This examination is worth 35 points. Take your time, read the questions carefully, and answer them concisely. **Be sure to answer the question I am asking**. Many questions have more than one possible correct answer. Be sure to explain your logic in arriving at the answer you give since explaining your thinking helps me to decide whether a wrong answer still might deserve partial credit. Good luck!

1. (4 points) Professor Jaroslav Flegr from the Charles University in Prague has an unconventional and controversial theory. He believes that infection with the protozoan parasite, *Toxoplasma gondii*, often communicated to humans through cat feces, is causing him (and presumably other people) to behave in unusual ways, compared with his younger, uninfected self. If Dr. Flegr is correct, infection with *T. gondii* is subtly manipulating his personality and might be responsible for psychological disorders in others. You are fascinated with host-parasite interactions and are intrigued by Flegr's theory together with evidence from other laboratories that appears to support his ideas. Therefore, you decide to focus your new lab on studying the molecular basis of *T. gondii*-induced neurological changes. One line of experimental evidence for the effects of *T. gondii* comes from rats where it was observed that infected rats were less afraid of cats than uninfected rats. You replicated these results in mice and identified 25 mRNA transcripts from the transcriptome of *T. gondii* infected neurons that were derived from *T. gondii*. **Next, 1) how will you determine which of these *T. gondii*-derived transcripts encode proteins that bind to mouse DNA and 2) identify what genes they might be targeting?**

There are several ways that you can determine whether any of these transcripts bind to DNA. Perhaps the most direct way would be to create antibodies against the proteins and perform ChIP from mouse chromatin (presumably neurons or brain extracts) to determine whether you can recover these from chromatin. ChIP-Seq will also identify the genes that any DNA binding proteins are binding. You could also have performed EMSA experiments to quickly determine which proteins could bind to DNA (with the caveat that this will not work if the protein requires other partners that are not present) and then make antibodies against those that bind DNA and use these in ChIP-Seq experiments.

2. (4 points) In support of Prof. Flegr's hypothesis, a group in France just (2016) published a paper showing that *T. gondii-infected* (TI) chimpanzees are attracted to the smell of leopard urine, but not to human or tiger urine. The hypothesized explanation is that since leopards are a natural predator of chimpanzees, whereas humans and tigers are not, the parasite has manipulated the olfactory preference of chimpanzees to favor them being eaten by leopards and the parasite reproducing and spreading. Another group suggests that differences in the microbiomes of infected individuals are responsible. **Your task is to determine whether, in fact, there are any differences in the microbiome composition of TI vs. control chimpanzees because chimps are endangered and we can't have leopards eating them. Discuss how you would accomplish your mission.**

This calls for a detailed analysis of the microbiome. You will want to collect feces from TI and control chimpanzees and then utilize 16S rRNA sequence analysis to determine how diverse the microbiome is in the different samples. If there are differences in the microbiome that can be linked to the attraction of chimps for leopard urine, then your experiments will have supported the hypothesis. Performing fecal transplants from TI to control chimps will allow you to determine whether this is sufficient to make the chimps attracted to leopard urine and would argue against T. gondii being the direct causal agent of the fatal attraction behavior.

3. (9 points) The incidence of *T. gondii* infection differs around the world. The French, who like to eat steak very rare, can have infection rates as high as 55%. In contrast only 10-20% of Americans and 30-40% of Czechs are infected. Flegr hypothesizes that his personal quirks (not being afraid to cross the street in dense traffic and criticizing the Communist government when they were in power) are the result of *T. gondii* infecting his brain cells, disrupting his attention to detail and making him less fearful.

a) (5 points). Since you have already shown that *T. gondii* causes reduced fear behavior in mice, you can use a mouse model to study how *T. gondii* affects vertebrate behavior. Research at Imperial College London showed that *T. gondii*-infected mice were not averse to the smell of cat urine. Instead, they were drawn to it, in a sort of "fatal attraction", like the chimps in question 2. Research at the University of Leeds showed that the *T. gondii* genome contains 2 genes that might increase production of dopamine in infected animals. You decide to identify mouse proteins that interact with these *T. gondii* proteins. **How will you identify all of the mouse proteins that interact with each of the 2 *T. gondii*-encoded proteins? Point out any limitations your method might have. How will you detect proteins that interact directly with the *T. gondii*-encoded proteins? How about those that interact indirectly?**

This question calls for a proteomic approach. You first need to either generate antibodies for the 2 T. gondii-encoded proteins, or create tagged constructs. These need to be transfected into mouse cells and the complexes collected either by immunoprecipitation, or via the tag. Identify the proteins by mass spectrometry. To determine which proteins interact indirectly, you first need to determine which interact directly. You could do this by a reverse tagging approach were you label other members of the complex and conduct the same sort of purification. Or you could directly test whether proteins interact using FRET or some other appropriate method. Those that are found in the larger complex but which you cannot demonstrate direct interactions for are likely to interact indirectly.

b) (4 points) You found that the dopamine D3 receptor is targeted by the *T. gondii* proteins. This receptor is expressed in the part of the brain that plays an important role in reward, pleasure, addiction and fear. It could certainly be that the *T. gondii* DNA-binding proteins are altering expression of this receptor, thereby changing how the brain responds to stimuli compared with wild type mice. **How might you prove that this receptor is necessary for the *T. gondii*-mediated "fatal attraction" behavior?**

This calls for creating a knock-out model. The simplest way to do this would be to check whether dopamine D3 receptor knockout mice are available. If so, you can use this model to test whether T. gondii infection leads to fatal attraction behavior. If not, you will need to generate one, either using standard knockout technology, or a Cre-lox based strategy that knocks out the dopamine D3 receptor in the reward/pleasure center of the brain. Again, once you have the knockout model, you can test whether T. gondii infection leads to fatal attraction behavior. A CRISPR-Cas 9 approach might also be useful, provided that it generates loss-of-function mutations in the dopamine D3 receptor. Knockdown methods such as RNAi are not acceptable answers.

4. (4 points) It turns out that animals that have been exposed to *T. gondii* produce offspring that have the same sort of "fatal attraction" behavior. This suggests that a heritable change has been made in the mouse genome that is propagated to subsequent generations. Therefore, you decide to identify potential epigenetic changes in the regulatory regions of genes you found in the dopamine pathway from 3b. Your new postdoc, Stephanie, is an expert in epigenetic analysis and suggests that you should utilize a whole genome analysis of histone acetylation in order to identify changes in these important genes. **Is she right? Why or why not? If she is correct, outline a method that would identify the appropriate epigenetic changes. If she is incorrect, outline a good approach to identify epigenetic changes in genes related to dopamine signaling.**

First of all, Stephanie is not right because histone acetylation, while an epigenetic phenomenon, is not heritable and you are tasked with studying heritable changes. The best approach would be to do a full panel of methyl histone ChIP experiments. Since you are supposed to be checking genes related to dopamine signaling, a whole-genome approach would not be required - you could simply look at the histone occupancy at promoters of genes in the dopamine signaling pathway in the offspring of T. gondii-exposed mice. However, if you decided to perform whole genome analysis, that would be OK. You could also perform bisulfite sequencing of the same gene promoters to identify if there were changes in DNA methylation associated with the phenotype in offspring.

5. (4 points) Your detailed observations suggest that not all infected mice are equally likely to display "fatal attraction" behavior. That is, while all of the infected mice are attracted to the smell of cat urine, some are less attracted to cat urine than others. You might expect that this group has some changes in the dopamine D3 receptor that are responsible for this altered behavior. Unfortunately, you sequence the D3 receptor in animals that are highly or moderately likely to display fatal attraction behavior and find no changes in the protein coding sequence. **Describe the experiments you would perform to determine whether the structure of the D3 promoter or upstream regulatory regions have been altered in infected vs. control mice.**

The question asks you to look at the promoter and upstream regulatory elements of the dopamine D3 receptor. I would perform methyl histone ChIP-QPCR experiments using the D3 promoter and upstream regulatory regions and compare what you see in infected vs. control mice. Since we are looking at the structure of the promoter, you might also want to perform an analysis such as 3C, 4C, etc to look for changes in the 3-dimensional organization of this gene.

6. (6 points) Uh, oh. Anderson Cooper is at it again. It was reported by the World Health Organization that children in Uganda, Liberia, Sudan and Tanzania are suffering from a mysterious and tragic syndrome colloquially called "nodding disease" (ND). It begins with epilepsy-like seizures that become progressively worse and is usually fatal. Some children wander off into the bush while others feel compelled to start fires and have been characterized by the media as "violent zombies". There is some evidence that ND is much more prevalent in areas where the parasitic worm *Onchocerca vovulus* is found. *O. vovulus* is usually transmitted by black fly bites. However, not all children with ND show signs of *O. vovulus* infection whereas others with ND exhibit some signs of nutritional problems such as Vitamin B6 deficiency (which has been linked with similar types of seizures). Anderson is in Uganda reporting on the story and a cloud of black flies is buzzing around him (attracted by his unusual fur), biting him repeatedly. **How would you test the hypothesis that deficiencies in certain metabolic pathways could cause some individuals to be susceptible to ND? How could you determine which of the children without symptoms might be at risk of developing ND? Can this analysis reveal whether Anderson will contract ND and die?**

This is a metabolomics/GWAS problem along the lines of the Illig paper. The first thing you might do is conduct a GWAS analysis of an affected and control population to ask whether there are any obvious links between the genome and the susceptibility. You will also want to perform non-targeted metabolomics from the blood of both cohorts and look for links between the presence or absence of particular metabolites and the symptoms of the disease. Children without symptoms who show the same profile as those with ND would be considered to be at risk. Your analysis cannot tell whether Anderson will contract ND and die, only whether he is at risk or not.

7. (4 points) Your research has demonstrated that it is not *O. vovulus*, per se, that is responsible for ND, but rather a protozoan parasite of *O. vovulus*, *Toxoplasma andersoncooperi*, that interacts with Vitamin B6 deficiency to predispose some children to develop ND. **Design a strategy to identify human genes that are required for *T. andersoncooperi* to infect neuroblast cell lines in Vitamin B6 deficient media.** Assume that you can readily detect infected cells by their fluorescence.

This will require an approach similar to a synthetic lethal screen, but you are not screening for lethality. Rather you will ask the question which genes when knocked down/out will prevent *T. andersoncooperi* infection of neuroblast cell lines in the presence or absence of vitamin B6 in the media. Perhaps the simplest approach would be to use a library of shRNAs delivered by a lentivirus vector to neuroblast cells in duplicate wells (one with B6, the other without). You would then assay by fluorescence which cells are infected (presumably nearly all wells). In some small number of wells, you may find reduced, or no fluorescence. These genes are candidates for being required for *T. andersoncooperi* infection. It would be a good idea to knock these genes out in a separate experiment using CRISPR-Cas9 to verify your findings.

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OPTIONAL – EXTRA CREDIT – 2 points.

Please show Ron that you have written something on this page so that he can record your 2 points, then tear it apart from the rest of the test to preserve your anonymity.

What suggestions do you have for improving this course? I changed a fair amount of the lecture material, dropped some old papers and added new ones for this year. I am particularly interested in knowing what parts of the course you found especially informative or beneficial, and which you think should be changed or replaced. Which papers did you particularly like or dislike?
Comments about Prof. Blumberg or Ron should be entered in the online evaluation, not here.

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