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The effects of *Toxoplasma* infection on rodent behavior are dependent on dose of the stimulus

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Abstract

Parasite *Toxoplasma gondii* blocks the innate aversion of rats for cat urine, putatively increasing the likelihood of a cat preying on a rat. This is thought to reflect an adaptive behavioral manipulation, because *Toxoplasma* can reproduce only in cat intestines. While it will be adaptive for the parasite to cause an absolute behavioral change, fitness costs associated with the manipulation itself suggest that the change be optimized and not maximized. We investigate these conflicting suggestions in the present report. Furthermore, exposure to cat odor causes long-lasting acquisition of learnt fear in the rodents. If *Toxoplasma* manipulates emotional valence of cat odor rather than just sensory response, infection should affect learning driven by the aversive properties of the odor. As a second aim of the present study, we investigate this assertion. We demonstrate that behavioral changes in rodents induced by *Toxoplasma* infection do not represent absolute all-or-none effects. Rather, these effects follow a non-monotonous function dependent on strength of stimulus, roughly resembling an inverted-U curve. Furthermore, infection affects conditioning to cat odor in a manner dependent upon strength of unconditioned stimulus employed. Non-monotonous relationship between behavioral manipulation and strength of cat odor agrees with the suggestion that a dynamic balance exists between benefit obtained and costs incurred by the parasite during the manipulation. This report also demonstrates that *Toxoplasma* affects emotional valence of the cat odor as indicated by altered learned fear induced by cat odor.

Keywords

fear; behavioral manipulation; dose-response; parasitism; parasite; conditioning

INTRODUCTION

The protozoan parasite *Toxoplasma gondii* infects a wide variety of warm-blooded organisms, including rodents (Dubey, 1998). Cat urine and body odors evoke strong and innate defensive response in rats (Blanchard et al., 2001, Dielenberg et al., 2001, Dielenberg and McGregor, 2001, Blanchard et al., 2003, Adamec et al., 2005, Apfelbach et al., 2005); an examples of

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kairomone action where semiochemicals emanating from one species has beneficial effects for the other species (Sbarbati and Osculati, 2006). Recent reports indicate that latent *Toxoplasma* infection results in entry of parasites into the brains of rodents and in loss of innate aversion in such animals towards cat kairomones (Berday et al., 2000, Vyas et al., 2007). This behavioral change is highly specific, sparing a wide range of behaviors related to olfaction, learning, conditioned fear, social status and mating success (Berday et al., 1995, Vyas et al., 2007, Webster, 2007). These observations are interesting because *Toxoplasma* can sexually reproduce only in the intestine of cats (Dubey, 1998). Furthermore, entry of parasites in sexual phase of life cycle also results in increased infectivity during the subsequent asexual phase. Thus, reproductive fitness of *Toxoplasma* is dependent upon predation of small warm-blooded animals by cats; and loss of the innate aversion is likely to increase predation and result in increased reproductive fitness for the parasite. This position agrees with the 'behavioral manipulation' hypothesis, which states that a parasite can change host behavior specifically to increase its own reproductive fitness (Vyas et al., 2007, Webster, 2007).

Although it is known that *Toxoplasma* infection causes behavioral change in rodents, it is not known if such behavioral change is an absolute all-or-none phenomenon or the change is relative and is dependent on the intensity of stimulus. It can be speculated that at lower doses of stimulus, manipulation of behavior is not beneficial for the parasite. For example, if cat odor is too weak, it is unlikely that a cat is nearby and parasite do not benefit from loss of aversion to cat odor. On the other hand, at very high doses of the stimulus, finite amount of behavioral manipulation induced by infection can probably be overwhelmed by innate aversion of the host to the intense cat odor. These different processes occurring at two extreme ends of stimulus-response curve could result in a non-monotonous function roughly resembling an inverted-U. As the first aim of this study, we measure relationship the between behavioral manipulation and strength of aversive stimulus presented, determining whether such an inverted-U occurs.

The response of rodents to cat kairomones is an innate and hard-wired behavior (Dielenberg and McGregor, 2001). Rodents retain an intense aversion to cat kairomones despite the fact that laboratory-bred animals have never been exposed to a cat or cat odors. In addition to the stereotypic innate defensive behaviors, exposure to cat odor also causes long-term acquired changes. An important facet of these acquired changes is the learning induced by predator odors. For example, when rats are exposed in a test situation to a collar previously worn by the cat, they subsequently show avoidance in the same situation when presented with unworn cat collar that is free of cat odor (Blanchard et al., 2001, Staples et al., 2005). If behavioral manipulation by *Toxoplasma* results in alteration of the emotional valence of cat odor, it is reasonable to expect that infection will affect learning driven by the aversive properties of the odor. As a second aim of the present study, we investigate this assertion by measuring conditioned fear in response to varying doses of cat body odor.

EXPERIMENTAL PROCEDURES

Experimental animals

Male Long-Evans rats (8 weeks old, Charles River Laboratories, 3 per cage) were used. Animals procured from this source tested serologically negative for *Toxoplasma*. Stanford University Administrative Panel for Laboratory Animal Care reviewed and approved all procedures related to animal maintenance and experimentation.

Toxoplasma culture

We employed a Prugniald strain of *Toxoplasma*. Parasites were maintained as tachyzoites by passage in human foreskin fibroblast monolayers.

Infection and Experimental groups

Infected fibroblasts were syringed-lysed using a 27-gauge needle to release tachyzoites. Animals, randomly assigned to experimental groups, were either infected i.p. with *Toxoplasma gondii* tachyzoites (10^7 per rat) or mock-infected with sterile PBS. All behavioral experiments were conducted between 7 to 8 weeks post infection, once a latent infection was established. Successful infection was ascertained by serology.

Behavioral measurements

Exploration by animals in a straight rectangular arena (length = 135 cm and width = 15 cm) was recorded (10 Hz during a 5-minute trial). Animals were introduced in one end of the arena and stimulus (when present) was put in the opposite end. Two opposing ends of the arena were made distinct using separate sets of visual and spatial cues. Occupancy of animals in the bisect containing the stimulus was measured as the endpoint. A customized tracking system embedded in NIH Image (<http://rsb.info.nih.gov/ni-image/Default.html>) was employed to generate a series of spatial coordinates. Endpoints were calculated using customized routines written in C++.

Behavioral testing (9 AM to 3 PM) was conducted in four sequential phases at the interval of twenty-four hours (one habituation phase followed by three test phases; trial duration = 5 minutes; inter-phase interval = 1 day). During the phase 1, animals were habituated to the arena. During the phase 2, aversion to bobcat urine was measured by placing bobcat urine at one end of the arena. Different experimental groups experienced varying doses of the urine. Specifically, 0.5, 1, 2 or 2.5 ml of undiluted bobcat urine was diluted to give final volume of 2.5 ml. In phase 3, a towel previously exposed to the cat served as the stimulus. Cat towel was obtained by keeping a cotton towel in contact with a female cat for 48 hours and then rubbing the towel vigorously at the neck of the cat for five minutes. Different experimental groups experienced varying doses of the towel. Specifically, towel size varied between 112, 225, 675 and 900 CM². Finally in the phase 4, memory of prior exposure to the cat towel was examined by measuring aversion to fresh towels never exposed to a cat. Same batch of bobcat urine (obtained from LegUp Enterprise Inc.; Lovell, ME) and cat towel (obtained from a domestic cat) was used throughout the experiment. Aversion was measured as occupancy in the bisect containing aversive stimulus during test phase relative to that in habituation phase (relative occupancy = occupancy in test phase/occupancy in habituation phase; chance level = 1; e.g. Figure 1A). Difference between control and infected animals was calculated by subtracting relative occupancy of control animals from that of infected animals (chance level = 0; e.g. Figure 1B).

It is important to note here that exposure to cat towel followed the exposure to bobcat urine in experimental procedure; raising a possibility that exposure to urine influenced the behavioral performance in subsequent trials. On the other hand, previous studies suggest that exposure of rats to excretory products of cat does not produce conditioning in rats (Blanchard et al., 2003). This is thought to reflect the fact that partial predator cues like urine and fecal odors do not predict certain bodily presence of the predator (Apfelbach et al., 2005). In agreement with these studies, we did not observe significant alteration in behavioral performance of animals previously exposed to bobcat urine. Control animals, when tested twenty-four hours after the exposure to 2 ml of bobcat urine, could not retain the memory of prior exposure (time spent in conditioned bisect = 49.2 ± 5.6 %, $n = 6$ animals; chance level = 50%). Hence, it is unlikely that exposure to bobcat urine influenced behavioral performance in subsequent trials.

Statistical analysis

Values are reported as mean \pm SEM. Two-way analysis of variance was conducted with dose and infection status as contributing sources of variability. In addition, planned comparisons

were made between control and infected animals using non-parametric MW tests. Difference between control and infected animals was analyzed using one-sample t-test (compared with chance level = 0).

RESULTS

The effect of infection on aversion to bobcat urine was dependent on dose of urine

The response of control and infected animals to varying doses of bobcat urine was examined. Relative occupancy was measured as occupancy during test trial relative to that during prior habituation. Exposure to urine produced significant aversion in control animals ($n = 43$), as manifested by reduced occupancy in the bisect containing the bobcat urine. Infection reduced the aversion to bobcat urine ($n = 42$), as demonstrated by increased relative occupancy (a 43% increase; $p < 0.001$, student's t-test) compared to control.

The response of both control and infected animals to the urine was further analyzed by measuring the relationship between aversion and amount of urine presented (Figure 1A). Response of control animals varied significantly with the dose (one way ANOVA, $p < 0.05$). An intermediate dose of the urine (1 ml) produced the most robust aversion. Planned comparisons revealed that the difference between control and infected animals was significant at the three higher doses of bobcat urine (Figure 1B; 1, 2 and 2.5 ml; $p < 0.05$). The magnitude of difference between control and infected animals was dependent on the dose of the urine (Figure 1B). The intermediate dose of bobcat urine (1 ml) produced the greatest magnitude of the behavioral difference between control and infected animals.

A two-way analysis of variance was conducted with dose of urine and infection status as sources of variance. It revealed a significant main effect of infection ($F_{1,63} = 23.8$, $p < 0.001$). Statistical significance was not achieved for the main effect of dose ($F_{3,63} = 2.4$, $p = 0.08$) and for the interaction between dose and infection ($F_{3,63} = 2.4$, $p = 0.08$).

The effect of infection on aversion to cat towel was dependent on size of towel

The response of control and infected animals to varying sizes of cat towel was examined. Exposure to cat towel produced a significant aversion in control rats, as demonstrated by reduced relative occupancy in the bisect containing the cat towel (Figure 2A; $n = 38$). We observed that cat towel was more aversive than bobcat urine for control rats. This is in agreement with previous reports that body odor is a better predictor of presence of predator and is more potent in evoking defensive response in rats compared to partial predator cues like excretory products (Jones and Dayan, 2000, McGregor et al., 2002). Animals exhibiting greater aversion to bobcat urine also exhibited greater aversion to cat towel (controls: $r = 0.36$, $p < 0.05$; infected: $r = -.38$, $p < 0.05$).

Previous reports indicate that defensive behavior elicited by cat towel depends on physical size or "dose" of the towel (Takahashi et al., 2005). Hence, the response of animals to the towel was further analyzed by measuring the relationship between aversion and size of towel presented (Figure 2A). Significant differences were present within control experimental group (one way ANOVA, $p < 0.05$). Relative occupancy in the towel bisect decreased monotonically with the increase in "dose" or size of the towel. Planned comparisons revealed that the difference between control and infected animals was statistically different only at one intermediate dose (Figure 2A; 675 CM²; $p < 0.05$). The difference between control and infected animals followed a rough inverse-U curve (Figure 2B), with one intermediate dose producing the highest magnitude of difference and more extreme doses producing lesser magnitudes. Surprisingly, the infection increased aversion by a small but significant degree ($p < 0.05$, one-sample t-test) at the lowest size of towel employed (112 CM²).

Two-way analysis of variance did not reveal significant main effect of infection ($F_{1,54} = 3.1$, $p = 0.09$). Statistically significant effects were observed for main effect of dose of towel ($F_{3,54} = 3.4$, $p < 0.05$) and for interaction between dose and infection ($F_{3,54} = 4.1$, $p < 0.05$).

Infection affected the strength of fear conditioning to prior exposure to cat towel, in a dose-dependent manner

Rats exposed to cat body odor are known to develop learned fear that can be expressed later in the absence of the actual aversive stimulus (Blanchard et al., 2001, Dielenberg and McGregor, 2001). We measured such a conditioned response of control and infected animals using varying doses of cat towel as the unconditioned stimulus and the relative occupancy in bisect containing sham towel as conditioned response. Conditioning in this paradigm is reflected as avoidance of the bisect that previously contained the cat towel; although no cat odor was present during the testing. An increase in learning or strength of conditioning due to infection is reflected as an increase in avoidance of the bisect or a decrease in relative occupancy.

Exposure to the cat towel produced significant conditioning when measured twenty-four hours later in control animals ($p < 0.001$, paired t-test). Such learned fear was reflected as avoidance of the conditioned bisect (lower relative occupancy). Furthermore, aversion to towel was significantly correlated with subsequent conditioning ($r = 0.52$, $p = 0.001$). As an important contrast to control animals, conditioning in infected animals was not significantly correlated to the aversion of cat towel observed during preceding trial ($r = 0.2$, $p = 0.2$).

As described earlier, the degree of aversion produced by exposure to cat towel was dependent on the “dose” or the size of the towel presented. This was further investigated by measuring the relationship between conditioning and size of towel presented during previous trial (Figure 3A) in control animals. Differences in conditioning observed in control animals at varying doses of unconditioned stimulus did not reach statistical significance (one way ANOVA, $p = 0.2$). Planned comparisons revealed that significant difference between control and infected animals were present at two of the four doses employed (Figure 3A; 225 CM² and 900 CM²; $p = 0.05$). Both the magnitude and direction of the infection effects were dependent on dose of cat towel employed as the unconditioned stimulus (Figure 3B). Differences between control and infected animals followed a rough inverted-U curve, with one intermediate dose producing compromised learning (reduced avoidance of conditioned bisect or increase in relative occupancy) and the highest dose producing increased learning (increased avoidance of conditioned bisect).

Two-way analysis of variance did not reveal significant main effects of infection ($F_{1,56} = 0.4$, $p > 0.5$) or of dose of unconditioned stimulus ($F_{3,56} = 1.3$, $p > 0.2$). Significant interaction between dose and infection was present ($F_{3,56} = 3.0$, $p < 0.05$). Exposure to the bobcat urine did not produce significant conditioning in control and infected animals (data not shown).

DISCUSSION

As a result of intense predation pressure, rodents have developed innate sensitivity to cat kairomones. Cat odors evoke immediate and intense defensive behaviors in laboratory rats, despite that fact that these laboratory rats are generations away from experiencing a real cat. Thus, it is surprising that infection with a parasite, *Toxoplasma*, reduces innate aversion to cat odors in rodents. Here we report that such loss of aversion is not absolute, but rather is dependent on strength of aversive stimulus used. We also report that in addition to affecting innate fear, *Toxoplasma* infection also affects learned fear expressed after conditioning to the cat odor.

The core of parasitism is the ability of an organism to exploit its host. According to the behavioral manipulation hypothesis, a parasite may be able to alter the behavior of its host for its own selective benefit (Thomas et al., 2005). Such selective behavioral change is proposed to increase reproductive success of the parasite, usually by enhancing its transmission efficiency. In case of *Toxoplasma*, loss of aversion to cat odors in infected rodents could cause an increase in predation rates and increase the transmission of *Toxoplasma* to cats, a necessary condition for its sexual reproduction (Dubey, 1998, Berdoy et al., 2000, Vyas et al., 2007). Furthermore, behavioral effects of *Toxoplasma* infection are highly specific to the innate aversion to the cat odor while a variety of health indicators and behavior remain unchanged (Vyas et al., 2007). These observations provide a strong support for the idea that *Toxoplasma* causes 'behavioral manipulation' of its rodent hosts.

Data in this report indicate that manipulation is relative and is observed only at intermediate stages of stimulus-response curve. It can be speculated that at lower doses of stimulus, manipulation of behavior is not beneficial for the parasite. For example, if cat odor is too weak, it is unlikely that a cat is in immediate vicinity and the parasite does not benefit from loss of aversion to cat odor. On the other hand, very high doses of the stimulus could induce intense innate aversion in the host, sufficient to overwhelm the finite amount of behavioral manipulation induced by the infection. These two different processes occurring at two extreme ends of stimulus-response curve could potentially explain the fact that magnitude of behavioral manipulation in this case follows a non-monotonous function roughly resembling an inverted-U.

It would obviously be adaptive to a parasite if it could overcome the innate aversion that is retained in response to very high intensities of stimulus. The fact that a parasite cannot do so may reflect the significant fitness costs involved in bringing about the manipulation itself. The true cost of the manipulation has recently been a matter of vibrant discussion (Poulin, 1994, Poulin et al., 2005, Thomas et al., 2005). Most direct cost for the parasite to cause the behavioral manipulation is related to consumption of energy and metabolites. Active secretion of chemicals is required for the most cases of behavioral manipulation, mere physical presence of parasites in brain or eyes not being sufficient to cause change in behavior (Hurd, 1990, Eberhard, 2000, Helluy and Holmes, 2005). Secretion of these factors requires energy consumption and represents a proximal cost of behavioral manipulation. More importantly, behavioral manipulation involves indirect and probabilistic costs. One form of such probabilistic cost is the possibility that the parasite will wind up in a non-host organism (Poulin et al., 2005). For example, behavioral manipulation of cockle *Austrovenus stutchburyi* by trematode *Curtuteria australis* results in loss of burrowing by the cockle. This makes cockles more susceptible to predation by avian definitive host of the parasite. As a side-effect of the manipulation, infected cockles also become more susceptible to predation by fish *Notolabrus celidotus* (Mouritsen and Poulin, 2003). Predation of infected cockles by non-host fish thus represents a probabilistic cost. Similarly, *Toxoplasma* infects a substantial portion of human population, with several behavioral effects of infection already reported (Flegr et al., 2002, Skallová et al., 2005, Flegr, 2007, Kanková et al., 2007). Since humans are not frequently preyed upon by cats, both the entry in humans and behavioral changes are probabilistic costs for the parasite. Another common source of cost for the behavioral manipulation is increased mortality of parasites during invasion of host organs necessary for the manipulation (Poulin et al., 2005). For example, the flatworm *Microphallus papillorobustus* infects amphipod *Gammarus* spp. If the flatworm manages to infect the cerebral region, it results in aberrant photic and mechanical response of the host (Helluy, 1983, Helluy and Thomas, 2003). This makes the intermediate host more vulnerable to predation by birds, which are the definitive hosts for the parasite (Helluy, 1984, Helluy and Thomas, 2003). Interestingly, parasites that enter the brain have higher mortality rates than those that remain in the abdomen and hence do not take active part in behavioral manipulation (Thomas et al., 2000). Furthermore, a few

Microphallus species other than *M. papillorobustu* infect the same individual amphipod. These species do not invade brain and remain in the abdomen, thus reaping the benefit of manipulation without producing it themselves. Mortality rate of these 'lucky passengers' is very low compared to manipulative *M. papillorobustu* individuals in the brain (Thomas et al., 2000, Poulin et al., 2005). This neatly demonstrates that there are substantial costs involved with the behavioral manipulation. For behavioral manipulation to be adaptive for the parasite, benefits obtained from the manipulation must exceed fitness costs incurred. Because of such dynamic balance between the cost and the benefit, it can be argued that manipulation will not be an absolute all-or-none phenomenon; magnitude of behavioral manipulation being optimized and not maximized.

Since the behavioral manipulation in this case is not absolute, it becomes important to ask if the modest behavioral manipulation observed is at all adaptive for the parasite. A direct experimental observation of increased transmission efficiency would require an ethically tenuous comparison of predation rates between control and infected animals. Nevertheless, mathematical models describing a similar prey-predator-parasite system demonstrate that even a small selective increase in susceptibility of infected prey population would be sufficient to cause significant increase of parasitic load in predator populations (Vervaeke et al., 2006). Hence an absolute behavioral manipulation might not be a precondition to obtain substantial fitness benefits.

What are the proximate mechanisms of behavioral changes induced by *Toxoplasma* infection? A small inventory of potential mechanisms could include inactivation of a few olfactory receptors important for cat odor detection, alteration in emotional valence of cat odor by brain rewiring, preferential localization of *Toxoplasma* to brain areas in septohippocampal or amygdalar regions or regions projecting to them, uniform distribution but with some brain regions being more sensitive to neuronal damage by *Toxoplasma* and diffusible substances emanating from infected cells or from *Toxoplasma*. These possibilities will need to be systematically tested in future studies. Observations described in the present report put an important constraint on the possible neurobiological mechanisms. Because the behavioral effects of *Toxoplasma* are specific and subtle, it is unlikely that sweeping changes in neural substrates are involved in bringing about these effects.

It is important to note here that concept of "dose" as a scalable measure of the stimulus can not be readily applied to cat odors. Despite continuous efforts to isolate aversive components of cat urine and body odors, we can only speculate about the actual active ingredient. This limits researchers to use arbitrary units like a pre-determined size of cat towel as the "dose" of the stimulus (Takahashi et al., 2005). Significant variation exists between different batches of cat odor and between odors collected from different cats in terms of aversion induced and probably in terms of actual aversive odor present. For this reason, we have employed the same batch of bobcat urine and cat towel throughout the experiment.

Exposure to cat and cat odor causes long-term changes in rodent behavior that can be observed in absence of subsequent exposures (Blanchard et al., 2001, Adamec et al., 2005, Staples et al., 2005). For example, exposure to cat produces long-term increase in anxiety when measured in elevated plus-maze (Adamec et al., 2005). This increase in unconditioned fear is associated with lasting changes in amygdalar neurotransmission, in stress hormone secretion and in pCREB content in brain regions related to regulation of stress (Adamec et al., 2006). Similarly, exposure to cat odor also causes acquired changes in the rodent behavior related to the learning. For example, when rats are exposed to a worn cat collar in a test situation, they subsequently show avoidance in the same situation when presented with unworn cat collar that is free of cat odor (Blanchard et al., 2001, Staples et al., 2005). This shows that rats have been conditioned

to the test situation; or in words animals demonstrate learned fear to a stimulus merely because of its historical association with cat odor and not because of actual odor present.

We report that infection not only reduces aversion to cat odors, but also affects learning driven by the cat odor. At intermediate dose of the cat towel (unconditioned stimulus) infection resulted in a decrease in learning or a decrease in avoidance of the conditioned bisect (conditioned response). At the highest dose used, infection resulted in enhanced learning or an increase in avoidance of the cat bisect. Thus, effects of infection on learned fear followed a non-monotonous inverted-U like function. We have previously reported that control and infected animals do not differ with respect to learning the association between footshock and a neutral tone or a neutral spatial context (Vyas et al., 2007). Thus, infection specifically affected learned fear when cat odor, and not the footshock, was used as the aversive stimulus. This further supports the idea that behavioral changes induced by *Toxoplasma* infection are specific to innate aversion to the cat odors. Since effects on learning reported here are not dependent on physical presence of odor during the testing, it can be argued that effects of *Toxoplasma* are not merely dependent on sensory perception of the odor or arising out of generic malaise. Effects of infection rather reflect a specific change in defensive and mnemonic behaviors. These observations support the behavioral manipulation hypothesis.

Defensive behaviors observed in a conditioned context are similar in nature to those observed during actual exposure to the cat odor. In addition, expression of conditioned fear also recruits many brain regions that would be normally involved in expression of innate fear to cat kairomones (Dielenberg et al., 2001, Li et al., 2004, Staples et al., 2005, Takahashi et al., 2007). In view of these shared neurobiological substrates, it is possible that same set of neural changes that induces loss of innate aversion is also responsible for alterations in learned fear. Interestingly, we previously observed that while *Toxoplasma* cysts could be observed in a variety of brain regions, density of cysts was significantly higher in amygdala, a brain region known to be important for both defensive behaviors and conditioning of the fear (Vyas et al., 2007).

Data presented in this report support the behavioral manipulation hypothesis by demonstrating that *Toxoplasma* affects learned fear induced by cat odor; an effect related to change in emotional valence of cat odor. This report also supports the notion that behavioral manipulation brought about by *Toxoplasma* is not an absolute all-or-none effect but is a rather subtle effect dependent on the amount of aversive stimulus present. This supports the notion that a dynamic balance exists for the parasite between costs and benefits of the manipulation and that magnitude of the manipulation is optimized in this context rather than maximized.

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ABBREVIATIONS

ANOVA

Analysis of variance

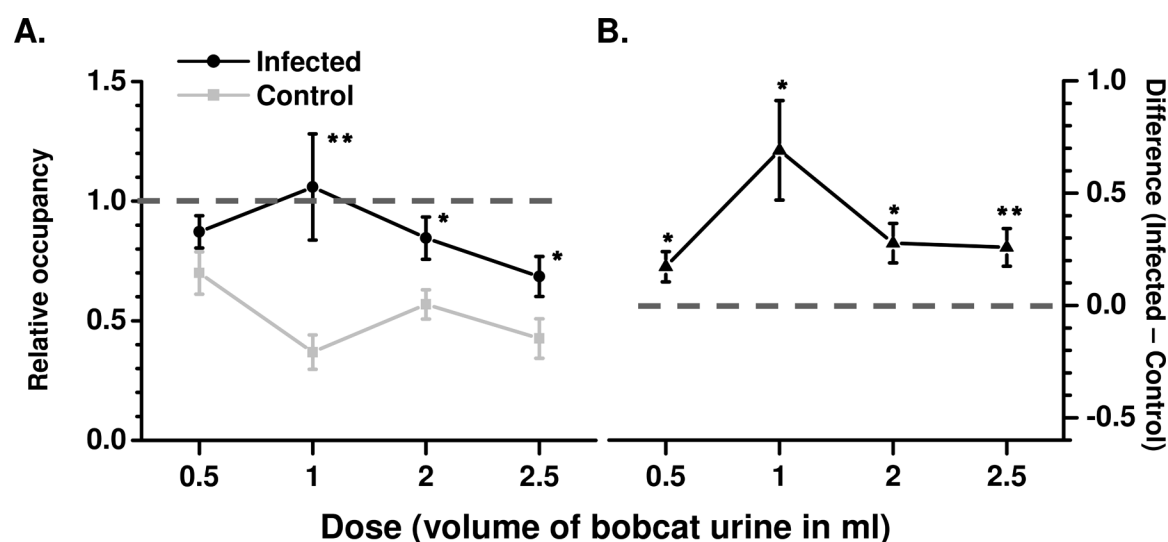


Figure 1.

Infection increased the relative occupancy in the bisect containing the urine in a dose-dependent manner. **A.** The ordinate depicts relative occupancy in bobcat bisect relative to occupancy in the same bisect during the habituation plus that during trial containing bobcat urine. Dotted line represents chance level. Abscissa depicts varying doses of the bobcat urine. *, $p < 0.05$; **, $p < 0.01$; planned comparison; MW test. **B.** Intermediate dose of bobcat urine produced a bigger magnitude of difference between control and infected animals. Ordinate depicts difference between control and infected animals in terms of relative occupancy in bisect containing bobcat urine. Data presented in this panel are derived from that in panel B. *, $p < 0.05$; **, $p < 0.01$; one-sample student's t -test, compared to chance level (depicted with dotted line).

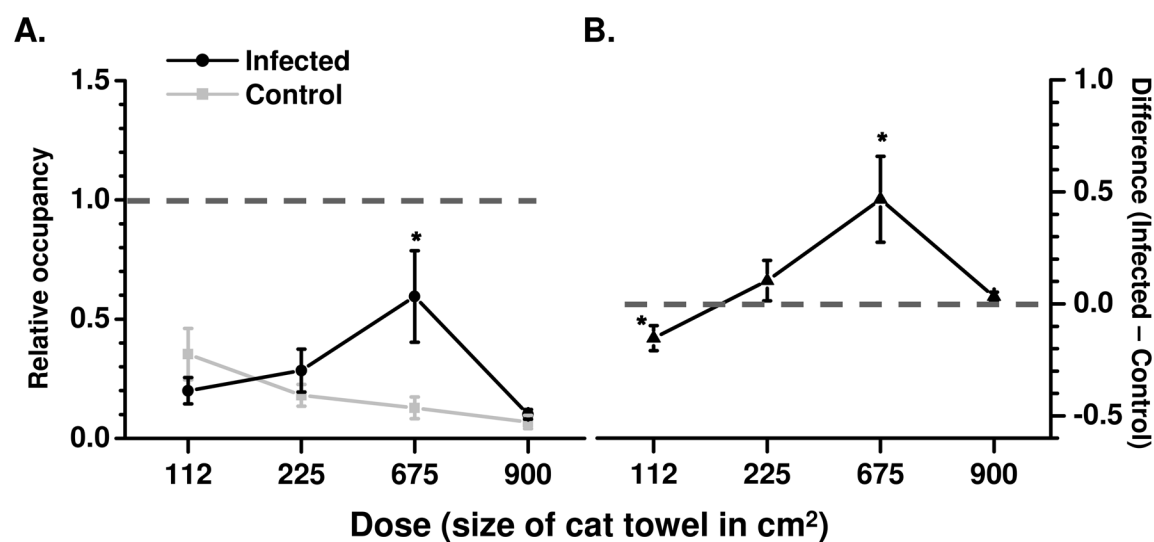


Figure 2.

Effect of infection on aversion to cat towel was dependent on size of the towel. **A.** Infection significantly increased relative occupancy in towel bisect at an intermediate dose. *, $p < 0.05$. **B.** Magnitude of difference because of infection was dependent on size of towel. Intermediate dose produced the biggest effect. Data presented in this panel are derived from that in panel A. *, $p < 0.05$.

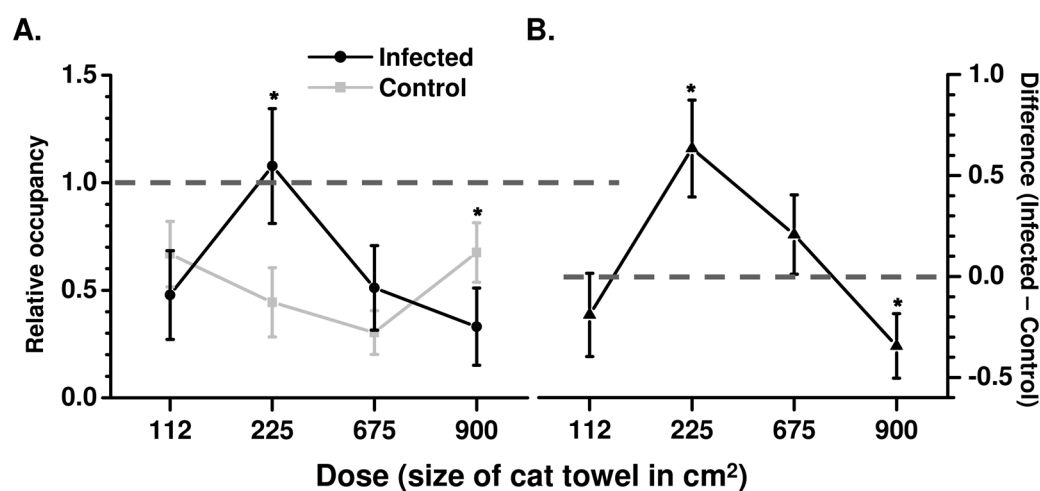


Figure 3.

Effects of infection on conditioned fear were dependent on size of the unconditioned stimulus, the cat towel **A**. Infection significantly increased relative occupancy in conditioned fear at an intermediate dose, but decreased relative occupancy at the highest dose. *, $p < 0.05$. **B**. Magnitude of difference because of infection was dependent on size of towel. Data presented in this panel are derived from that in panel A. *, $p < 0.05$.