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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.

Keywords: behavioral manipulation, DNA methylation, fear, MSRE, nonapeptide, parasite, testosterone

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Behavioral manipulation hypothesis posits that parasites can change host behavior in ways that benefit the parasite but not the host.[1] 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,

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 voles[12] 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] Posterodorsal 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 kairomones. 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 encompassing 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 programing 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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References

1. Thomas F, Adamo S, Moore J. Parasitic manipulation: where are we and where should we go? Behav Processes 2005; 68:185-99; PMID:15792688; http://dx.doi.org/10.1016/j.beproc.2004.06.010
2. Vyas A, Sapolsky R. Manipulation of host behaviour by Toxoplasma gondii: what is the minimum a proposed proximate mechanism should explain? Folia Parasitol 2010; 57:88-94; PMID:20608470; http://dx.doi.org/10.14411/fp.2010.011
3. Webster JP, McConkey GA. Toxoplasma gondii-altered host behaviour: clues as to mechanism of action. Folia Parasitol 2010; 57:95-104; PMID:20608471; http://dx.doi.org/10.14411/fp.2010.012
4. Berdoy M, Webster JP, Macdonald D. Fatal attraction in rats infected with Toxoplasma gondii. Proc Biol Sci 2000; 267:1591-4; PMID:11007336; http://dx.doi.org/10.1098/rspb.2000.1182
5. Vyas A, Kim S-K, Giacomini N, Boothroyd JC, Sapolsky RM. Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. Proc Natl Acad Sci 2007; 104:6442-7; PMID:17404235; http://dx.doi.org/10.1073/pnas.0608310104
6. Lim A, Kumar V, Hari Dass SA, Vyas A. Toxoplasma gondii infection enhances testicular steroidogenesis in rats. Mol Ecol 2013; 22:102-10; PMID:23190313; http://dx.doi.org/10.1111/mec.12042
7. Dass SAH, Vasudevan A, Dutta D, Soh LJT, Sapolsky RM, Vyas A. Protozoan parasite Toxoplasma gondii manipulates mate choice in rats by enhancing attractiveness of males. PloS One 2011; 6:e27229; PMID:22073295; http://dx.doi.org/10.1371/journal.pone.0027229
8. Vyas A. Parasite-augmented mate choice and reduction in innate fear in rats infected by Toxoplasma gondii. J Exp Biol 2013; 216:120-6; PMID:23225874; http://dx.doi.org/10.1242/jeb.072983
9. Meredith M. Vomeronasal, Olfactory, Hormonal Convergence in the Brain: Cooperation or Coincidence? a. Ann N Y Acad Sci 1998; 855:349-61; PMID:9929627; http://dx.doi.org/10.1111/j.1749-6632.1998.tb10593.x
10. Zhou L, Blaustein JD, De Vries GJ. Distribution of androgen receptor immunoreactivity in vasopressin-and oxytocin-immunoreactive neurons in the male rat brain. Endocrinology 1994; 134:2622-7; PMID:8194487
11. Wang Z, Bullock NA, De Vries GJ. Sexual differentiation of vasopressin projections of the bed nucleus of the stria terminals and medial amygdaloid nucleus in rats. Endocrinology 1993; 132:2299-306; PMID:8504734
12. Young LJ, Wang Z. The neurobiology of pair bonding. Nat Neurosci 2004; 7:1048-54; PMID:15452576; http://dx.doi.org/10.1038/nn1327
13. Koolhaas JM, Van den Brink T, Roozendaal B, Boorsma F. Medial amygdala and aggressive behavior: interaction between testosterone and vasopressin. Aggress Behav 1990; 16:223-9
14. Dass SAH, Vyas A. Copulation or sensory cues from the female augment Fos expression in arginine vasopressin neurons of the posterodorsal medial amygdala of male rats. Front Zool 2014; 11:42; PMID:24926317; http://dx.doi.org/10.1186/1742-9994-11-42
15. Auger CJ, Coss D, Auger AP, Forbes-Lorman RM. Epigenetic control of vasopressin expression is maintained by steroid hormones in the adult male rat brain. Proce

Natl Acad Sci 2011; 108:4242-7; PMID:21368111; http://dx.doi.org/10.1073/pnas.1100314108
16. Hari Dass SA, Vyas A. Toxoplasma gondii infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. Mol Ecol 2014; 23:6114-22; PMID:25142402; http://dx.doi.org/10.1111/mec.12888
17. House PK, Vyas A, Sapolsky R. Predator cat odors activate sexual arousal pathways in brains of Toxoplasma gondii infected rats. PLoS One 2011; 6:e23277; PMID:21858053; http://dx.doi.org/10.1371/journal.pone.0023277
18. Worth AR, Lymbery AJ, Thompson R. Adaptive host manipulation by Toxoplasma gondii: fact or fiction? Trends in Parasitol 2013; 29:150-5; PMID:23415732; http://dx.doi.org/10.1016/j.pt.2013.01.004
19. Bossdorf O, Richards CL, Pigliucci M. Epigenetics for ecologists. Ecol Lett 2008; 11:106-15; PMID:18021243