CORTISOL: FRIEND OR FOE IN THE DEVELOPMENT OF PTSD-ASSOCIATED SYNAPTIC PLASTICITY IN THE AMYGDALA?

SHEPHERD, RYAN
Neuroscience, College of Medical, Veterinary and Life Science

ABSTRACT

Post-traumatic stress disorder (PTSD) is a complex and debilitating psychiatric condition, associated with distinct changes in a region of the brain called the amygdala. These changes are associated with the recurrent 'flashback' experiences caused by the formation of pathologically strong fear memories. The human stress hormone cortisol plays a role in inducing these brain changes; however, evidence also suggests that cortisol may protect against these changes altogether. The exact role of cortisol in mediating PTSD-associated changes in the sensing nuclei of the amygdala is unclear, but this must be established if we hope to take advantage of this process therapeutically. Therefore, the following article reviews the evidence for the mechanism explaining the connection between cortisol and PTSD, drawing from relevant clinical and animal model literature.

Cortisol was found to have the potential to both exacerbate and prevent the development of PTSD behaviour and brain changes. The mechanism by which cortisol mediates these changes upon brain cells is complex and it involves interaction with several other chemical brain signals. Understanding this mechanism and its appropriate timings further will aid the development of preventative therapies for PTSD, and insight into fear memory formation as a whole.

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a complex anxiety disorder which may develop following exposure to a traumatic event. It is a relatively common disorder which significantly impacts working life and relationships of those affected. Around 3% of the population in England are estimated to suffer from PTSD (NHS Information Centre for Health and Social Care, 2009), and as much as 7.8% of young people are believed to develop PTSD by the age of 18 (Lewis et al., 2019). Following the traumatic event, people may experience sleep disturbance and anxiety. The memories formed during the initial traumatic event play a pathogenic role in PTSD where individuals report re-experiencing of the initial traumatic event via disturbing dissociative 'flashbacks' (WHO, 1992). These re-experiencing responses suggest impaired fear memory extinction, the process of fear memories fading with time, in PTSD patients. Mechanistically, these symptoms implicate dysregulation of fear memory circuitry as a key factor in the development of PTSD.

Fear helps protect individuals from harm, and fear-associated behaviours are present in everyday life. Consider a person bitten by a red spider; individuals may remember the feeling of being bitten by the spider when they see another red spider. From an evolutionary standpoint, this is the brain's means of keeping the person safe from subsequent spider bites. However, for an individual with PTSD, the fear memory may be so strong that even seeing the colour red, or any insect resembling a spider, might induce a harrowing flashback to the initial bite. In forming fear memories, individuals learn to avoid stimuli which cause stress or inflict pain—after which the stimuli or relevant contextual cues evoke a physiological response and a heightened emotional state in humans (Izquierdo et al., 2016).

The neural mechanisms responsible for the formation of fear memories have been a topic of vigorous research for decades, though the regions involved in these processes are well established (Johansen et al., 2011; Izquierdo et al., 2016). The process of storing memories associated with a traumatic event is complex and involves many different brain regions, including the hippocampus and amygdala. The hippocampus is widely accepted to be essential for the formation of new memories. The amygdala plays a key role in the stress response and is known to play a critical role in encoding of fear memories (Sanders et al., 2003; Andolina and Borreca, 2017; Chaaya et al., 2018).

The amygdala is involved in emotional regulation, particularly fear. During a traumatic event, the amygdala becomes activated. As time passes after the traumatic event, the cells which compose the amygdala become physically changed. These changes are thought to represent the formation of fear memories associated with the event (Chaaya et al., 2018). The parts of the brain which become activated when a memory is being formed are the amygdala's 'sensing nuclei' which send electrical signals to the 'activation nuclei.' The activation nuclei are responsible for causing us to feel anxious and afraid: for example, the 'fight, flight, freeze' response in the face of immediate danger. During a traumatising event, signals from the senses may cause neurons to fire in the sensing cells of the amygdala; fear memory formation is facilitated here by strengthening neural connections (Izquierdo et al., 2016). The amygdala's sensing nuclei are also involved in the encoding of contextual sensory cues associated with the initiating traumatic event. These nuclei communicate with the hippocampus to reinforce the creation of a permanent fear memory (Chaaya et al., 2018).

In the context of PTSD, changes in the cells of the amygdala's sensing nuclei after a traumatising event are thought to correlate with the strengthening of trauma-associated memory which causes recurrent flashbacks in patients (Zhang et al., 2019). The human stress hormone, cortisol, is likely to play a key role in these delayed structural adaptations given its role in the stress response and its known effects on memory-associated plasticity in other brain regions (Roozendaal et al., 2010). However, there remains a great deal of uncertainty regarding the effects of cortisol within the amygdala, and particularly the mechanisms which influence structural changes. Studies investigating the role and mechanisms by which cortisol influences structural changes during the development of PTSD are sparse, and at times contradictory. Some highlight the role of cortisol in strengthening connections (Karst et al. 2010; Atsak et al., 2014;

ryan-shep@hotmail.com

Di et al., 2016) and others insist they may actually play a role in preventing it (Chakraborty and Chattarji, 2019; Chakraborty et al. 2020). Put simply, PTSD-associated changes in the amygdala's sensing nuclei lead to pathologically strong fear memories and cortisol may be protective or pathological in causing these brain changes. An important gap in the literature is in elucidating the mechanism which leads to these two different outcomes.

Treatment for PTSD is primarily psychotherapeutic and uses trauma-focused cognitive-behavioural therapy to encourage extinction of fear memories. Currently, selective serotonin reuptake inhibitors (SSRIs) are the only approved pharmacological therapy prescribed to patients with PTSD for whom psychotherapy is ineffective (Friedman and Bernardy, 2017). A deeper understanding of the action of cortisol on cells of the amygdala's sensing nuclei may provide insights into new interventions for treatment-resistant PTSD. This review will explore recent findings on the link between amygdala plasticity and cortisol action during acute traumatic stress. Specifically, this review evaluates evidence for a potential mechanism in which cortisol action contributes to strengthening of connections in the sensing cells of the amygdala, and conversely, how it may prevent strengthening altogether.

BACKGROUND

Synaptic Plasticity Drives Fear Memory

Structural Plasticity in the Hippocampus

All forms of memory, including the strong fear memories which arise for individuals with PTSD, occur by synaptic plasticity. This is a process whereby brain cells physically change their shape in order to develop more connections (Chayya et al., 2018). In some brain regions, the tree-like dendrites of neurons exhibit small cellular protrusions known as dendritic spines, which allow for greater surface area and insertion of more receptors to the membrane and act as 'signal receivers'. This results in the strengthening of neural connections at the junctions between neurons (synapses), which exhibit plasticity. Synaptic plasticity is the means by which the brain changes itself at the cellular level, affecting connections between neurons. This may manifest as changes to dendritic spine density and morphology, which are mediated by complex intracellular signalling pathways (Sala and Segal, 2014). In short, complex signalling pathways lead to brain cells changing shape to allow for more connections between other cells, this makes the memory clearer and stronger. It is assumed that this process is similar for PTSD fear memories as more connections are created in the amygdala (Zhang et al., 2019).

To understand how cortisol changes cells in the amygdala's sensing nuclei, we first must explore how this change is brought about and how cortisol affects this process. Though this review focuses on the amygdala, the specific mechanisms underlying synaptic plasticity are best defined in the hippocampus. Therefore, by extrapolating from literature on hippocampal plasticity, we can begin to build a mechanism for the amygdala. There exists an established body of previous work exploring hippocampal plasticity in relation to the formation of fear-associated contextual cues (Kim and Fanslow, 1992; Phillips and LeDoux, 1995), and although cortisol action may differ in the sensing cells of the amygdala, there are likely shared mechanisms.

Generally, in the hippocampus, plasticity is mediated by the growth or retraction of small cell body protrusions known as dendritic spines, which are dense with receptors for the neurotransmitter glutamate—a chemical signal which excites neurons into firing (Sala and Segal, 2014). During memory consolidation, spine growth occurs as result of long-term potentiation (LTP): the process by which less excitatory input is required to cause a neuron to fire, due to increased stimulation over a short period of time. During the 'short lasting' early stages of LTP, new glutamate receptors are embedded into the membrane; after an hour or so, however, modulation of genes evokes protein synthesis to induce more permanent structural changes to brain cells (Chidambaram et al., 2019). These structural changes are thought to be mediated via upregulation of the transcription factor cAMP responsive element binding protein (CREB). This 'switches on' specific genes which control cell growth, such as brain derived neurotrophic factor (BDNF), and proteins responsible for remodelling of neuronal cytoskeleton, ultimately leading to cell changes which allow for stronger communication between neurons (Kida, 2012). CREB is activated by messenger signals such as calmodulin-dependent protein kinase IV (CAMKIV) and protein kinase A (PKA), which become more active when the cell receives excitatory signals from other neurons (Kida, 2020).

In addition to alterations in spine density, changes to cell shape are also relevant. Three distinct dendritic spine morphologies are typically observed: thin, stubby, and mushroom-shaped. Mushroom-shaped spines have a larger surface area and are considered 'mature' in comparison to thin or stubby spines (Sala and Segal, 2014). This process allows hippocampus brain cells to alter their communication with one another. If the cell changes shape to increase surface area, more neurotransmitter receptors can be inserted into the cell membrane. Think of this receptor insertion as opening a window in a house: if more windows are open the outside world will seem louder, and communication will be clearer. The louder this communication between brain cells, the stronger the fear memory.

Cortisol Influences Hippocampal Plasticity

Cortisol is a human steroid hormone released from the adrenal gland into the bloodstream in response to stressful events, carried by cortisol-binding-globulin into the brain (El-Farhan et al., 2017), where it may pass through neuronal cell membranes to act upon receptors within cells. The 'binding' of cortisol to cell receptors is like a key being inserted into a lock, which activates a chain reaction within brain cells. Cortisol acts on cells by activating two types of receptors within cells. It may bind to mineralocorticoid or glucocorticoid receptors, the former having greater affinity than the latter. This results in primarily mineralocorticoid receptor binding when blood-cortisol levels are low, and glucocorticoid receptor binding only when blood-cortisol levels are high, for example, during a stressful event (Mifsud and Reul, 2018). Both slow-acting intracellular receptors are thought to modulate gene expression, but the mechanism by which they regulate synaptic plasticity remains uncertain (Mifsud and Reul, 2018).

Evidence suggests cortisol may also bind to receptors on the outer surface of neurons to produce fast-acting changes to evoke synaptic plasticity, rather than slower-acting attachment sites found within neurons (Roozendaal et al., 2010). Cortisol is like a key which fits multiple locks, both inside (slow-acting) and on the outer surface of brain cells (fast-acting). The most interesting type of receptor for PTSD is the glucocorticoid receptor, which is most likely to be activated during stressful events (i.e., when cortisol levels are high).

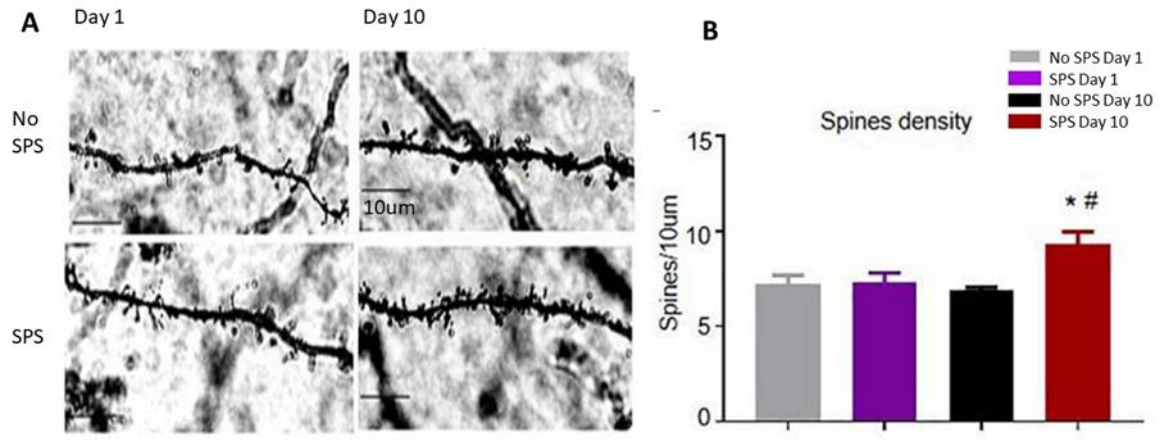
Cortisol is known to play a role hippocampal plasticity following acute stress (Roozendaal et al., 2010; Sandi, 2011). It is suspected that intracellular glucocorticoid receptor binding contributes to destruction of dendrites and weakening of neural connections (Sapolsky, 2000; Karst et al., 2010) in regions of

the hippocampus, perhaps leading to the memory recall impairments observed in PTSD patients.

In PTSD research, there appears to be an increasing focus on the role of membrane-bound glucocorticoid receptors following acute stress. For example, cortisol is thought to work with other chemical signals to influence receptor distribution (i.e., the 'opening of windows') on hippocampal neurons following an acute stress event, but the effect is too rapid to be mediated by receptors found within the cell, leading to the conclusion that cortisol binds on the cell surface to cause these changes (Zhou et al., 2011). So, during a traumatic event, cortisol 'unlocks' glucocorticoid receptors on the outside of amygdala brain cells. This eventually leads to the rapid strengthening of brain connections representing a fear memory which leads to the symptoms of PTSD.

Lastly, it is understood that the cortisol system is closely linked with the endocannabinoid system, which has been shown to play a role in regulating hippocampal plasticity in fear memory (Ruehle et al., 2011). Where the cortisol system acts to keep us alert and stressed, endocannabinoids normally work in opposition to this by acting as the brain's endogenous 'anti-stress' chemicals, bringing balance to the stressed brain (Morena et al., 2016). There are two key endocannabinoids implicated in modulating plasticity following stress: 2-Arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA), both of which bind to cannabinoid receptor 1 (CB1) (Ruehle et al., 2011). Endocannabinoids have been shown to play a role in dampening synapses where cells become less sensitive to signals from neighbouring cells, typically by reducing release of excitatory chemical signal from neurons after binding to a CB1 receptor on its membrane (Cohen et al., 2019). If inserting receptors to a neuron surface is similar to opening windows in a house, activating the endocannabinoid receptor CB1 causes the surrounding streets to become quieter. The neuron receives less 'noise', and its communication from neighbouring cells becomes weaker.

PTSD Induces Delayed Amygdala Plasticity



1. Effect of SPS paradigms on spine density of BLA pyramidal neurons by Zhang et al. (2019) under CC BY / Modified to show only parts B and C of the original figure. A: Representative image of amygdala neurons across SPS and non-SPS groups, at both day 1 and 10 following stress treatment. B: Bar graph showcasing quantified spine densities across experimental groups.

Synaptic plasticity in the amygdala's sensing nuclei has been a prominent focus in PTSD research recently given its role in fear memory formation (Izquierdo et al., 2016). A recent study by Zhang and colleagues (2019) used single prolonged stress in rats: assessing exploratory behaviours (which are dampened in fearful mice), selecting dendrites within the amygdala's sensing nuclei for spine quantification and measuring changes in electrical activity via cell recording to assess whether these selected cells exhibit altered electrical excitability. As depicted in Figure 1, no significant changes were observed on day 1 after stress, but increased spine density was observed at day 10 alongside increased excitability and heightened anxiety scores. There is evidence to suggest that this delayed strengthening is mediated by activation of glutamate receptors' sites on sensing neurons in the amygdala, similarly to that observed in other brain regions (Yasmin et al., 2016). As a result, if cortisol is able modulate chemical signalling in the amygdala, it may account for its role in amygdala plasticity in the formation of fear memory during traumatic stress. In short, cortisol encourages amygdala cells to physically grow more fear memory connections after a traumatic event; theoretically leading to pathologically strong fear memories in patients.

Considering exposure to psychological stress is shown to be followed by a delayed increase in cortisol levels (Takai et al., 2004), and the role it plays in hippocampal plasticity, it is likely they also play a role in this delayed increase of the amygdala's synaptic strengthening during PTSD, somehow causing the receptor 'windows' of the house to open. However, as previously mentioned there is evidence to suggest that while enhancing synaptic strengthening, cortisol may also play a role in protecting against the maladaptive strengthening of synapses which cause PTSD fear memories to become stronger; leading to uncertainty regarding the mechanism by which cortisol might modulate this effect.

METHODOLOGY

This review aims to address the gap in understanding of the cellular mechanisms for how cortisol causes or prevents changes to the sensing nuclei of the amygdala, which subsequently impacts upon pathological fear memory observed in PTSD. This involves reviewing established literature on synaptic plasticity in the hippocampus, and applying it to investigations involving PTSD, cortisol and the amygdala.

Literature investigating cortisol or corticosterone in conjunction with synaptic plasticity in the basolateral amygdala, or in the clinical context of PTSD, was selected from databases PubMed and Ovid. The evidence collated to produce a theoretical mechanism of cortisol action was selected exclusively from rodent studies, e.g. where cortisol-associated drugs or cortisol were administered directly to the basolateral amygdala via surgically inserted cannula prior to behavioural testing and analysis of plasticity changes. These rodent studies utilise models of PTSD, particularly the single prolonged stress model, outlined in Animal Models of PTSD. Evidence from similar rodent studies using endocannabinoid-associated drugs was collated to estimate the mechanism by which cortisol protects against PTSD, with supporting translational evidence from clinical trials.

Animal Models of PTSD

In neuroscience, some neurological or psychiatric disorders can be mimicked in animals (e.g., mice). This provides an opportunity to monitor the cellular and molecular brain changes which occur as the disorder manifests and cannot be obtained from patient data. Animal models of stress provide insight into how typical fear memories are formed; models of PTSD provide insight into how pathologically strong fear memories are formed. Before delving into literature on fear memory, it is important to outline some of the relevant models of stress which have been used to study PTSD over time, particularly as these models produce key evidence used to synthesise a summarised mechanism of cortisol action.

The single prolonged stress (SPS) model is the main animal model used in modern PTSD research. It more closely resembles human PTSD than other stress models and is also shown to stop fear memories from fading, which occurs in PTSD (Lisieski et al., 2018). In contrast to the acute restraint method (Mohammad et al., 2000; Wood et al., 2003), the SPS model evokes a stronger stress response via psychological, physical and pharmacological stressors, such as prolonged restraint, forced swim, and exposure to anaesthetic drugs respectively (Lisieski et al., 2018; Fang et al. 2018). Earlier studies make use of classical fear conditioning, which involves an unconditioned stimulus such as a foot shock, in conjunction with a tone to produce a conditioned fearful response (Phillips and Ledoux, 1992; Wiltgen et al., 2006), or inhibitory avoidance training where the animal learns to avoid a particular area or action via repeated foot shock (Atsak et al., 2014). Although not as relevant to PTSD as acute restraint or SPS, fear conditioning training allows for the specific study of how fear memory manifests in the amygdala's sensing nuclei, and how cortisol might influence the structural changes which accompany it.

MAIN FINDINGS

Cortisol Encourages PTSD-associated Amygdala Plasticity

Researchers generally agree that cortisol plays a role in regulating synaptic plasticity within the hippocampus, however the exact effects on the amygdala's sensing nuclei neurons following acute stress are undefined. Earlier findings have shown that chemical blockers of the glucocorticoid receptor, the main chemical attachment site for cortisol, can hinder memory consolidation when injected directly to the amygdala (Roozendaal and McGaugh, 1997), whereas glucocorticoid receptor-activating drugs produce the opposite effect (Akirav et al., 2004; Hui et al., 2004). An in-vitro study by Duvarci and Pace (2007) found that cortisol increases the excitability of amygdala neurons in rodent brain slices and dampens inhibitory signals from surrounding cells. Their findings suggest that these effects are mediated by glucocorticoid receptors, as the application of mineralocorticoid receptor blocker spironolactone does not hinder the effect at high cortisol concentrations. If the neuron is a house and the receptors are windows, 'excitability' represents how well an individual inside the house hears noise from outside. When cortisol interacts with glucocorticoid receptors, excitability increases.

The data from this study helps understand the role of cortisol in delayed synaptic strengthening. If cortisol can increase the excitability of these amygdala sensing nuclei neurons and dampen inhibitory input, synaptic strengthening is likely to follow, strengthening fear memory following a traumatic event, particularly as this will increase glutamate receptor activation which is deemed critical for amygdala synaptic strengthening (Zhang et al., 2019). More recent findings explore the importance of other neurochemical systems in the mechanism of cortisol action. For example, the noradrenergic system appears to contribute to this increased excitability induced by cortisol (Atsak et al., 2014) and the endocannabinoid system may play a role in modulating this cortisol-dependent dampening of inhibitory neural signals (Di et al., 2016). Inhibitory neural signals such as GABA (Gamma Aminobutyric Acid) act as a 'leash' for the electrical activity of neurons. If this leash stops functioning, neurons become excessively stimulated, driving plasticity and stronger neural connections (Owen and Kriegstein, 2002).

Interaction with Neurotransmitter Systems: GABA

Inhibitory neural signals are mediated by a neurotransmitter known as Gamma Aminobutyric Acid (GABA) (Owen and Kriegstein, 2002). If adding glutamate receptors is like opening windows in a house, adding GABA receptors is like installing sound-proofing panels to the walls. A more recent study by Di et al. (2016) describes a potential mechanism for the dampening effect of cortisol on GABA-releasing neurons which interact with the amygdala's sensing nuclei. As previously mentioned, it is well known that the 'anti-stress' endocannabinoid system is closely linked to cortisol release (Hill et al., 2009; Morena et al., 2016), and the activation of endocannabinoid receptors expressed on GABA-releasing neurons which transmit signals to amygdala neurons, has been shown to dampen GABA signals (Katona et al., 2001). Put simply, if cortisol somehow decreases the activity of GABA-releasing neurons, the leash on amygdala's sensing cells become looser, causing the sensing cells to fire more often, and causing PTSD connections to strengthen. To elucidate this mechanism of cortisol-mediated GABA-releasing cell suppression, electrophysiology was carried out on slices containing amygdala neurons from both non-stressed and stressed (via acute restraint) rats.

Neurons from non-stressed rats were treated with artificial cortisol (dexamethasone), which produced a significant decrease in inhibitory signals; a similar effect was observed in the stressed condition where endogenous cortisol would take effect. Subsequent washing of the slices did not affect this reduction in electrical activity, suggesting that cortisol triggers a downstream effect. Interestingly, the fast-acting nature of this cortisol effect, and that the application of gene transcription and protein synthesis inhibitors, as well as glucocorticoid and mineralocorticoid receptor blockers, do not prevent this reduction of inhibitory electrical activity: there is strong evidence to suggest that this mechanism occurs via membrane-bound receptors, similar to those thought to exist on hippocampal neurons (Roozendaal et al., 2010). In short, drugs which block the slow-acting cortisol receptors did not stop the reduced GABA inhibition effect; neither did washing the cortisol away from the cells. This implies that cortisol produces this effect by interacting with fast-acting receptors on the

amygdala cells' surfaces, not at slow-acting receptors within the cell.

The G-protein coupled receptor blocker GDP-B-S however, did abolish the suppressed inhibition, suggesting that this effect is mediated via a G-protein coupled receptor pathway. G-protein coupled receptors have a slower effect than other receptor types and cause a complex chain reaction to mediate their effects on cells after a receptor is activated on the outside of the cell (Yang et al., 2021). Application of an endocannabinoid receptor blocker also prevented the effect, as well as pharmacological inhibition of diaglycerol lipase, a key enzyme involved in the synthesis of the endocannabinoid messenger 2-AG. This would imply that endocannabinoid chemicals are involved in this chain reaction caused by cortisol, specifically 2-AG: this may seem counterintuitive as endocannabinoids are generally 'anti-stress' chemicals. In this case 2-AG is a chemical which 'relaxes' the GABA neurons which would typically protect us from developing PTSD fear memories, therefore indirectly contributing to PTSD symptoms.

To confirm that GABA cell inhibition was indeed an effect of endogenous stress in vivo, a drug was injected prior to restraint to prevent cortisol synthesis. This again blocked the effect, confirming this is indeed caused by cortisol. For behaviour experiments, an increased level of anxiety was inferred from the length of time spent at the centre of the open field during the open field test. Following acute restraint, drugs were injected directly to the amygdala via surgically inserted cannula. Disruption of the endocannabinoid system and 2-AG synthesis reduced anxiety behaviour; suggesting the reduction in synaptic inhibition exacerbates anxiety behaviour. Anxiety behaviour (indicative of strong fear memory) increases as amygdala connections strengthen, which occurs due to the loosening of the 'leash' kept by GABA-releasing neurons; a process which clearly involves cortisol and 2-AG.

Overall, it is thought that cortisol acts upon receptors on the cells of the amygdala, leading to an intracellular increase in the endocannabinoid 2-AG. This then binds an endocannabinoid receptor to reduce the release of GABA by an unknown mechanism. This reduction in synaptic inhibition likely increases the excitability of amygdala neurons, similar to the effect observed in the hippocampus induced by endocannabinoid receptor activators (Katona et al., 1999). In simpler terms, cortisol attaches to the surface of the cells of the amygdala. This leads to a chain reaction which causes the concentration of endocannabinoid chemical 2-AG to increase inside amygdala cells, which then release 2-AG. 2-AG interacts with endocannabinoid CB1 receptors on the GABA-releasing cells which typically 'leash' the activity of the amygdala, dampening their activity. As this leash loosens, the cells of the amygdala send more electrical signals during the traumatic event, leading to the formation of the strong fear memories and anxiety associated with PTSD.

Interaction with Neurotransmitter Systems: Noradrenaline

In addition to GABA and glutamate, there is another key chemical signal which interacts with the amygdala, noradrenaline, which is typically associated with alertness and memory formation (Ranjbar-Slamloo and Fazlali, 2020). An abundance of previous work investigates the role of the noradrenaline system in memory consolidation, both in the hippocampus (Kobayashi and Yasoshima, 2001; Hansen, 2017) and within the amygdala (Roozendaal et al., 2006). It is understood that the amygdala receives noradrenaline input from a region known as the locus coeruleus, which is thought to promote anxiety behaviour (McCall et al., 2017). There is evidence to suggest that the impact of noradrenaline upon amygdala plasticity is modulated by cortisol, again via the endocannabinoid system. Atsak et al. (2014) studied this relationship in greater depth using drugs administered to the amygdala's sensing nuclei via cannula following inhibitory avoidance training.

Throughout the investigation, the 'strength' of the fear memory induced by inhibitory avoidance was inferred by the 'retention latency': the period of time the rat would wait before entering the area where it previously received foot shock. If the rat waits longer, it is assumed it is more fearful of the foot shock; mirroring the strong trauma-associated fear memories experienced in PTSD. Similar to the findings of Di et al. (2016), injection of a glucocorticoid receptor activator resulted in dose-dependent enhancement of retention latency and therefore fear memory. This was prevented by administration of endocannabinoid receptor blockers, implying that endocannabinoids again play a maladaptive role in PTSD. Blocking glucocorticoid receptors and applying endocannabinoid receptor activators also enhanced fear memory, again demonstrating the importance of the relationship between cortisol and endocannabinoids in fear memory strengthening.

The administration of the membrane impermeable CORT-BSA (cortisol bound to cell serum molecules) produced a similar enhancement, suggesting that cortisol acts upon extracellular receptors to enhance memory in the amygdala, rather than their intracellular counterparts. For context, using CORT-BSA instead of regular cortisol is like making a cortisol key which cannot enter the house and can only be used at the front door, even if it technically fits other doors inside the house. Cortisol-driven endocannabinoid modulation of the noradrenaline system was investigated by injection of noradrenaline receptor blocker propranolol or activator clenbuterol. As expected, injection of the endocannabinoid activator increased retention latency, though co-administration with propranolol blocked this increase. Interestingly, clenbuterol (which mimics noradrenaline) alone increased retention but only at low concentration (1 ng); higher concentrations (10/100 ng) did not produce this effect, except when co-administered with a glucocorticoid receptor blocker where 100ng was required to increase retention. This effect was observed also with endocannabinoid blockers. In summary, activating endocannabinoid receptors seems to strengthen fear memory, but only when noradrenaline activates the amygdala's sensing cells. Fear memories are also strengthened by noradrenaline, but only at low concentrations, and it would appear cortisol works together with noradrenaline to produce this fear memory strengthening effect.

Cell staining was used to investigate the role of cortisol in facilitating changes in cell shape specifically, testing for proteins CAMKII and phosphorylated CREB (pCREB), as markers for amygdala sensory neurons and increased synaptic plasticity respectively. Subcutaneous injection of cortisol significantly increased the number of pCREB positive neurons, whereas co-administration with endocannabinoid blockers prevents this increase. Overall, cortisol increases synaptic plasticity in the sensing nuclei of the amygdala by activating CREB, and blocking the endocannabinoid system stops this process; emphasising the importance of endocannabinoids in the overall mechanism.

Interestingly, further study has revealed that noradrenergic modulation of amygdala plasticity may play a role in memory impairment associated with PTSD, leading to a weakening of neural connections at higher concentrations (Liu et al., 2019). This would confirm that the strengthening of fear memory occurs only at lower concentrations of noradrenaline, however, there is no note of decreased retention latency when a higher concentration of clenbuterol is administered (Atsak et al., 2014). Overall, this discrepancy is perhaps an indication of the

concentration and time-sensitive nature of PTSD plasticity in the amygdala, which may account for the seemingly contrasting evidence on the role of cortisol in amygdala plasticity in general. As well as the brain region itself, timing and concentration of cortisol release appear to be vitally important for determining the synaptic changes which occur.

Summarised Mechanism for The Harmful Effects of Cortisol

Combining findings from Atsak et al. (2014) and Di et al. (2016) described in Figure 2, it is likely that cortisol brings about adverse structural changes in the amygdala, both directly and indirectly via the endocannabinoid system, which impacts the release of other chemical brain signals, such as noradrenaline and GABA. Endocannabinoid receptors can be activated by multiple brain chemicals, but recent evidence reinforces the role of 2-AG specifically in mediating these harmful effects in the amygdala, as opposed to AEA (Di et al., 2016; Yasmin et al., 2020). Loosening the leash of GABA inhibition on the amygdala will likely increase excitation of amygdala neurons by both glutamate and noradrenaline, potentially contributing to the delayed structural effects observed by Zhang et al. (2019).

In summary, traumatic stress causes cortisol to enter the brain, which interacts with fast-acting glucocorticoid receptors on the surface of amygdala neurons. This interaction results in the amygdala synthesising more of the endocannabinoid chemical 2-AG, which then activates endocannabinoid receptors on GABA-releasing neurons to reduce GABA release and thus 'loosen the leash' on amygdala neurons; leaving amygdala neurons more open to receiving signals from other neurons and reducing inhibition of nearby noradrenaline-releasing neurons. This allows noradrenaline-releasing cells near the amygdala to release more noradrenaline. Noradrenaline combines with cortisol released during stress to increase the activity of messenger proteins PKA and cAMP within the amygdala cell. As the activity of these messengers increases, the gene regulator pCREB becomes more active in the cell: a protein which 'switches' genes on or off to suit the needs of the cell. In this case, it is likely that pCREB 'switches on' genes which allow the cell to grow more dendritic spines (Kida et al., 2012), allowing more connections between cells to be created. As more connections are formed in the amygdala, the fear memory associated with the traumatising event becomes stronger, contributing to the development of PTSD.

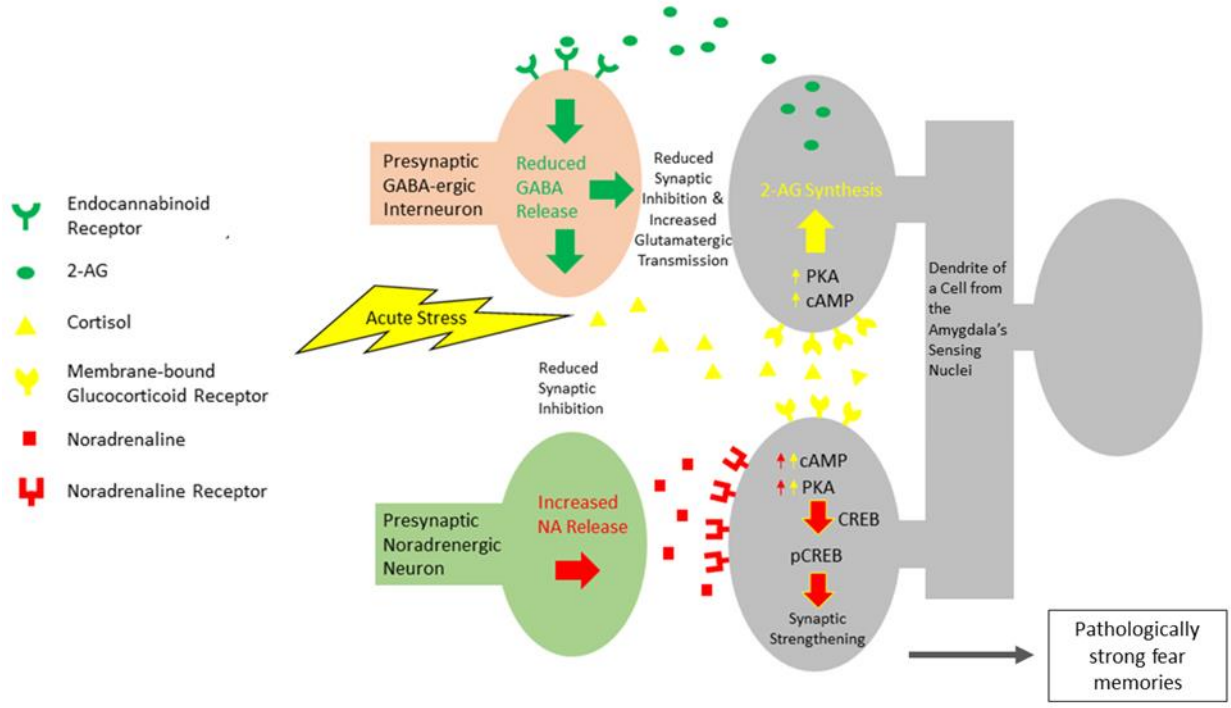


Figure 2: Action of cortisol on amygdala brain cells. Proposed mechanistic diagram of cortisol action on amygdala neurons to enhance synaptic strengthening, combining the findings of Di et al. (2016) and Atsak et al. (2014)—describing modulation of GABA

Cortisol "Protects" Against PTSD-associated Amygdala Plasticity

As mentioned previously, investigations into the effect of cortisol on amygdala plasticity can appear confusing and contradictory. Despite the clear mechanistic role of cortisol in facilitating PTSD-associated plasticity following acute stress, some recent studies suggest that under certain circumstances cortisol may prevent the delayed effects in the amygdala following acute stress (Chakraborty and Chattarji, 2019; Chakraborty et al., 2020) and therefore may have therapeutic potential in PTSD. This is supported by the lower baseline blood-cortisol levels in PTSD patients (Wahbeh and Oken, 2013), as well as lower endocannabinoid levels (Witteveen et al., 2010). Evidence from both rodent studies (Chakraborty et al., 2020) and human clinical studies suggest administration of cortisol shortly after a traumatic experience may prevent formation of a pathological fear memory (Zohar et al., 2011).

Corticosterone Helps Prevent Delayed Amygdala Plasticity in Rodents

Chakraborty et al. (2020) investigated the protective properties of cortisol following a 2-hour restraint stress in rats, measuring

anxiety behaviour, amygdala cell dendritic spine density and cortisol levels 10 days after acute stress, in accordance with previous studies investigating delayed amygdala plasticity (Zhang et al., 2019). Cortisol was administered 24 hours after immobilisation stress, orally via drinking water, as opposed to more stressful means of delivery which may interfere with naturally-occurring cortisol release. General anxiety and social anxiety behaviour were inferred from a maze exploration task as well as interactions with a juvenile rat, respectively. Stressed rats given the drug vehicle spent significantly less time in the open maze and interacted less with the juvenile rats, than stressed rats given cortisol treatment. This was reflected in the spine quantification analysis also, where the delayed increase in spine density at these neurons did not occur in the stressed cortisol group. Interestingly, blood-cortisol levels were also significantly lower in the stressed cortisol group than the vehicle group, suggesting that cortisol administration soon after a traumatic event may prevent delayed effects by disrupting delayed increase in cortisol levels, which would normally contribute to strengthened fear memory according to prior studies, as previously explored. This may account for some of the contrasting evidence involving the role of cortisol in PTSD pathology. Interestingly, a prior study produced similar findings, but administered cortisol 12 hours before acute stress rather than afterwards (Rao et al., 2012), providing some insight into the time window by which cortisol intervention might be protective.

A key difference between these studies and those mentioned when exploring how cortisol might enhance synaptic strengthening, is the delivery of cortisol. Injection directly to the sensing nuclei of the amygdala would not produce the same effect as oral administration, which would allow cortisol to interact with other systems and regions of the central nervous system before it reaches the amygdala. Unlike direct injection to the amygdala, this could allow for the effect on delayed cortisol release.

Involvement of Endocannabinoids in Cortisol Protection

As mentioned previously, acute traumatic stress specifically produces delayed synaptic strengthening in the amygdala (Zhang et al., 2019). Recent studies which investigate the mechanism by which cortisol may prevent this effect are sparse. However, perhaps this mechanism may be inferred by investigation into the role of the endocannabinoid system in this process given that it is likely that cortisol indirectly modulates plasticity after acute stress primarily through endocannabinoid release (Atsak et al., 2014; Di et al., 2016).

A recent study investigated the potentially protective role of endocannabinoid AEA in the delayed plasticity observed following acute stress (Yasmin et al., 2020). This may be useful as cortisol has previously been shown to increase amygdala AEA levels during stress (Hill et al., 2010). Similar to Atsak et al., (2014) acute restraint stress increased excitatory electrical activity within amygdala neurons shortly after stress which we understand leads to stronger connections between cells (Sala and Segal, 2014), effectively strengthening the fear memory. Although interestingly, blocking endocannabinoid receptors increased electrical activity in the amygdala in the absence of stress; suggesting that endocannabinoid receptor activation may have the opposite effect than initially suggested. Although perhaps this ‘stress-mimicking effect’ was exacerbated by the stress of injection itself. Overall, the endocannabinoid system is more complex than just an ‘anti-stress’ system, but there is evidence to suggest protective ‘anti-stress’ properties which stop pathologically strong fear memories being formed in PTSD.

Yasmin et al. (2020) focused on fatty acid amide hydrolase, an enzyme involved in the breakdown of AEA, exploring a potential separate mechanism from that seen with 2-AG. By inhibiting the chemical breakdown of AEA, the levels of AEA become increased in the brain, allowing its function to be investigated. Chemically preventing the synthesis of 2-AG ruled out the action of 2-AG, which did not significantly influence the increase in excitatory electrical activity observed.. Therefore it was concluded that this protective effect is mediated by AEA only.

Next, the AEA breakdown inhibitor was administered orally via food supply. This resulted in increased AEA levels within the isolated amygdala, which were initially found to be decreased following acute stress. To determine if AEA acts on endocannabinoid receptors to prevent the stress-induced excitation effect, an endocannabinoid receptor blocker was injected into rats previously injected with the AEA breakdown inhibitor as the restraint stress began. This is similar to having an abundance of keys, but the doors’ locks are all filled with cement; making unlocking the doors impossible despite the presence of fitting keys. The effect on the amygdala neurons was not significantly different from that seen in the stressed control rats without drug administration, indicating that the stress-protective effect is indeed mediated by AEA at endocannabinoid receptors. Interestingly, this treatment had no effect on the amygdala neurons’ excitatory electrical activity, reinforcing the proposed mechanism of GABAergic synaptic inhibition by 2-AG (see Figure 2), but not AEA, observed by Di et al., (2016), especially as 2-AG levels in the amygdala are raised following stress (Yasmin et al., 2020).

They then proceeded to determine the impact of AEA breakdown inhibition on the increased spine density observed 10 days later following acute stress; applying the AEA breakdown inhibitor for the 4 days approaching restraint stress, then counting the number of dendritic spines on the amygdala neurons. This treatment prevented the increase in the amygdala spine density caused by the restraint stress, suggesting that AEA action not only protects against the immediate effects of acute stress, but also the delayed effects associated with PTSD. AEA stops the amygdala’s cells from growing more connections after traumatic stress, which would imply the prevention of a strong fear memory being formed. To increase clinical relevance, it may have been useful to also conduct behavioural testing on day 10 to determine whether the effect of AEA breakdown inhibitors on synaptic strengthening correlates with anxiety behaviour.

Overall, it was concluded that AEA, which is intrinsically related to cortisol release (Hill et al., 2010), prevents the effects of acute stress by limiting glutamate release on the sensing neurons of the amygdala, such that cell excitability does not increase therefore preventing synaptic strengthening. This contrasts with 2-AG, which is thought to have a similar effect on GABA-releasing inhibitory neurons, subsequently increasing neuronal excitability (Di et al., 2016). It is suggested that this is due to AEA possessing a preference for neurons which release glutamate, whilst 2-AG prefers GABA-releasing neurons despite the cortisol-induced release of both (Yasmin et al., 2020).

Insights from Clinical Trials

Findings from PTSD clinical trials may not provide insight into the structural changes which occur in the amygdala, but they are the most relevant for investigating the potential therapeutic effects of cortisol in PTSD. A key human study involving patients admitted shortly after experiencing a traumatic event investigated the effects of injected cortisol on PTSD symptoms in a double-blind placebo-controlled trial (Zohar et al., 2011).

Patients would receive 4 psychological check-ups to assess PTSD symptom severity: prior to treatment, 2 weeks following treatment, 1 month following treatment, and 3 months following treatment. Severity was scored using the Clinician Administered PTSD Scale (CAPS). The CAPS scores were consistently lower for the cortisol-treated group in comparison to the placebo-treated group. In addition to this, fewer cortisol-treated patients (20%) were subsequently diagnosed with acute stress reaction than placebo (66.7%).

Though this study does not refer to the sensing nuclei of the amygdala, it was suspected that the injection of a high dose of cortisol (100-140mg) resets the brain's stress response via its negative feedback loop (Zohar et al., 2011), which could in turn prevent the delayed increase in cortisol associated with the synaptic strengthening in the amygdala. This idea is similar to a fuse protecting a computer from being overloaded by electricity, the system recognises it is receiving too much input and shuts off its access to the main power supply by burning the fuse. Overall, cortisol treatment seems to have potential, not only in preventing PTSD-associated synaptic changes in rodent models, but also in preventing the development of PTSD symptoms in human patients.

CONCLUSION

This review aimed to address the gap in understanding regarding the cellular and molecular action of the stress hormone cortisol, whose action leads to brain changes in the amygdala and subsequently affects the experience of fear memory in PTSD patients. Overall, the relationship between cortisol action and the PTSD-associated delayed synaptic strengthening observed in the amygdala is complex and likely dependent upon the timing and concentration of cortisol released during the traumatic event. Cortisol does appear to play a role in enhancing the delayed synaptic strengthening observed in the amygdala via interaction with the endocannabinoid system, upregulating synthesis and release of the endocannabinoid 2-AG after binding to slow-acting attachment sites on the cell surface. However, it is also true that cortisol administration before or shortly after acute stress prevents these delayed changes, likely by disrupting the delayed increase in cortisol levels seen after acute stress. Cortisol's relationship with the endocannabinoid system may also play a role in this, particularly AEA, but the extent to which it depends on cortisol needs clarification. Additionally, an explanation for such contrasting effects on amygdala synaptic strengthening could be the time points at which high concentrations of cortisol are released by the body following a traumatic event.

From the current literature, it appears the next steps in understanding how we might take advantage of the cortisol/endocannabinoid system therapeutically would be to identify the critical time points in which cortisol might be protective. Future studies may explore at which time point cortisol administration contributes to the enhancement of fear memory and therefore worsening of PTSD, as we know it does, rather than protecting against it. In addition to clarifying time-points, further studies should also investigate the long-term impact of the endocannabinoid-mediated mechanism, as opposed to immediate changes observed following acute stress. For example, it may be more useful to focus on changes in neuron dendritic spine density and shape, rather than measuring excitability in amygdala neurons 10 days after acute stress whilst administering drugs (via cannula) to target the endocannabinoid system during the consolidation phase. This would even allow for drug administration at different time points following acute stress, making it possible to build a timeline of amygdala synaptic strengthening and elucidate a potential window of opportunity for pharmacological intervention in the case of PTSD.

Moving towards exploring new PTSD therapies, it would also be useful to investigate the differences between endocannabinoid chemicals AEA and 2-AG in the process of fear memory formation, given their seemingly opposite effects despite both acting upon endocannabinoid receptors. Specifically, AEA breakdown inhibitors would be a candidate for preventive therapy following trauma. Although behavioural studies have not been carried out to determine whether this effect might reduce PTSD symptoms; further study is required. Overall, further study into the delayed neural changes in the amygdala as result of cortisol and endocannabinoids may allow for preventative pharmacological therapies which stop the formation of pathological fear memory shortly after trauma.

REFERENCES

Akirav, I. (2004). "A Facilitative Role for Corticosterone in the Acquisition of a Spatial Task Under Moderate Stress." *Learning & Memory* **11** (2), pp. 188–195.

Andolina, D., and Borreca, A. (2017). "*The Key Role of the Amygdala in Stress*" in *The Amygdala - Where Emotions Shape Perception, Learning and Memories,* IntechOpen, London. pp. 188–199.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., Fornari, R.V., and Roozendaal, B. (2014). "Endocannabinoid Signaling within the Basolateral Amygdala Integrates Multiple Stress Hormone Effects on Memory Consolidation." *Neuropsychopharmacology* **40** (6), pp. 1485–1494.

Chaaya, N., Battle, A.R., and Johnson, L.R. (2018). "An update on contextual fear memory mechanisms: Transition between Amygdala and Hippocampus." *Neuroscience & Biobehavioral Reviews* **92**, pp. 43–54.

Chakraborty, P., and Chattarji, S. (2019). "Interventions after acute stress prevent its delayed effects on the amygdala." *Neurobiology of Stress* **10**, article no: 100168 [no pagination].

Chakraborty, P., Datta, S., McEwen, B.S., and Chattarji, S. (2020). "Corticosterone after acute stress prevents the delayed effects on the amygdala." *Neuropsychopharmacology* **45** (13), pp 2139–2146.

Chidambaram, S.B., Rathipriya, A.G., Bolla, S.R., Bhat, A., Ray, B., Mahalakshmi, A.M., Manivasagam, T., Thenmozhi, A.J., Essa, M.M., Guillemin, G.J., et al. (2019). "Dendritic spines: Revisiting the physiological role." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **92**, pp. 161–193.

Cohen, K., Weizman, A., and Weinstein, A. (2019). "Modulatory effects of cannabinoids on brain neurotransmission." *European Journal of Neuroscience* **50** (3), pp. 2322–2345.

Di, S., Itoga, C.A., Fisher, M.O., Solomonow, J., Roltsch, E.A., Gilpin, N.W., and Tasker, J.G. (2016). “Acute Stress Suppresses Synaptic Inhibition and Increases Anxiety via Endocannabinoid Release in the Basolateral Amygdala.” *Journal of Neuroscience* **36** (32), pp. 8461–8470.

Duvarci, S., and Pare, D. (2007). “Glucocorticoids Enhance the Excitability of Principal Basolateral Amygdala Neurons.” *Journal of Neuroscience* **27** (16), pp. 4482–4491.

El-Farhan, N., Rees, D.A., and Evans, C. (2017). “Measuring cortisol in serum, urine and saliva – are our assays good enough?” *Annals of Clinical Biochemistry* **54** (3), pp. 308–322.

Fang, Q., Li, Z., Huang, G.-D., Zhang, H.-H., Chen, Y.-Y., Zhang, L.-B., Ding, Z.-B., Shi, J., Lu, L., and Yang, J.-L. (2018). “Traumatic Stress Produces Distinct Activations of GABAergic and Glutamatergic Neurons in Amygdala.” *Frontiers in Neuroscience* **12**, article no: 387 [no pagination].

Friedman, M.J., and Bernardy, N.C. (2017). “Considering future pharmacotherapy for PTSD.” *Neuroscience Letters* **649**, pp.181–185.

Hansen, N. (2017). “The Longevity of Hippocampus-Dependent Memory Is Orchestrated by the Locus Coeruleus-Noradrenergic System.” *Neural Plasticity* **2017**, pp. 1–9.

Hill, M.N., McLaughlin, R.J., Morrish, A.C., Viau, V., Floresco, S.B., Hillard, C.J., and Gorzalka, B.B. (2009). “Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* **34** (13), pp. 2733–2745.

Hill, M.N., Karatsoreos, I.N., Hillard, C.J., and McEwen, B.S. (2010). “Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo.” *Psychoneuroendocrinology* **35** (9), pp. 1333–1338.

Hui, G. (2004). “Memory enhancement of classical fear conditioning by post-training injections of corticosterone in rats.” *Neurobiology of Learning and Memory* **81** (1), pp. 67–74.

Izquierdo, I., Furini, C.R.G., and Myskiw, J.C. (2016). “Fear Memory.” *Physiological Reviews* **96** (2), pp. 695–750.

Johansen, Joshua P., Cain, Christopher K., Ostroff, Linnaea E., and LeDoux, Joseph E. (2011). “Molecular Mechanisms of Fear Learning and Memory.” *Cell* **147** (3), pp. 509–524.

Karst, H., Berger, S., Erdmann, G., Schutz, G., and Joels, M. (2010). “Metaplasticity of amygdalar responses to the stress hormone corticosterone.” *Proceedings of the National Academy of Sciences* **107** (32), pp. 14449–14454.

Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E.S., Mackie, K., and Freund, T.F. (1999). “Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **19** (11), pp. 4544–4558.

Katona, I., Rancz, E.A., Acsády, L., Ledent, C., Mackie, K., Hájos, N., and Freund, T.F. (2001). “Distribution of CB1 Cannabinoid Receptors in the Amygdala and their Role in the Control of GABAergic Transmission.” *The Journal of Neuroscience* **21** (23), pp. 9506–9518.

Kida, S. (2020). “Function and mechanisms of memory destabilization and reconsolidation after retrieval.” *Proceedings of the Japan Academy*, **96** (3), 95–106.

Kida, S. (2012). “A Functional Role for CREB as a Positive Regulator of Memory Formation and LTP.” *Experimental Neurobiology* **21** (4), pp. 136-140.

Kim, J., and Fanselow, M. (1992). “Modality-specific retrograde amnesia of fear.” *Science* **256** (5057), pp. 675–677.

Kobayashi, K., and Yasoshima, Y. (2001). “The Central Noradrenaline System and Memory Consolidation.” *The Neuroscientist* **7** (5), pp. 371–376.

Lewis, S.J., Arseneault, L., Caspi, A., Fisher, H.L., Matthews, T., Moffitt, T.E., Odgers, C.L., Stahl, D., Teng, J.Y., and Danese, A. (2019). “The epidemiology of trauma and post-traumatic stress disorder in a representative cohort of young people in England and Wales.” *The Lancet Psychiatry* **6** (3), 247–256.

Lisieski, M.J., Eagle, A.L., Conti, A.C., Liberzon, I., and Perrine, S.A. (2018). “Single-Prolonged Stress: A Review of Two Decades of Progress in a Rodent Model of Post-traumatic Stress Disorder”. *Frontiers in Psychiatry* **9**, article no: 196 [no pagination].

Liu, X.-H., Zhu, R.-T., Hao, B., Shi, Y.-W., Wang, X.-G., Xue, L., and Zhao, H. (2019). “Norepinephrine Induces PTSD-Like Memory Impairments via Regulation of the β-Adrenoceptor-cAMP/PKA and CaMK II/PKC Systems in the Basolateral Amygdala*.” Frontiers in Behavioral Neuroscience* **13**, article no: 43 [no pagination].

McCall, J.G., Siuda, E.R., Bhatti, D.L., Lawson, L.A., McElligott, Z.A., Stuber, G.D., and Bruchas, M.R. (2017). “Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior.” *ELife* **6**, article no: 18247 [no pagination]

Mifsud, K.R., and Reul, J.M.H.M. (2018). “Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain.” *Stress* **21** (5), pp. 389–402.

Mohammad, G., Chowdhury, I., Fujioka, T., and Nakamura, S. (2000). “Induction and adaptation of Fos expression in the rat brain by two types of acute restraint stress.” *Brain Research Bulletin* **52** (3), pp. 171–182.

Morena, M., Patel, S., Bains, J.S., and Hill, M.N. (2016). “Neurobiological Interactions Between Stress and the Endocannabinoid System.” *Neuropsychopharmacology* **41**, pp. 80–102.

NHS Information Centre for Health and Social Care (2009). “Adult psychiatric morbidity in England, 2007: results of a household survey.” (Leeds, NHS Information Centre for Health and Social Care.)

Phillips, R., and LeDoux, J. (1995). “Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning.” *The Journal of Neuroscience* **15** (7), pp. 5308–5315.

Phillips, R.G., and LeDoux, J.E. (1992). “Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning.” *Behavioral Neuroscience* **106** (2), pp. 274–285.

Ranjbar-Slamloo, Y., and Fazlali, Z. (2020). “Dopamine and Noradrenaline in the Brain; Overlapping or Dissociate Functions?” *Frontiers in Molecular Neuroscience* **12**, article no: 334 [no pagination].

Rao, R.P., Anilkumar, S., McEwen, B.S., and Chattarji, S. (2012). “Glucocorticoids Protect Against the Delayed Behavioral and Cellular Effects of Acute Stress on the Amygdala.” *Biological Psychiatry* **72** (6), pp. 466–475.

Roozendaal, B., and McGaugh, J.L. (1997). “Glucocorticoid Receptor Agonist and Antagonist Administration into the Basolateral but Not Central Amygdala Modulates Memory Storage.” *Neurobiology of Learning and Memory* **67** (2), pp. 176–179.

Roozendaal, B., Okuda, S., Van der Zee, E.A., and McGaugh, J.L. (2006). “Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala.” *Proceedings of the National Academy of Sciences* **103** (17), pp. 6741–6746.

Roozendaal, B., Hernandez, A., Cabrera, S.M., Hagewoud, R., Malvaez, M., Stefanko, D.P., Haettig, J., and Wood, M.A. (2010). “Membrane-Associated Glucocorticoid Activity Is Necessary for Modulation of Long-Term Memory via Chromatin Modification.” *Journal of Neuroscience* **30** (14), pp. 5037–5046.

Ruehle, S., Rey, A.A., Remmers, F., and Lutz, B. (2011). “The endocannabinoid system in anxiety, fear memory and habituation.” *Journal of Psychopharmacology* **26** (1), pp. 23–39.

Sala, C., and Segal, M. (2014). “Dendritic Spines: The Locus of Structural and Functional Plasticity*.” Physiological Reviews* **94** (1), pp. 141–188.

Sanders, M.J., Wiltgen, B.J., and Fanselow, M.S. (2003). “The place of the hippocampus in fear conditioning.” *European Journal of Pharmacology* **463** (1-3), pp. 217–223.

Sandi, C. (2011). “Glucocorticoids act on glutamatergic pathways to affect memory processes.” *Trends in Neurosciences* **34** (4), pp. 165–176.

Sapolsky, R.M. (2000). “Glucocorticoids and Hippocampal Atrophy in Neuropsychiatric Disorders.” *Archives of General Psychiatry* **57** (10), pp. 925-935

Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., and Nishikawa, Y. (2004). “Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults.” *Archives of Oral Biology* **49** (12), pp. 963–968.

Wahbeh, H., and Oken, B.S. (2013). “Salivary Cortisol Lower in Posttraumatic Stress Disorder.” *Journal of Traumatic Stress* **26** (2), pp. 241–248.

WHO (1992). “International statistical classification of diseases and related health problems: ICD-10.” (Geneva: World Health Organization).

Wiltgen, B.J. (2006). “Context Fear Learning in the Absence of the Hippocampus.” *Journal of Neuroscience* **26** (20), pp. 5484–5491.

Witteveen, A.B., Huizink, A.C., Slottje, P., Bramsen, I., Smid, T., and van der Ploeg, H.M. (2010). “Associations of cortisol with posttraumatic stress symptoms and negative life events: A study of police officers and firefighters.” *Psychoneuroendocrinology* **35** (7), pp. 1113–1118.

Wood, G.E., Young, L., Trevor., Reagan, L.P., and McEwen, B.S. (2003). “Acute and chronic restraint stress alter the incidence of social conflict in male rats.” *Hormones and Behavior* **43** (1), pp. 205–213.

Yang, D., Zhou, Q., Labroska, V., Qin, S., Darbalaei, S., Wu, Y., Yuliantie, E., Xie, L., Tao, H., Cheng, J., et al. (2021). “G protein-coupled receptors: structure- and function-based drug discovery.” *Signal Transduction and Targeted Therapy* **6**, article no. 7 [no pagination].

Yasmin, F., Saxena, K., McEwen, B.S., and Chattarji, S. (2016). “The delayed strengthening of synaptic connectivity in the amygdala depends on NMDA receptor activation during acute stress.” *Physiological Reports* **4** (20), article no. 13002 [no pagination].

Yasmin, F., Colangeli, R., Morena, M., Filipski, S., van der Stelt, M., Pittman, Q.J., Hillard, C.J., Teskey, G.C., McEwen, B.S., Hill, M.N., et al. (2020). “Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala.” *Proceedings of the National Academy of Sciences of the United States of America* **117** (1), pp. 650–655.

Zhang, H.-H., Meng, S.-Q., Guo, X.-Y., Zhang, J.-L., Zhang, W., Chen, Y.-Y., Lu, L., Yang, J.-L., and Xue, Y.-X. (2019). “Traumatic Stress Produces Delayed Alterations of Synaptic Plasticity in Basolateral Amygdala.” *Frontiers in Psychology* **10**, article no. 2394 [no pagination].

Zhou, M., Hoogenraad, C.C., Joëls, M., and Krugers, H.J. (2011). "Combined β-adrenergic and corticosteroid receptor activation regulates AMPA receptor function in hippocampal neurons." *Journal of Psychopharmacology* **26** (4), pp. 516–524.

Zohar, J., Yahalom, H., Kozlovsky, N., Cwikel-Hamzany, S., Matar, M.A., Kaplan, Z., Yehuda, R., and Cohen, H. (2011). "High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: Interplay between clinical and animal studies." *European Neuropsychopharmacology* **21** (11), pp. 796–809.