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Morbid attraction to leopard urine in *Toxoplasma*-infected chimpanzees

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Parasites are sometimes capable of inducing phenotypic changes in their hosts to improve transmission [1]. *Toxoplasma gondii*, a protozoan that infects a broad range of warm-blooded species, is one example that supports the so-called 'parasite manipulation hypothesis': it induces modifications in rodents' olfactory preferences, converting an innate aversion for cat odor into attraction and probably favoring trophic transmission to feline species, its only definitive hosts [2]. In humans, *T. gondii* induces behavioral modifications such as personality changes, prolonged reaction times and decreased long-term concentration [3]. However, modern humans are not suitable intermediate hosts because they are no longer preyed upon by felines. Consequently, behavioral modifications in infected people are generally assumed to be side effects of toxoplasmosis or residual manipulation traits that evolved in appropriate intermediate hosts. An alternative hypothesis, however, states that these changes result from parasite manipulative abilities that evolved when human ancestors were still under significant feline predation [3,4]. As such, *T. gondii* also alters olfactory preferences in humans; infected men rate cat urine, but not tiger urine, as pleasant while non-infected men do not [5]. To unravel the origin of *Toxoplasma*-induced modifications in humans, we performed olfactory tests on a living primate still predated by a feline species. We found in our closest relative, the chimpanzee (*Pan troglodytes troglodytes*), that *Toxoplasma*-infected (TI) animals lost their innate

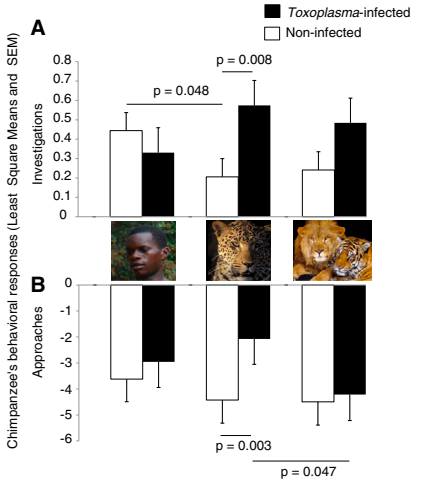

Figure 1. Comparison of the behavioral responses of 33 chimpanzees towards urine of human, leopard (natural predator) and tiger and lion (non-natural predators) during behavioral tests based on olfactory cues.
(A) Olfactory investigations performed towards urine source (for each urine type, n = 9 TI and n = 24 TN chimpanzees). (B) Approaches displayed towards urine source (for each urine type, n = 40 scans per individual). Least Square Means (LSM) and SEM are represented. White bars, TN chimpanzees; black bars, TI chimpanzees. Only significant differences in LSM ($p < 0.05$) are shown for biologically relevant two-by-two comparisons (intra-treatment comparisons: same urine type, different parasite status; and intra-parasite status comparisons: same parasite status, different urine type; see the Supplemental Information for a full set of biologically relevant comparisons.) For approach data, high negative values indicate fewer approaches towards urine source than low negative values.

aversion towards the urine of leopards (*Panthera pardus*), their only natural predator. By contrast, we observed no clear difference in the response of TI and *Toxoplasma*-non-infected (TN) animals towards urine collected from other definitive feline hosts that chimpanzees do not encounter in nature. Although the adaptive value of parasitically induced behavior should be assessed carefully, we suggest that the behavioral modification we report could increase the probability of chimpanzee predation by leopards for the parasite's own benefit. This possible parasite adaptation would hence suggest that *Toxoplasma*-induced modifications in modern humans are an ancestral legacy of our evolutionary past.

We performed collective olfactory tests on 33 chimpanzees (9 TI and 24 TN), living in five captive groups in

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Gabon. We conducted three 20-minute sessions per group (99 individual tests). To avoid mixing different olfactory cues, we presented one type of urine at a time, in random order: human urine, leopard urine, or urine from either tigers (*P. tigris*) or lions (*P. leo*), neither of which is a natural predator of chimpanzees. Indeed, tigers are Asiatic felines, and lions never lived in tropical rainforests, the natural habitat of chimpanzees. Using General(ized) Linear Mixed Models controlling for other fixed effects (individual rank and sex, and session number) and random effects (individual, group and session identities), we analyzed both the frequency of olfactory investigations performed towards a urine source (sniffing and licking) and the occurrence of approaches within a 1 meter radius from a urine source (scan sampling: '0' if absent and '1' if present; see Supplemental Experimental Procedures in the Supplemental Information). The interaction between the urine's origin and the chimpanzee's parasite status significantly influenced both variables (Table S1A,B: investigations, $F = 4.21$ and $p = 0.02$; approaches, $F = 10.22$ and $p < 0.001$). First, TN chimpanzees investigated leopard urine less frequently than human urine, as expected if chimpanzees avoid their predator based on odorant cues (Table S2A and Figure 1A; Difference of Least Square Means [DLSM]: $p = 0.04$). By contrast, TI chimpanzees both investigated and approached leopard urine more frequently than did TN individuals (Table S2A,B and Figure 1A,B; DLSM for investigations, $p = 0.008$; for approaches, $p = 0.003$). Surprisingly, TI animals tended to investigate more, but did not approach more frequently, urine collected from felines that are not their natural predators (Table S2A and Figure 1A,B) than TN chimpanzees did.

Even though our study is correlative and a pre-existing recklessness in TI animals may increase parasite encounter probability, several experimental studies have shown that a disruption of fear in infected rodents was directly caused by *T. gondii*, possibly through an epigenetic mechanism [6]. We therefore hypothesize that chimpanzees' behavioral modifications are a consequence, not a cause, of parasitism. *Toxoplasma*-induced changes could, however, result from side effects of infection. For example, *T. gondii* forms cysts in the brain [7], possibly to escape the host's immune system, and these cysts could cause, in turn, coincidental behavioral alterations in the host. However, this hypothesis fails to explain the increased attraction of TI animals towards leopard urine alone. Before concluding that parasitically induced behavior is a manipulated trait, several criteria need to be met [8]. In our case, providing formal proof that TI chimpanzees are preyed upon more than TN individuals, a finding that would strongly suggest parasite adaptation (sensu [1]), is impossible. Nevertheless, we hypothesize that behavioral modifications observed in TI chimpanzees could increase parasite fitness. This hypothesis is supported by the observation that leopards are responsible for an estimated individual predation risk of 30% per year in some populations of chimpanzees [9]. Furthermore, our own results showed that TN chimpanzees avoid leopard urine more than human urine. Finally, since leopards urine-mark their territory, a decreased avoidance of leopard urine in the wild may therefore increase the probability of prey–predator encounter.

Our study fuels an ongoing debate on the origin of *Toxoplasma*-induced behavior in humans. As feline predation pressure is virtually absent in modern humans, manipulation of a dead-end host such as our species should no longer benefit *T. gondii.* However, hominids have long coexisted with large carnivores and were preyed upon at rates comparable to those for extant primates [10]. When feline predation was still an evolutionary driver in our lineage, possible manipulative abilities may have evolved because early hominids were suitable intermediate hosts. Future research should focus on a wider range of species undergoing different predation pressures to shed light on the evolutionary history of *T. gondii* and unravel the circumstances under which host behavioral traits become extended phenotypes of parasites.

SUPPLEMENTAL INFORMATION

Supplemental information includes two tables, experimental procedures, author contributions, and supplemental references and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.12.020.

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