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THE EFFECTS OF EARLY LIFE STRESS ON LONG-TERM POTENTIATION IN PATHWAY FROM THE MEDIAL PREFRONTAL CORTEX TO THE BASOLATERAL AMYGDALA

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Abstract

A leading neurocircuitry model of emotional regulation points to the pathway from the medial prefrontal cortex (mPFC) to the basolateral amygdala (BLA). This pathway has been implicated in fear conditioning and extinction studies and its malfunction is hypothesized to underlie affective disorders such as PTSD and anxiety. Interestingly, the mPFC-BLA pathway shows delayed maturation in both humans and rats, rendering it vulnerable to early life stress (ELS). Indeed, several studies have linked ELS to emotional dysregulation as well as changes in the amygdala and PFC. However, no study has ever been done on the effect of ELS on long-term potentiation (LTP) in this pathway. In fact, very few studies on LTP in the mPFC-BLA pathway have been conducted at all which is surprising given LTP's role in learning and memory and given the mPFC-BLA pathway's proposed role in fear conditioning/extinction. Therefore, using electrophysiological methods in awake, freely behaving rats, the current study examined whether ELS in the form of neonatal isolation (ISO) affects LTP in the mPFC-BLA pathway. Results indicate that the mPFC-BLA pathway is resistant to LTP in both control and ISO rats following both sustained and theta burst high frequency stimulation (HFS). In fact, rats showed a tendency toward long-term depression (LTD) especially following sustained stimulation at 200 Hz. Small sample sizes prevented a meaningful comparison of LTD across ISO and control groups.

Introduction

The prevalence of affective disorders (e.g. anxiety, depression, PTSD) within our society has spurred a wealth of research on their underlying pathology. From this research, two brain structures---the amygdala and the prefrontal cortex (PFC)---have emerged as major players in the emotional game. The amygdala is well known for its involvement in generating emotions such as fear and anxiety. The prefrontal cortex, on the other hand, is a highly evolved brain structure associated with executive function, goal-directed behavior, and top-down cognition, and it is thought to be responsible for regulating emotions. The part of the PFC most connected to the amygdala is the medial PFC (mPFC) which synapses onto its basolateral aspects (BLA). Several lines of evidence support the idea that irregularities in mPFC-BLA communication may be the major underlying pathology of affective disorders. Interestingly, the PFC-BLA pathway shows delayed maturation and both brain structures continue to develop in early adolescence. This delayed developments renders the PFC-BLA pathway vulnerable to early life stress (ELS). Indeed, ELS has been linked to affective disorders in adulthood as well as abnormalities within and between the PFC and amygdala. Despite all of this, no study has ever been done on the effects of ELS on long-term potentiation in the mPFC-BLA pathway. LTP---an increase in synaptic strength following high frequency stimulation of presynaptic neurons---is a form of synaptic plasticity and is important because it is thought to be the cellular basis for learning and memory within the brain. Given the mPFC-BLA's role in emotional regulation and given that much of this regulation likely occurs at the level of learning (i.e. knowing when to feel fear and when not to feel fear), it seems plausible that at least one way ELS disrupts emotional regulation is via disrupting LTP in the mPFC-BLA pathway. However, this hypothesis remains to be tested.

The Amygdala and Emotion

The amygdala, an almond-shaped brain structure abutting the hippocampus within the temporal lobe, has a well-established role in mediating emotion, especially fear and anxiety (Lang & Davis, 2006; LeDoux, 2000; Gallagher & Chiba, 1996). The first indication of the amygdala's role in emotion comes from its neuroanatomical wiring: the amygdala is perfectly situated to receive sensory and memory-related information and ideally wired to then "respond" to that information via projections to parts of the brain capable of eliciting bodily changes associated with emotion (e.g. altered heart rate, respiration, blood flow, body-movement, etc.). Specifically, the basolateral amygdala (BLA) consisting of the lateral, basal, and basomedial nuclei receives input from the thalamus, sensory cortices, and hippocampus---parts of the brain associated with sensation and memory (McDonald, 1998). The BLA then sends excitatory synapses onto the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST; otherwise known as the extended amygdala) which are in turn capable of eliciting the bodily responses via connections with the hypothalamus and brain stem. For example, the hypothalamus via connections to the pituitary gland regulates release of a number of hormones (e.g. CRH, ACTH, glucocorticoids, etc.) responsible for sympathetic autonomic nervous system activation (e.g. fight or flight) and are associated with feelings of emotion, and the brainstem via projections through the spinal cord regulates heart rate, blood pressure, and respiration (LeDoux et. al., 1988; Schwaber et. al. 1982). The CeA and BNST also appear to be responsible for the heightened attention accompanying emotional responses. For instance, during emotional stimulation, projections from the CeA and BNST to the ventral tegmental area mediate increases in dopamine metabolism in the prefrontal cortex, which may play a role in feelings of anticipation (Goldstein et. al., 1996). Furthermore, the CeA increases norepinephrine release from the locus coeruleus, which is

associated with heighted attention (Redmond, 1977; Aston-Jones et. al., 1996). Lastly, the CeA directly projects to the lateral dorsal tegmental nucleus and parabrachial nuclei which have cholinergic projections to the thalamus and could mediate increased synaptic transmission of sensory relay neurons (Lang & Davis, 2006). This would explain the feelings of heightened sensation during emotional events. Finally, the CeA and BNST also regulate changes in motor activity associated with emotion. The CeA's projections to the ventral periacqueductal gray appear to regulate fight/flight responses including the classic "freezing" seen in rodents and humans (Fanselow, 1991). Moreover, since norepinephrine and serotonin facilitate excitation of motor neurons, rapid defensive action could be mediated by lateral BNST activation of the locus coeruleus and serotonin-containing raphe neurons (McCall and Aghajanian, 1979; White and Neuman, 1980). Thus, overall, it appears that the amygdala via the BLA is able to gather information from the senses and from memory and produce an emotional response via the CeA and BNST consisting of altered heart rate, respiration, autonomic nervous system activation, attention, orientation, and motor behavior.

Besides its neuroanatomy, a number of other clues indicate the amygdala's importance for emotional responses. Namely, much information can be gleaned from amygdala stimulation studies. In humans, electrical stimulation of the amygdala or abnormal electrical activation during temporal-lobe seizures produces autonomic changes and emotional behavior that people generally describe as fear or apprehension (Chapman et. al., 1954; Gloor et. al., 1981). In animals, electrical or chemical stimulation of the amygdala produces heightened attention/orienting behavior, cardiovascular effects, increased cortisol and epinephrine blood levels, sustained changes in respiration, and possibly even stomach ulcers if stimulation is prolonged (Lang & Davis, 2006). Electrical stimulation also activates facial motor neurons that elicit jaw movements and may

mediate facial expressions of emotion (Furuta & Murakami, 1989). In direct contrast to electrical stimulation, lesion studies offer more clues of the amygdala's role in emotion. In general, amygdala lesions have been shown to create an overall tameness in animals and an increased trust humans (Goddard, 1964; Adolphs et. al., 1998). Moreover, in both humans and animals, amygdala lesions strongly impair responses to fearful stimuli and block a type of associative learning called fear conditioning (Davis, 2000). In fear conditioning, an initially neutral stimulus (e.g. tone) is paired with an aversive unconditioned stimulus (US; e.g. electric shock) until the subject learns to fear the neutral stimulus by itself. Once the animal learns to fear the neutral stimulus, it is called a conditioned stimulus (CS). After amygdala lesion, the subject fails to pair the neutral stimulus with the unconditioned aversive stimulus. Specifically, the BLA appears to be responsible for pairing the conditioned and unconditioned stimuli, whereas the CeA is responsible for orienting behavior and attentional processing during conditioning (Hatfield & Gallagher, 1995; Hatfield et. al., 1996). Lastly, drug infusion studies have also shown the amygdala's role in emotion. Namely, infusion of the inhibitory neurotransmitter GABA or GABA agonists such as valium into the amygdala reduces fear responses (Lang & Davis, 2006). By contrast, local amygdala infusion of GABA antagonists increases fear responses (Davis, 2000). Thus, multiple lines of evidence point to the amygdala as a crucial brain structure involved in emotion.

Prefrontal Cortex and Emotion

In addition to the amygdala, the prefrontal cortex (PFC), the most anterior portion of the cerebral cortex, has emerged as a main brain structure involved in emotion (Dixon et. al., 2017; Fuster, 2008; Miller & Cohen, 2001). Experimenters often operationally define the PFC as the region of the frontal lobe innervated by the mediodorsal nucleus of the thalamus, although it has also been defined as the granular region of the primate frontal lobe and the part of the frontal lobe

for which electrical stimulation does not produce movement. Furthermore, the PFC is often divided into a number of sub-regions: dorsolateral, ventrolateral, dorsomedial, ventromedial, and orbitofrontal (orbital refers to the most ventral portion of the PFC). Not only are these areas densely interconnected with each other, but also collectively, these areas have interconnections with virtually all sensory systems, with cortical and subcortical motor systems, with limbic and midbrain structures involved in affect, memory, and reward (Fuster, 2008). Thus, the PFC is ideally located to coordinate a wide range of neural activity as it receives inputs from numerous brain areas providing information about many internal and external states and reciprocally innervates these brain areas to be able to influence brain activity (Miller & Cohen, 2001). Indeed, converging data from neuropsychological and neurophysiological studies point to the PFC's role as a sort of command center in the brain. More accurately, the functional role most often attributed to the PFC is executive function, the cognitive control of behavior necessary for attainment of chosen goals (Cohen & Servan-Schreiber, 1992; Passingham, 1993; Grafman, 1994; Wise et. al., 1996; Miller, 1999). Executive function is associated with a type of information processing called top-down processing. Bottom-up processing encompasses simple, automatic behaviors (e.g. orienting towards a novel stimuli) that are determined largely by sensory stimuli and well-established neural pathways. Top-down processing, on the other hand, encompasses more complex behaviors that are largely guided by internal states or goals. Two classic cognitive psychology tests, the Stroop task and the Wisconsin card sort task (WCST), portray the top-down executive functioning of the PFC (Stroop, 1935; MacLeod, 1991). In the Stroop task, subjects either read words or name the color in which the words are written. Executive function comes into play when a subject must identify a conflicting stimulus---for example saying the word GREEN displayed in red. Here the subject has to fight the more reflexive urge to say red and control his/her behavior to

meet the desired goal. In the WCST, subjects sort cards according to shape, color, or number of symbols on the card, and the sorting rule varies periodically. Here, executive function comes into play when the subject must change his or her sorting according to the new rule despite familiarity with the old rule. During these tasks, fMRI data show that subject's PFCs are extremely active (Cromheeke & Mueller, 2014). Furthermore, subjects with PFC damage perform very poorly in these tests (Perrett, 1974; Cohen & Servan-Schreiber, 1992; Vendrell et. al., 1995; Cohen et. al., 1999; Milner, 1963).

Given the PFC's role in top-down processing and its extensive interconnections with limbic areas such as the amygdala, hippocampus, and rhinal cortices, the PFC is thought to play a crucial role in emotional regulation (Ochsner & Gross, 2005; Dixon et. al., 2017). Evidence from a number of different areas support this hypothesis. For example, PFC lesions in animals often lead to aggressiveness and voracious appetite, indicating the PFC's role in inhibiting limbic structures associated with those behaviors (Langworthy & Richter, 1939; Soltysik & Jaworska, 1967; Shustin, 1959; Brutkowski & Mempel, 1961). Substantial evidence in this regard has come from rodent studies in which orbital frontal lesions increase aggressiveness and lower the threshold for emotional reactions (e.g. rage) induced by hypothalamic stimulation (De Bruin et. al., 1983; De Bruin, 1990; Sato, 1971; Sato, et. al., 1971). Conversely, rodent prefrontal stimulation suppresses attack behavior and raises the threshold for inducing that behavior by hypothalamic stimulation (Siegel et. al., 1974; Kruk et. al., 1979). In monkeys, PFC lesions to different PFC areas often result in opposing emotional effects, indicating a segregation of emotional control within the PFC. For instance, orbital PFC lesions in monkeys often create apathy, an emotional blunting associated with indifference to others, lack of facial expression, and social withdrawal (Jacobsen et. al., 1935; Crawford et. al., 1948; Deets et. al., 1970; Myers, 1972, Franzen & Myers, 1973). By contrast,

lesions to the dorsolateral PFC tend to increase emotionality and aggressiveness, indicating different emotional regulation roles in different parts of the PFC (Brody and Rosvold, 1952; Kling & Mass, 1974; Miller, 1976). Congruent with primate data, human PFC lesions/damage is associated with a number of emotional symptoms including apathy, depression, euphoria, mania, and disinhibition/impulsivity (Paradiso et. al., 1999; Holmes, 1931; Greenblatt et. al., 1950; Cummings, 1993, Drevets, 2000, Lishman, 1968, Rolls, et. al., 1994). Human PFC lesions are also commonly associated with inhibition of theory of mind (ToM), the ability to infer the feelings, motives, opinions, and emotions of another being. Theory of mind includes both a cognitive aspect (i.e. thinking other people's thoughts) and an emotional one (i.e. feeling what other people are feeling: empathy). Large prefrontal lesions impair both components of ToM, but orbitofrontal/ventromedial lesions specifically impair the empathic component (Stone et. al., 1998; Rowe et. al., 2001; Shamay-Tsoory et. al., 2004). Final evidence for the PFC's role in emotional regulation comes from the fact that essentially all affective disorders, disorders wherein emotional regulation has been disrupted (e.g. depression, anxiety, PTSD), are associated with prefrontal malfunction such as abnormal activity and reduced grey matter (Duval, 2015).

mPFC-BLA Pathway

A leading neurocircuitry model of the PFC's control over emotion points the medial PFC and its interconnections with the basolateral amygdala (Motzkin, 2015; Marek et. al., 2013; Drevets, 2008). Among the PFC subdivisions, the mPFC along with the orbital PFC has the strongest connectivity to the amygdala and other limbic structures (Ongur & Price, 2000). Importantly, neuroanatomical tracing studies show that the majority of the connectivity of the mPFC with the amygdala is within the BLA, not the CeA or BNST (McDonald et. al., 1996). Besides its anatomical wiring with the BLA, numerous lines of evidence point to the mPFC's

importance in emotional regulation. For example, substantial evidence for the mPFC'S role in emotional regulation comes from studies on fear extinction. Fear extinction is simply an extension of fear conditioning in which after fear training (i.e. pairing of CS with the aversive US), there is repeated presentation of the CS without the aversive US until the animal learns not to fear the CS any longer. In several rodent studies, scientists discovered that the mPFC is absolutely crucial for fear extinction. For example, lesion and pharmacological inhibition of the ventral portions of the mPFC (vmPFC) block or at the very least severely inhibit fear extinction (Morgan et. al., 1993; Quirk et. al., 2000). Conversely, vmPFC stimulation potentiates fear extinction (Milad et. al., 2004; Vidal-Gonzalez et. al., 2006). And lastly, fear extinction is associated with increased vmPFC single unit and local field activity (Milad & Quirk, 2002; Barrett et. al., 2003, Burgos-Robles et. al. 2007). These results indicate top-down inhibition of amygdalar output via the vmPFC. Interestingly, the mPFC may also be involved in top-down excitation of the amygdala, especially through its dorsal regions (dmPFC) (Morgan & LeDoux 1995; Arruda-Carvalho & Clem 2015). For example, in rodents, dmPFC stimulation augments fear expression and decreases fear extinction (Vidal-Gonzalez et. al. 2006), and dmPFC lesion augments fear extinction and decreases fear expression (Corcoran & Quirk, 2007; Sierra-Mercado et. al., 2011). However, the exact roles of the dorsal and ventral mPFC in fear expression and extinction remain hotly debated (Arruda-Carvalho & Clem 2015; see discussion). Despite these controversies, however, the mPFC has been inexorably linked to fear extinction.

More evidence for the importance of the mPFC-amygdala pathway in emotional regulation comes from psychiatric studies (Duval, 2015). Namely, nearly all affective disorders involve disruptions in medial-prefrontal and amygdalar activity. For example, fMRI studies show patients with panic disorder (PD), specific phobia (SP), social anxiety disorder (SAD), and post-traumatic

stress disorder (PTSD) all show amygdala hyperactivation in response to threatening stimuli compared to healthy controls (Kim et. al., 2012; Schweckendiek et. al., 2011; Lipka et. al., 2011; Lueken et. al., 2013; Bruhl et. al., 2011; Boehme et. al., 2014; Ball et. al., 2012; Klumpp et. al., 2010; Schmidt et. al., 2010; Milad et. al., 2009; Shvil et. al., 2013). The degree of amygdala activation is positively correlated with symptom severity (Lipka et. al., 2011; Ball et. al., 2012). Moreover, treatment with medication and psychotherapy usually results in decreased amygdala hyperactivation to threat (Lipka et. al., 2011; Labuschange et. al., 2010; Mansson et. al., 2013; Phan et. al., 2013; Taylor et. al., 2014; Zantvoord et. al., 2013). Conversely, PTSD and generalized anxiety disorder (GAD) are primarily associated with decreased mPFC activation (Patel et. al., 2012; Rougemont-Bucking et. al., 2011; Jovanovic et. al., 2011; Palm et. al., 2011; Etkin & Schatzberg, 2011; Price et. al., 2011; Schlund et. al., 2012). Moreover, pharmacotherapy and psychotherapy generally produce increases in mPFC activation in SAD and PTSD. Thus, the leading neurocircuitry model for affective disorders points to hyperactivation of the amygdala and hypoactivation of the mPFC, again suggesting top-down inhibition of the amygdala via the mPFC-BLA pathway. However, as indicated with studies on fear conditioning, the model may not be so simple as differing parts of the mPFC may have different roles in fear expression and extinction. In support of a more complex model, some studies on PTSD and SAD have shown increased mPFC activity compared to healthy controls (Fonzo et. al., 2010; Garrett et. al., 2012; Felmingham et. al., 2010; Blair et. al., 2011), and several studies on PD, SP, and SAD have reported no difference or decreased amygdala activity compared to healthy controls (Holzel et. al., 2013; Nitschke et. al., 2009; Boutros et. al., 2013; Ottaviani et. al., 2012; Lueken et. al., 2013; Klumpp et. al., 2013; Gimenez et. al., 2014). Nevertheless, regardless of nuances the neurocircuitry model of fear and emotion, overwhelming evidence for mPFC and amygdala imbalances in affective disorders points

to the importance of the mPFC-amygdala pathway in emotion. Indeed, more recent studies focusing specifically on functional connectivity between brain areas (as opposed to localized activity) have suggested a disruption in mPFC-amygdala connectivity in affective disorders. For example, SAD, PTSD, and GAD have all been associated with decreased functional connectivity (Dodhia et. al., 2014; Jovanovic et. al., 2011; Tromp et. al., 2012; Strawn et. al., 2012, 2013; Roy et. al., 2013). Furthermore, connectivity improves after treatment for GAD and SAD (Holzel et. al., 2013; Maslowsky et. al., 2010; Dodhia et. al., 2014). Thus, given its role in fear conditioning as well as disrupted activity and connectivity in affective disorders, the pathway from the mPFC to the BLA has emerged as a crucial player in emotional regulation.

Delayed Maturation and Early Life Stress

One of the most interesting components of the PFC-amygdala pathway is its relatively delayed maturation during development. In both humans and rats, the prefrontal cortex develops very slowly, not reaching peak maturation until adulthood (Caballero et. al., 2016). This maturation includes myelination, synapse formation, as well as pruning of neurons and dendritic spines. Regarding the PFC-amygdala pathway specifically, in rats, mPFC projections to the amygdala do not emerge unctil postnatal day (PD) 7 and continue to increase throughout neonatal and periweaning stages (Bouwmeester et. al., 2002). Subsequently, mPFC synapses within the amygdala are pruned to less than 50% of their starting density during late adolescents (Cressman et. al., 2010). By contrast, amygdalar projections to the mPFC appear at PD6 and their fiber density within layers II and V of the anterior cingulate cortex and infralmibic cortex actually increases during adolescents (Cunningham et. al., 2002). This density increase is accompanied by a linear rise in the number of axospinous and axodendritic synapses present in the neuropil. Further evidence for the mPFC-BLA's delayed development comes from human fMRI studies. For

example, normal connectivity between the mPFC and amygdala during viewing of fearful faces emerges only after age 10 and increases with age (Gee et. al., 2013b). Similar delayed mPFC-amygdala connectivity patterns have been noted for viewing images of pain administered to others and during response inhibition tasks (Decety et. al., 2011; Perlman & Pelphrey, 2011).

The late maturation of the PFC-amygdala circuit suggests that this circuit is particularly vulnerable to plastic changes in early life. Therefore, adverse early life experiences could negatively impact the mPFC-amygdala pathway and lead to psychiatric disorders in adulthood. Indeed, a growing number of epidemiological and clinical studies show that early life stress (ELS) increases the risk for anxiety and mood disorders and is linked to functional and structural changes in the amygdala and PFC in humans (Chocyk et. al., 2013a; Fareri & Tottenham, 2016). For example, a recent World Mental Health Survey revealed that 59.5% of childhood onset, 32.6% of adolescent-onset, and 13.6% of adult-onset mood disorders are associated with ELS (Kessler et. al., 2010). Furthermore, several neuroimaging studies in humans revealed altered activity and/or volume in the mPFC and amygdala in children, adolescents, and adults with a history of ELS (Hanson et. al., 2012; Rinne-Albers et. al., 2013; van Harmelen et. al., 2010; Wang et. al., 2013; Gee et. al., 2013a; Tottenham et. al., 2011). For example, ELS individuals show stronger amygdala activation to threatening stimuli such as fearful faces compared to controls (Gee et. al., 2013a; Tottenham et. al., 2011). Moreover, when studying functional connectivity between the mPFC and amygdala, Gee et. al. (2013a) found that higher demonstrations of separation anxiety in ELS individuals correlated with higher amygdala rather than PFC activity. Finally, ELS is associated with increased amygdala volume (Mehta et. al., 2009; Tottenham et. al. 2010) and decreased PFC volume (De Bellis et. al., 2002; Frodl et. al., 2010).

Substantial support for ELS's role in affective disorders and underlying neuropathology comes from rodent studies. Here, the typical form of ELS administered is maternal separation (MS) (Vetulani, 2013). MS is thought to mimic typical ELS in humans which often involves parental neglect or problems within the immediate family (Fareri & Tottenham, 2016). In both humans and rats, a neglectful and absent mother in early life can have severe developmental effects (Vetulani, 2013). For the first two weeks of life, the neonatal rat depends on its mother for thermoregulation, nutrition, the stimulation of urination, and protection (Sanchez et. al., 2001). The mother's tactile sensory input augments the pups' hypothalamic oxytocin concentrations which is required for the expression of filial huddling which in turn helps the mother recognize pups (Kojima et. al., 2012; Kojima et. al., 2011; Alberts, 2007). Moreover, licking and grooming of the pups stimulates their hippocampal development, spatial learning, and memory and reduces the hypothalamic-pituitary-adrenal (HPA) axis response to stress (Liu et. al., 2000; Liu et. al., 1997). ELS rats tend to have hyperactive HPA axes causing an increase in cortisol and other glucocorticoids throughout life which is believed to be the basis for many maladaptive changes within the brain. In addition to increased HPA axis response, MS also causes alterations in heart rate, circadian rhythms, and hormone levels in pups (Hofer, 1970; Hofer, 1975; Kuh, et. al., 1990; Meany et. al., 1991; Stanton & Levine, 1990).

A plethora of studies have linked ELS from MS paradigms to emotional/behavioral changes within the rodent. For example, several studies show that ELS induces anxiety-like and depressive behaviors in adult rats (Chocyk et. al., 2013b; Pascual & Zamora, 2007; Uchida et. al., 2010). Interestingly, ELS seems to increase measures of innate fear, such as the elevated plus maze or light/dark exploration test (Chocyk et. al., 2013b; Pascual & Zamora, 2007; Uchida et. al.,

2010), while decreasing measures of learned fear such as fear conditioning (Kato et. al., 2012; Stevenson et. al., 2009; Wang et. al., 2011).

Furthermore, a number of biological studies have revealed structural changes within the mPFC and amygdala in rodents following ELS. For example, ELS affects the morphology of the dendritic tree, the length of dendritic processes and spine/synapse density in pyramidal neurons of the mPFC (Bock et. al., 2005; Chocyk et. al., 2013b; Monroy et. al., 2010; Muhammad et. al., 2011; Pascual & Zamora, 2007). Importantly, both impairments and intensifications have been observed depending on the severity of ELS and the developmental time point at which ELS was delivered. Moreover, ELS influences the expression of neural cell adhesion molecules (NCAMs) in the mPFC which are used in structural plasticity processes such as neurite growth, axon guidance, and synapse stabilization (Chatterjee et. al., 2007; Chocyk et. al., 2010). Finally, ELS has been shown to decrease the number of S-100b and GFAP-immunoreactive flial cells in the mPFC (Braun et. al., 2009; Leventopoulos et. al., 2007; Musholt et. al., 2009). Structural changes from ELS have also been seen in the amygdala. For example, Johnson et. al. (2018) found amygdala hyperconnectivity with the prefrontal cortex and hippocampus in ELS rats compared to control animals. Moreover, Arnett et. al. (2015) found a decrease in glucocorticoid receptor mRNA in the amygdalas of ELS rats. Interestingly, lentiviral restoration of GRs within the amygdala reversed the changes in anxiety and social behaviors in these same rats.

Finally, a few studies have shown functional changes within the mPFC and following ELS in rodents. For example, ELS decreases metabolic activity in the mPFC of juvenile rats (Bock et. al, 2012). ELS also reduces basal unit activity and basal local field potential activity in the right and left mPFC. Moreover, MS attenuates hemispheric synchronization of the basal local field potential activity of the mPFC (Stevenson et. al., 2008). Lastly, Cohen et. al. (2013) discovered

that ELS causes increased c-fos expression in the rodent amygdala, indicating increased neural activity. Thus, overall ELS is a main risk factor for affective disorders and likely acts by altering the biology within the mPFC and amygdala.

Long-term Potentiation

Despite substantial evidence for ELS's effects on emotional disorders and structural and functional changes within the mPFC and amygdala, and despite the role of the mPFC-BLA pathway in emotional regulation, to our knowledge, no study has ever been done on ELS's effect on long-term potentiation (LTP) between the prefrontal cortex and amygdala. In fact, besides Maroun (2006), we were unable to find any groups who have studied LTP within this pathway at all which is surprising given LTP's role in learning and memory and given the mPFC-BLA's role in fear conditioning and extinction.

LTP, the leading cellular model for explicit memory within the brain, refers to a long-lasting increase in synaptic strength following high frequency stimulation of presynaptic neurons (Bailey et. al., 2015). For example, if LTP were to occur in the mPFC-BLA pathway, high frequency stimulation of mPFC (presynaptic) neurons would produce greater evoked responses from BLA (postsynaptic) neurons because the synapses between the mPFC and BLA would have been strengthened by the high frequency stimulation.

LTP is actually a specific subtype of the more broadly distributed phenomenon called synaptic plasticity i.e. changes in synaptic strength (Bailey et. al., 2015). In order to understand LTP's role in memory, one must first understand the role of synaptic plasticity in memory more generally. The idea that memory lies within the strength of synapses between neurons is an old one (Ramon y Cajal, 1984; Hebb, 1949), and it is an idea that is logically pleasing. For example, if one neuron is thought to store one bit of information and a second neuron another bit of

information, a strengthening of the connection between those neurons would seem to represent a strengthening of the association between those two bits of information. However, it was not until the 1970s that scientists actually mapped a form of memory called implicit (i.e. unconscious/procedural) memory onto synaptic plasticity in the sensory and motor neurons of several animals (Spencer et. al., 1966; Krasne, 1969; Alkon, 1974; Quinn et. al., 1974; Dudai et. al.; 1976; Menzel & Erber, 1978). The most famous of these animals is the *Aplysia* wherein scientists studied sensitization of the gill-withdrawal reflex: the reflexive withdrawal of the animal's gill after a light touch to the siphon. Sensitization refers to the unconscious learned fear that takes place after aversive tail shock and the enhanced gill-withdrawal reflex that accompanies it. Moreover, sensitization memory is graded: a single tail shock produces short-term sensitization that lasts for minutes and 5 repeated tail shocks produce long-term sensitization that lasts for up to several weeks. Conveniently, the *Aplysia's* nervous system is very small and the exact neurons responsible for this phenomenon could be easily identified and viewed under the microscope. In a series of elegant experiments, scientists discovered that short-term sensitization was accompanied by increased synaptic transmission between siphon sensory and gill motor neurons involving increased glutamate release from presynaptic neurons (Brunelli et. al., 1976; Castellucci & Kandel, 1976). Furthermore, long-term facilitation was accompanied by protein synthesis, remodeling of preexisting presynaptic compartments (e.g. increased number, size, and vesicle complement of active zones) and growth of new synapses between sensory and motor neurons (Bailey & Chen, 1983; 1988ab). Scientists labeled these forms of synaptic plasticity short-term facilitation (STF) and long-term facilitation (LTF), respectively (Bailey et. al., 2015).

Thus, based on experiments in *Aplysia* as well as a host of other animals, implicit memory had effectively been mapped onto functional changes at the synapse. However, it remained

unknown whether the same could be said for explicit memory (i.e. memorizing facts and figures or recounting a story from childhood). Nevertheless, a particular brain structure called the hippocampus was well-known for its association with the generation of new explicit memories. The hippocampus was made famous by the patient H.M. who had both his hippocampi surgically removed to save him from epileptic seizures (Scoville & Milner, 1957). Notably, H.M. failed to produce any new explicit memories after his surgery. He had to be reminded everyday why he was in the hospital. However, H.M. was able to remember memories from his past as well as acquire new implicit memories such as learning how to play a game, although he never explicitly remembered every having played the new game (Milner, 1962). A host of other studies also support the hippocampus's role in explicit learning and memory.

Crucially, in 1966, Terje Lomo discovered a form of functional synaptic plasticity within the hippocampus similar to STF and LTF in the *Aplysia* gill-withdrawal reflex. Specifically, Lomo noticed that high frequency stimulation of the performant path produced increased evoked responses from its downstream target neurons in the dentate gyrus (Lomo, 1966; Bliss & Lomo, 1973). This phenomenon became known as long-term potentiation or LTP. Interestingly, like LTF, LTP displays a short-term phase (lasting minutes to hours) associated with enhanced synaptic transmission and a long-term phase (lasting hours to days) associated with protein synthesis and possibly production of new synapses (Bailey et. al., 2015). Thus, LTP offers a basis for short-term and long-term memory. Moreover, LTP displays a number of other properties consistent with what one would expect for a cellular learning and memory model (Lynch, 2004). For example, LTP is input-specific meaning only the synapses that have received stimulation show potentiation (Bliss & Collingridge, 1993). Furthermore, LTP is associative meaning simultaneous strong stimulation

in one synapse and weak stimulation in another synapse can produce LTP in both synapses. Lastly, LTP is persistent potentially lasting for months or even a life time (Abraham, 1995).

The fact that LTP robustly occurs in a brain structure strongly associated with memory and displays a number of features consistent with expected learning and memory models makes it a strong candidate for a cellular model of how the brain learns. Indeed, several studies have established a link between LTP and memory. For example, it is well established that most forms of LTP in the hippocampus are mediated by the postsynaptic glutamate receptor NMDA (Bailey et. al., 2015). NMDARs are known as coincidence detectors because they require both the pre and postsynaptic cell to be active simultaneously in order to open. However, upon opening, NMDARs allow calcium influx and a cascade of cellular events leading to the increased synaptic transmission behind LTP. In 1986, Morris et. al. showed that infusion of the NMDAR antagonist APV into the hippocampus not only blocked LTP, but also impaired learning in the Morris water maze in which rats must learn which markings on the walls of a pool indicate the position of a standing platform. A criticism of this experiment, however, was that NMDARs might be involved in normal synaptic transmission in the hippocampus, not just LTP. Therefore, blocking NMDARs could have confounding effects. However, in 1998, Giese et. al. produced a knockin mouse containing the point mutant T286A of CaMKII which allowed for the disruption of the cellular cascade mediated by NMDARs responsible for LTP without affecting bassline NMDAR activity. In these knockin mice, LTP was absent in hippocampal slices and learning in the Morris water maze was strongly impaired. Further evidence for LTP's role in learning comes from studies showing that LTP and learning occlude one another (Barnes et. al., 1994; Moser et. al., 1998; Habib et. al., 2013; Takeuchi et. al., 2014). For example, a strong learning experience before testing for LTP in the hippocampus results in less LTP. Conversely, inducing hippocampal LTP in vivo inhibits

subsequent performance on learning tasks. Lastly, Whitlock et. al. (2006) showed that LTP and learning mimic each other. Namely, one-trial inhibitory avoidance learning in rats produces the same changes in hippocampal glutamate receptors as induction of LTP with high frequency stimulation and causes an increase in evoked synaptic transmission in the CA1 *in vivo* similar to those seen after induction of LTP with high frequency stimulation.

Importantly, LTP occurs in other brain areas besides the hippocampus such as the cortex, striatum, thalamus, and cerebellum (Lynch, 2004). Of particular note, LTP in the amygdala has been associated with fear conditioning (Sigurdsson et. al., 2006). Namely, auditory (e.g. tone) and somatosensory (e.g. foot shock) information representing the conditioned stimulus (CS) and unconditioned stimulus (US), respectively, reaches the lateral nucleus of the amygdala (LA) from both thalamic and cortical sources. Within the LA, particular dual-modality neurons receive information from both auditory and somatosensory neurons (Romanski et. al. 1993). As the current fear circuitry model suggests, synapses holding somatosensory information (i.e. pain) in the LA are strong and capable of eliciting the fear response, whereas synapses holding auditory information are relatively weak and normally incapable of eliciting the fear response. However, after fear conditioning, there is a pairing of auditory and somatosensory information such that auditory synapse become potentiated by the associative property of LTP (Sigurdsson et. al., 2006). Indeed, this model has received much support. For example, fear conditioning increases CS-evoked responses in the LA, suggesting that CS synapses become stronger after conditioning (Quirk et al., 1995; Rogan et. al., 1997; Collins & Pare, 2000; Repa et. al., 2001; Goosens et. al., 2003). Moreover, these increases in synaptic strength after fear conditioning mirror changes on the molecular scale induced by LTP (Miserendino et. al., 1990; Campeau et. al, 1992; Rodrigues et. al., 2001). Furthermore, LTP has been robustly induced in synapses transmitting auditory

information in the LA. For example, LTP has been demonstrated within the LA following high frequency stimulation of both thalamic and cortical auditory inputs *in vivo* in awake freely behaving rats (Doyere et. al., 2003). Moreover, LTP in the auditory thalamic-LA pathway was induced in vitro by pairing weak presynaptic stimulation with strong postsynaptic depolarization suggesting that LTP at auditory synapses can be induced via association with strong depolarization from somatosensory synapses (Weisskopf & LeDoux, 1999; Huang et. al., 2000). Other experiments have shown that auditory neuron-LA LTP is input specific which is consistent with the fact that animals can differentiate between tone frequencies that signify a US and tone frequencies that do not. (Weisskopft & LeDoux, 1999; Tsvetkov et. al., 2004). Importantly, LTP induction in the LA produces the same enhancement of CS-evoked field potentials as fear conditioning (Rogan & LoDoux, 1995). Lastly, several studies have shown that inhibiting LTP in the LA blocks fear conditioning (Campeau et. al., 1992; Miserendino et. al., 1990; Rodrigues et. al., 2001). Taking this idea one step further, in a stunning experiment utilizing the new technique of optogenetics, Nabavi et. al. (2014) were actually able to turn on and off the conditioned fear response to a tone by administering LTP or LTD to the lateral amygdala from auditory neurons in awake freely behaving animals. LTD, or long-term depression, is simply the reverse of LTP wherein low frequency simulation of presynaptic neurons weakens the synapse and is thus thought to underlie forgetting memories.

Despite all of this evidence for LTP's role in learning and memory, however, it is important to remember that memory in the mammalian brain is likely widely distributed across many synapses. Thus, even given such experiments as Nabavi et. al. (2014), it is hard to say what LTP at any one synapse exactly means behaviorally with regard to any one memory. Nevertheless, LTP

has been proven to be an essential brain function regarding learning and memory, and it is a phenomenon that deserves much study.

Current Experiment

The pathway from the mPFC to the BLA is clearly important in emotional regulation and has been implicated in forms of emotional learning such as fear expression and extinction. Given these findings and given evidence for LTP's role in learning and memory, it is extremely surprising that so few studies have been done on LTP in the mPFC-BLA pathway. Moreover, the mPFC-BLA pathway shows delayed maturation, leaving it vulnerable to ELS. Indeed, several studies have implicated ELS in functional and structural changes within the PFC and amygdala as well as disrupted emotional regulation later in life. Given this information, it seems very plausible that one way ELS causes emotional dysregulation in later life is through alteration of the mPFC-BLA pathway's ability to express LTP as learning and memory clearly has a huge role to play in proper emotional development. Therefore, we chose to study whether ELS effects LTP in the pathway from the mPFC to the BLA. In this study, an experimental group of rats labeled ISO (isolated) were neonatally isolated from their mother and from each other for 1 hour a day from PD 2-9. Control rats were raised normally and were only handled for regular cage cleaning, the same as ISO rats. Upon reaching adulthood (70-120 days) rats underwent stereotaxic surgery wherein a stimulating and recording electrode were placed and sealed within the mPFC and BLA, respectively. After 5 days of postsurgical recovery, LTP recordings were performed *in vivo* while rats were awake and freely behaving and levels of potentiation were compared across groups.

Methods

Animals and Housing

Sprague-Dawley breeders obtained from Charles River Laboratories (Wilmington, MA) were used for production of all experimental animals. Within, 24 hours of birth, litters were culled to 12 pups (8 males: 4 females). Culled liters were designated either control or isolated (ISO). ISO pups underwent ISO protocol (see below). Control rats were not handled except during periodic cage cleaning. All rats were weaned at 21 days of age at which time male littermates were housed two per cage. Only males were used from this point forward. Rats were kept in ventilated polycarbonate cages in a temperature (22 ºC) and pressure (1 atm) controlled room with 55% humidity and a 12-hour light/dark cycle (lights on at 7 AM). Standard rodent chow and tap water were provided *ad libitum*. All experimental protocols were approved by the Trinity College Institutional Animal Care and Use Committee (IACUC) and were in accordance with the US Public Health Service's Guide for the Care and Use of Laboratory Animals.

Neonatal Isolation (ISO)

From postnatal day (PD) 2-9, ISO pups underwent neonatal isolation. On the day of isolation treatment, the dam was transferred out of the nest into a clean cage and each pup was placed individually in clear plastic dishes (length, width, 14x5x5cm) spaced approximately 15cm apart within a sound-proof, humidity controlled, and heated (~30 ºC) isolation chamber. The chamber contained a white noise maker to mask the hearing of other pups' calls even in the ultrasonic range (Ultrasound Advice, London, UK). Heat was applied with an overhead heating lamp and regulated with a thermometer. Pups were kept in the isolation chamber for 1 hour, then immediately transferred back to the original nest along with the dam. Approximately 6 litters were isolated.

Stereotaxic Surgery

Between 70 and 120 days, rats were anesthetized intraperitoneally with a ketamine, xylazine, and acepromazine cocktail (25 mg/kg, 2.5 mg/kg, and 0.5 mg/kg, respectively) and placed in a stereotaxic surgical frame to immobilize the head. Measurements based off of Bregma were made to identify drilling sites for the medial prefrontal cortex (AP +3.0mm; LAT +0.4mm) and the basolateral amygdala (AP -2.8mm; LAT +5.0mm). After small holes were drilled into the skull at these locations, an epoxylite-insulated stainless-steel bipolar stimulating electrode was lowered into the mPFC (DV 5.0mm) and an epoxylite-insulated tungsten monopolar recording electrode was lowered into the BLA (DV 7.6-8.0mm). Evoked field potentials were monitored with a digital oscilloscope and the depth of the recording electrode was adjusted to where evoked responses were maximal. During surgery, the signal from the mPFC-BLA pathway manifested as a single negative peak with a latency around 26ms. Finally, electrodes were glued to the skull with dental acrylic, and the wound was sutured using biodegradable surgical threads.

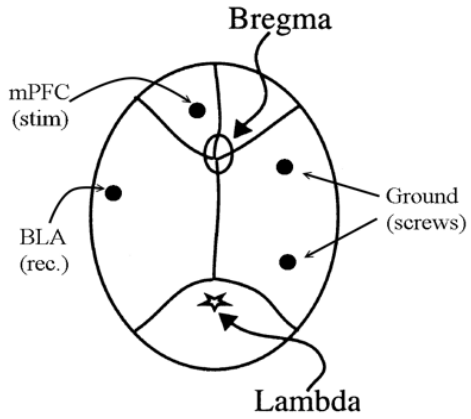

Figure 1. Image of rodent skull with coordinates for electrodes based off of Bregma. A stimulating electrode was placed in the medial prefrontal cortex (mPFC), a recording electrode in the basolateral amygdala (BLA).

Target Structure	AP (mm)	LAT (mm)	DV (mm)
mPFC	+3.0	+0.4	-5.0
BLA	-2.8	_5.0	-7.6

Table 1. Surgical coordinates for the mPFC and BLA. Values are given as millimeters from Bregma in either the AP (anterior/posterior), LAT (lateral), or DV (dorsal/ventral) direction.

Electrophysiological Recordings

After at least 5 days of postsurgical recovery, rats were individually placed in a noise-reducing recording chamber. After at least an hour of acclimation, rats were connected to recording instruments using long, noise-reducing wires to allow for free movement. Using a Grass S-88 stimulator, biphasic square wave pulses (pulse width = 0.25 ms, 50% duty-cycle) were delivered to the mPFC. Using a differential amplifier, evoked responses in the BLA were amplified 1000 fold, bandpass filtered from 1Hz-3kHz, notch filtered at 60Hz, and displayed on a BK precision digital oscilloscope. Signal population spike amplitudes (PSA) were measured and recorded using LabView computer software. Input/output curves were established for each animal by recording the PSA of evoked field potentials from varying input intensities. Specifically, starting from the lowest input intensity that elicited a response (typically 200 or 400 uA) and ending at 1500uA, PSA values were averaged across 10 trials for each input intensity (200, 400, 600, 800, 1000, 1200, 1400, and 1500 uA). Using the 50% maximum response current, baseline evoked field potential PSA values were determined using an average of 5 trials recorded every minute for 15 minutes. After establishment of baseline PSA values, high frequency stimulation (tetanization) was administered to induce LTP. Tetanization was delivered with either 5 Hz theta burst stimulation (10 bursts of 10 pulses at 400 Hz) or sustained stimulation (one burst of 900 pulses at 200 Hz) at a current of 800 uA. Immediately after tetanization, PSA values of evoked field potentials were recorded. The average of 5 trials was taken every minute for 15 minutes posttetanization and the average of 10 trials was taken at times 30 min, 60 min, 120 min, 180 min, 24 hr, and 48 hr posttetanization. Finally, the percent change of PSA values from the average baseline PSA value was determined for each recorded time point. A percent increase of 15% or more is typically considered LTP.

Results and Discussion

While this experiment encountered several difficulties in obtaining data, the lessons learned from experimentation as well as the trends seen in the small amount of acquired data are valuable and worth reporting. Therefore, the following section will detail problems encountered during experimentation including the small and inconsistent nature of the mPFC-BLA signal and the fact that the signal changes in morphology after surgery. This section will also present the input/output (IO) and LTP data for the 8 animals that were able to be recorded with reliable signals as well some interesting trends in this data such as the observations that the mPFC-BLA pathway seems to be resistant to LTP and prone to LTD and that the mPFC-BLA signal changes most dramatically in strength at the 24 and 48-hour time periods after high frequency stimulation. Regarding whether or not there were differences in control and ISO animals, however, small sample sizes simply prevented meaningful comparisons of plasticity across groups. Finally, we will give one speculation as to why the mPFC-BLA is so variable as well as review the recent findings that the prelimbic and infralimbic portions of the mPFC differ in functionality so that future experiments may take advantage of this information.

Small and Noisy Signal

When this project began, experimenters first used a DAM 50 differential amplifier to acquire biological signals evoked from the BLA during surgery. This amplifier not only removes noise from the signal by subtracting common frequencies from ground (reference) and recording electrodes, but also removes noise falling outside of its bandpass filter (1Hz-3KHz). However, even with these noise-reducing capabilities, the signal from the mPFC-BLA pathway during surgery was so small (typically 500 mV) as to be almost entirely masked by noise (Figure 2). Consequently, very few surgeries were successful in obtaining the desired signal, and for those

surgeries that were successful, none of these animals still retained a signal that was discernable over the noise after 5 days of postsurgical recovery. To overcome this difficulty, a Model 3000 A-M Systems Inc. amplifier was obtained which like the DAM 50 is also a differential amplifier with a bandpass filter; however, the Model 3000 amplifier also contains a notch filter for 60 Hz signals. This was important because, within the United States, electricity runs at 60 Hz within buildings, and much of the noise obtained during electrophysiology recording comes from this 60 Hz frequency. With the new amplifier, noise was significantly diminished and surgeries were much more successful in obtaining viable signals (Figure 2).

A) B)

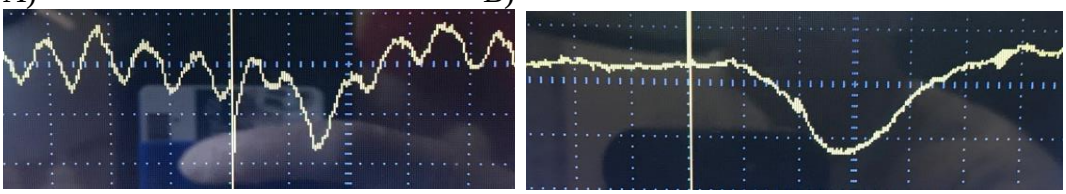

Figure 2. The mPFC-BLA signal acquired during surgery with **A)** the DAM 50 differential amplifier and **B)** Model 3000 A-M Systems Inc. differential amplifier

Signal Changes after Surgery

After solving the problem of acquiring such a small signal from the mPFC-BLA pathway during surgery, however, a new problem arose. Namely, upon checking the animals for signals after 5 days of recovery, the signal had changed in morphology (Figure 3). According to Maroun (2006) and Vouimba & Maroun (2011), the mPFC-BLA signal should manifest itself as a single negative peak at a latency around 25 ms. This is indeed the signal we obtained during surgery. However, the postsurgical signal contained a large positive peak around 8 ms followed by a negative peak at 16 ms and another negative peak at 32 ms. Unsurprisingly, this result caused much confusion and lead to rigorous testing of surgical and recording equipment. It was eventually concluded that either a) the signal changes after surgery or b) the recording equipment introduced a large stimulation artifact resulting in the large positive peak at 8 ms that shifts the signal latency

to the right. Regarding the first conclusion, it seems entirely possible that the signal morphology might change after the anesthetic from surgery has washed out of the brain. However, in Vouimba & Maroun (2011), awake, freely behaving rats showed the same signal morphology (namely a negative peak ~25ms) before and after surgery. Thus, we decided it was safest to assume our recording equipment had introduced some sort of stimulus artifact to the signal responsible for the large peak at 8 ms. Indeed, the small negative peak at 32 ms appears very similar in morphology and size to the signal obtained during surgery and removal of the large peak at 8 ms would shift the peak at 32 ms back to an expected latency of ~25 ms. Thus, we decided to record from the small negative peak at 32 ms.

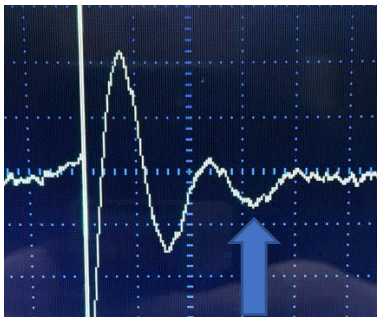

Figure 3. Post-surgical signal with a large positive peak around 8 ms and two negative peaks around 16 and 32 ms respectively after the stimulation artifact. The small negative peak at 32 ms (blue arrow) was chosen as the peak to measure during electrophysiological recordings.

With the morphology of the signal settled, we began LTP recordings. However, even with the improved amplifier, mPFC-BLA signals in awake, freely behaving animals were often weak and inconsistent if present at all after surgery. Consequently, only five control and three ISO animals were recorded with accurate, reliable data.

A) B)

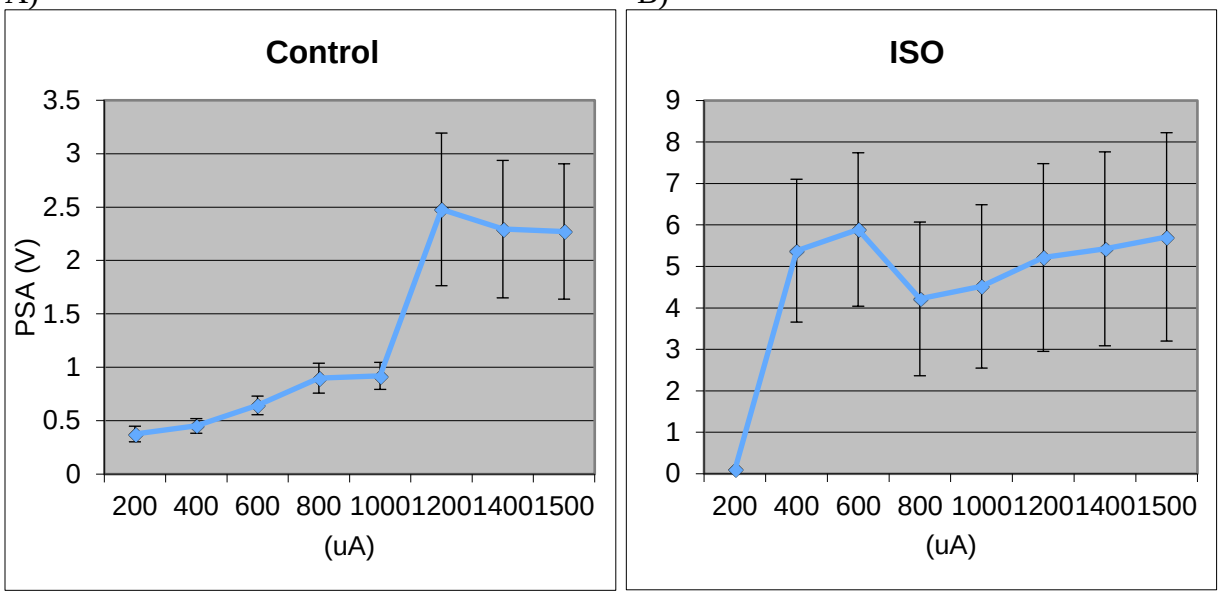

Figure 4. Input/Output curves for **A)** control and **B)** ISO animals. Horizontal-axis values are input currents in uA and vertical-axis values are population spike amplitudes (PSA) in the mPFC-BLA signal. Error bars represent +/- standard error.

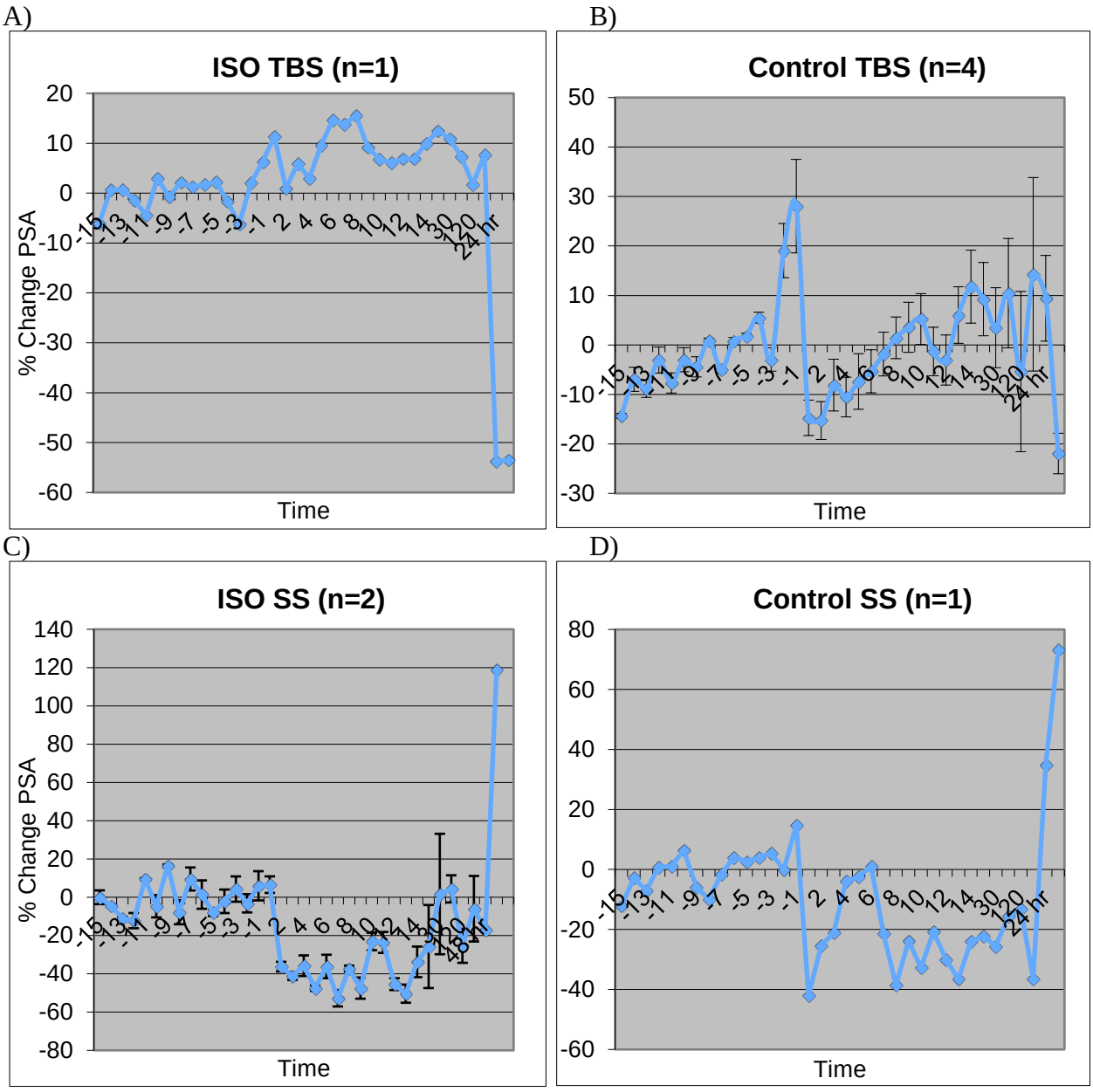

Figure 5. LTP data for four groups of rats: **A)** ISO-TBS (n=1) **B)** Control-TBS (n=4) **C)** ISO-SS (n=2) **D)** Control-SS (n=1). Time is given in minutes on the horizontal axis except for the 24 and 48 hour time points which are given in hours. Negative time points represent time before tetanization, and positive time points represent time after tetanization. Vertical-axis values are presented as percent changes in population spike amplitude (PSA) from baseline. Error bars represent +/- standard error. Abbreviations: TBS=theta burst stimulation; SS=sustained stimulation; n=sample size.

A) B)

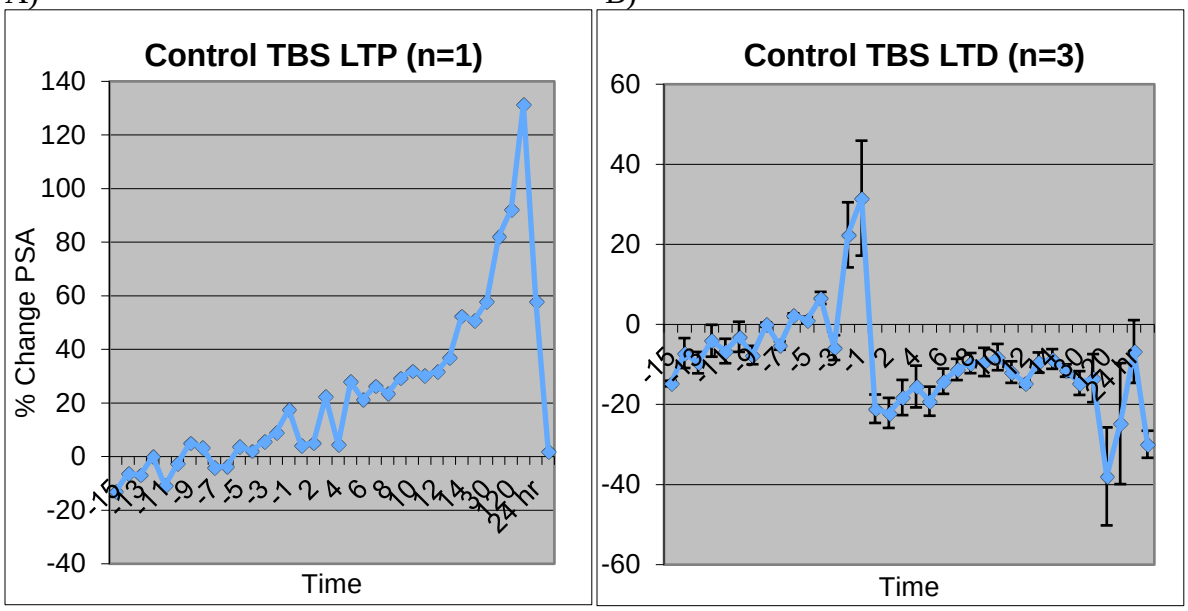

Figure 6. LTP data for two subsets of the Control-TBS animals. **A)** One animal within this group displayed LTP. **B)** The other three animals displayed LTD or no change.

IO Data

The input output (IO) curves for control and ISO animals are shown in Figure 4. On average, ISO animals showed stronger evoked potentials at 400-1500 uA. However, statistical tests of significance were not performed and it should be noted that there was great variability in population spike amplitude (PSA) within both groups as demonstrated by the large error bars. If larger sample sizes can be collected, future work should be done to test for baseline difference in mPFC-BLA signal strength across ISO and control animals. On this note, using very similar methodologies as used in our experiment, Vouimba & Maroun (2011) found changes in mPFC-BLA evoked potentials following fear conditioning, extinction, and reinstatement of fear. Specifically, fear conditioning, extinction, and reinstatement of fear were associated with potentiation, depression, and re-potentiation of mPFC-BLA evoked field potentials (EFPs), respectively. Thus, baseline EFP strength in the mPFC-BLA pathway may be a reflection of the level of innate fear or perhaps capacity for learned fear within the animal. Therefore, it is

conceivable that ISO and control animals would express different baseline mPFC-BLA signal strength, but definitive claims of differences in baseline signal strength across control and ISO animals cannot be made from this study.

LTP Data and Interpretations

The LTP data are shown in Figures 5. It was originally thought that only one stimulation protocol would be used: namely theta burst stimulation (TBS: ten 5Hz bursts of ten 400 Hz pulses). However, it quickly became apparent that TBS did not produce robust LTP in the mPFC-BLA pathway. Therefore, some animals were given sustained stimulation (SS: one burst of 900 pulses at 200 Hz) to see if a different stimulation protocol could induce LTP. Overall, 4 groups were created: ISO-TBS (n=1), Control-TBS (n=4), ISO-SS (n=2), Control-SS (n=1). Although small sample sizes negated the value of statistical tests of significance, several trends in the LTP data were observed. Namely, the ISO-TBS animal showed the most LTP-like changes following HFS with PSA increases of 10-15% at the 1, 6, 7, 8, 9, 30, and 60 minute marks. Of note, 15% is typically the minimum percent increase in PSA considered to be LTP. Conversely, every other animal (except for one animal from the Control-TBS group; see Figure 6) showed an initial decrease in PSA following HFS. A decrease in signal strength is usually caused by low frequency stimulation (e.g. 900 pulses at 1 Hz) and is called long-term depression (LTD). The fact that high frequency stimulation caused a decrease in mPFC-BLA signal strength is indeed a mystery. Interestingly, sustained stimulation at 200 Hz caused the greatest decreases in PSA (~40-50%) whereas TBS with 400 Hz pulses caused either small decreases in PSA or even slight increases in PSA (e.g. ISO-TBS). In fact, within the Control-TBS group, one animal displayed robust LTP while the other three animals showed either no change or small LTD-like plasticity (Figure 6). Thus, it may be that the mPFC-BLA pathway is biased towards LTD and that only extremely high

pulse frequencies (i.e. 400 Hz or greater) are capable of eliciting LTP in this pathway. Moreover, pulse frequencies lower than 400 Hz may actually cause LTD rather than LTP. Indeed, in anesthetized animals, Maroun (2006) also found that the mPFC-BLA pathway was resistant to LTP following high frequency TBS with pulses at 400 Hz and sustained stimulation with pulses at 100 Hz but readily susceptible to LTD following low frequency stimulation (900 pulses at 1 Hz). Future work should be done on determining which stimulation protocols are capable of eliciting LTP vs. LTD in the mPFC-BLA pathway.

Another interesting trend in the LTP data is the large change in synaptic strength at the 24 and 48 hr time periods across all groups. Curiously, for TBS, %PSA values at these time periods significantly decreased from baseline, whereas for SS, they significantly increased. It is almost as if the synapse rebounded in strength following LTD from the SS and shrunk following TBS at the 24 and 48 hr time points. The fact that the mPFC-BLA pathway shows such plasticity following HFS at the 24 and 48 hr time points is indeed intriguing. However, recent optogenetic experiments on the mPFC may help explain these results. Namely, in a 2015 study, Do-Monte et. al. found that optogenetic silencing of IL-mPFC neurons during extinction training disrupts fear extinction, but IL inhibition 24 hours or one week after extinction training has no effect on fear extinction. Similar results were reported when IL axon terminals specifically within the BLA were silenced with optogenetics (Bukalo et. al., 2015). These experiments implicate the importance of the IL-mPFC in the acquisition but not retrieval of extinction memory. Similar results have been reported in PL-BLA synapses but for fear expression rather than fear extinction (see below for discussion on IL/PL functional dichotomy) (Do-Monte et. al., 2015b). Thus, it may be that the memories for fear and fear extinction are initially acquired within mPFC-BLA synapses but are then moved to other synapses as time progresses. Therefore, within the context of fear conditioning and extinction

training, it makes sense that plasticity at the mPFC-BLA synapse might change dramatically 24 and 48 hours after HFS.

Finally, there is the question of whether control and ISO animals displayed any differences in plasticity. In both TBS and SS groups, ISO animals appear to have the greatest initial decreases in PSA following HFS. Thus, it might be said that ISO animals have the greatest tendency for LTD at this synapse. Moreover, ISO-TBS and ISO-SS animals showed the greatest changes in PSA at the 48 hr time point. Therefore, overall, the mPFC-BLA synapse in ISO animals may be more plastic than control animals. However, realistically, sample sizes are simply too small for a meaningful comparison of plasticity across ISO and control groups. Nevertheless, substantial evidence supports the idea that ELS might affect plasticity in this pathway. Namely, as mentioned in the introduction, ELS sensitizes the HPA axis causing increased cortisol, a stress hormone shown to affect LTP and LTD (Vetulani, 2013). For example, in the CA1 region of the hippocampus, cortisol suppresses induction of LTP and augments induction of LTD (Alfarez et. al., 2002). Cortisol has also been shown to affect LTP at many other synapses (Joels & Krugers, 2007). Thus, altered stress hormone levels from ELS likely effects LTP at many synapses in the brain. Indeed, ELS increases LTP in the Shaffer collateral-CA1 pathway of the hippocampus (Derks et. al., 2016) and the PL-IL pathway of the mPFC (Chocyk et. al., 2013b), increases both LTP and LTD in basolateral amygdala-dentate gyrus synapses (Blaise et. al., 2008), and decreases LTP in the ventral hippocampus-ILmPFC synapses (Baudin et. al., 2012). Moreover, and most crucial to the current experiment, ELS has been shown to affect plasticity in the amygdala. For example, Danielewicz & Hess (2014) found that ELS impairs LTP but enhances LTD in cortical-LA synapses and impairs both LTP and LTD in thalamic-LA synapses. Given the results of these

experiments, it seems likely that ELS would affect LTP in mPFC-BLA synapses, however, the current experiment cannot definitely confirm or deny this hypothesis.

State-Dependency of mPFC-BLA Signal

Although it is anecdotal, one piece of information may help shed some light on the results obtained in this study and the difficulty in collecting data. Namely, it was observed that mPFC-BLA signal strength was extremely dependent on the physiological state of the animal i.e. how awake or alert vs. restful or sleeping the animal was. Ideally, all animals are kept at the same physiological state---quiet waking---for the entire experiment. Indeed, certain precautions were taken to reach this ideal. Namely, all animals were given at least an hour to acclimate in the recording chamber before recording. Furthermore, recordings did not begin until the animal was in a quiet waking state i.e. resting on all four legs with eyes open. Moreover, if animals fell asleep during recordings, they were lightly prompted with loud noise or a light touch to wake them up. However, time constraints as well as practical considerations made it nearly impossible to be sure all animals were in the same physiological state for the entire recording. For example, tetanization often lifted animals out of quiet waking and caused them to become quite active---walking around and sniffing their environment. Furthermore, sometimes connecting wires would fall off of the animal's head and dangle in front of it, which often caused considerable alarm/arousal. The anecdotal state-dependency of the mPFC-BLA signal observed in this experiment is corroborated by results from Maroun (2006) who found that plasticity at the mPFC-BLA synapses changed depending on the physiological state the animal. Namely, as previously mentioned, anesthetized control rats in this experiment were resistant to LTP to but readily susceptible to LTD. However, after undergoing 30 mins of elevated platform stress just prior to anesthetization, rats exhibited LTP encompassing upwards of 40% increases in PSA after 100 Hz TBS and were resistant to LTD

following LFS at 1 Hz. Indeed, other experimenters have observed a change in amygdalar plasticity depending upon the physiological state of the animal (Vouimba et. al., 2004). Furthermore, given the amygdala and PFC's functional role in valuation of evolutionarily relevant stimuli, it makes some logical sense that the mPFC-BLA signal would change depending on physiological state of the animal.

The state-dependency of the mPFC-BLA signal may help explain several of the results obtained in our experiment. For example, as noted in the IO curves, baseline signal strength was far from constant in both control and ISO groups. Furthermore, one animal in the TBS-Control group exhibited a large spike in PSA just prior to tetanization which is the reason the LTP data for this group shows this large positive peak. Also, as previously mentioned, one animal within the TBS-Control group exhibited LTP whereas all of the other animals in this group showed either no change or LTD. Lastly, the large changes in plasticity at the 24 and 48 hr time points might also be explained by the state-dependency of the mPFC-BLA signal. Namely, the physiological state of the animal (e.g. how accustomed or aroused it was by its new environment) likely changed dramatically after 24 and 48 hours vs. the relatively short 3-hour time lapse in the initial LTP recording.

IL/PL Dichotomy

Lastly, even if large sample sizes of reliable LTP or LTD data had been obtained in this study, a comparison of plasticity at this synapse across ISO and control groups would still be hard to interpret given the recent conclusions over the functional roles of different subnuclei within the mPFC. Namely, according to Arruda-Carvalho & Clem (2015), scientists have concluded that the prelibic (PL) and infralimbic (IL) portions of the mPFC have differing roles in fear expression and extinction, respectively. For example, fear acquisition is positively correlated with increased PL

activity (Burgos-Robles et. al., 2009; Sotres-Bayon et. al., 2012; Courtin et. al., 2014) and fear extinction is positively correlated with increased IL activity (Milad & Quirk, 2002; Barrett et. al., 2003; Burgos-Robles et. al., 2007). Correspondingly, PL lesions inhibit fear acquisition (Corcoran & Quirk, 2007; Sierra-Mercado et. al., 2011) and IL lesions inhibit fear extinction (Quirk et. al., 2000; Chang & Maren, 2010; Fontanez-Nuin et. al., 2011; Sierra-Mercado et. al., 2011; Santini et. al. 2012), whereas PL stimulation augments fear acquisition (Vidal-Gonzalez et. al., 2006) and IL stimulation augments fear extinction (Milad et. al., 2004; Vidal-Gonzalez et. al., 2006; Kim et. al., 2010; Maroun et. al., 2012). Based on the methodologies used in this experiment, we cannot say for certain whether the IL or PL-mPFC was exclusively stimulated. A concentric bipolar stimulating electrode was typically placed 5 mm ventral to the surface of the brain (at AP +3.0mm; LAT +0.4mm) which according to the Rat Brain Atlas (Paxinos & Charles, 2007) would put the tip of the electrode inside the IL-mPFC. However, due to the uncontrollable nature of electrical stimulation, it cannot be said for certain whether or not the PL-mPFC was also stimulated. Future studies in this laboratory should be done on whether or not there are differences in mPFC-BLA plasticity depending on the dorsal/ventral positioning of the stimulating electrode within the mPFC. It would also be interesting to test fear conditioning and extinction alongside LTP/LTD protocols in the mPFC-BLA pathway. For example, it might be possible to occlude fear conditioning or extinction with PL and IL-BLA LTP, respectively. Conversely, perhaps fear conditioning and extinction could be augmented with LTD induction just prior to training.

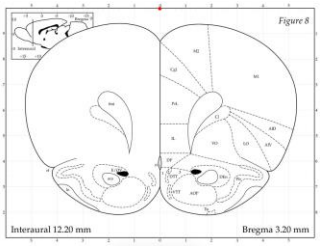

Figure 7. Coronal image of rodent mPFC including the prelimbic (PrL) and infralimbic (IL) sections (from: Paxinos & Charles, 2007)

Conclusion

In conclusion, due to several problems encountered during experimentation and the resulting small sample sizes of data obtained, this study cannot definitively say whether or not ELS affects synaptic plasticity at the mPFC-BLA synapses. However, with some certainty we can say that the mPFC-BLA pathway is resistant to the induction of LTP with both theta burst and sustained high frequency stimulation. Moreover, the mPFC-BLA pathway instead appears to be prone to LTD. Future studies should investigate which stimulation protocols are capable of eliciting LTP/LTD in this pathway. It may be that only an investigation of differences in LTD across control and ISO groups is worth investigating in this pathway. Furthermore, given the recent conclusions over the dichotomy of PL and IL mPFC function, future studies should be done to determine if there are differences in mPFC-BLA plasticity depending on whether the stimulating electrode is placed in the dorsal or ventral aspects of the mPFC.

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Works Cited

Abraham, W.C., Mason-Parker, S.E., Williams, J. & Dragunow, M. (1995). Analysis of the decremental nature of LTP in the dentate gyrus. *Brain Research Molecular Brain Research, 30,* 367-372.

Adolphs, R., Tranel, D. & Damisio, A.R. (1998). The human amygdala in social judgement. *Nature, 393,* 470-474.

Alberts, J.R. (2007). Huddling by rat pups: ontogeny of individual and group behavior. *Developmental Psychobiology, 49,* 22–32.

Alfarez, D.N., Wiegert, O., Joëls, M. & Krugers, H.J. (2002). Corticosterone and stress reduce synaptic potentiation in mouse hippocampal slices with mild stimulation. *Neuroscience,* 115(4), 1119–1126.

Alkon, D.L. (1974). Associative training of Hermissenda. *The Journal of General Physiology, 64,* 70-84.

Arnett, M.G., Pan, M.S., Doak, W., Cyr, P.E., Muglia, L.M. & Muglia, L.J. (2015). The role of glucocorticoid receptor-dependent activity in the amygdala central nucleus and reversibility of early-life stress programmed behavior. *Translational Psychiatry,* 5, e542.

Arruda-Carvalho, M. & Clem, R.L. (2015). Prefrontal-amygdala fear networks come into focus. *Frontiers in Systematic Neuroscience, 9,* 145.

Aston-Jones, G., Rajikowski, J. Kubiak, P. Valentino, R.J. & Shipley, M.T. (1996). Role of the locus coeruleus in emotional activation. *Progress in Brain Research, 107,* 379-402.

Bailey, C.H. & Chen, M. (1983). Morphological basis of short- and long-term memory in Aplysia. In *Perspectives on cognitive neuroscience* (ed. Weingartner H, Lister R), pp. 76-92. Oxford University Press, New York.

Bailey, C.H. & Chen, M. (1988a). Long-term memory in *Aplysia* modulates the total number of varicosities of single identified sensory neurons. *Proceedings of the National Academy of Sciences of the United States of America, 85,* 2373-2377.

Bailey, C.H. & Chen, M. (1988b). Long-term sensitization in Aplysia increases the number of presynaptic contacts onto the identified gill motor neuron L7. *Proceedings of the National Academy of Sciences of the United States of America, 85,* 9356-9359.

Bailey, C.H., Kandel, E.R. & Harris, K.M. (2015). Structural components of synaptic plasticity and memory consolidation. *Cold Spring Harbor Perspectives in Biology, 7*(7), a21758.

Ball, T.M., Sullivan, S., Flagan, T., et. al. (2012). Selective effects of social anxiety, anxiety sensitivity, and negative affectivity on the neural bases of emotional face processing. *Neuroimage. 59*(2), 1879-1887.

Barnes, C.A., Jung, M.W., McNaughton, B.L., Korol, D.L. Andreasson, K. & Worley, P.F. (1994). LTP saturation and spatial learning disruption: Effects of task variables and saturation levels. *Journal of Neuroscience, 14*, 5793-5806.

Barrett, D., Shumake, J., Jones, D. & Gonzalez-Lima, F. (2003). Metabolic mapping of mouse brain activity after extinction of a conditioned emotional response. *Journal of Neuroscience, 23*, 5740-5749.

Baudin, A., Blot, K., Verney, C., Estevez, L., Santamaria, J., Gressens, P., Giros, B. et. al. (2012). Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal cortex. *Neurobiology of Learning and Memory, 98*(3), 207-214.

Blair, K.S., Geraci, M., Otero, M., et al. (2011). Atypical modulation of medial prefrontal cortex to self-referential comments in generalized social phobia. *Psychiatry Research. 193*(1), 38–45.

Blaise, J.H., Koranda, J.L., Chow, U. & Haines, K.E. (2008). Neonatal isolation stress alters bidirectional long-term synaptic plasticity in amygdalo-hippocampal synapses in feely behaving adult rats. *Brain Research, 1193*, 25-33.

Bliss, T.V. & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the performant path. *Journal of Physiology, 232*, 357-374.

Bliss, T.V. & Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Bock, J., Gruss, M., Becker, S. & Braun, K. (2005). Experience induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cerebral Cortex, 15*, 802–808.

Bock, J., Riedel, A. & Braun, K. (2012). Differential changes of metabolic brain activity and interregional functional coupling in prefronto-limbic pathways during different stress conditions: functional imaging in freely behaving rodent pups. *Frontiers in Cellular Neuroscience, 6*, 19.

Boehme, S., Ritter, V., Tefikow, S., et. al. (2014). Brain activation during anticipatory anxiety in social anxiety disorder. *Social Cognitive and Affect Neuroscience, 9*(9), 1413-1418.

Boutros, N.N., Galloway, M.P., Ghosh, S., Gjini, K. & Bowyer, S.M. (2013). Abnormal coherence imaging in panic disorder: a magnetoencephalography investigation. *Neuroreport, 24*(9), 487-491.

Bouwmeester, H. Smits, K. & Van Ree, J.M. (2002). Neonatal development of projections to the basolateral amygdala from prefrontal and thalamic structures in rat. *Journal of Computational Neurology, 450,* 241-255.

Burgos-Robles A., Vidal-Gonzalez I., Santini E. & Quirk G. J. (2007). Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron, 53*, 871–880.

Burgos-Robles, A., Vidal-Gonzales, I. & Quirk, G.J. (2009). Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *Journal of Neuroscience, 29,* 8474-8482.

Braun, K., Antemano, R., Helmeke, C., Buchner, M. & Poeggel, G. (2009). Juvenile separation stress induces rapid region- and layer-specific changes in S100ss- and glial fibrillary acidic protein-immunoreactivity in astrocytes of the rodent medial prefrontal cortex. *Neuroscience, 160,* 629-638

Brody, E.G. & Rosvold, H.E. (1952). Influence of prefrontal lobotomy on social interaction in monkey group. *Psychosomatic Medicine, 14,* 406-415.

Bruhl, A.B., Rufer, M., Delsignore, A., Kaffenberger, T. Jancke, L & Herwig, U. (2011). Neural correlates of altered general emotion processing in social anxiety disorder. *Brain Res, 1378,* 72-83.

Brunelli, M., Castellucci, V. & Kandel, E.R. (1976). Synaptic facilitation and behavioral sensitization in Aplysia: possible role of serotonin and cyclic AMP. *Science, 194,* 1178-1181.

Brutkowski, S. & Mempel, E. (1961). Disinhibition of inhibitory conditioned responses following selective brain lesions in dogs. *Science, 134,* 2040-2041.

Bukalo O., Pinard C. R., Silverstein S., Brehm C., Hartley N. D., Whittle N., et al. (2015). Prefrontal inputs to the amygdala instruct fear extinction memory formation. *Science Advances, 1,* e1500251.

Caballero, A., Granberg, R. & Tseng, K. (2016). Mechanisms contributing to prefrontal cortex maturation during adolescence. *Neuroscience & Behavioral Reviews, 70,* 4-12.

Campeau, S., Miserendino, M.J. & Davis, M. (1992). Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience, 106,* 569e574.

Castellucci, V. & Kandel, E.R. (1976). Presynaptic facilitation as a mechanism for behavioral sensitization in Aplysia. *Science, 194,* 1176-1178.

Chang, C. H. & Maren, S. (2010). Strain difference in the effect of infralimbic cortex lesions on fear extinction in rats. *Behavioral Neuroscience, 124,* 391–397.

Chapman, W.P., Shroeder, H.R., Guyer,, G., Brazier, M.A.B., Fager, C., Poppen, J.L., Solomon, H.C. & Yakolev, P.L. (1954). Physiological evidence concerning the importance of the amygdaloid nuclear region in the integration of circulating function and emotion in man. *Science, 129*, 949-950.

Chatterjee, D., Chatterjee-Chakraborty, M., Rees, S., Cauchi, J., de Medeiros, C.B. & Fleming, A.S. (2007). Maternal isolation alters the expression of neural proteins during development: 'Stroking' stimulation reverses these effects. *Brain Research, 1158*, 11-27

Chocyk, A., Dudys, D., Przyborowska, A., Maækowiak, M. & Wêdzony, K. (2010). Impact of maternal separation on neural cell adhesion molecules expression in dopaminergic brain regions of juvenile, adolescent and adult rats. *Pharmacological Reports, 62*, 1218-1224.

Chocyk, A., Majcher-Maslanka, I., Dudys, D., Przyborowska, A. & Wedsony, K. (2013a). Impact of early-life stress on the medial prefrontal cortex functions: a search for the pathomechanisms of anxiety and mood disorders. *Pharmacological Reports, 65*, 1462-1470.

Chocyk, A., Bobula, B., Dudys, D., Przyborowska, A., Majcher-Maslanka, I., Hess, G. & Wedzony, K. (2013b). Early-life stress affects the structural and functional plasticity of the medial prefrontal cortex in adolescent rats. *European Journal of Neuroscience, 38*, 2089-2107.

Collins, D.R. & Pare, D. (2000). Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(-). *Learning and Memory, 7*, 97e103.

Cohen, J.D. & Servan-Schreiber, D. (1992). Context, cortex and dopamine: a connectionist approach to behavior and biology in schizophrenia. *Psychological Review, 99*, 45-77.

Cohen, J.D., Barch, D.M., Carter, C.S. & Servan0Schreiber, D. (1999). Schizophrenic deficits in the processing of context: converging evidence from three theoretically motivated cognitive tasks. *Journal of Abnormal Psychology, 108*, 120-133.

Cohen, M.M., Jing, D., Yang, R.R., Tottenham, N., Lee, F.S. & Casey, B.J. (2013). Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America, 110*(45), 18274-18278.

Corcoran, K.A. & Quirk, G.J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *Journal of Neuroscience, 27*, 840-844.
Courtin J., Chaudun, F., Rozeske, R. R., Karalis, N., Gonzalez-Campo, C., Wurtz H., et al. (2014). Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. *Nature, 505*, 92–96.

Crawford, M.P., Fulton, J.F., Jacobsen, C.F., & Wolfe, J.B. (1948). Frontal lobe ablation in chimpanzee: a resume of "Becky" and "Lucy". *Res. Publ. Assoc. Nerv. Ment. Dis*., 27, 50-58.

Cressman, V.L., Balaban, J., Steinfeld, S., Shemyakin, A., Graham, P., Parisot, N. & Moore, H. (2010). Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. Journal of Computational Neurology, 518, 2693-2709.

Cromheeke, S. & Mueller, S.C. (2014). Probing emotional influences on cognitive control: an ALE meta-analysis of cognition emotion interactions. *Brain Structure and Function, 219*, 995-1008.

Cummings, J.L. (1993). Frontal-subcortical circuits and human behavior. *Archives of Neurology*. 50, 873-880.

Cunningham, M.G., Bhattacharyya, S. & Benes, F.M. (2002). Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. *Journal of Computational Neurology, 453*, 116-130.

Danielewicz, J. & Hess, G. (2014). Early life stress alters synaptic modification range in the rat lateral amygdala. *Behavioral Brain Research, 265*, 32-37.

Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In: J.P. Aggelton (Ed.), *The Amygdala* (pp. 213-287). Oxford, UK: Oxford University Press.

De Bellis, M.D., Keshavan, M.S., Shifflett, H., Iyengar, S., Beers, S.R., Hall, J., et al. (2002). Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. *Biological Psychiatry, 52*, 1066-1078.

De Bruin, J.P.C., Vanoyen, H.G.M. & Vandepoll, N. (1983). Behavioral changes following lesions of the orbital prefrontal cortex in male rats. *Behavioral Brain Research, 10*, 209-232.

De Bruin, J.P.C. (1990). Social behavior and the prefrontal cortex. In: H.B.M. Uylings, C.G. Van Eden, J.P.C. De Bruin, M.A. Corner, and M.G.P. Feenstra (eds), *The prefrontal cortex: Its structure, Function and Pathology*. Amsterdam: Elsevier, pp. 485-497.

Decety, J., Michalska, K.J. & Kinzler, K.D. (2011). The Contribution of Emotion and Cognition to Moral Sensitivity: A Neurodevelopmental Study. *Cerebral Cortex, 22*, 209-220.

Deets, A.C., Harlow, H.G., Singh, S.D., & Blomquest, A.J. (1970). Effects of bilateral lesions of the frontal granular cortex on the social behavior of rhesus monkeys. *Journal of Comparative and Physiological Psychology, 72*, 452-461.

Derks, N.A.V., Krugers, H.J., Hoogenraad, C.C., Joels, M. & Sarabdjitsingh, R.A. (2016). Effects of early life stress on synaptic plasticity in the developing hippocampus of male and female rats. PLOS ONE, 11(10), e0164551.

Dixon, M.L., Thiruchselvam, R., Todd, R. & Christoff, K. (2017). Emotion and the prefrontal cortex: An integrative review. *Psychological Bulletin, 143*(10), 1033-1081.

Dodhia, S., Hosanagar, A., Fitzgerald, D.A., et al. (2014). Modulation of resting-state amygdala-frontal functional connectivity by oxytocin in generalized social anxiety disorder. *Neuropsychopharmacology, 39*(9), 2061–2069.

Do-Monte F. H., Manzano-Nieves G., Quinones-Laracuente K., Ramos-Medina L. & Quirk G. J. (2015a). Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *Journal of Neuroscience, 35*, 3607–3615.

Do-Monte F. H., Quiñones-Laracuente K. & Quirk G. J. (2015b). A temporal shift in the circuits mediating retrieval of fear memory. *Nature, 519*, 460–463.

Doyere, V., Schafe, G.E., Sigurdsson, T. & LeDoux, J.E. (2003). Longterm potentiation in freely moving rats reveals asymmetries in thalamic and cortical inputs to the lateral amygdala. *European Journal of Neuroscience, 17*, 2703e2715.

Drevets, W.C. (2000). Neuroimaging studies of mood disorders. *Biological Psychiatry, 48*, 813-829.

Drevets, W.C., Price, J.L. & Furey, M.L. (2008). Brain structural and functional abnormalities in mood disorders: implications for microcircuitry models of depression. *Brain Structure and Function, 213*(1-2), 93-118.

Dudai, Y., Jan, Y.N., Byers, D., Quinn, W.G. & Benzer, S. (1976). Dunce, a mutant of Drosophila deficient in learning. *Proceedings of the National Academy of Sciences of the United States of America, 73*, 1684-1688.

Duval, E.R., Javanbakht, A. & Liberson, I. (2015). Neural circuits in anxiety and stress disorders: a focused review. *Therapeutics and Clinical Risk Management, 11*, 115-126.

Etkin, A. & Schatzberg, A.F. (2011). Common abnormalities and disorder-specific compensation during implicit regulation of emotional processing in generalized anxiety and major depressive disorder. *American Journal of Psychiatry, 168*(9), 968-978.

Fanselow, M.S. (1991). The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: A. Depaulis & R. Bandler (Eds.), *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization* (pp. 151-173). New York, NY: Plenum Publishing Co.

Fareri, D.S. & Tottenham, N. (2016). Effects of early life stress on amygdala and striatal development. *Developmental Cognitive Neuroscience, 19*, 233-247.

Felmingham, K., Williams, L.M., Kemp, A.H., et. al. (2010). Neural responses to masked fear faces: sex differences and trauma exposure in posttraumatic stress disorder. *Journal of Abnormal Psychology, 119*(1), 241-247.

Fontanez-Nuin, D. E., Santini, E., Quirk, G. J. & Porter, J. T. (2011). Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. *Cerebral Cortex, 21*, 727–735.

Fonzo, G.A., Simmons, A.N., Thorp, S.R., Norman, S.B., Paulus, M.P. & Stein, M.B. (2010). Exaggerated and disconnected insular-amygdalar blood oxygenation level-dependent response to threat-related emotional faces in women with intimate-partner violence posttraumatic stress disorder. Biological Psychiatry, 68(5), 433-441.

Franzen, E.A. & Myers, R.E. (1973). Age effects of social behavior deficits following prefrontal lesions in monkeys. *Brain Research, 54*, 277-286.

Frodl, T., Reinhold, E., Koutsouleris, N., Reiser, M. & Meisenzahl EM. (2010). Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. *Journal of Psychiatric Research, 44*, 799-807.

Furuta, A. & Murakami, T. Modulation of the jaw muscle activity during the rhythmical jaw movement by stimulation of the cortical masticatory area and amygdala in the rabbit. *Shigaku, 77*(2), 607-617.

Fuster, J.M. (2008). *The Prefontal Cortex*. London, UK: Elsevier.

Gallagher, M. & Chiba, A. A. (1996). The amygdala and emotion. *Current Opinion in Neurobiology, 6*, 221-227.

Garrett, A.S., Carrion, V., Kletter, H., Karchemskiy, A., Weems, C.F. & Reiss, A. (2012). Brain activation to facial expressions in youth with PTSD symptoms. *Depression and Anxiety*, *29*(5), 449-459.

Gee, D.G., Gabard-Durnam, L.J., Flannery, J., Goff, B., Humphreys, K.L., Telzer, E.H., Hare, T.A., Bookheimer, S.Y. & Tottenham N. (2013a). Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proceedings of the National Academy of Sciences of the United Sates of America*, Epub.

Gee, D.G., Humphreys. K.L., Flannery, J., Goff, B., Telzer, E.H., Shapiro. M., Hare, T.A., Bookheimer, S.Y. & Tottenham N. (2013b). A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. *Journal of Neuroscience, 33*, 4584-4593.

Giese, K.P., Fedorov, N.B., Filipkowski, R.K., & Silva, A.J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science, 279*, 870–873.

Giménez, M., Ortiz, H., Soriano-Mas, C., et al. (2014). Functional effects of chronic paroxetine versus placebo on the fear, stress and anxiety brain circuit in Social Anxiety Disorder: initial validation of an imaging protocol for drug discovery. *European Neuropsychopharmacology, 24*(1), 105–116.

Gloor, P., Olivier, A. & Quesney, L.F. (1981). The role of the amygdala in the expression of psychic phenomena in temporal love seizures. In: Y. Bren-Ari (Ed.), *The Amygdaloid Complex* (pp. 489-507). North-Holland, New York: Elsevier.

Goddard, G.V. (1964). Functions of the amygdala. *Psychological Bulletin, 62*, 89-109.

Goldstein, L.E., Rasmusson, A.M., Bunney, B.S. & Roth, R.H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *Journal of Neuroscience*, 16(15), 4787-4789.

Goosens, K.A., Hobin, J.A. & Maren, S. (2003). Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? *Neuron, 40*, 1013e1022.

Grafman, J. (1994). Alternative frameworks for the conceptualization of prefrontal functions. In: F. Boller. & J. (Eds.), *Handbook of Neuropsychology* (pp. 187). Amsterdam: Elsevier.

Greenblatt, M., Arnot, R., & Solomon, H.D. (1950). *Studies in Lobotomy*. New York, NY: Grune & Stratton.

Habib, D., Tsui, C.K., Rosen, L.G. & Dringenberg, H.C. (2013). Occlusion of low-requency-induced, heterosynaptic long-term potentiation in the rat hippocampus in vivo following spatial training. *Cerebral Cortex, 24*, 3090-3096.

Hanson, J.L., Chung, M.K., Avants, B.B., Rudolph, K.D., Shirtcliff, E.A., Gee, J.C., Davidson, R.J. & Pollak, S.D. (2012). Structural variations in prefrontal cortex mediate the relationship between early childhood stress and spatial working memory. *Journal of Neuroscience, 32*, 7917-7925.

Hatfield, T. & Gallagher, M. (1995). Taste-potentiated odor conditioning: impairment produced by infusion of an N-methyl-D-aspartate antagonist into basolateral amygdala. *Behavioral Neuroscience, 109*(4), 663-668.

Hatfield, T., Han, J.S., Conley, M., Gallagher, M. & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central amygdala interfere with Pavlovian second-order conditioning and reinforce devaluation effects. *Journal of Neuroscience, 12*, 5256-5265.

Hebb, D. O. (1949). *The organization of behavior: A neuropsychological theory*. Mahwah, NJ: John Wiley & Sons Inc.

Hofer, M.A. (1970). Physiological responses of infant rats to separation from their mothers. *Science, 168*, 871–873.

Hofer, M.A. (1975). Studies on how early maternal separation produces behavioral change in young rats. *Psychosomatic Medicine, 37*, 245–264.

Holmes, G. (1931). Mental symptoms associated with brain tumours. *Lancet, 1*, 408-410.

Hölzel, B.K., Hoge, E.A., Greve, D.N., et al. (2013). Neural mechanisms of symptom improvements in generalized anxiety disorder following mindfulness training. *NeuroImage Clinical, 2*, 448–458.

Huang, Y.Y., Martin, K.C., Kandel, E.R. (2000). Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. *Journal of Neuroscience, 20*, 6317e6325.

Jacobsen, C.F., Wolfe, J.B. & Jackson, T.A. (1935). An experimental analysis of the functions of the frontal association areas in primates. *Journal of Nervous and Mental Disease, 82*, 1-14.

Joels, M. & Krugers, H. (2007). LTP after stress: Up or down? *Neural Plasticity, 2007*, 93202.

Johnson, F.K., Delpech, J.C., Thompson, G.J; Wei, L., Hao, J., Herman, P., Hyder, F. & Kaffman, A. (2018). Amygdala hyper-connectivity in a mouse model of unpredictable early life stress. *Translational Psychiatry, 8*, 49.

Jovanovic, T & Norrholm, S.D. (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience, 5*, 44.

Kao, G.S., Cheng, L.Y., Chen, L.H., Tzeng, W.Y., Cherng, C.G., Su, C.C., Wang, C.Y. & Yu, L. (2012). Neonatal isolation decreases cued fear conditioning and frontal cortical histone 3 lysine 9 methylation in adult female rats. *European Journal Pharmacology, 697*, 65–72.

Kessler, R.C., McLaughlin, K.A., Green, J.G., Gruber, M.J., Sampson, N.A., Zaslavsky, A.M., Aguilar-Gaxiola, S. et al. (2010). Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *The British Journal of Psychiatry, 197*, 378–385.

Kim, S. C., Jo, Y. S., Kim, I. H., Kim, H., Choi, J. S. (2010). Lack of medial prefrontal cortex activation underlies the immediate extinction deficit. *Journal of Neuroscience, 30*, 832–837.

Kim, J.E., Dager, S.R. & Lyoo, I.K. (2012). The role of the amygdala in the pathophysiology of panic disorder: evidence from neuroimaging studies. *Biology of Mood and Anxiety Disorders*, 2(1), 20.

Kling, A. & Mass, R. (1974). Alterations of social behavior with neural lesion sin nonhuman primates. In: R.T. Holloway (ed.), *Primate Aggression, Territoriality, and Xenophobia*. New York, NY: Academic Press, pp. 361-386.

Krasne, F.B. (1969). Excitation and habituation of the crayfish escape reflex: the depolarizing response in lateral giant fibers of the isolated abdomen. *Journal of Experimental Biology, 50*, 29-46.

Labuschange, I., Phan, K.L., Wood, A., et. al. (2010). Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. *Neuropsychopharmacology, 35*(12), 2403-2413.

Lomo, T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiologica Scandinavia, 68,* 128.

Lynch, M.A. (2004). Long-term potentiation and memory. Physiological Reviews, 84(1), 87-136.

Klumpp, H., Angstadt, M., Nathan, P.J. & Phan, K.L. (2010). Amygdala reactivity to faces at varying intesnsities of threat in generalized social phobia: an event-related functional MRI study. *Psychiatry Research, 183*(2), 167-169.

Klumpp, H., Post, D., Angstadt, M., Fitzgerald, D.A. & Phan, K.L. (2013). Anterior cingulate cortex and insula response during indirect and direct processing of emotional faces in generalized social anxiety disorder. *Biology of Mood and Anxiety Disorders, 3*(1), 7.

Kojima, S. & Alberts, J.R. (2011). Oxytocin mediates the acquisition of filial, odor-guided huddling for maternally-associated odor in preweanling rats. *Hormones and Behavior, 60,* 549–558.

Kojima, S., Stewart, R.A., Demas, G.E. & Alberts, J.R. (2012). Maternal contact differentially modulates central and peripheral oxytocin in rat pups during a brief regime of mother-pup interaction that induces a filial huddling preference. *Journal of Neuroendocrinology, 24,* 831–840.

Kruk, M.R., Van Der Poel, A.M. & DeVos-Frerichs, T.P. (1979). The induction of aggressive behavior by electrical stimulation in the hypothalamus of male rats. *Behavior, 70*, 317-221.

Kuhn, C.M., Pauk, J. & Schanberg, S.M. (1990). Endocrine responses to mother-infant separation in developing rats. *Developmental Psychobiology, 23,* 395–410

Lang, P.J. & Davis, M. (2006). Emotion, motivation, and the brain: reflex foundations in animal and human research. *Progress in Brain Research, 156,* 3-29.

Langworthy, O.R. & Richter, C.P. (1939). Increased spontaneous activity produced by frontal lobe lesion in cats. *American Journal of Physiology, 126,* 158-161.

LeDoux, J.E. (1998). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *Journal of Neuroscience, 8,* 2517-2529.

LeDoux, J.E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23,* 155-184.

Leventopoulos, M., Ruedi-Bettschen, D., Knuesel, I., Feldon, J., Pryce, C.R. & Opacka-Juffry, J. (2007). Long-term effects of early life deprivation on brain glia in Fischer rats. *Brain Research, 1142,* 119–126.

Lipka, J., Miltner, W.H.R. & Straube, T. (2011). Vigilance for threat interacts with amygdala responses to subliminal threat cues in specific phobia. *Biological Psychiatry, 70*(5), 472-478.

Lishman, W.A. (1968). Brain damage in relation to psychiatric disability after injury. *British Journal of Psychiatry, 114*, 373-410.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S. et al. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science, 277*, 1659–1662.

Liu, D., Diorio, J., Day, J.C., Francis, D.D. & Meaney, M.J. (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience, 3*, 799–806.

Lueken, U., Hilbert, K., Stolyar, V., Maslowski, N.I., Beesdo-Baum, K. & Wittchen, H.U. (2013). Neural substrates of defensive reactivity in two subtypes of specific phobia. *Social Cognitive and Affective Neuroscience*, Epub.

MacLeod, C.M. (1991). Half a century of research on the Stroop effect: an integrative review. *Psychological Bulletin, 109*, 163-203.

Mansson, K.N.T., Carlbring, P., Frick, A., et. al. (2013). Altered neural correlates of affective processing after internet-delivered cognitive behavior therapy for social anxiety disorder. *Psychiatry Research, 214*(3), 229-237.

Marek, R., Strobel, C., Bredy, T.W. & Sah, P. (2013). The amygdala and medial prefrontal cortex: partners in the fear circuit. *The Journal of Physiology, 591*(10), 2381-2391.

Maroun, M. (2006). Stress reverses plasiticity in the pathway projecting from the ventromedial prefrontal cortex to the basolateral amygdala. *European Journal of Neuroscience, 24*(10), 2917-2922.

Maroun, M., Kavushansky, A., Holmes, A., Wellman, C. & Motanis, H. (2012). Enhanced extinction of aversive memories by high-frequency stimulation of the rat infralimbic cortex. *PLoS One, 7*, e35853.

Maslowsky, J., Mogg, K., Bradley, B.P., et al. (2010). A preliminary investigation of neural correlates of treatment in adolescents with generalized anxiety disorder. *Journal of Child and Adolescent Psychopharmacology, 20*(2), 105–111.

McCall, R.B. & Aghajanian, G.K. (1979). Serotonergic facilitation of facial motoneuron excitation. *Brain Research, 169*, 11-27.

McDonald, A.J., Mascagni, F. & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience, 71*, 55-75.

McDonald, A.J. (1998). Cortical pathways to the mammalian amygdala. *Progress in Neurobiology, 55*, 257-332.

Meaney, M.J., Viau, V., Bhatnagar, S., Betito, K., Iny, L.J., O'Donnell, D., Mitchell, J.B. (1991). Cellular mechanisms underlying the development and expression of individual differences in the hypothalamic–pituitary–adrenal stress response. *Journal of Steroid Biochemistry and Molecular Biology, 39*, 265–274.

Mehta, M.A., Golembo, N.I., Nosarti, C., Colvert, E., Mota, A., Williams, S.C., et al. (2009). Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. *Journal of Child Psychology and Psychiatry, 50*, 943-951.

Menzel, R. & Erber, J. (1978). Learning and memory in bees. *Scientific American, 239*, 80-87.

Milad, M.R. & Quirk, G.J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature, 420*, 70-74.

Milad, M.R., Vidal-Gonzalez, I. & Quirk, G.J. (2004). Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioral Neuroscience, 118*, 389-394.

Milad, M.R., Pitman, R.K., Ellis, C.B., et. al. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 22*(12), 1075-1082.

Miller, M.H. (1976). Dorsolateral frontal lobe lesions and behavior in the macaque: Dissociation of threat and aggression. *Physiology & Behavior., 17*, 209-213.

Miller, E.K. (1999). The prefrontal cortex: complex neural properties for complex behavior. *Neuron, 22*, 15-17.

Miller, E.K. & Cohen, J.D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience, 24*, 167-202.

Milner, B. (1962). In: Physiologie de l'hippocampe. Passouant P, editor. Centre National de la Recherche Scientifique; Paris. pp. 257–272.

Milner, E.K. (1963). Effects of different brain lesions on card sorting. *Archives of Neurology, 9*, 90.

Miserendino, M.J., Sananes, C.B., Melia, K.R. & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMD antagonists in the amygdala. *Nature, 345*, 716-718.

Monroy, E., Hernandez-Torres, E. & Flores G (2010). Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. *Journal of Chemical Neuroanatomy, 40*, 93–101.

Morgan, M.A., Romanski, L.M. & LeDoux, J.E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neuroscience Letters, 163*(1), 109-113.

Morgan, M.A. & LeDoux, J.E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral Neuroscience, 109*, 681-688.

Morris, R.G., Anderson, E., Lynch, G.S. & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*, 774-776.

Moser, E.I., Krobert, K.A., Moster, M.B. & Morris, R.G. (1998). Impaired spatial learning after saturation of long-term potentiation. *Science, 281*, 2038-2042.

Motzkin, J.C., Philippi, C.L., Wolf, R.C., M.K., Baskaya, M.K. & Koenigs, M. (2015). Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. Biological Psychiatry, 77, 276-284.

Muhammad, A. & Kolb, B. (2011). Maternal separation altered behavior and neuronal spine density without influencing amphetamine sensitization. *Behavioral Brain Research, 223*, 7–16.

Musholt, K., Cirillo, G., Cavaliere, C., Rosaria, B.M., Bock, J., Helmeke, C., Braun, K. & Papa, M. (2009). Neonatal separation stress reduces glial fibrillary acidic protein- and S100bimmunoreactive astrocytes in the rat medial precentral cortex. *Developmental Neurobiology, 69*, 203–211.

Myers, R.E. (1972). Role of prefrontal and anterior temporal cortex in social behavior and affect in monkeys. *Acta Neurobiologiae Experimentalis, 32*, 567-579.

Navabi, S., Fox, R., Proulx, C.D., Lin, J.Y., Tsien, R.Y. & Malinow, R. (2014). Engineering a memory with LTD and LTP. *Nature, 511*, 348-352.

Nitschke, J.B., Sarinopoulos, I., Oathes, D.J., et al. (2009). Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *American Journal of Psychiatry, 166*(3), 302–310.

Ochsner, K.N. & Gross, J.J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences, 9*(5), 242-249.

Ongur, D. & Price, J.L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys, and humans. *Cerebral Cortex, 10*, 206-219.

Ottaviani, C., Cevolani, D., Nucifora, V., et al. (2012). Amygdala responses to masked and low spatial frequency fearful faces: a preliminary fMRI study in panic disorder. *Psychiatry Research, 203*(2–3), 159–165.

Palm, M.E., Elliott, R., McKie, S., Deakin, J.F.W. & Anderson, I.M. (2011). Attenuated responses to emotional expressions in women with generalized anxiety disorder. *Psychological Medicine, 41*(5), 1009-1018.

Paradiso, S., Chemerinski, E., Yazici, K.M., Tartaro, A. & Robinson, R.G. (1999). Frontal lobe syndrome reassessed: comparison of patients with lateral or medial frontal brain damage. *Journal of Neurology, Neurosurgery, and Psychiatry, 67*, 664-667.

Pascual, R., Zamora-Leon, S.P. (2007). Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. *Acta Neurobioligae Experimentalis, 67*, 471–479.

Passingham, R. (1993). *The Frontal Lobes and Voluntary Action*. Oxford, UK: Oxford Univ. Press.

Patel, R., Spreng, R.N., Shin, L.M. & Girard, T.A. (2012). Neurocircuitry models of posttraumatic stress disorder and beyond: a meta-analysis of functional neuroimaging studies. *Neuroscience and Behavioral Reviews, 36*(9), 2130-2142.

Paxinos, G. & Watson, C. (2007). The Rat Brain in Stereotaxic Coordinates. London: Academic Press.

Perlman, S.B. & Pelphrey, K.A. (2011). Developing connections for affective regulation: age-related changes in emotional brain connectivity. *Journal of Experimental Child Psychology, 108*, 607-620.

Perrett, E. (1974). The left frontal lobe of man and the suppression of habitual responses in verbal categorical behavior. *Neuropsychologia, 12*, 323-330.

Phan, K.L., Coccaro, E.F., Angstadt, M., et. al. (2013). Corticolimbic brain reactivity to social signals of threat before and after sentraline treatment in generalized social phobia. *Biological Psychiatry, 73*(4), 329-336.

Price, R.B., Eldreth, D.A. & Mohlman, J. (2011). Deficient prefrontalattentional control in late-life generalized anxiety disorder: an fMRI investigation. *Translational Psychiatry, 1*(10), e45.

Quinn, W.G., Harris, W.A. & Benzer, S. (1974). Conditioned behavior in Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America, 71*, 708-712.

Quirk, G.J., Repa, C. & LeDoux, J.E. (1995). Fear conditioning enhances shortlatency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron, 15*, 1029e1039.

Quirk, G.J., Russo, G.K., Barron, J.L. & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *Journal of Neuroscience, 20*, 6225-6231.

Ramon y Cajal, S. (1894). Croonian lecture. La fine structure des centres nerveux. *Proc R Soc London, 55*, 444-468.

Redmond, D.E. (1977). Alteration in the function of the nucleus locus: a possible model for studies on anxiety. In: I.E. Hanin. & E. Usdin, (Eds.), *Animal Models in Psychiatry and Neurology* (pp. 292-304). Oxford, UK: Pergamon Press.

Repa, J.C., Muller, J., Apergis, J., Desrochers, T.M., Zhou, Y. & LeDoux, J.E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nature Neuroscience, 4*, 724e731.

Rinne-Albers, M.A., van der Wee, N.J., Lamers-Winkelman, F. & Vermeiren, R.R. (2013). Neuroimaging in children, adolescents and young adults with psychological trauma. *European Child & Adolescent Psychiatry*, Epub.

Rodrigues, S.M., Schafe, G.E. & LeDoux, J.E. (2001). Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. *Journal of Neuroscience, 21*, 6889e6896.

Rogan, M.T., Staubi, U.V. & LeDoux, J.E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature, 390*, 604-607.

Rogan, M.T. & LeDoux, J.E. (1995). LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. *Neuron, 15*, 127e136.

Rolls, E.T., Hornak, J., Wade, D. & McGrath, J. (1994). Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *Journal of Neurology, Neurosurgery, and Psychiatry, 57*, 1518-1524.

Romanski, L.M., Clugnet, M.C., Bordi, F. & LeDoux, J.E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behavioral Neuroscience, 107*, 444-450.

Rougemont-Bucking, A., Linnman, C., Zeffiro, T.A., et. al. (2011). Altered processing of contextual information during fear extinction in PTSD: an fMRI study. *CNS Neuroscience & Therapeutics, 17*(4), 227-236.

Rowe, A.D., Bullock, P.R., Polkey, C.E, & Morris, R.G. (2001). Theory of mind impairments and their relationship to executive functioning following frontal lobe excisions. *Brain, 124*, 600-616.

Roy, A.K., Fudge, J.L., Kelly, C., et al. (2013). Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(3), 290–299.

Sánchez, M.M., Ladd, C.O. & Plotsky, P.M. (2001). Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Developmental Psychopathology, 13*, 419–449.

Santini, E., Sepulveda-Orengo, M. & Porter, J. T. (2012). Muscarinic receptors modulate the intrinsic excitability of infralimbic neurons and consolidation of fear extinction. *Neuropsychopharmacology, 37*, 2047–2056.

Stanton, M.E. & Levine, S. (1990). Inhibition of infant glucocorticoid stress response: specific role of maternal cues. *Developmental Psychobiology, 23*, 411–426.

Sato, M. (1971). Prefrontal cortex and emotional behaviors. *Folia Psychiatrica Et Neurologica Japonica, 25*, 69-78.

Sato, M., Onishi, J. & Otsuki, S. (1971). Integrating functions of the prefrontal cortex on emotional behaviors. *Folia Psychiatrica Et Neurologica Japonica, 25*, 283-293.

Schlund, M.W., Verduzco, G., Cataldo, M.F. & Hoehn-Saric, R. (2012). Generalized anxiety modulates frontal and limbic activation in major depression. *Behavioral and Brain Functions, 8*(1), 8.

Schmidt, S., Mohr, A., Miltner, W.H.R. & Straube, T. (2010). Task-dependent neural correlates of the processing of verbal threat-related stimuli in social phobia. *Biological Psychology, 84*(2), 304-312.

Schwaber, J.S., Kapp, B.S., Higgins, G.A., & Rapp, P.R. (1982). Amygdaloid basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. *Journal of Neuroscience, 2*, 1424-1438.

Schweckendiek, J., Klucken, T., Merz, C.J., et. al. (2011). Weaving the (neuronal) web: fear learning in spider phobia. *Neuroimage, 54*(1), 681-688.

Scoville, W.B. & Milner, B. J. (1957). *Journal of Neurology, Neurosurgery, and Psychiatry, 20*, 11–21

Shamay-Tsoory, S.G., Tomer, R., Holdsher, D., Berger, B.D., & Aharon-Peretz, J. (2004). Impairment in cognitive and affective empathy in patients with brain lesions: anatomical and cognitive correlates. *Journal of Clinical and Experimental Neuropsychology, 26*, 1113-1127.

Shustin, N. A. (1959). *Physiology of frontal lobes: An experimental investigation.* Leningrad: Medgiz.

Shvil, E., Rusch, H.L., Sullivan, G.M. & Neria, Y. (2013). Neural, psychophysiological, and behavioral markers of fear processing in PTSD: a review of the literature. *Current Psychiatry Reports, 15*(5), 358-374.

Siegel, A., Edinger, H., & Lowenthal, H. (1974). Effects of electrical stimulation of the medial aspect of the prefrontal cortex upon attack behavior in cats. *Brain Research, 66*, 467-479.

Sierra-Mercado, D., Padilla-Coreano, N.Q. & Quirk, G.J. (2011). Dissociable roles of prelimbic and infalimbic cortices, ventral hippocampus and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology, 36*, 529-538.

Sigurdsson, T., Doyere, V., Cain, C.K. & LeDoux, J.E. (2006). Long-term potentiation in the amygdala: A cellular mechanism of fear learning and memory. *Neuropharmacology, 52*(1), 215-227.

Soltysik, S. & Jaworska, K. (1967). Prefrontal cortex and fear-motivated behavior. *Acta Biotheoretica Experimentalis*, 27, 429-448.

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., Quirk, G. J. (2012). Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron, 76*, 804–812.

Spencer, W.A., Thompson, R.F. & Nielson, D.R. (1966). Decrement of ventral root electrotonus and intracellularly recorded PSPs produced by iterated cutaneous afferent volleys. *Journal of Neurophysiology, 29*, 253-274.

Stevenson, C.W., Halliday, D.M., Marsden, C.A. & Mason, R. (2008). Early life programming of hemispheric lateralization and synchronization in the adult medial prefrontal cortex. *Neuroscience, 155*, 852–863.

Stevenson, C.W., Spicer, C.H., Mason, R. & Marsden, C.A. (2009). Early life programming of fear conditioning and extinction in adult male rats. *Behavioral Brain Research, 205*, 505–510.

Strawn, J.R., Bitter, S.M., Weber, W.A., et al. (2012). Neurocircuitry of generalized anxiety disorder in adolescents: a pilot functional neuroimaging and functional connectivity study. *Depression and Anxiety*, *29*(11), 939–947.

Strawn, J.R., Wehry, A.M., Chu, W.J., et al. (2013). Neuroanatomic abnormalities in adolescents with generalized anxiety disorder: a voxel-based morphometry study. *Depression and Anxiety, 30*(9), 842–848.

Stone, V.E., Baron-Cohen, S. & Knight, R.T. (1998). Frontal lobe contributions to theory of mind. *Journal of Cognitive Neuroscience, 10*, 640-656.

Stroop, J.R. (1935). Studies of interface in serial verbal reactions. *Journal of Experimental Psychology, 18*, 643-662.

Takeuchi, T., Duszkiewicz, A.J. & Morris, R.G. (2014). The synaptic plasticity and memory hypothesis: Encoding, storage and persistence. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 369*, 20130288.

Taylor, C.T., Aupperle, R.L., Flagan, T., et. al. (2014). Neural correlates of a computerized attention modification program in anxious subjects. *Social Cognitive and Affective Neuroscience, 9*(9), 1379-1387.

Tottenham, N., Hare, T.A., Quinn, B.T., McCarry, T.W., Nurse, M., Gilhooly, T., Millner, A., et. al. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Developmental Science, 13*, 46-61.

Tottenham, N., Hare, T.A., Millner, A., Gilhooly, T., Zevin, J.D., Casey, B.J. (2011). Elevated amygdala response to faces following early deprivation. *Developmental Science, 14*, 190-204.

Tromp, D.P., Grupe, D.W., Oathes, D.J., et al. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of General Psychiatry, 69*(9), 925–934.

Tsvetkov, E., Shin, R.M. & Bolshakov, V.Y. (2004). Glutamate uptake determines pathway specificity of long-term potentiation in the neural circuitry of fear conditioning. *Neuron, 41*, 139e151.

Uchida, S., Hara, K., Kobayashi, A., Funato, H., Hobara, T., Otsuki, K., Yamagata, H. et al. (2010). Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. *Journal of Neuroscience, 30*, 15007–15018.

van Harmelen, A.L., van Tol, M.J., van der Wee, N.J., Veltman, D.J., Aleman, A., Spinhoven, P., van Buchem, M.A. et al. (2010). Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment. *Biological Psychiatry, 68*, 832–838

Vendrell, P., Junque, C., Pujol, J. Jurado, M.A., Molet, J. & Gragman, J. (1995). The role of prefrontal regions in the Stroop task. *Neuropsychologia, 33*, 341-352.

Vetulani, J. (2013). Early maternal separation: a rodent model of depression and a prevailing human condition. *Pharmacological Reports, 65*, 1451-1461.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L. & Quirk, G.J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learning and Memory, 13*, 728-733.

Vouimba, R.M. & Maround, M. (2011). Learning-induced changes in mPFC-BLA connections after fear conditioning, extinction, and reinstatement of fear. *Neuropsychopharmacology, 36*, 2276-2285.

Vouimba, R.M., Yaniv, D., Diamond, D. & Richter-Levin, G. (2004). Effects of inescapable stress on LTP in the amygdala versus the dentate gyrus of freely behaving rats. *European Journal of Neuroscience, 19*, 1887-1894.

Wang, L., Jiao, J. & Dulawa, S.C. (2011). Infant maternal separation impairs adult cognitive performance in BALB/cJ mice. *Psychopharmacology (Berl), 216*, 207–218.

Wang, L., Paul, N., Stanton, S.J., Greeson, J.M., Smoski, M.J. (2013). Loss of sustained activity in the ventromedial prefrontal cortex in response to repeated stress in individuals with early-life emotional abuse: implications for depression vulnerability. *Frontiers in Psychology, 4*, 320.

Weisskopf, M.G. & LeDoux, J.E. (1999). Distinct populations of NMDA receptors at subcortical and cortical inputs to principal cells of the lateral amygdala. *Journal of Neurophsyiology, 81*, 930e934.

White, S.R. & Neuman, R.S. (1980). Facilitation of spinal motoneuron excitability by 5-hydroxytryptamine and noradrenaline. *Brain Research, 185*, 1-9.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., & Bear, M.F. (2006). Learning induces long-term potentiation in the hippocampus. *Science, 313*, 1093-1097.

Wise, S.P., Murray, E.A. & Gerfen, C.R. (1996). The frontal-basal ganglia system in primates. *Critical Review of Neurobiology, 10*, 317-356.

Zantvoord, J.B., Diehle, J. & Lindauer, R.J.L. (2013). Using neurobiological measures to predict and assess treatment outcome of psychotherapy in posttraumatic stress disorder: systematic review. *Psychotherapy and Psychosomatics, 82*(3), 142-151.