Toxoplasma and reaction time: role of toxoplasmosis in the origin, preservation and geographical distribution of Rh blood group polymorphism

M. NOVOTNÁ[1], J. HAVLÍČEK[2], A. P. SMITH[3], P. KOLBEKOVÁ[1], A. SKALLOVÁ[1], J. KLOSE[4], Z. GAŠOVÁ[5], M. PÍSAČKA[5], M. SECHOVSKÁ[6] *and* J. FLEGR[1]*

[1] *Department of Parasitology, Charles University, Viničná 7, Prague 128 44, Czech Republic*
[2] *Department of Anthropology, Faculty of Humanities, Charles University, Husnikova 2075, Prague 15800, Czech Republic*
[3] *Centre for Occupational and Health Psychology, School of Psychology, Cardiff University, Tower Building Park Place, CF10 3AT, UK*
[4] *Central Medical Psychology Department, Central Military Hospital, U vojenské nemocnice 1200, Prague 169 02, Czech Republic*
[5] *Institute of Haematology and Blood Transfusion, U nemocnice 1, 120 00 Prague, Czech Republic*
[6] *Transfusion Unit, General Teaching Hospital, K Interně 640, Prague 156 00, Czech Republic*

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SUMMARY

The RhD protein which is the *RHD* gene product and a major component of the Rh blood group system carries the strongest blood group immunogen, the D-antigen. This antigen is absent in a significant minority of the human population (RhD-negatives) due to *RHD* deletion or alternation. The origin and persistence of this RhD polymorphism is an old evolutionary enigma. Before the advent of modern medicine, the carriers of the rarer allele (e.g. RhD-negative women in the population of RhD-positives or RhD-positive men in the population of RhD-negatives) were at a disadvantage as some of their children (RhD-positive children born to pre-immunized RhD-negative mothers) were at a higher risk of foetal or newborn death or health impairment from haemolytic disease. Therefore, the RhD-polymorphism should be unstable, unless the disadvantage of carriers of the locally less abundant allele is counterbalanced by, for example, higher viability of the heterozygotes. Here we demonstrated for the first time that among *Toxoplasma*-free subjects the RhD-negative men had faster reaction times than Rh-positive subjects and showed that heterozygous men with both the RhD plus and RhD minus alleles were protected against prolongation of reaction times caused by infection with the common protozoan parasite *Toxoplasma gondii*. Our results suggest that the balancing selection favouring heterozygotes could explain the origin and stability of the RhD polymorphism. Moreover, an unequal prevalence of toxoplasmosis in different countries could explain pronounced differences in frequencies of RhD-negative phenotype in geographically distinct populations.

Key words: heterozygous advantage, balancing selection, evolution, blood antigen, parasite, Rhesus factor, blood group system.

INTRODUCTION

The RhD protein, which is the *RHD* gene product and a major component of the Rh blood group system, carries the strongest blood group immunogen, the D-antigen. This antigen is absent in a significant minority of the human population (RhD-negatives) due to *RHD* deletion. The origin and persistence of this RhD polymorphism, especially the high frequency of RhD negatives in Caucasian population associated with the spreading of a single haplotype (cde) with a deletion of the whole RHD gene (Wagner and Flegel, 2000), is an old evolutionary enigma (Haldane, 1942; Hogben, 1943; Fisher *et al*. 1944; Li, 1953). Before the advent of modern medicine, the carriers of the rarer allele (e.g. RhD-negative women in the population of RhD-positives or RhD-positive men in the population of RhD-negatives) were at a disadvantage as some of their children (RhD-positive children born to pre-immunized RhD-negative mothers) were at a higher risk of foetal or newborn death or health impairment from haemolytic disease. Therefore, the RhD-polymorphism should be unstable, unless the disadvantage of carriers of the locally less abundant allele is counterbalanced by, for example, higher viability of the heterozygotes (Feldman *et al*. 1969). Such heterozygote advantage is known to be responsible for polymorphism in haemoglobin genes (and existence of sickle disease) in areas with endemic malaria (Allison, 1954). The possible role of the parasites in the origin and persistence of the RhD polymorphism is suggested by pronounced differences in frequency of particular

* Corresponding author: Department of Parasitology, Charles University, Viničná 7, CZ-128 44 Praha 2, Czech Republic. Tel: +420 221951821. Fax: +420 224919704. E-mail: flegr@email.cz

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RhD phenotypes in different geographical areas. The frequency of RhD-negative subjects is about 15% in Caucasians, 8% in Africans and 1% in Asians, which corresponds with the allele frequencies of 40%, 28% and 10%, respectively.

An infectious agent which could fulfil such a role is the most common protozoan parasite worldwide *Toxoplasma gondii*, which infects 20–70% of inhabitants in different developed countries (and as many as 90% in some developing countries). Toxoplasmosis does not occur in the islands where definitive hosts of *Toxoplasma* (cats) are absent (Dubey *et al*. 1997). Post-natally acquired toxoplasmosis in immunocompetent subjects causes mild disease, acute toxoplasmosis, which turns spontaneously into life-long latent toxoplasmosis. Latent toxoplasmosis is characterized by the presence of dormant stages of the parasite in cysts, mostly in the neural and muscular tissues, and immunity against new *Toxoplasma* infections (Remington and Krahenbuhl, 1982). Latent toxoplasmosis in humans is considered as clinically asymptomatic (Markell *et al*. 1999; Roberts and Janovy, 2000). However, infected people have impaired reaction times (Havlicek *et al*. 2001) and about 2·6 times higher risk of traffic accidents (Flegr *et al*. 2002; Yereli *et al*. 2006), possibly as a result of manipulation activity of *Toxoplasma* aimed to increase the chance of transmission from the intermediate to the definitive host (from any bird or mammal species to any feline species (Berdoy *et al*. 2000)). The possible protection of the Rh heterozygotes against *Toxoplasma*-induced impairment of reaction times could explain the primary spreading of the RHD gene (originated by a duplication of RHCE gene) in the RHD-negative population of our (mostly *Toxoplasma*-infected) ancestors. Shorter reaction times of *Toxoplasma*-free, RhD-negative homozygotes could explain secondary spreading of the RHD deletion in regions with low occurrence of cats and therefore low prevalence of toxoplasmosis, like in prehistoric Europe. In the present study, we examined a possible association between the RhD phenotype (or RhD genotype) and toxoplasmosis-associated impairment of psychomotor performance (reaction times) in 4 independent populations.

MATERIALS AND METHODS

Subjects

The 3 studies were carried out on 4 different population samples: 3 groups involving voluntary thrombocyte and blood donors and 1 group of military conscripts. Group 1 (UHKT1) consisted of 69 men and 45 women tested between 1998 and 2000 at the blood transfusion department of the Institute of Haematology and Blood Transfusion, Prague. The mean age of the study participants was 36·4 years (37·5 in men and 34·8 in women). The overall seroprevalence of *Toxoplasma* infection was 36% (34·8% in men and 37·8% in women) and 27·2% of participants were RhD-negative (33·3% in men and 17·8% in women). Group 2 included 89 men and 37 women tested at the same blood transfusion department between 2004 and 2006 (UHKT 2) and group 3 included 221 men and 92 women tested at the blood transfusion department of the Zbraslav University Hospital in Prague between 2002 and 2006 (Zbraslav). The mean age of participants was 34·1 years (34·8 in men and 32·4 in women). The overall seroprevalence of *Toxoplasma* infection was 34·4% (34·5% in men and 34·1% in women) and 26·5% of men and 24% of women were RhD-negative. For unknown reasons, the proportion of *Toxoplasma*-infected subjects in blood donors is usually higher than the prevalence of toxoplasmosis observed in the Czech population (Skallová *et al*. 2005; Kolbeková *et al*. 2007). Very high frequency of RhD-negative subjects reflected the higher demand for RhD-negative blood and therefore preferential selection of RhD-negative donors rather than the frequency of the RhD minus allele in the general population. The testing procedure was conducted under standard laboratory conditions between 08:00 and 11:00, either prior to (Zbraslav) or after (UHKT1 and UHKT2) the blood donation session. Group 4 comprised 464 men tested for psychomotor performance during regular psychological examinations of conscripts, who were training to either be drivers, guards or presidential guards. About 80% of the conscripts from the July 2005 draft consented to the use of their test results for the research project purposes and provided 5 ml of blood for serological testing. The mean age of participants was 19·7 years. The overall seroprevalence of *Toxoplasma* infection was 30·2% and 18·4% of subjects were RhD-negative. The proportion of *Toxoplasma*-infected subjects and RhD-negative subjects in the population of conscripts corresponded well with frequencies expected in the general population. All study subjects (blood donors and draftees) were screened for health status prior to their enrolment in the study. The recruitment of study subjects and data handling were performed in compliance with Czech legislation in force and all participants voluntarily signed the informed consent form. The Institutional Review Board of the Faculty of Science, Charles University (ethical review board) approved the project.

Psychomotor test

Reaction time was measured by the two computer versions of a simple reaction time test (Smith *et al*. 1989). A white square (1 × 1 cm) appeared in the centre of the black computer display at irregular intervals ranging from 1 to 8 sec; the length of the test was 3 min. The subject had to respond to the square

immediately after it appeared on the display by pressing a key on a special keyboard. At the time of testing neither the subjects nor the researcher were aware of the results of the immunological assessment of toxoplasmosis. For the blood donors, the computer measured and recorded reaction times in each trial. The blood donors were tested individually under supervision of an assistant. After the experiment, the mean reaction time for each minute of the test was computed, omitting all outliers, i.e. values more than 2·5 S.D. away from the raw total mean reaction time for the particular subject. The conscripts were tested in groups of 15–20 and instructions about how to perform the test were written on the first (introductory) computer screen. In contrast to the blood donors who were assessed individually, the conscripts were instructed as a group about the importance of responding to visual signals with their maximum possible speed. The conscripts responded by pressing the left button of the computer mouse and the program calculated the mean reaction times from all reaction times achieved during a 1-min simple reaction time test; all other conditions of the test were the same as before. The simple reaction time test was part of a 3-h personality, intelligence and psychomotor performance testing session. As the motivation of some conscripts to achieve fast responses in the simple reaction task was rather low, only 315 subjects whose mean reaction time had been lower than 550 ms and who had had less than 4 premature responses were included in the study. (The data had an approximately Gaussian distribution under the 550 ms cut point.) To follow the method used in previous studies, the raw reaction times were log transformed; however, the results of all analyses were approximately the same when performed with raw data.

Serological tests

Serological tests for toxoplasmosis were carried out in the National Reference Laboratory for Toxoplasmosis of the National Institute of Public Health, Prague. Specific IgG and IgM antibody titres for toxoplasmosis were determined by ELISA (IgG: SEVAC, Prague, IgM: TestLine, Brno), optimized for the detection of acute toxoplasmosis (Pokorný *et al*. 1989), and complement fixation test (CFT) (SEVAC, Prague). The decrease in CFT titres is more regular and therefore better reflects the length of *T. gondii* infection (Warren and Sabin, 1942). CFT titres of antibodies to *Toxoplasma* in sera were measured at dilutions between 1 : 8 and 1 : 1024. The subjects with negative results of IgM ELISA (positivity index $<0{\cdot}9$) and absorbance in IgG ELISA $>0{\cdot}250$, i.e. approximately 10 IU/ml, were considered latent-toxoplasmosis positive.

The RhD blood group type (presence of the RhD antigen on the erythrocyte membrane) was determined in all serum samples using the human monoclonal anti-D reagents (Seraclone®, ImmucorGamma Inc.) in the blood transfusion service laboratories. The complete Rh phenotype was determined using the human monoclonal anti-C, -c, -E and -e reagents (Dynex). The DD-Dd-dd genotype was presumed based on the knowledge of population frequencies of genotypes corresponding to each phenotype (for examples D + C + c-E-e + phenotype is encoded in 95·5% by DD and in 4·5% by Dd (Daniels, 2002)). The presence of some misclassified subjects in our large experimental set could only add a random noise to the data. Such random noise could increase the probability of false negative results of the study but not of false positive results.

Statistical analysis

The SPSS 12.0 and Statistica 6.0 programs were used independently and in parallel for all statistical testing including General Linear Model test (GLM), Kendall nonparametric test, chi^2 test, logistic regression and testing of statistical test assumptions (normality of data and normality of residuals with Shapiro-Wilks tests and residual graphs and homogeneity of variances with Levene's test for homogeneity of variances). To control for the effect of the blood transfusion unit and age in the Kendall regression test, we used residuals computed by GLM with independent factors age and set (hospital) instead of raw data in the analysis. The effects of RhD and toxoplasmosis on reaction time were examined using GLM (study 3) and GLM repeated measures test (studies 1 and 2) with the dependent variables being reaction time in minutes 1, 2 and 3 of the test (continuous variables), and the independent variables RhD (binary variable RhD or categorical factor RhD genotype: DD, Dd, dd), toxoplasmosis (binary variable toxo), age (continuous variable age), blood transfusion unit (binary variable set) and within-subject factor of minute of the test (3-level categorical variable R1). Only the relevant effects and interactions are reported in the text; complete results of GLM are shown in the supplementary information at: http://natur.cuni.cz/flegr/supplement2.pdf.

RESULTS

In the original study (Havlíček *et al*. 2001), the subjects with anamnestic titres of anti-*Toxoplasma* antibodies (*Toxoplasma*-infected subjects) had significantly longer reactions, especially in minute 2 of the test, suggesting that they lost concentration earlier or got tired more quickly than *Toxoplasma*-free subjects. Since the subjects of the original study published in 2001 were thrombocyte donors, they were already tested for the RhD phenotype. In search for possible biological effects of the RhD

phenotype, we reanalysed the original data, entering the information on RhD phenotype into the statistical models. The experimental set of voluntary thrombocyte donors (UHKT1) consisted of 69 men and 45 women. Previous studies suggested that males and females could respond differently (often in an opposite way) to toxoplasmosis (Lindová *et al.* 2006, for review see Flegr, 2007). Therefore, we analysed men and women separately.

The results show statistically significant interactions between test duration (minutes 1, 2 and 3), RhD phenotype and toxoplasmosis ($F_{2,128}=3{\cdot}25$; $P=0{\cdot}042$; $\eta^2=0{\cdot}048$), suggesting that the RhD-positive and RhD-negative males were differently influenced by toxoplasmosis in particular minutes of the test (Fig. 1). In the RhD-negative subset, the *Toxoplasma*-infected males had non-significantly longer reaction times than *Toxoplasma*-free subjects (1st minute: $F_{1,20}=2{\cdot}64$, $P=0{\cdot}120$, $\eta^2=0{\cdot}144$; 2nd minute: $F_{1,20}=0{\cdot}60$, $P=0{\cdot}448$, $\eta^2=0{\cdot}02$; 3rd minute: $F_{1,20}=2{\cdot}64$, $P=0{\cdot}120$, $\eta^2=0{\cdot}068$), while in RhD-positive subset, any effect of *Toxoplasma* infection was absent (1st minute: $F_{1,43}=1{\cdot}67$, $P=0{\cdot}203$, $\eta^2=0{\cdot}037$; 2nd minute: $F_{1,43}=0{\cdot}01$, $P=0{\cdot}913$, $\eta^2<0{\cdot}001$; 3rd minute: $F_{1,43}=0{\cdot}26$, $P=0{\cdot}610$, $\eta^2=0{\cdot}006$). The results obtained for females were qualitatively the same (Table 1), the statistical significance of the interaction was lower while the effect size (η^2) was higher ($F_{2,80}=3{\cdot}18$; $P=0{\cdot}047$; $\eta^2=0{\cdot}074$). Due to the low number of subjects, separate analyses of 8 RhD-negative and 37 RhD-positive women provided only non-significant results.

To reveal possible difference in reaction times between RhD-positive homozygotes and heterozygotes, we repeated the study on a larger scale. We collected new data (UHKT2) in the same blood transfusion facility 4 years after the end of the previous study (89 male and 37 female thrombocyte donors not involved in the previous study) and in the blood transfusion department of the Zbraslav University Hospital (313 blood donors). For technical reasons, at the Institute of Hematology and Blood Transfusion, the performance tests were carried out after the blood donation session (as in the study of Havlíček *et al.* 2001) while the Zbraslav participants were tested prior to the session. Therefore, we also included the blood transfusion facility as one of the independent factors in the analyses. The results for men showed a statistically significant effect of toxoplasmosis ($F_{1,301}=5{\cdot}61$; $P=0{\cdot}019$; $\eta^2=0{\cdot}018$) and interaction between RhD phenotype and toxoplasmosis ($F_{1,301}=5{\cdot}71$; $P=0{\cdot}017$; $\eta^2=0{\cdot}019$), suggesting that toxoplasmosis prolonged reaction times, and RhD-negative subjects responded differently (more strongly than RhD-positive) to the infection (Fig. 1). In the RhD-negative subset, the *Toxoplasma*-infected males had significantly longer reaction times in all 3 mins of the test (1st minute: $F_{1,76}=10{\cdot}61$, $P=0{\cdot}002$, $\eta^2=0{\cdot}116$;

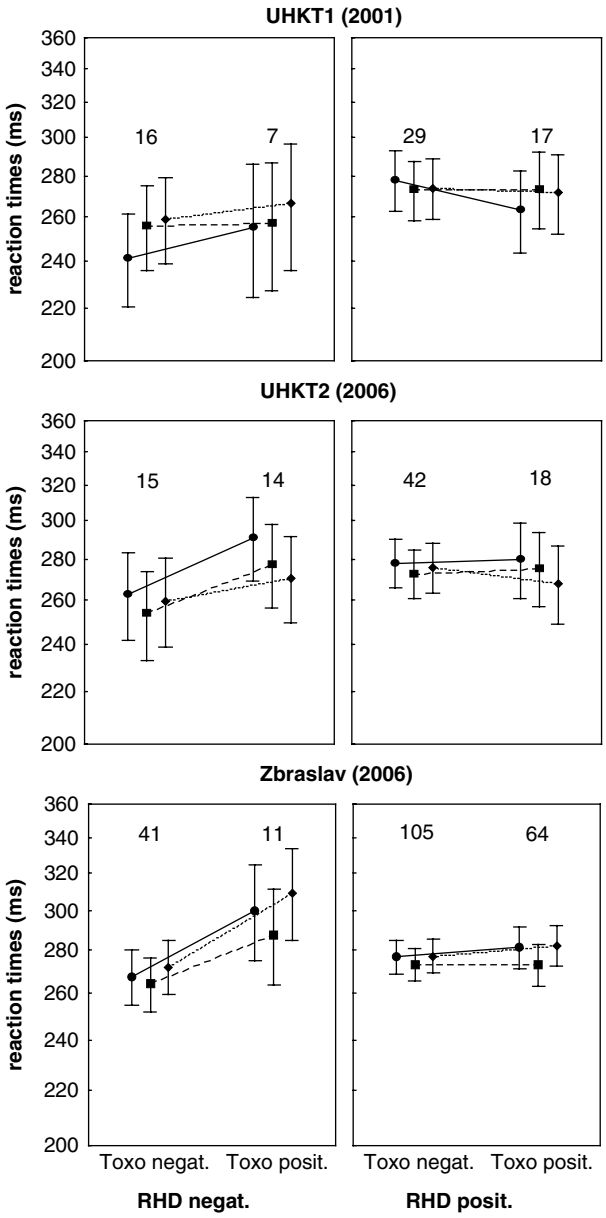

Fig. 1. Simple reaction times in 3 populations of men. The y-axis shows mean reaction times (in msec) of *Toxoplasma*-free (left part of each panel), *Toxoplasma*-infected (right part of each panel), RhD-negative (left panels) and RhD-positive (right panels) men in minutes 1 (circles), 2 (squares) and 3 (diamonds) of the test; the spreads indicate 95% confidential intervals computed for non-transformed data, figures indicate the numbers of *Toxoplasma*-free and *Toxoplasma*-infected men.

2nd minute: $F_{1,76}=7{\cdot}89$, $P=0{\cdot}006$, $\eta^2=0{\cdot}093$; 3rd minute: $F_{1,76}=5{\cdot}59$, $P=0{\cdot}021$, $\eta^2=0{\cdot}064$) than *Toxoplasma*-free subjects. In the RhD-positive subset, the effect of *Toxoplasma* infection was non-significant in any minute of the test (1st minute: $F_{1,224}=0{\cdot}05$, $P=0{\cdot}822$, $\eta^2=0{\cdot}019$; 2nd minute: $F_{1,224}=0{\cdot}03$, $P=0{\cdot}867$, $\eta^2<0{\cdot}001$; 3rd minute: $F_{1,224}=0{\cdot}09$, $P=0{\cdot}765$, $\eta^2=0{\cdot}014$). Except for age, no other main effect was significant. In a separate analysis of the women, these effects were not significant (Table 1).

Table 1. Reaction times of *Toxoplasma*-free and *Toxoplasma*-infected, RhD-positive and RhD-negative subjects

(The table also shows P values for effect of toxoplasmosis and Toxo-Rh interaction in particular minutes of the test.)

			mean reaction time (ms) and standard deviation		
		N	1st min	2nd min	3rd min
1st study: Blood donors (UHKT1 Havlíček *et al.* 2001)					
MEN					
RhD neg.	Toxo free	16	240·9 (30·0)	255·4 (26·8)	258·9 (25·8)
	Toxo infected	7	255·1 (15·7)	257·1 (11·0)	266·1 (17·8)
RhD pos.	Toxo free	29	277·7 (49·4)	272·8 (45·0)	273·9 (39·6)
	Toxo infected	17	262·9 (27·8)	273·2 (29·1)	271·4 (27·8)
	P_{Toxo}/P_{ToxoRh}		0·837/0·072	0·798/0·647	0·697/0·274
WOMEN					
RhD neg.	Toxo free	3	239·1 (9·2)	264·7 (8·1)	264·5 (8·6)
	Toxo infected	5	249·7 (33·3)	257·4 (31·1)	276·5 (25·0)
RhD pos.	Toxo free	25	260·4 (27·1)	270·0 (29·2)	283·9 (27·9)
	Toxo infected	12	265·2 (29·7)	283·9 (44·4)	272·8 (33·4)
	P_{Toxo}/P_{ToxoRh}		0·574/0·685	0·947/0·493	0·930/0·234
2nd study: Blood donors 2002–2006 (UHKT2 and Zbraslav)					
MEN					
RhD neg.	Toxo free	56	266·1 (40·7)	261·3 (33·6)	268·6 (42·5)
	Toxo infected	25	294·9 (48·7)	281·7 (40·0)	287·4 (54·2)
RhD pos.	Toxo free	147	277·1 (41·1)	272·9 (43·7)	276·7 (41·4)
	Toxo infected	82	281·1 (41·4)	273·6 (41·0)	279·1 (41·1)
	P_{Toxo}/P_{ToxoRh}		0·006/0·010	0·039/0·055	0·081/0·034
WOMEN					
RhD neg.	Toxo free	19	306·4 (99·1)	308·3 (102·2)	299·1 (66·0)
	Toxo infected	11	278·5 (18·6)	280·6 (41·2)	284·8 (33·7)
RhD pos.	Toxo free	66	289·1 (54·9)	281·9 (52·2)	287·0 (53·2)
	Toxo infected	33	301·8 (59·8)	298·0 (58·6)	301·6 (61·6)
	P_{Toxo}/P_{ToxoRh}		0·148/0·093	0·521/0·184	0·794/0·386
3rd study: Conscripts 2001					
RhD neg.	Toxo free	43	394·3 (72·9)	nd.[1]	nd.
	Toxo infected	15	440·3 (82·9)	nd.	nd.
RhD pos.	Toxo free	177	392·3 (62·2)	nd.	nd.
	Toxo infected	80	378·4 (65·1)	nd.	nd.
	P_{Toxo}/P_{ToxoRh}		0·208/0·007		

[1] not determined.

Because of linkage disequilibria, the presumed RhD genotype (heterozygote/homozygote) of subjects can be estimated on the basis of the complete Rh phenotype of each subject and RhD haplotype frequencies in European populations (Daniels, 2002). The complete Rh phenotype data were available for 340 subjects (250 men and 90 women) of groups UHKT2 and Zbraslav. In the subsequent analysis, the presumed RhD genotype (categorical: DD, Dd, dd) instead of the binary variable RhD phenotype was included into the models. The results for 250 men showed no significant main effect but a significant interaction between toxoplasmosis and RhD genotype ($F_{2,237}=3{\cdot}375$; $P=0{\cdot}036$; $\eta^2=0{\cdot}028$), suggesting that in contrast to both RhD-negative and RhD-positive homozygotes, the heterozygotes were protected against *Toxoplasma*-induced impairment of reaction times (Fig. 2). The interaction between

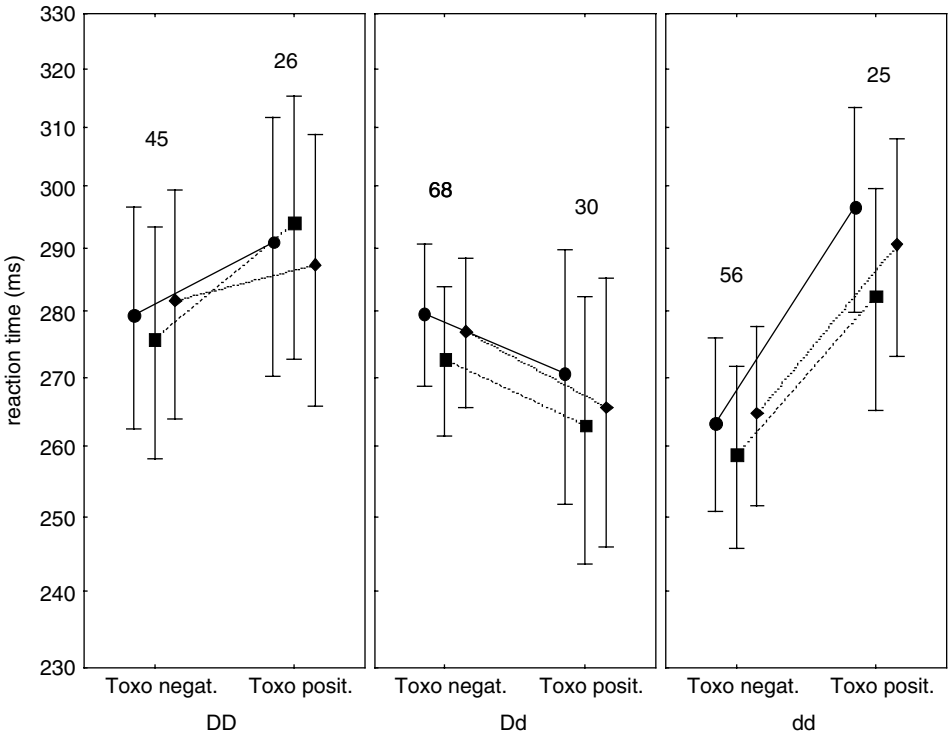

Fig. 2. Simple reaction times of blood donors (men, UHKT2 and Zbraslav). The y-axis shows mean reaction times (in msec) of *Toxoplasma*-free (left part of each panel), *Toxoplasma*-infected (right part of each panel), RhD-positive homozygotes (DD, left panel), RhD-positive heterozygotes (Dd, middle panel) and RhD-negative homozygotes (dd, right panel) in minutes 1 (circles), 2 (squares) and 3 (diamonds) of the test; the spreads indicate 95% confidence intervals computed for non-transformed data, figures indicate the numbers of *Toxoplasma*-free and *Toxoplasma*-infected men.

test duration (minutes 1, 2 and 3), RhD phenotype and toxoplasmosis was not significant ($F_{4,474}=0{\cdot}37$; $P=0{\cdot}827$; $\eta^2=0{\cdot}004$). The interaction between toxoplasmosis and RhD genotype was not significant for women, which could be due to either the smaller number of experimental subjects or, possibly, confounding effects of fluctuating levels of hormones across the menstrual cycle (Havlicek *et al*. 2005).

The negative influence of toxoplasmosis on reaction times is known to increase with the duration of *Toxoplasma* infection (Havlíček *et al*. 2001). Results of previous studies show that the duration of *Toxoplasma* infection cannot be estimated from the highly fluctuating anti-*Toxoplasma* antibodies measured with ELISA; however, the estimation can be based on more or less regularly decreasing titres as determined by a complement fixation test (Flegr *et al*. 2000). Fig. 3 shows that there was a negative relation between concentrations of anti-*Toxoplasma* antibodies and reaction times in RhD-positive men, gradually increasing from minute 1 to minute 3 of the test. This effect was statistically significant for minute 3 of the test (Kendall $Tau=-0{\cdot}161$; $P=0{\cdot}018$, one-tailed test) as well as for the mean reaction time in all 3 minutes (Kendall $Tau=-0{\cdot}137$; $P=0{\cdot}036$, one-tailed test). No such effect was observed in RhD-negative men (Fig. 3) and women (not shown). The separate regression for particular genotypes (DD, Dd and dd) provided no significant results. However, the Kendall *Tau* was around zero for Dd and dd and around $-0{\cdot}15$ for DD, suggesting that RhD-positive homozygotes were responsible for the progressive increase in reaction times with duration of the infection in RhD-positive subjects. The data shown in Fig. 3 indicate that the absence of the effect of length of infection in Dd heterozygotes might be explained by their resistance to pathological effects of toxoplasmosis, while in dd homozygotes it possibly results from their immediate response to the infection (rapidly increased reaction times after the infection).

In our third study, we analysed reaction times of 315 male military conscripts. The analysis showed that the RhD-negative subjects had longer reaction times than RhD-positive subjects (Rh main effect: $F_{1,310}=7{\cdot}85$; $P=0{\cdot}005$; $\eta^2=0{\cdot}025$), and after the infection, the RhD-negative subjects had non-significantly longer ($F_{1,55}=3{\cdot}76$, $P=0{\cdot}057$, $\eta^2=0{\cdot}064$) and RhD-positive subjects non-significantly shorter ($F_{1,254}=3{\cdot}42$, $P=0{\cdot}065$, $\eta^2=0{\cdot}013$) reaction times than the corresponding non-infected subjects (Toxo-RhD interaction: $F_{1,310}=7{\cdot}83$; $P=0{\cdot}007$; $\eta^2=0{\cdot}024$) (Table 1 and Fig. 4).

The GLM analysis of pooled data of all 4 populations of men (dependent variable being reaction time in the 1st minute of the test, independent variables Toxo, RhD, Set, Age) showed a significant effect of Toxo ($F_{1,850}=6{\cdot}06$, $P=0{\cdot}014$, $\eta^2=0{\cdot}003$)

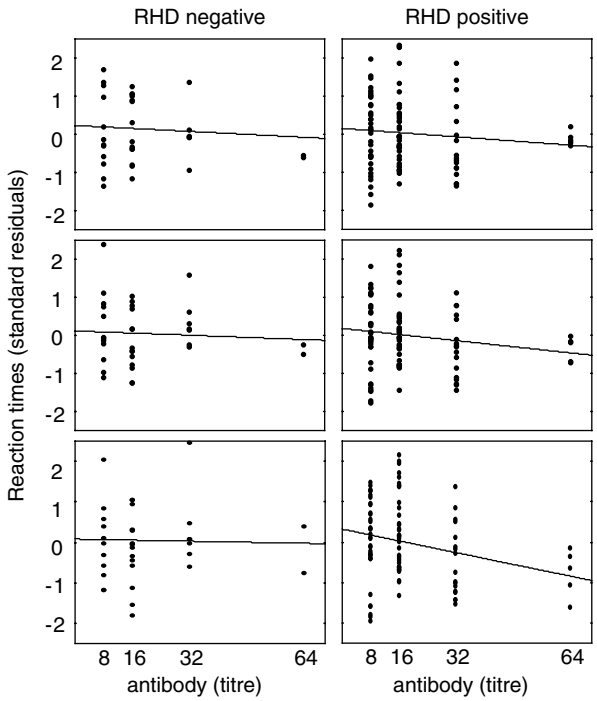

Fig. 3. Negative correlation between concentrations of anti-*Toxoplasma* antibodies and reaction times of blood donors (men, UHKT1, UHKT2 and Zbraslav). The y-axis shows mean reaction times (in standard residuals) in minutes 1, 2 and 3 of the test in RhD-positive men (right panels) and zero correlation among RhD-negative men (left panels). The standard residuals of logarithmic reaction times were GLM with age and hospital (UHKT1, UHKT2 and Zbraslav) as independent variables. The group with the lowest antibody titres measured with CFT (1 : 8) includes subjects with the longest duration of *Toxoplasma* infection.

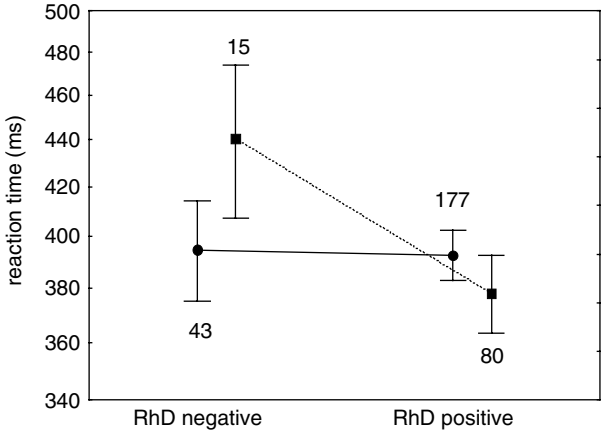

Fig. 4. Simple reaction times of blood conscripts. The y-axis shows mean reaction times (in msec) of *Toxoplasma*-free (circles), *Toxoplasma*-infected (squares), RhD-negative (left), RhD-positive (right) conscripts in one minute-test; the spreads indicate 95% confidential intervals computed for non-transformed data, figures indicate the numbers of *Toxoplasma*-free and *Toxoplasma*-infected men.

and a highly significant effect of Toxo-RhD interaction ($F_{1,850}=11{\cdot}53$, $P=0{\cdot}001$, $\eta^2=0{\cdot}006$). The GLM repeated measures test for pooled data of all 3 populations of male blood donors (dependent variables being reaction time in minutes 1, 2 and 3 of the test, independent variables Toxo, RhD, Set, Age) showed a significant effect of Toxo ($F_{1,366}=3{\cdot}93$, $P=0{\cdot}048$, $\eta^2=0{\cdot}011$) and a Toxo-RhD interaction ($F_{1,539}=5{\cdot}96$, $P=0{\cdot}015$, $\eta^2=0{\cdot}016$). These effects were non-significant in similar analysis of pooled data of female subjects. The GLM repeated measures test for pooled data of all male and female blood donors (dependent variables being reaction time in minutes 1, 2 and 3 of the test, independent variables Toxo, RhD, Set, Sex and Age) showed a significant effect of Sex-Toxo-RhD ($F_{1,528}=5{\cdot}11$, $P<0{\cdot}024$, $\eta^2=0{\cdot}010$).

DISCUSSION

Observed effects of the RhD phenotype on *Toxoplasma*-induced deterioration of psychomotor performance could explain not only the origin of the RhD blood group polymorphism, but also the geographical differences in frequency of RhD-minus alleles in human populations. The possible physiological mechanism of the effect of toxoplasmosis on psychomotor performance and the protective role of the RhD protein can only be speculated on at this point. The structure homology data suggest that the RhD protein acts as an ion pump of uncertain specificity and unknown physiological role (Kustu and Inwood, 2006; Biver *et al*. 2006). The molecule could either play a direct role in signal processing or in ontogenetic processes shaping the signal processing machinery. Several independent indices suggest that toxoplasmosis might affect the neurotransmitter levels, most probably that of dopamine, in certain parts of the brain (Stibbs, 1985; Flegr *et al*. 2003; Novotná *et al*. 2005; Skallová *et al*. 2006; Hodková *et al*. 2007). Dopamine and its agonists and antagonists are known to influence not only reaction times (Courtiere *et al*. 2003) but also performance in many attention-related tasks and recognition of new stimuli (Kahkonen *et al*. 2002; Rihet *et al*. 2002; Nieoullon, 2002). However, until now, no link has been reported to exist between dopamine (or any other component or function of the neural system) and the RhD phenotype.

Among *Toxoplasma*-free men in study 1 (UHKT1) and study 2 (UHKT2 and Zbraslav) and *Toxoplasma*-free women in study 1 (UHKT1), the RhD-negative subjects had faster simple reaction times than RhD-positive subjects. In the study 2 (UHKT2 and Zbraslav) the RhD-negative *Toxoplasma*-infected women were faster than RhD-negative *Toxoplasma*-free women (significantly better in the 1st and 2nd minute of the test, data not shown) and in the *Toxoplasma*-free conscripts the psychomotor performance of RhD-negative and

RhD-positive subjects was approximately the same. As far as we know, this effect of Rh factor on psychomotor performance has not been observed or at least reported in the scientific literature. Prevalence of latent toxoplasmosis is approximately 30% worldwide and no psychomotor performance study was probably performed on a *Toxoplasma*-free subpopulation.

It is possible that the better psychomotor performance of RhD-negative subjects in the *Toxoplasma*-free population could be the reason for spreading of the 'd allele' (deletion) in the European population. In contrast to the situation in Africa and certain (but not all) regions of Asia, the abundance of wild cats (definitive hosts of *Toxoplasma gondii*) in the European territory was very low before the advent of the domestic cat (Torrey and Yolken, 1995). Therefore, the prevalence of latent toxoplasmosis in the prehistoric European population was also probably negligible. Currently, the frequency of RhD-negative subjects in the Caucasian population (with about 30% prevalence of toxoplasmosis) is about 20% while in the African population with a very high prevalence of toxoplasmosis (Roever-Bonnet, 1972) it is only 5% (Daniels, 2002). Theoretically, we could expect the decrease of the RhD-minus allele in the European population after the advent of the domestic cat; however, this event was relatively recent and probably coincided with relaxation of many forms of natural selection.

The protective effect of RhD against the *Toxoplasma*-induced impairment of psychomotor performance could explain not only the differences in frequency of RhD-negative subjects in toxoplasmosis-low and toxoplasmosis-high regions, but also the origin and primary spreading of RHD gene (RHCED duplication) in our African ancestors. A comparison of mean reaction times between infected dd homozygotes and Dd heterozygotes shows that the protective effect of the RhD genotype on *Toxoplasma*-induced reaction time changes is relatively strong. Reaction times play an important role in interactions with prey and predators as well as in intraspecies combats. In our evolutionary past, the frequency of toxoplasmosis in populations of our ancestors was probably rather high due to consumption of raw or undercooked meat and generally lower hygiene standards. Under such conditions, the RhD heterozygotes with the shortest reaction times were probably favoured by natural and sexual selection, which could only partly be counterbalanced by the selection against RhD-negative women with lower reproductive success. It must be kept in mind that in addition to longer reaction times, the 'asymptomatic' latent toxoplasmosis in humans has also been shown to have several other effects, including those on behaviour and personality, length of pregnancy, probability of birth of male offspring and testosterone concentration (Flegr *et al.* 1996; Kaňková *et al.* 2007; Kaňková and Flegr, 2007; Flegr *et al.* 2008). The protective role of Dd phenotype against these effects of latent toxoplasmosis and probably also of other parasites or even other factors (aging, stress) should be addressed in future studies.

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