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Influence of a Parasite on a Phenotype of a Host – Startle Reaction

(Vliv parazita na fenotyp hostitele – úleková reakce)

Bakalářská práce

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Abstract: This work deals with the possible use of prepulse inhibition experiments in the research of parasites manipulating human behavior. The works begins with explanation of the term "manipulation hypothesis" and description of chosen studies within the paradigm. Next we will write about startle response and that will eventually lead us to prepulse inhibition and its consequences for neurobiological research. We will also mention *Toxoplasma gondii* as a delegate of manipulative parasites and its connection with schizophrenia.

Keywords: manipulation hypothesis, prepulse inhibition, schizophrenia, startle response, *Toxoplasma gondii*

Abstrakt: Tato práce se zabývá možným použitím testů prepulsní inhibice při výzkumu parasitů ovlivňujících lidské chování. Začneme vysvětlením pojmu manipulační hypotéza a probereme vybrané studie pracující v rámci tohoto paradigmatu. Potom se zmíníme o úlekové reakci a toto téma nás nakonec zavede k prepulsní inhibici a jejím důsledkům pro výzkum v oblasti neurověd. Zmíníme se také o protistním organismu *Toxoplasma gondii*, zástupci parazitů ovlivňujících lidské chování, a jeho spojení se schizofrenií.

Klíčová slova: manipulační hypotéza, prepulsní inhibice, schizofrenie, úleková reakce, *Toxoplasma gondii*

Content

1. Introduction

When searching for useful sources I found out that not much research had been done on the main topic of my thesis. There exists a quantity of studies on manipulation hypothesis and another stack of them on the startle response but putting those two together is apparently an unfashionable affair. I was utterly amazed by the situation because startle response is something so much connected with anti-predation behavior and that is in turn connected with the usual topic of the manipulation hypothesis studies, that I couldn't believe that almost no one has been working with the possibility that parasites can manipulate his host's ability to escape the predator simply by weakening his reflexes preparing him for the run. Of course, there is always a bunch of analysis of the reaction times in the bounderies of manipulation hypothesis paradigm but that is not exactly the same. Reaction times are too general. Although I have also mentioned them in this work, it was not sufficient.

I started to read more about the startle response on its own and also about the prepulse experiments in which scientists manage to increase or decrease the startle response by preceding it with another (usually weaker) stimulus. I heard about the prepulse inhibition once and it has fascinated me ever since. This brought me to the decision that I would definitely want to try this kind of experiment in my future research to test the possibility of our favorite parasite *Toxoplasma gondii* changing not just the very startle reflex but also the sensorimotor gating (examined by the prepulse) in human. I have a reason to believe this to be true based on possible connections between *T. gondii* and at least some types of schizophrenia, as schizophrenia patients – among which there has repeatedly been found an increased prevalence of latent toxoplasmosis – have been known to have their prepulse inhibition changed in comparison with healthy population.

So here I am. I started with manipulation hypothesis and ended with schizophrenia, though I hope that just metaphorically; and I want this thesis to follow my progress.

In the next chapter, I will write about the manipulation hypothesis because it is the intended topic of this thesis. In chapter three I will briefly mention the startle response itself and than I will finally turn to the description of parameters and use of prepulse inhibition.

2. The Manipulation Hypothesis

As we all know, parasites and other pathogens can change their host behavior. We stay at home when we are suffering from cold. We are in bed, running a temperature and we start blowing our nose full of phlegm. A cricket infected with a Gordian worm commits suicide by jumping into water (e.g. Biron et al., 2005). Snail's tentacles inhabited by sporocystes of digenean worm *Leucochloridium paradoxum* change their color and shape and even start to pulsate thus perfectly imitating caterpillars, favorite food of many birds. I can go on naming many other examples of shifts in host behavior and appearance but I rather want to concentrate on a few special topics.

2.1 I'll Make You Fulfill My Interests: Are All the Changes Adaptive?

In a manipulation hypothesis paradigm officially introduced by Holmes & Bethel´s article in 1972 we often consider the behavioral shifts to be adaptive for the parasite and ever since Richard Dawkins published his book titled *The Extended Phenotype* (1982) we have been well disposed toward viewing them as manifestations of parasite genes in its host's body. Organisms behave in certain way because their parasites want them to do so. Gordian worm needs to be transported into the water and so cricket serves him as a well-controlled vehicle. *L. paradoxum* can only proceed with its life cycle in birds and snail tentacles are nothing more than a decorated plate on which the worm can be presented as a good offer of bird´s food. This could be actually true in many cases. For example *Toxoplasma gondii* is believed to manipulate its temporary host's behavior by changing its hormonal levels. This belief was recently supported by Gaskell et al. (2009). Gaskell and her team 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. This discovery is a real surprise for the scientific community as tyrosine hydroxylases have never been described in protists before but have been only known in animals in which they serve as a rate-limiting step in synthesis of dopamine.

No matter how tempting these explanations seem to be not all changes must necessarily be adaptive – it is true at least for the parasite. If we stay at home when sick we will probably not infect other people. This behavior could be viewed as adaptive in our

population and may, in fact, be observed as an important factor in the evolution of virulence[1]. Third explanation is also possible. Shift in host behavior may simply be a side effect of the infection and has therefore no adaptive value either for host or for parasite.

According to Poulin (1995) alterations in host behavior can only be considered adaptive if they satisfy certain below stated conditions:

1. Complexity: simple traits can also be adaptations but they can as well arise by chance;
2. Purposiveness of design: adaptive characters are well fitted for their function and their environment;
3. Convergence: they are more likely to be adaptations if they have arisen independently in several lineages of hosts or parasites;
4. Fitness effect: they must be the cause of increased fitness of either the host (Poulin unlike many other scientists discusses adaptability for hosts and for the parasite together. I mention it here for the sake of completeness though I do not personally think that adaptability for the host should be considered as a part of manipulation hypothesis.) or the parasite.

In his review Poulin skeptically notes that host behavioral changes thought to facilitate parasite transmission from host to host are often simple, fitted to their presumed purpose but lacking in the experimental support, so we should be careful when taking them for really adaptive; he nevertheless states that he personally believes that at least some of the discussed changes are adaptive. Poulin is quite productive in reviewing and analyzing data acquired on the field of manipulation hypothesis and so I decided to submit two of his interesting studies in Box 1.

[1] For a closer look on the evolution of virulence and impact of host mobility on pathogen transmission see for example Read (1994), Ewald (1995) or Frank (1996)

Box 1: Manipulation Meta-Analyzed

In 1994 Robert Poulin decided to find answers to three fundamental questions of parasitic helminthology by carrying out a meta-analysis of studies concerning manipulation hypothesis in helminth taxa. The three questions were:

1. Is there variation among helminth taxa in the ability of parasites to alter their host behavior?
2. Is the magnitude of behavioral changes different in systems involving vertebrate and invertebrate hosts?
3. Are behavioral changes more pronounced in systems where the parasite relies on its current host being preyed upon for transmission?

According to expectations the answer to the first question was YES; the magnitude of the effect of parasites indeed varies among helminth taxa.

The second answer was more problematical since the data suggested that though the effects on host activity were similar in both vertebrates and invertebrates, the effect on microhabitat choice appeared much more severe in vertebrates. This contradicts the author's assumption that control of host behavior by a parasite would be easier to achieve if the difference in size between host and parasite was small[2] or if they were taxonomically and biochemically related. Poulin then concluded that the impact of parasites on vertebrate behavior might be greater than previously believed which is actually very probable in spite of studies published since that time (however, I haven't found any recent meta-analysis being performed on this particular aspect).

Finally we are approaching the most problematical result of the study. The author in accord with common belief predicted that behavioral changes in infected hosts would be more apparent in hosts serving as intermediate links in the parasitic life cycle than in hosts whose behavior was unlikely to influence parasite transmission. This surprisingly proved to be incorrect, making the author consider possible bias caused by the fact that all parasites transmitted by means of food chain were nematodes, whose taxon as a whole has the strongest effect on its hosts. Poulin provides further two possible explanations for his amazing results; first one saying that parasites not transmitted through predation can obtain other benefits by

[2] The difference in size between helminthes and their vertebrate hosts is, of course, greater than the difference between parasites and invertebrates.

altering the behavior of their hosts (as we can see for example in *Sacculina sp.*). The second explanation takes into account that severity of the effect on host might be disadvantageous to its parasitic passenger itself, as it most probably doesn't want its comfortable vehicle to die on pathogenicity before it is consumed by the parasite's next host.

Manipulation Hypothesis in Crisis?

In 2000 Poulin is meta-analyzing again, this time using his results as support for weakening of the manipulation hypothesis paradigm. On the basis of his meta-analysis he reported strong negative correlation between parasite-effect size and the year of publication of the study. The results were significant in the parasites infecting an intermediate host from which they are transmitted to their next host by means of predation. The correlation surprisingly disappears in the behavioral changes that have no obvious adaptive value. Poulin suggests a handful of explanations including my favorite one that the spectacular effects were stressed at first for the manipulation phenomenon to be recognized whilst weaker evidences are studied nowadays when the strong ones have already been well described. This explanation contradicts the author's null hypothesis that the magnitude of published effects remains the same through time. All the same, the phenomenon of parasite-induced changes in host behavior really exists and has been acknowledged even by Poulin himself. In my opinion we should adopt from his work the idea that we have to be more careful when handling a new paradigm – those astounding results might not be true for the whole nature. But we should have known this long time ago!

2.2 Trophic Transmission: Eat Me or I'll Die!

Let's presume the shifts in host's behavior are adaptive for the parasite, i.e. the parasite so to say follows its own interest by changing its host's phenotype. What interest could be in play? The Gordian worm in cricket wants to be transported into water, so transport to parasite's usual environment can be considered one type of interest. Parasitic crustacean *Sacculina* uses its crab as a better transporter – not only for one trip but for its whole life. A crab doesn't reproduce itself but eats enough to bring its parasitic guest sufficient food and cares for him similarly as for its own eggs. This is in my view our usual idea of the common parasite: a guest feeding and reproducing itself in its host's body. Other interest could be

found in parasites using blood-sucking insects as vectors for transmission. They usually obstruct suction organism of their host which therefore uses much more of them thus efficiently transporting manipulative inhabitants to many new homes. The most discussed interest within the paradigm of manipulation hypothesis is, however, the trophic transmission from one host to another, i.e. the situation when parasite wants its temporary host to be eaten by certain predator which is incidentally also the parasite's next host.

To give some examples, I would start with the usually mentioned case of trematode parasite *Diplostomum spathaceum* changing vision of its intermediate fish host causing the fish to be more vulnerable to its predation by birds, the parasite´s final host. Seppälä et al. (2004) found out and described in their study that infected fishes and control ones did not differ in their preference for the surface layers but infected fishes showed less escape behavior in laboratory conditions. Interesting findings were, however, later presented by Seppälä et al. (2005) when conducting a field study on infected fishes and control species predated by wild birds. The experiment resulted in infected fishes showing no difference in the predation vulnerability from that of the controls. The results were considered to be caused by the experimental set-up allowing gulls to feed on the prey in very unnatural manner (standing on the edges of the cages).

Another example is the bivalve *Austrovenus stutchburyi* and its trematode parasite *Curtuteria australis.* The cockles infected by this parasite showed changes in burrowing behavior leading to increased risk of predation by aquatic birds. It seems that infection with *C. australis* in young cockles can modify growth of their feet making moving in the sediment impossible for them. Cockles with relatively small foot cannot completely hide from the eyes of the predators. Although this seems to be a very good example of the manipulation hypothesis other explanation is also possible. The causality could be reversed here. It may be that relatively small foot is a mark of cockles in poor condition which are therefore more susceptible to the parasitic infection. In this case, higher possibility of parasite transmission to its next host, aquatic bird, could be a mere coincidence. (Thomas & Poulin, 1998).

Yet another example is the freshwater amphipod *Gammarus pulex* infested by the fish acanthocephalan *Pomphorhynchus tereticollis.* The non-infested amphipods seek refuge more frequently in the presence of the predator (in case of this experiment the bullhead *Cottus gobio*) compared with controls without fish cues. On the contrary, *G. pulex* infested by *P. tereticollis* shows no increased use of refuge when the predator is present. This could most probably be the reason (or at least one of the reasons) why the prevalence of the parasite in its amphipod host was found to be ten times higher when the amphipods were collected from the

stomach contents of their bullheads predators compared with free ranging individuals collected in the same river (Perrot-Minnot et al., 2007).

Before coming closer to the very topic of this work, i.e. to manipulating the reaction of host to the predator, let us say, that the evolution of trophic transmission is well described in Lafferty's review (1999). I found interesting especially the part about possible interaction of two parasites in the same host. In Box 2 a closer view of another example of trophic transmission is given.

Box 2: To Be or Not to Be Eaten

When considering the manipulation hypothesis we are interested mostly in the parasites with multi-host life cycles, especially those in which the transmission occurs when their temporary host is eaten by the next – usually the final one. Such, in fact, might not be the only cases where some manipulation comes into consideration. In Milinski, 1985 we can see a possible effect of a parasite not wanting to be eaten on its host behavior. Milinski conducted an experiment comparing effects of two different parasites (plerocercoid larvae of the cestode *Schistocephalus solitude* and the microsporidian sporozoan *Glugea anomala*) on the three-spined stickleback *Gasterosteus aculeatus L.* The sticklebacks infected by the plerocercoid larvae behaved in accord with the assumption of the manipulation hypothesis (stating that the host inhabited by a parasite wanting to be munched by a predator will show a stronger willingness to take a higher risk of predation) and showed no fear in the presence of a "curious" cichlid *Tilapsia mariae*. More surprisingly, the fish infected by the sporozoan competed out the parasite-free fish from the safest feeding zone (most distant from the predator). These results suggest that even parasites that mustn't be transmitted to the different species by means of predation could also manipulate their host – to avoid the unpleasant event of being enjoyed as a lunch.

Though this seems to be a very interesting topic, I have found no other studies at all dealing with the possibilities of parasites manipulating their hosts to avoid being digested. There exist, however, some studies implying that the parasitic-induced changes in host behavior need not always be general but can display certain rate of predator-specificity. As we already know from Vyas, 2007, the behavioral changes induced by *Toxoplasma gondii* can be highly predator-specific; the rodents infected by *T. gondii* show decreased sensitivity specifically to cat odors, thus increasing their chance to be eaten by our domestic pets. Now,

are there any other cases in which a parasite might choose by what kind of predator it really doesn't want to be eaten[3]? The answer is yes, there are. For example an acanthocephalan parasite *Acanthocephalus lucii* changes the behavior of its isopod hosts towards higher susceptibility to predation by predators; however, when exposed to two types of predators, one of which is the parasite definitive host (the perch) and one of which is not (a dragonfly larvae in the study in question), the infected isopod shows greater increase in the vulnerability to predation towards perch (Seppälä et al., 2008). The mechanisms underlying the different susceptibilities of infected isopods to predation by perch and dragonfly larvae are not clear, but according to the authors of the study they are likely to be dependent on behavioral differences between the two predators.

A wide specter of questions still remains to be answered in this part of the manipulation hypothesis paradigm.

For the sake of completeness I wish to note that parasites not transmitted by means of trophic transmission can dispose with other ways how to escape from predation of their hosts. For example the above mentioned Gordian worm *Paragordius tricuspidatus* can escape from fish or frogs eating its host by wriggling out of the mouth, nose or gills of the predator that had consumed its cricket vehicle (Ponton et al., 2006).

2.3 Oh No, You Are so Slow!

The way a parasite manipulates its host could often manifest itself by slowing the host down or otherwise altering its predator-avoidance mechanisms. Of course, this slowing down could be just a side-effect of the infection. When I return to the example of a human being having cold, we are usually not much interested in running when we are sick. Also both sticklebacks (those infected by *Schistocephalus solitude* as well as those infected by *Glugea anomala*) from Box 2 (Milinski, 1985) showed some difficulties in movement. These difficulties were caused just by the mass of parasites present in the host body. It could be problematical to name these cases as the very manifestations of the manipulation hypothesis in spite of the fact that they show certain possible adaptive effect. However, there are changes in host behavior, the causes of which cannot be explained simply by the presence of parasites in host body.

[3] It should be noted that the cichlid from the Milinski's experiment wasn't the definitive host of the *S. solitude* (the fish-eating birds are), which means the study had just shown that the predation risk was higher for the sticklebacks infected by the cestode on the whole with no reference to the predator-specificity.

A change in escape response can be found e.g. in the cockroach *Periplaneta americana* infected by the acanthocephalan *Moniliformis moniliformis.* The infected insect shows a decrease in wind-evoked escape responses, a predator avoidance behavior consisting of fewer escape responses, longer latency and higher threshold for escape behavior. The escape response is modified in ways that probably increase predation risk for infected animals. Though there is always a possibility that the parasite decreased its host's stamina on the whole, other behavioral tasks suggest that this is not the case; the parasite does not damage any internal tissues and the infected cockroaches live as long as the non-infected ones (Libersat & Moore, 2000).

Other example of altering host reactions and activity can be found in *Toxoplasma gondii.* Mice infected with *T.* gondii were shown to be more active (Hutchinson et al., 1980, Hay et al., 1983, Hay et al., 1985) but to have prolonged reaction times in certain phases after post-inoculation. The increase in the reaction times, however, corresponds with the peak of tissue cysts development in brain and might be therefore caused by side-effects of the infection pathology (Hrdá et al., 2000). Relatively different results were found in human beings when *Toxoplasma*-positive probands showed significantly prolonged reaction times in comparison with the controls. Furthermore, positive correlation was reported between length of infection and mean reaction time. These findings suggest that this is caused rather by cumulative effects of latent toxoplasmosis than by one-step pathological effects of acute phase of the disease (Havlíček et al. 2001).

Changes in fast-start escape responses caused by cestode *Schistocephalus solidus* were found in three-spined sticklebacks *Gasterosteus aculeatus* L. Maximum velocity and acceleration decreased with increasing parasite index. No significant differences in maximum velocity and acceleration were, however, found in the fishes infected with *Bunodera* spp., a parasite in which the trophic transmission by means of predations doesn't apply (Blake et al. 2006).

Interestingly, effects of parasite on its host abilities needn't always be negative. Effect of the acanthocephalan *Polymorphus minutus* on its intermediate crustacean host *Gammarus roeseli* is manifested in increased swimming speed in comparison with the uninfected control. Its swimming speed increases even more when the infected amphipod is confronted with the non-host crustacean predator *Dikerogammarus villosus.* This could be another good example of the inverted manipulation hypothesis – yet another parasite could manipulate its host with the aim not to be eaten, at least not by the non-host predator (Medoc & Beisel, 2008).

3. Startle Response

Startle response observed usually in mammals is a reaction to unexpected stimuli. Certain types of the startle response are observed in dependence on the stimuli character. It can be elicited by acoustic, visual or tactile stimuli, but in experimental conditions the acoustic stimulus is used in most of the studies. A primary acoustic startle stimulus seems to be quite simple with only a few synapses involved. The experimental data suggest that the circuit in rats consists of auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticualris pontis caudalis, spinal interneuron, lower motor neuron and muscles (Davis et al., 1982).

The reason why I mention this relatively simple circuit consisting probably of five synapses and the neuromotoral junction is that it is used as a model system for studying habituation, sensitization, prepulse inhibition, classical conditioning, fear or anxiety, and drug effect on behavior. All those mechanisms (except for the drug effects) are often involved in antipredator behavior and therefore can possibly be altered by the parasite manipulating its host to increase the probability of trophic transmission. I found no studies bringing together the two phenomena, startle response and manipulation hypothesis, but I think that this area of experimental research could be very promising in the future.

I shall not deal in great detail with the startle response as my main interest lays in one specific part of the phenomenon, i.e. in the prepulse inhibition of the startle response. I will, however, mention several studies in which the model of startle response was used.

3.1 Don't Scare Me or What Is This Startle Response all about and Where Do We Use It?

The acoustic startle response (ASR) is a relatively simple reflex characterized by a rapid contraction of facial and skeletal muscles following after an unexpected and intense acoustic stimulus. It was described in many mammalian species somewhere nearly a hundred years ago (e.g. Prosser & Hunter, 1936) and it probably protects the organism from injury by a predator or by a blow. The startle pattern consists of eye-lid-closure and contraction of facial, neck and skeletal muscles, as well as stopping of ongoing activity and acceleration of the heart rate. The startle has non-zero baseline which means that it can be altered by many

external and internal conditions; that is the main reason for it being used as a valuable behavioral tool to asses mechanisms of sensorimotor response plasticity (Koch, 1999).

The ASR is often used in human when dealing with certain types of psychiatric disorders because it can help to understand underlying mechanisms of the diseases. An exaggerated ASR was for example found in patients with Gilles de la Tourette syndrome which is a lifelong fluctuating neuropsychiatric disease characterized by the presence of motor and vocal tics and a range of associated behavioral problems that include anxiety, attention-deficit, hyperactivity, obsessions and compulsions (Gironell et al. 2000).

Another study was designed to test startle potentiality in adolescents and adults with spectrum of autistic disorders. While healthy population shows decreased startle response when exposed to a positive stimulus, patients with spectrum of autistic disorders exhibit increased startle when exposed to both negative and positive stimuli. This suggests a disruption in basic affective processes in spectrum of autistic disorders at the level of the early motivational response (Wilbarger et al., 2009).

4. Prepulse Inhibition

As discussed in the previous chapter when a strong intense stimulus is presented mammals show a startle response of the whole body including the blink reflex. If a weak prepulse signal precedes the strong stimulus by approximately 100 milliseconds (time gaps of 30 to 500 milliseconds are actually used) the startle response is inhibited or gated. This is called a prepulse inhibition (PPI) of the startle response. The measures of prepulse inhibition and habituation of the startle reflex were found to represent relatively stable neurobiological markers (Cadenhead et al., 1999), allowing therefore the use of PPI changes as markers for schizophrenia (e.g. Braff et al., 1999) and many other diseases. Besides PPI prepulse facilitation (PPF) can be also present in dependence on the prepulse nature; in this case, the startle response to the main stimulus is increased instead of decreased.

Many studies dealt with the methodological needs of proper experiments because the results are highly dependent on the specific parameters as the background noise (Hsieh et al., 2006), prepulse intensity – prepulse of the intensity of 2 dB above background noise shows no effect on the startle stimuli as shown for example in Swerdlow (1993) while with increasing intensity of the prepulse also increases the probability of prepulse-elicitated startle reactions (Dahmen and Corr, 2004) – or even the phase of the day (as reported in female rats by Adams et al., 2008).

4.1 Proband Requirements and Gender Differences

In human studies the experiment usually consists of one session in which a proband sits in a sound-attenuated room with headphones on his/her ears. The eye blink component of the startle reflex is measured using electromyography of the orbicularis oculi muscle. The proband must also meet certain requirements to be allowed to participate in the studies. As the startle stimulus is often acoustic, subjects with hearing impairment have to be excluded from the study as well as patients with specific neuropsychological disorders known to alter the PPI. 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.

There is also a problem of gender difference. Women show significantly lower PPI than men especially for the weaker prepulse (Swerdlow, 1993). Swerdlow showed that too week prepulse (i.e. 2 dB above background noise) exhibits prepulse inhibition neither in men nor in women whilst 4 dB does. Trials with prepulses at the intensity of 4, 8 and 16 dB above background noise showed similar results with significant role of gender and of the prepulse intensity. The role of gender is clearly visible in schizophrenia patients where the weakness of PPI in women in combination with less severe disease manifestations could confuse interpretation of the results. Irrespectively of this, there is a PPI deficit found both in male (Braff et al., 1999) and female (Braff et al., 2005) schizophrenia patients. No gender differences were noted in the amplitude, habituation or latency of the startle reflex.

According to the abstract by Braff et al. (1995) the schizophrenia linked loss of PPI was attributable to the profound loss of PPI/gating in male schizophrenia patients which means that the reported increased virulence of schizophrenia in males may relate to the greater loss of PPI that is seen in schizophrenia male patients. The authors state that PPI and related cognitive functions in female schizophrenia patients should be assessed with the knowledge that menstrual cycle/hormonal variability is an important variable since cognitive functions dependent on dopamine tone may be very sensitive to menstrual cycle phase. Swerdlow et al. (1997) in fact proved those expectations two years later in their study which revealed significant changes across the menstrual cycle in women with peak PPI values in the early follicular phase.

These changes, however, may be problematic when dealing with other mammalian species for there were no gender differences proven in rats (Swerdlow et al., 1993). Experiments with Sprague-Dawley female rats (Adams et al., 2008), though revealing

significant differences in PPI in dependence on the time of the day (light or dark phase), showed no significant difference in PPI (or other measure used, such as baseline startle, habituation or activity during testing) in dependence on the estrous stage. The author claims that his results are consistent with several other studies though there exist also several studies suggesting different results in connection with the estrous phase. Those could be probably explained by differences in the experiment design as some conditions can increase and other decrease the impact of estrous stage.

4.2 Experiment Characteristics

The dependence of PPI and PPF results on the experimental conditions is shown in Hsieh et al. (2006), where two different noise backgrounds and few different types of prepulse are used and compared. Lower background noise (54 dB vs. 70 dB) increased the PPI effect probably because of bigger difference between the prepulse itself and the background noise. As for the types of prepulse, both the discrete 86 dB prepulse emitted 120 milliseconds prior to the 115 dB startle stimulus eliciting the PPI and continuous or discrete 2000 milliseconds or 4500 milliseconds long prepulses eliciting PPF were used.

In Table 1 I compared parameters used in the several randomly chosen studies I write about in this paper. Two of them were conducted by the same author and the second one was done four years later. Four different teams of scientists published another four studies. As you can see, the characteristics of the experiments are relatively similar which means that the results of all of them (and of many other studies of this type as well) should be easily comparable. The reliability of the data from prepulse inhibition studies carried out at several different places by several different teams of scientists was in fact tested by a separate study by Swerdlow et al. (2007). The multi-site experiment showed that although some measures can vary depending on the team and other testing conditions, careful attention to methodology enables acquiring of reliable data. The study was in fact focused on multi-site testing with the aim to obtain one common data set. I think, however, that the variations in methodology used on different sites may serve as a guideline for preparation of a new study in an institute where prepulse inhibition was never used in the past (i.e. for our work). Now we know what to be careful about.

As you can see in the table each session takes approximately half an hour so as to prevent tiredness of probands as well as too strong effect of habituation. In each session

several types of trials are usually presented. Habituation is measured on single pulse trials in the beginning and at the end of the experiment (e.g. in Cadenhead et al., 1999 and Feifel et al., 2009), single pulse trials are also presented randomly among trials with prepulse. According to Dahmen and Corr (2004) trials with single prepulse stimulus without the main stimulus should be also present to exclude prepulse-elicitated startle. It is also standard procedure to present prepulse stimuli of a few different levels of intensity (e.g. at 4, 8 and 16 dB above background-noise). As noted above, background-noise level is also an important characteristic capable to bias results of the experiment (Hsieh et al., 2006); white noise at the intensity of 70 dB is typically used. Length of the prepulse is usually 20 milliseconds while length of the main stimulus is set to 40 milliseconds. In my randomly chosen sample of studies, the time span between the prepulse and the main stimulus varied between 30 and 120 milliseconds with at least two lengths often tested. The intensity of the main stimulus was 118 dB in Swerdlow's experiments and 115 dB in all the others.

4.3 Schizophrenia or Why Bother?

The PPI is used as a tool for deeper understanding of many neuropsychological diseases, one of which, namely schizophrenia, was subject of many independent studies. The etiology of the disease is not yet thoroughly understood. It seems that many different factors (genetics, sociological, psychological and others) might be involved. The main symptoms are considered to be thinking and behavioral disorders. For schizophrenia to be diagnosed at least one of the following symptoms is required to be present for at least one month:

a) Hearing one's own thoughts, intrapsychological hallucinations (removing, inserting or emitting thoughts);
b) Delusions of control and influence, experience of passivity or belief that one is controlled by psychotic experience;
c) Hallucination of voices commenting patient's behavior or talking about the patient;
d) Delusional belief out of line of one's culture.

In absence of those symptoms at least two of the following ones must be present for at least one month:

a) Persisting hallucinations;
b) Formal thinking disorders (incoherency, thought "jumps" between unrelated topics, often with few seconds pause in talking, neologisms);

c) Catatonic manifestations;
d) Negative symptoms in form of apathy, alogia (poor speech, autism, flat affect – showing no emotions);
e) Conspicuous behavioral changes (loss of emotional relations and interests, social withdrawal, idleness, aimlessness…).

It is usual to divide symptoms as negative (apathy, alogia etc.) and positive (hallucinations, delusions, obvious behavioral changes, formal thinking disorders).

(diagnostical criteria from Raboch & Pavlovský, 2001)

The reason I write about schizophrenia in this work is that there are some suspected connections between schizophrenia and presence of *Toxoplasma gondii* cysts in the human brain. Certain data of ours and of other scientists´ (Torrey & Yolken review, 2003; Brown, 2006; Bachmann et al., 2005; Flegr et al., 2003) indicate a possibility that exposure to *T. gondii* could be a risk factor for development in schizophrenia as well as that *T. gondii* may play a role in the clinical manifestation of schizophrenia psychopathology in a subgroup of patients with schizophrenia. The theory is supported for example by (1) increased prevalence of toxoplasmosis in schizophrenia patients in comparison with healthy population (Wang et al., 2006), (2) the fact that exposure to *Toxoplasma gondii* in early childhood is one of the risk factors for appearance of schizophrenia later in life (Brown, 2006) or (3) similar effects on dopamine circuits in brain (Flegr, 2006). Our latest not yet published results from MR show visible difference between brain images of schizophrenia patients with and without latent toxoplasmosis; this difference between toxoplasma-postitive and toxoplasma-negative brain images, however, disappears in non-schizophrenic population. We presume that this might be caused by *T. gondii* making the pathological effects of schizophrenia stronger by joint impact of both factors. Dopamine can be a linkage between schizophrenia and the parasite, as schizophrenia is no doubt connected with altered levels of dopamine in certain parts of the brain, although we are still in search of the exact locations of the changes (e.g. Kegeles et al., 2006); I wrote about the connection between *T. gondii* and dopamine in the chapter about manipulation hypothesis. As schizophrenia decreases the levels of prepulse inhibition both in male and female patients, I think it could be advisable to use prepulse inhibition experiment on healthy human subjects with latent toxoplasmosis to find whether *T. gondii* also changes the PPI levels in non-schizophrenia patients or whether it could be used as a diagnostic criterion when judging the risk of schizophrenia in *T. gondii* infected population. Because the *T. gondii* infection in the latent phase of the disease has also been found to increase reaction times in healthy population (Novotná et al., 2008), the PPI test could probably also bring new

relevant information on changes in this highly predator-avoidance connected behavior by measuring the differences between the startle latency in infected and non-infected healthy population.

5. Conclusion

In this work I attempted to provide a brief view of one possibility of how the manipulation hypothesis can be measured in humans by a methodology based on physiological measures and not only on psychological questionnaires and ethological experiments. Manipulation hypothesis has been tested many times on various types of organisms in the role of both hosts and parasites. Although certain research results suggest that manipulation does not occur in all cases where it was supposed to be found, there is still enough indicia that the manipulation exists as a powerful factor in evolution of many types of parasite-host coexistence with a strong impact on ecology. However, it is not just because of these ecological and evolutional aspects that it is advisable to seek for the deeper understanding of the parasite-host interactions; as we can see from the connections between *Toxoplasma gondii* and (at least some types of) schizophrenia, future research in this area could have some promising implications for human medicine, especially for neurobiology and psychiatry. Although I think that the data collected by the questionnaire methodology and ethological experiments, some of which were also done in our laboratory, can be useful we need some validation of our results. Prepulse inhibition can be just the experimental method we were seeking for and therefore I hope it will be possible to use it in my experimental work for the diploma thesis. I am aware of the fact that the prepulse inhibition experiments – in spite of their simple outlook – can be rather demanding on fulfilling all conditions, e.g. strength of background noise, state of probands, technical parameters of stimuli. However, it will certainly be worth the effort particularly because of the connection between (at least some types of) schizophrenia and *Toxoplasma gondii* infections promises a very interesting field for another research and possible application in deeper understanding of mechanisms underlying schizophrenia pathology.

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7. Appendix - Table 1

study	Swerdlow et al., 1993	Swerdlow et al., 1997	Cadenhead et al., 1999	Dahmen and Corr, 2004	Braff et al., 2005	Feifel et al., 2009
study aims	To examine gender differences in sensorimotor gating (I use the human part only)	To demonstrate changes in sensorimotor inhibition across the menstrual cycle in women	To examine the stability of startle response measures	To find potential confound in measurement and interpretation of PPI, examination of "nonstartling" prepulses	To determine differences in PPI in healthy women and female schizophrenia patients	To compare PPI in both male and female adults diagnosed with ADHD and the normal population
aaclimation period	5 min	5 min	5 min	3 min	5 min	5 min
background noise	70 dB	70 dB	70 dB	70 dB	70 dB	70 dB
types of trials	pulse alone, prepulse+main stimulus, no stimuli trials	pulse alone, prepulse+main stimulus, no stimuli trials	pulse alone, prepulse+main stimulus, no stimuli trials	prepulse+main stimulus, main stimulus alone, prepulse alone	pulse alone, prepulse+main stimulus, no stimuli trials	pulse alone, prepulse+main stimulus
session length	N/A	N/A	25 min	23 min	N/A	N/A
number of trials	72	72	96	70	48 prepulses, 34 pulses alone and some no-stimuli trials.	74
noise distributed by	headphones	headphones	headphones	N/A	headphones	headphones
prepulse length	20 msec	20 ms	20 msec	20 ms	20 msec	20 msec
prepulse force	72 (no effect), 74, 78 or 86 dB	72 (no effect), 74, 78 or 86 dB	78 - 86 dB	80, 85, 90 dB	78 or 86 dB	86 dB
distance between prep. and stimuli	100 msec	60 msec	30 or 120 msec	120 msec	30 or 120 msec	30, 60 or 120 msec
stimuli length	40 msec	40 msec	40 msec	40 msec	40 msec	40 msec
stimuli force	118 dB	118 dB	115 dB	115 dB	115 dB	115 dB
other characteristics	36 trials in pseudorandom order repeated twice	36 trials in pseudorandom order repeated twice	4 blocks, the first and the last 5 pulses alone each, the second and the third 12 pulses alone and 6 prepulses+main stimuli each	10 blocks, all 7 trial types each (3x prepulse alone, 3x prepulse + pulse, 1x pulse alone)	similar to the Cadenhead experiment	4 blocks, 5 puls alone trials blocks one and four, each; blocks two and three 32 trials each.

Table 1: Comparison of the parameters of randomly chosen studies on prepulse inhibition.