Supplemental Material

CBE—Life Sciences Education

Nelms and Segura-Totten

Appendix 1

Student Demographic survey

Name:

Student ID number:

Address (where we should mail your gift card if you win the raffle):

Major:

Age:

Are you: male / female (circle one)

Race: White / African American or Black / American Indian or Alaska Native / Asian / Native Hawaiian or Other Pacific Islander (circle one)

Ethnicity: Hispanic or Latino / Not Hispanic or Latino (circle one)

Answer the following questions to the best of your recollection:

Approximately how many of the biology courses you have taken include reading research papers?

Please list the courses and when you took them (freshman, sophomore, junior, senior year)

Estimate how many research articles you have read in college:

Are you a: freshman / sophomore / junior / senior (circle one)

Expected graduation date:

GPA:

Appendix 2 – Research article for think aloud exercise with line numbers.

THE ROYAL SOCIETY

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Fatal attraction in rats infected with *Toxoplasma gondii*

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We tested the hypothesis that the parasite *Toxoplasma gondii* manipulates the behaviour of its intermediate rat host in order to increase its chance of being predated by cats, its feline definitive host, thereby ensuring the completion of its life cycle. Here we report that, although rats have evolved anti-predator avoidance of areas with signs of cat presence, *T. gondii*'s manipulation appears to alter the rat's perception of cat predation risk, in some cases turning their innate aversion into an imprudent attraction. The selectivity of such behavioural changes suggests that this ubiquitous parasite subtly alters the brain of its intermediate host to enhance predation rate whilst leaving other behavioural categories and general health intact. This is in contrast to the gross impediments frequently characteristic of many other host–parasite systems. We discuss our results in terms of their potential implications both for the epidemiology of toxoplasmosis and the neurological basis of anxiety and cognitive processes in humans and other mammals.

Keywords: *Rattus norvegicus*; *Toxoplasma gondii*; parasite manipulation; cat odours; anxiety; predation

1. INTRODUCTION

According to the manipulation hypothesis, a parasite may alter the behaviour of its host for its own benefit, usually by enhancing its transmission rate. The hypothesis implies that such host behaviour modification represents a sophisticated product of parasite evolution aimed at host manipulation, rather than an accidental side-effect of infection (Barnard & Behnke 1990; Poulin 1994). Parasites that are transmitted through the food chain constitute classic examples of such manipulation: the parasite is immature in the intermediate host and must be eaten by a predatory definitive host before it can reach maturity and complete its life cycle. Unfortunately, however, many studies have either attached little importance as to whether the host in question normally carries the parasite and/or studied hosts maintained under highly unnatural laboratory conditions. The transferability of such studies and their applicability to the epidemiology and evolution of disease in the wild may thus be open to question (Moore & Gotelli 1990; Webster *et al.* 2000).

The host–parasite system *Rattus norvegicus–Toxoplasma gondii* provides a convenient model in which to examine such questions. *T. gondii* is an intracellular protozoan (Beverley 1976) capable of infecting all mammals. Its associate disease, toxoplasmosis, is of significant economic, veterinary and medical importance (Luft & Remington 1986; Schmidt & Roberts 1989) and has sparked renewed interest due to its debilitating reactivation in AIDS and other immunosuppressed patients (Luft & Remington 1986). *T. gondii* has an indirect life cycle, where members of the cat family are the definitive hosts of the parasites and the only mammals known to shed *T. gondii* oocysts with their faeces (Hutchinson *et al.* 1969). If the oocysts are ingested by another mammal such as a wild rodent (the intermediate host) small thin-walled cysts form in various tissues, most commonly the brain. Such cysts remain viable for the life of the host (Remington & Krahenbuhl 1982). A cat can therefore become infected by either of two routes: it may directly ingest oocysts shed from another cat in the environment, or it may ingest cysts when eating infected intermediate-host prey (Hutchinson *et al.* 1969).

Previous field and experimental studies demonstrated that wild rats represent a significant and persistent intermediate-host reservoir for *T. gondii*, with a mean prevalence of 35% across all populations irrespective of environmental conditions and maintained, at least in part, through congenital transmission (Webster 1994*a*). It may thus be feasibly expected to benefit the *T. gondii* parasite if it could somehow enhance the transmission rate from this large intermediate-host reservoir to the cat definitive host, and so complete its life cycle. Moreover, since sexual reproduction of *T. gondii* can be accomplished only in the feline, there might be strong selective pressure on the parasite to evolve such a mechanism.

Indeed, there are several reasons to predict that the *T. gondii* parasite may be able to achieve this. Principally, the formation of parasitic cysts in the brain of its host places *T. gondii* in a privileged position to manipulate behaviour (Werner *et al.* 1981). Accordingly, recent studies on both wild and wild–laboratory hybrid rats have demonstrated that *T. gondii* causes an increase in activity (Webster 1994*b*) and a decrease in neophobic (fear of novelty) behaviour (Webster *et al.* 1994; Berdoy *et al.* 1995*b*), both of which can be argued to facilitate transmission to the felid definitive host. In contrast, other costly behavioural patterns such as competition for mates and social status (Berdoy *et al.* 1995*a*), which do not have any obvious impact upon cat predation rate, are left unaltered by the parasite (Berdoy *et al.* 1995*b*).

For any small mammal under heavy predation pressure, the capacity to detect and avoid areas associated with high predation risk is likely to be of strong selective advantage. Rats have evolved an innate and pronounced defensive reaction to predator odours, including cat

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(Vernet-Maury *et al.* 1984; Blanchard *et al.* 1990; Berdoy & Macdonald 1991; Klein *et al.* 1994). Even naive laboratory rats that have not been in contact with cats for several hundred generations still show strong aversive reactions when confronted with cat odours. Such innate anti-predator behaviour and the inherent anxiety that signs of cat presence seem to engender (Blanchard *et al.* 1990) is, from the parasite's point of view, an obvious obstacle militating against its successful transmission to its cat definitive host. Here we investigate whether the parasite is able to interfere with the rat's innate reaction to potential predation risk by cats.

STOP. PLEASE ANSWER QUESTIONS 1 AND 2 BEFORE PROCEEDING TO THE NEXT SECTION.

2. MATERIAL AND METHODS

Observations were carried out on adult Lister–hooded laboratory rats, which were outbred four generations previously with male rats trapped from rural UK farms. Laboratory–wild hybrids, rather than pure wild rats, were used so as to ensure known parasitic and social histories of individuals, whilst still obtaining behavioural patterns comparable to those of their wild counterparts. The Lister–hooded laboratory strain was chosen because of its reported behavioural similarity to wild rats (Mitchell 1976). The laboratory rat population was serologically and parasitologically *T. gondii* negative. All rats were also treated with ivermectin anthelmintic (MSD-Agvet Ltd, Hoddesdon, UK) in order to ensure freedom from helminthic or ectoparasitic infections that could bias the data (Ostlind *et al.* 1985).

Experimental rats ($n = 32$) were orally infected with 20 cysts of the low-virulence cyst-forming RRA (Beverley) strain in isotonic saline. This strain had been maintained by continuous passage of infective brain homogenate in outbred AA strain mice bred in house at the University of Strathclyde (precise details are published in Webster 1994*b*). Control rats ($n = 32$) were sham inoculated with isotonic saline. At the end of the study the rats were killed with carbon dioxide. *T. gondii* antibodies were determined by the IgG indirect latex agglutination test (Toxoreagent; Eiken, Tokyo, Japan; Tsubota *et al.* 1977). Titres $\geq$ 1:32 were considered positive (Webster 1994*a*,*b*; Webster *et al.* 1994). *T. gondii* brain cysts were determined by microscopic examination of macerated brains in phosphate-buffered saline. Data from any exposed rat found to be serologically or parasitologically *T. gondii* negative at the end of the study were excluded from analysis. Thus the final sample size for analysis consisted of 23 infected rats and 32 uninfected rats.

To test the potential effect of *T. gondii* on the rat's perception of predation risk we observed the nocturnal exploratory behaviour of rats in outdoor pens (2 m × 2 m). The ground was covered with a layer of white sand to provide a homogeneous and neutral surface that could be cleaned between each test. The pens were enriched with a labyrinth of bricks dividing the area into an array of 16 cells. Each corner contained seven drops of one of four distinct odours deposited on and within wooden nest-boxes: the rat's own smell (own straw bedding), neutral smell (fresh straw bedding treated with water), cat odour (fresh bedding treated with undiluted cat urine) and rabbit odour (fresh bedding treated with undiluted rabbit urine). Rabbit odour served as a control for a mammalian non-predator. The position of the four smells (own, water, rabbit and cat) was changed between each test in order to avoid positional biases. Each of the scented areas also contained a water and food bowl covered by a transparent plastic cover.

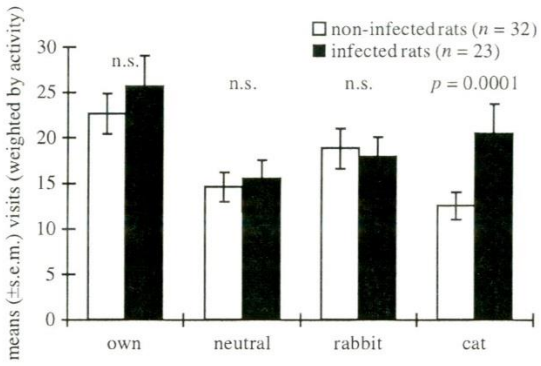

Figure 1. Mean (± s.e.m.) numbers of visits (weighted by overall rat activity) to the four scented areas in the outdoor pens over one night. Uninfected and *T. gondii*-infected rats differ only in their response to areas associated with high predation risk ($F_{1,54} = 22.03$, $p = 0.0001$).

Each rat was tested singly and videotaped from dusk to dawn with a low-intensity camera fixed on a scaffolding 3 m above the test pens. The pens were illuminated from above with two 1 kw halogen lamps to which the rats had completely habituated (Berdoy 1994).

The effect of infection status on visits to the four scented areas was tested using a profile analysis in the General Linear Model procedure in SAS (SAS 1988) to take into account the fact that responses to the four areas are linked. Since the number of cells visited is proportional to rat activity (only rats who emerge from their nest-boxes will show a preference or avoidance to smells) the test of parallelism was carried out on means weighed by overall cell use after checking that there was no difference between infected and uninfected rats ($F_{1,54} = 0.85$, $p = 0.4$). Residuals were tested for normality. The level of aversion or preference to cat areas was tested by comparing (*t*-test) the relative visits to cat versus rabbit areas (cat minus rabbit).

STOP. PLEASE ANSWER QUESTIONS 3 AND 4 BEFORE PROCEEDING TO THE NEXT SECTION.

3. RESULTS

The rats' nocturnal behaviour in the outdoor pens (total of 670 rat-hours of observation) revealed a significant divergence between infected and uninfected rats in their overall response to the smells (GLM repeated measures, $F_{3,159} = 9.19$, $p = 0.0001$), which was caused by a differential response to cat odours ($F_{1,54} = 22.03$, $p = 0.0001$; figure 1). Uninfected rats exhibited a healthy aversion of cat-scented areas ($n = 32$, $t = -3.33$, $p = 0.002$). Infected rats, however, were significantly less averse ($n = 23$, $t = 2.36$, $p = 0.002$) and showed no overall avoidance of areas with signs of cat presence ($t = 0.21$, $p = 0.8$). Alterations induced by *T. gondii* infection were confined to the predator's odour, as both types of rats behaved similarly with respect to areas containing their own smell (which was preferred by both), neutral smell and rabbit odour (figure 1).

Since the number of cells visited is proportional to exploratory activity, the impact of *T. gondii* was predictably more visible amongst rats who explored the pen more intensively ($n = 55$, $F_{1,54} = 27.38$, $p = 0.0001$). Thus, amongst the most active animals (top 25%, $n = 14/55$; seven infected and seven uninfected), control rats

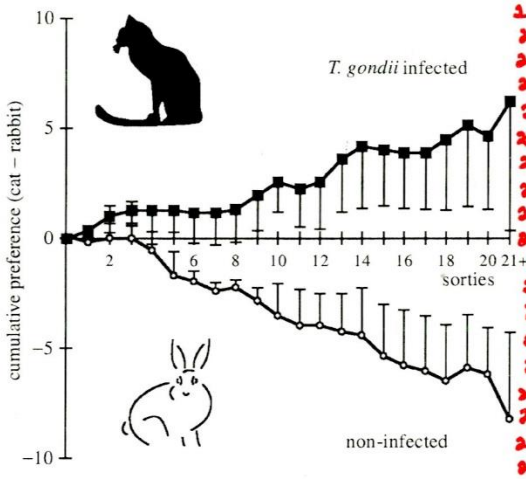

Figure 2. Development of preference or avoidance throughout the night exhibited by the 25% most active rats ($n = 14$, seven infected rats, seven uninfected). Results are shown as the mean cumulative number of cat cells minus the number of rabbit cells visited during each sortie. The data above the x-axis therefore represent a relative preference for the cat areas whilst data below the x-axis indicate avoidance. Vertical bars describe 95% confidence intervals. Time on the x-axis is represented in terms of sorties within the night. Sorties are characterized by bursts of rat activity separated by intervals when the rats shelter into a nest-box for a minimum of 1 min. The rising line for uninfected rats indicates a prolonged, and sensible, avoidance of cat-scented area that is essentially maintained throughout the night. In contrast, *T. gondii*-infected rats tend to exhibit a preference for predator-scented areas. The difference between uninfected and *T. gondii*-infected rats is significant from the third sortie onwards.

continued to exhibit a stable avoidance of cat-scented areas throughout the night, whereas *T. gondii*-infected rats showed a preference for areas with signs of cat presence (figure 2).

STOP. PLEASE ANSWER QUESTIONS 5 AND 6 BEFORE PROCEEDING TO THE NEXT SECTION.

4. DISCUSSION

Inherent within the parasite manipulation hypothesis is the premise that behavioural modification represents a sophisticated product of parasite evolution rather than an accidental side-effect of infection (Barnard & Behnke 1990). However, in the few cases where the relationship between physiology and behaviour has been investigated, clinical parasitism is usually evident and has caused the complete loss of a particular behaviour rather than a modification of a specific complex behavioural pattern as illustrated here (e.g. Rau 1983, 1984). Even studies indicating that parasites can affect host learning and spatial performance (e.g. Stretch *et al.* 1960; Kvalsvig 1988; Nokes *et al.* 1992) have been confounded by parasite-induced disruptions of overall host health status (Thompson & Kavaliers 1994). The same does not appear to be true of subclinical *T. gondii* infection. We found that infected individuals show no difference from uninfected individuals in terms of general health status (Webster 1994*b*; Berdoy *et al.* 1995*b*), and behavioural categories unlikely to influence predation rate, even when energetically costly, appear unaltered (Berdoy *et al.* 1995*a*). Moreover, we found here that the alterations induced by *T. gondii* infection were confined to the predator's odour, as both types of rats behaved similarly with respect to areas containing their own smell (which was preferred by both), neutral smell and rabbit odour (figure 1). This suggests that the potentially fatal attraction exhibited by infected rats was not caused by a gross impairment of olfactory faculties. Instead, manipulation by *T. gondii* appears to alter subtly the cognitive perception of the host in the face of predation risk. As with any evidence of host behavioural alterations, further investigations should now ideally incorporate the outcome of real predation rates by the appropriate definitive host as the yardstick of advantage to the parasite (Webster *et al.* 1994, 2000; Poulin 1992; Moore & Gotelli 1990). Nevertheless, whilst direct predation studies are fraught with practical as well as some ethical difficulties, we have shown previously that *T. gondii*-infected rats are indeed more likely to be caught by traps in the wild (Webster *et al.* 1994).

In addition to the implications raised here for the epidemiology of *T. gondii* in the wild in terms of increased transmission rates, the results of this study may also have causal and functional implications.

From a causal view point, our findings may have implications for the study of the neurological basis of behaviour. Indeed, the reaction by potential prey to cat stimuli is used to study the neurological basis of anxiety and the mechanisms of anxiolytic (anxiety relieving) drugs. Such studies have found, for example, that blocking the normally anxiogenic NMDA receptors in the amygdala causes rats to approach cats 'fearlessly' (Adamec *et al.* 1999) in much the same way as our infected rats approached the areas treated with cat urine. One could speculate that such an effect might imply an anxiolytic action of *T. gondii*. Likewise, exposure of laboratory rats to predator odours, but not other noxious odours, induces fast wave activity in the dentate gyrus of the hippocampus (File *et al.* 1993; Hogg & File 1994). Such a response can be blocked by serotonin (5-HT) antagonists (Blanchard *et al.* 1990; Kavaliers & Colwell 1991) or even by the presence in these mice of another protozoan, *Eimeria vermiformins* (Kavaliers & Colwell 1994). Such observations could suggest that some parasitic infections, such as *T. gondii* and *E. vermiformins*, may be able to attenuate the 5-HT-sensitive predator-induced response, thereby reducing the accompanying anxiety-related anticipatory defence reactions of a host to a predator.

Finally, we believe that these results may also provide a functional explanation of the altered brain function in infected humans, where *T. gondii* prevalence has been found to range from 22% in the UK to 84% in France (Desmonts & Couvreur 1974). Although humans represent a dead-end host for the parasite, our results could suggest that the reports of altered personality and IQ levels in *T. gondii*-infected patients (Burkinshaw *et al.* 1953; Flegr & Hrdy 1994) represent the outcome of a parasite evolved to manipulate the behaviour of another mammal. It is noteworthy that rat behaviour is often viewed as the outcome of a conflict between pronounced neophobic reactions and strong exploration tendencies characteristic

of opportunistic omnivores. The uneasy balance between these conflicting motivations, very pronounced in rats but also visible in humans ('the omnivores paradox', Rozin 1976), may thus provide a particularly fertile ground for manipulation by *T. gondii*.

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PLEASE ANSWER QUESTION 7.

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Appendix 3

Discussion questions participants answered while reading the article, "Fatal attraction in rats infected with Toxoplasma gondii" (Berdoy, Webster, & Macdonald, 2000)

Question	**Placement in article (at the end of section indicated)**	**Level of Bloom's taxonomy**
Summarize the life cycle of *T. gondii*.	Introduction	Comprehension
Given the information presented in the Introduction, do you think that *T. gondii* will interfere with the rat's innate reaction to potential predation risk by cats? Briefly explain your answer.	Introduction	Analysis
Predict how the results would change if the authors had used laboratory rats instead of laboratory-wild rat hybrids.	Materials and Methods	Analysis
How might the rats' reaction to cat urine be related to predation risk?	Materials and Methods	Analysis
In Figure 1, which of the four scented areas in the outdoor pens showed a significant difference between visits by affected rats compared to unaffected rats?	Results	Analysis
What do the results of Figure 2 suggest about *T. gondii*'s effect on rat behavior? Explain your answer in terms of the data shown in the figure.	Results	Analysis
Using what you have learned from this research study, what would be your next experimental steps to continue this research?	Discussion	Synthesis

Appendix 4. List of all the themes encountered during qualitative analysis. The themes arising from qualitative analysis are shown in the shaded boxes. Subthemes are arranged below the themes in open boxes. The percent of participants demonstrating a subtheme as well as the average instance of that subtheme for the group are shown. N = 6 (faculty); N = 11 (students). For subthemes that have codes denoting understanding and lack of understanding (+/-), percent and averages are found in parenthesis in the same order. SEM, standard error of the mean.

Theme 1: Thinking Tools								
Subtheme	Working definition	Example	Faculty			Students		
			# out of 6	Percent	Average instance ± SEM	# out of 11	Percent	Average instance ± SEM
Assimilating academic language	While reading, participants would encounter technical language, known or unknown, and subsequently use it in their think aloud process.	P: (Pause) So it's saying that it [*T. gondii*] affects the brain, but not the rest of the body. And, they're wanting to discuss their results in terms of humans and other mammals. (Pause) hmm (student).	1	17	0.17 ± 0.17	6	55	0.73 ± 0.24
Correcting statement	In the event that a participant expressed a detail in error, they followed up with a correction to their statement.	They won't come out of anybody else despite infections occurring in other animals. (Pause-finding spot) Oh excuse me, I said all animals, all mammals (faculty).	3	50	0.50 ± 0.22	0	0	0

Creating or using mental and physical visuals "Creating visuals"	Participants wrote, drew, or verbally described.	Draws life cycle out on smart pad (faculty).	4	67	2.12 ± 0.79	1	5	0.45 ± 0.37
Deeming information as significant or interesting "Interesting"	As a participant read and thought aloud, they commented that the information was significant and/or interesting.	Um (underlines "Titres > 1:32 were considered positive" after reading) gondii brain cy-that will be important for interpreting the figures. Maybe (faculty).	4	67	2.17 ± 0.79	2	18	0.45 ± 0.37
Doing follow-up literature search "Literature research"	Participants referred to initial sources in the paper when they wanted further clarification on something they read.	So I'm gonna check if I can see if another source has said the same thing. Umm, [inaudible segment]…Okay, yeah, congenital transmission occurs in one percent to ten percent of children born to infected mothers. So, I guess that's what it	1	17	0.17 ± 0.17	2	18	18 ± 12

		means (student).						
Looking up terms/defining through own etymology "Looking up terms"	When a participant encountered an unfamiliar term, they would determine its meaning from its root words or by looking it up on a computer.	F-E-L-I-D. So not field, felid. I don't know what that means. Um I'm guessing it means related to cats because feline, F-E, so (student).	4	67	3.17 ± 1.57	7	64	1.45 ± 0.61
Recalling information previously read in the article "Recalling"	As a participant read along and thought aloud, s/he may comment about a previous portion of the article.	They said earlier in their (Pause) in their abstract that it did not change any other behavioral… categories (faculty).	4	67	0.83 ± 0.23	1	9	0.18 ± 0.18
Relying on definition of term provided in article "Relying on definition provided"	In the event that a term was described in the text, a participant indicated that they either understood it or noticed it.	Oh, so that's what they mean by laboratory wild hybrids (student).	0	0	0	5	45	0.45 ± 0.16

Re-reading text one or more times "Re-reading"	The participant commented that s/he reread a portion of the text.	I'm gonna go back to the last sentence (student).	6	100	33 ± 8.65	11	100	10 ± 3.13
Searching article for answer to a question "Searching article"	As the participant encountered questions related to the article, s/he searched through the article for information to aid his/her response.	Now I'm trying to figure out if they've said any difference between the wild, or the wild-laboratory hybrids or the lab rats. But I don't see them specifying, it just says infected and uninfected (student).	5	83	2.33 ± 0.88	5	45	0.64 ± 0.28
Summarizing or recapping "Summarizing"	The participant summarized a portion of the text.	So cats, they can reproduce out of cats, but they can infect all mammals. So everybody gets sick, but only cats can allow them to complete the lifecycle (faculty).	6	100	14.7 ± 4.8	11	100	4.6 ± 1.1

Taking notes	The participant wrote down notes.	So, I'm gonna write on the side, uhh, let me see, parasites…found… are transmitted through food…transmitted …through… food…exhibit…uhh, manipulation hypotheses (student).	4	67	5.2 ± 2.7	5	45	2 ± 0.89
Underlining a key piece of information "Underlining"	The participant underlined a portion of the text.	And whenever I'm reading papers I like to underline like the summary sentences (student).	4	67	8 ± 3.4	7	64	6.9 ± 3.2
Using a reference point / prior knowledge "Prior knowledge"	The participant exhibited prior knowledge or used a reference point in the text while thinking aloud.	The wild animal, um, it's really hard to collect and have any kind of consistency with wild animals because they come from so many different unknown social backgrounds. And	6	100	7.5 ± 1.9	6	55	2 ± 0.86

		when you're studying behavior, that's a really important thing to consider (faculty).						
Using context clues in the text "Context clues"	The participant used other words in the reading to determine an unknown word.	I don't really know what a latex agglutination test is but I can tell from the context of the sentence that they're determining the amount of infection of gondii so I'm not gonna look that up (faculty).	4	67	0.83 ± 0.31	2	18	0.27 ± 0.19
Theme 2: Science Literacy and Process Skills								
Analysis (+/-)	The participant verbalized at least one of the following: thoughts indicating that s/he understood relationships in the information	Kay, so it's saying the T. gondii infected cats had a preference for the cat side is the uh, as opposed to the rabbit side. (Pause) I mean I see what it's	(6/5)	(100/83)	(13.6 ± 0.71/1 ± 0.26)	(8/7)	(73/64)	(5.6 ± 0.97 /1.6 ± 0.51)

	presented in the article, analysis of the data the graph depicted, or understanding and interpretation of statistical analysis.	saying, but that graph for some reason isn't, doesn't really help me too much. I think the, the wording was best (student).						
Evaluating a scientific argument "Evaluating"	The participant judged the quality of the research or methods in the article and provided a justification.	No. Uh, and and to explain my answer, there was not enough concrete behavioral evidence to support it. They make statements about studies without really providing any of the evidence that is in those papers. So I don't have enough to go on to actually make that call. In fact I'm a little, little bit, I'm a little suspicious of the whole, of the whole thing. I	6	100	9.2 ± 2.1	2	18	0.55 ± 0.37

		think that was obvious when I was talking about the lab rats that they used (faculty).						
Expressing familiarity with the layout of research articles "Article layout"	The participant made a comment that indicated his/her familiarity with research articles.	I figure they're just gonna go ahead and explain that through the rest of the article, so it's okay that I don't understand (student).	1	17	0.17 ± 0.17	2	18	0.27 ± 0.19
Identifying rationale or big picture of study. "Identifying rationale"	The participant voiced a statement that indicated they understood the rationale or big picture of the study.	So we're looking to see if that's going to take place here. If the, uh, the parasite is gonna behaviorally alter that rat, um, for its own ends (student).	2	33	0.5 ± 0.34	4	36	0.36 ± 0.15
Making connections with real life applications of science that are not featured in	As participants read and thought aloud, they connected what the read to real life applications of	Okay, so it's saying that we could potentially use this science to relieve anxiety because they were able to	2	33	0.50 ± 0.34	3	27	0.27 ± 0.14

article "Making connections"	science they were familiar with.	change rats from not being afraid of like their, the cat (student).						
Reaching conclusions	While reading and/or responding to questions, participants verbalized conclusions from reading information that was implied or inferred within the article.	mmm, congenital transmission by protozoan parasite [inaudible segment] public health problem [inaudible segment] umm, congenital infection affects a mother and a fetus or a newborn, it is still surprising that despite the abundant immunoepidemiology knowledge of congenital transmission of a protozoan parasite, no definitive etiology or predictive diagnostic tests have been identified	2	33	0.33 ± 0.21	4	36	0.36 ± 0.15

		(student).						
Seemingly procedural method to read the graph/figure "Procedure for reading graph"	While reading a graph, participants described their process for reading a graph.	So, the first thing I do is I look at the axes. Alright they've got own, neutral, rabbit, and cat. Ok and there's-another thing I look for is what do the standard-what do the bars represent-standard error, ok (faculty).	2	33	0.50 ± 0.34	1	9.1	0.18 ± 0.18
Understanding research design (+/-) "Research design"	The participant indicated their understanding or lack of understanding of research design. Although understanding of research design could be dependent on the existing prior knowledge	I thought, we'll get to the t-test later then won't we to compare the two corners. So they did it the way I would have done it, which is a factorial design. (Tilts head to read Figure 1) And you've got infected versus non-	(6/3)	(100/50)	(10.9 ± /0.50 ± 0.22)	(11/9)	(100/82)	(3.1 ± 0.73/1.73 ± 0.38)

	(Thinking Tools), we separated these two subthemes because the understanding of research design involves an important and distinct type of prior knowledge that falls within the science literacy skill set.	infected and you've got the four corners. And it is a repeated measure in that case. (Nodes head) Sure, because each rat is going to invest, could potentially go into all four corners and if they don't go into a corner, they get, they just get a zero (faculty).						
Theme 3: Comprehension Difficulties								
Due to unknown vocabulary/jargon "Jargon"	Participants did not understand the reading because they were unfamiliar with the vocabulary or jargon being utilized.	I don't know what sorties is (faculty).	4	67	1.50 ± 0.73	8	72	3.40 ± 1.2
Due to lack of knowledge/incorrect knowledge "Lack of	Participants expressed that they did not know something and/or	So I-V-E-R-M-E-C-T-I-N ivermeectin A-N-T-E-H-E-L-M-I-N-T-I-C,	4	67	0.67 ± 0.21	6	55	0.73 ± 0.24

knowledge”	speculated about the meaning of it.	anthelmintic. Uh, MSD-Agvet limited [inadubilbe segment]. I have, its clearly some type of chemical agent. I do not know what it is (student).						
Due to wording/sentence structure “Wording”	The wording and/or sentence structure of the article created comprehension difficulties for participants.	Including cat, okay. That’s fine, it just started with a bunch of sources and it like threw me off (student).	1	17	0.17 ± 0.17	6	55	0.73 ± 0.31
Participant becomes distracted focusing on a small detail “Distracted”	Instead of continuing their reading, a participant would become distracted or focused on a small detail that would cause them to not follow through with expressing their understandings	parasitic… gondiai [/gondii/]…I don’t know how to say it. I: Gondii is how I say it. Would say it. P: Gondii. I: Yeah. Gondii? Gondiai? I don’t know (student).	2	33	0.33 ± 0.21	2	19	0.27 ± 0.19

	aloud.							