20.020 Spring 2009
Team: Sleep-away
Final Report

Narrative

Problem: Sleeping

The problem we are addressing is sleep. On its face, sleep seems like such a waste of time: why do people need to spend a third of their day (much less here at MIT) being unproductive? Life would be so much better – and more practical – if our time was spent PSetting or studying rather than lying in bed. (5000 undergrads x 6 hrs/day x 350 days/yr = approximately 10 million undergrad-hours/yr… we could have cured cancer by now or something!)

Because much is still unknown about sleep – including the fundamentals of its function/purpose – trying to "cure" sleep would be a little overambitious. We decided to start by tackling a smaller problem: temporarily extending the time which people could stay awake.

Of course, there already exist solutions to this problem – stimulant drugs such as Modinafil and caffeine. Caffeine, for example, has been proven effective in live trials every day (and probably is the only reason half the class isn't passed out during the presentation); it is also cheap and easily obtainable. However, one problem with caffeine is the body's development of a tolerance for the drug: because it works by blocking adenosine receptors, the body responds by increasing the number receptors in the central nervous system so that increasing doses of the drug are needed. This increase also causes withdrawal symptoms from caffeine.

Our proposed system will work in similar ways to caffeine – by manipulating the adenosine-receptor system in the brain. Instead of blocking the receptors with a foreign molecule, however, we will focus on preventing adenosine from reaching the receptors in the first place.

Adenosine is produced in the brain when cells burn ATP to produce energy. Adenosine is a byproduct of the reaction; it builds up inside the cell until the concentration gradient is enough for the cell to pump it out. It then attaches to receptors on the outside of the cell, which then inhibit the production of stimulants and induces tiredness. When the brain is resting, the adenosine is gradually broken down into inosine by enzymes.

Our system consists of an immunoliposome – which can pass through the blood-brain barrier and deliver the system into the brain – enclosing a group of adenosine receptors and adenosine deamilase, the enzyme which breaks down adenosine. Once inside the brain, the immunoliposome releases its contents, and the receptors sequester the adenosine outside the cell before it attaches to the real receptors on the cell. The enzyme then breaks down the adenosine so the receptors can capture more molecules.

Eventually, we expect the levels of adenosine production in brain cells to overtake the rate at which our system can capture it, either through degradation of our parts or pure stoichiometry. Thus, our system will serve more as an adenosine buffer, delaying the rate at which the brain becomes tired (like a spare tank of gas). If this system is successful, we can further develop it into a long-term solution for sleepiness.

The initial impact of this project might be limited – this system is essentially a replacement for caffeine, which serves its purpose effectively. Although it does not have the side effects of caffeine, its relative cost and complexity would hinder its practicality as a replacement. As longer-term systems are developed, however, its potential impact is huge (as shown by the ten million undergrad-hours per year calculation earlier).

There are still many unanswered questions about the workings of our system. For example, we must consider the possible side effects of continuously sequestering adenosine. The actual reaction of our system is safe – we are simply emulating a process which occurs naturally in the brain, and caffeine is deemed safe – but sleep is more than a chemical process involving adenosine. Until more is known about sleep itself, we can't really determine the effects of our system on the brain without in vivo testing. In addition, we must always be cautious when delivering substances into the brain, taking care to avoid contamination or other errors.

Since the theory behind our system is fairly straightforward, our work plan will consist of constructing and testing our system.

Technical Documentation

Our device is a coding sequence for adenosine deaminase (ADA) in *e. coli*. This sequence consists of 1002 base pairs, numbers 1724186 to 1725187 in the *e. coli* genome. ADA, found in many organisms – including humans - is the enzyme which breaks down adenosine into inosine. We are especially interested ADA for our project because adenosine plays a key role in inducing tiredness. By creating a system to sequester and break down adenosine inside the brain, we hope to disrupt the adenosine-receptor system and delay the onset of tiredness. Because of the difficulty of creating proteins extracellularly and the risk of inserting foreign cells into the brain, we plan to produce ADA outside the body through *e. coli* or other cells, and deliver them to the brain via immunoliposomes.

Adenosine Deaminase in e. coli (http://partsregistry.org/wiki/index.php/Part:BBa_M12000)

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   1 atgattgata ccaccctgcc attaactgat atccatcgcc accttgatgg caacattcgt
  61 ccccagacca ttcttgaact tggccgccag tataatatct cgcttcctgc acaatccctg
 121 gaaacactga ttccccacgt tcaggtcatt gccaacgaac ccgatctggt gagctttctg
 181 accaaacttg actggggcgt taaagttctc gcctctcttg atgcctgtcg ccgcgtggca
 241 tttgaaaaca ttgaagatgc agcccgtcac ggcctgcact atgtcgagct gcgtttttca
 301 ccaggctaca tggcaatggc acatcagctg cctgtagcgg gtgttgtcga agcggtgatc
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 901 tcccgcgagc aaatccgcca ggcacagatt aatggtctgg aaatggcttt cctcagcgct
 961 gaggaaaaac gcgcactgcg agaaaaagtc gccgcgaagt aa
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