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Úleková reakce u osob s latentní toxplasmosou

Startle Reaction in Subjects with Latent Toxoplasmosis

Diplomová práce
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Vedoucí závěrečné práce: Prof. RNDr. Jaroslav Flegr, CSc.

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Prohlášení:

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V Praze, 29. 08. 2011

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ABSTRACT

Possible connection between latent toxoplasmosis and schizophrenia is a very interesting and medically important topic. In this thesis I tried to map current state of knowledge in the interdisciplinary research of schizophrenia and *Toxoplasma gondii* and their possible connections as well as to show differences in responses between *Toxoplasma*-positive and *Toxoplasma*-negative subjects using simple computer-administered tests of prepulse inhibition of startle reaction (PPI). Such differences would suggest another similarity between schizophrenia patients and subjects with latent toxoplasmosis as the sensorimotor gating responsible for PPI was found to be disrupted in schizophrenia patients. Side goal of the study was to test newly developed PC software for testing PPI and to determine its applicability in further research.

Subjects for the tests were recruited among adepts of professional military service; 409 subjects completed the test of acoustic PPI and 276 subjects completed the test of visual PPI. All the subjects were tested on presence of specific anti-*Toxoplasma* IgG in their blood serum.

Both tests revealed significant ($p<0.001$) differences between responses on prepulse-preceded stimuli and plain stimuli without prepulse, no significant results were, however, gained for the effects of latent toxoplasmosis neither in response times nor in reactions on prepulse-preceded stimuli. One of the possible explanations is the effect of positive RhD factor in vast majority of experimental subjects; inadequate number of RhD negative subjects with latent toxoplasmosis disallowed further analysis of differences between reactions of RhD positive and RhD negative subjects with latent toxoplasmosis.

KEYWORDS

Manipulation hypothesis, latent toxoplasmosis, startle response, prepulse inhibition, schizophrenia, reaction times

ABSTRAKT

Možné propojení mezi latentní toxoplasmozou a schizofrenií je velmi zajímavé a z lékařského hlediska důležité téma. V této práci jsem se pokusila zmapovat současný stav poznání v této mezioborové oblasti. Neméně důležitým cílem práce bylo i poukázat, za použití jednoduchých počítačových testů prepulsní inhibice úlekové reakce, na rozdíly v reakčních časech a zpracování signálů s prepulsem u *Toxoplasma*-pozitivních a *Toxoplasma*-negativních pokusných osob. Rozdíly v rychlosti reakce u signálů s prepulzem by mohly poukazovat na další propojení mezi schizofrenií a latentní toxoplasmosou neboť senzorimotorické zpracování je u schizofrenních pacientů narušeno. Dalším z cílů práce bylo otestovat funkčnost nově vyvinutých počítačových programů k testování prepulsní inhibice a stanovit jejich použitelnost v dalších experimentech.

Pokusné osoby byly získány mezi uchazeči o profesionální vojenskou službu v Armádě ČR. Test zvukové inhibice úlekové reakce vyplnilo 409 pokusných osob, 276 pokusných osob dokončilo test vizuální prepulsní inhibice. Pokusné osoby byly rovněž otestovány na přítomnost specifických IgG protilátek proti parazitnímu organismu *Toxoplasma gondii* v krevní plasmě.

Oba testy poukázaly na signifikantní rozdíl ($p<0,001$) mezi reakcemi na signály bez prepulsu a signály, kterým předcházel slabší prepulsní signál. Efekt toxoplasmosy na reakční čas ani na rozdíl ve zpracování signálů s a bez prepulsu nebyl prokázán. Jedním z možných vysvětlení pro negativní výsledky sledované hypotézy je možný vliv krevních Rh faktorů, které významně interagují s dosud sledovanými efekty latentní toxoplasmosy. Převážná většina pokusných osob s latentní toxoplasmosou byla Rh pozitivní, a nebylo tedy možné posoudit rozdíly mezi reakcemi Rh pozitivních a Rh negativních osob s latentní toxoplasmosou.

KLÍČOVÁ SLOVA

Manipulační hypotéza, latentní toxoplasmosa, úleková reakce, prepulsní inhibice, schizofrenie, reakční časy

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1. INTRODUCTION

It is over one hundred years since the discovery of the protozoan parasite *Toxoplasma gondii* in the tissues of hamster-like rodent, gundi (*Ctenodactylus gundi*) (Nicolle & Mancaux, 1908 and 1909 in Dubey, 2008) and the database of our knowledge on the subject has been steadily growing ever since, yet some parts of its biology and interaction with its hosts remain a mystery. While most of the nowadays research deal with the parasite's interaction with human immunity as well as with other aspects important to clinical medicine, e.g. acute phase of toxoplasmosis during pregnancy or eye form of toxoplasmosis which may lead to blindness, my interest was from the first moment focused on the manipulative part of the parasite, i.e. its extraordinary ability to alter behavior of its hosts including human beings. The more I have discovered about this aspect of the organism, the more I was pushed back into the realm of clinical medicine following the researchers' journey from discovering *Toxoplasma* as a potential example of host-manipulation hypothesis to modern hypotheses pointing out possible connections between the parasite and various psychiatric and neurological disorders. Today, I'm looking on the word *Toxoplasma* and it is schizophrenia what is actually perceived by my mind.

In this thesis, I would like to pursue the journey again may be with several stops and excursions into affiliated topics. There are in fact several main areas touched by the work and named in the Keywords section of this thesis:

1. Manipulation hypothesis, the theory that parasitic organisms can, as a part of their extended phenotype, manipulate behavior of their hosts to increase their odds for survival and reproduction;

2. *Toxoplasma gondii*, one of the manipulative protist parasites supposed to be changing behavior of its intermediate hosts to increase its chance to be transmitted to its definitive host, a feline;
3. Reaction times, one of the characteristics shown to be affected by *Toxoplasma gondii* in both human and mice studies;
4. Startle reaction, a characteristics suggested to be affected by *Toxoplasma gondii* by previous questionnaire surveys;
5. Prepulse inhibition of startle reaction, a characteristics connected with sensorimotor gating and disturbed in schizophrenia patients as well as in patients with other mental disorders, human and animal subjects under effects of pharmaceutical substances and by many other conditions such as day period or menstrual cycle in female subjects;
6. Schizophrenia, a group of mental disorders with severe positive and negative symptoms and many possible causes. The *Toxoplasma gondii* is one of the suspects of being a cause of severe type of schizophrenia and possibly it is also directly responsible for some of the symptoms previously accredited to schizophrenia itself.

The voyage in the course of which I will try to explain how I traveled from traffic accidents to one of the most feared mental illnesses will be concluded with my own small contribution to the research, i. e. with the experimental part of this thesis. It is, however, more of a beginning of a new chapter than the end because the experimental part is mostly a pilot study and first observation of the studied phenomenon from the point of view of methodology that has never been used in *Toxoplasma* research before.

1.1 STRUCTURE OF THE THESIS

As it was implied before, the text has two main parts, first being a background research and second being the experimental part of my work.

In the first part, I am going to introduce the contemporary stage of the research and it is therefore divided into subchapters corresponding with main subjects of the thesis. The subchapter Manipulation Hypothesis starts the whole part exploring the history and current knowledge on the ability of parasites to manipulate their hosts.

The manipulation hypothesis part is followed by a chapter on one of the most famous representatives of the manipulative parasites and simultaneously the main topic of this thesis, *Toxoplasma gondii.* While this subchapter is concluded by several references on hypotheses connecting the organism with various psychiatric disorders, next chapter follows up discussing schizophrenia as one of the most important diseases that were already persuasively linked with the parasite.

The chapter about reaction times is rather short and focused mainly on studies of *Toxoplasma gondii*-induced changes in the characteristics. The reaction times studies were already conducted in our laboratory and this thesis is aimed to push the research further so I limited the text on reaction times only to a brief summary of previous results with a quick introduction into the topic.

The fifth subchapter applies to the startle reaction, an escape mechanism observed mostly in mammals. As the thesis is mainly oriented on the prepulse inhibition of the startle reaction, an introduction and summary of the current knowledge of this topic is presented as a basis for the next chapter oriented on the very subject of prepulse inhibition.

There are many diagnostic methods that can be and are used to diagnose, model and study schizophrenia in all its forms. Among them there is

one that seemed to fit best our research needs and possibilities in our effort to learn more about the connections between latent toxoplasmosis and schizophrenia. The method is prepulse inhibition of startle reaction and it is described and supplied with several examples of use in the sixth subchapter of the first part.

The seventh and the last subchapter strives to mix all the previous ingredients together and explain why we chose the experiments we used to study the topics we studied and otherwise prepares ground for the experimental part. In this part there are also presented ideas and background for further research as this thesis is only a small part in the continuing studies of *Toxoplasma gondii* and its effects on human personality, behavior and psychical health. I am aware that these issues are usually considered to be part of a Discussion section of the experimental part I have however decided that as the experimental part of this thesis makes only a small (and mostly pilot) section of the research pursued in our laboratory, it would be more suitable to set it into some well-explained context with discussion section briefly mentioning only some of the possible research development directly connected with the presented experiment. I am also aware that the seventh subchapter includes paragraphs discussing issues such as development of our test batteries and our experience as well as our future plans which usually are not part of a diploma thesis. If the reader tends to think these paragraphs are inappropriate and only wasting his/her time, I beg him/her to skip the last two parts of the seventh subchapter. Main references to further research will be briefly noted in the discussion and other issues contained in this chapter are by no means necessary to understand and evaluate the thesis. I do, however, think that these paragraphs might be useful in setting this work into wider context and explaining why and how the programs presented in the experimental parts were developed, and how do they fit into long-term research plan conducted by my colleagues and me in search for *Toxoplasma*-induced behavioral and pathological changes and their underlying mechanisms.

The main beauty as well as challenge of our research lies probably in its multidimensionality. We deal with cross over issues between parasitology, etology, psychiatry, psychology and other branches of biological and medical research making us dig deeply into the fields and learn new information on everyday basis. The advantage of the research, however, turns into huge disadvantage when writing a limited thesis. I have tried to review all the areas connected with the presented research but only brief mention is ultimately dedicated to each topic. Whole books or reviews could be and were published about most of the paragraphs and subchapters included in the work and they were written by much more knowledgeable authorities than me. I have mentioned interesting reviews as well as original studies in each part of the text and resign to the effort to replace them. I am able to recommend heaps of further reading in any area mentioned in the text.

1.2 AIMS OF THE THESIS

As it is implied in former description of the thesis, my general goal was to push recent research of behavioral changes caused by latent toxoplasmosis in human subjects more into the realm of psychiatry and neurobiology both by theoretical survey into performed research made by our team as well as by other scientists and by the experimental part where I tried to map differences between subjects with and without latent toxoplasmosis using a method similar to our previous tests of reaction times as well as to a method of prepulse inhibition used in schizophrenia research.

More specifically, my goals were

1. To test whether our newly developed method of PC administered prepulse inhibition can be used in *Toxoplasma*-schizophrenia research and to determine further development of the method;
2. To study differences in reaction times between *Toxoplasma*-positive and *Toxoplasma*-negative subjects in altered conditions where a disturbing preceding signal is present;
3. To present a theoretical survey into the method of measuring the prepulse inhibition of startle reaction in humans that can be used as a theoretical background for our further research using the original method of prepulse inhibition measurement.

2. THEORETICAL PART

To get oriented in the lagoon of *Toxoplasma*-connected research is one of the most difficult objectives I have ever imposed upon myself. There are tens of thousands results for the term *"Toxoplasma gondii*" on Google Scholar even when the search is limited only to most recent years. The same is true for the term "schizophrenia" and "prepulse inhibition" turns out only a little bit better. For the purpose of this thesis, I have limited my background research only to areas strongly connected with the studied topics therefore omitting the newest development in the field of *Toxoplasma*'s interactions with human immune system or pharmaceutical approaches to schizophrenia therapy. Although many of the omitted topics are deeply interesting and worth of long and knowledgeable discussion I tried to keep this thesis as focused on the main theme as the multidimensionality of the presented research allowed.

2.1 MANIPULATION HYPOTHESIS

In 1972, Holmes and Bethel published an idea that parasites might be able to change their host's behavior to better suit their needs and opened a long term discussion whether the parasite's influence is an adaptive part of its extended phenotype (Dawkins, 1982) or whether the changes are caused just by the side-effects of the infection. While the debate never really stopped and we have some persuasive reports that some of the behavioral changes are of course just caused by the worsened health of the host organism and other manifests of its illness, other behavioral changes seem to be parasites' way to enhance their odds for survival and reproduction. As an example of the former case it is possible to mention Gourbal et al. (2001) who showed that although mice

infected with a parasite *Taenia crassiceps* demonstrate altered behavior in confrontation with the parasite's final host, a cat, the alteration might be caused secondarily by the parasite's interactions with its host's physiology and immunity. Rather sceptical remains also the recent review of manipulation hypothesis in parasites with complex life-cycles (Cézilly et al., 2010) with authors stating that our current knowledge of mechanisms standing behind the manipulation is still too weak for the authors to be absolutely certain about the true adaptiveness of behavioral changes induced by the parasite and concluding that although the spectacular life cycles can be very suggestive about the adaptive evolution, further research is needed to ultimately determine whether the alterations are really an adaptive manifestation of parasite's extended phenotype or simply a by-effect of the infection. Authors also suggest that the host's vulnerability to predation might be non-specific attempt of the parasite to avoid death inside its intermediate host rather than effort to transmit itself to the specific final host. In this place I should, however, remark, that according to several studies (e.g. Berdoy et al., 2000); some of the behavioral changes seem to be very predator-specific indeed.

While acknowledging the mentioned remarks as well as other studies such as the Robert Poulin's metaanalysis of research reporting host-manipulative behavior of parasites pointing out strong negative correlation between parasite-effect size and the year of publication of the study (Poulin, 2000), for the rest of the thesis I will assume that some level of adaptive host-manipulating behavior exists at least in case of *Toxoplasma gondii*. Next part of this subchapter will be focused on research conducted to learn more about the host-manipulation hypothesis and on observed examples of such phenomenon.

The behavioral changes usually perceived as adaptive are mostly seen in parasites with complex life-cycles facilitating their transfer from one environment (the host one) to another which could be either a new host or a free ecosystem. While as an example for the host-to-host transmission the life-cycle of *Toxoplasma gondii* is usually described, transmission to non-host

environment is often studied in crickets infected by hairworms. I'll use this example to demonstrate several aspects of manipulation hypothesis research focusing on four main questions:

1. Do we have some confirmed cases of altered behavior?
2. Is there an adaptive explanation for altered behavior?
3. How complex are the parasite-produced changes and what mechanisms lay behind them?
4. Is there something the host can do to increase his own chances for survival and reproduction after the infection?

2.1.1 ALTERED BEHAVIOR CONFIRMED

Parasite-induced changes in host behavior and phenotype have been observed in many host-parasite associations thorough many branches of organisms (Poulin & Thomas, 1999). Visible changes in host phenotype can be observed for example in snails (mainly *Succinea* sp.) infected by a digenean parasite *Leucochloridium paradoxum* (e.g. Robinson, 1947); insects can be manipulated by particular fungi to die in a position that is enhancing wind-facilitated spread of the spores (e.g. Maitland 1994). Behavioral changes are reported for example in an experiment conducted by Edward P. Levri who suggested that the freshwater snail *Potamopyrgus antipodarum* infected by the trematode parasite *Microcephallus* sp. is more vulnerable to predation by parasite final host and the altered behavior thus indeed enhances parasite transmission (Levri, 1999).

Furthermore, one parasite can do more than affect just one of his host's traits. As an example we can use cestode parasite *Schistocephalus solidus* altering body coloration (LoBue & Bell, 1993), response to large fish (Milinski, 1985) and vertical distribution (Smith & Kramer, 1987) of its fish host, a stickleback.

According to the authors' statement the three-part study conducted by Frédéric Thomas and his team was the first attempt to determine how

widespread is the suicidal behavior of insects infected by a water-seeking worm. Their field observations showed behavioral differences between infected and uninfected crickets (*Nemobius sylvestris)* leading the infected individual to be more likely to end in water. 50% of the infected individual stayed, however, on dry land. The authors suggest that the parasite-induced behavior may not lead the cricket to water but induce errand behavior taking the cricket away from its natural environment thus increasing the chance of encountering water ecosystem. Once a water stream is encountered, behavior of infected individuals changes again and the crickets jump straight into water while uninfected individuals keep dry (Thomas et al., 2002).

2.1.2 ADAPTIVE OR NOT?

The biggest question concerning the very basis of manipulation hypothesis is probably whether the observed changes are true parasitic adaptation enhancing its further development or just by effect of an infection. I have already mentioned some objections against adaptiveness, more positive view can be found in Poulin's review (Poulin, 1995) in which the author presented conditions under which certain behavior can be considered adaptive.

1. Complexity: Simple traits can easily evolve by chance and cannot be considered an evidence for adaptiveness of the behavioral changes;
2. Purposefulness of design: Adaptive characters should be well fitted for their environment and function;
3. Convergence: Traits that have arisen independently in various lineages of hosts and their parasites are more likely to be adaptations;
4. Fitness effect: The discussed traits must cause an increase of fitness in the parasite (or, according to Poulin, either the parasite or the host).

Poulin states that although there is not yet enough evidence for adaptiveness of behavioral changes enhancing parasite transmission, he personally believes in adaptiveness of at least some of the cases mentioned in his review.

Field evidence for adaptiveness of the parasitic manipulation was brought by a study published by a research group from Bourgogne University (Lagrue et al., 2007). The study worked with an acanthocephalan parasite *Pomphorhynchus laevis* which alters behavior of his intermediate amphipod host *Gammarus pulex* but not that of its sympatric host, an introduced species *Gammarus roeseli*. Authors found 26.3—28.3 times higher proportion of parasitized *G. pulex* in the stomach contents of one of its several definitive hosts *Cottus gobio* in comparison to the arthropods found in benthos, while no such difference was found for the introduced species. Their results also suggested behavioral changes specific to the definitive host rather than increased rate of predation by other non-host species. An adult frog *Rana esculenta* was used as a control predator.

According to a study published by Seppälä et al. (2008), although the behavioral changes induced in host (isopods from Lake Jyväsjärvi in Central Finland) by its parasite (acanthocephalan *Acanthocephalus lucii*) might lead to higher vulnerability to predation by both parasite next host (perch *Perca fluvialis*) and other non-host predators (such as a dragonfly larvae), the vulnerability might be biased toward the definitive host thus suggesting that despite the costs of non-hosts predation, host manipulation might still be an adaptive trait advantageous for the parasite.

Surprisingly enough, even if the parasite encapsulated in body of its host is predated by a non-host organism, it still has a chance for survival. Escaping behavior was observed under laboratory conditions in Gordian worms when their cricket vehicle was predated by generalist predators such as fish (*Oncorhynchus mykiss)*, perch (*Lepomis gibbosus*), bass (*Micropterus salmoides*) or frog (*Rana erythraea*). According to the observation: “the worm escaped predation by wriggling out of the mouth, nose or gills of the predator that had consumed its host” (Ponton et al., 2006).

There's however a situation, in which the adaptiveness of parasitic manipulation gets exploited by non-host predator feasting on easy prey. This was reported by Mouritsen & Poulin (2003) in intertidal New Zealand cockle (*Austrovenus stutchburyi*) manipulated by echinostome trematode (*Curtuteria australis*) which is predated both by avian hosts and by benthic feeding fish (*Notolabrus celidotus*) which constitutes a death end for the parasite. The authors estimate that while 2.5% of parasitized cockles are successfully transmitted into their avian host, 17.2% are lost to fish. Adaptive significance of the system depends mainly on the feeding behavior of the definitive host i.e. the percentage of its food that is constituted by cockles.

2.1.3 ON UNDERLYING MECHANISMS

Very little is known about mechanisms responsible for behavioral changes in parasite-manipulated organisms. Sometimes, the cause lies in simple physiological change induced by a parasite—this applies for example in case of *Diplostomum spathaceum* (Trematoda), a parasite that impairs eyesight of its fish intermediate hosts thus impairing also its escape behavior (Seppälä et al., 2004). In most cases of manipulation hypothesis, the manipulation is much more complicated and we are still only beginning to discover what are the underlying mechanisms.

First attempts were already made in explanation of behavioral changes in crickets infected by hairworms. Sanchez et al. (2008) found out, that the behavioral changes have in fact two separate parts. Firstly, the parasite induces erratic behavior of the cricket. Suicidal behavior is induced afterwards and this change is probably unidirectional – no decrease in suicidal behavior was observed in crickets in this phase of manipulation.

Thomas et al. (2003) were trying to discover the very molecules responsible for the behavioral changes in infected crickets. The study reports decreased levels of various aminoacids in the cricket's body implying possible

competition for nutrition between the parasite and its host. More interesting from the point of view of the manipulation hypothesis paradigm were the changes in taurin, valin and tyrosine. Authors haven't suggested any explanation for increased levels of valin and tyrosine, taurin, however, might be responsible for the cricket feeling thirsty and therefore trying to find some water.

2.1.4 HOST'S PART IN THE PLAY

Poulin et al. (1994) in their article published in a Forum section of the journal Oikos speculate that the outcome of parasite-host manipulation is in fact result of long-term evolutionary arms race in which both the parasite and the host want to dominate over its adversary and the race is responsible for high variability in effects of parasitic manipulation—some hosts learn to cope with infection better than others. In 2005, Biron et al. asked, whether the host got some advantage from collaboration with its manipulator or whether it all ended always in the same way: with loss on host's side. The result of their study on crickets infected with hairworms suggested unfortunately that at least in this case, the chance for reproduction after releasing the parasite into water is increased neither by collaboration (bringing the worm to water) nor by resisting the manipulation: all the male crickets are castrated regardless of their behavior. In females, albeit the collaborative ones managed to produce eggs and attract males as opposed to their non-collaborative counterparts, the difficulties with mounting males, taking spermatophores and ovipositing prevent them from successful reproduction.

In order not to close this chapter with such pessimistic prognosis, recent study by Ponton et al. (2011) shows that at least some of the changes induced by Gordian worm in its cricket hosts may be reversible and return to normal after the worm is released. The study also indicate that changes induced by manipulative parasites in their host are of very subtle, complex and

multidimensional nature and further research will be probably challenging yet immensely interesting.

. . . . . .

I could go on naming many other studies conducted within the framework of manipulation hypothesis paradigm and possibly fill several volumes of pages specified for an average diploma thesis, the conclusions would be, however, always the same: Much work has been already done to discover all the details of manipulative interactions between parasite and its host, much more work is yet to be done. As we are also aiming to add another brick to the collective task, I'll rather over to another topic of this thesis.

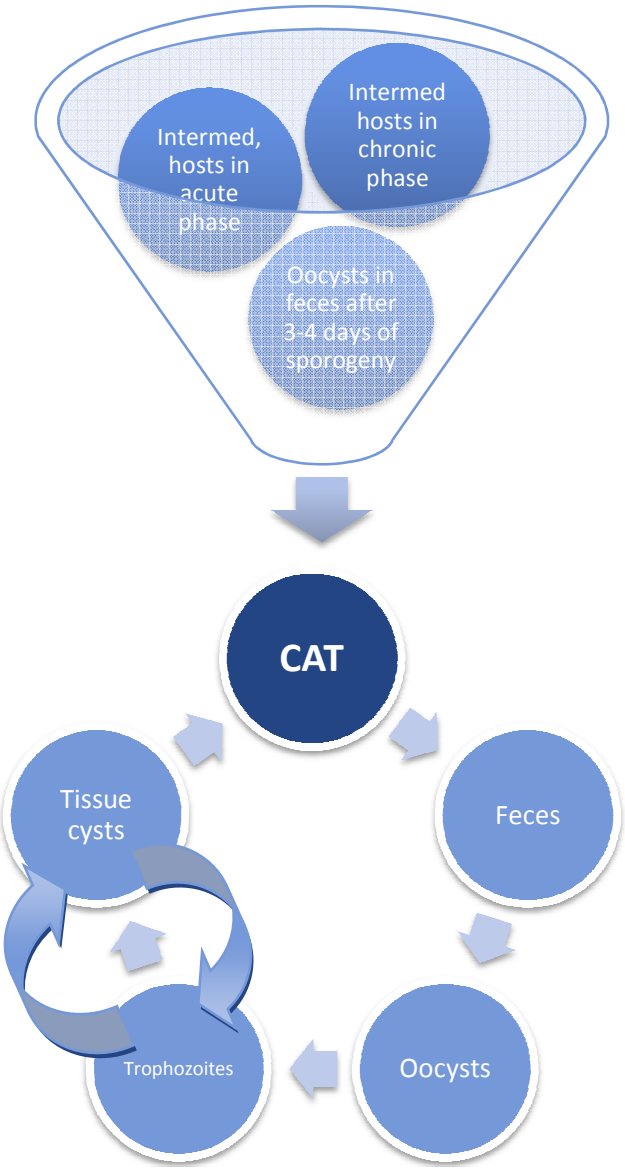
2.2 *TOXOPLASMA GONDII*

While the first subchapter of the theoretical part was intended to be a short introduction into the manipulation hypothesis paradigm with several hints and examples of topics that are currently being studied, this chapter is focused solely on one of the best known examples of manipulation hypothesis, *Toxoplasma gondii*. Not only is this parasite interesting because of wide variety of behavioral changes that it is capable to induce in its intermediate hosts, it is also an important human pathogen known from everyday clinical practice of many physicians. In following paragraphs I'll try to explain the life cycle of unicellular organism, its importance in clinical medicine as well as current state of knowledge of its manipulative abilities.

2.2.1 ON MICE, HUMANS AND *TOXOPLASMA GONDII*

Toxoplasma gondii (Apicomplexa) is a unicellular obligatory parasite of endothermic vertebrates—active particularly in mammals but can be found also in birds (Miller et al., 1972). It was first discovered in gundi (Nicolle and Mancaux, 1908) and shortly afterward in rabbit (Splendore, 1908) and in 1923 it was identified as human parasite by a Czech physician Josef Janků in an eye of a child during autopsy (Janku, 1923). In the following years, mainly the association between *Toxoplasma* and human diseases was discovered including identifying meat as a source of the infection (Jacobs et al., 1960; Desmonts et al., 1965). Other risk factors for toxoplasmosis have been, however, found since that time such as having a cat or frequent consumption of raw vegetables outside the home (Baril et al, 1999). A cat nematode *Toxocara cati* was originally suspected of transmission of the disease (Hutchinson, 1967). Further investigations, however, dismissed this suggestion (Sheffields & Melton, 1969) and in 1970, the *T. gondii* life-cycle with cats as definitive hosts (see Img. 1) in which *Toxoplasma* pursue sexual reproduction was finally discovered (Frenkel et al., 1970).

IMG 1. *TOXOPLASMA GONDIE* LIFECYCLE



Toxoplasma gondii's final host, a cat (either domestic or other *Felinae* species), excrete feces with oocystes. After the oocystes are consumed by other mammals, trophozoites cause acute phase of the infection but wander from the reach of strong immune system into tissues. *T. gondii* cysts then wait for the host to be predated by their next host causing so called chronic phase of the infection. *T. gondii* can be also transmitted congenitally in acute (humans) or chronic (mice) phases of the infection.

Although the best intermediate host *Toxoplasma* can choose in Europe is probably mouse because this way its trophic transmission to domestic cats

living all around us is relatively simple, the means of its transmission often cause the oocysts to end up in completely wrong host such as man.

2.2.2 TOXOPLASMOSIS CLINICAL

Although the life-cycle of *Toxoplasma gondii* had not been known before the seventies of the twentieth century, it has been recognized as a human pathogen for many more years. The eye form of toxoplasmosis discovered by Josef Janků in 1923 is not the only disease caused by the parasite. Probably the most severe problem is the congenital toxoplasmosis transmitted from pregnant mother to the developing fetus as reviewed for example in Wong & Remington (1994). In spite of many years of knowledge of congenital toxoplasmosis, proper diagnosis still might by a challenge even today. Possible approach was published by Magi & Migliorini (2011) showing that it is possible to diagnose congenital toxoplasmosis in cases where classical serology techniques didn't work properly by Western blotting.

Although congenital toxoplasmosis in mice doesn't seem to be as severe as in human, earlier and intermediate maternal infection can cause conditions such as stillbirth or non-viability, and learning or memory capability damage in the offspring (Wang et al.,2011). According to the mentioned study, positive offspring of mice infected in later phases of pregnancy (15 days after gestation in contrast to the mice infected at day 5 and 10 after gestation) had lesser number of cysts in their brain tissue and also showed a longer latency and lower number of errors in the step-through passive avoidance test.

Latent toxoplasmosis returning into its acute phase is still a severe problem for the patients after transplantation. The toxoplasmosis can be either transmitted directly from the donor but reactivation of the old infection is possible, too. Renoult et al. (1997) in their article on toxoplasmosis in kidney transplant recipients report 64.5% mortality rate in observed patients and

conclude that an early diagnosis and therapy are essential for the patients' survival as ten of eleven patients given specific treatment survived the toxoplasmosis onset. Main clinical features shown in the observed patients mostly within 3 months after the transplantation were fever, neurological disturbances and pneumonia.

2.2.3 TOXOPLASMOSIS MANIPULATIVE

Slightly less studied than clinical cases of toxoplasmosis are behavioral changes caused by the parasite in the latent phase of the infection. During the last twenty years several major discoveries were conducted mostly on mice and human subjects revealing shifts in behavioral traits (Flegr & Hrdý, 1994) and reaction times (Havlíček et al., 2001) in humans as well as changed behavioral traits (Skallová et al., 2006) and lesser fear of cat odor (Vyas et al., 2007) in mice. Manipulation hypothesis paradigm associated with latent toxoplasmosis has been reviewed many times before for example by Joanne P. Webster (2007) and it is therefore unnecessary to repeat what was already established. It is, however, necessary to pay attention to the important context of the seemingly unimportant changes in human: the slower reaction times can lead to severe consequences for example in traffic as was shown in studies focused on toxoplasma-positive victims of traffic accidents (Flegr et al., 2009).

2.2.4 TOXPLASMOSIS PSYCHOTIC

The least studied but highly important not only from the point of view of this thesis is the possibility of *Toxoplasma*-induced mental disorders. Connection between congenital toxoplasmosis and mental retardation is a long known issue (e.g. Caiaffa et al., 1993), but other role of *T. gondii* in etiology of mental illnesses had not been studied until recently. In previous years, studies were published linking mental disorders such as schizophrenia with prenatal infections by various infectious agents (Brown & Derkits, 2010) including *Toxoplasma gondii* (Brown et al., 2005). In Miman et al. (2010) study the

toxoplasmosis is considered to be possibly connected with obsessive-compulsive disorders with prevalence of toxoplasmosis significantly higher in OCD patients in comparison to healthy population. Prandota (2011) suggests that autism-specter disorders or Alzheimer disease could be also connected to the congenital or acquired toxoplasmosis and Jones-Brando et al. (2003) showed that drugs used for treating schizophrenia as well as bipolar disorder inhibit the replication of *T. gondii* in vitro thus connecting toxoplasmosis also with bipolar disorder though not necessarily as a cause if the illness.

In a large study on 900 psychiatric patients and more than 200 controls Hinze-Selch et al. (2010) showed that an additional diagnosis of personality disorder was associated with increased probability of *T. gondii* infection. This is in accordance with previously found shifts in personality traits in healthy individuals with latent toxoplasmosis (e.g. Flegr et al., 2000) and can be explained by interactions of toxoplasmosis with neurotransmitter levels participating in development of personality disorders. The authors, however, state that though their data showed significant correlation between *T. gondii* infection and personality disorders, they are unable to say whether it is *T. gondii* causing the disorders or whether the disorders facilitate *T. gondii* infection.

Further studies will be probably needed to determine the causality of latent toxoplasmosis and psychiatric disorders and possibly distinguish between the disorders caused, correlated and interacting with the infection as well as to determine whether the changes in etiology of mental disorders are connected specifically with the manipulative and pathogenic activity of *Toxoplasma gondii* or whether there are more pathogens such as cytomegalovirus causing similar effects in humans by a more general mechanisms. There is, however, one mental disorder which is probably the one most connected with toxoplasmosis and that is schizophrenia. General information about this disease will be presented in the next subchapter and connections to toxoplasmosis will be reviewed in the subchapter 2.7.

2.3 SCHIZOPHRENIA

Schizophrenia or schizophrenia-like mental disorders as we see it today was discovered as a discrete mental illness by German psychiatrist Emil Kraepelin at the end of 19th century as dementia praecox (in Jablensky, 2007) and renamed to schizophrenia in 1911 by Eugen Bleuler (in Bleuer, 1950). It is a persistent and often chronic mental disorder affecting a variety of aspects of behavior, thinking, and emotion. Variety of symptoms can be seen in psychiatric patients diagnosed with schizophrenia the prevalence of which varies between 0.4 and 2.2 patients in 100 individuals with significant heterogeneity of prevalence and incidence among different world populations (Goldner et al., 2002).

2.3.1 DIAGNOSIS

According to American Psychiatric Association whose diagnostic manuals (Diagnostic and statistical manual of mental disorders) are often used around the world to diagnose variety of mental illnesses, the diagnostic criteria for schizophrenia are as follows (an internet version based on the newest DSM-IV-TR manual from the BehaveNet® was used, see the References; this version is in accordance with criteria named in Schizofrenie: Neurobiologie,klinický obraz, terapie by Motlová & Koukolík, 2004):

- *Characteristic symptoms (Criteria A)*: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):
    1. Delusions
    2. Hallucinations
    3. Disorganized speech (e.g., frequent derailment or incoherence)
    4. Grossly disorganized or catatonic behavior
    5. Negative symptoms, i.e., affective flattening, alogia, or avolition

Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts, or two or more voices conversing with each other.

- *Social/occupational dysfunction*: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).
- *Duration*: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
- *Schizoaffective and Mood Disorder exclusion*: Schizoaffective Disorder and Mood Disorder With Psychotic Features have been ruled out because either (1) no Major Depressive, Manic, or Mixed Episodes have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.
- *Substance/general medical condition exclusion*: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.
- *Relationship to a Pervasive Developmental Disorder*: If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of Schizophrenia is made only if prominent delusions

or hallucinations are also present for at least a month (or less if successfully treated).

Classification of longitudinal course (can be applied only after at least 1 year has elapsed since the initial onset of active-phase symptoms):

- **Episodic With Interepisode Residual Symptoms** (episodes are defined by the reemergence of prominent psychotic symptoms); also must be specified if: **With Prominent Negative Symptoms**
- **Episodic With No Interepisode Residual Symptoms**
- **Continuous** (prominent psychotic symptoms are present throughout the period of observation); also must be specified if: **With Prominent Negative Symptoms**
- **Single Episode In Partial Remission**; also must be specified if: **With Prominent Negative Symptoms**
- **Single Episode In Full Remission**
- **Other or Unspecified Pattern**

2.3.2 NEUROTRANSMITTER AND CYTOKINE HYPOTHESES

Schizophrenia specter disorders are connected with shifts in several main neurotransmitters as well as with other molecules such as cytokines. Although for our purposes the dopamine role in schizophrenia etiology is probably the most important one as shifts in dopamine are supposed to be caused by *T. gondii* in brain tissues, other main molecules associated with schizophrenia will be briefly mentioned:

- **Cytokines**, i.e. molecules connected with inflammatory immune reactions are suspected to have a vital role in schizophrenia etiology based both on human studies and animal models. Blood cytokines might penetrate the brain blood barrier and bind to neuron receptors altering normal intracellular signaling and transmission, neural circuit formation and synapse maturation. Abnormalities in this process might be

responsible for impairment of brain functions and ultimately lead to the development of schizophrenia (reviewed by Watanabe et al., 2010).

- **Glutamate.** Medial frontal region glutamate and glutamine are changed in schizophrenic patients and changes in glutamate and glutamine concentration are decreasing at faster rate with age in schizophrenic controls in compare with healthy individuals (Marsman et al., 2011). Pharmaceutical studies also suggest the role of NMDA receptors and glutamate in schizophrenia (Seeman, 2009).
- **Serotonine.** Potential involvement of serotonin in the pathogenesis and neurodevelopmental diathesis of schizophrenia was suggested more than ten years ago noting also that 5-HT could play an important role in the modulation of pathologic neurochemical systems (mostly DA) and thereby provide a target for therapeutic agens (Lieberman et al., 1998).
- **GABA.** The role of GABA in schizophrenia was suggested in 70's (Roberts, 1972) and it has been studied with varied intensity ever since. One of the more recent studies in vivo for example shows correlation between decreased GABA levels in neocortex in schizophrenia patients in compare with healthy controls leading to impaired cortical inhibition (Yoon et al., 2010).
- **Dopamine.** In the review published in 1976 by Meltzer and Stahl the authors stated that the evidence of a role for DA in the pathophysioloy of schizophrenia was compelling but not irrefutable. 35 years later the dopamine is still considered to be an important part of schizophrenia pathology as can be seen for example in the article by Heinz and Schlagenhauf (2010) on dysregulation of the mesolimbic dopamine system in schizophrenia patients. Current approach to dopamine hypothesis of schizophrenia is probably best reviewed in Howes & Kapur (2009) linking the schizophrenia risk factors to increased presynaptic striatal dopaminergic function.

As can be clearly seen from the publication years of the mentioned studies and reviews, the research is still in process and no one can probably point out a single molecule that is the most important in the schizophrenia development. Moreover, the studies suggest that many different substances are probably involved in the process and years of studies will be needed to recognize their unique roles and interactions between them.

One of the methods that are often used to study schizophrenia and its pharmaceutical models is measuring of prepulse inhibition of startle reaction. The method and examples of its usage will be described in the next chapter.

2.4 REACTION TIMES

Reaction times (RT) on diverse stimuli are often measured in order to determine extension of impairment in patients after traumatic brain injury as well as in patients with various diseases. It has been shown that including reaction time tests into diagnostic batteries could help in distinguishing between subjects without brain damage and patients whose brain was damaged but whose cognitive functions are intact (Collins & Long, 1996). Reaction times measures showed to be a strong diagnostic tool in discriminating between patients with different mental disorders as was shown by Fleck et al. (2001) on patients with schizophrenia and bipolar disorder with schizophrenia patients slower than both bipolar patients and healthy volunteers. This is in accordance with other studies such as Vinogradov et al. (1998) suggesting that reaction times in schizophrenic patients are longer and more variable than in healthy controls.

From the point of view of this thesis, the impairment of reaction times in schizophrenic patients is just one part of the importance of reaction times measures. RT are an important component of escape behavior when encountering the predator, making thus a perfect target for manipulative endeavor of parasites transmitted through the means of predation. Unsurprisingly, the parasite-induced reaction times changes can be indeed found in humans infected by *Toxoplasma gondii* (Havlíček et al., 2001) or in mice though there the prolonged reaction times are more probably connected with the acute phase of infection rather than with manipulation in latent phase (Hrdá et al., 2000).

2.5 STARTLE RESPONSE

Startle reaction or startle response is a simple reflex evoked by an unexpected stimulus protecting an animal from injury caused by a predator or by a blow. Although observed mainly in mammals (e.g. Prosser & Hunter, 1936) where it is characterized by a coordinated rapid contraction of facial and skeletal muscles following after the stimuli, similar types of escape behavior can be found in other vertebrates (e.g. Davis et al. (1976) describing startle reaction and its changes in fish with telencephalic lesions) as well as in invertebrate species (e.g. startle reaction of the locust *Locusta migratoria* stimulated by a heath source in Robertson et al., 1996). In mammals, whole body startle (contraction of facial, neck and skeletal muscles, suppression of any ongoing activity, acceleration of the heart rate and closure of eye-lids) can be observed and measured while in human the eye-blink component of the reaction is usually measured. The startle can be elicited by different type of stimuli including acoustic, visual and tactile signals while in experimental studies the acoustic startle is used preferably.

This simple circuit which, according to experimental data gained in rats, consists of auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticularis pontis caudalis, spinal interneuron, lower motor neuron and muscles (Davis et al., 1982) is an important key to research in many neurobiological fields and models because it has a non-zero baseline meaning that it could be easily modulated by various factors ranging from emotions, day phase, menstrual cycle to influence of administered drugs or preceding disturbing stimuli (Koch, 1999). It was used in studies of habituation and sensitivity and classical conditioning and, when preceded by weaker stimuli, it plays an important role in studies of sensorimotor gating (see next subchapter on the prepulse inhibition of startle reaction).

The acoustic startle reaction (ASR) is often used in research of underlying mechanism of certain psychiatric diseases such as autistic specter disorders

(Wilbarger et al., 2009) or Gilles de la Tourette Syndrome (Gironell et al., 2000). It can be also used to determine the size and time span of neurobiological changes caused by drug addiction as was studied for example in cocaine-dependent subjects during prolonged abstinence (Corcoran et al., 2011). The study showed that neural changes including decrements in ASR accompanying cocaine dependency really persist beyond the acute withdrawal period.

2.6 PREPULSE INHIBITION

In recent studies, a specific modification of startle stimuli is often used to determine more precisely changes induced by above mentioned conditions such as drug use or mental illnesses. The modification consists in alternating the classical startle stimuli with signals preceded by a weaker (leading) signal. This so called prepulse modification of startle reaction with special focus on more widely used prepulse inhibition will be reviewed in following paragraphs. Although the prepulse and startle signals might be presented in acoustic, visual or tactile forms, only the acoustic PPI will be discussed due to its wide usage in psychiatry and because our own future experiments will be based mainly on acoustic PPI.

2.6.1 PPI: WHAT IS IT

When a startle stimuli is preceded by a weak prepulse in a short interval of several tens of milliseconds, startle reaction is altered either upward (prepulse facilitation of startle reaction) or downward (prepulse inhibition of startle reaction, PPI) in dependence on modulation circumstances such as the length and strength of the prepulse signal, interval between prepulse and the main stimuli and all the factors mentioned above as factors influencing startle reaction.

As it was shown in Plappert's study on mice (Plappert et al., 2004), prepulse inhibition and prepulse facilitation are two independent processes that together produce the final response. According to the study, the prepulse inhibition and prepulse facilitation depend on:

1. the interpulse interval between prepulse and startle stimulus,
2. prepulse intensity,
3. long-term experience,
4. and mouse strain in the mice studies.

More specifically, there are three independent types of reaction on prepulse stimulus preceding the main signal:

1. **Amplitude facilitation** / prepulse facilitation (e.g. Flaten & Blumenthal 1996);
2. **Latency facilitation** (e.g. Braff et al., 1978 showing the differences between both amplitude and latency of the blink reaction in groups of schizophrenic patients and healthy subjects). Results from several studies indicate that latency and amplitude of the reaction in prepulse-led stimulus are two independent processes with different developmental course (e.g. Ornitz et al., 1986) and affected differently by various startle and prepulse stimuli parameters (Blumenthal and Levey, 1989).
3. **Amplitude inhibition** / prepulse inhibition described variety of experimental subjects such as children (Balaban et al., 1989), nonhuman animals (Ison & Hoffman, 1983) or adults with mental disorders (Braff et al., 2001).

2.6.2 PPI: WHERE & WHY WE USE IT

According to Blumenthal (1999), the prepulse modification of startle "is a response measure that has several obvious advantages, including:

1. Availability of animal models, leading to an understanding of the neurological mechanisms underlying the effect;
2. Availability of developmental models;
3. Minimal compliance and motivation required of the subject;
4. Sensitivity to manipulation of the sensory, cognitive, social, and pharmacological environment;
5. An effect size that is great enough that even rather large changes in methodology cannot obscure this effect;
6. Functional significance in the life of the organism.

What more can we ask of a response measure?"

Exactly so, the prepulse modification (especially the inhibition) of startle response have been found to represent relatively stable neurobiological markers (Cadenhead et al., 1999) allowing thus the use of PPI measures to study schizophrenia (Braff et al., 1999), autism (Perry et al., 2007), dementia (Ueki et al., 2006), and other mental and cognitive disorders.

2.6.3 PPI: HOW DO WE USE IT

The experimental session in human subjects is usually carried on in calm environment with subject sitting in experimental room either looking into white wall or with closed eyes, headphones on his/her ears further isolating the subject from outside noise and presenting the acoustic signals on the background of white noise. Electromyography of the orbicularis oculi muscle is used to measure blink component of the startle response and further measures of vital functions such as blood pressure, pulse, electrical resistance of the skin or EEG might be also recorded. It is advisable to perform a toxicological analysis first to detect possible drug abuse or use of other medicines because many pharmaceutics were found to bias the PPI results, this part is, however, omitted from part of the studies due to administrative and ethical reasons.

The experimental session should be conducted at the same day-time in all of the experimental subjects due to changes in PPI in dependence on the day phase (Adams et al., 2008 as measured in rats).

Modalities of prepulse-led and baseline stimuli should remain the same in all the experimental subjects because little changes in prepulse-pulse intervals or intensity may cause big alterations of the responses sometimes even causing the prepulse inhibition to be switched to prepulse facilitation. This problem is most effectively solved presenting the experimental subjects with always the same row of stimuli sorted in pseudorandom order. This way the habituation of startle reaction might be also measured on a row of about six

stimuli without prepulse that are presented in the beginning and at the end of experimental sessions. Stimuli are usually also preceded by several minutes of undisturbed background noise for the subject to acclimatize to the experimental conditions.

Parameters of experimental sessions used in PPI studies (based on Swerdlow et al., 1993; Swerdlow et al., 1997; Cadenhead et al., 1999; Dahmen & Corr, 2004; Braff et al., 2005; Feifel et al., 2009):

- Acclimatisation period: 3-5 minutes,
- Background: White noise at about 70 dB,
- Prepulse-intensity: 2-16 dB above the background noise, usually presented in several alterations such as 4, 8 and 16 dB prepulse-trials; prepulses at the intensity of 2 dB above the background noise showed, however, no prepulse effect where used (Swerdlow et al., 1993),
- Prepulse length: 20 ms,
- Stimulus intensity: 115-120 dB,
- Stimulus length: 40 ms,
- Interval between prepulse and main stimulus: 30-120 ms; again, several alterations (such as 30, 60 and 100 ms) were usually used during the course of prepulse session
- 70 – 100 stimuli were used in reviewed experimental sessions, often sorted in several blocks (habituation part – main experiment – habituation part) or with the 36 stimuli design repeated twice in one session. As discovered in personal experience as well as based on verbal warning of a researcher experienced in the field, results from longer experimental sessions may be affected by the experimental subject falling asleep.

Adherence to basic methodological rules of experimental design was shown to be sufficient for gaining reliable results across multiple testing sites

(Swerdlow et al., 2007) allowing therefore comparison of results gained by multiple research groups and laboratories.

2.6.4 PPI: SUBJECT REQUIREMENTS

Beside parameters of the experimental session, attention must be paid also to the experimental subjects. Subjects with hearing deficits should be obviously excluded from studies; gender differences (Swerdlow, 1993) and menstrual cycle in female subjects (Swerdlow et al., 1997) should be considered when evaluating the data.

As mentioned before, drug abuse as well as prescribed medication often alters PPI results and subjects under the effects of any pharmaceutical substances (Braff et al., 2001) should be excluded from the experiment. Even cigarette smoking was found to interact with lower dosage of nicotine increasing the PPI in rats (Acri et al., 1994) as well as in human subjects (Della Casa et al., 1998). Cigarette smoking also reduced startle amplitude during the first 6-7 minutes of post-smoking session (Kumari et al., 1996). Considering the results of these studies, smokers should be probably also excluded from PPI experiments or at least forbidden to smoke during the defined time interval preceding experimental session.

2.7 LINKING SCHIZOPHRENIA WITH TOXOPLASMOSIS USING PPI

Following the hints scattered in previous texts, some kind of linkage between toxoplasmosis and schizophrenia might seem obvious and hidden at the same time. Where is a direct causality? Why don't we see articles claiming to unfold all of the schizophrenia mysteries by proving that schizophrenia is nothing but another phase of latent toxoplasmosis? Is there any connection between the two diseases, after all? Supposing latent toxoplasmosis is capable of inducing schizophrenia under specific conditions, is there a way to determine risk for schizophrenia in Toxoplasma-positive individuals using methods, such as PPI, pointing out differences between healthy controls and schizophrenic patients? Well, the issue is too much complicated to give simple answers to any of these questions.

2.7.1 SCHIZOPHRENIA & LATENT TOXOPLASMOSIS

As was already mentioned, the first studies pointing out on the possible links between schizophrenia and *Toxoplasma gondii* spoke mainly about effects of infection in mothers during the pregnancy (Brown et al., 2005; Brown, 2006) later including also the infection in early childhood among the schizophrenia risk factors (Mortensen et al., 2007). Prevalence of latent toxoplasmosis in schizophrenia patients in compare with healthy population has been studied since 50's (Kozar et al., 1953; Wende, 1956; Jírovec et al., 1957) or more recently for example by Wang et al. (2006) who showed that schizophrenia patients had higher levels of IgG anti-*Toxoplasma* antibodies in compare with control group and *Toxoplasma*-positive schizophrenia patients displayed in some aspects different clinical manifestation of the mental disease than seronegative patients. Clearly different display of the disease among seronegative and seropositive group of schizophrenia patients was described in Horáček et al. (2011) with *Toxoplasma*-positive patients showing significant reduction in gray matter volume bilaterally in the caudate, median cingulate, thalamus and occipital cortex and in the left cerebellar hemispheres.

Epidemiologically there are several similarities between schizophrenia and toxoplasmosis. (1) Both schizophrenia (reviewed in Kendler & Diehl, 1993) and toxoplasmosis (Sacks et al., 1982; Stagno et al., 1980) seem to run in families though in schizophrenia a genetical risk factor is supposed while in toxoplasmosis the exposition to cats and similar food habits is probably responsible for infection of several members of a family. (2) The age of onset of both schizophrenia (Watt & Szulecka, 1979) and toxoplasmosis (Ryan et al., 1995) is higher in females than in males. (3) Both diseases are more frequent in poorer and more crowded households (schizophrenia: Regier et al., 1993; toxoplasmosis: Kruszon-Moran & McQuillan, 2005). Other epidemiological areas do not back up toxoplasma and schizophrenia similarities or are in direct opposition to the theory. Another evidence for the connections can be found in pharmaceutical studies showing that drugs used for schizophrenia treatment often inhibits also growth of *T. gondii* and other protists (Jones-Brando et al., 2003).

The possible way for *T. gondii* to cause schizophrenia in infected humans is through shifts in neurotransmitter levels, especially the dopamine. It was already mentioned that dopamine has probably a crucial role in schizophrenia etiology. In subjects with latent toxoplasmosis, shifts in dopamine levels are suspected cause of personality changes (Flegr et al., 2003) and proper “tools” were already found in *T. gondii* genome by Gaskell et al. (2009) who found two genes encoding tyrosine hydroxylase in T. gondii genome. The encoded enzymes metabolize phenylalanine as well as tyrosine catabolizing phenylalanine to tyrosine and tyrosine to L-DOPA, dopamine precursor.

2.7.2 SCHIZOPHRENIA & PPI

The PPI deficit was found both in male (Braff et al., 1999) and female (Braff et al., 2005) schizophrenia patients with much stronger effect in males (Braff et al., 1995). Pharmacological treatment with certain antipsychotics may decrease the schizophrenia effect on sensorimotor gating gaining normal responses in

PPI tests (Leumann et al., 2002). Pharmaceutical and animal (Swerdlow et al., 1990; Gururajan et al., 2011) models of schizophrenia are also tested using PPI measurement. The idea behind this thesis and further research plans consist in possibility that *Toxoplasma*-positive healthy subjects may differ in PPI from seronegative subjects either generally or in a subgroup of subjects with increased risk of schizophrenia development in following years. The idea is based both on high sensitivity of PPI and evidences that *T. gondii* is able to alter psychomotor functions of otherwise healthy subjects as was already shown in studies of reaction times.

2.7.3 PREVIOUS RESEARCH – TOXOPLASMOSIS & REACTION TIMES

The motor performance studies previously conducted in our laboratory on human subjects (mostly biology students of our faculty) were based on completing PC administered simple reaction time task. Experimental subjects were asked to press button on the screen (either by clicking on touchpad, mouse button or by pressing the enter key) each time a signal (white square on the black background) is presented on the experimental screen. Results of the experiments suggested differences between *Toxoplasma*-positive and *Toxoplasma*-negative subjects with slower reaction in *Toxoplasma*-positive subjects (Havlíček et al., 2001). These results were further backed up by studies in military drivers as well as in civil victims of traffic accidents both suggesting prolonged reaction times in *Toxoplasma*-positive subjects and possible interactions with Rhesus factor (Flegr et al., 2002; Novotná et al., 2008; Flegr et al., 2008; Flegr et al., 2009).

Other studies focusing on latent toxoplasmosis induced changes in human personality traits, pregnancy course or 2D:4D digit ratio were also conducted in our laboratory, these are, however, too distant to the main topic of this thesis and are therefore omitted from the review.

2.7.4 CURRENT RESEARCH – TOXOPLASMOSIS & PC TESTS

After acknowledging possibilities of involving PPI into *Toxoplasma* research, the basic question was how exactly will be the method implemented in our current testing sessions. The main idea was, of course, to get our own system for PPI measurement but meanwhile we decided to further develop our PC administered tests of reaction times that were previously successfully used in mapping some of the *Toxoplasma gondii*-induced changes in human (Novotná et al., 2008). Some of the results are presented in the experimental part of this thesis while other experiments conducted on biology students of our faculty are still in the testing process with data collected from several tens of experimental subjects. Beside the two experimental programs presented below, other programs were developed to interconnect Stroop task used in schizophrenia research (e.g. Perlstein et al., 1998) with reaction times and prepulse inhibition of startle response. Preliminary results of those experiments are anticipated in the beginning of 2012.

2.7.5 FUTURE RESEARCH – TOXOPLASMOSIS & PPI

The planned research consists of three main parts that will be tested and evaluated independently and eventually put together to compare differences and similarities between all the subject groups.

First part is already conducted in the Prague Psychiatric Center and works with a pharmaceutical model of schizophrenia induced by ketamine. Healthy subjects undergo a PPI experiment both under and without the effect of administered drug. Our aim is to collect blood samples of tested subjects, test them on presence of *Toxoplasma*-antibodies in the blood serum and determine whether there is an effect of *Toxoplasmosis* on subjects' PPI after the drug was administered. Control experimental subjects with administered placebo will be used to compare results of our own experiments on healthy subjects which come under the second part of the planned research.

Second part of the project which should be started in October 2011 at our department consists of PPI testing of healthy individuals, students of our faculty, tested on latent toxoplasmosis. We are anticipating a case-control study investigating differences between *Toxoplasma*-positive and *Toxoplasma*-negative subjects in several different traits including changes in sensorimotor gating and startle reactions (measured by the PPI), personality changes (further studies using psychological questionnaires such as Cattel 16PF, Clonninger TCI, and the Big Five), and performance changes in reaction times and reaction times with disturbing stimuli (the tests will be further developed with changes based on observation of the results described in this thesis as well as with consideration of the new data gained when using the standard PPI test).

Finally, in the third part of the project we plan extended collaboration with the Prague Psychiatric Center. We would like to determine whether the altered results of PPI in schizophrenic patients are connected with the mental disease or whether there are not in fact caused by latent toxoplasmosis. In this part, we'll need to examine schizophrenic patients using PPI and take their blood samples to be examined for *Toxoplasma* antibodies. Although this part of the study will be probably the most interesting and clinically important section of the whole project, ethical and methodological issues involved are challenging enough for me not to dare speculating about particular schedule of the research. I am, however, personally sincerely looking forward both to conducting the experiments as well as to the gained data.

With such inspiring project in mind, I unfortunately have to return to the present which is still marked mainly with pilot experiments and future planning. The experiment presented in this thesis is not, as I do sincerely believe, bad or unworthy of any attention, it is, however, one of the first steps in a long and interesting journey in which I'd love to be participating in my future years.

3. EXPERIMENTAL PART

The experimental part is structured as a standard research article with the exception of introduction which was replaced by the theoretical part. Conclusion is intended to be a conclusion of the whole thesis and is therefore contained in a chapter of its own at the end of the experimental part.

3.1 MATERIALS AND METHODS

Experimental subjects. Test subjects were recruited during interviews for adepts of professional military service in the Armed Forces of the Czech Republic held from March till May 2011. Experimental subjects underwent medical examination including blood sampling by a medical assistant. Following medical examination, the test subjects completed several PC administered tests including the two tests important for this study; the test of visual prepulse inhibition was completed by 340 male and 69 female participants and the test of acoustic prepulse inhibition was completed by 225 male and 51 female participants all aged 18 to 47 years.

Experimental subjects were asked to fill in their name, surname and birth number in the tests as well as during the blood sampling. These data were used to link blood results of individual test respondents with their test results; after identification, the data set was anonymized and further research was conducted on the anonymized data set.

Laboratory tests. All the blood samples were examined for blood types and Rh factors in the place of bleeding and for presence of antibodies against *Toxoplasma gondii* in the National Reference Laboratory for Toxoplasmosis of

National Institute of Public Health, Prague. Specific anti-Toxoplasma IgG in all subjects and IgM in high IgG subjects were determined by Enzyme-linked immunosorbent assay (ELISA) (Pokorný et al., 1989). Complement fixation test (CFT) was also used to determine *Toxoplasma*-positivity in tested subjects, because it is supposed to be more reliable in establishing "old" T. gondii infections (Warren & Sabin, 1942).

Subjects with missing blood samples, contradictory results of tests on toxoplasmosis as well as two subjects considered to be in post-acute phase of toxoplasmosis were not included in the study focusing on the effects of latent toxoplasmosis, they were, however, included in the analysis of the effects of prepulse inhibition.

Computer tests. Experimental subjects completed tests of acoustic and visual prepulse inhibition. These tests were developed on the basis of the simple tests of reaction times used previously in our laboratory (e.g. Flegr et al., 2008).

Common characteristics of both tests. The experimental program consists of an introductory screen asking for personal information such as name, birth number or other numerical identifier, sex and age. After the form is completed, instructions and three trial signals are demonstrated. Experimental data are collected from the beginning of the session starting right after the trial run.

Test of acoustic prepulse inhibition. Test subject sits in front of a simple grey screen with only one button and he/she (hereinafter named only he, his) is instructed to click the mouse each time he hears stimuli that were previewed to him in the trial section. Stimuli are presented through headphones with volume set on maximum. All the subjects are equipped with the same headphones. 32 prepulse-preceded stimuli and 28 plain stimuli are presented in pseudorandom order ensuring similar representation of plain and prepulse-preceded stimuli in the first, middle and last part of the program run. Program ends after the experimental subject responded to the last stimulus.

Stimuli, prepulses and background noise of the computer program were adopted from prepulse inhibition testing sessions of Prague Psychiatric Center in Bohnice with the permission of responsible researchers.

Test of visual prepulse inhibition. Tested subject sits in front of a simple grey computer screen with square white field in the middle and smaller square box in the center of the field. The field stays white during the trial run while during the experiment noise randomly displayed small grey boxes fill the white field. Main stimulus—red square—is presented in the central square box which is not disturbed by the visual noise. Green box outside the central square is presented as visual prepulse stimulus shortly before some of the main stimuli. The program consists of 28 plain stimuli and 32 prepulse-preceded stimuli distributed evenly in pseudorandom order thorough the program run. Program ends after the experimental subject responds to the last presented stimulus.

Data analysis. Programs Excel 2007 and Statistica v.8.0 were used for processing and the statistical analysis of the gained data. Raw data sheets were transformed using Excel functions into a treatable table, all the outliers and nonsensical values were manually deleted. Results from both computer tests were divided into three groups and average values of reaction times were counted separately for the first, middle and last parts of the experimental session as well as for prepulse-preceded stimuli and stimuli without prepulse.

T-test for dependent samples was used to determine differences between stimuli with and without preceding prepulse, factorial ANOVA was used to analyze effects of latent toxoplasmosis.

3.2 RESULTS

Latent toxoplasmosis tests. 35 male and 6 female subjects who took test of visual prepulse inhibition were found to be *Toxoplasma*-positive while 263 males and 60 females were *Toxoplasma*-negative. Test of acoustic prepulse

inhibition was taken by 28 *Toxoplasma*-positive males, 3 *Toxoplasma*-positive females and 220 *Toxoplasma*-negative subjects (173 males, 47 females).

Rh factor was planned to be included in the study as a co-factor when estimating the effect of toxoplasmosis on reaction times and prepulse inhibition but as only one woman and no man was both *Toxoplasma*-positive and Rh negative in the acoustic prepulse test and in the visual prepulse test the same was true for 2 females and 1 male, Rh factor wasn't used in the analysis. Precise numbers are as follows:

In acoustic prepulse test, 211 subjects (171 males and 40 females) were Rh positive and 40 subjects (30 males and 10 females) were Rh negative.

In visual prepulse test, 317 subjects (256 males and 52) females were Rh positive while 57 respondents (43 males and 14 females) were Rh negative.

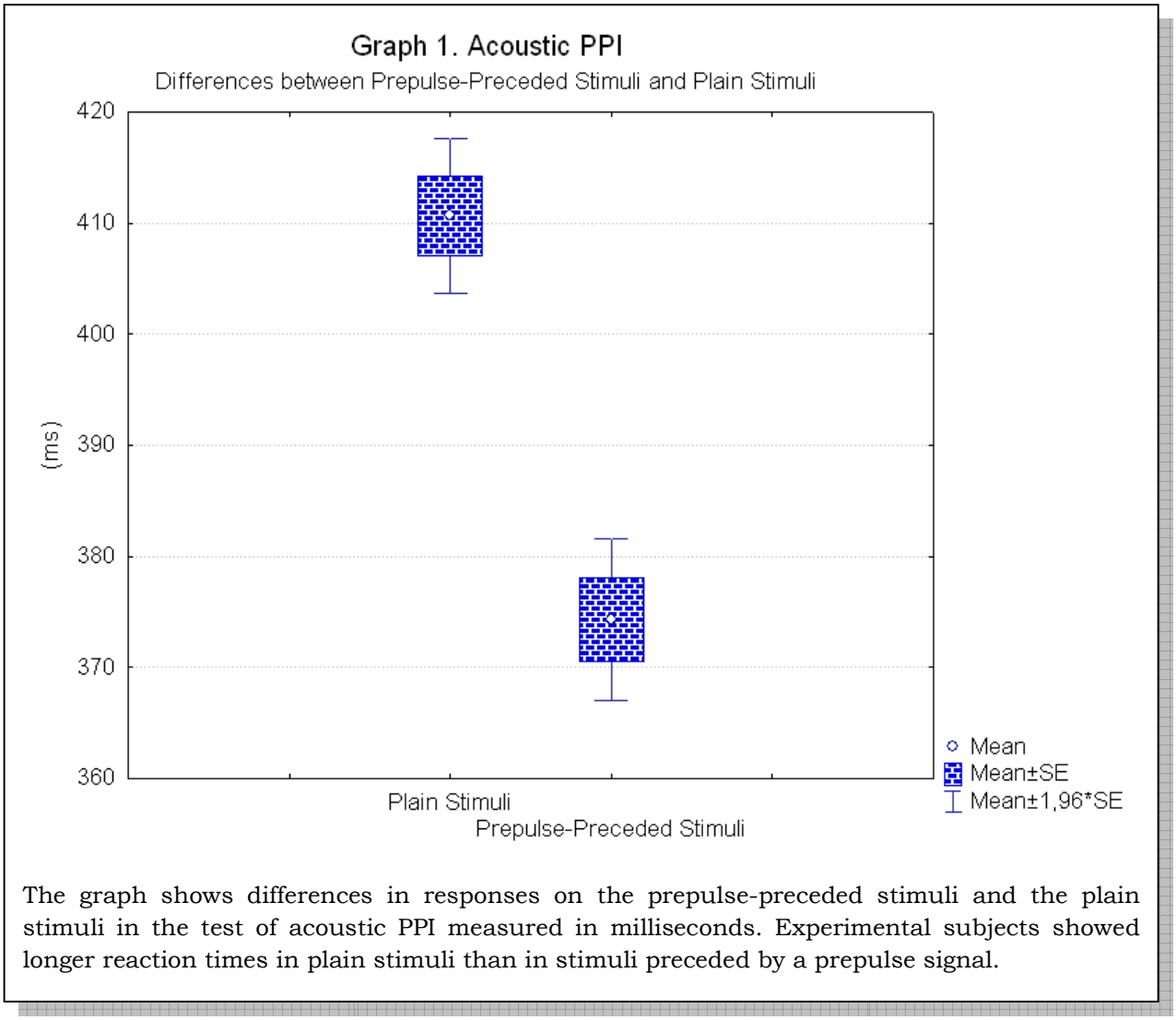
Differences between plain and prepulse-preceded stimuli in tests of acoustic prepulse inhibition. Significant difference ($p<0.001$) was found between prepulse-preceded stimuli (Mean 374.30 ms, $\sigma=61.75$) and plain stimuli without prepulse (Mean 410.68 ms, $\sigma=59.10$). The difference was significant for averages counted from the whole test (see Graph 1) as well as for the first, middle and third part of the experiment run counted separately (see Tab. 1).

Differences between plain and prepulse-preceded stimuli in tests of visual prepulse inhibition. Significant difference ($p<0.001$) between prepulse-preceded stimuli and plain stimuli was found separately in each of the three parts of the experiment but not thorough the whole program run. The differences were contradictory for the middle part of the experiment, where prepulse-led stimuli had shorter responses than plain stimuli without prepulse while for the other two parts the results where inversed. Results for the three parts of the experiment are as follows:

- 1st part prepulse: mean 287.48 ms, σ=51.94, 1st part without prepulse: mean 275.68 ms, σ=42.12;

- 2nd part prepulse: mean 277.16 ms, σ=43.18, 2nd part without prepulse: mean 292.26, σ=49.07;

- 3rd part prepulse: mean 285.03 ms, σ=42.88, 3rd part without prepulse: mean 277.59 ms, σ=43.8 (see Tab. 1).



The graph shows differences in responses on the prepulse-preceded stimuli and the plain stimuli in the test of acoustic PPI measured in milliseconds. Experimental subjects showed longer reaction times in plain stimuli than in stimuli preceded by a prepulse signal.

TABLE 1. DIFFERENCES BETWEEN PREPULSE-LED AND PLAIN STIMULI

	With prepulse		Without prepulse		t	p
	Mean	St. Dev.	Mean	St. Dev.		
Whole acoustic	**374.30**	**61.75**	**410.68**	**59.10**	**-25.03**	0.0000
1st third	**361.24**	**63.19**	**402.26**	**64.85**	**-17.17**	0.0000
2nd third	**384.26**	**74.75**	**413.00**	**65.51**	**-10.57**	0.0000
3rd third	**376.84**	**65.10**	**418.10**	**64.19**	**-20.86**	0.0000
Whole visual	283.47	42.35	282.59	42.01	1.08	0.2810
1st third	**287.48**	**51.94**	**275.68**	**42.12**	**5.30**	0.0000
2nd third	**277.16**	**43.18**	**292.26**	**49.07**	**-7.93**	0.0000
3rd third	**285.03**	**42.88**	**277.59**	**43.86**	**5.35**	0.0000

Summary results of all t-tests conducted to determine differences between prepulse-preceded and plain stimuli. First four rows show results for the acoustic PPI test and another four rows for the visual PPI. All the differences were significant ($p<0.001$) except for the overall program run of the visual PPI test.

Effects of latent toxoplasmosis on the prepulse inhibition. Although mean reaction times in *Toxoplasma*-positive subjects were mainly in the acoustic PPI test slightly higher than in *Toxoplasma*-negative subjects, the effect was never significant (nor close to it). For the entire mean values see Tab. 2 (acoustic PPI test) and Tab. 3 (visual PPI test).

3.3 DISCUSSION

Difference between prepulse-preceded and plain stimuli. Our experimental results showed that there indeed are differences between prepulse-preceded stimuli and stimuli without prepulse, the differences between the visual and the acoustic part as well as changed latencies in reactions on baseline and prepulse-led signals need to be, however, discussed more closely.

TABLE 2. VIZUAL PREPULSE INHIBITION - REACTION TIME MEANS (MS)

	1st third		2nd third		3rd third		All 60 stimuli	
	Plain	Prepulse	Plain	Prepulse	Plain	Prepulse	Plain	Prepulse
Toxo- Males	276,4558	287,2205	290,5981	277,9936	275,1873	284,3692	281,9142	283,1506
Toxo- Females	274,4819	290,3701	297,0041	272,4771	286,0600	289,9862	287,4612	287,8303
Toxo+ Males	272,8331	284,2594	291,5290	278,5322	277,4298	281,8816	282,2101	280,6173
Toxo+Females	278,6550	294,0242	307,9570	297,8592	301,1625	289,9572	302,3677	297,9797
Toxo- All Subjects	276,0969	287,7932	291,7629	276,9906	277,1642	285,3904	282,9227	284,0015
Toxo+ All Subjects	273,6850	285,6884	293,9331	281,3606	280,9029	283,0634	285,1600	283,1582

TABLE 3. ACOUSTIC PREPULSE INHIBITION - REACTION TIME MEANS (MS)

	1st third		2nd third		3rd third		All 60 stimuli	
	Plain	Prepulse	Plain	Prepulse	Plain	Prepulse	Plain	Prepulse
Toxo- Males	398,9922	357,1467	408,9056	378,8796	413,2983	372,5834	406,7648	369,7451
Toxo- Females	394,7929	360,6842	406,6960	380,9983	415,3100	374,9001	404,8926	372,3539
Toxo+ Males	400,8321	356,7258	415,6008	382,4730	421,6815	379,5359	411,6853	373,4296
Toxo+Females	445,2667	394,3333	459,8000	432,2407	474,6250	416,6667	458,8452	414,6378
Toxo- All Subjects	398,0992	357,8990	408,4357	379,3302	413,7261	373,0761	406,3666	370,2999
Toxo+ All Subjects	405,1323	360,3652	419,8781	387,2892	426,8051	383,1292	416,2492	377,4175

Considering the character of the used experimental programs, the visual and the acoustic tests should be discussed separately because while the acoustic signal could be described as a classical startle stimulus, the visual program is rather a reaction-time test supplemented by a preceding disturbing stimulus.

In **acoustic test**, the reactions on the prepulse-led stimuli were shorter than reactions on the plain stimuli without prepulse which is not in accordance with previously published studies suggesting that prepulse has no effect on startle-induced reaction time shortening (e.g. Valls-Solé et al., 2005). Possible

explanation could be that in our experiment, the prepulse stimulus played, in fact, the role of signal for reaction. Although we have presented prepulse stimulus separately during the test session to ensure this is not the case and recorded number of error reactions (number of clicks not following the main signal was low in most of the subjects), we cannot completely cast out the possibility that some of the subjects reacted on the prepulse instead of on the main stimuli. In further future experiment, this can be determined by comparing our results from this study with a new experiment presenting a signal different from the startle stimuli to which the subject's reaction is due and leaving the startle and prepulse-led startle stimuli just as distraction signals. This experimental design was used in Valls-Solé at al. (2005) study as well as in several other studies focused on determining the startle effect on reaction times (e.g. Valls-Solé et al., 1995 used a visual signal to which the subject reacted and Carlsen et al., 2003 used an audio signal). In the study by Dahmen and Corr (2004) main stimuli without prepulse included in the experimental session also prevent prepulse-elicitated startle.

In **visual test** the experimental results differ in the course of the experimental session with responses on prepulse-led stimuli longer in first and third parts and shorter in the middle part. Although the results counted in separated parts are significant, differences between the reaction times are not very high and overall result of the experimental session showed no significant results. Previous studies suggested that disturbing signals have robust distracting effects on reaction times for example in drivers where the effect is important for driving safety (e.g. Martens & Van Winsum, 2000 using the Peripheral Detection Task) and that both acoustic and visual distraction signals can modify reaction times of experimental subjects with differences in visual and audatory distraction (Corneil & Munoz, 1996; Berti and Schröger, 2001). The reason why our test brought confusing results might lie in the intensity of distracting signal which was relatively small in comparison with the main stimuli (smaller green square on grey rectangles background vs. bigger red

square in the middle of the working screen on the white undisturbed background). Habituation to the distracting stimulus may also play role in the experiment. Although startle response is not habituated in reaction time tasks (Carlsen et al., 2003), neither the prepulse nor the main stimuli were causing startle in this experiment and it is possible that the experimental subjects habituated on the disturbing signal during the first third of the experiment demonstrating shorter reaction times in the middle part. The elongated reaction times in the third part cannot, however, be explained in this way.

***Toxoplasma*-induced changes in prepulse inhibition.** Our experiment did not, unfortunately, show any effects of latent toxoplasmosis on prepulse inhibition in PC administered tests nor did it show any difference in reaction times in both plain and prepulse-preceded stimuli between *Toxoplasma*-positive and *Toxoplasma*-negative subjects. This comes as a surprising finding because differences in reaction times were already suggested by several studies both on victims of traffic accidents (Flegr et al., 2002; Kocazeybek et al.,2009) and using simple PC administered test of reaction times on which our two new programs are based (Havlíček et al., 2001). The test of reaction times itself was successfully used in several other experiments (e.g. Hall & Smith, 1996). Two main causes might be responsible for the lack of positive results. First, the test might not be lengthy enough to test effects of toxoplasmosis which lie, as it was shown in above mentioned study by Havlíček et al., mainly in earlier increase of the reaction times in *Toxoplasma*-positive subjects in comparison with *Toxoplasma*-negative controls. Second and probably more satisfactory explanation may be in the lack of Rh-positive *Toxplasma*-negative subjects.

The D antigen which is carried by the RhD protein is the strongest blood group immunogen; it is, however, missing in about 15% of Caucasian population. The RhD protein presumably acts as a gas channel (Kustu & Inwood, 2006) but except for its well known role in hemolytic disease of the fetus and the newborn (reviewed in Urbaniak & Hreiss, 2000) no effects of the absence or presence of the RhD protein on the surface of erythrocytes had been

known until recently. In previous years several studies were published suggesting protective role of Rh-positive phenotype against *Toxoplasma*-induced changes in personality traits (Flegr et al., 2010), weight gain in pregnant women (Kaňková et al., 2010) or risk of traffic accidents (Flegr et al., 2009). Moreover, the protective role of Rh-positive phenotype was found using the very same PC administered test of reaction times on which our tests are based (Flegr et al., 2008). Regarding all these studies and considering that only one *Toxoplasma*-positive, Rh-negative experimental subject completed the test of acoustic PPI and only 3 *Toxoplasma*-positive, Rh-negative subjects completed the test of visual PPI, the lack of results supporting our hypothesis might be caused largely by the bias in representation of Rh-negative phenotype in the study. Another cross-sectional study on larger population sample or a case control study with even representation of both phenotypes in male and female subjects is needed to determine the latent toxoplasmosis effects.

Another problem might be associated with fearfulness or other personality traits of experimental subjects. It was already shown that experimental subjects with latent toxoplasmosis develop different personality traits than uninfected subjects (e.g. Flegr et al., 1996). Studies of startle reaction conducted by Edwin W. Cook III (Cook et al., 1991 and Cook et al., 1992) show different latency of cardiac acceleration and blink magnitude in subjects with low and high fear as measured by Fear Survey Schedule Scores. Affective modulation of startle was enhanced among high fear subjects compared to low fear subjects with the biggest differences between groups when using aversive slides viewed by the experimental subjects. Interactions between personality traits changes induced by *Toxoplasma gondii* and reaction times affected by startle stimuli might be therefore responsible for insignificant results of our tests and further modulations of experimental programs as well as including personality factors and fear-determining questionnaires could help to find all the pieces of puzzle creating the final picture of *Toxoplasma*-induced

changes in human reaction times, startle responses and their prepulse modifications.

4. CONCLUSIONS

The theoretical part of the study attempted to provide a review of current research on latent toxoplasmosis and its effect on reaction times, startle reaction and prepulse inhibition of startle reaction as well as on its possible responsibility for certain type of schizophrenia and symptoms previously attributed to schizophrenia itself. Numerous this year's studies suggest that the research of manipulation hypothesis including effects of *Toxoplasma gondii* on human behavior is still very active and the database of knowledge is growing every day; very little is, however, still known about the parasite as a cause of schizophrenia. Some studies imply general effects of viral or parasitic infections in pregnant women or in early childhood on the development of schizophrenia in adult age, others point directly to *T. gondii*.

Although the main hypothesis of the study, differences in responses and sensorimotor gating between subjects with and without latent toxoplasmosis, was not backed up by significant results, previous similarly oriented experiments as well as hints from theoretical background suggest the need of further research in this field. Significant differences between responses on prepulse-preceded stimuli and plain stimuli without preceding signal show that the newly developed programs are capable of measuring differences in reactions on stimuli and can be used in further experiments or further developed by setting prepulse-pulse interval and other characteristics to levels that allow more precise measurements. The theoretical study of previously conducted PPI experiments allows deeper preparation for follow-up research and will be used as a starting point for adjusting the design of PPI measurements sessions.

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