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Title Page

Infection of male rats with *Toxoplasma gondii* results in enhanced delay aversion and neural changes in the nucleus accumbens core

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Summary

Rats infected with the protozoan parasite *Toxoplasma gondii* exhibit reduced avoidance of predator odors. This behavioral change is likely to increase transmission of the parasite from rats to cats. Here we show that infection with *Toxoplasma gondii* increases the propensity of the infected rats to make more impulsive choices, manifested as delay aversion in an intertemporal choice task. Concomitantly, *Toxoplasma gondii* infection causes reduction in dopamine content and neuronal spine density of the nucleus accumbens core, but not of the nucleus accumbens shell. These results are consistent with role of the nucleus accumbens dopaminergic system in mediation of choice impulsivity and goal-directed behaviors. Our observations suggest that *Toxoplasma gondii* infection in rats causes a syndromic shift in related behavioral constructs of innate aversion and making foraging decisions.

Introduction

Toxoplasma gondii is a protozoan parasite of the rat (*Rattus novergicus*) and many other animals. This host-parasite association has been widely studied as an example of parasitic manipulation of host behavior. Rats infected with *Toxoplasma gondii* exhibit greater exploration of spaces containing cat odors [1-3]. This behavioral change is thought to increase parasite transmission because cats are the ultimate host of this parasite [4, 5].

Innate aversion to predators is a flexible behavior. Odors that are more predictive of immediate predator presence evoke a stronger aversion compared to partial cues like urine or feces [6]. Similarly, more concentrated cat odors elicit a larger fear response [6]. Apart from conditional dependence on predator cues, innate aversion is also in contiguity with other behaviors like searching for food. For example, laboratory ant colonies (*Lasius pallitarsis*) unequivocally choose feeding sites offering more concentrated sugar solutions. In the presence of predators, the preference for concentrated sugar is diminished; yet the devaluation for the richer feeding site becomes more blunted as the concentration of sugar solution offered is increased [7]. This and several similar observations, [8] suggest that foraging decisions and predator aversion are related behavioral constructs. Foraging decisions in the laboratory settings have often been framed in terms of an intertemporal choice between larger later and smaller sooner food receipts. In a delay discounting task where delays to larger later rewards are progressively varied while keeping intervals between successive trial initiations constant within a block, consistent choice of larger later reward represents an economically rational choice. In this setting, choice for smaller sooner rewards demonstrates an intolerance to delay and thus, is interpreted as an impulsive choice. In light of these observations, we investigated if *Toxoplasma gondii* infection led to more impulsive delay-averse foraging decisions in the infected rats.

The midbrain dopaminergic system is critical for evaluating salience of various options [9]. This system pivots around nucleus accumbens, which receives dense dopaminergic inputs from the ventral tegmental area. The nucleus accumbens interacts with limbic regions involved in emotional valence like the amygdala [10]; and also with frontal cortical regions involved in executive functions like the orbitofrontal, anterior cingulate, prelimbic and infralimbic cortices [11]. Nucleus accumbens also influence goal-directed behaviors through its projections to globus pallidum and hypothalamic nuclei. Consistent with this, selective excitotoxic lesions or quinolinic acid-induced lesions of nucleus accumbens core, but not shell, enhance impulsive choice in rats [12-14]. These observations suggest that the dopaminergic signaling in the nucleus accumbens acts as the interface between salience of various challenges and opportunities; and the resultant behavioral output. In view of its central role in choice impulsivity, we also investigated changes in neuronal morphology and dopamine content of nucleus accumbens.

Materials and Methods

Adult male Wistar rats were employed. All animal procedures were approved by NTU-IACUC. Animals were either infected with tachyzoites (5×10^6, *i.p.*) or mock-infected with sterile saline and tested >8 weeks post-infection.

Procedure to measure delay averseness was adopted from [15] (Figure 1). Animals were tested daily for six days per week (one session per day). Each session consisted of sixty choice trials executed at 100s interval, consisting of five delay blocks of ten trials each (delays = 0, 10, 20, 40 and 60 s). One of the lever delivered the smaller-sooner-reward (SSR; 1 pellet, immediate) and the alternative lever delivered the larger-later-reward (LLR; 4 pellets, after an appropriate delay). The number of LLR choice was used as a measure of impulsivity. Data presented depict an average of three day block. Subsequently, sucrose preference was measured by giving rats a choice between two bottles containing either tap water or 1% sucrose (test duration = 2h). The consumption was measured by weighing the bottles.

Dopamine and 5-HT levels were measured by HPLC in brain punched obtained from 500 µm thick sections. For spine density measurements, brains were processed for rapid Golgi staining [16, 17]. Spines on a continuous 80 µm of secondary dendrites were counted at 1000X magnification.

Analysis of variance (ANOVA) was used to estimate statistical significance of main effects and interactions. Spine density and neurotransmitter content within nucleus accumbens were analyzed using Mann-Whitney U test.

Results

Infection increased delay aversion.

Control (14 animals) and infected (12 animals) subjects were tested for their propensity to choose between SSR and LLR (Figure 1).

Figure 2A depicts the choice exhibited by the animals for LLR (% of total trials) over successive delays. Both control and infected animals preferred the larger reward in absence of delay (Figure 2A, 0s; one-sample t-test against chance of 50%; $p < 0.0001$; control: $|t_{13}| = 19.2$, infected: $|t_{11}| = 12.7$). Control and infected animals did not significantly differ in choice of the larger reward when the delay was set to zero (independent sample t-test; $|t_{24}| = 1.3$, $p > 0.2$). As the delays increased, animals progressively reduced their preference for the LLR (repeated measure ANOVA, Table 1). Control animals preferred LLR at all delays examined, except at 60s (Figure 2A; one-sample t-test against chance; $|t_{13}| \geq 4.19$, $p \leq 0.001$, Bonferroni correction applied post-hoc to correct alpha probabilities for multiple testing of five delays). In contrast, preference for LLR was statistically insignificant at all delays greater than 0s for infected animals ($|t_{11}| \leq 2.07$, $p \geq 0.31$). Between the two experimental groups, infected animals exhibited greater intolerance to the delay of rewards (ANOVA: Table 1; main effect of infection status: $p = 0.024$). Post-hoc analysis revealed statistical significant differences between control and infected at delays of 40 s and 60 s (Figure 2A; LSD: $p < 0.05$, Bonferroni correction applied to correct for multiple comparisons).

The sensitivity of rats to the delay in reward receipt is typically time inconsistent, in that devaluation rate of the reward is not constant across proximal and distal time delays [18]. To recapitulate this, we fitted the mean number of LLR choice obtained for each group at the various delays to a hyperbolic model using nonlinear regression (Figure 2A, solid lines; Table

2; V = D0/(1 + k*D), where D0 is preference for LLR at zero delay). Infected animals exhibited a steeper coefficient of discounting, suggesting a greater sensitivity of the reward value to the delay in its receipt (fit model and group parameters in Table 2). For the individual data, the rate of hyperbolic decay (*k*) was calculated. Only individuals with $R^2 > 0.8$ were included in the analysis. Distribution of parameter *k* was non-normal; hence a non-parametric test was used to compare experimental groups (Shapiro-Wilk test: $p < 0.001$). Infected subjects exhibited greater coefficient of discounting (Mann-Whitney U test; $|Z| = 2.05$, $p = 0.0093$; n = 8 control and 7 infected animals).

Approximately 50% of control animals did not reach a point of indifference even during the longest delay used in the experiment (Figure 2B). The mean choice of the control animals was above the point of indifference at all delays. In contrast, more of the infected animals reached the point of indifference at much shorter delays (Figure 2B; two sample Kolmogorov-Smirnov test: $|Z| = 2.33$, $p < 0.001$).

Consistent with the preference for SSR, infected animals earned fewer food pellets during the task (pellets earned during session, mean ± SEM: control 189 ± 7, infected = 158 ± 11; $|t_{24}| = 2.5$, $p < 0.05$). Despite being delay averse, the infected animals were less likely to engage in premature or persistent responding measured by the number of inter-trial interval nose-pokes (Figure 2C and Table 1). The latency to initiate rewarded trials through nose-poke did not differ significantly (Figure 2D and Table 1). Thus, the behavior of infected rats in this task was guided by intertemporal choice impulsivity without a probable contribution from generic or motor impulsivity.

Infection did not reduce sensitivity to the reward.

We further tested whether infection altered the sensitivity of the animal to reward (N = 14 animals for control and 12 animals for infected). When provided with water and 1% sucrose

simultaneously, the preference for sucrose was more pronounced in infected animals (Figure 3A; independent sample t-test: $|t_{24}| = 3.15$, $p = 0.004$). Infected animals consumed greater amount of sucrose (Figure 3B; $|t_{24}| = 3.43$, $p = 0.002$), whereas the total consumption was not significantly affected by the infection ($|t_{24}| = 1.66$, $p = 0.11$). Results for sucrose preference were in direct contrast with changes in intertemporal choice. Despite a greater preference for rewards, infected animals exhibited a reduced tendency to wait for larger rewards when delays were imposed. Control and infected animals gained comparable body weight during the experimental period ($|t_{26}| = 0.89$; $p > 0.3$, independent sample t-test).

Infection reduced spine density of the neurons in the nucleus accumbens core.

We quantified the number of spines over 80 µm segment for neurons of nucleus accumbens core (AcbC) and shell (AcbSh). The infection caused a marked reduction in the number of spines for AcbC neurons (Figure 4, left; Mann Whitney U test: $|Z| = 2.57$, $p = 0.01$; N = 6 control and 6 infected animals). In fact, the minimum observed value of spine density for the control group was still greater than in 5 out of 6 infected animals. Similarly, the maximum observed value of the infected group was observed to be below the median of the control animals. The spine density of AcbSh neurons did not significantly differ between control and infected animals (Figure 4, right; $|Z| = 0.16$, $p = 0.87$), suggesting that effects of the infection were specific to the core sub-region of the nucleus accumbens. Figure 4B depicts representative examples of AcbC dendrites.

In order to preclude a generalized change in the spines, we also quantified spine density in brain regions anatomically connected to the nucleus accumbens (basolateral amygdala, anterior cingulate cortex, orbitofrontal cortex and medial prefrontal cortex). Infection did not

significantly alter spine density in these brain regions (Mann-Whitney U test; $|Z| < 1.14$; $p > 0.15$).

Infection reduced dopamine content in the nucleus accumbens core.

We quantified the amount of dopamine and 5-HT in tissue micro-punches obtained from AcbC and AcbSh. Apart from causing changes in spine density measurements, the infection caused a statistically significant decrease in dopamine content of the AcbC (Figure 5, left; Mann Whitney U test: $|Z| = 2.07$, $p = 0.039$; N = 8 control and 6 infected animals; control = 9.82 ± 0.70 ng/mg, infected = 5.99 ± 1.59 ng/mg). The dopamine content of AcbSh did not significantly differ between control and infected animals (Figure 5, right; $|Z| = 0.52$, $p = 0.62$; control = 6.40 ± 0.95 ng/mg, infected = 4.69 ± 1.21 ng/mg). The infection did not cause statistically significant difference in 5-HT content of either AcbC (control = 3.29 ± 0.41 ng/mg, infected = 3.08 ± 0.57 ng/mg; $|Z| = 0.26$, $p = 0.80$) or AcbSh (control = 1.74 ± 0.18 ng/mg, infected = 1.45 ± 0.20 ng/mg; $|Z| = 0.78$, $p = 0.44$); with the exception of BLA (control = 1.72 ± 0.19 ng/mg, infected = 1.19 ± 0.14 ng/mg; $|Z| = 2$, $p = 0.0426$). AcbC dopamine content was not significantly correlated with discounting constant k or preference for LLR at zero delay ($p > 0.75$).

In order to preclude a generalized change, we also quantified dopamine content in brain regions that send dopaminergic projections to and from the nucleus accumbens (ventral tegmental area, basolateral amygdala, medial amygdala, ventral pallidum, anterior cingulate cortex, posterior cingulate cortex, caudate putamen dorsal, caudate putamen ventral and medial prefrontal cortex). Infection did not significantly alter dopamine content in these brain regions (Mann-Whitney U test; $|Z| > 1.16$; $p > 0.245$).

Discussion

Earlier work demonstrates that rats infected with *Toxoplasma gondii* lose their innate aversion to cat odors. In this report, we show that the infection with *Toxoplasma gondii* creates delay aversion in male rats by increasing steepness of the discounting for receipt of larger rewards at increasing delays. This is reflected as preference for smaller sooner rewards. Behavioral changes within an infected individual have often been viewed as a collection of independent phenotypes arising in isolation to each other. A contrarian view posits that multi-dimensional behavioral changes in the host reflect a syndrome arising because of inter-connected biological imperatives [19]. We propose that the host behavioral change after *Toxoplasma gondii* infection is not a monolithic reduction of the innate fear. Instead it comprises of a behavioral syndrome consisting of reduced innate fear, increased sexual attractiveness and greater delay aversion; all hallmarks of a "carpe diem" animal personality [20-22]. Biological imperatives that bind these behavioral changes remain presently unknown, although a plausible and untested speculation can be offered. Several studies cutting across phylogenetic boundaries show that a shortening of life-span results in greater "carpe diem" impulsivity [20, 22, 23]. However, metabolic investment resulting in current payoffs often exists in a tradeoff with future/residual payoffs [24-26]. We speculate that delay aversion and loss of innate fear are contiguous behavioral changes reflecting an expedited life-history for the host. This notion agrees with the observations that the infected host increases current metabolic investment in the form of androgen and sexual pheromone production [4, 27, 28]. Similarly, the presence of *Toxoplasma gondii* cysts in mice brain increases exploration of open and exposed regions of an arena, suggesting a change in perceived risk [29].

The concept of impulsivity has often been divided into motor impulsivity and choice impulsivity [30]. Motor impulsivity is typically characterized as a reduced ability to stop an

ongoing motor response or to withhold from making a new motor response. Choice impulsivity, on the other hand, refers to cognitive decisions made under risk/uncertainty or when delays to receipt are involved. Specifically, choice impulsivity manifests itself as a reduced tolerance for delayed gratification; characteristics similar to those exhibited by *Toxoplasma gondii* infected rats. Within the delay discounting task, infection reduced nose pokes during inter-trial interval when receipt of food was not possible. Nose-pokes during the inter-trial interval might reflect either a failure to inhibit premature responding or viewed as persistent action in absence of reinforcement. In contrast, the latency of nose-poke to initiate a trial remained unaffected. We suggest that the increase in impulsivity of *Toxoplasma gondii* infected rats is restricted or at least more pronounced in the domain of choice rather than motor phenotypes. This agrees with observations in human subjects, showing greater ability of the infected individual to inhibit a pre-potent motor response [31], though choice impulsivity in infected humans subjects have not yet been tested. As an important caveat, we have not explicitly tested for motor impulsivity in this report. It is possible that nose pokes during inter-trial interval may be influenced by choice in the preceding trial thus, might not be an independent measure of motor impulsivity. A split-sample analysis conducted on our dataset indeed demonstrated that number of nose pokes at zero delay and discounting of nose poke numbers across delays could be predicted from choice made in the preceding trial.

The infection did not diminish preference for food when delays were not involved, as demonstrated by the increased preference for sucrose post-infection. This suggests that *Toxoplasma gondii* infection did not alter overall appetite as measured by food intake (which could have explained a preference for the smaller reward outcome), nor did it bias the animals away from high-calorie food (as indicated by an increased preference for sucrose over water compared to controls). These observations agree with prior observations that animals infected with *Toxoplasma gondii* retain comparable body weights [2], have similar

food consumption after deprivation [2] and continue to perform energetically expensive behaviors [32] as compared to uninfected controls.

The mesolimbic dopamine system is involved in mediating impulsivity in delay discounting tasks [9]. This system pivots around the nucleus accumbens, receiving dopaminergic projections from the ventral tegmental area. In rats, bilateral excitotoxic lesions of AcbC increase delay aversion in discounting tasks; while lesions of AcbSh do not affect this behavior [12]. This is consistent with our observations that the delay aversion in the infected rats is accompanied by a reduced spine density in AcbC but not in AcbSh. A pharmacological decrease in dopaminergic transmission by receptor antagonism increases delay aversion in rats [33, 34]. This is consistent with our observations that the infection-induced increase in impulsivity is concomitant with a reduction in dopamine levels in the AcbC. Interestingly, effects of the infection on innate fear can be rescued by haloperidol, an inverse agonist of dopamine receptors [35].

Thus, reduced dopamine content and spine density of the nucleus accumbens agrees well with increased delay aversion post-infection. What remains an unresolved surprise is the fact the *Toxoplasma gondii* infection is previously suggested to *increase* dopamine, in contrast to the present report ([36], but also see [37]). For example, *in vitro* infection of mammalian dopaminergic cells by the parasite results in robust increase of dopamine synaptic release [36]. Indeed *Toxoplasma gondii* genome contains two amino acid hydroxylase genes that are surprisingly similar in sequence to mammalian tyrosine hydroxylase, a rate-limiting enzyme in dopamine synthetic pathway [38]. The protein product of these parasite genes has been demonstrated in infected mice brains, and parasitic cysts in mice brain exhibit robust immunoreactivity to dopamine antibodies [36]. It is unknown if the decrease in the nucleus accumbens dopamine reported by us is derived from the host or the parasite tyrosine hydroxylase.

Like other neurotransmitters, the effects of dopamine on the behavior are intricately dependent on the brain region. For example, administration of atomoxetine, resulting in increased dopamine in the prefrontal cortex but not the nucleus accumbens, decreases impulsivity in delay discounting task and 5-choice serial reaction time task [39]. Administration of amphetamine leads to more widespread dopaminergic stimulation, resulting in increased impulsivity in the 5-choice serial reaction time task [40] and decreased impulsivity in the delay discounting task [41]. Moreover, the effects of atomoxetine on 5-choice serial reaction time task can be reversed by selective dopamine antagonism in the AcbC [42]. These observations suggest that the site of dopamine change in the brain has significant effect on the behavior. We report a region-specific rather than a generalized alteration in dopamine content. It is presently unclear how a generalized supply of tyrosine hydroxylase like genes from *Toxoplasma gondii* can result in sub-region specific changes in dopamine concentrations [43]. This is pertinent because *Toxoplasma gondii* does not exhibit an exclusive tropism to nucleus accumbens or its sub-regions [44].

Toxoplasma gondii has earlier been reported to cause structural changes in neurons of host brain [45]. In the present report, we show that the infection reduces neuronal spine density in AcbC. It is plausible that the reduced spine density of AcbC neurons results in a decrease in inward synaptic current and resultant firing rates experienced by these neurons. This could potentially result in increased impulsivity through weaker dis-inhibition of efferent brain regions. This possibility remains currently unstudied. In both cases of dopamine and spine density, the infection induced effects remain more pronounced in AcbB compared to AcbSh. Mechanisms of such anatomically restricted changes remain presently unknown. It has earlier been suggested that Toxoplasma gondii preferentially concentrates in certain brain regions; and this tropism can explain behavioral changes post-infection through local manipulation of neuronal signaling and/or damage [2, 46, 47]. Two earliest studies in this regard reported a

rather wide-spread occurrence of tissues cysts in a variety of brain regions [2, 47]. Both of these reports suggested a mild tropism to nucleus accumbens, ventromedial hypothalamus or amygdala. Core and shell divisions of nucleus accumbens were not analyzed separately in these studies. More recent studies have failed to reveal any substantial tropism in any of these three brain structures in mice and rats, instead reporting a rather "probabilistic" spread of the parasites [29, 48]. Another potential source of region-specific effects could arise because of the selective innervation from vasopressinergic fibers, rather than tropism of the *Toxoplasma gondii* cysts themselves. Within the nucleus accumbens, most of the arginine vasopressin containing fibers terminate in the core rather than the shell [49]. Arginine vasopressin neurons in the medial amygdala have been previously shown to be preferentially activated by cat odor [27]; an atypical event because these neurons are typically activated during sexual signaling [50]. The atypical recruitment of arginine vasopressin neurons is mediated by an epigenetic event dependent on a parasite-induced increase in testosterone synthesis [51]. It is plausible - and yet unproven - that stronger post-infection vasopressinergic inputs coupled with tendency of these fibers to terminate in core but not in shell could lead to selective anatomical/neurochemical effects within nucleus accumbens.

The concurrent changes in choice impulsivity, AcbC spine density and AcbC dopamine point towards a concerted shift in the behavior of the infected rats. The concordance between these variables suggests a "conformity to *a priori* expectations based on purported function" [52]. In other words, it is unlikely that the increase in choice impulsivity is an accidental by-product of the infection, because it is accompanied by non-generalized changes in biological substrates known *a priori* to be involved in the behavior.

Finally, the data presented here provide an impetus to integrate parasitic changes in host behavior with trade-offs that are commonly pre-existing in life-history choices. In addition, these changes provide us with a useful paradigm to better understand neuropathology in

conditions characterized by increased impulsivity like substance abuse, gambling, attention related disorders and high-risk behaviors [41].

Data Accessibility

All raw data is available in Dryad repository (doi:10.5061/dryad.r33n9).

Journal editors and anonymous peer reviewers may view the submission for review purposes using the following url: http://dx.doi.org/10.5061/dryad.6s5vv

Competing Interests

We have no competing interests.

Authors' Contributions

DT designed and conceptualized experiments; conducted delay discounting experiment, parts of sucrose preference, dopamine quantification and spine density experiments; conducted data collection and statistical analysis and wrote the paper. LJTS conducted parts of delay discounting and spine density experiment. LWL conducted parts of sucrose preference and dopamine quantification experiments. TCWD conducted parts of dopamine quantification experiment. XZ conducted parts of dopamine quantification experiment. 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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Financial Disclosures

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

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Figure Legends

Figure 1. Procedure employed for quantifying delay aversion, depicting a single trial. A progressive delay protocol was used, whereby delay across trials within a block monotonically progressed from 0 to 60 s [15].

Figure 2. Infection induced impulsive choice by increasing delay aversion, without affecting motor impulsivity. **(A)** Control animals chose larger-later rewards (LLR) more frequently than infected animals. The ordinate depicts the number of choices made for LLR (mean ± SEM) for a series of sequentially larger delays (depicted in abscissa). Solid lines represent a hyperbolic discount curve fitted to the data. $V = D0/(1 + k*D)$, where D0 is preference for LLR at zero delay. *, $p < 0.05$, post-hoc test between control and infected, Bonferroni's correction for multiple tests applied. The dotted gray line parallel to abscissa depicts the point of indifference. **(B)** More of the infected animals reached the point of indifference at shorter delays to reward. The point of indifference is defined as the earliest delay when an animal chose a smaller-sooner reward (SSR) in five or more trials (out of ten). Animals that did not reach the point of indifference at the highest delay used (60s) were ascribed a value of >60s. Median is depicted by the dotted gray line. **(C)** Infected animals executed fewer redundant nose-pokes during the inter-trial interval, suggesting that the enhanced choice impulsivity is not a generalized phenomenon. *, $p < 0.05$, post-hoc test. **(D)** Latency to initiate rewarded trials through nose-poke was not different between control and infected animals. N = 14 for control and 12 for infected.

Figure 3. Infection increased sensitivity to rewards. **(A)** Infected animals exhibited greater preference for 1% sucrose reward, compared to water (%, relative to sucrose + water consumption). **, $p < 0.01$, independent t-test. **(B)** Infection increased sucrose consumption,

but total consumption remained unchanged. N = 14 animals for control and 12 animals for infected group.

Figure 4. Infection reduced spine density of the neurons in nucleus accumbens core (**A**), but not in shell. Bars depict medial and inter-quartile range. *$P < 0.05$ for comparison between control and infected animals. N = 6 animals for control and infected group. (**B)** Representative examples of AcbC dendrites.

Figure 5. Infection reduced dopamine content in nucleus accumbens core (*left*), but not in shell (*right*). Bars depict medial and inter-quartile range. *$P < 0.05$ for comparison between control and infected animals. N = 8 animals for control and 6 animals for infected group.

Table 1. Analysis of variance for infection status and delay aversion.

	df	*F*	*p*
Choice for larger later reward			
Infection status	1,24	5.79	0.024
Delay	4,96	38.92	< 0.0001
Interaction	4,96	2.98	0.023
Number of nose pokes			
Infection status	1,24	6.51	0.018
Delay	4,96	52.09	< 0.0001
Interaction	4,96	5.31	0.0007
Latency to nose-poke initiation			
Infection status	1,24	0.21	0.648
Delay	4,96	18.92	< 0.0001
Interaction	4,96	0.09	0.986

Between subject source of variance: control or infected; within-subject: delay = 0, 10, 20, 40 or 60s. n = 14 control and 12 infected animals

Table 2. Hyperbolic discounting model for control and infected groups.

Fit for means of two group at various delay

	k	*95% confidence interval of k*	R^2
*Hyperbolic discounting; V = D0/(1 + k*D), where D0 is preference for LLR at zero delay; df =4*			
Control	0.00909	0.00745 to 0.01072	0.973
Infected	0.02913	0.02498 to 0.03327	0.991

Delay = 0, 10, 20, 40 or 60s. n = 14 control and 12 infected animals.

Fit for individual animal performance at various delay

	D0	*k*	R^2
*Hyperbolic discounting; V = D0/(1 + k*D), where D0 is preference for LLR at zero delay; df =4*			
Control animals	9.292 ± 0.353	0.01630 ± 0.00432	0.829 to 0.965
Infected animals	9.047 ± 0.256	0.2976 ± 0.1933	0.919 to 0.999
Inter-group comparisons Mann-Whitney U test	$\|Z\|$ = 0.98, p = 0.3162	$\|Z\|$ = 2.05, p = 0.0093	

Delay = 0, 10, 20, 40 or 60s. n = 8 control and 7 infected animals. Only individuals with $R^2 >$ 0.8 were included in the analysis.

Figure 1

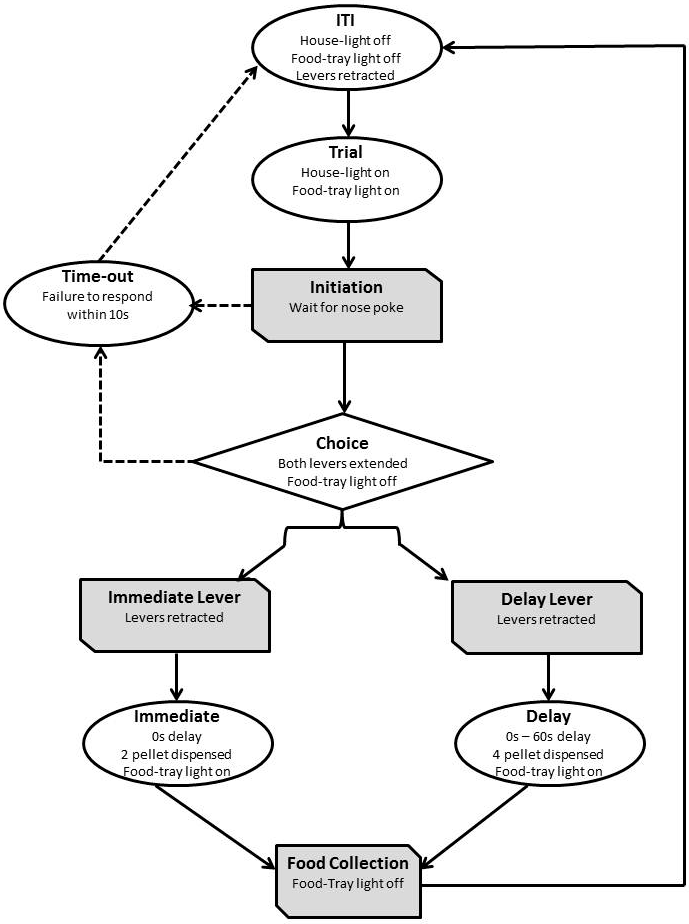

ITI
House-light off
Food-tray light off
Levers retracted
Trial
House-light on
Food-tray light on
Time-out
Failure to respond
within 10s
Initiation
Wait for nose poke
Choice
Both levers extended
Food-tray light off
Immediate Lever
Levers retracted
Delay Lever
Levers retracted
Immediate
0s delay
2 pellet dispensed
Food-tray light on
Delay
0s – 60s delay
4 pellet dispensed
Food-tray light on
Food Collection
Food-Tray light off

Figure 2

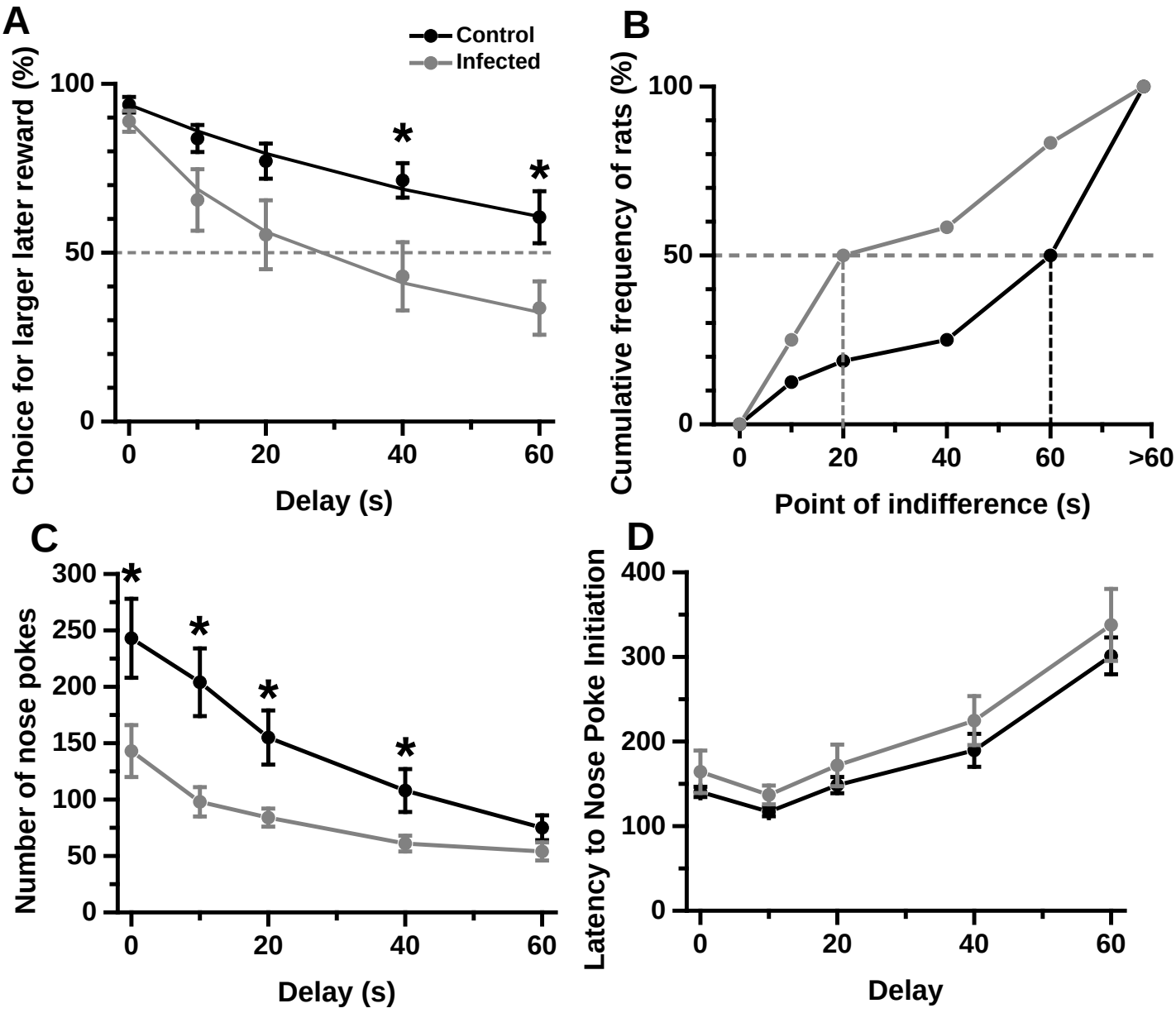

A
Control
Infected
Choice for larger later reward (%)
100
50
0
0
20
40
60
Delay (s)
*
*
B
Cumulative frequency of rats (%)
100
50
0
0
20
40
60
>60
Point of indifference (s)
C
Number of nose pokes
300
250
200
150
100
50
0
0
20
40
60
Delay (s)
*
*
*
*
D
Latency to Nose Poke Initiation
400
300
200
100
0
0
20
40
60
Delay

Figure 3

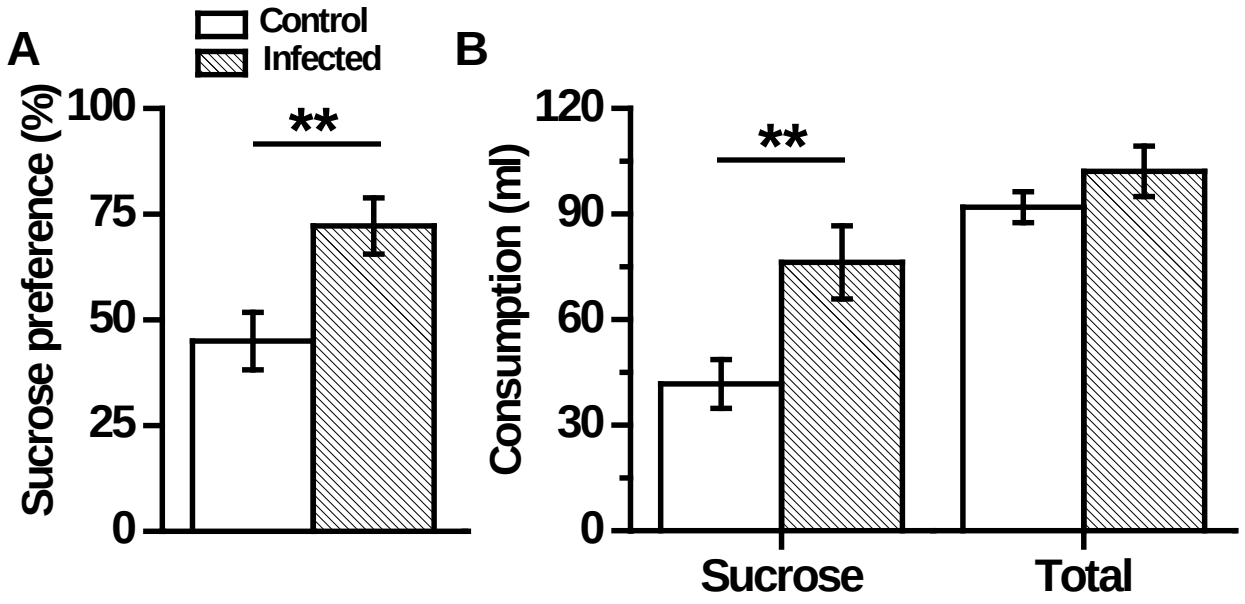

A
Control
Infected
Sucrose preference (%)
100
75
50
25
0
**
B
Consumption (ml)
120
90
60
30
0
**
Sucrose
Total

Figure 4

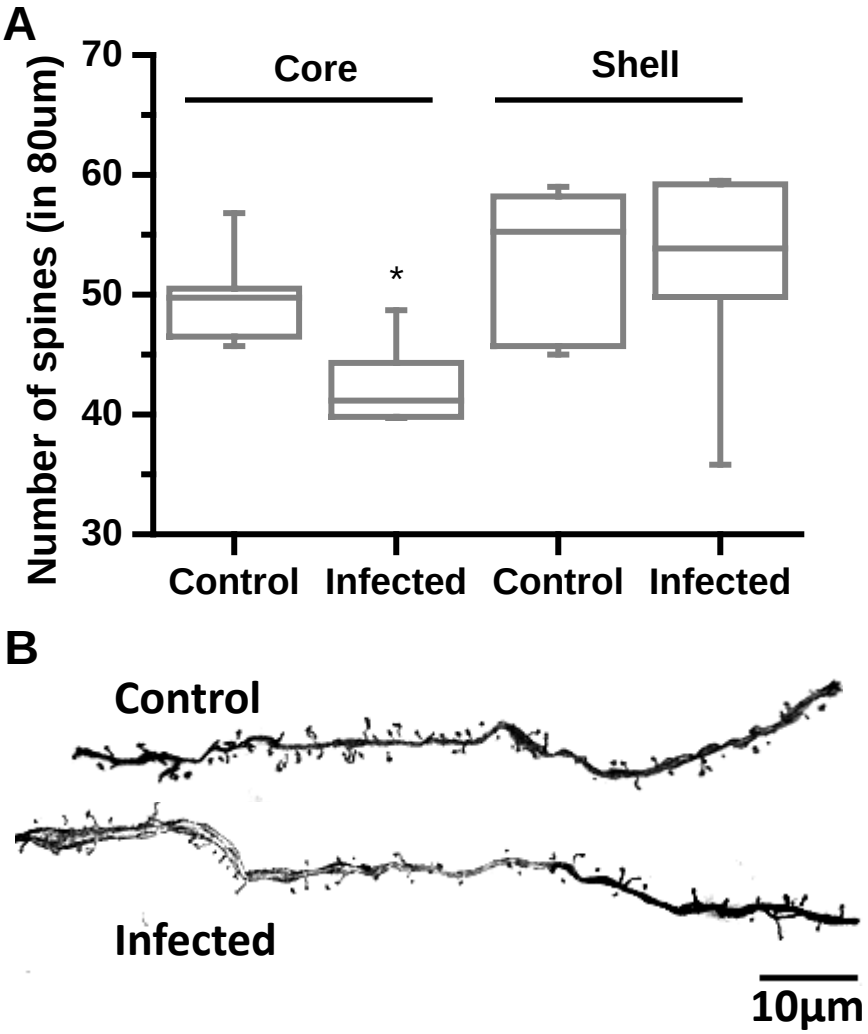
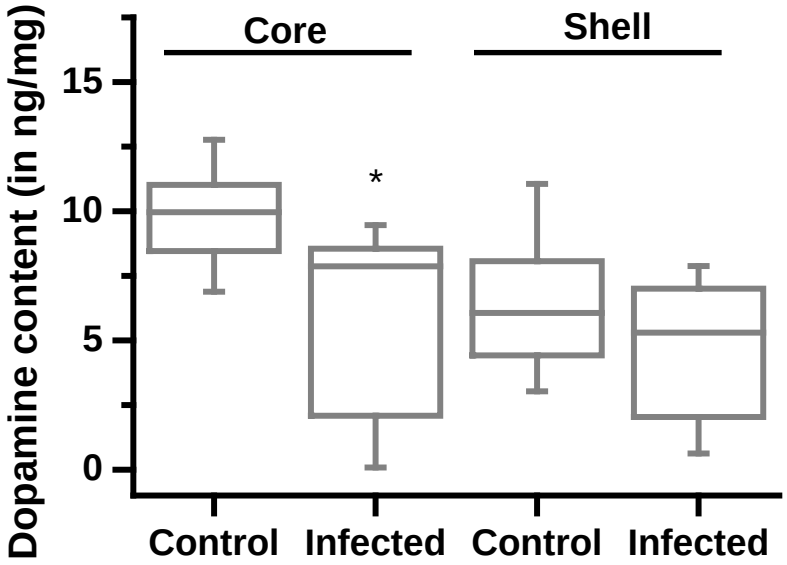

A
Number of spines (in 80um)
70
60
50
40
30
Core
Shell
*
Control
Infected
Control
Infected
B
Control
Infected
10µm

Figure 5


Core
Shell
Dopamine content (in ng/mg)
15
10
5
0
*
Control
Infected
Control
Infected

Supplementary Materials and Methods

Animals and Infection

Male Wistar rats were used (8 weeks old; housed 2/cage; 12 hours light-dark cycle, lights on at 7 AM; *ad libitum* food and water except during operant testing). During operant experiments, rats were maintained on a restricted diet at 80-85% of their free-feeding weight but were allowed to gain 3 – 5 g per week. In addition to the food rewards obtained during experiments, the rats were supplied with a portion of standard laboratory rat chow in their home cage within 1 h post-testing. Animals were obtained from the vivarium of National University of Singapore. All animal procedures were approved by Nanyang Technological University's institutional animal care and use committee. *Toxoplasma gondii* tachyzoites were maintained in human foreskin fibroblast cultures. Infected fibroblasts were syringe-lysed to release tachyzoites. Animals were either infected with tachyzoites (5×10^6, *i.p.*) or mock-infected with sterile phosphate buffered saline. Eight weeks elapsed between infection and start of behavioral experiment. The same set of animals was used for all experiments described below, except for a separate set of animals employed for measurement of spine density.

Delay aversion

Procedure to measure delay averseness was adopted from Evenden and Ryan, 1996 [1] (Figure 1). Operant chambers contained a houselight, internal stimulus lights, food-delivery magazine and two retractable lever positioned to the left and right of the magazine (30**x**24**x**30 cm; Med-Associates, St Albans VT). Chambers were enclosed in a sound-attenuating and ventilated outer cabinet. Operation of the pellet dispenser delivered 45 mg food pellets (formula 5TUM; TestDiets, Richmond, USA) into the food receptacle. Masking noise was provided by operation

of ventilating exhaust fans mounted on the outer cabinet (88 dB). The front panel of each operant chamber was equipped with two retractable stainless-steel response levers mounted 8.5 cm above the floor, and 7 cm off to either side of the centerline.

Initial phase of the training involved extension of one lever and delivery of one pellet for each lever press made by the subject. The procedure was repeated for the other lever. This phase of training continued until the animal completed >60 rewarded lever presses in 30 minutes for each lever. In the next phase of training, both levers were retracted before placing the animal in the operant box. Every 40 s, the start of a trial was cued by switching on of houselight and food magazine light. The subject was required to make a nose-poke within 10s, resulting in presentation of a single lever. A lever press within 10s of presentation resulted in immediate delivery of one food pellet. A failure to respond within 10s to trial initiation or lever presentation resulted in the abortion of that trial. When the rat had completed at least 60 successful nose-poke initiation trials in one hour, it was progressed the final stage of delayed discounting task.

In the final stage animals were tested daily for six days per week (one session per day, session length = 100 minute; repeated until inter-day variation in the performance became stable). Each session consisted of sixty choice trials executed at 100s interval and consisting of five delay blocks of twelve trials each (delays = 0, 10, 20, 40 and 60 s). Each block started with two forced trials in which only one lever was presented (one trial per lever, in random order), followed by ten free-choice trials. Each trial began with the illumination of the houselight and the food magazine light. The rat was required to make a nose-poke response, ensuring that it was centrally located at the start of the trial. If the rat did not respond within 10 s of the start of the trial, the operant chamber was reset to the intertrial state of total darkness until the next trial began and the trial was scored as a missed trial. Upon a successful nose-poke initiation, the food magazine light

was extinguished and levers were extended. One of the lever delivered the smaller-sooner-reward (SSR; 1 pellet, immediate) and the alternative lever delivered the larger-later-reward (LLR; 4 pellets, after an appropriate delay). Designation of lever with respect to reward magnitude was counterbalanced between cage-mates and kept constant for any particular test subject. Number of SSR and LLR choices made during ten free-choice trials for each delay was recorded and the number of LLR choice was used as a measure of impulsivity. Other trial parameters including latency to initial a trial as well as number of nose-pokes during the inter-trial intervals were also recorded. Data presented depict an average of three day block.

Preference to sucrose reward

Three days after termination of delay discounting task, animals were tested for sucrose preference. Food restriction continued during the intervening period. 24 hours prior to testing, rats were individually housed and introduced to two bottles containing tap water. Next day, rats were given a choice by presenting them with two bottles containing either tap water or 1% sucrose (test duration = 2h). Initial location of bottles was counterbalanced across animals and switched after 1h during the test. The consumption was measured by weighing the bottles.

Dopamine measurement

After decapitation, brains were rapidly frozen in slurry of isopentane + dry ice and subsequently stored at -80^{0}C. Tissue micro-punches were obtained using 10-gauge needles from 500 µm thick brain section. Harvested tissue fragments were weighed and homogenized in 0.1 N perchloric acid, centrifuged at 13200 rpm for 5 min at 4°C, and supernatants were filtered by Ultrafree-MC (0.1 µm) centrifugal devices (Millipore). Dopamine and 5-HTlevels were measured by HPLC using UltiMate® 3000 System with Coulochem III electrochemical detector (Thermo Fisher Scientific). The HPLC mobile phase consisted of 90 mM sodium phosphate monobasic

dihydrate, 50 mM citric acid, 2.1 mM 1-octanesulfonate monohydrate, 0.1 mM EDTA, and 12.5% acetonitrile (pH 3.0). Samples were separated on a MD-150 analytical column (3 mm x 15 cm, Thermo Fisher Scientific). The amount of dopamine and 5-HT was normalized to the weight of tissue.

Spine density measurement

Brains were quickly removed post-decapitation and processed for rapid Golgi staining as described before [2, 3].

Individual neurons were quantified at 1000X magnification (Olympus BX43 microscope, 100X objective). Spines were defined as all protrusions in direct continuity with the dendritic shaft, irrespective of their morphological characteristics. Dendrites directly originating from cell soma were classified as primary dendrites. The first branch emanating from a primary dendrite was defined as a secondary dendrite. All quantifications were restricted to secondary dendrites. Starting from the origin of the branch, and continuing away from the cell soma, spines were counted along an 80-μm stretch of the dendrite.

Statistics

Analysis of variance (ANOVA) was used to estimate statistical significance of main effects and interactions. Figures represent mean ± SEM. Spine density and neurotransmitter content within nucleus accumbens were analyzed using Mann-Whitney U test. Figures represent median and inter-quartile range. Number of animals is noted in figure legends.

References for supplementary materials and methods

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