Title Page

Toxoplasma gondii infection and testosterone congruently increase tolerance of male rats for risk of reward forfeiture

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Abstract

Decision making under risk involves balancing the potential of gaining rewards with the possibility of loss and/or punishment. Tolerance to risk varies between individuals. Understanding the biological basis of risk tolerance is pertinent because excessive tolerance contributes to adverse health and safety outcomes. Yet, not much is known about biological factors mediating inter-individual variability in this regard. We investigate if latent *Toxoplasma gondii* infection can cause risk tolerance. Using a rodent model of the balloon analogous risk task, we show that latent *Toxoplasma gondii* infection leads to a greater tolerance of reward forfeiture. Furthermore, effects of the infection on risk can be recapitulated with testosterone supplementation alone, demonstrating that greater testosterone synthesis by the host post-infection is sufficient to change risk tolerance. *Toxoplasma gondii* is a frequent parasite of humans and animals. Thus, the infection status can potentially explain some of the inter-individual variability in the risky decision making.

Introduction

Animals and humans typically make decisions in ambivalent situations and under risk of forfeiture. Biological factors play an important role in such decision making. Two such biological factors have attracted greater scientific interest: first, mesolimbic dopaminergic system which pivots around nucleus accumbens; and second, steroid hormones secreted by peripheral glands. Testosterone secreted by male gonads enhances risk-taking behavior in human subjects (Coates and Herbert, 2008; Cooper et al., 2014; Peper et al., 2013; Stanton et al., 2011). Extraneous testosterone can be used as a positive reinforcement in rodents (Wood, 2004; Wood et al., 2004), suggesting its ability to intersect with dopaminergic reward system in the brain. Consistent with this, placement of testosterone or its metabolites in nucleus accumbens facilitates conditioned place preference (Frye et al., 2002), again suggesting that testosterone can activate mesolimbic dopaminergic pathways involved in decision making under risk.

Interestingly a widely prevalent protozoan parasite (Jones et al., 2014), *Toxoplasma gondii*, alters both testosterone synthesis and nucleus accumbal dopamine content in laboratory rats (Lim et al., 2013; Tan et al., 2015). *Toxoplasma gondii* invades testes in this animal model (Hari Dass et al., 2011; Vyas, 2013), resulting in a long-term increase of testosterone synthesis (Lim et al., 2013). In addition, the infection results in greater synthesis of arginine vasopressin in brain regions afferent to nucleus accumbens (Hari Dass and Vyas, 2014), structural diminution of nucleus accumbens neurons and decrease in total dopamine concentration (Tan et al., 2015). Retrospective studies suggest that chronic *Toxoplasma gondii* infection enhances behaviors reminiscent of risk-taking in human subjects like being involved in traffic accidents (Flegr et al., 2002; Flegr et al., 2009; Yereli et al., 2006).

63 These observations suggest that *Toxoplasma gondii* increases tolerance to reward forfeiture
64 through associated increase in testosterone availability. In this report, we experimentally test
65 this hypothesis.

66 # Materials and Methods

67 ## Animals

68 Male Wistar rats were used. Rats were 8 weeks of age at the start of experiments, housed 2
69 per cage with 12 hours light-dark cycle (lights on at 7 AM). Rats were provided with *ad*
70 *libitum* access to food and water, except during operant experiments when rats were
71 maintained on a restricted diet to 85% of their free-feeding weight and allowance of 3 – 5 g
72 per week body weight gain. Animals were obtained from the vivarium of National University
73 of Singapore. All animal procedures were approved by Nanyang Technological University's
74 institutional animal care and use committee.

75 ## Parasites

76 *Toxoplasma gondii* tachyzoites of type 2 Prugniaud strain were maintained in human skin
77 fibroblast cultures. Infected fibroblasts were syringe-lysed to release tachyzoites. Animals
78 were either infected with tachyzoites (5×10^6, intraperitoneal) or mock-infected with sterile
79 phosphate buffered saline. Eight weeks elapsed between infection and the start of behavioral
80 experiment; an incubation period consistent with the presence of chronic infection and
81 absence of acute parasitic proliferation (Vyas et al., 2007a).

82 ## Castration and Testosterone treatment

83 Surgery was performed using aseptic techniques under isoflurane anesthesia (2.5% gaseous
84 isoflurane with pure O_2). After placing animals in dorsal recumbency, testes were approached
85 through a mid-scrotal incision. Testes, vas deferens and testicular fat pad were bilaterally
86 removed followed by suturing of spermatic blood vessels. Scrotum was subsequently sutured.
87 One micro-infusion pump was placed subcutaneously supplying either vehicle (grape seed oil)
88 or testosterone cypionate. Microinfusion pumps (iPRECIO SMP-200; Durect) delivered their
89 cargo for several months requiring only monthly refills through the septum of the pumps

accessed through subcutaneous route. Pumps were programed to deliver 0.8 µl/day of vehicle or testosterone cypionate (200 mg/ml dissolved in grape seed oil; Pfizer) at a constant rate. This dose of the testosterone is in slight excess to physiological norms of circulating testosterone (Aubele et al., 2008).

Animals were given pre-operative prophylaxis antibiotic (Baytril 10mg/kg, sc; Bayer) and pain relief (Carprofen 5 mg/kg, sc; Pfizer). After surgery, animals were housed singly for >3 days with supplemental pain relief daily (Carprofen 5 mg/kg, sc). Animals were re-housed with prior cage-mates once wound healing was visually confirmed. At least one week elapsed between surgery and start of food restriction for operant testing. Pumps were programed to start infusion only after the recovery period.

Quantification of serum Testosterone levels

The method was modified from French (2013). 98 µL of serum and 2 µL of internal standard (10 ng/mL Testosterone-2,3,4-$^{13}C_3$ in acetonitrile) were suspended in 1.1 mL of hexane:ethyl acetate (90:10 v/v). The mixture was vortex-mixed and centrifuged at 3000 rpm for 10 min at 4°C. The aqueous layer was frozen on dry ice and the supernatant was pipetted into in a clean tube. The solvent was then evaporated to dryness. Extracted testosterone was reconstituted in 100 µL of 20% acetonitrile. After reconstitution, the extracted sample was centrifuged at 13200 rpm for 10 min at 4°C and 85 µL of the supernatant was transferred to HPLC vials for liquid chromatography electrospray tandem mass spectrometry (LCMS/MS) analysis for the detection of testosterone. Detection of testosterone using LCMS/MS spectrometry is described in Takyi-Williams et al. (2015). The quantitation limit of the method was 0.06 ng/mL and the method was linear within a range of 0.06 ng/mL to 1.95 ng/mL

Balloon analogous risk task

Operant performance under risk of reward forfeiture was measured using a balloon analogous risk task, adapted from Jentsch et al. (2010) (Figure 1). Operant chambers used for training and testing were provisioned with a house light and an internal stimulus lights (30x24x30 cm, Med-Associates; programmed using K-Limbic, Conclusive Solution). Chambers were enclosed in a sound-attenuating and ventilated outer cabinet. Ventilating exhaust fan mounted on the outer cabinet provided a masking white noise (88 dB, linear scale). Operation of the pellet dispenser delivered 45 mg food pellets (formula 5TUM; TestDiets) into the food receptacle within the operant chamber. In addition, two retractable stainless steel response levers were mounted on either side of the food delivery receptacle (8.5 cm above the floor, 7 cm lateral to the outer edge of food tray).

During initial training, rats were individually placed in the operant chambers and one of the levers was extended for 30 minutes (phase 1). Each operant response of one lever press was reinforced with the delivery of one food pellet. The process was repeated for the other lever and animals were subsequently returned to their home cage. This phase of training was repeated daily until all rats committed ≥ 60 responses for each lever during a 30-minute session.

Subsequently, one of the levers was randomly designated as the ‘add’ lever (phase 2). The left or right lever was designated as the ‘add’ lever in a counterbalanced manner across animals; and kept consistent for each animal across training and testing. Rats were trained to increase lever presses on the ‘add’ lever by successively increasing requisite responses from 1 to 3 and then 10 before delivery of one food pellet ensued (session duration = 20 minutes; 1 trial/day). This phase of the training continued till individual animals accumulated ≥ 30 lever presses per session.

Next, subjects were trained in sessions comprising 54 trials (phase 3; 1 session/day). The 'add' lever was presented. Animals were required to accumulate a pre-determined number of lever presses (varied randomly between 2 to 15) before the 'add' lever was retracted and an alternative lever designated as the 'cash-out' was presented. Pressing the 'cash-out' lever resulted in delivery of food pellets equal in number to presses of the 'add' lever required for that trial.

After completion of training in the phase 3, rats began daily testing on the actual task (Figure 1; 54 trials/session, 1 session/day). Initially animals did not encounter any risk of reward forfeiture (baseline). During each trial, 'add' and 'cash-out' levers were presented simultaneously. Animals were required to execute >1 lever presses on the 'add' lever and follow it up by pressing 'cash-out' lever. This resulted in delivery of delivery of food pellets equal in number to the total number of 'add' lever presses. Pressing the 'cash-out' lever before the 'add' lever resulted in an aborted trial without delivery of food. Failure to respond within 3s resulted in a mistrial. Both mistrial and aborted trials resulted in zero yields. Only gainful trials were included in the analysis. The process was repeated daily till they reached a stable baseline ($p > 0.05$ for mean lever presses on 'add' lever when analyzed for three consecutive days). Stable baseline was observed after 12-18 successive sessions had elapsed.

Once a stable baseline had been achieved, animals were tested under a risk of forfeiture. Each successive press of the 'add' lever added one pellet to the accrued reward, but also linearly increased the probability to total forfeiture. Three forfeiture probabilities were used (Δ increase in forfeiture probability per 'add' press: 0, 0.111 and 0.167; assigned pseudo-randomly and non-alternating; one session per day). For experiments involving castrated animals with/without testosterone supplementation, only 0 and 0.167 risk schedule was used. Trials comprising of zero risk of reward forfeiture were signaled by the illumination of a house light during sessions. Response of animals in zero forfeiture trials before introduction

of risk (baseline) was compared with zero risk trials after introducing risk of forfeiture (probe). Trials comprising of risk of reward forfeiture were signaled by the illumination of a distinct stimulus light within the operant chamber. Mixed-risk sessions continued till stable responding was achieved after 12 to 18 successive sessions. Probe trials with zero risk of reward forfeiture were interspersed with forfeiture risk trials in a pseudorandom manner. Mean lever presses during gainful trials was used as the endpoint during both baseline and mixed-risk sessions.

Animals were assigned in the groups in a random manner. Training and subsequent testing for control and infected animals was conducted >7 weeks post-infection. All animals in control and infected groups were tested using continuous reinforcement schedule (FR1). For experiment involving testosterone supplementation, gonad-intact animals were first trained to a stable baseline before surgery. After at least one week of post-surgery recovery, animals were again trained till stable baseline and then testing commenced. Castrated animals with or without testosterone supplementation were tested at continuous reinforcement schedule (FR1), although these animals had been initially trained on intermittent schedule (FR3) and then shifted to FR1 till stable baseline had been achieved.

Statistics

Analysis of variance (ANOVA) was used to estimate the statistical significance of main effects and/or interactions. Cumulative frequency distributions were fitted with Boltzmann sigmoidal function. Paired t-test was used for orthogonal comparisons between baseline and probe trials. Effect sizes (eta squared, η^2) for ANOVA were calculated as well as Cohen's d for pairwise comparison (>0.8 interpreted as a large effect) (Cohen, 1977). Number of animals is noted in figure legends.

Results

We quantified decision making under risk using a rodent version of the balloon analogous risk task (BART, Figure 1) (Jentsch et al., 2010). Animals were first trained to press an "add-lever" that increased the size of the upcoming reward (one food pellet per press) and a "cash-out" lever that delivered the accrued reward.

Even in baseline conditions before introduction of risk, control animals executed relatively few lever presses (n = 14 animals). For example, more than 50% of animals exhibited <3 lever presses per gainful trial in pre-risk episodes (Figure 2A, *black*; Table 1). We further introduced risk whereby each press of the add-lever increased the probability of reward forfeiture akin of a balloon being burst under increasing inflation. The probabilities of balloon bursting were fixed at 0.111 or 0.167 per additional press during two separate sessions (or an 11.1% and 16.7% incremental risk). Probe trials with zero risk of reward forfeiture were interspersed with 11.1% and 16.7% forfeiture trials in a pseudorandom manner. The mean numbers of add-lever presses, during baseline and in probe trials, were quantified.

Performance of control and infected animals was compared at baseline and during post-risk probe trials (repeated measure ANOVA: risk as within-subject and infection status as between-subject source of variance). Analysis of variance showed significant main effects of risk in interceding trials ($F_{1,24} = 57.05$, $p < 0.001$, $\eta^2 = 0.10$). Main effect of infection status was not statistically significant ($F_{1,24} = 0.57$, $p = 0.457$, $\eta^2 = 0.02$). ANOVA revealed significant interaction between risk and infection status ($F_{1,24} = 18.71$, $p < 0.001$, $\eta^2 = 0.03$). Post-hoc comparisons demonstrated significant suppression of lever pressing by control animals during probe trials, compared to baseline (Figure 2B, *left*; paired student t-test: $|t|_{13} = 5.15$, $p = 0.0002$; effect size: Cohen's d = 1.11). In contrast, lever pressing by the infected

animals during probe trials was comparable to paired baseline measurements (Figure 2B, *right*; $|t|_{11} = 1.1$, $p = 0.295$; statistical power = 0.05). All animals gained comparable body weight during the experimental period (independent sample t-test: $|t_8| = 0.203$; $p = 0.845$; effect size: Cohen's d = 0.13).

In view of greater testosterone synthesis by *Toxoplasma gondii* infected animals (Lim et al., 2013), we subsequently tested if testosterone was sufficient to increase tolerance to reward forfeiture congruent to effects of the infection. Uninfected rats were castrated and implanted with micro-infusion pump delivering either vehicle (n = 7 rats) or testosterone cypionate (n = 9 animals; dose = 160 µg/day). We quantified amount of the testosterone circulating in blood serum in vehicle- and testosterone-treated castrates. Castration reduced serum testosterone levels in vehicle-treated rats to 0.137 ± 0.0262 ng/mL while supplementation restored serum testosterone levels in testosterone-treated rats to 0.709 ± 0.105 ng/mL. (independent sample t-test: $|t|_{13} = 4.35$, $p = 0.0008$; effect size: Cohen's d = 2.52). Operant responses during baseline were recorded (Figure 3A, Table 2), followed by trials with risk of reward forfeiture (16.7%) and probe trials containing zero risk. Probe trials were interspersed with forfeiture trials in a pseudorandom manner.

Performance of vehicle- and testosterone-treated animals was compared at baseline and during post-risk probe trials (repeated measure ANOVA). Analysis of variance showed significant main effects of risk in interceding trials ($F_{1,14} = 15.55$, $p = 0.001$, $\eta^2 = 0.15$). Main effect of testosterone status was not statistically significant ($F_{1,14} = 0.21$, $p = 0.651$, $\eta^2 = 0.05$). ANOVA revealed significant interaction between risk and testosterone status ($F_{1,14} = 5.19$, $p = 0.039$, $\eta^2 = 0.03$). Post-hoc comparisons demonstrated significant suppression of lever pressing by vehicle-treated animals during probe trials, compared to baseline (Figure 3B, *left*; paired student t-test: $|t|_6 = 5.21$, $p = 0.002$; effect size: Cohen's d = 1.57). In contrast, lever pressing by testosterone-treated animals during probe trials was not significantly

different compared to paired baseline measurements (Figure 3B, *right*; $|t|_8 = 1.3$, $p = 0.229$; statistical power = 0.158). All animals gained comparable body weight during the experimental period (independent sample t-test: $|t_8| = 0.149$; $p = 0.885$; effect size: Cohen's d = 0.14). Thus, testosterone treatment induced risk tolerance, similar to *Toxoplasma gondii* infected animals.

Performance of control uninfected intact rats (Figure 2A, baseline and probe) was compared with castrated animals receiving vehicle supplementation (Figure 3A, baseline and probe). A two-way ANOVA revealed significant main effects of risk ($F_{1,38} = 13.96$, $p = 0.0006$, $\eta^2 = 0.26$). Main effect of experimental treatment was not statistically significant ($F_{1,38} = 0.32$, $p = 0.575$, $\eta^2 = 0.59$). Similarly no interaction between risk and experimental group was evident ($F_{1,38} < 0.00$, $p = 0.995$, $\eta^2 < 0.01$).

We also compared operant responding of the treated and untreated animals during trials with 16.7% risk of reward forfeiture (frequency distribution depicted in Figure 2 and 3, blue). Effect of *Toxoplasma gondii* infection on mean number of lever presses during 16.7% risk trials did not reach statistical significance (% change relative to baseline trials pre-risk, mean ± SEM: -40.1 ± 4.8 % for control and -28.0 ± 5.1 % for infected; independent t-test: $|t|_{24} = 1.73$ p = 0.096). Similarly, effect of testosterone supplementation did not reach statistical significance when compared to respective vehicle supplemented animals (mean ± SEM: -39.7 ± 4.1 % for vehicle and -29.2 ± 4.8 % for testosterone; $|t|_{14} = 1.59$, p = 0.135). Baseline and probe trials in this experimental design do not have determinate theoretical optima. In contrast, trials with 16.7% risk of reward forfeiture represent theoretical optima of 3 to 4 lever presses per trial (Figure 4, left; gray line). For each individual animal, we calculated ratio of trials with optimal operant responding (3 or 4 lever presses) relative to total number of gainful trials. Trials with 1 or 7 lever presses were omitted from this calculation because these trials are characterized by certain outcomes. *Toxoplasma gondii* infection increased

trials with economically optimal responses (Figure 4A, right; independent t-test: $|t|_{24} = 3.34$, $p = 0.0027$; effect size: Cohen's $d = 1.32$). Similarly, testosterone supplementation in non-infected animals resulted in increased frequency of optimal responding (Figure 4B, right; $|t|_{14} = 2.51$, $p = 0.0243$; effect size: Cohen's $d = 1.23$).

Discussion

Several paradigms have been used to quantify decision making under risk in animals and humans. As an endpoint, these tasks typically use suppression of operant responding due to a decrease in probability of reward (Stopper and Floresco, 2011), an increase in probability of aversive outcome (Simon et al., 2009) or an increase in risk of forfeiture (Jentsch et al., 2010; Lejuez et al., 2002). Amongst these tests, BART exhibits good test-retest validity in both human and rodent studies (Jentsch et al., 2010; White et al., 2008). Moreover, BART exhibits considerable predictive validity in humans. For example, individual variation of riskiness in BART can explain significant variation in self-reported risky behaviors including drug abuse, gambling attitudes, driving sans seatbelts and sexual intercourse with multiple partners sans protection (Hunt et al., 2005; Lejuez et al., 2003a; Lejuez et al., 2003b; Lejuez et al., 2002). Similar predictive studies for risk-taking operant behavior have not yet been conducted; barring a demonstration that pharmacological inactivation of orbitofrontal cortex reduces risk-taking in this task (Jentsch et al., 2010). This is an important gap in the knowledge because animal studies can provide a robust avenue to delineate biological mechanisms of inter-individual variability in risk taking. In this backdrop, we describe that a frequent parasitic infection of humans and animals (Jones et al., 2014; Webster, 1994) can decrease risk aversion in a rodent model. Furthermore we show that sustained testosterone rise, akin to that observed in the infected animals and humans (Flegr et al., 2008; Lim et al., 2013), is sufficient to reduce risk aversion.

In this context some limitations of the current study are noteworthy. Our conclusions are based on the observations that unlike corresponding controls *Toxoplasma gondii* infection or testosterone treatment does not reduce operant responding in probe trials after intervening exposure to the risk. Our observations do not provide unequivocal evidence of reduced operant responding in the treatment groups during trials containing forfeiture risk. Moreover,

we did not measure testosterone levels in current cohort of infected animals and corresponding controls. Our assumption of congruence between the infected animals and testosterone supplemented animals is thus based on historical observations of greater testosterone synthesis after the infection (Lim et al., 2013).

A significant number of human beings are infected with *Toxoplasma gondii* (Hill et al., 2005). Traditionally, this infection has been thought of being asymptomatic and of little clinical interest in immune-competent hosts. This traditional thought has been recently challenged by observations that latent *Toxoplasma gondii* infection in healthy individuals leads to personality changes and increased testosterone (Flegr, 2013; Flegr et al., 2011; Flegr et al., 2008). Retrospective studies show that the infected individuals are more likely to be involved in situations reminiscent of risk like traffic accidents (Flegr et al., 2002; Flegr et al., 2009; Yereli et al., 2006). These studies suggest that the infection is associated with risk taking, but their experimental design does not confirm the directionality of cause-and-effect. For example, it can be alternatively argued that individuals with greater risk taking propensity engage in dietary practices that later increase their possibility to become infected. Using a prospective design, the present study confirms that *Toxoplasma gondii* infection *causes* risky behaviors, and that the increase can be recapitulated by rise in testosterone post-infection. Given the high incidence of *Toxoplasma gondii* infection, this infection can possibly explain part of the variance observed between individual for risky behaviors.

We note that the concept of risk has been used in economic and biological literature in two divergent manners (Schonberg et al., 2011). The economic concept of risk centers on the greater variability of outcomes around the same central tendency. In contrast, biologists have often taken risk to reflect instrumental responses when reward must be balanced with probability of aversive outcomes like punishment or loss. In this report, we use the later articulation, showing greater tolerance of the infected or testosterone-treated male rats to risk

313 of reward forfeiture. Congruent to prior observations (Jentsch et al., 2010; Lejuez et al.,
314 2002), we also show that untreated animals are risk-averse and do not respond optimally as
315 an economically rational actor would in the face of the risk. Thus, effects described here
316 should be viewed as a reduction in risk aversion rather than an increase in risk seeking.

317 Several studies show that rats infected with *Toxoplasma gondii* lose their innate aversion to
318 cat odors (Berdoy et al., 2000); a phenotype that is believed to increase trophic transmission
319 of the parasite from rats to its definitive cat host (but also (Worth et al., 2013)). This
320 observation has often been presented as a specific and isolated behavioral change (Berdoy et
321 al., 1995a; Lamberton et al., 2008; Vyas et al., 2007a; Vyas et al., 2007b). Yet current
322 observations show that *Toxoplasma gondii* increases risk tolerance, in addition to increasing
323 approach to potentially risky cat odors. We posit that effects of infection represent a
324 behavioral syndrome represented by coordinated increase in risky behaviors rather than a
325 constrained reduction in kairomonal aversion only (Cézilly and Perrot- Minnot, 2010;
326 Thomas et al., 2010). Apropos, prior studies show that treatments that increase testosterone
327 also 'embolden' mice by increasing approach to predator odors (Kavaliers et al., 2001).
328 Moreover, effects of *Toxoplasma gondii* infection on aversion to cat odor can be rescued by
329 castration pre-infection (Lim et al., 2013), suggesting that effects of testosterone on risk
330 tolerance presented here are syndromically related to predator avoidance in this host-parasite
331 association. On the other hand, a more parsimonious account of observations reported here
332 will include the fact that we have used exogenous vehicle or testosterone supply in otherwise
333 castrated animals here. This binary comparison might not faithfully capture an incremental
334 change in testosterone concentration caused by *Toxoplasma gondii* infection.

335 Increased operant responding of the infected animals can be alternatively explained as
336 reflection of greater metabolic demands leading to greater motivation to seek food. Several
337 strands of evidence contradict this alternative. Control and *Toxoplasma gondii* infected

338 animals consume equal amount of food after twelve hours of food deprivation (Vyas et al.,
339 2007a). In addition the infection does not alter body weight gain (Thomas et al., 2010) and
340 urinary creatinine levels (Vasudevan et al., 2015), suggesting non-difference in anabolic and
341 catabolic processes. This is supported by continued investment of the infected rats in
342 energetically expensive behaviors like dominance and competition (Berdoy et al., 1995b).

343 Operant responding of untreated animals in this study remains below theoretical optima. This
344 suggests that effects of the infection or testosterone treatment reflect an increase in risk
345 tolerance; rather than instituting a risk preference over prior indifference. It should be noted
346 that BART uses operant responding as a proxy for decision making. The strength of this
347 proxy depends on construct validity of the BART, i.e. ability of operant responding in this
348 task to accurately reflect the construct of decision making under risk. This is supported by
349 positive association between BART responding and use of habit-forming substances,
350 gambling or unprotected sexual intercourse (Lejuez et al., 2002).

351 Testosterone is reported to have several cognitive effects. Subcutaneous supplementation of
352 testosterone in male rats result in reduced behavioral flexibility, manifested as a reduced
353 ability to shift from previously learned operant rules (Wallin and Wood, 2015). Similarly,
354 chronic testosterone increases instrumental responses in rats when greater rewards co-occur
355 with a greater risk of punishment (Cooper et al., 2014). These studies involve administration
356 of testosterone in gonad-intact male rats. Exogenous testosterone in these cases can result in
357 negative feedback on endogenous androgen production (Swerdloff et al., 2002), thus
358 potentially complicating interpretations. Human subject with greater testosterone levels take
359 greater financial risk in the laboratory (Apicella et al., 2014) and accumulate greater financial
360 payoff during risky transactions on the real-world trading floor (Coates et al., 2009; Coates
361 and Herbert, 2008). Adult men with greater endogenous testosterone take greater risk in the
362 Iowa gambling task (Stanton et al., 2011) and adolescent boys with greater endogenous

363 testosterone exhibit a greater tolerance to risk-taking in BART (Peper et al., 2013). Cause and
364 effect relationships in these human studies remain understudied because of methodological
365 constraints. In this backdrop, we use castrated animals without endogenous source of
366 testosterone, thus circumventing the possibility of interaction between exogenously supplied
367 testosterone and testicular steroidogenesis. Thus, we show that the rise in testosterone is
368 *casually* linked to a greater risk tolerance. The effects of testosterone in this study could be
369 either due to its direct interaction with androgen receptor or its aromatization and subsequent
370 interaction of resulting estrogen with its receptors (Kavaliers et al., 2012; Kavaliers et al.,
371 2008). Testosterone is also known to be metabolized to 5α-dihydrotestosterone, a potent
372 agonist of androgen receptors.

373 The data presented here suggest that *Toxoplasma gondii* infection results in a coordinated
374 increase in risk-taking behavior of the host. This provides us with a useful paradigm to better
375 understand biological changes mediating risky behaviors underpinning substance abuse,
376 pathological gambling, attention related disorders and high-risk behaviors (Winstanley,
377 2011).

Data Accessibility

All raw data will be made available in Dryad repository upon publication.

Competing Interests

We have no competing interests.

Authors' Contributions

DT designed and conceptualized experiments; conducted BART; conducted data collection and statistical analysis. AV took part in conceptualization; conducted statistical analysis and wrote the paper. All authors commented and gave final approval for publication.

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All authors declare that they have no conflict of interest or financial disclosures.

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Figure Legends

Figure 1. Procedure employed for testing risk aversion using balloon analog risk task. Adapted from Jentsch et al. (2010). Rats were trained on a stable baseline at 0% risk before introduction of a mixed-risk schedule (probe trials interspersed with 11.1% and/or 16.7% forfeiture probabilities) until stable performance.

Figure 2. Performance of control rats and *Toxoplasma gondii* infected rats in absence and presence of risk of forfeiture (**A**). Only gainful trials are included, excluding mistrials and trials that resulted in forfeiture. Both baseline (*black*) and probe trials (*red*) were identical, except that probe trials were interspersed with trials containing risk of reward forfeiture (*green* and *blue*) in a pseudorandom manner. The ordinate depicts the cumulative frequency (%) for the mean number of lever presses during gainful trials. Solid lines represent a Boltzmann sigmoidal fit of the data (minima and maxima constrained at 0% and 100%, respectively; fit characteristics in Table 1). N = 14 for control and 12 for infected groups. Presence of risk of reward forfeiture in interceding trials suppressed operant responding in control, but not infected, animals (**B**). ***, $p < 0.001$, paired Student's t-test with Bonferroni correction. **Inset:** Temporal sequence of the experiment.

Figure 3. Performance of castrated rats supplemented with vehicle or with testosterone, in absence and presence of risk of reward forfeiture (**A**). Only gainful trials are included, excluding mistrials and trials that resulted in forfeiture. The ordinate depicts the cumulative frequency (%) for the mean number of lever presses. Solid lines represent a Boltzmann sigmoidal fit of the data (minima and maxima constrained at 0% and 100%, respectively; fit characteristics in Table 2). N = 7 for vehicle and 9 for testosterone supplemented animals. Presence of risk of reward forfeiture in interceding trials suppressed operant responding in

549 vehicle-treated, but not testosterone-treated, animals (**B**). **, $p < 0.01$, paired Student's t-test
550 with Bonferroni correction. **Inset:** Temporal sequence of the experiment.

551 **Figure 4.** Performance during trials with 16.7% risk of forfeiture for control and *Toxoplasma*
552 *gondii* infected rats (**A**); and for castrates with vehicle or testosterone supplementation (**B**).
553 Only gainful trials are included, excluding mistrials and trials that resulted in forfeiture.
554 Panels in left depict mean actual yield of pellets experienced by individual subjects vis-à-vis
555 theoretical yield for 16.7% incremental risk as a function of lever presses. Ordinate in right
556 panels depict ratio of trials with optimal operant responding (3 or 4 lever presses) relative to
557 total number of gainful trials. Trials with 1 or 7 lever presses were omitted from this
558 calculation because these trials are characterized by certain outcomes. *, $p < 0.05$, **, $p <$
559 0.01, independent Student's t-test.

Table 1. Sigmoidal fit for cumulative frequencies of mean lever presses by control and infected animals.

	Slope	*V50*	R^2	*P (Kolmogorov-Smirnov test)*
Baseline				
Control	0.43	2.71	0.99	0.739
Infected	0.49	2.71	0.97	
Probe				
Control	0.32	1.94	0.99	<0.0001
Infected	0.51	2.33	0.99	
Risk = 11.1%				
Control	0.18	1.64	0.96	<0.0001
Infected	0.36	1.90	0.97	
Risk = 16.7%				
Control	0.18	1.55	0.98	0.979
Infected	0.19	1.80	0.98	

Boltzmann model; $Y = Bottom + ((Top-Bottom)/(1+ \exp^{(V50-X/Slope)}))$

Table 2. Sigmoidal fit for cumulative frequencies of mean lever presses by castrated and testosterone-supplemented animals.

	Slope	*V50*	R^2	*p (Kolmogorov-Smirnov test)*
Baseline				
Castrated	0.35	2.59	0.98	0.997
Supplemented	0.35	2.42	0.97	
Probe				
Castrated	0.23	1.96	0.91	<0.0001
Supplemented	0.45	2.19	0.98	
Risk = 16.7%				
Castrated	0.14	1.60	0.98	0.401
Supplemented	0.16	1.72	0.98	

Boltzmann model; $Y = Bottom + ((Top-Bottom)/(1+ \exp^{(V50-X/Slope)}))$

Figure 1

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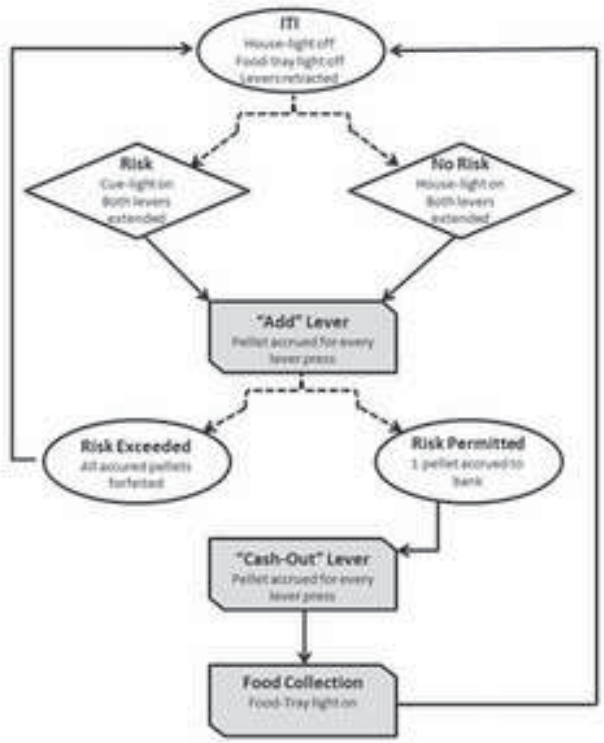

Figure 1

Figure 2

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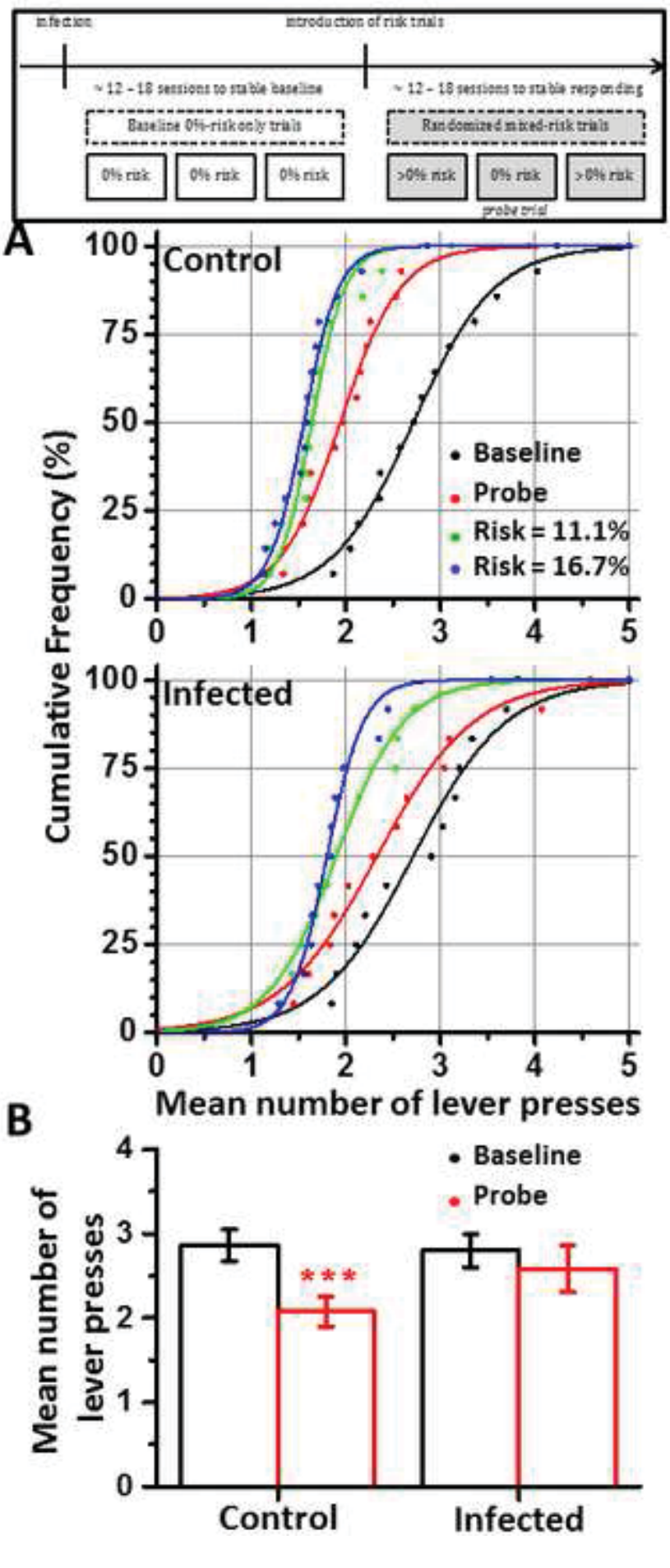

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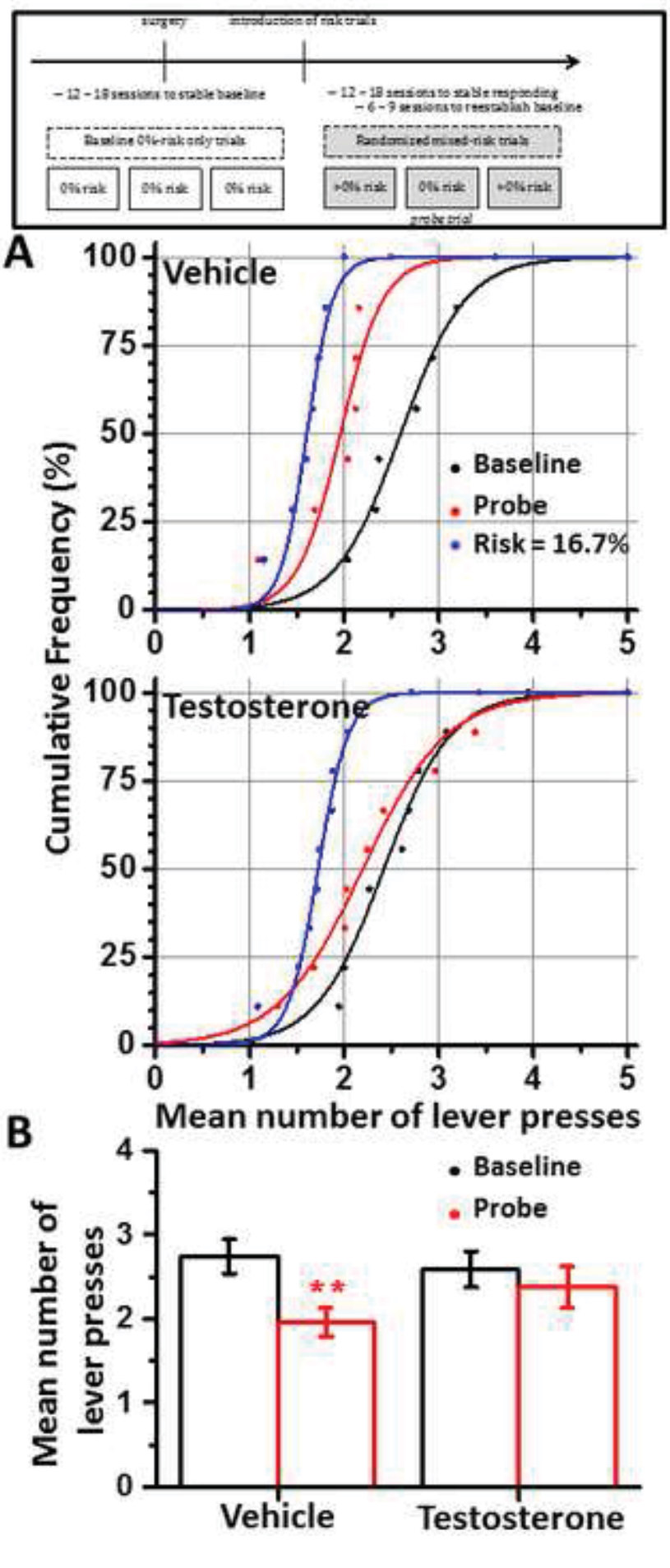

Figure 3

Figure 4

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