# Toxoplasma gondii induced shift in decision making and impulsive behaviours in infected male rats (Rattus novergicus)

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NANYANG TECHNOLOGICAL UNIVERSITY

# *Toxoplasma gondii* induced shift in decision making and impulsive behaviours in infected male rats (*Rattus novergicus*)

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**SCHOOL OF BIOLOGICAL SCIENCES**

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A thesis submitted to the Nanyang Technological University in partial fulfilment of the requirement for the degree of Doctor of Philosophy

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# Abbreviations

| | |
|---|---|
| 5-HT | 5-hydroxytrytophan |
| 5CSRT | five-choice serial reaction time task |
| AAH | amino acid hydroxylase |
| ACC | anterior cingulate cortex |
| ADHD | attention deficit hyperactivity disorder |
| ANOVA | analysis of variance |
| BART | balloon analogous risk task |
| BIS-11 | 11-point Barratt Impulsiveness Scale |
| BLA | Basolateral amygdala |
| COMT | catechol-O-methyltransferase |
| CPP | conditioned place preference |
| DA | Dopamine |
| GABA | Gamma-aminobutyric acid |
| HR | High-Reward arm |
| ICSS | intracranial self-stimulation |
| LLR | larger-later reward |
| LR | Low-Reward arm |
| mPFC | medial prefrontal cortex |
| MSN | medium spiny neurons |
| NAc | nucleus accumbens |
| OFC | orbital prefrontal cortex |
| PET | positron emission tomography |
| PFC | prefrontal cortex |
| SNc | substantial nigra |
| SSR | smaller-sooner reward |
| SSRT | stop-signal reaction time |
| VP | ventral pallidum |
| VTA | ventral tegmental area |

# Abstract

Rats infected with *Toxoplasma gondii* lose their innate aversion to cat odours and instead develop an atypical attraction to cat urine. This phenomenon has been used widely as a model system for behavioural manipulation hypothesis. This hypothesis posits a naturally selected ability of the parasites to change host behaviour in ways that are beneficial for parasite transmission but detrimental to the host itself. The dominant narrative in this regard posits that *Toxoplasma gondii* changes fear response of the host, in order to increase parasite transmission to its definitive felid hosts. Yet, fear of predators is not a monolithic behavioural construct. It instead exists in a continuum with approach behaviours due to constant weighing of options required in an ambivalent and probabilistic environment. In this framework, I demonstrate that *Toxoplasma gondii* infection alters decision making in infected rats by instituting more impulsive, delay-averse and risk-seeking choices. In addition, I show concomitant changes in mesolimbic dopamine system that might underlie this behavioural shift. Finally, I extend these observations by linking observed behavioural changes to the endocrine environment akin to those observed during *Toxoplasma gondii* infection. These observations suggest that changes in host behaviour post-infection are part of a wider behavioural syndrome that targets negotiation of trade-offs between the current and residual fitness of animals.

# Chapter 1 Introduction

***

*'Choices are the hinges of destiny'*

— PYTHAGORAS

***

This thesis is arranged as a compilation of individual collections of results forming chapters that focus on the central theme of post-infection effects of *Toxoplasma gondii* on decision making. Chapter one provides a broad introduction to the fields of parasitism and decision making – topics central to this thesis; and follows with the specific aims of this thesis and approaches that were employed. Chapters two to four describe sets of experiments, which address each specific aim as detailed in Chapter one. They are meant to read as *results* chapters and will have their own introduction, methods and discussion. Chapter five concludes this thesis and reviews the overall findings. It also includes a review on the present research and the upcoming theme of multi-dimensionality that spans the topics of choice impulsivity and parasite manipulation.

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## Behavioural Manipulation and *Toxoplasma gondii*

Parasites can alter host phenotypes. Alterations of host morphology can result in snails (*Batillaria cumingi*) infected by trematode parasites (*Cercaria batillariae*) growing to abnormally large sizes. Such morphological change resulting in gigantism enables the parasite to increase host biomass, which allows an increase in asexual production of parasite larval stages [1, 2]. Parasitized ants can end up looking like berries when a nematode parasite (*Myrmeconema neotropicum*) turns the abdomen of its intermediate ant host bright red [3]. This

plausibly tricks the fruit-eating birds into consuming the ant and spreading the parasite through its droppings to other ants and insects. In some cases, changes in host physiology due to parasitism result in a reduction in host fertility. A relevant example is vitellogenesis and down-regulation of egg production in the mealworm beetle (*Tenebrio molitor*) after a tapeworm (*Hymenoleis diminuta*) infection [4]. The net result of this change is that host reduces its allocation to reproduction and instead reallocates resources and available nutrients to somatic maintenance for parasite usage [5]. Parasites can also cause changes in host behaviour. For example, mice infected with tapeworms (*Taenia crassiceps*) show inhibition of mating behaviour, taking more time to mount, intermit and ejaculate during intercourse [6]. Tapeworms require estradiol for its own reproduction and the increase of estradiol (a female reproduction hormone) in the infected host causes the observed feminization in male mice [6-8].

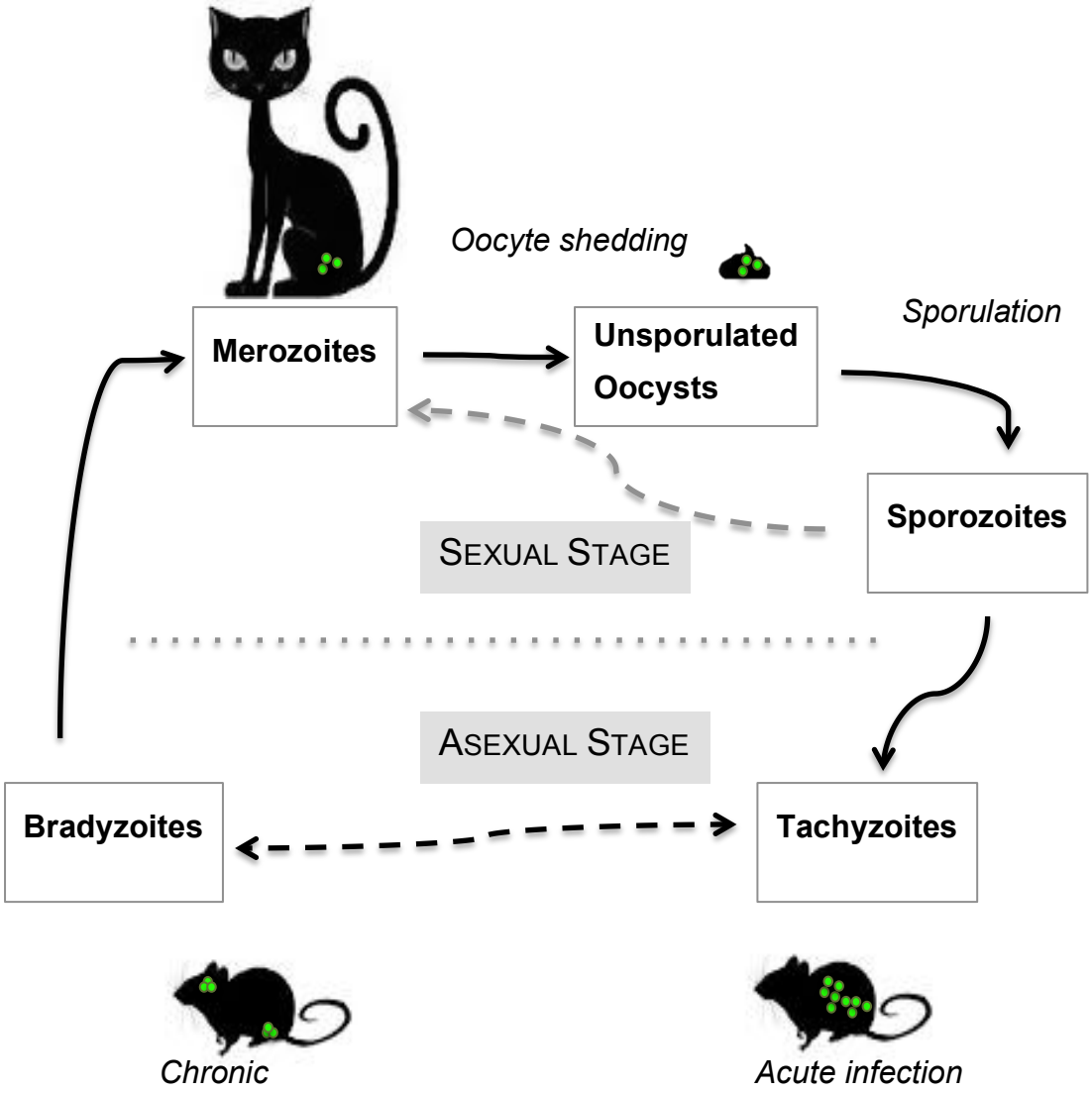
There are three alternative explanations for host behavioural change post parasitism [9-11]. First, it could be a direct and adaptive manipulation by the parasite. In other words, this view posits that prior natural selection has resulted in the current ability of the parasite to create an extended phenotype in the host [12]. Such change is likely to increase fitness of the parasite; and is often termed ‘behavioural manipulation’, in the strict sense. Second, change in host behaviour could reflect host adaptation. As the host tries to defend itself from the parasitism, it could take recourse to a variety of behavioural alterations that help clear itself of infection or to minimise the negative effects. For example, lethargy in an infected host could help conserve resources and re-route them to fight the infection. This is likely to increase host fitness. Third, change in host behaviour could be a fortuitous outcome that might help parasite transmission or could be a by-product of pathology caused by infection. For example, a parasitized fish might need greater energy in view of on-going immune assault and thus lead to more oxygenated surface water where predation of fish by parasite’s definitive host is more likely. In this view, change in behaviour does not reflect a historical natural selection.

An observer typically does not have an experimental overview of the historical natural selection. Hence it becomes difficult to differentiate a ‘manipulation’ of host behaviour vis-à-vis other possibilities like adaptive host response and by-

product/fortuitous outcome. A few criteria have been formalized to experimentally make this distinction. Apart from enhanced fitness of the parasite, these include 1) complexity; 2) purposiveness; and, 3) convergence [9, 10].

Based on the framework of this thesis, I would like to articulate these criteria using *Toxoplasma gondii* – *Rattus novergicus* association (see Box 1 for details for life cycle of this parasite). In short, *Toxoplasma gondii* is a widely prevalent protozoan parasite of rats and humans [13-15]. While asexual stages of its life cycle can be sustained in a variety of warm-blooded organisms, it can undergo sexual reproduction only in the intestines of a cat. Cats are infected by Toxoplasma when they eat infected prey. The parasite undergoes gametogenesis in cells of the cat intestines, resulting in the eventual shedding of faecal oocysts that are ingested by intermediate hosts. These events are important for the parasite because they permit a) sexual recombination; b) infection of herbivore hosts who are unlikely to be infected through carnivory; and, c) big inoculum of cysts that are recalcitrant to environmental factors. Yet, a major limitation in this scheme is entry of the parasite in the cat itself. Preys of cats avoid cats and cat odours [16]. Apropos, *Toxoplasma gondii* infection leads to reduced aversion of rodents to cat odours [17]. A subset of animals also develops an atypical and 'fatal' attraction. These behavioural observations suggest, but do not prove, that the parasite creates an extended phenotype in the host behaviour. The qualification in the preceding sentence is necessary because it is yet unknown if infected rodents are indeed predated more frequently by cats. In this setting, it is interesting to ask if reduced aversion to predator odour in *Toxoplasma gondii* infected rats represents a behavioural manipulation as described in the previous paragraph.

**Box 1 The complex life cycle of *Toxoplasma gondii***



**Box 1 The complex life cycle of *Toxoplasma gondii*.**

*Toxoplasma gondii* is a protozoan in the tissue cyst-forming coccidian subclass belonging to the phylum Apicomplexa. It is an obligate intracellular parasite which means it cannot reproduce outside of host cells and is dependent on the host's intracellular resources. Coccidian parasites generally infect intestinal tracts and have a two-stage life cycle comprising of an asexual and sexual stage. In *Toxoplasma gondii*, both cycles occur in different hosts. Felid host which excretes the oocyst is the definitive host whereas other non-feline hosts in which the asexual stage occurs are the intermediate hosts.

Merogony refers to replication of merozoites that takes place in the intestines of the cat where gametes are formed. The fusion and fertilization of gametes occurs, with resultant unsporulated oocysts being shed in the faecal material. Sporulation occurs after exposure to favourable environmental conditions resulting in the formation of sporozoites. Sporozoites can either infect cats or other animals when the environmentally resistant sporulated oocysts are ingested.

In intermediate hosts, asexual replication occurs, forming fast-replicating tachyzoites during the acute infection stage. Chronic infection occurs when bradyzoites, a slow-replicating form, are formed within tissue cysts. When the intermediate hosts are predated by the cat, and tissue cysts are ingested, the life-cycle of *Toxoplasma gondii* is complete [18-20].

There are at least 15 known lineages, classified into 6 main groups [21] of *Toxoplasma gondii* – of which three main genetic strains types I, II and III [22] make up the majority of human infections in the northern hemisphere. In laboratory conditions, type I strains are lethal to rodents, type II has an intermediate virulence and type III is avirulent [23]. Type II is the most widely used strain in experimental studies as well as the most common strain of infection in the United States and Europe.

A complex behavioural change requires an organizing principle and is less likely to be an accidental effect. For example, if *Toxoplasma gondii* infection merely led to blockade of nasal passage resulting in a generalized loss of response to all odours, the behavioural effects post-parasitism will be less inspiring. Yet, *Toxoplasma gondii* infection does not result in a generalized olfactory change. Instead, the parasitism leads to a very specific recruitment of arginine vasopressin neurons in the medial amygdala, neuronal populations that are typically dedicated to sexual pheromones in uninfected animals [24, 25]. This atypical recruitment then likely results in a shift of behaviour away from avoidance and towards approach. The complex cross wiring described above is less likely to arise merely as a side effect, by-product or host sickness. This mechanism also demonstrates purposiveness, another characteristic of behavioural manipulation. A gentle shift in behaviour towards approach is 'too well fitted' to its purpose to arrive by chance. In other words, there is conformity between the observed change and 'a priori design specification that an engineer might use' [9]. Convergence, that is repeated and an independent establishment of the trait in disparate phylogenies, is less clear in the present example. Host behaviours that increase predation are common in several parasites with complex life cycle requiring trophic transit between two hosts [26-28]. Yet, convergence at the level of mechanisms is not yet well supported. Similarly, it has been notoriously difficult to show that host behavioural change demonstrably results in greater reproductive fitness for the parasites. Thus, to summarize, *Toxoplasma gondii* blocks the innate aversion of infected rats to cat odours. This change in behavioural manipulation appears to be a specific manipulation exerted by the parasite on host fear response.

However, fear response is not a binary response but is a graduated reaction norm titrated against the strength of the cue. Innate aversion to predators, therefore, is a flexible behaviour. As a starting example, rats react more strongly to predator cues that are more predictive of cat presence like cat fur or cat collars, rather than partial cues like urine or faecal material [29]. In the few paragraphs that follow, I discuss the broader context of the fear response and how it relates to other host behaviours.

## Fear is an adaptive and dynamic response to environment

Predator fear is not an inflexible behaviour. If animals were maximally defended, the prey will constantly hide, seeking perpetual safety from its predator, and in the process forfeiting foraging and mate-seeking. Such maximal defence is not an optimal solution because benefits of safety do not always outweigh opportunity costs of lost payoffs. It is therefore important to balance safety from predators with searching for food (and mates) in order to maximise fitness.

Animals will tend to avoid an environment where predators are present [30, 31]. Yet they are more willing to risk predator encounter if the habitat is rich in resources. As an example, laboratory ant colonies (*Lasius pallitarsis*) unequivocally choose feeding sites that offer more concentrated sugar solutions [32]. The same colony faces a trade-off when a better feeding site also contains a predator with non-zero and non-certain probability of attack (*Formica subnuda*). In this situation, effects of the predator on foraging can be viewed as an archetypical continuum. At one extreme, the ant colony can enhance current foraging effort with immediate reproductive pay-off, but resulting in lower life span and reduced residual fitness (‘me-now’). At the other extreme, the foraging prey can accept a mediocre foraging payoff of a lower quality feeding site in return for the enhanced probability of survival from predation and future reproductive gains (‘me-later’). Consistent with this continuum, *Lasius pallitarsis* reduces its preference for concentrated sugar when the predator is present [32]. Moreover, reduction in the choice for a better feeding site becomes more blunted as sugar solution offered becomes more concentrated. Similar trade-offs between foraging efficiency and predation risk have been observed in voles, grey squirrels, deer mice, kangaroo rats and many other animals (reviewed in [33]).

Other studies have also shown that animals alter their anti-predator response in various situations depending on cues that predict predator encounters. Factors include direct or indirect cues; interval between predator exposures; type of predator; and even the current state of the animal itself. Desert rodents change their avoidance response depending on either direct predation risk by the

presence of barn owls or indirect predation risk during simulated full moon illumination [34]. Gerbils respond according to the rate of perceived predation by owls or snakes [35]. Grasshoppers adjust their avoidance depending on the risk of aerial or ground predation by birds or lizards [36]. Salmon vary their avoidance response depending on their hunger state [37]. Thus, it is clear that animals balance their defensive behaviours according to predation threat and internal milieu.

Animals also calibrate reproductive efforts in accordance with predator avoidance. Spiders reduce oviposition and egg-laying in response to predator cues [38]. In fish, reproductive investment is similarly altered with predation risk [39]. Interestingly, animals have evolved strategies to compensate for anti-predator behavioural responses. This is especially apparent in female mate-choice during increased predation risk. Females mating with fitter males (likely to advertise more) are at increased risk if predators are drawn to the more conspicuous males during matings. As such, females might choose less preferred males when under high predation risk [40-43].

These examples show that fear, or anti-predator, behaviour in animals is a titrated response and not merely all-or-nothing behaviour. Within a landscape of fear, animals need to decide whether to forego opportunities for safety or approach rewards while simultaneously risking predation. At this juncture, I have summarized 1) change in host fear response after *Toxoplasma gondii* infection; and, 2) the idea that fear is a flexible behaviour that is best described as an approach-avoidance continuum. I will now turn my focus to decision-making and its relationship with the approach-avoidance continuum.

## Choice / Decisions

In the previous section, I summarized fear as a condition-dependent and flexible behaviour. The same idea can also be articulated in terms of choices or decisions. This articulation is poignantly captured in two axioms of the protagonist in Gardner 's wonderful fiction 'Grendel': namely 'everything fades'; and, 'alternatives exclude' [44]. For each morsel of food regurgitated by parents for the brood, there is one less morsel available to the parents for their survival. For each decision to create brighter plumage, there is a risk that predators will also be attracted by this sexual advertisement. In short, current fitness cannot be maximized without forgoing future fitness and survival. Alternatives of 'me-now' and 'me-later' exclude each other. Animals typically negotiate these trade-offs using conditional behaviours, or 'if-then-else' clauses. Flexibility of fear can thus be viewed as part of this broader ecological constraint on animals that result in condition-dependence of the behaviour. Put simply, fear is conceptually part of a whole suite of choice/decision behaviours whereby animals attempt to optimize their behaviour in accordance with incipient ecological conditions. I use decision-making and choice interchangeably because a decision is the same as 'choosing' one option over several others (Latin dēcīdere: to cut off, as in cut off alternative options). I would also note that I do not use decision/choice to mean a *conscious* process. I merely use these constructs to mean condition-dependent manifestation of alternative behaviours.

Ecological decisions by the animal in the wild can be categorised under three broad classes: decisions about options that differ in 1) effort; 2) delay of the receipt; and, 3) risk involved. A few pertinent examples follow. Western gulls (*Larus occidentalis*) and Western crows (*Corvus caurinus*) feed on shelled prey [45]. They pick up whelk, fly with a vertical ascent and drop the shell. The larger the molluscs, the higher the bird needs to ascend. Clark's nutcrackers (*Nucifraga columbiana*) and scrub jays (*Aphelocoma californica*) face a delay in consuming food if they decide to cache it [46, 47]. Juncos (*Junco phaeonutus*) take more risk between choosing certain or variable food options during foraging depending on energy budgets and expenditures [48].

Decision making is central to almost every aspect of human activity. It is therefore not surprising that interest in decision or choice spans an impressive array of distinct disciplines, including economics, psychology, game theory, medicine, law, addiction, evolutionary biology, political science and accounting, to name a few [49-51]. I will now attempt to provide an overview on the broader scope of decision making before focusing on my intended approach.

Economic theories are useful in providing an initial understanding of how decisions are made. They provide normative standards, which allow a comparison of decisions or comparison of actions to standards. These standards, in turn, allow evaluation of the decision to arrive at the 'best' or economically rational choice. I will briefly discuss risk-based decisions as an exemplar of economic orientation. In economics, risk is defined as greater *dispersion* around same central tendency; hence an alternative option of 9 and 11 at 50% probability is less risky than an option between 5 and 15 at 50% probability. Expected value theory suggests that decisions are made based on choosing the possibility that maximises returns (value x probability) [52]. Thus, in the example of options above, choice of an economically rational subject would be indifferent regardless of choice between 9 and 11 or 5 and 15. Yet, the decision in this case frequently departs from the theoretical expectation of the indifference. Failure of this theory to explain subjectivity in value-derivation gives rise to 'expected utility theory' where desirability of returns influences decisions by devaluating or adding value to one alternative [53]. In other words, expected utility adds a decision weight function, which accounts for preference or differential in risk-appetites. Choices are made based on interactions between subjective values (utility) - where decision makers place a higher 'arbitrary' value on a choice – and objective values; this introduces the terms 'risk-seeking' and 'risk-averse' [54].

Thus economics has traditionally treated decisions as an exercise in rational optimization. Neuroscience addresses decision-making processes from the perspective of proximate mechanisms that are under historical selection pressure; are constrained by phylogenies or co-option; and are thus not necessarily rational/optimum in a strict economic sense [55]. A detailed review of mechanisms is provided in the next section. The unifying goal across these disciplines is to understand the decision-making process by providing some

standard by which decision are made; and by taking into account the desirability of outcomes.

Before moving to a discussion of biological substrates of decision making, I think it is important to expand on the concept of 'impulsivity', a concept that is routinely used in the neurobiology of decisions and choice [56].

Let's start with an economic concept of 'value'. Since value is a subjective concept, an economist might infer it indirectly by studying its discount or rate at which value drops when it is delayed or needs more effort or becomes subject to probability of forfeiture. By studying the degree of discounting to the utility of a gain/loss as its discounter (time, effort or risk) varies systematically, an observer can extrapolate or at least compare the value of a desired outcome. I will attempt to make this concept more palpable by using delay discounting as an exemplar. What is the 'value' of two pellets of food for a rat? The value depends on subjective 'utility' of these two pellets for the individual rat, and thus must be inferred indirectly through its behaviour [57]. If rat is given a choice between receipt of x pellets 'now' or x+2 pellets 'later'; value of 2 pellets will be discounted due to delay in receipt. If the delay is systematically varied, discount to the value will also vary in a mathematically coherent way. For example, a constant rate of discount will result in exponential decay for choice of larger later receipt with the coefficient of decay used to infer the value [58-61]. Yet rats, and a multitude of other animals, show time-inconsistent discount in such experiments [62-64]. Observed discount rates for animals typically become larger as delays become longer, resulting in a hyperbolic rather than an exponential curve. Moreover, the same delay can cause different discounts depending on the framing or time horizon. Thus, the choice for two pellets now or four pellets after 1 minute is decided differently than the choice for two pellets after 60 minutes and four pellets after 61 minutes. This is despite the indifference between two scenarios from a purely economic viewpoint – in both cases, the discount should be related to the 1-minute time difference in the receipt of two available options. This is, thus, referred as 'impulsive' behaviour.

'Delay discounting' is a term commonly used to describe the inter-temporal preference for the x pellets 'now' over the x+2 pellet 'later' [65]. Economists, on

the other hand, are more specific and distinguish between ‘time discounting’ and ‘time preference’ [66]. ‘Time discounting’ is a broader term to explain placing less value or caring less about a later outcome which includes factors that diminish the expected utility such as uncertainty or changing tastes due to the delay. ‘Time preference’ is used specifically for the preference for immediate utility over delayed utility, in what a neurobiologist would refer to as ‘delay discounting’. In this thesis, I use the term ‘delay discounting’. In general terms, impulsivity is used to refer to choice or decisions that do not maximize net returns or minimize net losses over a long time frame.

The construct of choice impulsivity has been used with slight distinction in the fields of behavioural economics and psychology. The term ‘choice’ in economics refers to purchasing decisions made by a consumer with a finite and constrained budget, which in turn reflects the ‘revealed preference’ for the underlying utilities [67]. In psychology, choice merely reflects choosing between two disparate payoffs associated with different costs pertaining to time. Choice for a smaller-sooner reward refers to in this case to an impulsive choice. This framework has been widely used in psychological research dealing with socioeconomic status, personality and ageing. In the present thesis, I use the construct of choice as it refers to its prior and established use in psychological literature [56]. The same framework has also been used in neurobiological research pertaining to decision making.

Impulsive choices are widespread in animals including humans. This tendency seems economically irrational at first look and yet it likely reflects naturally selected constraints on the decision making. Because current and residual fitness are typically in conflict with each other, they cannot be maximized simultaneously. Thus the horizon of the future is often limited and clouded by investment in the present. A ‘four-pellets-over-two-pellets’ decision in the distant future is resolved differently than the same decision with an immediate outcome. That is because non-clarity of the future imposes a greater cost on delayed gratification. The paradigm of delay discounting has also been widely used in economics to model discounted utility [66]. This model attempts to integrate varied social and psychological motives or influencers into a single parameter of

discounting rate, akin to *k* used in the result section of chapter 3 (see page 66 Table 3-2 Hyperbolic-discounting model for control and infected groups.

From a clinical perspective, impulsivity is an important concept. In his work on personality traits, Eysenck describes a three-dimensional model of which one primary measure is impulsivity [68, 69]. High levels of impulsivity are implicated in a number of clinical psychiatric disorders including attention deficit hyperactivity disorder (ADHD), bipolar disorder, mania, pathological gambling, personality disorders, eating disorders and substance abuse. For example, ADHD patients show increased impulsivity (reviews by [70, 71]) being less able to inhibit impulsive responses in the stop-signal reaction time (SSRT) task [72] as well as demonstrating increased delay-aversion in the delay-discounting task [73-76]. Substance abuse users also show a preference for the immediacy, discounting future yet larger rewards in the delay-discounting task [77-80]. Thus, understanding the biological mechanism underpinning impulsivity has important implications for better understanding psychiatric disorders and abnormality in neural circuits involved.

# Neurobiology of decision-making

My thesis focuses on impulsivity, which is a subset of reward-based decision making. In this section, I review key factors affecting such goal-directed decision making with an emphasis on choice impulsivity. Various parts of the brain are important in specific aspects of decision making. This includes mesolimbic dopaminergic system that spans area of the frontal lobes, nucleus accumbens (NAc), ventral tegmental area (VTA) and nodes of limbic system like the hippocampus and amygdala. Dopamine (DA) is the key modulator within this circuit.

### *Role of dopamine in reward and goal-directed behaviour*

Dopamine (DA) is a major neurotransmitter within the reward pathway in the brain and is involved in movement, emotions, rewards and motivation. Many studies have focused on the role of DA in substance abuse and addiction; reward incentive learning; motivation and goal-directed behaviour and also in decision making [81-83]. Substance of abuse act via their action of triggering dopamine increase in the brain, especially in the NAc [84]. In humans, stimulant drug use reduced the availability of the DA receptor when measured by positron emission tomography (PET), implying increased DA release and binding to its receptors [85].

DA neurons are crucial in the reward system that forms the basis of reinforcement learning. This is supported by the increase in firing rate of DA neurons when rewards are uncertain [86]. Phasic DA firing also encodes information about reward and prediction error [87]. This suggests that DA neurons respond to reward cues rather than the actual receipt. Separately, it was also shown that dopaminergic signals in the NAc are dynamically regulated in response to cues and rewards. In the absence of a cue, dopamine release corresponds to reward delivery and when the reward-predictive cue is present, dopamine release shifts to the predictor instead [88]. In another study where DA was depleted by 6-OHDA lesions within the NAc, DA-depleted rats showed a specific difference in motivation to seek food while general ability to distinguish between novel food remained unchanged [81]. Thus DA parses rewards into its

components of motivation/incentive salience (‘want’), incentive learning and emotional affect (‘like’) rather than affecting sensory perception itself [81, 89]. The incentive salience of DA forms the core of goal-directed behaviour. In intracranial self-stimulation (ICSS) studies, rats will press a lever that delivers current to stimulate the DA reward pathways and administering the DA receptor antagonist blocks ICSS. These examples support the role of DA in motivation and in reward-related incentive learning [81-83, 90] and provide evidence for a role of dopamine in reward.

**Box 2 The dopamine systems.**

Dopamine (DA) is a monoamine member of the catecholamine family of neurotransmitters (see [91] for a review). There are two main groups of dopaminergic neurons in the central nervous system, comprising of main cell bodies in substantial nigra (SNc) and ventral tegmental area (VTA), respectively. Dopaminergic projections from the SNc to the rest of the brain make up the nigrostriatal pathway that is involved in motor functions. Projections from the VTA to frontal parts of the brain make up the mesocortical pathway. The VTA also provides efferent connections to limbic and medial-frontal brain areas via the nucleus accumbens. This is known as the mesolimbic pathway. It plays an important role in rewards and motivation [92-94]. See Figure 1-1, red line for a schematic on the mesolimbic pathway. The dopaminergic system has been implicated in predictive reward signalling, motivational salience and reinforcement learning [86, 89, 95] – all intrinsically linked to reward. Mesolimbic dopaminergic system exists at the interface of action selection and rewards. This intersection asserts the role of dopamine in facilitating reward-related effort [96], and in goal-directed behaviour [90, 97].

There are at least 5 sub-types of DA receptors classified into two main classes – D1-like and D2-like [98]. The effect of DA on D1 and D2-receptors affect NAc differently: D1 stimulation provides an excitatory glutametergic drive whereas D2 activation inhibits NAc neurons [99]. Antagonistic studies on the receptor types confirm these observations: D1 antagonists decrease cell excitability; D2 antagonists increase neuronal firing [100].

### *Cortical-striatal-limbic network*

The nucleus accumbens (NAc) is a key structure in the cortico-striatal-limbic network that is involved in impulsive decision making [101]. The NAc has reciprocal connectivity with multitude of nodes in the mesolimbic dopaminergic system; congruent with its important influence on decisions/choice [102]. Positioned at the interface between the cortical/limbic regions, it receives convergent inputs from a variety of brain regions [103, 104], including the prefrontal cortex (PFC) and limbic structures such as the hippocampus and amygdala [105]. See Figure 1-1 for nodes of the cortico-striatal-limbic network. Amongst various brain regions afferent/efferent to the NAc, the prefrontal cortex guides behaviour through executive control [106]; the amygdala mediates effects of emotions including conditioning (affective signalling) [107]; and the hippocampus processes contextual and spatial information [105].

In this paragraph, I briefly touch on the dysfunction of brain regions in humans and associated impulsivity, to introduce the role of each associated brain regions in impulsive decision making. Patients with attention deficit and hyperactivity disorder (ADHD) appear to have frontal cortex dysfunction [108] and have been shown to make more impulsive decisions [74-76]. Patients with damage to the ventral medial part of the frontal cortex demonstrate impulsive disadvantageous choices in the Iowa Gambling Task [109, 110]. A growing number of studies point towards the involvement of the frontostriatal system in impulsive behaviour [111-113]. In a functional imaging study in healthy volunteers, a reduction in NAc activation was observed in impulsive individuals [114]. These human studies have been supplemented by lesion studies in animals that allow specific and experimental ablation. As an exemplar, I will now summarise the current understanding from lesion work in the domain of delay-based impulsivity task where the rat is allowed the choice of an immediate smaller reward or delayed larger rewards with delays progressively increased over trials.

#### *Prefrontal cortex*

The mPFC and the orbital prefrontal cortex (OFC) interact to form a larger network of the PFC [115] and have been implicated as components of the circuit. Initial work in the prelimbic and infralimbic cortices show a flattened discounting

curve [116]. Lesions in the anterior cingulate cortex (ACC) and the medial prefrontal cortex (mPFC) did not have an effect on delay discounting in rats [116]. The lack of a clear effect on impulsivity of these subregions within the mPFC cortex is plausibly caused by insensitivity to the shifts in delay contingencies during the trials [116, 117]. Studies on OFC lesions showed contrasting results and upon closer inspection, the difference lies in the timing of the lesion – whether it was made prior to task acquisition or after. The OFC lesioned-rats, which acquired the task after the lesions, increased impulsive choice [118, 119] whereas those that reached stable baseline performance prior to lesions retained their preference for the delayed choice being less impulsive [117]. These findings suggest that the OFC is involved in updating the value of a reward and damage to this brain structure disrupts the devaluation of future rewards. The rats in the Winstanley [117] study were unable to update the reduced valuation of the future rewards resulting in a persistent choice for the larger delayed reward [117, 119, 120]. This also reflects 'perseverative behaviour' which is also associated with OFC lesions [121, 122]. In other words, cognitive flexibility mediated by the prefrontal cortex, particularly the OFC, is a critical component influencing decision making (see [110] for a review on the role of prefrontal cortex in decision making)

*Amygdala*

Connections between amygdala and PFC relay information regarding reward magnitude to the PFC and are important for goal-directed behaviour [123]. Basolateral amygdala (BLA) lesions increase impulsivity in the delay-discounting task [117]. BLA inactivation also increases impulsivity in the effort domain [124]. This suggests a role of the BLA in incentive/reward representation. The BLA is involved in associative encoding of rewards and forms part of the neural circuit important in using incentive processes to guide behaviour [125]. Thus a functional BLA is crucial in forming associations between a response and the incentive value of the outcomes of the responses [125-128]. Together, these studies suggest a role of the BLA in biasing choice toward [117] high cost rewards [129] and in reward representation and valuation [130-132]. In other words, affective processing mediated by the amygdala is a critical component

influencing decision making (see [133] for a review on the role of amygdala in decision making)

*Nucleus Accumbens*

The location of the NAc suggests it functions as an “interface” or the site of integration between signals from the cortical and limbic regions [116, 117, 129] and the connections between the NAc and BLA provides an important pathway in which limbic systems can drive action and behaviour [117, 134]. Studies in rats with excitotoxic lesions in the NAc core region increased impulsive choice in rats tested in the delay-discounting assay [116]. The NAc core contributes to instrumental learning and responding for delayed reinforcements tasks as NAc-lesioned rats showed no impairment in learning when no delay is involved [135]. These effects are specific to the core subregion of the NAc as lesions in the corresponding shell subregion showed no impairments in the delayed-reinforcement tasks and goal-directed behaviour [136]. Thus, a functional NAc core region is necessary in bridging the delays between actions and delayed outcomes.

*Connections*

I now shift the focus from the NAc as an anatomical entity to its connections within the cortico-striatal-limbic network, discussing how the brain integrates and balances the processes and communication within this network. Ambroggi [137] showed that limbic inputs from the BLA drive NAc neuronal firing activity and correspondingly change reward-seeking behaviour. Electrophysiological recording in rats engaged in reward discrimination tasks revealed that reward-predictive cues initiate activity in BLA neurons that precedes NAc activity. While connections between the prefrontal cortical regions and the NAc are established [138, 139], functional studies (similar to Ambroggi) have not been performed in the prefrontal cortex. Thus how a disruption of PFC/OFC inputs to NAc may affect behaviour and associated neural activity is currently unknown.

The role of DA in rewards and goal-directed behaviour has been briefly discussed in the preceding section. DA release in the NAc can be conditioned to reward-predictive cues [88]. Thus, DA projections from the ventral tegmental area (VTA)

are also important. Gamma-aminobutyric acid (GABA) agonist infusions in the VTA blocked behavioural responses in rats trained to lever-press for rewards. This is accompanied by a reduction in cue-evoked firing in the NAc [140]. While DA is necessary, it is insufficient to induce a change in neuronal activity in the NAc and acts as a modulator of neuronal excitability [124, 141, 142]. This relationship of the dopaminergic projections and various brain structures is essential for NAc dopamine release [137, 143]. Interrupting communication between the BLA and the DA projections within the NAc with GABA-agonist and DA-antagonists infusions, in respective regions, revealed that both DA inputs and BLA inputs to NAc core are required to drive approach responses to the rewarding cues [137, 144]. Thus, it is evident that the interaction of the BLA, the NAc and the DA system is essential for appropriate action-selection to incentive stimuli or goal-directed actions [137, 144, 145]. The spines of the medium spiny neurons in the NAc receive synaptic inputs from cortical afferents with co-convergence from amygdala projections. DA has also been suggested to synapse onto the medium spiny neurons (MSN) spines [146-148]. This forms the triad of synaptic connection: spines, glutamate (excitatory) synapse and DA synapse, and this configuration supports the possibility for DA to modulate excitatory glutamate transmission [149].

In summary, current findings implicate the nodes of the cortico-striatal-limbic network in mediating impulsivity – the OFC involved in updating reward valuations; the BLA in reward valuation and maintaining an ‘online’ representation of rewards; the NAc as the interface integrating such representations; with the DA systems in modulating responsiveness and excitability in the NAc. Thus, using the *Toxoplasma gondii* infected rat as an impulsive rat model might allow an understanding the interaction of the brain regions underpinning impulsivity and reveal unifying insights for reward-based goal-directed decision making.

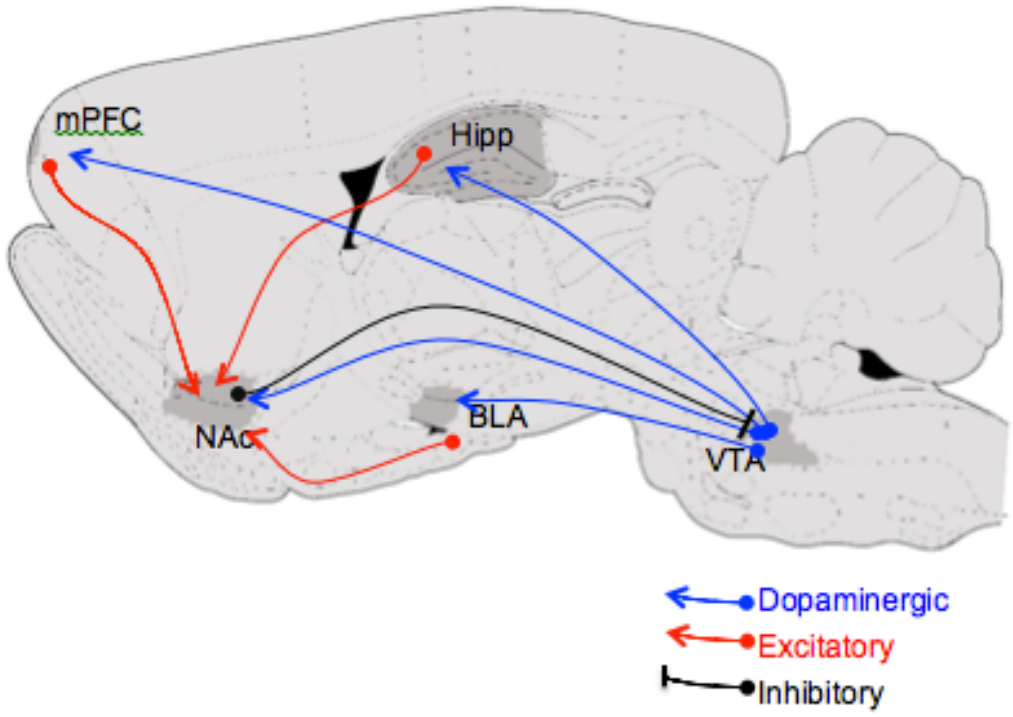



**Figure 1-1 A simplified schematic of the cortico-straital-limbic network in the rodent brain. Dopaminergic, excitatory and inhibitory connections to and from the ventral tegmental area (VTA) and within main nodes of the cortico-striatal-limbic network are shown. The primary reward circuit includes dopaminergic projections from the VTA to the nucleus accumbens (NAc), which release dopamine (DA) in response to reward-related stimuli. The ventral pallidum (VP) is the main efferent of the NAc.**

**Box 3 The cortico-striatal-limbic connection.**

*Nucleus accumbens*

The NAc is divided into two main regions. The core of the NAc comprises of a more dorsal part that is continuous with the dorsal striatum. The shell consists of medial and ventral parts of the NAc [102]. Majority of NAc cells are medium spiny neurons (MSN) [150]. The spines of the MSN receive synaptic inputs from cortical afferents with co-convergence from amygdala and hippocampus projections. Axons containing DA from VTA also terminate onto the MSN spines. Cross talk between MSN spines, afferent glutamatergic synapses and dopaminergic synapses allows DA to modulate excitatory glutamate transmission [149]. Ventral pallidum (VP) is one of the major efferent of the NAc.

*Ventral tegmental area*

Dopamine (DA) neurons make up 60 – 65% of the VTA cells [151]. These dopaminergic neurons contribute to goal-directed behaviours and the release of dopamine in forebrain regions are involved in responding to rewards [86, 152] as well as facilitating motivation (incentive behaviour) [81, 89]. The rest of the VTA consist of 30- 35% gamma-aminobutyric acid (GABA)-ergic interneurons and a very small percentage (2 – 3%) of glutamatergic neurons [151].

The DA and GABA neurons in the VTA project to the frontal cortices (of which PFC is in relevance to decision making circuitry). VTA receives inputs from different brain nuclei including PFC to medullary brainstem and lateral hypothalamus [91]. A major VTA-DA excitatory afferent is from the PFC, which loops back to the PFC whereas, the VTA-GABA afferent from PFC projects to the NAc instead. The NAc (shell) and VP provide the major inhibitory GABAergic signals to the VTA [153, 154]. This suggests that VTA activity is regulated by an integrated network of inputs rather than discrete brain structures [155].

**Box 3 The cortico-striatal-limbic connection (cont'd).**

*Hippocampus*

The hippocampus interacts with the NAc to provide environmental and spatial information for state modulation [105]. The connections within the hippocampus from various nuclei (*e.g.* amygdala and mPFC) directly and indirectly allow it to provide contextual information to the NAc. In addition, the projection starting from the hippocampus, via the NAc and VP, also provides the context-dependant activation of VTA-DA neurons.

*Amygdala*

Amygdala's role in affective behaviour [107] is widely studied and the connections from the amygdala (more specifically the basolateral region) to the NAc relay this information and provide emotional salience to the NAc. Numerous findings show the amygdala's role in fear conditioning and this is crucial in affective-salience information in adaptive decision making.

*Prefrontal cortex*

Prefrontal cortical controls behavioural flexibility and promotes goal-directed behaviour [156]. Connections between amygdala and PFC relay information regarding reward magnitude to the PFC. Inputs from this region provide guiding and supervisory control for behaviour [157]. This is supported by studies showing that damage to this region affects the ability to anticipate future outcomes and thus resulting in 'inflexibility' of choice without affecting immediate reward consequence [110].

## Specific Aims and Content

In this chapter:

- I started with the description of behavioural manipulation hypothesis in the context of loss of fear in *Toxoplasma gondii* infected rats;
- Next, I strived to put fear in a broader context of conditional behaviours or decisions/choice;
- And, expanded on current biological concepts in decision/choice;
- Finally, I summarized the neurobiology of decision making.

Within this framework, I will now present specific aims of my thesis and their rationale.

As detailed in the previous sections, rats infected with *Toxoplasma gondii* exhibit reduced or even reversed fear to predator odours. This phenomenon has been presented as a classic case of behavioural manipulation; a hypothesis that parasites can change the behaviour of their host in a way that benefits them but not the host. In other words, genes of a parasite can produce a phenotype in the host. The genes of the parasites have 'extended' beyond physical confines of the parasite's body [12].

The narrative of my thesis starts from the effects of parasitism on fear and aims to extend these observations in the domain of choice or decisions. To reiterate what I wrote earlier, choice or decision is not used here in its common sematic sense of *conscious deliberation*. Rather I refer to decision or choice as a conditional behaviour where alternative options are weighed and chosen depending on incipient ecological and/or physiological parameters.

The rationale behind my effort to link fear changes in the host to choice is the inter-connectedness between these two constructs. As detailed in the previous chapter, fear is a flexible behaviour that often exists in a continuum. Condition-dependence of the fear suggests that it is part of a larger syndrome of conditional behaviours, selected to negotiate trade-offs between current and residual fitness [30, 32, 33]. See page 19 for a detailed articulation in this regard.

My thesis aims to extend *Toxoplasma gondii – Rattus novergicus* association beyond the current focus on innate fear manipulation and towards a choice or decision paradigm.

Decisions in humans and animals have often been examined in the context of 'discounting' (see page 23 in the introduction for description of discounting). The discounting refers to situations when the 'value' of a positive reinforcement is diluted due to greater efforts required and greater delays or greater risks involved in the receipt. Thus I compare decision making in control and infected animals in response to effort, delay and risk (three subsequent chapters). The mesolimbic dopamine system is known to mediate decision making in rats and in humans [86, 96, 152, 158-162]. Please also see page 26 for an introduction on the dopaminergic system. In view of this, I also examine the effects of *Toxoplasma gondii* infection on dopamine and associated monoamine – 5-hydroxytrytophan (5-HT) (Chapter 4). *Toxoplasma gondii* infection enhances testicular steroidogenesis [163]. In view of this, I examine if testosterone is able to recapitulate the effects of the infection on decision/choice (Chapter 5).

**Submitted to Brain Research**

# Chapter 2 Infection of male rats with *Toxoplasma gondii* induces effort-aversion in a T-maze decision-making task

*'Choose the best for the least'*

*— ANONYMOUS*

Donna Tan[1], Ajai Vyas[1*]

## Summary

*Toxoplasma gondii*, a protozoan parasite, alters aversion to predators in infected rats. This change is thought to increase transmission to the final host of the parasite, *i.e.* the cat. Prior behaviour research has mainly focused on the loss of fear, or 'fatal attraction', after *Toxoplasma gondii* infection [17, 164]. Nonetheless, fear response is not a monolithic behaviour. It is a conditional behaviour that is responsive to trade-offs between cost/benefit of approach and avoidance [30, 32, 33]. The change in fear post-infection can thus also be viewed as a shift in the negotiation of the trade-off towards approach and away from avoidance behaviours. In other words, the loss in fear or the gain of attraction can be viewed as a choice or a decision (the etymology of the word 'decide', meaning 'letting go of alternatives').

Decisions or choices serve to provide behavioural optimization of costs/benefits, by using disparate behavioural strategies in the face of changes in ecological situations. In this chapter, I analyse this process from a vantage point of efforts. I do this by comparing effects of *Toxoplasma gondii* infection on choice for instrumental responses contingent upon larger-harder rewards compared to

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DT designed and conceptualized experiments; conducted the effort T-maze; conducted data collection, statistical analysis and wrote the paper. AV took part in the conceptualization and wrote the paper.

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those of smaller-easier option. In other words, I examine if the infected animals exhibit lesser or greater effort aversion in their valuation of choices.

Apropos, behavioural change after the infection is accompanied by dendritic atrophy of principal neurons in the basolateral amygdala (BLA) [165]; a brain region that mediates emotional valence and the influence of emotions on decision making [132]. Inactivation of the BLA results in effort aversion in choice tasks [124, 166]. As effort aversion results in lower economic payoff in these tasks, the change in behaviour after BLA lesions has been referred as ‘impulsive choice’ [118, 167]. Diminution of the BLA suggests that *Toxoplasma gondii* infection could result in effort aversion, similar to that observed after pharmacological suppression of this structure [165].

I hypothesize that *Toxoplasma gondii* infection will shift decisions towards impulsive choice (smaller-easier rather than larger-harder).

## Materials and Methods

### *Animals and Infection*

Male Wistar rats were obtained from the vivarium of National University of Singapore and housed in the animal care facility (12 weeks of age; housed 2/cage; 12 hours light-dark cycle, lights on at 7 AM; provided *ad libitum* access to water except during operant testing). During operant experiments, all rats were maintained on a restricted diet at 85% of their free-feeding weight but were allowed to gain 3 – 5 g per week. In addition to the food rewards obtained during testing, the rats were supplied with a portion of standard laboratory rat chow (Purina) in their home cage, within 1 h post-testing. All animals were provided with *ad libitum* food and water when operant experiments were not on going. All animal procedures were approved by the Nanyang Technological University's institutional animal care and use committee. All procedures related to animals were in accordance with National Institute of Health guidelines for ethical use of animals in experimentation.

A *Prugniaud* strain of *Toxoplasma gondii* was used. This strain is similar to the strain used for prior work with rats [164]. *Toxoplasma gondii* tachyzoites were maintained in human skin fibroblast cultures. Infected fibroblasts were syringe-lysed by using a 27-gauge needle to release tachyzoites. Animals were either infected with tachyzoites ($5\times10^6$, *i.p.*) or mock infected with sterile phosphate buffered saline after they had achieved a stable baseline during the effort-discounting task. Typically, an incubation period of six to eight weeks is required for onset of chronic infection and absence of acute parasitic proliferation [164].

### *Effort Aversion Training*

The procedure to measure delay averseness was adopted from [168].

An elevated T-maze with a centre runway attached to two choice arms (70**x**12**x**30 cm) was placed 75 cm above floor (Figure 2-1A). One arm was designated the High-Reward (HR) arm and the other the Low-Reward (LR) arm (counter-balanced between left and right across animals). A circular receptacle

(2.5 cm in diameter) containing food pellets was placed 2 cm away from the end of each arm (45 mg food-reinforcement pellets, formula 5TUM; TestDiets, Richmond, USA). A three-dimensional right-angled triangular-shaped block was used as a barrier. Animals had to climb over the vertical edge of the barrier in order to obtain rewards. The height of the barrier was increased successively over the course of the training. A divider (12**x**30 cm) was used to block access to choice arms during forced trials. Animals were always placed in the centre runway for all trials and removed after five minutes or after they had successfully completed each stage.

During the two-day habituation, animals were allowed to explore the maze for five minutes. During the first phase of training, animals were exposed to the differential baiting of the pellet container with four pellets in the HR arm and two pellets in the LR arm. Rats were allowed to consume pellets from both arms. They were given five training trials each day over two days.

During the second phase of training which was similar to the first except that animals were removed immediately after entering one choice arm and consuming the pellet in that arm. From this phase onwards, each session consisted of 12 trials presented continuously. Each session began with two initial forced trials in which entry to only one arm was allowed, followed by ten free-choice trials. Animals were progressed to the next stage as a group when all animals achieved a 90% choice of the HR arm during free-choice trials.

During the third phase of the training, a 15-cm barrier was placed in the centre of the HR arm. Animals were removed from the maze only after they had climbed the barrier in the HR arm and consumed their reward. They were given five training trials. For all subsequent trials, animals were removed once they had entered a choice arm and consumed the pellet in that arm. During training trials, if an animal entered a LR arm, a barrier was placed in the LR arm in the next trial to force it to learn to enter the HR arm. Animals were trained on each barrier over three days before the height of the barrier was increased (20 cm, 25 cm and 30 cm, successively). After 12 days, the animals progressed to the actual phase of testing in the effort-discounting task. In the final testing phase, barrier height was

maintained at 30 cm and test sessions were similar to the training phase. Animals were given 12 trials each day (with the first two trials being forced trials into each choice arm) and tested over blocks of three days. HR choice was averaged over the three-day period. The schedule of barrier training is shown (Figure 2-1B).

### *Effort Aversion*

Rats were first trained to stable baseline with the 30 cm barrier on the effort-discounting task. They were then injected with either *Toxoplasma gondii* or vehicle after pseudo-random group assignment. Baseline performance was matched between groups; and cage-mates were kept in the same group assignment. Animals were tested in three-day blocks with one pre-infection testing block and subsequent blocks during week two, five and eight post-infection to assess changes in baseline choices over the course of the infection (Figure 2-1B).

### *Statistics*

Statistical analysis was conducted using Graphpad Prism 6. The number of HR choice was averaged from sessions within a three-day block, excluding forced trials. 2-way mixed model repeated measures analysis of variance (ANOVA) was used to estimate statistical significance of main effects and interactions (between subject source of variance = infection status and within-group = time post-infection). Bonferroni multiple comparisons were used for post-hoc analysis. Effect sizes were estimated using Cohen's d [169], with a value of >0.8 interpreted as a strong relationship. Figures represent mean ± SEM. The number of animals is noted in figure legends.

# Results

I tested choice between larger-harder and smaller-easier rewards in control and *Toxoplasma gondii* infected rats in an effort-based decision-making task (Figure 2-1A). Before infection, all rats were trained to climb barriers in order to obtain rewards. Choosing an arm without a barrier allowed access to two pellets of food rewards. The alternative option of climbing a 30cm barrier in the opposing arm resulted in access to four pellets of food rewards. After the initial training period, all rats chose the HR arm on at least 80% of the trials. Rats were assigned to experimental groups in a pseudorandom manner. At the time of assignment there were no inter-group differences between animals assigned to control and infected categories (10 control and 14 infected animals; independent sample t-test: $t_{22}$ = 0.5233, $p$ = 0.6060; statistical power = 0.92; effect size: Cohen's d = 0.216).

### *Infection increased effort aversion*

Analysis of variance was conducted to dissect variance ascribed to within-group source (time post-infection), between-group source (infection status) and their interaction. ANOVA revealed statistically non-significant interaction ($F_{(3,66)}$ = 2.196, 0.097). Main effects of both time post-infection and infection status was statistically significant (within-subject: $F_{(3,66)}$ = 2.839; $p$ = 0.045; between-subject: $F_{(1,22)}$ = 6.562; $p$ = 0.018; Figure 2-2A).

Upon infection through the intraperitoneal route, *Toxoplasma gondii* proliferates in the peripheral organs for the initial 1-2 weeks. Tachyzoites of the parasite go through cycles of cell invasion, asexual replication and then lysis of the host cell. This results in a steady increase in parasite burden. In response to the resulting immune challenge, tachyzoites then migrate to immune-privileged organs like brain and convert to slowly dividing bradyzoites. Multitudes of bradyzoites form tissue cysts that go through recrudescence with relatively slow turnover rates. The cystic phase of the infection is characterized by shallower host immune response and lack of any sickness behaviour. By 30 days post-infection, most of the parasite burden is found in immune-privileged organs with much clearance of the parasite from the periphery.

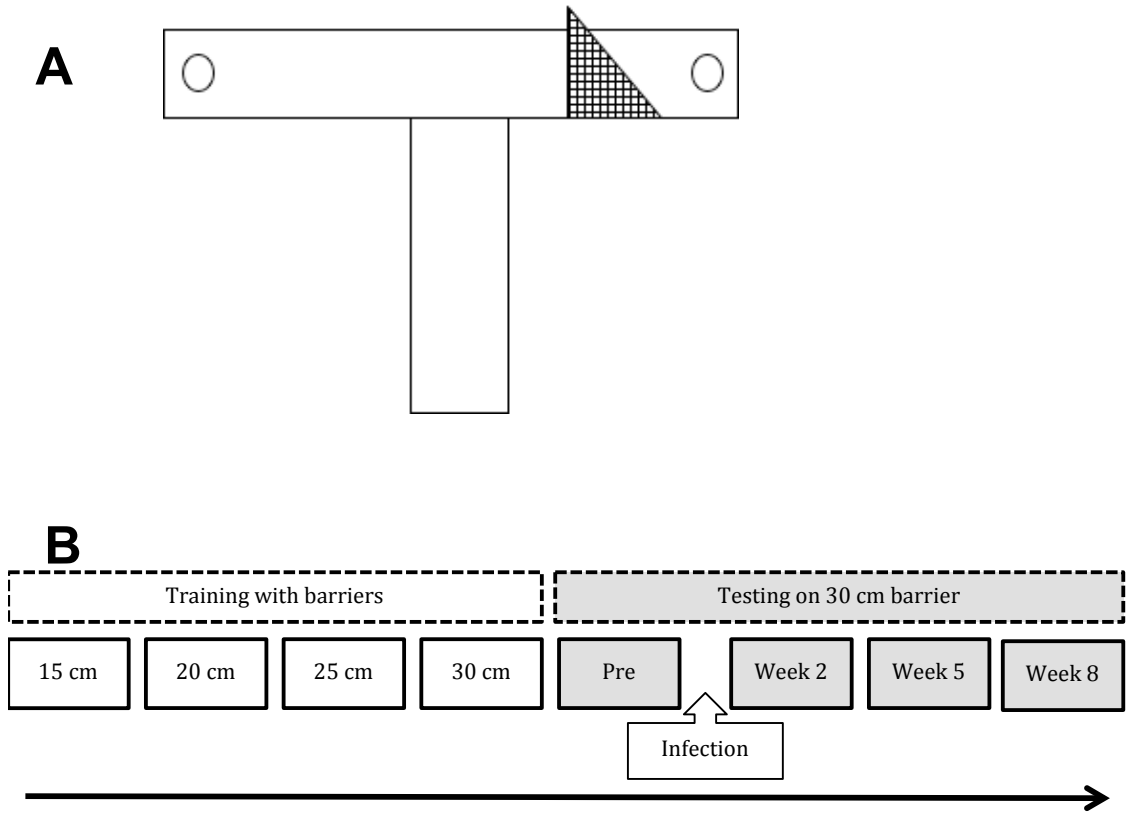


**Figure 2-1 Effort-based decision task (A) Schematic view of the T-maze used for effort-based decision-making, adapted from [168]. Rats were trained to choose between climbing a barrier in order to obtain 4 pellets (HR, harder-larger reward) or receive 2 pellets by entering in opposing arm without a barrier (LR, easier-smaller reward) (B) Experimental overview showing various stages of training following by testing timeline over course of the infection. Height of the barrier was progressively increased from 15 cm to 30 cm during training. The 30 cm barrier was used in the final stage of testing. Acute phase testing was carried out 2 weeks post-infection and the chronic phase 5 weeks and 8 weeks post-infection.**

I was interested to determine if effects of the infection were specific to its chronic phase that entails access to the central nervous system and not to any unobserved immune upheaval or sickness behaviour during acute infection. In view of biological differences between acute and chronic phases of the infection, I statistically analysed effects of the infection on effort aversion at 2, 5 and 8 weeks post-infection. Non-orthogonal planned comparisons were made between experimental groups at these time-points.

During the acute phase of the infection (week 2), *Toxoplasma gondii* infection did not result in any change in effort aversion (Figure 2-2B, *left;* choice for larger-harder reward: $t_{88}$=1.039, $p$ > 0.9999, statistical power = 0.87; effect size: Cohen's d = 0.217). In contrast, the infection significantly increased effort aversion at 8 weeks post-infection (Figure 2-2B, *right*; $t_{88}$=3.337, $p$ = 0.005; Cohen's d = 1.099).

In order to preclude effects of the infection on motor or spatial behaviour, I tested if equalization of efforts required to gain larger or smaller rewards also rescued choice of the infected animals for the smaller rewards. A second identical barrier was placed in the LR arm. Thus animals could choose either smaller or larger rewards by scaling identical barriers with equal efforts. In response, the infected animals switched their performance to larger rewards. Analysis of variance was conducted to dissect variance due to within-group source (presence of additional barrier), between-group source (infection status) and their interaction. ANOVA revealed statistically significant interaction ($F_{(1,22)}$ = 4.329, 0.049). Main effects of both presence of barrier and infection status were statistically significant (within-subject: $F_{(1,22)}$ = 4.964; $p$ = 0.036; between-subject: $F_{(1,22)}$ = 6.822; $p$ = 0.016).

In contrast to the choice observed in the 1-barrier, infected rats switched their preference and chose the HR arm when the effort needed was equalized in both HR and LR arms in the 2-barrier test (Bonferroni's multiple comparison: $t_{22}$ = 3.337, $p$ = 0.006). Control rats performance was not affected by the presence of the additional barrier (Figure 3-2B, left; $t_{22}$ = 0.964, $p$ > 0.9999). There was no significant difference between control and infected rats in their HR choice in the

2-barrier test (Figure 2-2C; independent sample t-test; $t_{22}$ = 1.314, $p$ = 0.202; statistical power = 0.681; effect size: Cohen's d = 0.579).

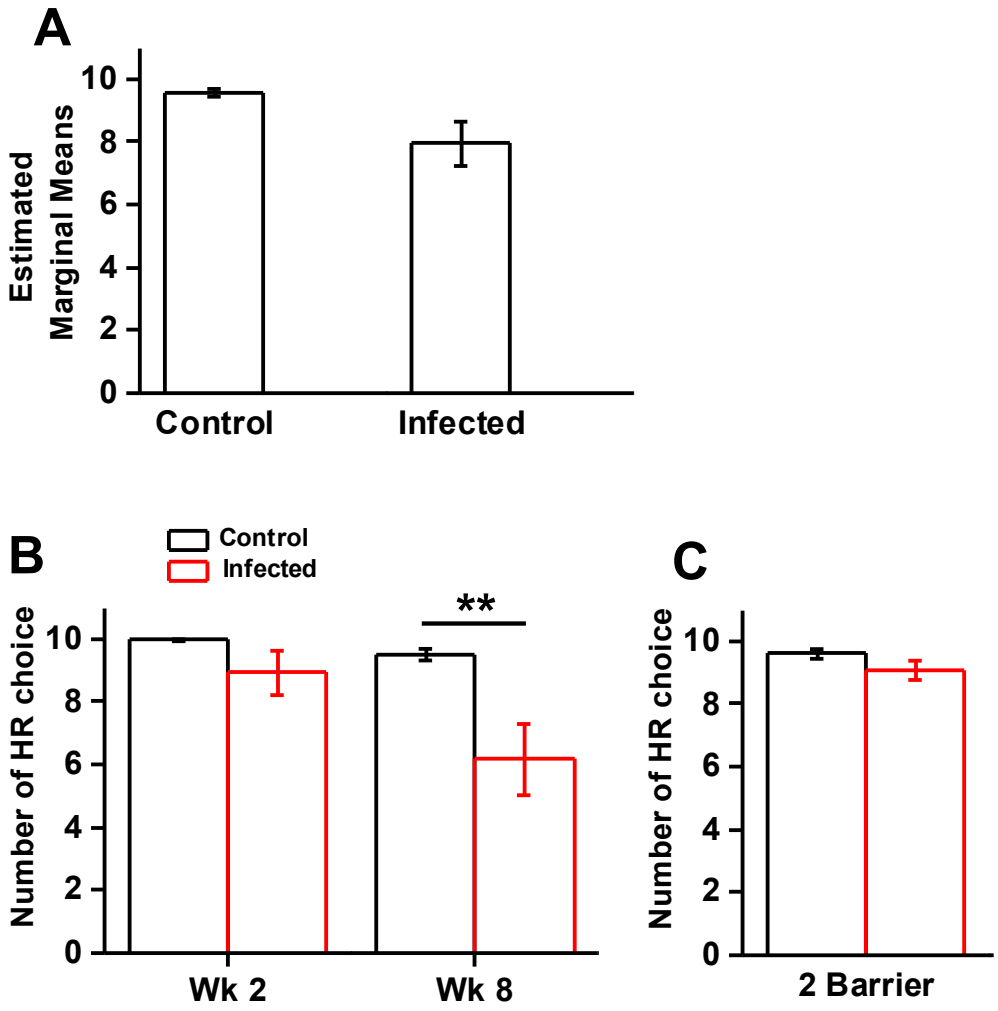


**Figure 2-2 Infection increased effort-aversion during the chronic phase. (A) Estimated means of infection effect on number of HR choice. (B) Infected rats reduced their choice of HR arm during the chronic stage of infection (week 8 post-infection) whereas this aversion to effort was not seen during the acute phase (week 2 post-infection). (C) Infected rats chose the HR arm when the effort needed was equalised in both HR and LR choice arms. The ordinate depicts mean ± SEM number of HR choice (out of 10 free-choice trials) by animals; abscissa depicts the progression of infection. **, *p* < 0.01, post-hoc test, compared to controls. N = 10 for controls animals and 14 infected animals.**

## Discussion

Results presented in this chapter show that *Toxoplasma gondii* infection leads to effort aversion in the infected animals. This was demonstrated by reduction of preference for larger-harder rewards. The experimental task required animals to climb a barrier with a vertical face of 30 cm in order to gain access to larger reward; a feat that might require motor and spatial dexterity. It can be argued that when the rats are exerting physical effort in overcoming the barrier, an inevitable delay might have been incurred. However, the time taken to scale the barrier to receive the larger reward was negligible (<1 s) in my experiments as the rats were well trained and skilfully climbed the barrier during the testing phase. Therefore, the difference in the HR and LR choice is the result of the physical effort needed to scale the barrier and not due to a possible delay involved in climbing the barrier. In addition, reduced preference for larger-harder rewards post-infection is unlikely to be due to deficits in motor or spatial behaviours. This is borne out by the observation that when similar barriers were placed in both counter-opposing arms containing larger or smaller rewards, the infected animals consistently choose larger rewards. This also demonstrates that the infection does not diminish motivation to obtain positive reinforcement.

Tasks used here have been previously used to study effort-based choices in rodents. This assay was developed to circumvent the need of operant responding while dissecting the role of dopamine in motor and motivational processes [168]. Other groups adapted this task extending its use to effort-based decision making – focusing instead on pharmacological manipulation; brain lesions; and inactivation [124, 170, 171]. This task exhibits good construct validity. This is borne out by the observations that haloperidol, a D2 receptor antagonist, creates effort aversion in this task consistent with the role of dopamine in goal-directed behaviours. Similarly, dopamine depletion within the NAc using pharmacological approach results in effort aversion, congruent with role of the NAc in decision making. Neither D2 receptor antagonism nor DA depletion results in a loss of preference for higher rewards under equal-effort [172]. This shows that instrumental behaviour in this task can be used to measure effort discounting

without confounds of changes in motivation or sensitivity to the rewards. This task also shows good test-retest validity. This is borne out by previous studies and also by the stable performance of uninfected animals over 10 weeks of testing in this chapter.

Impulsivity is typically defined as the lack of forethought resulting in non-optimal choices in dichotomous or discounting variants of cost-benefit analysis [118, 173, 174]. This includes effort as one of the domains of choice. In that respect, I broadly refer to an increased preference for the less effortful less optimal option as an impulsive choice. This reduced preference to exert effort is sometimes specifically referred to as apathy [175, 176]. Patients of psychiatric disorders and smokers demonstrate increased impulsivity in their decisions [DSM-IV 167, 173, 177, 178-181]. For example, schizophrenic patients exhibit steeper discount to more effortful rewards [DSM-IV 167, 173, 177, 178-181]. Similarly, human subjects undergoing cigarette withdrawal also show an increase in effort discounting [DSM-IV 167, 173, 177, 178-181].

Neural substrates of effort impulsivity have been previously studied. The medial prefrontal cortex, basolateral amygdala and the nucleus accumbens as well as the BLA-ACC connections have been implicated in modulating effort-based decision making. Destruction of dopaminergic terminals of the nucleus accumbens in rats increases impulsivity in effort-based tasks and excitotoxic lesions within the anterior cingulate region of the medial prefrontal cortex show similar effects in reducing rats' preferences to exert more effort for a larger reward [168, 171]. Reversible bilateral inactivation of the basolateral amygdala with bupivacaine also changes choice behaviours in rats biasing them against the more effortful option. The role of the BLA in sustaining effortful choice is further established in functional disconnection studies between the BLA and ACC [124, 170, 171]. In these experiments, preference for the larger-harder option is diminished in unilateral inactivation of the BLA and ACC performed contra-laterally. Further studies show that neurons of the BLA encode anticipatory information about rewards magnitude [126] and as such, a reduction in BLA neuronal activity is likely to affect information relay about the expected reward

magnitude of options [182]. Furthermore, lesions of the BLA compromise influence of motivational salience on instrumental responses. For example, BLA lesions abolish typical devaluation of preferred food reward observed in response to pre-feeding [125, 183]. The established role of the BLA in effort discount is congruent with effects of *Toxoplasma gondii* infection on the amygdala neurons.

*Toxoplasma gondii* infection in rats results in a dendritic retraction in the BLA, accompanied by a reduction in basal corticosterone secretion [165]. This is in stark contrast to higher stress hormones and amygdalar hypertrophy observed in a variety of environmental perturbations that result in enhanced emotionality [184]. Related to this, individual variation in basolateral dendritic architecture is related to aversion to predator odour [185, 186]. Events within the BLA are also involved in mediating conditional behaviours that have emotional or arousal components [107, 187]. For example, rat pups form classical conditioning in a conditional manner dependent on the presence or absence of the mother [188]. In the absence of mothers, aversive unconditioned stimuli result in corticosterone-mediated recruitment of pup BLA. This is reflected in readily established aversive conditioning to co-occurring sensory stimuli. In the presence of mothers, BLA remains non-recruited and an appetitive conditioning to co-occurring sensory stimulus is instead formed even in presence of aversive unconditioned experience. This phenomenon is a strong example of conditional behaviour or ‘if-then-else’ nature of many biological programs. The defining role of the BLA in this conditionality is congruent with simultaneous effects of *Toxoplasma gondii* infection on effort impulsivity and BLA dendrites. Since rescue and/or recapitulation experiments have not been performed in this chapter, the association between the amygdala and impulsivity remains a speculation, albeit a strong one, congruent with previously known role of this brain structure in effort discounting.

In short, the data presented in this chapter present a first glance at the possibility that *Toxoplasma gondii* infection could change decision-making or choices of rat hosts. In this way, these observations extend the present paradigm beyond the perception of a monolithic change in fear. These observations begin to steer

*Toxoplasma gondii – Rattus novergicus* association towards a landscape of multi-dimensional change in conditional behaviours.

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# Chapter 3 Infection of male rats with *Toxoplasma gondii* results in enhanced delay aversion and diminution of nucleus accumbens core

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## Summary

Rats infected with the protozoan parasite *Toxoplasma gondii* exhibit a reduced avoidance of predator odours. This behavioural change is likely to increase the transmission of the parasite from the rats to cats. In this chapter, I 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 a reduction in dopamine (DA) content and neuronal spine density of the nucleus accumbens (NAc) core, but not the nucleus accumbens shell. These results are consistent with the role of the NAc dopaminergic system in the mediation of choice impulsivity and goal-directed behaviours. These observations suggest that

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DT designed and conceptualized all 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.

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*Toxoplasma gondii* infection in rats causes a syndromic shift in related behavioural constructs of innate aversion and making foraging decisions.

The *Toxoplasma gondii – Rattus novergicus* parasite-host association has been widely studied as an example of parasitic manipulation of host behaviour. Rats infected with *Toxoplasma gondii* exhibit a greater exploration of spaces containing cat odours [17, 164, 189]. This behavioural change is thought to increase parasite transmission because cats are the ultimate host of this parasite [163, 190].

As mentioned earlier (page 19), the innate aversion to predators is a flexible behaviour. Odours that are more predictive of immediate predator presence evoke a stronger aversion compared to partial cues like urine or faeces [191]. Similarly, more concentrated cat odours elicit a larger fear response [191]. Apart from conditional dependence on predator cues, innate aversion is also in contiguity with other behaviours 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 [32]. This and several similar observations, [33] suggest that foraging decisions and predator aversion are related behavioural constructs. Foraging decisions in 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 rewards represent an economically rational choice. In this setting, the choice for smaller-sooner rewards demonstrates an intolerance to delay and thus, is interpreted as an impulsive choice. I 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 the salience of various options [113]. This system pivots around the NAc, which receives dense

dopaminergic inputs from the ventral tegmental area [91, 139, 192]. The NAc interacts with limbic regions involved in emotional valence like the amygdala [132]; and also with the frontal cortical regions involved in executive functions like the orbitofrontal, anterior cingulate, prelimbic and infralimbic cortices [150]. The NAc also influences goal-directed behaviours through its projections to the globus pallidum and the hypothalamic nuclei. Consistent with this, selective excitotoxic lesions or quinolinic acid-induced lesions of the NAc core, but not the shell, enhances impulsive choice in rats [116, 136, 193]. These observations suggest that the dopaminergic signalling in the NAc acts as the interface between salience of various challenges and opportunities; and the resultant behavioural output. In view of its central role in choice impulsivity, I also investigated changes in neuronal morphology and DA content of the NAc.

## 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. Details of animals and infection methods are as previously described in chapter 3 (see page 41). Eight weeks elapsed between infection and the start of behavioural experiment. The same set of animals was used for all experiments described below, except for a separate set of animals employed for the measurement of spine density.

### *Delay aversion*

The procedure to measure delay averseness was adopted from [194] (Figure 3-1). Operant chambers contained a house light, 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 centreline.

The initial phase of the training involved the extension of one lever and the 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 the switching on of the house and food magazine light. The subject was required to make a nose-poke within 10 s, resulting in the presentation of a single lever. A lever press within 10 s of presentation resulted in the 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 progressed the final stage of the delayed discounting task.

In the final stage, animals were tested daily for six days per week (one session per day, session length = 100 minutes; repeated until inter-day variation in the performance became stable). Each session consisted of sixty choice trials executed at 100 s intervals 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 house light 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 levers delivered the smaller-sooner reward (SSR; one pellet, immediate) and the alternative lever delivered the larger-later reward (LLR; four 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. The 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 initiate a trial as well as the number of nose-pokes during the inter-trial intervals were also recorded. Data presented depict an average over a three-day block.

***Preference to sucrose reward***

Three days after the 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 = 2 h). The initial location of the bottles was counterbalanced across animals and switched after 1 h during the test. The consumption was measured by weighing the bottles.

***Monoamines 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 sections. 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 (DA) and serotonin (5-HT) levels 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 DA or 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 [184, 195].

Medium spiny neurons (MSNs) from the core and shell regions of the nucleus accumbens were selected as these neurons represent the major neurons population (~95%). Our analysis of nucleus accumbens neurons was restricted to those located between bregma 1.08 mm and 2.28 mm [196]. Golgi-cox

impregnated MSNs were identified by their spiny nature and characteristic dendritic architecture as described by Robinson and Kolb [197]. The criteria used to select neurons for reconstruction were as described by Vyas [184] and selected MSNs were fully impregnated with Golgi stain and had clearly visible spines with minimal or absent obstruction by neighboring Golgi-stained cells or blood vessels. Six neurons from each region of each hemisphere per animal were selected using camera lucida at a low-powered magnification of 100× (Olympus BX43 microscope, 10X objective) by a person blinded to experimental groups. Individual neurons were quantified at 1000X magnification (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 the NAc were analysed using the Mann-Whitney U test. Figures represent the median and inter-quartile range. The number of animals is noted in figure legends.

# 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 3-1). Figure 3-2A 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 3-2A, 0 s; 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 4-1). Control animals preferred LLR at all delays examined, except at 60s (Figure 3-2A; one-sample t-test against chance; $|t_{13}| \geq 4.19$, $p \leq 0.001$, Bonferroni correction applied post-hoc to alpha probabilities for multiple comparison at five delays). In contrast, preference for LLR was statistically insignificant at all delays greater than 0 s for infected animals ($|t_{11}| \leq 2.07$, $p \geq 0.31$). Between the two experimental groups, infected animals exhibited a greater intolerance to the delay of rewards (ANOVA; Table 4-1; main effect of infection status: $p = 0.024$). Post-hoc analysis revealed statistical significant differences between control and infected animals at delays of 40 s and 60 s (Figure 3-2A; LSD: $p < 0.05$, Bonferroni correction applied to correct for multiple comparisons).

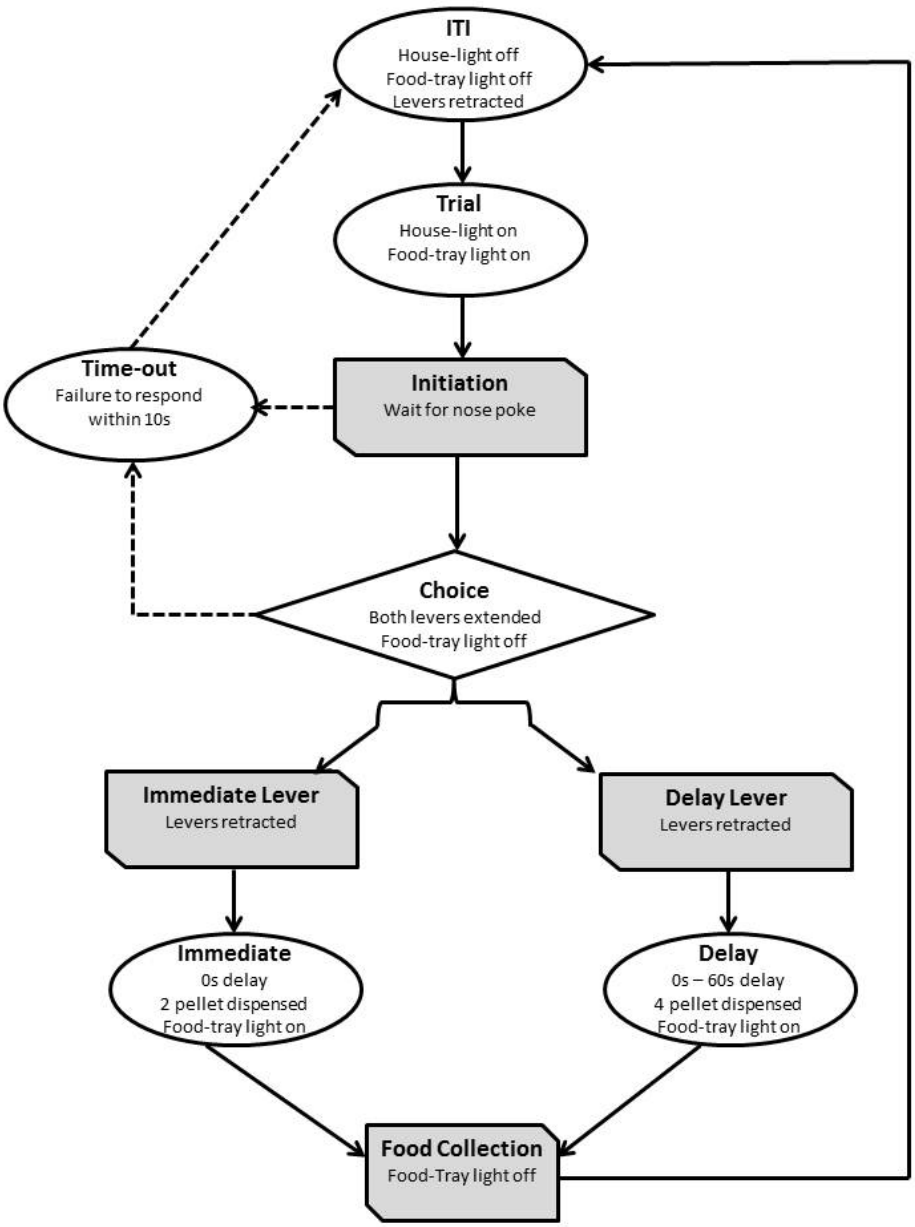



**Figure 3-1 Procedure employed for quantifying delay aversion, depicting a single trial whereby delay across trials within a block monotonically progressed from 0 to 60 s [194].**

**Table 3-1 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 60 s. n = 14 control and 12 infected animals

**Part 1 of 2 for Figure 3-2**

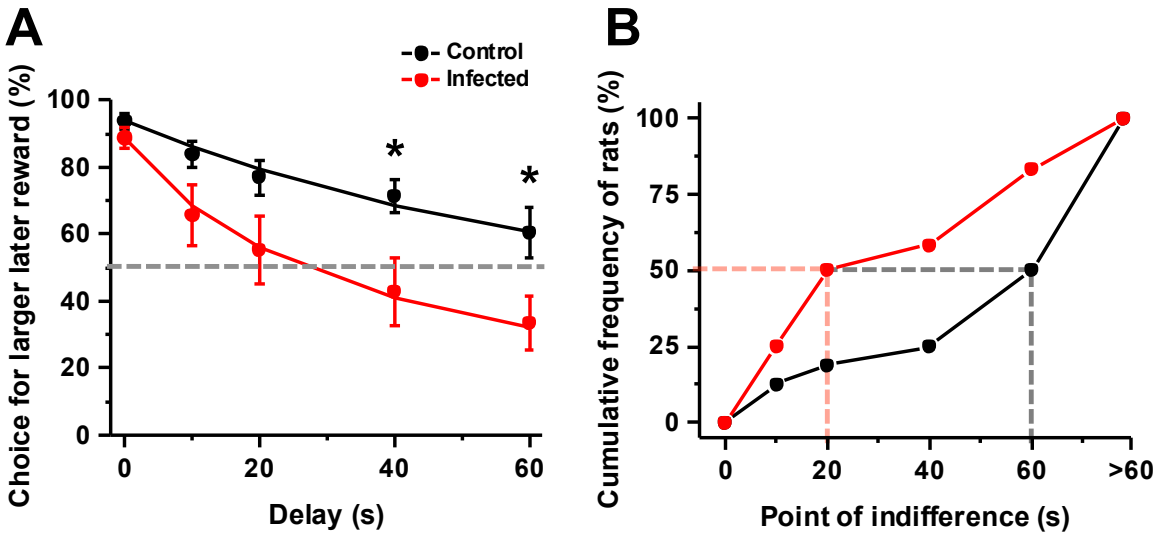



**Figure 3-2 Infection induced impulsive choice by increasing delay aversion, without affecting motor impulsivity. (A) Control animals chose LLR more frequently than infected animals. The ordinate depicts the number of choices made for LLR (mean ± SEM) for a series of sequentially larger delays (depicted 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 grey 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 SSR in five or more trials (out of ten). Animals that did not reach the point of indifference at the highest delay used (60 s) were ascribed a value of >60 s. Median is depicted by the dotted grey line.**

**Part 2 of 2 for Figure 3-2**

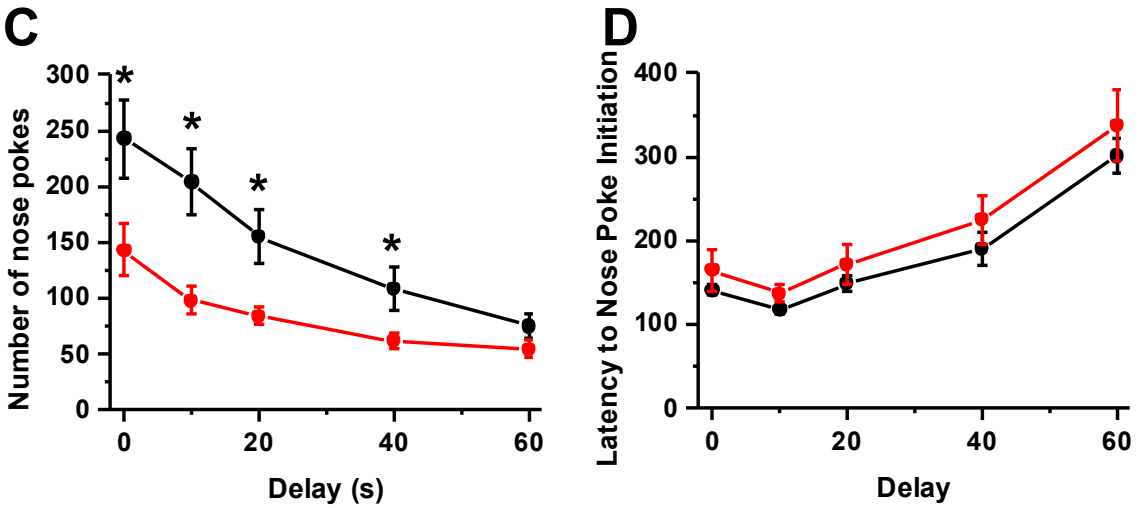



**Figure 3-2 Infection induced impulsive choice by increasing delay aversion, without affecting motor impulsivity. (C) Infected animals executed fewer redundant nose-pokes during the inter-trial interval, suggesting that the enhanced choice impulsivity is not a generalized phenomenon. *, $p < 0.05$, 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.**

The sensitivity of rats to the delay in reward receipt is typically time inconsistent, in that the devaluation rate of the reward is not constant across proximal and distal time delays [64]. To recapitulate this, I fitted the mean number of LLR choice obtained for each group at the various delays to a hyperbolic model using nonlinear regression (Figure 3-2A, solid lines Table 3-2; 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 Table 3-2). For the individual data, the rate of hyperbolic decay (*k*) was calculated. Only individuals with $R^2 > 0.8$ were included in the analysis (fit model and individual parameters in Table 3-2; V = D0/(1 + k*D), where D0 is preference for LLR at zero delay). 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 a 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 3-2B). 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 3-2B; 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 3-2C and Table 4-1). The latency to initiate rewarded trials through nose-poke did not differ significantly (Figure 3-2D and Table 4-2). Thus, the behaviour of infected rats in this task was guided by intertemporal choice impulsivity without a probable contribution from generic or motor impulsivity

**Table 3-2 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 60 s. 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 60 s. n = 8 control and 7 infected animals. Only individuals with $R^2 > 0.8$ were included in the analysis.

***Infection did not reduce sensitivity to the reward***

I further tested whether infection altered the sensitivity of the animal to reward (N = 16 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 3-3A; independent sample t-test: $|t_{24}|$ = 3.15, $p$ = 0.004). Infected animals consumed greater amounts of sucrose (Figure 3-3B; $|t_{24}|$ = 3.43, $p$ = 0.002), whereas the total consumption was not significantly affected by the infection ($|t_{24}|$ = 1.66, $p$ = 0.11). The 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).

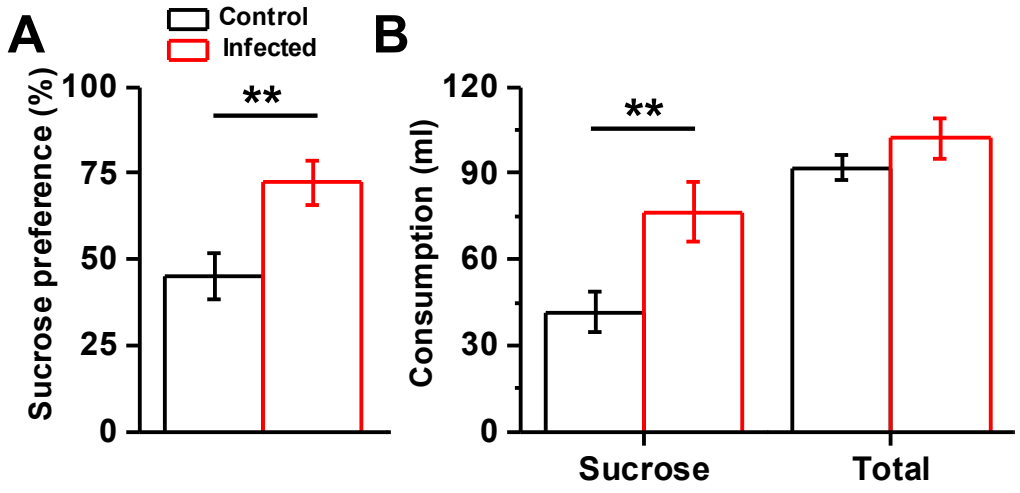



**Figure 3-3 Infection increased sensitivity to rewards. (A) Infected animals exhibited greater preference for a 1% sucrose reward, compared to water (%, relative to sucrose + water consumption). **, $p < 0.01$, independent t-test. (B) Infection increased sucrose consumption *(left)*, but total consumption remained unchanged *(right)*. N = 14 animals for control and 12 animals for infected group.**

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

I quantified the number of spines over 80-μm segments for medium spiny neurons of NAc core and shell. The infection caused a marked reduction in the number of spines for NAc core neurons (

Figure 3-4, *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 control group was still greater than 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 the NAc shell neurons did not significantly differ between control and infected animals (

Figure 3-4, *right*; $|Z| = 0.16$, $p = 0.87$), suggesting that effects of the infection were specific to the core sub-region of the NAc.

Figure 3-4B depicts representative examples of NAc core dendrites.

In order to preclude a generalized change in the spines, I also quantified spine density in brain regions anatomically connected to the NAc (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; $|Z| < 1.14$; $p > 0.15$).

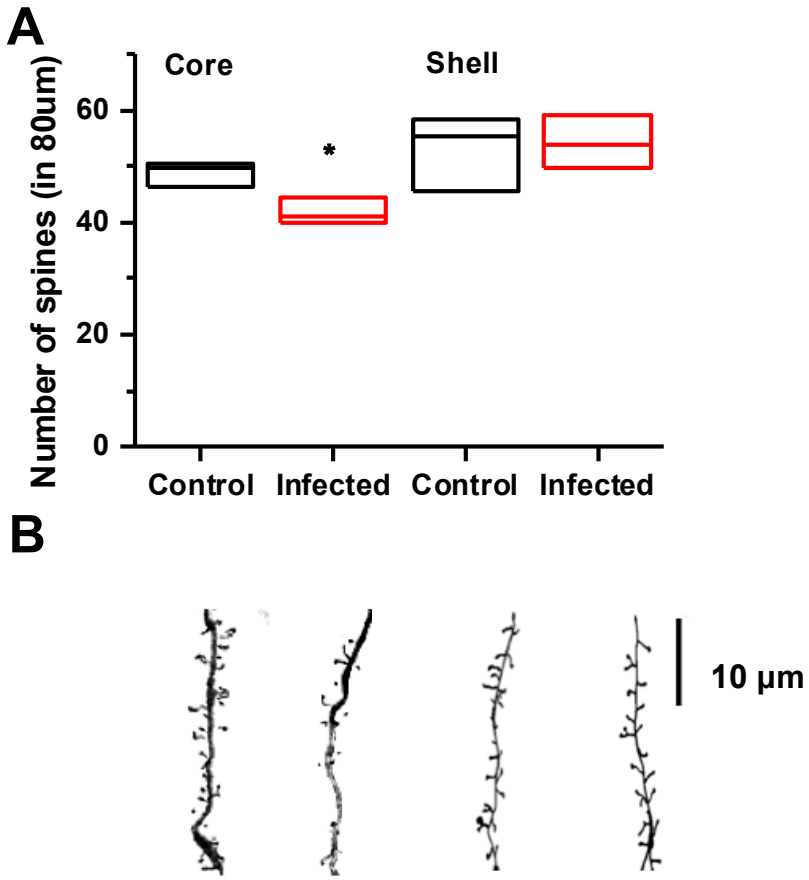
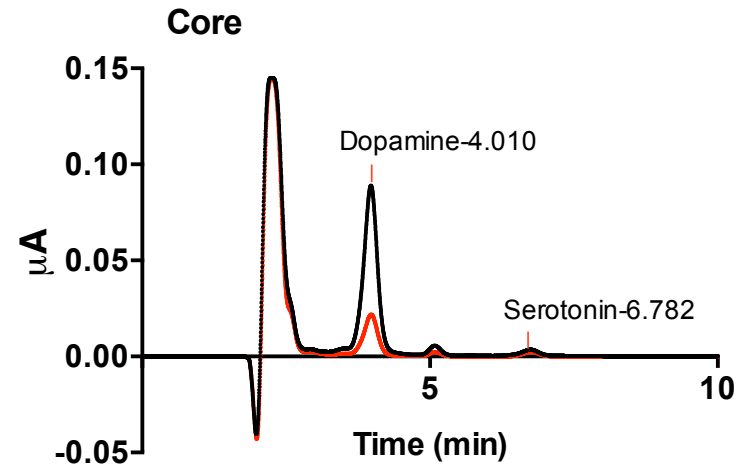


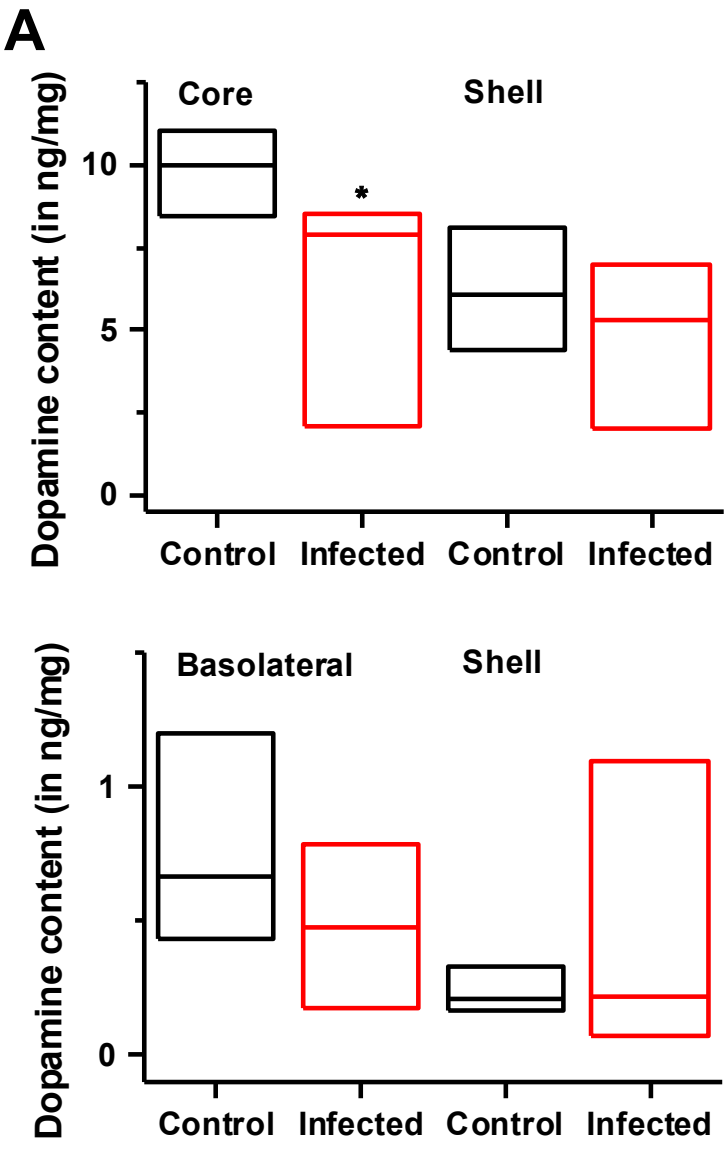

**Figure 3-4 Infection reduced spine density of the neurons in NAc core (A, *left*), but not in shell (*right*). Bars depict medial and inter-quartile range. *, $p < 0.05$ for comparison between control and infected animals. (B) Representative examples of NAc core and shell dendrites. N = 6 animals for control and 6 animals for infected.**

***Infection reduced dopamine content in the nucleus accumbens core***

I quantified the amount of DA and 5-HT in tissue micro-punches obtained from the NAc core and the NAc shell. Apart from causing changes in spine density measurements, the infection caused a statistically significant decrease in DA content of the NAc core (Figure 3-5A, 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 DA content of the NAc shell did not significantly differ between control and infected animals (Figure 3-5A, 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 the NAc core (control = 3.29 ± 0.41 ng/mg, infected = 3.08 ± 0.57 ng/mg; $|Z| = 0.26$, $p = 0.80$) or the NAc shell (control = 1.74 ± 0.18 ng/mg, infected = 1.45 ± 0.20 ng/mg; $|Z| = 0.78$, $p = 0.44$); with the exception of the BLA (Figure 3-5B; control = 1.72 ± 0.19 ng/mg, infected = 1.19 ± 0.14 ng/mg; $|Z| = 2$, $p = 0.0426$). The NAc core DA content was not significantly correlated to the discounting constant k or a preference for LLR at zero delay ($p > 0.75$).

**Part 1 of 2 for Figure 3-5**



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**B**

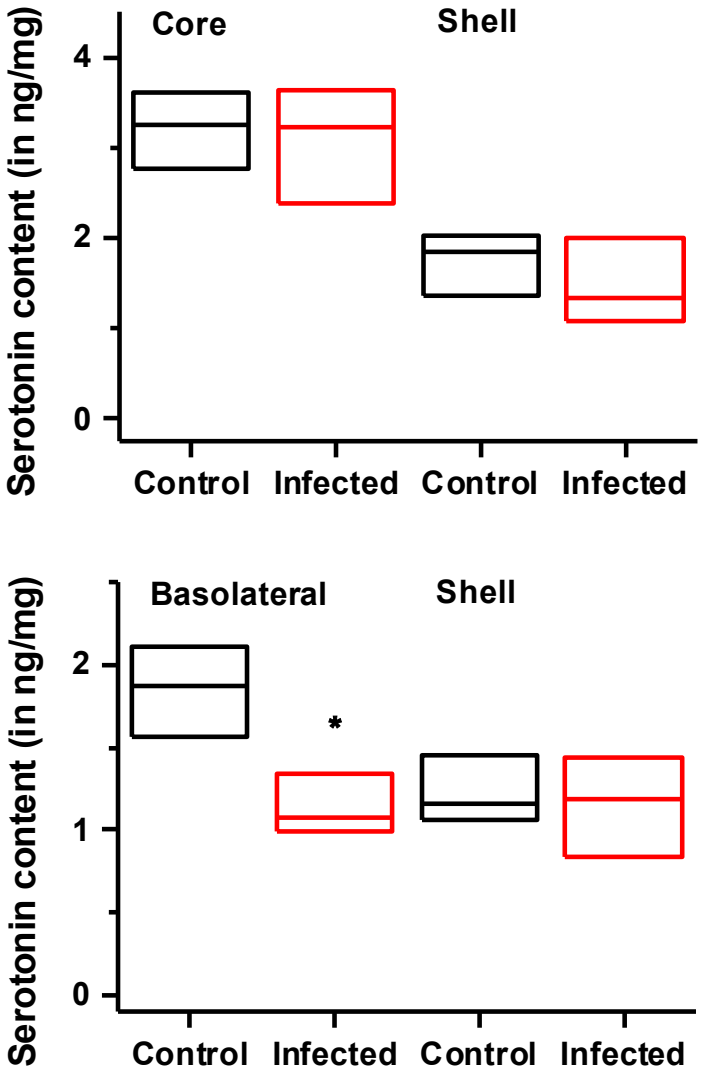



**Figure 3-5 Infection changed neurotransmitter content in specific brain regions. (A) DA content is reduced in NAc core (*left*), but not in shell (*right)*. Bars depict medial and inter-quartile range. *, $p < 0.05$ for comparison between control and infected animals. (B) 5-HT content is reduced in amygdala, basolateral region (left), but not in medial (right). Inset: Representative HPLC trace for analysis of dopamine and serotonin. N = 8 animals for control and 6 animals for infected group.**

## Discussion

Earlier work demonstrates that rats infected with *Toxoplasma gondii* lose their innate aversion to cat odours. In this chapter, I show that the infection with *Toxoplasma gondii* creates delay aversion in male rats by increasing the steepness of the discounting for receipt of larger rewards at increasing delays. This is reflected as a preference for smaller-sooner rewards. Behavioural 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 behavioural changes in the host reflect a syndrome arising because of inter-connected biological imperatives [198]. I propose that the host behavioural change after *Toxoplasma gondii* infection is not a monolithic reduction of the innate fear. Instead it comprises of a behavioural syndrome consisting of reduced innate fear, increased sexual attractiveness and greater delay aversion; all hallmarks of a 'carpe diem' animal personality [199-201]. Biological imperatives that bind these behavioural 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 [199, 201, 202]. In contraposition, metabolic investment resulting in current payoffs often exists in a trade-off with future/residual payoffs [203-205]. I speculate that delay aversion and the loss of innate fear are contiguous behavioural 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 [163, 206]. 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 [207].

The concept of impulsivity has often been divided into motor impulsivity and choice impulsivity [208]. Motor impulsivity is typically characterized as a reduced ability to stop an on-going motor response or to withhold from making a new motor response. The five-choice serial reaction time task (5CSRT), go/no-go and stop-signal reaction time task (SSRT) are the three most commonly used assays

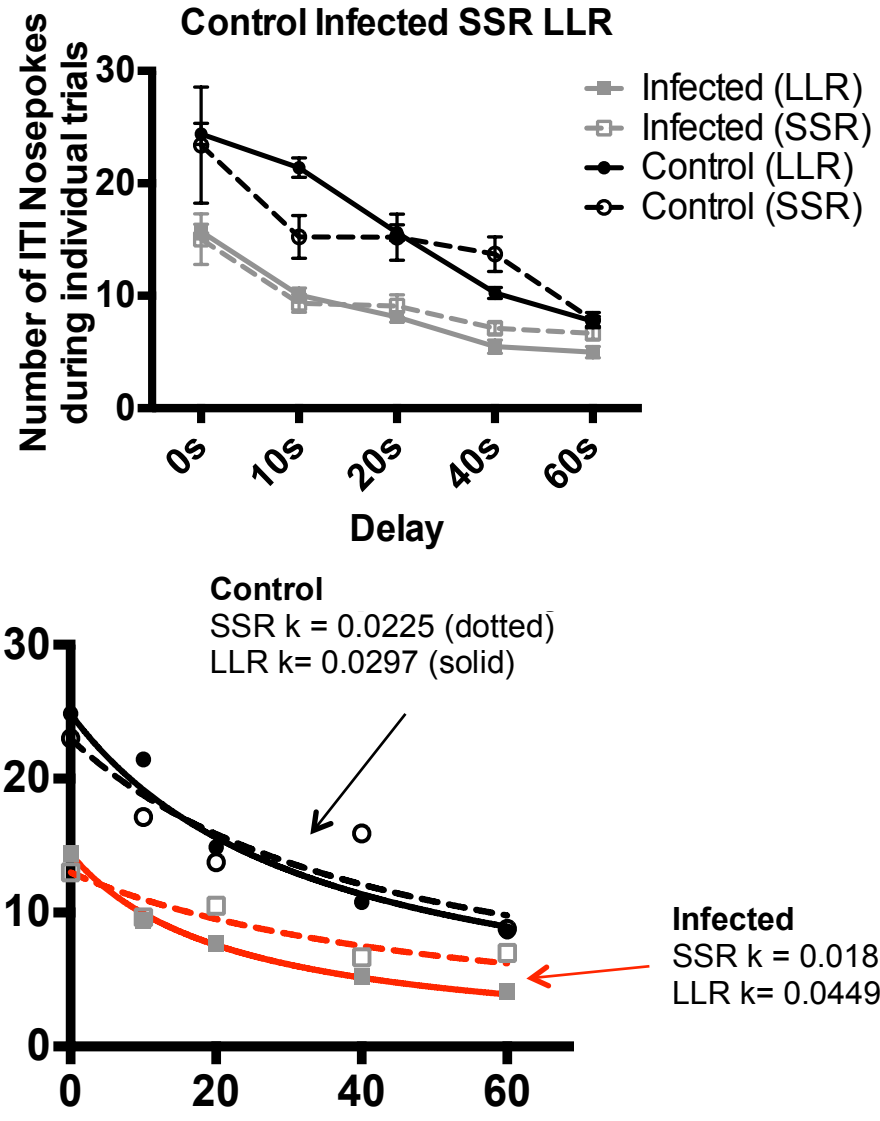
to assess motor impulsivity (see [209] for a recent review). In the 5CSRT [210], a nose-poke in the appropriate aperture is required when a brief stimulus light is presented (randomly in 1 of 5 apertures). Action restrain is required to withhold from premature responding during the inter-trial intervals. In the go/no-go test [211, 212], 2 signals are used to signal appropriate responses and when the no-go signal is delivered, action restrain is required to inhibit motor response before any action has been initiated. In the SSRT [209, 213-215], similar to the go/no-go test, 2 signals are used but instead of a no-go signal, a stop signal is presented in a subset of go trials where action cancellation is required instead, to stop the motor response after the action has been initiated.

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 the inter-trial interval when the 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. I 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 [216], though choice impulsivity in infected human subjects has not yet been tested. As an important caveat, I have not explicitly tested for motor impulsivity in this chapter as the differences between uninfected and infected nose-poke behaviour was a post-hoc observation. To further delineate the differences in motor impulsivity observed would require additional tests as described in the paragraph above. I did not do so as motor impulsivity was not the main measure intended, thus I used it as an indirect measure instead. Nose-pokes during the inter-trial interval can reflect either a failure to withhold premature responding; or as persistent actions. This is because responses made during the inter-trial interval will not yield rewards as

opposed to the required action of nose-poke initiation when cued by the switching on of the houselight. An analogy of this unrewarded action is that just as repeatedly pressing the elevator button will not make the elevator arrive faster, repeated nose-poking will not initiate the trial earlier. This also draws parallels with measures of unrewarded or unnecessary actions measured during motor impulsivity tests. It is also possible that nose pokes during the inter-trial interval may be influenced by choice in the preceding trial thus, might not be an independent measure of motor impulsivity.

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), not did it bias the animals away from high-calorie food (as indicated by in increased preference for sucrose over water compared to controls). Our observations in this regard agree with prior observations that animals infected with *Toxoplasma gondii* retain comparable body weights [164], have similar food consumption after deprivation [164] and are involved in continuation of energetically expensive behaviours [217] as compared to uninfected controls.

**Box 4 Split-sample analysis of nose-poke behaviour of control and infected rats**




**Nose-poke performance across delays can be predicted from choice of preceding trial.(A) Nose-poke behaviour of control and infected rats during choice of SSR or LLR. (B) Theoretical and predicted fit of a split-sample analysis of nose-poke behaviour corresponding to SSR or LLR. Nose pokes were tested if it could be predicted from the outcome of previous trial. Control and infected animals were examined separately. Dyads of choice and nose pokes were tabulated for each delay.**

**Box 4 Split-sample analysis of nose-poke behaviour of control and infected rats**

A split-sample analysis conducted on my 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 (Box 4). In order to carry out the split-sample analysis, approximately 50% of dyads at each delay were partitioned into a 'training set'; the other 50% consisted of a 'testing set'. Training set data was used to derive a hyperbolic discounting of the number of nose-pokes. Dyads containing smaller-sooner rewards (SSR) and larger-later rewards (LLR) were analysed separately. The resultant curves are depicted in above.

All 4 models explained more than 78% of variation ($R^2$ values between 0.78 to 0.99).

Next I examined the generality of these models by asking if these equations could predict the relationship between nose pokes and trial outcomes in the testing set. Dyads in the testing set did not significantly deviate from corresponding models obtained using the training set. The theoretical model explained testing set with $R^2$ greater than 0.67 (*P*s > 0.5). The main message from this analysis is that there are group differences in nose pokes; and that choice changes the relationship between delay and nose pokes.

The mesolimbic DA system is involved in mediating impulsivity in delay-discounting tasks [113]. This system pivots around the NAc, receiving dopaminergic projections from the ventral tegmental area. In rats, bilateral excitotoxic lesions of the NAc core increase delay aversion in discounting tasks; while lesions of NAc shell do not affect this behaviour [116]. This is consistent with our observations that a reduced spine density in the NAc core, but not in the NAc shell accompanies the delay aversion in the infected rats. A pharmacological decrease in dopaminergic transmission by receptor antagonism increases delay aversion in rats [160, 218]. This is consistent with our observations that the infection-induced increase in impulsivity is concomitant with a reduction in DA levels in the NAc core. Interestingly, effects of the infection on innate fear can be rescued by haloperidol, an inverse agonist of DA receptors [219].

Thus, reduced DA content and spine density of the NAc agrees well with increased delay aversion post-infection. What remains an unresolved surprise is the fact the *Toxoplasma gondii* infection is previously postulated to *increase* DA, in contrast to the present chapter [220]. For example, *in vitro* infection of mammalian dopaminergic cells by the parasite results in a robust increase of DA synaptic release [220]. Indeed *Toxoplasma gondii* genome contains two amino acid hydroxylase (AAH) genes that are surprisingly similar in sequence to mammalian tyrosine hydroxylase, a rate-limiting enzyme in the DA synthetic pathway [221]. The protein product of these parasite genes has been demonstrated in infected mice brains, and parasitic cysts in mice brain exhibit robust immunoreactivity to DA antibodies [220].

In a later gene knockout study where one of the AAH gene was disrupted, no increase in dopamine levels were found with infection and with the gene-knockout, both *in vitro* and *in vivo* [222]. The discrepancies of dopamine levels in the *in vivo* mouse infection studies [220, 222-225] suggest that the phenotype is not stable [222] or that experimental procedures differ across labs [226]. Martin [227] reported that while dopamine amount in PC12 cells is increased, this did not translate to an increase in spontaneous release. Comparing dopamine release triggered by high potassium conditions, the percentage increase in DA

release relative to total DA synthesized was lower in infected cells as only a small portion of the increased DA was released. In addition, the host machinery required for DA synthesis was recruited into the parasite's compartment past its cyst wall. Broadly, this could imply that dopamine is needed by the parasite. Importantly, all of the *in vivo* studies were carried out in the mice infection model and analysed for whole brain dopamine content. My study is the first to analyse dopamine content in various brain regions in rats and the decrease in dopamine was specific to the NAc core. It is unknown if the decrease of DA in the NAc reported by my study is derived from the host or the parasite tyrosine hydroxylase. It is speculated/plausible that the decrease in dopamine content, observed in this study, could be due to the parasite's requirement for additional dopamine [227].

Like other neurotransmitters, the effects of DA on the behaviour are intricately dependent on the brain region. For example, administration of atomoxetine, resulting in increased DA in the prefrontal cortex but not the NAc, decreases impulsivity in delay-discounting tasks and 5-choice serial reaction time tasks [228]. The administration of amphetamine leads to more widespread dopaminergic stimulation, resulting in increased impulsivity in the 5-choice serial reaction time task [229] and decreased impulsivity in the delay-discounting task [230]. Moreover, the effects of atomoxetine on 5-choice serial reaction time task can be reversed by selective DA antagonism in the NAc core [231]. These observations suggest that the site of DA change in the brain has a significant effect on behaviour. I report a region-specific rather than a generalized alteration in DA 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 DA concentrations [232]. This is pertinent because *Toxoplasma gondii* does not exhibit an exclusive tropism to the NAc or its sub-regions [233].

*Toxoplasma* gondii has earlier been reported to cause structural changes in the neurons of host brain [165]. In this chapter, I show that the infection reduces neuronal spine density in the NAc core. It is plausible that the reduced spine density of NAc core neurons results in a decrease in the 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 DA and spine density, the infection-induced effects remain more pronounced in the NAc core compared to the NAc shell. The 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 behavioural changes post-infection through the local manipulation of neuronal signalling and/or damage [164, 234, 235]. Two earliest studies in this regard reported a rather widespread occurrence of tissues cysts in a variety of brain regions [164, 235]. Both of these reports suggested a mild tropism to the NAc, ventromedial hypothalamus or amygdala. Core and shell divisions of the NAc were not analysed 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 [207, 236]. Another potential source of region-specific effects could arise because of the selective innervation from vasopressinergic fibres, rather than from tropism of the *Toxoplasma gondii* cysts themselves. Within the NAc, most of the arginine vasopressin containing fibers terminate in the core rather than the shell [237]. Arginine vasopressin neurons in the medial amygdala have been previously shown to be preferentially activated by cat odour [206]; an atypical event because these neurons are typically activated during sexual signalling [238]. The atypical recruitment of arginine vasopressin neurons is mediated by an epigenetic event dependent on a parasite-induced increase in testosterone synthesis [24]. It is plausible, and yet unproven, that stronger vasopressinergic inputs post-infection coupled with tendency of these fibres to terminate in the core but not the shell, could lead to selective anatomical/neurochemical effects within the NAc.

The concurrent changes in choice impulsivity, the NAc core spine density and the NAc core DA point towards a concerted shift in the behaviour of the infected rats. The concordance between these variables suggests a 'conformity to *a priori* expectations based on purported function' [9]. In other words, it is unlikely that

the increase in choice impulsivity is an accidental by-product of the infection, by virtue of it being accompanied by non-generalized changes in biological substrates known *a priori* to be involved in the behaviour.

Finally, the data presented here provide an impetus to integrate parasitic changes in host behaviour 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 behaviours [230].

# Chapter 4 *Toxoplasma gondii* infection and testosterone congruently increase tolerance of male rats for forfeiture risk

Donna Tan[1], Ajai Vyas[1*]

## Summary

Decision making under risk involves balancing the potential of gaining rewards with the possibility of loss and/or punishment. Tolerance to such risk varies between individuals, genders and age groups. Understanding the biological basis of risk tolerance is pertinent because excessive tolerance contributes to adverse health and safety outcomes including addiction, sexually transmitted diseases and pathological gambling. Yet, not much is known about biological factors mediating inter-individual variability in this regard. I investigate if latent *Toxoplasma gondii* infection can cause risk tolerance, using a rodent model of the balloon analogous risk task (BART). 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 risky decision making.

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DT designed and conceptualized experiments; conducted BART and surgeries; conducted data collection, statistical analysis and wrote the paper. AV took part in conceptualization; conducted statistical analysis and wrote the paper.

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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, the mesolimbic dopaminergic system, which pivots around the nucleus accumbens; and second, steroid hormones, secreted by peripheral glands. Testosterone secreted by male gonads enhances risk-taking behaviour in human subjects [239-242]. Extraneous testosterone can be used as a positive reinforcement in rodents [243, 244], suggesting its ability to intersect with the dopaminergic reward system in the brain. Consistent with this, intracranial delivery of testosterone or its metabolites in the nucleus accumbens facilitates conditioned place preference, increasing time spent in the non-preferred, drug-paired side of the conditioned place preference (CPP) apparatus [245]. Animals initially received testosterone or its metabolites prior to being placed in a non-preferred chamber of the CPP apparatus, alternating with receiving vehicle prior to being placed in the preferred chamber during conditioning training days. This suggests that testosterone can activate the mesolimbic dopaminergic pathways involved in decision making under risk.

Interestingly a widely prevalent protozoan parasite [14], *Toxoplasma gondii*, alters both testosterone synthesis [163] and nucleus accumbal dopamine content (see Chapter 3 in this thesis) in laboratory rats. *Toxoplasma gondii* invades the testes in this animal model [206, 246], resulting in a long-term increase of testosterone synthesis [163]. In addition, the infection results in greater synthesis of arginine vasopressin in brain regions afferent to the nucleus accumbens [24], the structural diminution of nucleus accumbens neurons and a decrease in total dopamine concentration [247]. Retrospective studies suggest that chronic *Toxoplasma gondii* infection enhances risky behaviours in human subjects including a propensity to be involved in traffic accidents [248-250].

These observations suggest that *Toxoplasma gondii* increases risky behaviour and impulsivity through the associated increase in testosterone availability. In this chapter, I focus on using the BART to test this hypothesis.

## Materials and Methods

### *Animals and infection*

Male Wistar rats were used. The method of infection used is detailed in chapter 2 and details of animal husbandry in chapter 3 (see pages 41 and 56).

### *Castration and Testosterone treatment*

Surgery was performed using aseptic techniques under isoflurane anaesthesia (2.5% gaseous isoflurane with pure $O_2$). After placing animals in dorsal recumbency, the testes were approached through a mid-scrotal incision. Testes, vas deferens and testicular fat pad were bilaterally removed followed by suturing of spermatic blood vessels. The scrotum was subsequently sutured. One micro-infusion pump was placed subcutaneously supplying either vehicle (grape seed oil) or testosterone cypionate. Microinfusion pumps (iPRECIO SMP-200; Durect) delivered their cargo for several months requiring only monthly refills through the septum of the pumps accessed through subcutaneous route. The 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 the physiological norms of circulating testosterone [251].

Animals were given pre-operative prophylaxis antibiotic (Baytril 10 mg/kg, *s.c.*; Bayer) and pain relief (Carprofen 5 mg/kg, *s.c.*; Pfizer). After surgery, animals were housed singly for >3 days with supplemental pain relief daily (Carprofen 5 mg/kg, *s.c.*). 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.

### *BART procedures*

Operant performance under risk of reward forfeiture was measured using a balloon analogous risk task (BART), adapted from [252] (Figure 4-1). Operant

chambers used for training and testing are similar to the delay-discounting paradigm and detailed dimensions are provided in Chapter 3 (see page 56)

During the 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 one to three and then ten 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; one session/day). The 'add' lever was presented. Animals were required to accumulate a pre-determined number of lever presses (varied randomly between two to fifteen) 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 the completion of training in the phase 3, rats began daily testing on the actual task (Figure 4-1; 54 trials/session, one 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 the 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 3 s

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 analysed for three consecutive days). A stable baseline was observed after 12 to 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 ($\Delta$ 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 signalled by the illumination of a house light during sessions. Trials comprising of risk of reward forfeiture were signalled 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. Mean lever presses during gainful trials were used as the endpoint during both baseline and mixed-risk sessions.

***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-tests were used for orthogonal comparisons between baseline and probe trials. Effect sizes were estimated using Cohen's d [169], with a value of >0.8 interpreted as a strong relationship. The number of animals is noted in figure legends.

# Results

### *Infection increased risk-appetite*

I quantified decision making under risk using a rodent version of the BART (Figure 4-1, [252]). 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 the 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 4-2A, *black*; Table 4-1). I 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.

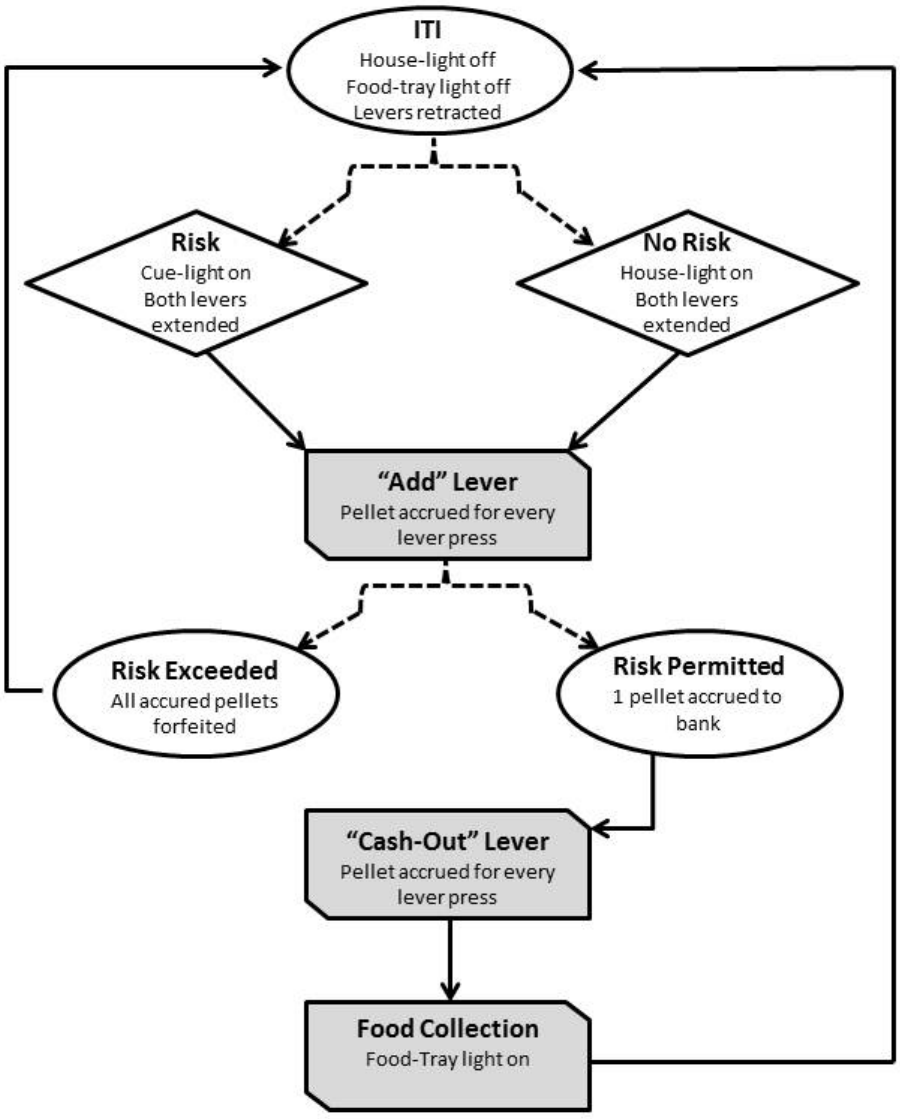



**Figure 4-1 Procedure employed for testing risk aversion using BART. Adapted from [252]. Rats were trained on a stable baseline at 0% risk before introduction of a mixed-risk schedule (probe trials interspersed with 11.1% and/or 16.7% forfeiture probabilities) until stable performance.**

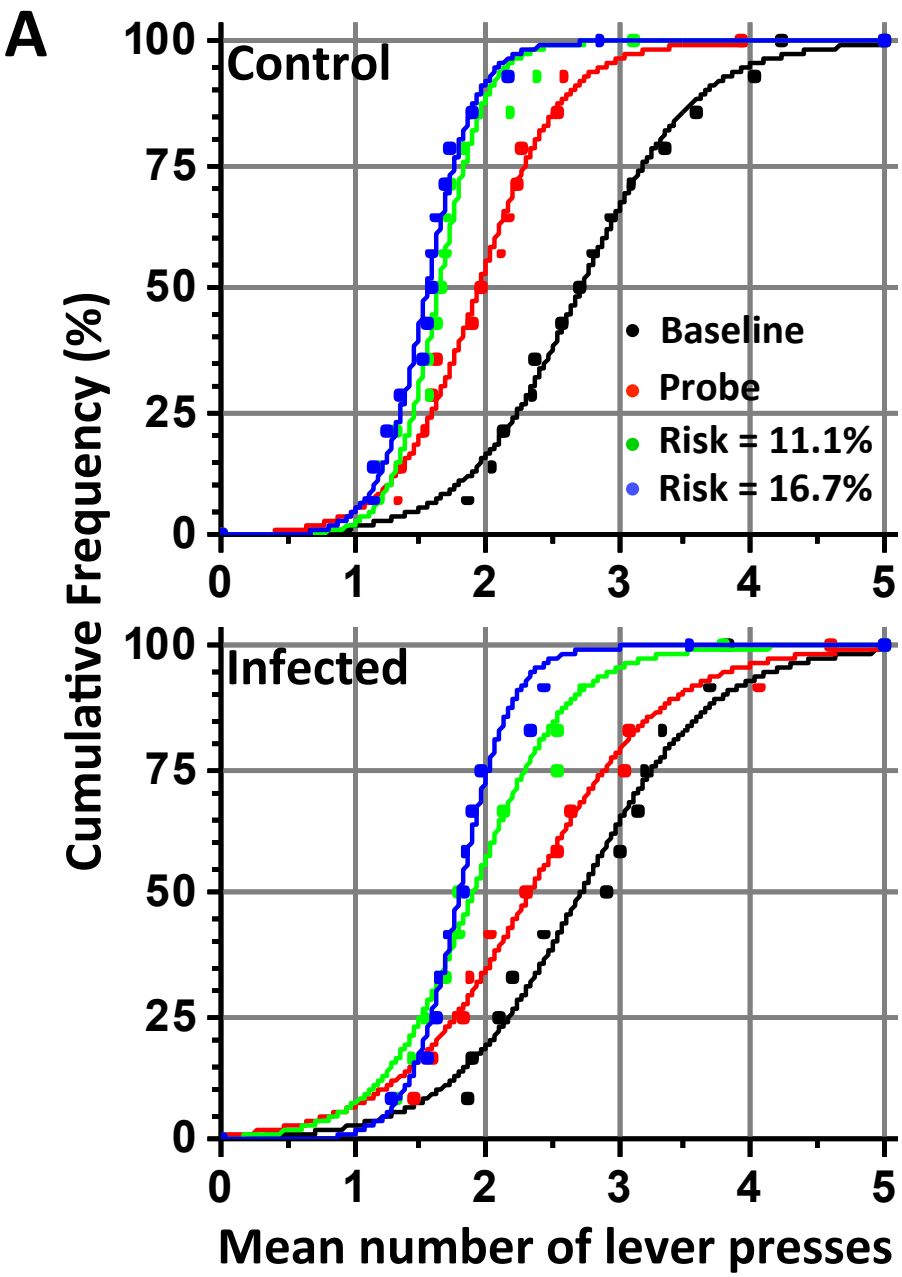
**Table 4-1 Sigmoidal fit for cumulative frequencies of mean lever presses by control and infected animals.**

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

The 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). Main effects of infection status was not statistically significant ($F_{1,24}$ = 0.57, $p$ = 0.457). ANOVA revealed significant interaction between risk and infection status ($F_{1,24}$ = 18.71, $p$ < 0.001). Post-hoc comparisons demonstrated significant suppression of lever pressing by control animals during probe trials, compared to baseline (Figure 4-2B, *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 4-2B, *right*; $|t|_{11}$ = 1.1, $p$ = 0.295; statistical power = 0.95).

**Part 1 of 2 for Figure 4-2**



**Part 2 of 2 for Figure 4-2**

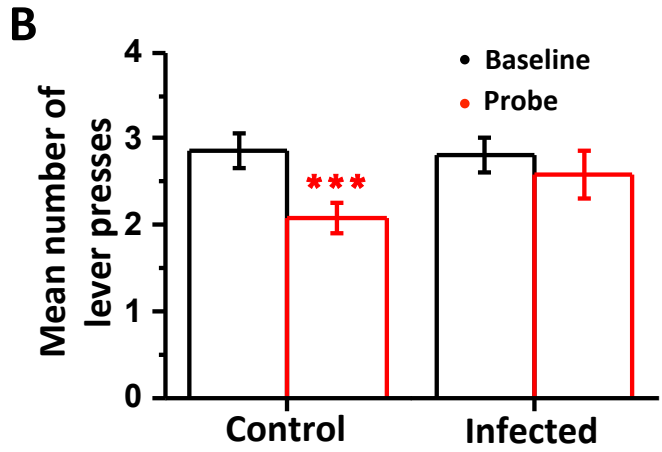



**Figure 4-2 Performance of control rats and *Toxoplasma gondii* infected rats in absence and presence of risk of forfeiture. (A) Only gainful trials are included, excluding mistrials and trials that resulted in forfeiture. Both baseline (*black*) and probe trials (*red*) were identical, except that 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 5-1). 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.**

***Testosterone-supplementation increased risk-appetite***

In view of greater testosterone synthesis by *Toxoplasma gondii* infected animals [163], I 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). Operant responses during baseline were recorded (Figure 4-3A, *black*; Table 4-2), 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.

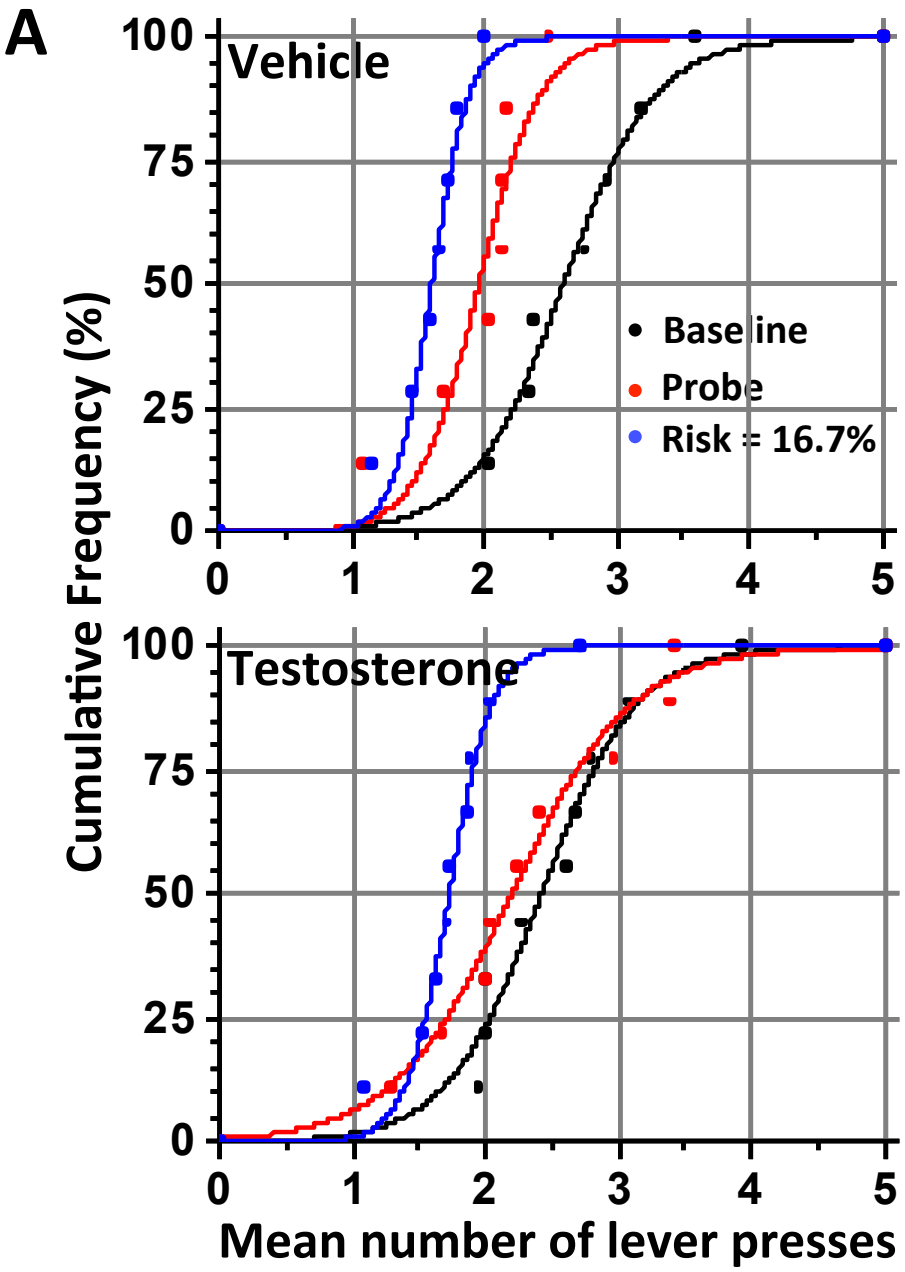
The performance of vehicle- and testosterone-treated animals was compared at baseline and during post-risk probe trials (repeated measure ANOVA). Analysis of variance showed significant main effects of risk in interceding trials ($F_{1,14}$ = 15.55, $p$ = 0.001). Main effect of testosterone status was not statistically significant ($F_{1,14}$ = 0.21, $p$ = 0.651). ANOVA revealed a significant interaction between risk and testosterone status ($F_{1,14}$ = 5.19, $p$ = 0.039). Post-hoc comparisons demonstrated significant suppression of lever pressing by vehicle-treated animals during probe trials, compared to baseline (Figure 4-3B, *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 4-3B, *right*; $|t|_8$ = 1.3, $p$ = 0.229; statistical power = 0.84). Thus, testosterone treatment induced a tolerance to reward forfeiture, similar to *Toxoplasma gondii* infected animals.

**Table 4-2 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; $Y = Bottom + ((Top-Bottom)/(1+ \exp^{(V50-X/Slope)}))$

**Part 1 of 2 for Figure 4-3**



**Part 2 of 2 for Figure 4-3**

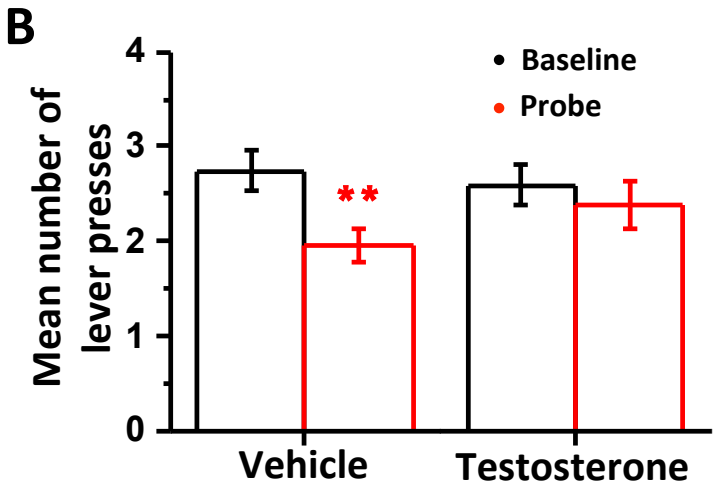



**Figure 4-3 Performance of castrated rats supplemented with vehicle or with testosterone, in absence and presence of risk of reward forfeiture. (A) Only gainful trials are included, excluding mistrials and trials that resulted in forfeiture. The ordinate depicts the cumulative frequency (%) for the 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 4-2). N = 7 for vehicle and 9 for testosterone supplemented animals. Presence of risk of reward forfeiture in interceding trials suppressed operant responding in vehicle-treated, but not testosterone-treated, animals. (B) **, $p < 0.01$, paired Student's t-test with Bonferroni correction.**

## 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 spending in response to a decrease in the probability of reward [253], an increase in the probability of aversive outcome [254] or an increase in risk of forfeiture [252, 255]. Amongst these tests, BART shows good test-retest validity in both human and rodent studies [252, 256]. Moreover, BART exhibits considerable predictive validity in humans. For example, individual variation of riskiness in BART can explain significant variation in self-reported risky behaviours including drug abuse, gambling attitudes, driving sans seatbelts and sexual intercourse with multiple partners sans protection [255, 257, 258]. Similar predictive studies for risk-taking operant behaviour have not yet been conducted; barring a demonstration that pharmacological inactivation of the orbitofrontal cortex reduces risk-taking in this task [252]. 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. Within this framework, I describe that a frequent parasitic infection of humans and animals [14, 259] can decrease risk aversion in a rodent model. Furthermore I show that sustained testosterone rise, akin to that observed in the infected animals and humans [163, 260], is sufficient to reduce risk aversion. 20% to 40% of human beings are infected with *Toxoplasma gondii* [13] with prevalence rates ranging between 10% in the US to 80% in Latin America [261] (17% in Singapore [262, 263]). Traditionally, this infection has been thought of 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 [260, 264, 265]. Retrospective studies show that the infected individuals are more likely to be involved in risky situations like traffic accidents [248-250]. These studies suggest that the infection is associated with risky behaviour, 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 behaviours, and that the increase is related to rise in testosterone post-infection. Given the high incidence of *Toxoplasma gondii* infection, this infection can possibly explain part of the variance observed between individuals for risky behaviours.

I note that the concept of risk has been used in economic and biological literature in two divergent manners [266]. The economic concept of risk centres 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 the probability of aversive outcomes like punishment or loss [266]. In this chapter, I use the later articulation, showing greater tolerance of the infected or testosterone-treated male rats to risk of reward forfeiture. Congruent to prior observations [252, 255], I also show that untreated animals are risk-averse and do not respond optimally as an economically rational actor in the face of the risk. Thus, the effects described here should be viewed as a reduction in risk aversion rather than an increase in risk seeking.

Several studies show that rats infected with *Toxoplasma gondii* lose their innate aversion to cat odours [17]; a phenotype that is believed to increase trophic transmission of the parasite from rats to its definitive cat host (but also [267]). This observation has often been presented as a specific and isolated behavioural change [164, 217, 268, 269]. Yet current observations show that *Toxoplasma gondii* decreases risk-aversion, in addition to increasing approach to potentially risky cat odours. I posit that the effects of infection represent a behavioural syndrome represented by a coordinated reduction in risky behaviours rather than a constrained reduction in kairomonal aversion only [198, 270]. Apropos, prior studies show that treatments that increase testosterone also ‘embolden’ mice by increasing approach to predator odours [271]. Moreover, effects of *Toxoplasma gondii* infection on aversion to cat odour can be rescued by castration pre-infection [163], suggesting that effects of testosterone on risk tolerance presented here are syndromically related to predator avoidance in this model.

Testosterone is reported to have several cognitive effects. Subcutaneous supplementation of testosterone in male rats results in reduced behavioural flexibility, manifested as a reduced ability to shift from previously learned operant rules [272]. Similarly, chronic testosterone increases instrumental responses in rats when greater rewards co-occur with a greater risk of punishment [239]. These studies involve administration of testosterone in gonad-intact male rats. Exogenous testosterone in these cases can result in negative feedback on endogenous androgen production [273], thus potentially complicating interpretations. Human subjects with greater testosterone levels take greater financial risk in the laboratory [92] and accumulate greater financial payoff during risky transactions on the real-world trading floor [242, 274]. Adult men with greater endogenous testosterone make more disadvantageous choices in the Iowa Gambling Task, shown by their preference toward high, immediate, and uncertain rewards despite experiencing larger losses [241]. Adolescent boys with greater endogenous testosterone exhibit a greater tolerance to risk-taking in BART [240]. Cause and effect relationships in these human studies remain understudied because of methodological constraints. Thus, I use castrated animals without an endogenous source of testosterone, thus circumventing the possibility of interaction between exogenously supplied testosterone and testicular steroidogenesis. Thus, I show that the rise in testosterone is *casually* linked to a greater risk tolerance.

The data presented here suggest that *Toxoplasma gondii* infection results in a coordinated increase in the risk-taking behaviour of the host. This provides us with a useful paradigm to better understand biological changes mediating risky behaviours underpinning substance abuse, pathological gambling, attention related disorders and high-risk behaviours [230].

# Chapter 5 Synthesis & Discussion

*Toxoplasma gondii* infection was previously reported to result in loss of fear to predator odours; an effect that has been postulated to increase parasite transmission at the cost of the host. In this thesis I set out to place the effects of the infection on fear in a wider context of decision making or choice. I have discussed specific results and their interpretations in the preceding three chapters. In this chapter, my goal is to provide an overarching interpretative structure for my results.

## Infection and Choice Impulsivity

In this section, I first posit that *Toxoplasma gondii* infection creates impulsivity of choice in the infected male rats. I further present an alternative view that the infection status in humans and animal models explain some of inter-individual variance in decision-making and associated psychiatric disorders.

Impulsivity is often construed as a behaviour that is 'poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation that often result in undesirable consequences', [275] and which 'imperil long-term goals and strategies for success' [276]. Several authors have further differentiated between motor impulsivity and choice impulsivity (see page 74 for more discussion on this). Motor impulsivity refers to the inability to stop a pre-potent motor response. Motor impulsivity is typically measured by the stop-signal task and the go/no-go task where animals are required to withhold from making a prepotent motor response. Choice impulsivity, on the other hand, refers to an inability to optimize outcomes in lieu of immediate gratification. Discounting tasks are generally used for this measure of impulsivity, of which the time domain has been the most-well studied.

In this thesis, I have concentrated on the impulsivity of choice. Briefly, male rats infected with *Toxoplasma gondii* exhibit the following behavioural changes:

a) Effort aversion – increased choice for smaller-easier payoffs;
b) Delay aversion – increased preference for smaller-sooner payoffs; and,

c) Risk tolerance – increased propensity to take risk of reward forfeiture.

All three outcomes above are typically interpreted as impulsive choice in previous literature. This leads me to conclude that *Toxoplasma gondii* infection in male rats leads to increase in choice impulsivity. This conclusion is supported by convergent outcomes from three disparate tasks. Thus, it is more parsimonious to conclude that 'choice impulsivity' is a consilient construct post-infection, rather than three separate changes in the behaviour.

Choice impulsivity has been previously associated with poor medical and psychiatric outcomes. High levels of impulsivity among subjects with psychiatric and personality disorders, substance abuse disorders, pathological gambling disorders and high-risk behaviours have been observed. Impulsivity can be assessed in a variety of ways, generally by the use of self-report personality questionnaires and behavioural tasks. The 11-point Barratt Impulsiveness Scale (BIS-11) is one of the more widely used personality measures amongst self-report questionnaires. Regarding laboratory-based behavioural tasks, delay discounting is the most commonly used and sometimes referred to as inter-temporal choice. Probability discounting and BART have also attracted attention in recent literature. Impulsivity or a lack of impulse control is a prominent feature in psychiatric disorders, typically in attention deficit hyperactivity disorder (ADHD) and bipolar patients (see reviews by [230, 277-280]). These patients have deficits in multiple domains of impulsivity - decreased attention and behavioural inhibition and a large reduction in tolerance for delays [281]. Children with ADHD show a slower response inhibition [282] and ADHD patients show steeper discounting curves in the impulsive choice task [74-76]. Patients with bipolar disorder have higher BIS-11 scores [280] and the impulsive behaviours extend to delay discounting during episodes of depressive and manic period [283].

Substance abusers of stimulants like cocaine or methamphetamine and opiates like heroin report higher impulsivity scores and demonstrate increased discounting manifested as preference for smaller immediate options [77, 78, 284, 285]. Alcohol misuse is similarly associated with the impulsive traits. Both substance and alcohol abusers show high-risk sexual and suicidal behaviours

[286-289]. Such impulsive behaviours also extend to cigarettes and food in smokers and binge-eating disorder patients, respectively [167, 173, 290, 291]. There is robust evidence for steeper discounting and an increase in risky decision-making in pathological gamblers [112, 292]. One general theme across these broad categories show that an impulsive behaviour trait, or the lack of forethought or the willingness to forego long-term (health) benefits for the immediacy of pleasure, might present as an increased vulnerability to addictive behaviours or underlie psychiatric disorders.

The phenotype of impulsivity shows remarkable variation between both humans and rodents. Biological factors underpinning these variations presently remain unclear. The change in choices after *Toxoplasma gondii* infection leads me to speculate that this infection can explain part of inter-individual variance in the impulsive phenotype. Some support for this speculation is available in human literature. Multiple studies conducted across almost 90 countries, including the Czech Republic, Turkey and Mexico, have reported an association between latent Toxoplasmosis and the risk of being involved in a traffic accidents [248-250, 293-295]. Most evidence, thus, relates to traffic accidents and is retrospective in design (barring two prospective studies: [250, 294]). The connection of these studies to impulsivity is based on a purely hypothetical assumption that increased risk-taking results in a greater number of traffic accidents. Thus, the connection of human literature to choice impulsivity cannot be confirmed as yet. I posit that future prospective work will show greater delay-aversion, effort-aversion and risk tolerance in human subjects latently infected with *Toxoplasma gondii*. Moreover, I hypothesize that greater impulsivity in humans will be consequent to prior *Toxoplasma gondii* infection during prospective studies, rather than simultaneous or pre-existing.

*Toxoplasma gondii* infection has been linked as a risk factor to several psychiatric disorders including schizophrenia, bipolar disorder and Parkinson's disease (reviewed in [296-302]. First, being infected puts individuals at greater odds of developing psychiatric disorders (reviewed in [296]). The reported odds ratios range from 1.8 to 2.7 for Schizophrenia, 1.5 for bipolar disorder and 3.4 for

obsessive-compulsive disorder [296, 303]. These odds ratios are based on the concordance between serological evidence of the infection and presence of the disorder. In the case of schizophrenia, these retrospective estimates have been confirmed by at least two prospective studies. In US military personnel, for example, researchers found a positive association between rising *Toxoplasma gondii* IgG antibodies and later diagnosis of the schizophrenia [304]. The same study did not find a meaningful relationship between schizophrenia and herpes or influenza viruses; suggesting that the relationship was specific to the *Toxoplasma gondii* and not related to generalized immune competency. Similarly, a prospective investigation using routine screening of expectant mothers found positive odds of *Toxoplasma gondii*-positive mothers for future development of schizophrenia [305]. Moreover, the infected schizophrenia patients are reported to exhibit more severe symptoms of psychosis than uninfected patients [306, 307].

This also leads me to suggest that future studies should attempt to delineate effects of prior *Toxoplasma gondii* infection on psychiatric conditions known to co-elute with impulsivity, *e.g.* addiction, pathological gambling, risky sexual behaviours and financial risks.

Aspects of choice impulsivity are moderately heritable [308-314]. One common approach to study heritability is by using twins or family associations to examine if an observed behaviour is more likely to be concordant in genetically similar individuals. In longitudinal twin studies of delay discounting and BART, biometric genetic analysis was performed on choice behaviour to estimate the genetic and environmental contributions across ages. In these two studies, increased impulsivity measures were found to be correlated within monozygotic (but not in dizygotic) twin pairs across ages tested. Similarly, animal studies of delay discounting and BART show significant variability between inbred rat strains. Since inbred strains are assumed to be genetically identical, differences in discounting or risk-taking behaviours suggest a genetic contribution to choice impulsivity [308-314]. Heritability of choice impulsivity does not preclude the notion that *Toxoplasma gondii* infection predisposes individuals towards

impulsive behaviours. Heritability measurements are specific to environmental context. Moreover, heritability of impulsivity could be linked to heritability of predisposition to infection.

## Post-infection impulsivity as a behavioural syndrome

In the previous section, I argued that *Toxoplasma gondii* infection in male rats increases impulsivity of choice. I further proposed that future work might show that the infection status in humans and animal models explain some of inter-individual variance in decision-making and associated psychiatric disorders. In this section I present my observations in the context of the possible adaptive value of the impulsivity. Subsequently, I posit that choice impulsivity post-infection is related to previously reported effects of *Toxoplasma gondii* infection in a syndromic manner, rather than these being independent effects of the infection.

Institution of choice impulsivity after the infection does not automatically equate with a mal-adaptive outcome for the host. Firstly, the impulsivity itself could reflect a naturally selected optimization of the behaviour. When seen in isolation, delay aversion and effort aversion indeed look like sub-optimal strategies. For subsequent discussions, I will use delay aversion as an exemplar for the discussion, although similar logic can also be extended to effort aversion. For example, an economically rational actor will consistently choose larger later rewards in delay discounting model used for my experiments. As the start of each successive trial remains constant regardless of choice exerted in the previous trial, the total payoff is always higher when delayed gratification is preferred. And yet, the decisions in this task must be viewed in the context of foraging decisions in naturalistic settings [315]. Stevens and Stephens [55] summarize this argument eloquently. Using a patch-exploitation paradigm often used in the discussion of optimal foraging, these authors examine a strategy that results in ‘long-term rate maximization’. The underlying assumption is that historical natural selection will optimise strategies that maximize the amount of food gathered per unit of time over a long time frame – a reasonable assumption that would predict choice for the delayed reward for the task used in this thesis. Stevens and Stephens [55] highlight that unlike constant inter-trial intervals experimentally imposed in laboratory experiments, both travel time between patches; and delays between successive payoffs within same patch, vary in the case of patch exploitation. Depending on the richness of environment, rate maximization might

be achieved at any point of the continuum between a delay-averse 'skim-the-surface' to a delay-tolerant 'lick-the-bowl' strategy. Thus delay aversion (or delay-tolerance) is not inherently a non-optimal solution. Rather, environmental conditions dictate the economic optimization.

Environmental influences on the adaptive value of impulsivity thus, lead to a second important direction. In situations where the environmental context is predictable between generations over a long time, selection pressure might be expected to shift the decision making towards extremes of continuum between delay-aversion and delay-tolerance. An extreme form of this adaptation is found in star-nosed moles where selection for progressively smaller prey with lower handling time has reached adaptive perfection constrained only by anatomical and physiological considerations [316]. However, if the environment itself fluctuates between richness and sparseness through time, selection pressure is unlikely to be consistent. Several strands of convergent evidence show that selection pressure in these cases lead to conditional decisions; or 'if-then-else' kind of programs executed during the ecological lifetime of an individual organisms. In other words, natural selection acts on the flexibility of decision making rather than the presence or absence of impulsivity *per se*. To make this point palpable, let us consider growth rates in adolescent tree squirrels (*Tamiasciurus hudsonicus*). Availability of food for squirrels varies between years. This environmental difference leads to yearly variation in population density, further leading to variation in inter-individual competition [317-319]. During episodes of high population density, adolescent squirrels grow rapidly and reach sexual maturity earlier [317, 320]. This can be viewed as a delay-averse strategy that increases current sexual payoff at the cost of future/residual reproductive payoffs [321]. Interestingly, endocrine, rather than genetic differences underlie this shift. Exposure to maternal environment of actual, or perceived, high density predisposes F1 offspring to faster growth [317]. In other words, prior selection has likely resulted in a conditional behaviour ('if' high population density, 'then' grow faster, 'else' slower), rather than any particular extreme of growth rate itself [322]. Similar examples can be found in cases of maternal care by rat dams, classical conditioning in rat pups and stress reactivity in humans [188, 323-327].

Effects of *Toxoplasma gondii* on rat impulsivity should be seen in that context. It is likely that rats have a pre-existent flexibility, rather than a fixed strategy, in decision making. This flexibility results in impulsive choices, manifested here as excessive preference for 'me-now' behaviour. This collection of behaviour plausibly maximizes current fitness at the cost of future fitness. In reverse, assemblage of 'me-later' decisions maximizes future at the opportunity cost of today [55, 328]. Host behaviour post-infection can then be seen as a syndromic shift of behaviours on this continuum. It will be interesting to view the shift from vantage points of the host and the parasite separately. *Toxoplasma gondii* is a trophically transmitted parasite, associated with a lower fear of intermediate hosts to definitive predator host. Increase in trophic transmission by risk-prone choices by the host will likely decrease host longevity [28, 329]. Decrease or perceived decrease in life span is known to make animals more impulsive [321, 330]. For example, fatally infected hosts often resort to a 'terminal investment' in testosterone [331, 332]. This is because current investment in testosterone often trades off with longevity itself. This terminal investment is interpreted as manifestation of 'me-now' conditional behaviour in face of bleak 'me-future'. Similarly, human beings in poorer health, lower survival probabilities and lower social economic status (lower income, lower education) exhibit more impulsive choices in the delay-discounting task [333-337]. This suggests that the perceived length of residual life expectancy rather than the accumulated biological/psychological changes during ageing explains the shift in impulsive choice. Related to this, experimentally induced greater brood size in European starlings (*Sturnus vulgaris*) congruently leads to greater telomere attrition (an integrative marker of lower residual life) and delay aversion [330]. In this backdrop, I suggest that lower life expectancy associated with *likely* lower fear to predator odours in the infected rats drive impulsive decisions. In other words, 1) lower fear to predators and greater impulsivity reflect a behavioural syndrome; and that 2) such a syndromic shift reflects a conditional programme that maximizes host payoffs in face of increased mortality post-infection.

The chances of a successful transmission for the parasite increases when more dimensions of host behaviour are being manipulated or changed by the parasite

itself [270]. Focus on ‘multidimensionality’ in host manipulation has centred around two main themes [198, 270, 338, 339]. A panselectionist-based narrative laces its focus on the independent alterations that increase the parasite’s fitness. In contrast, a syndromic narrative takes into account the cascade of infection effects in approaching the topic of ‘multidimensionality’ (see [270, 340, 341] as well as [198, 339] for in-depth discussions of both definitions). For example, ants infected by *Dicrocoelium dendriticum* are manipulated on various levels, including a propensity to climb higher on the tree cover and a morphological change with its belly turning bright red. Both phenotypic manipulations have adaptive values for the parasite. In another instance, *Gammarus pulex* infected with *Pomphorhynchus laevis*, manipulation occurs across various levels resulting in phototaxis, activity, geotaxis and attraction to conspecifics resulting from a syndrome of serotonin sufficiency [342]. These multitudes of changes can either be seen as independent responses to natural selection arising separately (panselectionism) or consilient outcomes for a single cascade connected through shared proximate mechanisms and driven concurrently during selection (*i.e.* a syndrome). Observations in this thesis lead me to propose a syndromic interpretation as discussed below.

The post-infection syndrome of reduced fear and greater impulsivity affects multiple dimensions of host behaviour. Further aspects of change in the infected rats show higher activity level, reduced fear of novel stimuli and decreased predator avoidance/vigilance behaviour [15, 17, 219]. Other than the range of behavioural changes observed in the infected animal, atypical activation of arginine vasopressin neurons in the medial amygdala in response to cat odour, higher levels of male urinary proteins and testosterone [24, 163, 343] and a reduction in total nucleus accumbens core dopamine levels add to the multitude of observed changes in the *Toxoplasma gondii*-infected intermediate host. These lend support that multidimensionality could arise partly from a neuromodulatory disruption by the parasite in the host’s neural circuitry that results in a coordinated shift of behavioural and physiological phenotypes in a syndrome of reduced fear, increased sexual attractiveness and greater impulsivity [198, 270, 339].

## Endocrine Mechanisms

In previous two sections, I have tried to present my observations in a wider context of impulsive choices. In this section I will discuss potential hormonal substrates of impulsive choice. In the next section, I will focus on neural substrates.

*Toxoplasma gondii* invades immune-privileged organs during the latent phase of the infection. Much of the work on behavioural manipulation has focused on the invasion of the central nervous system. For example, several studies have carefully analysed potential tropism (or lack of tropism) of *Toxoplasma gondii* cysts for specific brain regions [164, 207, 233, 235, 236, 344]. Subsequently it became apparent that *Toxoplasma gondii* also invades testes in male rats [163, 206]. Similar observations were also reported in dogs, sheep and rabbits. Like the brain, testes are immune-privileged organs [345-347]. Because developing haploid sperms have different antigenic personality than diploid male, spermatogenesis requires a sustained suspension of the immune system. This is achieved by the production of testosterone by Leydig cells in the testes; testosterone being an immune-suppressive steroids. Additionally a tight blood-testes barrier prevents easy access to developing sperms by immune cells. Thus, the entry of *Toxoplasma gondii* in the testes presents the parasite an opportunity of evasion from the immune system. In fact, cyst burden in the testes of male rats exceeds that in the brain [163, 206].

After encystment, *Toxoplasma gondii* increases steroidogenesis in Leydig cells of the testes [163]. This is achieved by increased abundance of key enzymes involved in the reception of endocrine signals from the pituitary and also in enzymes mediating the conversion of cholesterol to testosterone. Autocrine and/or paracrine mechanisms involved in this metabolic effect presently remain unknown. The rise in testosterone is necessary for behavioural effects of the infection. This is supported by the observations that *Toxoplasma gondii* infection does not result in a loss of fear in castrated animals [163]. Retrospective cohort-based studies show that *Toxoplasma gondii* infection in immune-competent

humans is also associated with greater circulating testosterone [260, 348]. Infected male humans also exhibit anatomical features reminiscent of greater testosterone [349], like greater dominance perceived by females which is likely mediated by facial symmetry which is a sexual advertisement in male humans [350].

In the rat model, infected rats show greater testosterone during the chronic phase of the infection. This is supported by direct quantification of the steroid in blood and testes [163]. This increase is also supported by parallel evidence that biological substrates responsive to testosterone also change. This includes an increase in sexual pheromones produced by the liver and extra-hypothalamic arginine vasopressin in the brain [24, 351]. These changes are known to reflect an increased supply of testosterone in rats.

Interestingly, testosterone has been known to increase risk tolerance. For example, chronic testosterone increases instrumental responses in rats when greater rewards co-occur with a greater risk of punishment [239]. Human subjects with greater testosterone levels take greater financial risks in the laboratory and accumulate greater financial payoffs during risky transactions on the real-world trading floor [242, 352]. Adult men with greater endogenous testosterone make more impulsive risky choices in the Iowa Gambling Task and adolescent boys with greater endogenous testosterone exhibit a greater tolerance to risk of reward forfeiture in the Balloon Analogous Risk Task (BART) [240, 353]. The role of testosterone in the delay-discounting task has been investigated in gonad-intact adolescent rats. In this paradigm, exogenous testosterone seems to reduce impulsive decisions by increasing choices of larger-later rewards [354]. This discrepancy could result from the negative interaction between exogenous testosterone and gonadal steroidogenesis [273].

Using BART, I show that exogenous testosterone in castrated rats is sufficient to institute risk tolerance in adult male rats. Interestingly observations from my colleagues in the laboratory show that sustained intra-cranial delivery of testosterone in castrated rats also results in reduced fear to predators akin to that observed post-infection. These observations show that testosterone is *sufficient*

to induce behavioural effects found after *Toxoplasma gondii* infection. It is also plausible that testosterone is the common link between the syndromic shift in the behavioural change in fear and impulsivity. In fact, greater testosterone and/or sexual advertisement, known to be dependent on high testosterone, seem to co-elute with myopic life histories characterized by an emphasis on 'me-now' over 'me-later' [355-360]. While I demonstrate *sufficiency* of testosterone for at least one form of choice impulsivity, my work does not prove that rise in testosterone after the infection is *necessary* for the behavioural syndrome. That experiment would involve surgical intervention after the infection has reached its latent phase. Surgical stress at that phase is likely to result in the reactivation of acute infection, thereby confounding the interpretation. Future work should aim to circumvent this limitation.

The focus of discussion on testosterone raises an important question. Testosterone is primarily a male steroid; adrenals and ovaries in females synthesize very small amounts. One would assume the blunting of behavioural change in the females if such effects are dependent on the testosterone. Only one rat study has addressed this question, showing that the infection does result in behavioural change in females, akin to that in males [361] (see [362-365] for work done in female mice). Mechanisms of behavioural change in females remain much understudied. This puzzle is ripe for future experimental work.

*Toxoplasma gondii* infection also results in lower basal corticosterone levels in male rats [165]. This endocrine effect is congruent with dendritic atrophy in basolateral amygdala [165], a brain region exquisitely responsive to circulating corticosterone. Corticosterone is primarily synthesised in the adrenal glands, an endocrine organ that is not immune-privileged and does not harbour parasites during latent infection. One possibility is that reduced corticosterone reflects negative feedback on androgen along the hypothalamic-pituitary-adrenal axis. The existence of such a negative feedback is well known in prior literature [366, 367].

## Neural Mechanisms

In the previous section, I have summarized and discussed possible relationships between hormones and the observed impulsive choices post infection. Here, I will shift the focus of discussion to the neural substrates of impulsivity.

The quality of decisions has a direct impact on the quality of life and impulsive choices generally result in non-optimal rewards outcomes. Thus, a main focus of the neuroscience of decision making is in defining neural elements and delineating the neural circuitry involved in choice impulsivity. Impulsivity is a multi-faceted concept (see pages 74 and 101 for more discussion on this) and in this section, I will focus my discussion only on choice impulsivity. Much of the work identifies a network of cortico-straital-limbic connections important in mediating choice (see page 29 in the introduction for a brief description of the organization of the cortico-striatal-limbic network). One of the central nodes within this circuit is the nucleus accumbens (NAc), postulated to be the site of signal convergence and integration of the reward network. The NAc is made up of two sub-regions – core and shell, with each sub-region having discreet roles in impulsivity. Evidence for the role of the NAc in decision making comprises of lesions and pharmacological studies showing changes in the impulsivity.

### *Nucleus Accumbens*

Much of the work on the NAc has focused on either the core sub-region or the NAc as a whole. Excitotoxic lesions and pharmacological inactivation studies of the core sub region of the NAc reduced preference for the effortful choice [368, 369]. Rats with inactivated or lesioned NAc were less willing to climb a barrier for a greater reward. The NAc has been shown to influence choice between different effort costs with a functional NAc being necessary to overcome higher costs. NAc neurons are responsive to food and rewarding stimuli [370, 371]. Neuronal firing activity in discreet subgroups within this nuclei have been shown to respond differently to various stages in decision making, primarily during cue-presentation; response initiation or reward delivery and action of choice [372]. Electrophysiological recordings were analysed for changes in firing pattern during

the three behavioural events, relative to the stages of the decision process. A subgroup of NAc neurons demonstrated phasic increases in firing rate during cue presentation. Importantly, this large population of neurons showed increased activity during low-cost trials, over high-cost trials, even before a response was initiated. Other subgroups maintained changes in firing while completing the action required for the higher-value/cost. These studies show that neurons within the NAc are sensitive to the subjective value (cost-discounted) of the costly option [372-375] as well as the act of choosing (choice selection) the higher effort option [369, 372]. In addition, these neurons also encode anticipatory responses and reward expectations [141, 376, 377] thus the normal functioning of the NAc is necessary for reward representation especially for an effortful choice.

NAc lesions alter delay-based decision making toward impulsive choices [116]. Lesioned rats preferred the smaller sooner reward to the larger later reward. Interestingly yet when a variant of the delay-discounting task is used where instead of having a fixed smaller sooner reward with progressive increase in delay, the amount of the small reward is adjusted based on the preceding choice with delay fixed, NAc-lesioned rats did not show this increase in impulsivity and performed the same as sham-treated rats [378]. Acheson suggests that NAc lesions impaired the ability of rats to adapt to changes rather than the ability to tolerate longer delays. However there are other differences in the both Cardinal's and Acheson's studies other than the delay-discounting task. The area of lesion was different and Acheson included the shell regions. The positive reinforcement used also differed; food was used as a reward in the former whereas water was used in the latter. NAc lesions, in addition to influencing impulsive choice, impair learning in tasks when delays are imposed between action and reinforcing outcome [135]. This delay-imposed learning impairment highlights the role of the NAc in time-based choice and learning tasks [116, 135, 136].

Studies of NAc involvement in risk-based decision making tasks have shown that lesions or inactivation of this nuclei results in a risk-averse choice in probabilistic-discounting tasks where rats chose small-certain over large-risky choice in probabilistic discounting tasks [253, 379]. NAc's role in risk-based decision

making is postulated to be less of overcoming risk-aversion but more of influencing future risky choice due to an response action bias that is dependent on the history of rewarded actions [253, 379].

The direction of behavioural effects observed in my thesis after *Toxoplasma gondii* infection is similar to that observed after NAc lesions or inactivation. Infected rats, like the rats with inactivated or lesioned NAc, were less willing to climb a barrier or lever-press more for a greater reward. This thesis also presents evidence that the infection leads to reduction in spine density of NAc neurons, in addition to reducing dopamine content in this brain region. While a decrease in spine density is not exactly the same as an inactivation or lesion, it is plausible that the reduced spine density of NAC core 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.

Thus prior work with NAc damage agrees well with diminution of NAc post-infection, in domains of their effect on effort and delay aversion. In contrast, the infection increases risk tolerance in my observations, while prior studies report reduced risk tolerance after NAc damage/inactivation. These studies differ in their design from the task used in my thesis. Many studies have used probability-discounting tasks comparing options with different dispersion in outcomes around same central tendency. This conception of risk is similar to the classical sense of risk in economic literature (see page 99 for a discussion on this). Instead, I use risk of reward forfeiture measures in a balloon analogous risk task. This is because this task uses the concept of risk as a behaviour that results in probable loss and resonates with naturalistic concept of risk-taking. Tasks based on the economic concept of risk as variance have enjoyed only limited success in predicting inter-individual variability in naturalistic risk taking in humans [266]. It is likely that the incongruence between my observations and previously reported effects of the NAc on risk reflect differences in conception of the risk as forfeiture probability and as variance, respectively.

The NAc has numerous connections to other brain regions within the cortico-straital-limbic circuit. The effects of *Toxoplasma gondii* infection on neural substrates include changes in two brain regions – nucleus accumbens and basolateral amygdala (BLA). Both of these are critical nodes within the cortico-striatal-limbic circuit, and are also key neural elements involved in decision making. I will now discuss the role of BLA in impulsive decision making.

***Basolateral Amygdala***

Similar to the findings presented for the NAc, pharmacological inactivation of the BLA increases impulsive choice and reduces the preference for the larger effortful reward regardless in tasks that involve climbing barrier or repeatedly pressing levers [124, 160]. The BLA is involved in associative encoding of rewards and forms part of the neural circuit important in using incentive processes to guide behaviour. Thus a functional BLA is crucial in forming associations between a response and the incentive value of the outcomes of the responses [125-128]. Together, these studies suggest a role of the BLA in biasing choice toward effortful rewards [124] and in reward representation and valuation [130-132].

Delay-discounting studies have demonstrated that the loss of function of the BLA results in an increase in impulsive choice [117]. In a delay-discounting assay, lesioned rats chose the smaller immediate reward more often. The results of the BLA lesion mirror NAc lesions in that it increases impulsive choice in rats [116]. Role of the BLA has been suggested in maintaining the representation of the reinforcer (i.e. the larger reward) over the duration of the delay and the ability to maintain this ‘online’ representation of the larger reward helps guide behaviour [130-132].

My finding of effort-aversion and delay-aversion post-infection is similar to other studies investigating the role of BLA in impulsive decision making. Plausibly, dendritic retraction in the BLA due to *Toxoplasma gondii* infection plays a role in this congruency, in addition to the infection effects in the NAc. Interestingly, NAc and BLA are inter-connected through reciprocal axons. For example, interruption

of communication between the BLA and NAc with GABA agonists infusions leads to the conclusion that both BLA and dopaminergic inputs are required to augment NAc neuronal activity which, in turn drives behavioural responses to rewarding cues [137, 144].

***Interactions with the Dopaminergic system***

In *Toxoplasma gondii*-infected rats, brain neurochemistry is altered with a reduction in total content of dopamine, specifically in the nucleus accumbens core but not in the immediate neighbouring regions. The exact mechanism for dopamine changes is currently unknown (see page 79 for a discussion on the neurotransmitter change in chapter 3).

However it was recently reported that *in vitro* dopamine amount in infected cells increased yet this did not translate to an increase in spontaneous release, resulting in an overall net decrease in dopamine release (spontaneous release relative to the total dopamine synthesized) from infected cells [222]. In addition, host machinery required for DA synthesis was recruited into the parasite's compartment past its cyst wall [227]. Future studies along this line of investigation would unravel the mechanism of dopamine changes in the infected rat.

Studies in animals implicate the role of dopamine in impulsive decision making. The results have been conflicting. Haloperidol, a dopamine antagonist, also increases impulsive choice in the delay discounting task [380]. Drugs that increase dopamine release like amphetamine and methylphenidate, typically decrease impulsivity in the delay discounting tasks [63, 160, 381-385] yet the effects of these drugs have also been shown to increase impulsivity in the same task [386-389]. Dalley [390] reported that D2 receptors are reduced in the NAc in impulsive rats. In line with this, D1 and D2 receptor antagonist administered peripherally increase impulsivity [160, 382, 384]. In humans, greater catechol-O-methyltransferase (COMT) activity – a dopamine-degrading enzyme – is associated with increased impulsivity [391]. Administration of tolcapone, a COMT inhibitor, which increases dopamine levels, reduces impulsivity. Despite the conflicting results with amphetamine, generally decreasing dopamine levels show

an increase in delay-based impulsivity. The effects of dopamine on effort-based impulsivity are less conflicting – an increase in dopamine leads to an increased willingness for high effort/reward choice thus a decrease in dopamine results in increased impulsivity [159, 172]. This agrees with the observed reduction in dopamine content in the impulsive infected rats.

The impact of dopamine on cognitive functions has been suggested to be in an inverted U-shape where both low and high levels of dopamine result in impaired performance in working memory tasks (see review [392]). This suggests that there is likely an optimal level of dopamine associated with peak performance and Floresco [393] similarly reviewed that the effect of a change in dopamine levels is not a linear function. The ambiguity in the dopamine-impulsivity literature might possibly be better examined for an optimal range rather than focusing on a linear relationship of dopamine and performance in decision-making tasks.

Intriguingly, *Toxoplasma gondii* genome contains two amino acid hydroxylase genes that are surprisingly similar in sequence to mammalian tyrosine hydroxylase, a rate-limiting enzyme in the dopamine synthetic pathway [221]. The protein product of these parasite genes has been demonstrated in infected mice brains, and parasitic cysts in mice brains exhibit robust immunoreactivity to dopamine antibodies [220]. It is unknown if the decrease in the nucleus accumbens dopamine reported by us is derived from the host or the parasite tyrosine hydroxylase. *In vitro* infection of mammalian dopaminergic cells by the parasite results in a robust increase of dopamine synaptic release [220] (see page 79 for a detailed discussion of this anomaly).

# Conclusion

The *Toxoplasma gondii* – *Rattus novergicus* parasite-host association has been widely studied as an example of parasitic manipulation of host behaviour. The focus has been on the fear manipulation hypothesis and the 'fatal attraction' in *Toxoplasma gondii* infected rats [17, 164, 189] to cats. The central theme in my thesis is developed around decision making as I attempt to shift the narrative of parasite manipulation from fear towards choice impulsivity. I conclude this thesis with the main findings of my research as defined in my specific aims and propose two new approaches for interpreting future *Toxoplasma gondii* research.

1. **Infection caused a change in decision making in males rats. Infected males are effort-averse, delay-averse and more willing to take more risk** (see chapters 2, 3 and 4)**.**

This is the first demonstration that Toxoplasma gondii infection changes host behaviour in decision making. The impulsive choices demonstrated by the infected host are the result of a discreet preference in not wanting to work harder or wait longer for a larger reward. This includes a preference for accepting risk. I have extended the present infection-induced behaviour paradigm of fear towards choice

2. **Infection caused a change in the neural substrates of decision making. Infected males have reduced dopamine levels and spine density, specific to the nucleus accumbens core** (see chapter 3)**.**

I show that the concomitant change in neural substrates is specific to the mesolimbic dopamine system. This further supports my hypothesis that *Toxoplasma gondii* infection affects decision making. The mesolimbic dopaminergic system is involved in rewards, motivation and mediating choice impulsivity [113]. The nucleus accumbens is a central node within this network postulated for its role in signal integration.

3. **Risk impulsivity can be mediated by sustained increase in testosterone. Infected males and non-infected castrates supplied with exogeneous testosterone show increased risk-tolerance** (see chapter 4)**.**

I have demonstrated 'sufficiency' that increased testosterone can result in increased risky choice behaviour, similar to *Toxoplasma gondii* infected rats. This also identifies another biological substrate mediating the post-infection shift in impulsive behaviour.

My research on '*Toxoplasma gondii'*-induced changes in 'decision making', spans two disciplines. These two fields are almost as different as *different* can be. Parasitologists study parasites, hosts and their interaction, focusing on ecology. Behavioural neurobiologists study the brain and the biology of the nervous system, looking at connections, neuronal activities and how these drive behaviour. Decision making spans research in many fields, from politics to finance and mental health. My focus on decision making – a field that spans even more disciplines – it further opens the divide between these fields. Yet based on my findings, I propose an opportunity to marry these disparate fields. I also include two considerations for future approaches towards using *Toxoplasma gondii* as a perturbation model, in respective fields.

4. ***Toxoplasma gondii* infection can be viewed as a syndrome of 'multidimensional' change. Infection affects a change in multiple dimensions of host behaviour. Infected males show a coordinated shift in behaviours and physiology** (discussed in chapter 5)**.**

My findings, on the whole, introduce another dimension of host-parasite manipulation. My observations suggest that changes in host behaviour post-infection are part of a wider behavioural syndrome providing evidence for a host infection syndrome induced by *Toxoplasma gondii*. I propose that correlative comparisons on effect size on individual behaviour traits altered by *Toxoplasma gondii* should provide further evidence for the syndromic narrative.

5. ***Toxoplasma gondii* infected rats as an impulsive phenotype. Infected males present an ensemble of choice impulsivity and decision making neural correlates** (discussed in chapter 5)**.**

Prior studies on decision neuroscience have focused on the role of specific brain regions and connectivity between regions using lesions and inactivations. While these methods have been successful, the effects of total 'knock out' are drastic. In addition, results are incomplete and sometimes conflicting [117, 118]. My results show concurrent changes in choice impulsivity, with dopamine and neuro-structural changes converging on nucleus accumbens core. Together with the dendritic retraction observed in the basolateral amygdala [165], the effects of *Toxoplasma gondii* infection have highlighted changes in two brain regions – nucleus accumbens core and basolateral amygdala. These are the main nodes, and provide important connections, within the cortico-striatal-limbic circuit. Dopamine is a key neurotransmitter involved in decision making. The concordance between these variables suggests a 'conformity to *a priori* expectations based on purported function' [9]. In other words, it is unlikely that the increase in choice impulsivity is an accidental by-product of the infection, by virtue of it being accompanied by non-generalized changes in biological substrates known *a priori* to be involved in the choice behaviour. I propose the use of *Toxoplasma gondii* as a naturally occurring parasitic perturbation model for choice impulsivity. As cliché as it sounds, perhaps the parasite knows more about *impulsivity* than the neuroscientist. It has had the help of 'natural selection' over millions of years refining the mechanisms to control host behaviour [394]. The *Toxoplasma gondii – Rattus novergicus* model is currently under-explored yet holds much potential in understanding the neural circuitry and mechanisms of decision making.

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*'Parasites: Evolution's Neurobiologists*

*— SHELLEY ADAMO [394]*

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