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Aggression Priming by Potentiation of Medial Amygdala Circuits

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Review of Nordman et al.

Aggression is a complex social behavior that is necessary for survival and protecting territories/resources but can be destructive when it is expressed inappropriately. Short-term escalation in aggression is observed in many species, including humans, after an initial exposure to a conspecific intruder. This phenomenon is referred to as aggression priming. Using cell type-specific labeling and functional manipulations in different brain regions, considerable progress has been made to identify the medial amygdala (MeA), ventromedial hypothalamus (VMH), and bed nucleus of stria terminalis (BNST) as critical nodes for producing aggression (Choi et al., 2005; Lin et al., 2011; Unger et al., 2015; Miwako et al., 2016; Falkner et al., 2020). Olfaction has a widely accepted role in rodent aggression, and it has been shown that olfactory cues converge on MeA from the olfactory bulb, which then relays this information to multiple brain regions, including VMH and BNST (Hashikawa et al., 2016; Chen and Hong, 2018). Nevertheless, the neural basis of experience-dependent aggression priming remains unknown.

To investigate aggression priming, in a recent study Nordman et al. (2020) modified the standard resident–intruder aggression behavior paradigm for rodents. In their task, a male mouse was first placed in a neutral arena for habituation. They then measured the resident's baseline level of aggression by introducing a male intruder, which resulted in offensive aggression behaviors by the resident mouse. Following this baseline test, a second intruder was introduced into the arena, which elicited more intense aggression behaviors as quantified by shorter attack latencies and increased attack probabilities. Using optogenetic stimulation and *in vivo* electrophysiology, the authors then searched for specific circuits underlying aggression priming.

Although previous studies have found that MeA plays a critical role in aggression, whether it is also involved in aggression priming had not been explored. To answer this question, Nordman et al. (2020) used optogenetic protocols that induce either long-term depression (LTD) or long-term potentiation (LTP) between MeA neurons and their downstream targets during behavior, by expressing channelrhodopsin-2 (ChR2) in CaMKIIα-expressing MeA neurons. Low-frequency optogenetic stimulation of ChR2-expressing MeA cell bodies—a protocol expected to induce LTD at synapses between MeA and their downstream postsynaptic targets—suppressed the effects of previous aggression priming, as revealed in a subsequent aggression test session. Conversely, when high-frequency optogenetic stimulation was used to induce LTP in MeA in the absence of a natural aggression priming session, it led to an increase in subsequent aggression behaviors, suggesting that this manipulation mimics natural aggression priming. Furthermore, systemic injection of an NMDA receptor antagonist (MK-801) eliminated the LTP-induced enhancement of aggression, suggesting that NMDA-dependent plasticity contributes to aggression priming.

The authors next searched for the downstream target regions of MeA that contribute to aggression priming. The MeA sends prominent projections to VMH and BNST, two areas that have previously been implicated in aggression. Therefore, the authors asked whether either or both of these pathways undergo synaptic strengthening during aggression priming. Indeed, synaptic strength, which was measured by *in vivo* field recordings from VMH or BNST neurons receiving ChR2-expressing MeA fibers before versus after behavior, was enhanced in both pathways after aggression priming. Furthermore, high-frequency optogenetic stimulation of ChR2-expressing MeA terminals in VMH or BNST induced LTP at MeA → VMH or MeA → BNST synapses, respectively, which promoted aggression behavior. Importantly, low-frequency optogenetic stimulation of ChR2-expressing MeA terminals in VMH or BNST right after the high-frequency stimulation protocol induced LTD at MeA → VMH or MeA → BNST synapses, respectively, which blocked

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the aggression-priming effect of optogenetically inducing LTP in MeA terminals. These experiments provided strong evidence that synaptic plasticity in divergent MeA pathways plays a key role in aggression priming in rodents.

Finally, Nordman et al. (2020) asked whether the observed circuit plasticity underlying aggression priming might be a common feature of enhanced aggression induced by different experiences. To test whether the identified MeA pathways are involved in trauma-induced overaggression, the authors adopted a protocol that used mild footshocks to induce aggressive behaviors. These experiments showed that traumatic stress potentiates synaptic transmission between MeA and its synaptic partners VMH and BNST. Optogenetic LTD in MeA blocked trauma-induced overaggression, supporting the idea that MeA is involved in enhanced aggression induced by different types of experiences.

The study by Nordman et al. (2020) fills a critical gap in our understanding of circuit mechanisms underlying aggression priming. Using a combination of optogenetics, physiology, and behavior, the authors identified two divergent MeA pathways, MeA → VMH and MeA → BNST, that undergo synaptic potentiation after experience-dependent aggression escalation, and they showed that this potentiation is necessary for aggression-priming behavior. Notably, a previous study found that inhibiting MeA impaired both social defeat priming and future social defensive behaviors, but blocking protein synthesis in the MeA had no effect on either behavior (Markham and Huhman, 2008). That result suggests that in the case of social defeat, MeA is an important gateway for sensory information, but is not a site of critical experience-dependent plasticity, which requires local protein synthesis. Consistent with this notion, optogenetic induction of LTD in MeA terminals abolished aggression priming, while LTP terminal stimulation induced NMDA receptor-dependent aggression priming. Together with the fact that MeA projection neurons do not exhibit significant local synaptic connectivity (Keshavarzi et al., 2014), it might be that MeA-driven aggression priming relies on potentiation of MeA pathways outside of the MeA, but not input synapses.

How heterogenous subpopulations are embedded into MeA circuits and contribute to aggression priming is unclear and, therefore, represents an important subject for further studies. The posterior portion of MeA is subdivided into dorsal and ventral subnuclei. The ventral portion of MeA (MeApv) is activated by defensive stimuli (Choi et al., 2005). Notably, within MeApv, there is a subpopulation of dopamine D_1 receptor-positive neurons that differentially innervate VMH and BNST, with excitatory projections to VMH and inhibitory projections to BNST that elicit opposing behavioral responses to threatening stimuli (Miller et al., 2019). In contrast, the study by Nordman et al. (2020) found that MeApv sends an excitatory projection to BNST, which contributes to aggressive behaviors. Together, these findings support the hypothesis that MeApv sends both excitatory and inhibitory inputs to BNST, which would arise either from different MeApv subpopulations or a single population that is capable of releasing both excitatory and inhibitory neurotransmitters. Similarly, heterogeneity within MeApv-recipient BNST and VMH neurons may be expected. For instance, the expression level of oxytocin receptors in a BNST subpopulation is associated with aggression (Calcagnoli et al., 2014). Within the VMH, glutamatergic neurons coexpressing estrogen receptor 1 and the progesterone receptor have been identified as being necessary and sufficient for aggression (Lee et al., 2014; Yang et al., 2017), and steroidogenic factor 1-expressing neurons are recruited for defensive behaviors (Kunwar et al., 2015). Additional studies are needed to determine whether these aggression-related cell types in BNST and VMH participate in modulating aggression priming, which is a likely possibility. Also, since VMH receives strong inputs from BNST (Lo et al., 2019), whether the BNST → VMH circuit is involved in the regulation of aggression priming remains an open question.

Nordman et al. (2020) revealed that synaptic plasticity in MeA outputs play a key role in aggression priming. A remaining intriguing question is what pathways enable aggression experiences to modify synaptic transmission within MeA → VMH and MeA → BNST circuits. One possibility is that the attack experience elicits testosterone release that strengthens these synapses. Indeed, research suggests that winning fights elicits testosterone release, which reorganizes the aggression system and promotes further winning (Fuxjager et al., 2011). In the aggression-priming assay, Nordman et al. (2020) observed a behavioral correlate of this effect (i.e., animals increased aggressive behaviors toward a submissive mouse once winning was assured). Notably, both androgen and estrogen receptors are highly enriched in VMH and BNST (Davis and Moore, 1996). Testosterone release induced by aggression could lead to an increase in estrogen in the brain, which would act through estrogen receptors in VMH and BNST to enhance synaptic plasticity of MeA inputs (McEwen et al., 1991). This offers a promising direction for the study of aggression experience-dependent plasticity. Future studies that combine RNA sequencing with pharmacological manipulations will enable a more comprehensive exploration of the molecular mechanisms underlying aggression priming. Overall, research investigating the role of divergent MeA circuits in regulating aggression priming has the potential to shed light on the treatment of post-traumatic stress disorder and other related psychiatric diseases in which excessive aggression plays a role.

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