Title: Gender differences in behavioural changes induced by latent toxoplasmosis

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Abstract

There is growing evidence that the protozoan *Toxoplasma gondii* modifies behaviour of its intermediate hosts including humans, where it globally infects about 20-60 % of the population. Although it is considered asymptomatic in its latent stage, it was previously found to have remarkable and gender different effects on the personality factors A (warmth), G (rule consciousness), L (vigilance, mistrust) and Q3 (self control, self image) from Cattell's 16PF Questionnaire. We performed a double blind experiment testing 72 and 142 uninfected and 20 and 29 infected men and women in order to behaviourally verify these gender differences. Our composite behavioural variables Self Control and Clothes Tidiness (analogue both to the 16PF factors G – conscientiousness and Q3 – self control) showed a significant effect of the toxoplasmosis-gender interaction with infected men scoring significantly lower than uninfected men and a trend in the opposite direction in women. The effect of the toxoplasmosis-gender interaction on our composite behavioural variable Relationships (analogue to factor A – warmth) approached significance; infected men scored significantly lower than uninfected men whereas there was no difference in women. In the composite behavioural variable Mistrust (analogue to factor L), the pattern was affected by living environment (rural vs. urban). Possible interpretations of the gender differences are discussed.

Key words: human, *Toxoplasma*, 16PF, manipulation hypothesis

1. Introduction

Latent toxoplasmosis is a common infection in both developed and developing countries, occurring in about 20 - 60 % of the population. In medical terms it is considered asymptomatic in immunocompetent persons (Remington and Krahenbuhl, 1982), but there is growing evidence about its effects on human behaviour.

The coccidian parasite *Toxoplasma gondii* uses felids as definitive hosts and practically all warm-blooded animals as intermediate or secondary hosts. In the latent stage in its intermediate and secondary hosts, it is encysted in the brain and muscular tissue presumably for the host's lifetime. The infection is known to modify the behaviour of rodents, causing decreased anxiety (Hutchison et al., 1980*b*), decreased reaction speed (Hrdá et al., 2000), decreased neophobia (Webster et al., 1994), impaired motor performance (Hay et al., 1983), lower ability to discriminate between familiar and novel surroundings (Hutchison et al., 1980a; Hay et al., 1984a), deficits in learning capacity and memory (Witting 1979), reduced specific predator avoidance (Berdoy et al., 2000), increased activity (Webster, 1994; Hutchison et al., 1980*a*; Hay et al., 1984b) and aggressiveness (Arnott et al., 1990). These behavioural changes are often interpreted in terms of the manipulation hypothesis, which proposes that an animal serving as an intermediate host is manipulated by the parasite to behave in a way to increase the probability of being transmitted to the definitive host by predation (Barnard and Behnke, 1990; Dawkins, 1982). Indeed, it was observed in rats that they can be more easily trapped when infected by *Toxoplasma*, a situation which could simulate predation by the felid final host (Webster et al. 1994). It was also shown that infection by related coccidian species, e.g. *Frenkelia* and *Sarcocystis*, increases the predation risk of parasitized rodents (Voříšek et al., 1998; Hoogenboom and Dijkstra, 1987). In humans, a higher number of traffic accidents were found to occur to *Toxoplasma*-infected subjects (Flegr et al., 2002). This study can be also considered an analogy of a predation test.

Toxoplasma was found to both impair the psychomotor performance (prolong simple reaction times) of human subjects (Havlíček et al., 2001) and affect the human personality profile. In contrast to the behavioural changes observed in animals and to the psychomotor performance changes in humans, an obvious gender difference appeared in the modification of the personality traits measured by a questionnaire. Researchers using Cattell's 16 Personality Factor Questionnaire (16 PF) (a widely used and standardized psychological diagnostic tool) on a sample of biology students and academic staff from Charles University found an opposite toxoplasmosis-induced shift in men and women in four personality factors, namely the factors A (the high pole of this dimension can by characterized e.g. as warmth), G (rule consciousness), L (vigilance, mistrust) and Q3 (self control, self image) (Flegr et al., 1996). More specifically, infected men scored significantly lower in the factor G (disregards rules, expedient), higher in the factor L (suspecting, jealous, dogmatic), and non-significantly lower in the factors A (reserved, detached, critical) and Q3 (uncontrolled, lax, follows own urges) (Flegr et al., 1996; Flegr and Hrdý, 1994). Infected women scored significantly higher in the factor A (warm-hearted, outgoing), non-significantly higher in the factors G (conscientious, persistent, moralistic, staid), Q3 (controlled, exacting will power, socially precise), and significantly lower in the factor L (trusting, accepting conditions, tolerant) (Flegr et al., 1996). Moreover, another study found a correlation between the duration of toxoplasmosis and the level of the above mentioned factors (A, G, L and Q3) in two sets of women infected by *Toxoplasma* (Flegr et al., 2000). The same was reported for men in the factor G (Flegr et al., 1996). These findings provided experimental support for the suggestion that the personality change was the result of infection rather than subjects with a specific personality profile being more prone to infection.

There are two possible explanations as to why the personality shifts occurred in an opposite direction in men and women. Firstly they could be actual gender differences or secondly it could be that the questionnaire reflected real changes in one gender only, but that the same changes had been masked in the other gender for some reason (e.g. a tendency to

deny an unwelcome personality change resulting in a stylisation in the questionnaire). The only way to distinguish between these two alternatives is to conduct a behavioural test. Thus, in our study we assessed behavioural manifestations of the four 16PF factors A (warmth), G (rule consciousness), L (vigilance, mistrust) and Q3 (self control, self image) to investigate if we were dealing with a profound infection based gender difference or merely a superficial difference in the ability or willingness to report the truth about one's own personality (undesirable change) in a questionnaire.

2. Methods

2.1. Subjects

The sample consisted of 92 male and 171 female students of the Faculty of Science, Charles University, with the mean age of 21.1 years (s.d. = 1.8). The sample was thus homogenous concerning both the course of the study and age. All subjects were asked to voluntarily participate in the research project and to sign an informed consent form. They were informed that after providing 2 ml of blood for serological analysis, they would another day undergo psychological and ethological testing without further specification of what they will be assessed on. Simultaneously, they were given the 16PF Questionnaire to fill out at home. The testing proceeded in up to about 6 months after recruitment during the years 2002 to 2004.

2.2. Immunological tests for toxoplasmosis

Specific anti *Toxoplasma* IgG and IgM antibodies concentrations were determined by ELISA (IgG: SEVAC, Prague, IgM: TestLine, Brno), optimized for early detection of acute toxoplasmosis (Pokorny et al., 1989), and with the complement fixation test (CFT) (SEVAC, Prague) which is more sensitive and therefore more suitable for the detection of old *Toxoplasma*

infections (Warren and Sabin, 1942). The titre of anti-*Toxoplasma* antibodies in sera was measured in dilutions between 1:8 and 1:1024. Subjects with negative results of IgM ELISA (positivity index<0.9) and having CFT titres higher than 1:8 were considered latent-toxoplasmosis positive.

2.3. Behavioural tasks

All subjects participated in a double blind one-hour testing session focused on a number of behavioural variables using questionnaire and interview techniques, clothing assessment and simple experiments (the sequence of the tests within the session is given in Fig. 1). The computer administered questionnaire and the interview contained questions relating

Figure 1. around here

to the 16PF factors A (warmth) and G (rule consciousness) or Q3 (self control, self image), which were phrased so as to ask about specific previous behaviour rather than a general attitude (e.g. “How many times have you been cleaning at home during last week?” instead of “Do you often clean at home?” or “Do you like cleaning?”). The interview as well as the clothing observation was standardized and in both cases carried out by the same female experimenter in all testing sessions. The simple experiments were designed to relate to the 16PF factors G (rule consciousness), Q3 (self control, self image) or L (vigilance, mistrust). They were administered so as to hide the purpose they were applied for. (E.g. in the experiment designed to measure the willingness to taste a strange liquid, the subjects were told they would be assessed on the inherited ability to discriminate the bitter taste of phenylthiocarbamide).

The behavioural data were categorized into four behavioural composite variables: "Relationships" contained variables relating to the quality (the subvariable Warmth) and quantity (the subvariable Friends) of interpersonal relationships, and was designed to correspond to the 16PF factor A (warmth), "Self-Control" combined data from written or oral questions concerning rule regardance (the subvariable Self-Control in Self-Report), questions about the care of own

appearance (the subvariable Self-Control in Care of Appearance) and simple experiments concerning orderliness, reliability, conscientiousness (the subvariable Self-Control in Experiments), and was intended to correspond to the factors G (rule consciousness) or Q3 (self control, self image); "Clothes Tidiness" was composed from variables obtained observing the clothing of the subjects and was similarly expected to correlate with the factors G or Q3. Finally "Mistrust" contained data from experiments designed to measure vigilance or mistrust and was expected to correlate with the factor L (vigilance, mistrust). Table 1 shows a detailed composition of the behavioural composite variables.

Table 1 around here

Continuous variables were adjusted to reach values 0 to 5 and to have the normal distribution. Ordinal or categorical variables were scored as given in Table 1. All variables in Mistrust were converted to z-scores. A mean of given variables was then counted to create a composite behavioural subvariable (variable). Finally, a mean of behavioural subvariables composed the ultimate composite behavioural variables.

2.4. Statistical analysis

Statistica v. 6.0 general linear module was used for all statistical testing, i.e. for ANOVA, ANCOVA and linear regression. The results of testing of ANCOVA assumptions, namely of the testing of normality of data distribution, normality of residuals and homogeneity of variances were nonsignificant for all studied models. All four behavioural composite variables were tested for the effect of the toxoplasmosis-gender interaction with ANOVA. In all ANOVA tests, we included one potential categorical and two continual confounding factors, namely place of residence, age and ordering number of the testing session. Because we tested four behavioural variables, we used the stepdown Bonferroni correction for four tests (Holm, 1979).

3. Results

We performed a correlation analysis of the four behavioural composite variables Relationships, Self-Control, Mistrust and Clothes Tidiness, and all 16 factors from the 16 PF Questionnaire. Relationships were associated with the factor A (warmth; b=0.30, r^2=0.091, p<0.001); Self-Control correlated with the factor Q3 (self control; b=0.18, r^2=0.033, p=0.005), and in women also with the factor G (rule consciousness; b=0.19, r^2=0.038, p=0.014). Mistrust and Clothes Tidiness expressed no correlation with the 16 PF factors.

Latent toxoplasmosis was diagnosed in 20 men (21.7 %) and 29 women (17 %). There was a different effect of the latent toxoplasmosis infection on women and men in all four behavioural composite variables.

In Relationships, the effect of the toxoplasmosis-gender interaction nearly reached the formal level of statistical significance ($F_{1,244}$=3.79, p=0.053). Infected men scored significantly lower (1.93) in Relationships than uninfected men (2.26, $F_{1,83}$=4.67, p=0.033), whereas the score of infected women (2.55) did not significantly differ from the score of uninfected women (2.60, $F_{1,158}$=0.15, p=0.695). Further, we examined the two Relationships subvariables separately. The effect of the toxoplasmosis-gender interaction on the first subvariable Warmth was significant ($F_{1,244}$=5.12, p=0.025). Infected men had significantly lower scores (1.90) than uninfected men (2.28, $F_{1,83}$=5.73, p=0.019), whereas the scores of infected (2.95) and uninfected (2.88) women did not differ ($F_{1,158}$=0.13, p=0.718). The effect of the toxoplasmosis-gender interaction on the second subvariable Friends was not significant ($F_{1,244}$=0.63, p=0.427).

The effect of the toxoplasmosis-gender interaction on Self-Control was highly significant ($F_{1,242}$=12.27, p<0.001), and remained significant also after Bonferroni correction for multiple tests. Infected men reached lower scores in Self-Control (2.03) than uninfected men (2.24, $F_{1,81}$=6.16, p=0.015) whereas infected women scored non-significantly higher (2.55) than uninfected women (2.42, $F_{1,159}$=2.54, p=0.113). The strongest gender difference in the

Toxoplasma induced shifts appeared in the subvariable Self-Control in Experiments. The effect of infection was significant both in men ($F_{1,81}$=7.35, p=0.008) and women ($F_{1,159}$=7.20, p=0.008), with infected men scoring lower (1.97) than uninfected men (2.21), and infected women scoring higher (2.60) than uninfected women (2.29). The effect of the toxoplasmosis-gender interaction was again highly significant ($F_{1,242}$=15.66, p<0.001).

Similarly, the effect of the toxoplasmosis-gender interaction on Clothes Tidiness was significant ($F_{1,250}$=6.39, p=0.012), and remained significant also after Bonferroni correction for multiple tests. Infected men scored lower in Clothes Tidiness (3.57) than uninfected men (3.88, $F_{1,85}$=4.80, p=0.031), whereas infected women reached non-significantly higher scores (4.18) than uninfected women (4.03, $F_{1,163}$=1.93, p=0.167).

Figure 2 around here

The pattern in Mistrust was somewhat more complicated. There was a significant effect of the three-way interaction - toxoplasmosis-gender-place of residence ($F_{2,248}$=4.32, p=0.014). Infected men scored higher (0.28) in Mistrust than uninfected men (-0.31), when their permanent residence was in the countryside ($F_{1,11}$=2.89, p=0.117), but they scored significantly lower (-0.32) than uninfected men (-0.03), when their permanent residence was in a small or middle-sized city ($F_{1,73}$=4.74, p=0.033). In contrast, infected women scored non-significantly lower (-0.07) than uninfected women (0.10), when their permanent residence was in the countryside ($F_{1,25}$=1.64, p=0.212), but higher (0.20) than uninfected women (0.05), when their permanent residence was in a city ($F_{1,137}$=1.40, p=0.239). When we compared subjects with a rural permanent residence only, the effect of the toxoplasmosis-gender interaction was significant ($F_{1,38}$=5.13, p=0.029). The same was true for the effect of the toxoplasmosis-gender interaction in subjects with a urban permanent residence ($F_{1,212}$=4.47, p=0.036).

4. Discussion

The aim of using behavioural data was to confirm (or refute) the existence of an opposite effect of latent toxoplasmosis on men and women in the personality characteristics Relationships (warmth), Self-Control (rule consciousness), Clothes Tidiness and Mistrust (vigilance). In all four behavioural composite variables studied, we found a significant or nearly significant gender difference in how latent toxoplasmosis influenced men and women. Specifically, we found significantly lower scores in infected men compared to uninfected men in Relationships, Self-Control and Clothes Tidiness, and non-significantly higher scores in the same variables in infected women compared to uninfected women (with the exemption of Relationships, where the scores of infected and uninfected women did not differ). This is in agreement with previous studies using Cattell's 16 Personality Factor Questionnaire, where the *Toxoplasma* induced shifts in factors A (warmth), G (rule consciousness), and Q3 (self control) showed a very similar pattern (Flegr et al., 1999; Flegr et al., 2000; Flegr et al., 1996). In Mistrust, we replicated previous findings only for subjects living in the countryside. In this subsample, infected men scored significantly higher than uninfected men, and infected women scored non-significantly lower than uninfected women (similarly to the results obtained with the 16 PF for the factor L (vigilance), Flegr et al., 1999). The subsample of subjects living in a town or city behaved in the opposite way - infected men scored lower than uninfected men and infected women scored higher than uninfected women.

These behavioural findings confirmed and extended the results of earlier work. Until now, it has not been possible to decide if the latent-toxoplasmosis induced changes are profound changes of personality traits or if infected people merely alter their self-image, and so they score differently in a questionnaire. It could have been possible, for instance, that women deny a personality change occurring in a direction we can observe in men, and present themselves rather as having an opposite personality. Our results in the behavioural composite variable Self-

Control provide important evidence for a real personality change. Out of the 3 Self-Control subvariables, the greatest gender difference was obtained for the Self-Control in Experiments, which is composed of real situation experiments (e.g. magnitude of the experimental session delay, or putting crayons back in their case and ordering them, after using them in another experiment, vs. not doing so) and does not include any self-assessment. Thus it excludes the possibility that the gender differences are an artefact of how (differently) men and women see themselves.

Although we recorded a borderline significant difference for the effect of toxoplasmosis on Relationships in men and women, we did not repeat the finding of increased scores in infected women compared to uninfected women observed in previous research (Flegr et al., 1996; Flegr et al., 1999). The number of experimental subjects in the present behavioural study was relatively large; however, it was still about three times lower then in the previous questionnaire-based studies. Therefore the probability of the type 2 error (false negative result) was relatively high. We believe that future work with a different experimental population or with a refined set of behavioural tasks could confirm higher warmth in *Toxoplasma*-positive women.

Because Clothes Tidiness did not correlate with the factor G (conscientious, persistent, moralistic, staid) nor Q3 (controlled, exacting will power, socially precise) as expected, we cannot relate it to the results of previous research. The seemingly plausible explanation that untidiness of clothes indicating low hygienic standard is a risk factor for acquisition of disease is, however, not very likely since the positive association was found only for men. In women in contrast, there was a slight trend in the opposite direction. This would be quite difficult to explain if we proposed that untidy clothes are related to higher probability of infection.

The effect of residency (rural vs. urban), which was found in Mistrust, has appeared in a previous study of the relationship of latent toxoplasmosis and the psychobiological factor Novelty Seeking (Novotná et al., 2005). It is possible that the birth environment type influences the way people respond to specific tasks, such as those we have assessed. At present, we have

no suitable explanation for biological or sociological backgrounds of this phenomenon. A more extensive assessment of Mistrust, using items (experiments) having the same meanings for persons from both the countryside and cities, is needed to shed light on how Mistrust is influenced by toxoplasmosis.

There can be several explanations why *Toxoplasma* induces opposite behavioural shifts in men than in women. We offer an evolutionary, neurophysiological and a psychological perspective to account for this phenomenon.

Toxoplasma-induced differences in rodents are often interpreted in the theoretical frame of the manipulation hypothesis. According to this theory, infected intermediate hosts are manipulated by the parasite to behave in a way, which makes them an easier prey for the final host. In primitive human societies, being reserved, detached and critical to other people (low Relationships), being uncontrolled, lax, disregarding rules (low Self Control) and untidily dressed would isolate males from a group, making them a more probable prey for a felid. The composition of food of today's wild felids, such as tiger and leopard, is known to contain a large portion of primates, which suggests our animal ancestors were frequently hunted by large cats (Zugerbühler and Jenny, 2002; Karanth and Sunquist, 1995). A different case for females could be where the parasite benefits from being congenitally transmitted to offspring. Congenital transmission of *Toxoplasma* in its latent stage is usually considered impossible in humans. However, it is common in other mammal species (Owen & Trees, 1998). There is also some indirect evidence that the activation of latent infection and transmission of the parasites into the foetus during pregnancy could occur in humans (Hostomská et al. 1957). In contrast to children of women who acquired toxoplasmosis primary during pregnancy, children of women with activated latent toxoplasmosis could be asymptomatic and could escape attention of clinicians. The influence of toxoplasmosis on women behaviour could be just a by-product of the infection. However, we can also speculate that the parasite could try to manipulate women to be more conscientious, persistent, controlled, socially precise (Self-Control), tidily dressed and warm-

hearted or outgoing (which we assume on the basis of the previous questionnaire findings of higher factor A, not confirmed here), in order to make her more attractive for potential sexual partners, and in such a way increase the rate of its transmission into the offspring.

The mechanism behind the neurological and behavioural effect of *Toxoplasma* infection is unknown, but there is some evidence that dopamine could be the main molecule included in this process. An increased level of dopamine was observed in the brains of mice chronically infected with *Toxoplasma* (Stibbs, 1985). *Toxoplasma*-infected men have a decreased level of Novelty Seeking (Flegr et al., 2003), which is expected to be associated with high dopaminergic baseline activity and postsynaptic downregulation (Cloninger, 1998; Hansenne, 2002). Interestingly, female rodents have a higher dopamine concentration compared to males in diencefalon, mesencefalon (Beyer et al., 1991), and the striatum (Walker et al., 2000; Morissette and Di Paolo, 1993). Women also have a higher synaptic concentration and a higher synthesis capacity of dopamine in striatum (Laakso et al., 2002). Furthermore, female sex hormones estrogen and progesterone are known to alter the dopamine activity in striatum and nucleus accumbens (Becker, 1999; Miller et al., 1998), and to have a neuroprotective effect working through the dopaminergic system causing a lower prevalence of degenerative illnesses such as Alzheimer disease, Parkinson disease or psychoses in women (Lindamer et al., 1997; Dluzen and McDermott, 2000). So the less severe or even opposite effect of toxoplasmosis on women could be caused by the protective effect of female sex hormones on the dopaminergic activity.

The psychological explanation of the gender difference assumes that men and women use a different strategy to cope with nonspecific stressors (which latent toxoplasmosis qualifies as). There is some evidence that during lifetime, men are socialized to cope with stress differently from women. Men are expected to use more problem-focused forms of coping, while women are brought up to cope with an emotion-focused style (Berns and Johnson, 1989; Carver et al., 1989). Thus coping with toxoplasmosis, men would be expected to withdraw from the

329 society in order to individually concentrate on the “problem”, whereas women would be expected
330 to turn to the society, where they can express emotions about it.
331 In conclusion, this study has shown that latent toxoplasmosis probably causes rather
332 profound changes of human behaviour, predominantly the behaviour directed to other people
333 and the society. Therefore, *Toxoplasma* must be considered one of the biological factors that
334 are able to affect the key traits of human personality.

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Legends to figures:

Figure 1. Sequence of tests in the behavioural testing session. Numbers in brackets denote concrete behavioural variables measured in the particular part of the experiment. Behavioural variables are described in Table 1.

Figure 2. Relationships (a), Self Control (b) and Clothes Tidiness (c) in men and women infected with *Toxoplasma gondii* (Toxo.pos.) and in uninfected controls (Toxo.neg.).