the absence of receptor binding inhibition, so while the stress response is decreased in magnitude, the inability to prevent the catecholamine binding in the absence of the antibody treatment allows for catecholamine uptake.
Normal mice, no traumatizing or administration of treatment.	No changes in concentration of catecholamines is expected within the margin of error.
Treated with 25mg of Zoloft in 20 µL of water (drug comparison daily).	The change in catecholamines concentration will be around the same as the starting trial concentration.

Table 2: Anticipated neurochemical responses to each treatment course.

Anticipated Outcomes for Aim 2: Behavioral Evaluation

The physical symptoms that each group is expected to exhibit are included in table 3 below. Though these observations may not easily be observed, and rats not continuously monitored, it is anticipated that the 24/7 video monitoring of each rat will be performed and the footage reviewed every day for cataloging of symptom development.

Treatment Group	Expected Outcomes

Untreated PTSD.	Freezing, hyper arousal, isolation, decreased movement, all in episodes and general skittishness.
Treated with 10 µL antibody infusion.	Semi-normal behaviors, slight hyperarousal-like residual behaviors.
Treated with 10 µL antibody infusion and fear extinction.	Normal behaviors as observed at the beginning of experiment, if not more traits of resilience and exploration comfortability.
Traumatized and untreated but undergo fear extinction.	Slight general skittishness may be observed.
Normal mice, no traumatizing or administration of treatment.	Normal behavioral reactions.
Treated with 25mg of Zoloft in 20 µL of water (drug comparison daily).	Semi- normal behavior with episodes of depression and or isolation with decreased movement.

Table 3: Expected behavioral outcome to each treatment course.

CHAPTER 6: CONCLUSION, DISCUSSION, AND FUTURE STUDIES

By reviewing multiple past and proposed studies for the treatment of PTSD it is very apparent the availability of treatments specifically for the treatment of fear modulation and hyperarousal in PTSD is lacking. Understanding the effects of catecholamine binding to adrenaline receptors during a traumatic 'fight or flight' event, this pathway can be targeted and manipulated to prevent such interaction from occurring and if performed as anticipated by the inhibition of actin reduction proteins, reduce dendritic plasticity and therefore fear modulation.

Both specific aims 1 and 2 looked at the different changes that could occur from the proposed biologic antibody treatment as well as current pharmaceutical options used to treat the symptoms of PTSD. While no effective treatment for the elimination of hyperarousal from fear modulation currently exists, as outlined in both of the experimental methods and anticipated outcomes, it is expected that the trial consisting of the rats treated with the proposed polyclonal antibody for the alpha-2A adrenergic receptors in the spinophilin cofilin axis and fear extinction method that mimics the use of cognitive behavioral therapy in humans are to show best recovery and reduced response to a conditioned traumatic stimuli. This group is anticipated to show decreased concentrations in catecholamines due to a reduce stress response, as well as decreased behavioral symptoms due to the inability of catecholamines to shift the dendritic plasticity activity of previously modulated pathways within the spinophilin cofilin axis.

While the exact procedures for the production of the antibody is not fully outlined, by creating a specifically targeted antibody to the spinophilin cofilin axis, altering both the permeability and reactivity with certain nucleic acids and biological marker series the inhibition of only this regions receptors should produce the preferred response with the surrounding receptors unaffected. This method allows for a much more effective, holistic and longitudinal

drug delivery method to prevent the injection of artificially synthesized compounds into the body when the use of the B cells and antigens already in your body can do the job just as well.

Other than the technical specificity of the antibody production still needing to be specifically outlined, the actual delivery of the eventually developed biologic to the spinophilin cofilin axis through the blood brain barrier (BBB) is the largest limitation to this proposal.[48,49] More current studies have also determined this issue in various other chronic neurodegenerative conditions as well.[49] A proposed combat to this issue is the formation by modification of the antibody of interest into a blood brain barrier friendly IgG fusion protein-like biomolecule.[49] By engineering the preexisting biologic to favorably fuse by the same pathway as fusion proteins, there is promise that a blood brain barrier entry solution for large molecule treatments will be developed in the near future to eliminate this limitation.[49]

For aim 1 the neurochemical changes were focused on. For the second aim however, data is much more subjective in that the observed data was just that, observational; based on general social behaviors and reactions to environmental changes. The best behavioral anticipated outcomes are expected to be observed in the group of which receives the antibody treatment and fear extinction treatment which would cause subjects to hopefully exhibit very few, if none of the behaviors that the untreated, control group 1 would exhibit. This would mean that they are not hyper aroused by unfamiliar movement, as well as have a very low, if not no response to the startle box behavioral analysis.

Most recent literature has been looking at adrenergic receptor targeting drugs.[29] The proposed biologic would mimic the studies' mechanism of delivery but rather utilize an antibody instead of a pharmaceutical. In the future the specific study of the mechanism of the newer developed drugs, Guanfacine of which corresponds with and depresses Clonidine would give a

better idea of the intensity of the role of catecholamines on adrenergic receptors and neurochemical binding affinities to surrounding regions.[29] Of all of the future studies needed the most, the overall better understanding of fear modulation should be prioritized to have a clearer idea of the specificity of various associated protein activities involved in the pathway to modulate, emotionally, a fearful and traumatic stimuli.

Expansion of this experiment could include a third aim which would observe long-term cognitive effects and longevity of the various treatment groups. The elevated pulse maze test (EPM) as discussed in chapter 4 could be beneficial in the observation of new skill and memory consolidation and provide a way in which each therapy affects the wear done on the whole body. This experimental aim along with further testing could aid in determining the longevity and temporal changes the proposed and similarly analyzed treatments produce. Though the use of a rat will never come close to the complexity of a fully functioning and traumatized brain, it is a start to a longer lasting, further reaching understanding and study to serve a large demographic of hurting individuals.

PTSD is debilitating, it doesn't discriminate against character or class, no one thing makes someone more at risk of developing or modulating traumatic responses to fear and no one individual will ever be able to predict who will suffer from it before it happens. Some may never come close to knowing the effect it has on a life but an illness so nondiscriminatory is important to understand and combat because even if not personally suffering with PTSD, everyone will at some point in their life meet someone who does. I started this process trying to target specifically PTSD in veterans but realized that even though a large population of suffering individuals are from that demographic, PTSD can affect the youngest, the oldest, and the richest or poorest. An illness that could take down the strongest of soldiers should be faced with full force. Developing

a treatment should put into frame an individual's whole body for restoration of the broken, eliminating the cycle of unnecessary drug induced declines.

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