Book Chapter

Molecular Correlates of Stress-Induced Increases in Fear Memory Consolidation within the Amygdala

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Abstract

Stress can significantly modulate brain function and increase the risk for developing various psychiatric disorders. Many of the brain regions that are implicated in psychiatric disorders and are vulnerable to the effects of stress are also involved in mediating emotional learning. Emotional learning has been a subject of intense investigation for the past 30 years, with the vast majority of studies focusing on the amygdala and its role in associative fear learning. However, the mechanisms by which stress affects amygdala-dependent memories remain unclear. Here we review the literature on the enhancing effects of acute and chronic stress on the acquisition and/or consolidation of a fear memory, as measured by auditory fear conditioning, and discuss potential mechanisms by which these changes occur in the amygdala. We hypothesize that stress-mediated activation of the glucocorticoid receptors (GR) and norepinephrine release within the amygdala leads to mobilization AMPA receptors to the synapse, which underlies stress-induced increases in fear memory. We discuss the implications of this hypothesis for understanding how stress affects fear extinction and the development of treatments for anxiety disorders. Understanding how stress-induced changes in glucocorticoid and norepinephrine signaling might converge to affect emotional learning by increasing the trafficking of AMPA receptors and enhancing amygdala excitability is a promising area for future research.

Introduction

Stress is defined as any threat or perceived threat that disturbs an organism's ability to maintain homeostasis. Although activation of the stress response is initially adaptive, long-term exposure to stress poses a significant risk for the development of numerous psychiatric disorders [1-5] Many of the brain regions that are implicated in psychiatric disorders and are vulnerable to the effects of stress are also involved in mediating emotional learning, alterations of which have been suggested to contribute to the onset and maintenance of these disorders [6]. Emotional learning has been a subject of intense investigation for the past 30 years, with the vast majority of studies focusing on the

amygdala and its role in associative fear learning. While this has led to identification of many of the underlying mechanisms, it remains unclear how stress affects these mechanisms. A greater understanding of this process is of great clinical relevance particularly for pharmacological interventions that are provided in conjunction with learning-based treatments [7]. Here we review the literature on the cellular and molecular mechanisms mediating associative learning, as measured by Pavlovian fear conditioning. We then summarize the effects of acute and chronic stress on the acquisition and consolidation of a fear memory and discuss potential mechanisms by which these changes occur in the amygdala. All the studies we review used auditory fear conditioning, unless otherwise specified. We hypothesize that stress-mediated activation of the glucocorticoid receptors (GR) and norepinephrine release within the amygdala leads to mobilization AMPA receptors to the synapse, which underlies stress-induced increases in fear memory. We discuss the implications of these hypotheses for understanding how stress affects fear extinction and the development of treatments for anxiety disorders.

Pavlovian Fear Conditioning as a Model of Associative Learning

Pavlovian fear conditioning has been used extensively to study the cellular and molecular mechanisms of associative learning. In Pavlovian fear conditioning, a neutral stimulus, such as a tone (conditioned stimulus, CS) is paired with an aversive stimulus, such as a foot shock (unconditioned stimulus, US). After the animal learns to associate the CS with the US, presentation of the tone alone elicits defensive responses that are characteristic of fear, such as freezing [8] This process of fear conditioning involves distinct stages of memory. Acquisition refers to the initial learning of the CS-US association, and is followed by consolidation, which is the 24-h period during which the memory is thought to become stable. Long-term memory is expressed the next day when the CS is presented in the absence of the US, leading to retrieval of the fear memory. Interestingly, retrieval renders the memory labile again and reconsolidation is required to put it back into long-term storage. When retrieval

involves multiple presentations of the CS alone, the CS gradually loses the ability to evoke a conditioned response, demonstrating acquisition of extinction. This form of learning also requires consolidation so that the extinction memory can be retrieved at a later time point.

Numerous studies have established a functional role for specific sub nuclei in the amygdala in mediating fear conditioning. These subnuclei include the lateral nucleus (LA), which is the main input nucleus and a key site of plasticity underlying associative learning [9-10], the basal nucleus (B), and the central (CeA) nucleus, which is the main output nucleus of the amygdala. During acquisition, the CS causes glutamate release from pre-synaptic terminals where it binds to AMPA and NMDA receptors located on principal cells in the LA. These same cells in the LA are depolarized by somatosensory inputs activated by the foot shock (US) [11]. Strong depolarization removes the Mg^{+} block from NMDA receptors, allowing for calcium entry [12]. Calcium in turn activates the Ca^{2}/calmodulin dependent protein kinase II (CAMKII), which modulates AMPA receptors by increasing their conductance [13,14] and/or by increasing their trafficking to the synapse [15]. Specifically, CAMKII increases the conductance of GluA1-containing AMPA receptors via phosphorylation mechanisms [13,14] and mobilizes GluA1 to the synapse [15]. During the acquisition of a conditioned fear memory, GluA1 is driven into the synapses of dendritic spines of principal neurons in the LA [16], a process that is necessary for fear learning [17]. For the memory to be consolidated, activation of protein kinases, such as protein kinase A and mitogen-activated protein kinase (MAPK), trigger gene transcription and the translation of new proteins [18-20], including GluA1 and GluA2-containing AMPA receptors [21]. During consolidation, GluA2 subunits replace the newly inserted GluA1 subunits, thereby stabilizing the memory for later retrieval [22,23]. Maintenance of GluA2 in the synaptic membrane has been shown to be dependent upon interactions with protein kinase M zeta (PKMζ), which together underlie the long-term persistence of conditioned fear memories in the LA [24].

Efects of Acute Stress on Fear Memory: Potential Mechanisms in the Bla

Acute stress rapidly activates the autonomic nervous system, which results in the release of catecholamines, such as norepinephrine, from the adrenal medulla. The release of norepinephrine acts directly on the cardiovascular system triggering peripheral responses, such as an increase in heat rate, respiration, and blood pressure [25]. Stress also leads to the release of norepinephrine into multiple brain regions through projections from the locus coeruleus [26]. In addition, stress engages the HPA axis by stimulating the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, which causes the pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH in turn stimulates the synthesis and release of glucocorticoid hormones from the adrenal cortex [27], which bind to mineralcorticoid receptors (MR) and glucocorticoid receptors (GR) located throughout the body and brain. Acute activation of the stress response primarily leads to the binding of glucocorticoids to low affinity GRs rather than high affinity MRs, which are occupied under basal conditions [28].

Converging lines of evidence indicate that acute stress leads to changes in auditory fear conditioning by activating GRs and stimulating the local release of norepinephrine in the lateral and basal nuclei (BLA) of the amygdala. The most commonly reported effect of stress on learning is an enhancement in memory consolidation. For example, acute stress or a single systemic injection of the glucocorticoid hormone corticosterone administered immediately after fear conditioning improves long-term memory [29-32]. These enhancing effects of a post-training injection of corticosterone on memory consolidation are blocked by a local infusion of the β-adrenergic receptor antagonist atenolol into the BLA, indicating a necessary role for norepinephrine in the amygdala in this corticosterone-induced response [32]. Interestingly, infusion of β-adrenergic receptor antagonists into the BLA did not affect consolidation of a conditioned fear memory when administered in the absence of corticosterone [32,33], indicating that norepinephrine only plays

this role when the stress response is activated. Evidence also supports a role for direct activation of GRs in the amygdala on memory consolidation. This is based on the observation that blunting the activity of GRs in the BLA with a viral vector impairs long-term memory without affecting acquisition or postshock freezing [34].

Further support for the roles of GR activation and NE release in the amygdala on stress-induced increases in memory consolidation come from studies using inhibitory avoidance. During inhibitory avoidance training, an animal is placed in a brightly lit compartment, with access to a dark chamber. Entering the dark chamber leads to a foot shock and subsequent avoidance of this area. Latency to enter the dark chamber the next day is used to measure retrieval of the fear memory. Like Pavlovian fear conditioning, activity of NMDA and AMPA receptors in the BLA is required for the acquisition and consolidation of this fear memory [35]. Inhibitory avoidance studies reveal that fear memory consolidation is enhanced by a single post-training systemic injection of the GR agonist dexamethasone, effects that are replicated by directly infusing the same GR agonist into the BLA [36]. Furthermore, an intra-BLA infusion of propranolol is sufficient to block both the systemic and local effects of dexamethasone, demonstrating that norepinephrine signaling within the BLA is required for GR-mediated enhancement in memory consolidation [37]. Activation of GR is also required for norepinephrine-mediated increases in memory consolidation in the inhibitory avoidance task. This was demonstrated by a study in which the enhancing effects of a post-training intra-BLA infusion of a β-adrenergic receptor agonist on consolidation was blocked by pretreatment with an intra-BLA infusion of the GR-antagonist RU 38486 [37]. In addition to interacting with norepinephrine signaling within the amygdala, glucocorticoids are also able to influence the release of norepinephrine by binding to GRs in the locus coeruleus [38]. As a result, infusion of the GR agonist RU 28362 into the locus coeruleus enhances consolidation of an inhibitory avoidance memory [39]. This effect is blocked by intra-BLA infusion of the β-adrenergic receptor antagonist atenolol, providing further evidence of a role for norepinephrine

signaling in the BLA in stress-induced enhancement of memory consolidation [40].

While the above studies demonstrate that activation of the GR and the β-adrenergic receptor enhances consolidation of BLA-dependent memories, the downstream signaling pathways are unknown. Based on the known role of the GluA2 subunit of the AMPA receptor in the consolidation of a BLA dependent memory [24], we hypothesize that stress enhances memory consolidation by promoting mobilization of the GluA2 subunit to the synapse in the BLA. Evidence in support of this hypothesis comes from studies demonstrating that administration of corticosterone increases surface expression of GluA2 in primary hippocampal cell cultures by activating GR [41,42]. Like memory consolidation, this effect is dependent on protein synthesis [42]. Similarly in the intact animal, acute platform stress has been shown to increase synaptic GluA2 levels in the hippocampus, as measured by co-localization of GluA2 and PSD-95 [43]. Furthermore, stress increases co-localization of GluA2 and PKMζ, indicating that PKMζ may be involved in maintaining GluA2 at the synapse [43] similarly to how it is known to function during the consolidation of a spatial memory [44]. In accordance with these findings, stress-induced improvement in long-term memory in the Morris water maze is associated with increased synaptic expression of GluA2 in the hippocampus, a change that requires activation of GR [45]. Although stress-induced changes in the mobilization of GluA2 have not been directly assessed in the amygdala, there is indirect evidence that acute stress within the BLA may affect AMPA receptors in a similar manner. In a study using auditory fear conditioning, animals exposed to acute platform stress immediately after a three-tone retention test demonstrated enhanced freezing to presentations of the tone the next day, indicating that stress enhanced reconsolidation of the fear memory. This behavioral effect was associated with stress-induced increases in the density of mushroom spines within the BLA [46]. Given the necessary involvement of GluA2 trafficking in spine expansion ([47]; Passafario et al., 2003), these results are consistent with effects of acute stress on mobilization of this subunit in the BLA under conditions in

which stress enhances reconsolidation of a memory. Additional work using slice electrophysiology has shown that application of corticosterone persistently increases the frequency mEPSCs in principal cells of the BLA, the maintenance of which is dependent on GR activation [48]. While increases in mEPSC frequency may be attributed to increases in the presynaptic release of glutamate, it can also be the result of an increase in the trafficking of AMPA receptors to release sites. Future studies are needed to test whether corticosterone-mediated increases in glutamatergic transmission involve mobilization of GluA2 to the synapse and whether this is a mechanism by which stress could enhance memory consolidation in the amygdala.

Norepinephrine signaling has also been shown to affect mobilization of AMPA receptors. A role for stress in this process is suggested by the finding that acute predator stress increases GluA1 trafficking to the synapse via phosphorylation of Ser845 in the hippocampus [49]. In hippocampal cultures, the surface expression of both GluA1 and GluA2 is enhanced by application of a β-adrenergic receptor agonist isoproterenol [50]. Interestingly, a lower dose of the same β-adrenergic receptor agonist did not have this effect unless it was combined with corticosterone [50], consistent with a facilitative effect of glucocorticoids on norepinephrine signaling. Based on these findings in the hippocampus we hypothesize that NE increases trafficking of GluA2 to the synapse in the BLA in a GR-dependent manner. This idea is supported by behavioral studies mentioned above in which norepinephrine-induced increases in memory consolidation were dependent upon glucocorticoids in the BLA [37]. Supportive evidence is also found in studies using slice electrophysiology that show activation of the β-adrenergic receptor increases AMPA-mediated current and induces LTP in the LA-BLA pathway [51,52]. However, contrary to our hypothesis, pre-treatment with corticosterone did not promote this effect and instead suppressed the facilitative effect of β-adrenergic stimulation on LTP [52]. This discrepancy may be accounted for by the dose of corticosterone used, which was three times higher than the dose used in the Zhou et al., study to facilitate the effects of a β-adrenergic receptor agonist in hippocampal cell culture. Given the opposing effects that can be

elicited by different doses of corticosterone [53], it is possible that a lower dose of cortisterone would facilitate the effects of norepinephrine on LTP induction. Additional studies are needed to test whether norepinephrine signaling in the BLA promotes trafficking of GluA2 to the synapse. Furthermore, the mechanisms by which corticosterone and norepinephrine interact to stimulate mobilization of GluA2 to the synapse are important topics for future research. Experiments utilizing reduced preparations would benefit from mimicking the relative timing of NE and corticosterone release, such that NE signaling is released first, as it is in the intact animal [54].

Effects of Chronic Stress on Fear Memory: Potential Mechanisms in the Bla

In contrast to acute stress, which promotes immune function and metabolism, chronic stress has been shown to suppress immune function and negatively affect metabolism, which increases the risk for cardiovascular disease [25]. Interestingly, chronic stress leads to adaptations within the HPA axis, such that the response to subsequent stressors is exaggerated [55]. For example, exposure to numerous tail shocks for three days followed by one week of recovery leads to an augmented corticosterone response to one additional tail shock [56]. Chronic stress also sensitizes other components of the stress response, including the noradrenergic system. This has been shown in several studies demonstrating that exposure to chronic cold stress prior to acute immobilization stress or exposure to electric shock further enhances the release of norepinephrine in multiple brain regions, including the hippocampus, prefrontal cortex, and bed nucleus of the stria terminalis [57-59]. Although the same experiments have not been conducted in the BLA, there is evidence that chronic cold stress does enhance the effects of norepinephrine on BLA excitability [60], indicating that chronic stress may exacerbate responses to a future stressor through norepinephrine signaling in the BLA.

Given the known effects of foot shocks on activation of the stress response and the necessary use of these shocks during fear conditioning, sensitization of the stress response by pre-exposure

to chronic stress may contribute to the effects of chronic stress on fear learning. Numerous studies using auditory fear conditioning have shown that chronic stress before training, either with or without a period of recovery, enhances long-term memory [61-67]. While some report a moderate effect of chronic stress on acquisition [62,63,66,68], others only report effects on memory consolidation [61,64,65,67]. Stress-mediated increases in both processes may work together to improve retention of the fear memory.

Chronic stress-induced enhancement of fear memory has been attributed to the effects of chronic stress on morphological and physiological changes within the amygdala. For example, chronic immobilization stress increases dendritic arborization of excitatory (principal and stellate) but not inhibitory neurons within the BLA [69]. The same chronic stressor also increases the density of spines on primary and secondary dendrites of principal neurons in the BLA [65,70,71]. Additional studies show that chronic immobilization stress enhances long-term potentiation at thalamic inputs to principal neurons in the LA, indicating that chronic stress may affect network excitability in the LA in such a way that it becomes more responsive to future emotionally salient events [65]. Such changes in excitability, in conjunction with stress-induced sensitization of the stress response (see above) may contribute to the enhancing effects of chronic stress on the acquisition and consolidation of a conditioned fear memory.

An investigation into the synaptic changes leading to stress-induced enhancement of LTP revealed that chronic stress increases the ratio of NMDA receptor to AMPA receptor-EPSCs in the amygdala. In addition, chronic stress lowers the coefficient of variation of NMDA receptor mediated responses, indicating an increase in the number of synaptic NMDA receptors mediating EPSCs. In addition to targeting NMDA receptors, there is evidence that chronic stress targets AMPA receptors, as demonstrated by stress-induced increases in GluA1-containing AMPA receptors in LA spines [64,72]. We hypothesize that the increase in the available pool of GluA1 would lead to more GluA1 trafficking during learning, which may contribute to

stress-enhanced memory acquisition. After learning, the replacement of GluA1 by GluA2 would then result in enhanced synaptic GluA2, which may be a mechanism by which chronic stress enhances memory consolidation. Although it has not been established that an increase in synaptic GluA1 would necessarily lead to an increase in synaptic GluA2, our hypothesis is in line with the proposal that GluA1 insertion creates synaptic placeholders that reserve the space for later insertion by GluA2 [73,74]. Interestingly, it has been shown that chronic stress has no effect on the coefficient of variation of AMPA receptors [65], indicating no effect of stress on the number of AMPA receptors contributing to the EPSC. In contrast to our hypothesis, one interpretation of these findings is that chronic stress does not increase the number of AMPA receptors and instead produces silent synapses that are devoid of AMPA receptors. An alternative interpretation is that stress does increase the number of AMPA receptors, but unlike NMDA receptors, they are not as functionally involved in mediating current, because they are consistently trafficked between the synaptic and extra-synaptic membrane in newly formed synapses. Such changes in AMPA receptor mobilization within nascent spines have been demonstrated using quantum dot imaging to track individual receptors [75,76]. However, the possibility remains that during learning, these receptors are incorporated into the synapse, where they mediate current and contribute to the acquisition of a conditioned fear memory. Furthermore, the observed stress-induced increase in the NMDAR/AMPAR ratio remained below 1 [65], indicating that even though stress led to an increase in NMDA receptor mediated current, it was still less than AMPA receptor mediated current, which may also have been increased. In support of this idea is the finding that four days of foot shock stress has been found to increase AMPA-mediated mEPSCs in the LA [72]. Future studies are needed to directly test the role of stress-induced changes in AMPA receptors, NMDA receptors, and cell excitability in the BLA on stress-induced enhancement of memory acquisition and consolidation. It should be noted that all studies described in this section have quantified molecular changes in chronically stressed animals that have not been fear conditioned. Given that fear conditioning alone increases expression of GluA1 as early as 5 minutes after conditioning

[23], understanding how chronic stress affects this process requires examining GluA1 in stressed and non-stressed fear conditioned animals shortly after conditioning. Similarly, a relevant time point for evaluating the replacement of GluA1 by GluA2 would be 24 hours after conditioning in stressed and non-stressed mice. Understanding how chronic stress leads to changes in norepinephrine and glucocorticoid signaling that might contribute to changes in NMDA and/or AMPA receptor function would also be an interesting subject for future research.

Implications for Fear Extinction

The inability to extingush memories associated with an aversive experience is commonly found in stress-related psychiatric disorders. As a result, understanding how stress affects extinction has garnered a lot of attention in the past decade [77]. The vast majority of studies investigating the effects of stress on extinction involve exposing animals to chronic stress prior to fear conditioning and report stress-induced increases in conditioned responses the next day [66,68,8-80]. Although it is possible that this increase in freezing reflects a stress-induced impairment in extinction learning, this interpretation is confounded by the well established effects of stress on acquisition and consolidation. In other words, animals that learn the CS-US association better will take longer to learn that the CS no longer predicts the US during extinction training.

The relationship between initial acquisition of the fear memory and later extinction of that memory is particularly important given the differential role AMPA receptor subunits play in each process in the amygdala. During acquisition, GluA1 subunits are rapidly trafficked to the synaptic membrane, and are later replaced by GluA2 subunits during memory consolidation [23]. During extinction learning, synaptic GluA2 subunits in the LA are internalized [81-83]. This process is also required for retrieval of an extinction memory [84]. The opposing roles of synaptic GluA2 in initial consolidation and subsequent extinction is especially relevant when considering how exposure to stress prior to fear conditioning affects each process. If the hypothesis proposed here is correct, and chronic stress increases

the amount of GluA2 incorporated into the synapse, then subsequent removal of GluA2 from the synapse during extinction would be expected to take longer, leading to impairments in extinction learning and retention of the extinction memory. While these results indicate that stress-induced enhancement in the acquisition and/or consolidation of a fear memory may render that memory resistant to extinction, it does not address whether stress directly affects the acquisition or retention of an extinction memory. Such studies would involve fear conditioning animals, allowing the memory to be consolidated, and then exposing them to stress prior to extinction learning or retrieval of the extinction memory. To our knowledge, one study tested the effects of stress on extinction in this way and found that acute platform stress two weeks after extinction training impaired the recall of an extinction memory [85]. Given the known role of the medial prefrontal cortex (mPFC) in the retention of extinction memories [86], these results indicate a detrimental effect of stress on this brain region. Future studies are needed to investigate the direct effects of stress on the acquisition of extinction. Additional studies are also needed to address how stress affects extinction by modifying the network activity of the amygdala and mPFC.

Potential Clinical Implications

Several lines of evidence implicate the amygdala in the pathophysiology of numerous psychiatric disorders, including PTSD [87-89] depression [90,91], social phobia [92], and specific phobias, such as arachnophobia [93]. Given that stress is a risk factor for the development of psychiatric disorders [1-5], understanding how stress affects the functioning of the amygdala may provide insight into the etiology and treatment of these disorders. In this paper, we have reviewed two components of the acute stress response involved in mediating enhanced consolidation of a BLA-dependent memory: activation of the GR and increases in norepinephrine signaling. Pharmacological interventions targeting these systems may have therapeutic value, particularly when combined with cognitive behavioral therapy [7]. For example, pharmacologically enhancing memory consolidation by activating GR or NE receptors could strengthen

memories formed during therapy. In support of this idea are studies demonstrating that activation of GRs via ingestion of hydrocortisone prior to exposure therapy improves outcome, as measured by a reduction in symptoms in patients with PTSD [94], acrophobia [95], social phobia [96], and arachnophobia [96]. Similarly, increasing the release of norepinephrine with yohimbine, an α2-adrenergic receptor antagonist, prior to exposure therapy has been shown to be effective in treating social phobia [97] and claustrophobia [98]. Alternatively, blocking memory consolidation by targeting these same components of the stress response could be used to disrupt the formation of a traumatic memory. Studies utilizing this approach provided propranolol shortly after exposure to a traumatic event and found that it decreased the later development of PTSD [99], an effect that was dependent on high drug adherence [100]. However, propranolol did not affect PTSD symptoms when it was administered to patients up to 48 hours after physical injury [101], a result that may be accounted for by the narrow time window during which memories are consolidated. It is possible that consolidation does not last 48 hours and targeting consolidation with propranolol is only effective if it is given closer to the time of trauma. This possibility highlights the importance of tracking the time between trauma exposure and propranolol administration. Consistent with this idea is one study that did not monitor the time between drug administration and trauma and found no effect of propranolol on the development of PTSD [102]. It should be noted that the vast majority of studies targeting consolidation with propranolol have been conducted in adults and propranolol may not be therapeutically effective in children [103]. Another strategy for disrupting traumatic memories with propranolol has been to interfere with memory reconsolidation, in which case patients are treated after a traumatic memory has already been consolidated. Administration of propranolol during or immediately after retrieval of the traumatic memory has been shown to impair reconsolidation, leading to a decrease in PTSD symptoms [104-106]. It is currently unknown whether blocking GR activation immediately after trauma or during retrieval would be similarly beneficial. Together these studies indicate that pharmacologically targeting GR activation or

norepinephrine signaling to either enhance memories formed during therapy or impair memories formed during a traumatic event are promising treatment strategies.

Conclusions and Future Directions

Understanding how stress affects amygdala-dependent memories is an area of ongoing research. Converging lines of evidence indicate that acute stress enhances consolidation of a fear memory by activating GRs and stimulating the local release of norepinephrine in the amygdala. However, the downstream signaling pathways have not been identified. Based on the known role of GluA2-containing AMPA receptors in the BLA in memory consolidation, we hypothesize that stress enhances consolidation by promoting mobilization of the GluA2 subunit to the synapse. In contrast to the acute effects of stress, chronic stress has been shown to enhance both the acquisition and consolidation of a fear memory. Based on evidence indicating that chronic stress increases the available pool of GluA1 in the BLA, we hypothesize that there are consequent increases in GluA1 trafficking during learning that contribute to the effects of chronic stress on acquisition. After learning, the replacement of high levels of synaptic GluA1 with GluA2 may be a mechanism by which chronic stress enhances memory consolidation. Understanding how stress-induced changes in glucocorticoid and norepinephrine signaling might affect emotional learning by increasing the trafficking of AMPA receptors and enhancing amygdala excitability is an interesting area for future research.

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