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Extended epigenotype in a *Rattus novergicus* – *Toxoplasma gondii* association

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Several studies demonstrate that rats (*Rattus novergicus*) infected with protozoan parasite *Toxoplasma gondii* exhibit lesser fear to cat odors. This is thought to increase transmission of the parasite to its definitive hosts, *i.e.* cats. This is an example of extended phenotype where a gene of an organism allegedly creates a phenotype in another organism. We examined a possible proximate mechanism for this phenotype, describing an epigenetic change in arginine vasopressin gene in medial amygdala of male rats. Exogenously mimicking medial amygdala DNA hypomethylation resulted in reduction of fear to cat odors in uninfected animals, thus suggesting sufficiency. Systemic blockade of infection-induced DNA hypomethylation countermanded infection-induced behavioral change, thus suggesting necessity. This leads us to propose an epigenetic basis for this extended phenotype.

Behavioral manipulation hypothesis posits that parasites can change host behavior in ways that benefit the parasite but not the host. Such manipulation can be viewed as an extended phenotype, *i.e.*, a case where parasite’s genes exert a behavioral phenotype in the host body rather than the body bearing the genes themselves. The idea of extended phenotypes has been rhetorically used to demonstrate that natural selection acts on genes and not on individuals. *Toxoplasma gondii* (henceforth *Toxoplasma*) and *Rattus novergicus* (*rats*) association has been widely studied in this regard.2,3 Plethora of pre-existing knowledge about rat biology makes this system especially suitable to dissect proximate mechanisms of the manipulation.

Asexual stage of Toxoplasma can be sustained in a variety of hosts including rats, but sexual stage is restricted to cat intestines. Such two-stage life cycles take advantage of ubiquitous trophic interactions between predators and prey. And yet such a strategy is constrained by evolved innate aversion that prey animals exhibit to cues of predatory presence. Toxoplasma, and presumably many other parasites, softens this constraint by manipulating predator aversion in the infected hosts. In case of Toxoplasma, a subset of infected rats also develops an attraction to cat odors.4,5

*Toxoplasma* invades rat brain and forms long-lasting cysts there. Intriguingly, brain is not the only part that Toxoplasma invades. Testes of male rats provide another immune-privileged refuge to the parasite. Inside testes Toxoplasma increases synthesis of the testosterone, a hormone required for secondary sexual characters and associated behaviors.6 For example, greater testosterone could underlie greater attractiveness accorded by sexually receptive females to infected males.7,8 Since Toxoplasma can also travel through ejaculate, such gain of attractiveness could increase horizontal parasite transmission.

Interestingly testosterone and/or its metabolites bind to their receptors spread in a wide variety of brain regions. From perspective of this report, medial amygdala is especially important among these. Medial amygdala is the first neural site where olfactory information coming from main and accessory olfactory system intersects.9 Medial amygdala neurons process information from sexual pheromones and then project to downstream regions that are involved in sexual motivation and consummation. Within medial amygdala,

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testosterone increases abundance of arginine vasopressin (AVP).10,11 This nine amino acid peptide is crucial for a variety of social and sexual behaviors, ranging from pair-bonding between sexual partners in voles12 to inter-male aggression in rats.13 These neurons are also activated during exposure of male rats to females.14 The effect of testosterone on medial amygdala AVP is thought to involve a hypomethylation event at 2 promoter sites upstream of the gene, resulting in enhanced transcription.15

In short, Toxoplasma increases testosterone; testosterone binds to its receptors in medial amygdala; testosterone can increase AVP transcription through DNA hypomethylation; and, AVP neurons are activated during exposure to sexual pheromones. This provided the backdrop to the research paper discussed here.16 Postero-dorsal aspect of the medial amygdala (henceforth MePD) is particularly rich in AVP. This sub-region is also selectively activated in response to sexual odors. Thus the report concentrated on this particular brain region. The report showed that promoter sites for AVP gene in MePD were relatively hypomethylated in the Toxoplasma infected male rats. This was congruent with greater mRNA abundance for AVP in these animals, suggesting more transcription. When uninfected males were exposed to cat odor, only a few MePD-AVP neurons expressed Fos protein, an immediate early gene marker for recent activation. This is not surprising because MePD is selectively involved in processing of sexual pheromones and not predator kairromones. In fact, the level of activation observed for control animals after cat odor was comparable to that observed during exposure to a neutral odor during another study.14 In striking contrast, Toxoplasma infected animals exhibited a robust activation of MePD-AVP neurons. This increase was of similar magnitude as seen in uninfected male rats that have recently copulated or have been exposed to female pheromones.14 The speculative proposition here is that the infection somehow created leakiness, allowing predator odor to tap into neurons that routinely signal sexual opportunities.17 This mismatch could then explain loss of fear to cat odors in infected males. The idea is even more appealing because it could also explain why a subset of animals additionally gains an attraction. Further pharmacological experiments showed that DNA hypomethylation was necessary for the behavioral change post-infection. Moreover, externally instituted DNA hypomethylation in the MePD was sufficient to cause reduction in fear, in absence of the parasite.

There are a few ambiguities associated with the above interpretation. Firstly, the observations do not absolutely prove necessity and sufficiency of a restricted epigenetic event in MePD-AVP gene only. This is because the pharmacological agents used in the experiments were not specific to AVP gene alone. This leaves a possibility that hypomethylation of MePD-AVP could be a non-consequential epigenetic change while another epigenetic change in hitherto unknown locus might underlie the phenotype. For example, inhibitors of DNA methyltransferase could have instituted hypomethylation in any gene within MePD. Similarly, blockade of post-infection hypomethylation involved a systemic approach enveloping the whole body. Future experiments are necessary in this regard. Secondly, there is no definitive proof that change in rat behavior after Toxoplasma infection actually results in greater transmission of the parasite. Such an effect will be predicted by behavioral manipulation hypothesis, yet the debate about this possibility remains presently unresolved.18

Within the constraints of these limitations, experiments in this paper suggest an intriguing possibility. Epigenetic changes are important mechanisms when an ecological memory is required to be retained for long time.19 These modifications allow animals and plants to create conditional behaviors; flexible strategies that can be calibrated according to incipient ecological conditions. The work discussed here presents a possibility that parasites might have access to conditional prograning of the host. Thus genes of the parasites can create epigenetic change in the host molecular machinery; resulting in a behavioral manipulation. These experiments suggest, but do not conclusively prove, an extended epigenotype for an extended phenotype.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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