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Imprinting: When Early Life Memories Make Food Smell Bad

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A recent study has found that pathogen exposure early in the life of the nematode *Caenorhabditis elegans* leads to a long-lasting aversion that requires distinct sets of neurons for the formation and retrieval of the imprinted memory.

Early life experiences can make memories that last a lifetime. One of the most striking examples is imprinting: the rapid learning that occurs during a critical period early in life, and establishes a long-lasting behavioral response to a specific stimulus. A classic example was first described by Konrad Lorenz [1], who noticed that newly hatched geese form parental attachments to the first moving object that they see. Imprinting has since been found in many other animals. For instance, salmon form an olfactory memory that guides return to their native stream to spawn [2], and mammals strongly favor food odors that they experience around birth [3]. While imprinting is a universal learning process, there are fundamental aspects that remain poorly understood. What are the sites of memory formation and retrieval? And is there a difference between imprinted memories and memories that form later in life? A recent paper by Jin *et al.* [4] demonstrates that exposure of the nematode *Caenorhabditis elegans* to pathogenic bacteria early in life leads to specific, long-lasting imprinted aversion. Moreover, this study shows that neurons involved in aversive memory formation are distinct from those implicated in memory retrieval, and that the neural circuits for imprinting are similar, but not identical, to those required for adult learning.

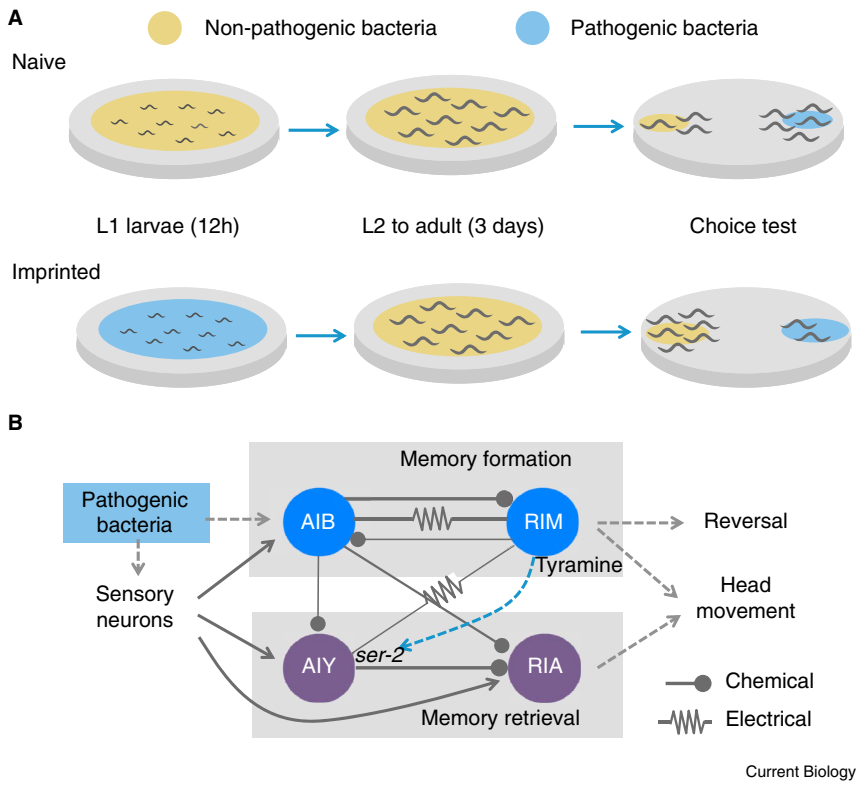

Figure 1. Pathogen imprinting early in the life of *C. elegans* induces long-term aversive memory.
(A) Schematic illustration of the imprinting protocol during the first larval stage and adult choice assay between pathogenic and non-pathogenic bacteria. Naïve animals have a slight preference for the odor of pathogenic bacteria. Exposure of newly hatched larvae to pathogenic bacteria results in the avoidance of these bacteria in the adult stage. (B) Wiring diagram of interneurons implicated in imprinted memory formation and retrieval.

Although *C. elegans* has an adult nervous system of merely 302 neurons, it can modify its preference for odors, tastes and temperatures based on its experience. This plasticity is crucial for survival as it allows the worm to distinguish, for instance, nutritious and potentially life threatening bacteria in its habitat. *C. elegans* may initially prefer the smell of pathogenic bacteria, but upon ingestion and subsequent malaise, adult worms learn to avoid the smell of these pathogens [5]. This short-term aversive memory can last up to 24 hours. In the new study, Jin *et al.* [4] found that exposure to pathogenic bacteria in the first larval stage L1 leads to a long-lasting aversive memory, maintained into adulthood (Figure 1A) [4]. Strikingly, animals exposed to the pathogen at later larval stages do not develop this long-lasting aversion. Thus, the earliest developmental L1 stage defines a

critical period for the formation of imprinted aversive memories.

What is the neural coding for aversive imprinting? The simplicity of the worms' nervous system allowed Jin *et al.* [4] to address this question with exquisite resolution. Silencing either of a pair of interneurons, called AIB and RIM, during early life abolished imprinted aversion to pathogenic bacteria in the adult; however, silencing the same neurons in the adult did not affect imprinted aversion (Figure 1B). Thus, AIB and RIM are important for memory formation but dispensable for memory retrieval. The opposite picture emerged for two other interneurons, AIY and RIA: silencing of these during the early larval stage had no effect on memory formation, but they were found to be indispensable during memory retrieval in the adult.

While the same sets of neurons are important in adult-learned aversion, the individual AIB and AIY neurons are not required for adult aversion [6]. Furthermore, imprinted aversion shows a clear distinction between the memory formation and retrieval phases of learning. The study of the famous patient HM already suggested that the formation and retrieval of long-term memories in humans are mediated by distinct circuits [7]. The new study [4] refines this concept with extraordinary single-cell precision and furthers the notion that learning is a universal property of any nervous system.

Adult-learned aversion and imprinting also share molecular components: both forms of learning require serotonergic and glutamatergic signaling [4,5]. But differences in the molecular players exist here as well. For instance, the AMPA-type receptor GLR-1 is only needed for imprinted aversion, whereas the NMDA-type receptor NMR-1 is involved only in adult learning. In addition, long-term imprinted aversion, but not short-term adult aversion, requires CRH-1, the *C. elegans* orthologue of the cAMP response element-binding protein (CREB). This may come as no surprise, as the transcription factor CREB is known to be a universal key player in long-term memory formation in many animals [8–10]. In *C. elegans*, CREB is also required for long-term habituation and appetitive olfactory memory [11,12]. It will be interesting to determine in which cells CREB is required and what genes are regulated during imprinted aversion.

What is the molecular signal that relays information of the pathogenic infection from the site of memory formation to the site of retrieval? RIM, one of the memory-formation neurons, releases tyramine, the invertebrate counterpart for (nor-) epinephrine [13]. Tyramine is needed during the first larval stage, but not at later stages, for imprinted aversion. SER-2, a G-protein coupled receptor for tyramine, specifically required for imprinted aversion, seems to impart the signal required for recall. SER-2 expression is required in the AIY retrieval neuron, thus providing a bridge between memory formation and retrieval. In mammals, epinephrine and norepinephrine released onto the amygdala play a central role in stress-induced long-term adaptive responses [14]. Tyramine release in response to pathogenic infections may serve a similar role in the formation of enhanced aversive long-term memories in the worm.

Jin *et al.* [4] used calcium imaging to analyse the functional consequences of imprinting on the memory circuit. Differences between naïve and imprinted animals were detected in AIB, AIY and RIA neurons. Calcium transients in the AIB and AIY neurons of imprinted animals were more pronounced in response to switches between non-pathogenic and pathogenic bacterial odors. Interestingly, RIA calcium transients appear to change polarity in imprinted animals. The RIA neuron receives inputs from several sensory and head motor neurons that guide navigation [15]. The authors hypothesize that, during imprinting, changes in the relative weight of the excitatory and inhibitory inputs to RIA may ultimately lead to different responses to bacterial odors.

While this study [4] lays the groundwork, several questions remain. First, where and how are these memories stored? Imprinted aversion results in changes to neural responses at multiple sites in the circuit. This may suggest that memory storage is a distributed property of the nervous system. The ability to manipulate and monitor the activity of individual neurons should allow a dissection of memory with a new level of precision. Second, what makes the early larval stage susceptible to imprinting? During the first larval stage synaptic connections are remodeled [16] and 80 new neurons are integrated into the existing circuit [17]. Therefore, this 'critical period' in development could be especially prone to sensory input and modifications in the development and refinement of synaptic connections. At first sight no obvious differences in specification or connectivity of the imprinted neurons were detected using fluorescent synaptic markers. But a closer comparison of structural and functional connectivity would be required to test if differences exist in naive and imprinted animals. Serial reconstruction of the imprinted connectomes seems a daunting task. But if it is possible in any organism, it must be the worm; *C. elegans* has been there before [18].

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Evolution: Bacterial Territoriality as a Byproduct of Kin Discriminatory Warfare

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Recent work suggests that the inability of genetically distinct colonies of the bacterium *Bacillus subtilis* to freely merge is often a byproduct of microbial warfare mediated by divergent suites of chemical weaponry. Any effects of such kin-discriminatory antagonisms on levels of within-group cooperation at other traits remain unclear.

Humans and many other animals, some plants and even microbes are often more helpful, or less antagonistic, toward individuals with whom they share a high degree of genetic kinship than toward less-related individuals. Such 'kin discrimination' can be defined as simply the differentiation of behavior (broadly defined) as a function of genetic relatedness among social interactants [1,2], independently of ultimate evolutionary or proximate molecular causes. In microbes that engage in cooperative group motility, one form of kin discrimination is the inability of genetically distinct colonies to merge freely into a larger social group (Figure 1). Two recent studies of the model bacterium *Bacillus subtilis* — one a screen of colony-interaction phenotypes among natural isolates [3] and the other a screen of genetic mutants and genome content [4] — together suggest that variable sets of anti-competitor toxins may often be the proximate molecular cause of such colony-merger incompatibilities in this species.

Several bacterial species exhibit colony-merger incompatibilities, including *Proteus mirabilis* (the species in which such incompatibilities were first discovered) [5] and *Myxococcus xanthus* [2,6]. Although the fine-scale spatial distribution (or 'microbiogeography') of distinct incompatibility types (or 'allotypes') in natural populations of these species remains poorly understood, small patches of soil can harbor high levels of allotype diversity. For example, a centimeter-scale soil patch in Germany was found to contain at least 45 distinct swarming allotypes of *M. xanthus* [6], and just one cubic centimeter of soil in Slovenia was shown to harbor at least a dozen *B. subtilis* motility allotypes [3]. By extrapolation, the total numbers of swarming allotypes in these species, worldwide, appear to be immense.

Evolutionarily, kin-discrimination phenotypes might exist either because they are directly favored by selection or rather as a byproduct of something else [7]. Are traits that prevent bacterial colonies from freely merging ever directly favored by selection specifically because of such non-merger effects? Perhaps, as strains that would be poor competitors specifically in chimeric groups would benefit from the territorial exclusion of other strains that might only outcompete them were their colonies to merge [6]. However, natural selection for colony-merger prevention *per se* (as distinct from selection for competitive traits that cause non-merger phenotypes as indirect side effects) remains undemonstrated and the inability of colonies to merge may often evolve nonadaptively. Indeed, a recent experimental-evolution study with *M. xanthus* indicates that a molecularly diverse range of non-merger incompatibilities easily evolve as