

More Stories!

Forum

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The Chemotrode

Chapter Three

by **Robert J. O'Connell**

“How did you like the Parent’s meeting?”

Eileen sat next to the Doctors desk and replied, “We enjoyed meeting the whole group. It really is a comfort to know that there are other parents with smooth brain children and to hear how well they are coping.”

The Doctor smiled and said, “The results of your blood tests are in now and you should be relieved to know that neither of you have a defective gene. So John’s problem is not inherited but is likely a spontaneous mutation. Unfortunately, we do not know how this happens but it is such a rare event in the whole population that I can’t imagine that it could happen again in your family.”

I know that this all is a lot for you to consider, but now that you have heard our research plans in the parents meeting let me give you some additional literature on the smooth brain defects, a list of future meeting dates and a description of the risks and benefits of joining my study. At our next appointment we can review the results of the genetic screen of John’s blood that we took two weeks ago and you can let me know what your wishes are as we proceed. If you agree to this we can schedule a visit to my lab at the same time.”

“Can I see your mice then?”

He replied “If you come Monday morning next week we can show you the testing we are doing in the animal quarters. We are not allowed to keep animals in the research lab. The medical school has climate controlled rooms that are in a different restricted area and are on a different light cycle so we can test the animals when they think it is dark out.”

“Why is that? Aren’t they asleep?” She asked.

“No mice are like a number of other small animals whose activity is determined by light cycle. They get very alert and active at sundown and again at sunup. In nature wandering around looking for food in the daylight is too dangerous. We fool them by making them live in rooms where the lights are controlled to go on and off when we want. For all of them they never see the sun, but don’t seem to mind. This way we can make them alert at the time we want to test them.”

“Are they white mice? I think they are so cute.”

“No their coats are black. As you already know mice are very important animals for research. It is possible to buy mice from suppliers who have been inbreeding them for so long that they are basically clones of each other. That is, for a particular strain like the ones we use, they all have the same genes and bodily functions. This degree of standardization is required in order to reduce the amount of variability one expects in any animal experiment. In this strain which is known as C57BL6J we know the whole genome and can manipulate parts of it to create a series of animals with different phenotypes. I think that we now have made one whose brain is smooth and fails to fill the cranium like John’s. So next we need to see what its behavioral phenotype is like and then if we can find a defect, try to develop a way to fix it. I think that we will be at it for a number of years.”

A decade passes and John, his younger sister and his mother return to the doctor’s office for John’s annual appointment. After saying, “hello” the children go back to the Doctor’s waiting room

which is equipped with all sorts of games and amusements. Dr. Neville is a little more stooped, but time has improved Eileen considerably. She now has the polished look of a young matron, adept, confident, and accustomed to getting her own way.

Under questioning Mrs. Kelly reported that, “John is a little behind in school but gets along well with the other students. He is very attached to his sister. She is very good with him. When you watch them together you would never suspect that John has any difficulty at all. He is also a whiz at cards and computer games, although I worry a little about the amount of time he spends in front of the monitor.”

The Doctor made a few additional notes in the thick file on his desk and replied, “This is all good news. Don’t worry about the computer time, as long as he is doing OK in school. The focus, coordination and problem solving skills he uses in the computer games are important uses of his brain. The more cognitive work he does, the better.

This time I want to bring you up to date on the exciting progress we have made since I last saw you. As I said when you visited the lab two years ago we initially concentrated our efforts on experiments with our smooth brain mice. As you recall, John has a defect in a gene which we now know a little better. It is now called *doublecortin*, (*Dcx*) which is brain-specific. In John’s case this appears to be a new mutation in your family as neither you nor your husband are carriers of this defect.”

“Yes we were very happy to learn that some years ago and as you can see our beautiful daughter is the result of that good news.”

Dr Neville continued, “It has now been determined that the *Dcx* gene’s normal function is to guide the manufacture of a protein called the microtubule associated protein nicknamed MAP. MAP proves to be a necessary cog in the protein motor in neurons that allows them to move through the brain. As a consequence of this defect in John, we can

conclude that most of the neurons that should have arrived in the upper layer of the cortex of his brain can’t move as well as they should so they got stuck in lower regions of the cortex making it thicker and the surface smoother than normal. Because of this misplacement most of these neurons were instructed to die making this lower layer thinner than normal and even eliminating it in some regions. In the mouse we have to knockout *Dcx* and a related gene called *Dclk2* to produce the same anatomical phenotype as in the human. In the resulting smooth brain mouse the brain abnormality leads to a complex variety of motor and spatial defects in behavior. One of the simplest to describe is revealed in the forced swimming test you watched several years ago.”

“Oh yes, I still tell friends about the mice. I called my favorite one floaty although your name for him was 210. It was fun to watch him get better over time.”

“Yes it does appear that practice makes perfect, or at least it helps. In our version of this test, normal and mutant mice are evaluated daily in a small diameter round swimming chamber about three mouse body lengths wide and several deep. A normal mouse placed in this stressful environment every day spends most of the few minutes of exposure actively swimming around and trying to climb the smooth walls of the chamber. Mutant mice on the other hand after their first exposure act depressed and give up quickly, spending most of their time immobile, simply floating at the surface. When we remove their brains and make thin sections stained with a special dye to reveal the location of new neurons we can see under the microscope that there is a very robust negative correlation between the number of new cells in the layers of the cortex which should express *Dcx* and the amount of time spent immobile. Sections of the mutant mouse brain have only a few stained cells and the number we can see that make it to the cortex is negatively correlated with the duration of immobility. A small number of new cells were seen

in animals with the longest periods of immobility. These results told us several things. First, the fact that we could see any stained cells at all in the mutants meant that there is a background amount of normal cortical development going on in the adults and that some of the stem cells remaining in the mutant brain are capable of producing new normal neurons. Some of these are able to get to the correct place and make the right connections. We thought for a long while that there must be something the matter with this observation since conventional wisdom at the time stated that new neurons were not produced in the adult brain. With the progress made in studies of stem cells we now know that this is not true.”

“It always amazed me that such a complex change in behavior could be caused by the absence of a few cells in the cortex,” she said

“Well it really is more than a few if you look at the whole brain. At the time I thought, if we could increase the number of dividing stem cells in John’s brain we could, on a statistical base, drive the cortex towards normalcy. Neurons that make it correctly will live. Incorrect ones will die. We have since discovered the protein substance, which we named stem cell activating protein nicknamed SCAP that drives stem cells to divide in the normal embryo and found that it did the same thing to a lesser degree in mutant adult mice. It also has the ability to reactivate the defective *Dcx* gene in a large fraction of the daughter cells that are born from the remaining stem cells. These modified cells then can try to go to their proper place in the cortex. The next problem then became getting this substance into the right place in the defective brain.”

“So if you could increase the number of dividing stem cells in the mutant brain then some of them would get to the right place and make the right connections and maybe the animal’s behavior would improve.”

“Exactly, unfortunately, substances placed in the gut or blood supplies of the body are actively

blocked from entering the brain so a simple vascular route of delivery was out of the question. We first solved this problem directly with the design of a delivery device we called a chemotrode. It is a small diameter hollow metal tube packed solid with SCAP. The free end is inserted through a hole we make in the skull and targeted to the cerebral ventricle of the animal. This ventricle is the fluid filled chamber in the adult that remains from the inside of the original hollow tube in the embryo. The chemotrode is then fixed to the skull with dental cement. Mice tolerate this treatment very well. SCAP in the chemotrode dissolves slowly in the cerebral spinal fluid and diffuses to the few stem cells that still line the ventricle. We quickly learned that to be effective this tube had to be removed and refilled frequently because of the proteases normally found in cerebral spinal fluid. These enzymes chop up any free proteins that are present in the brain. Although mutant animals treated with the chemotrode got better, as measured by the decrease in their immobility scores, and the increase in *Dcx* positive cortical cells we saw in slices of their brains it seemed unlikely that this delivery system would be useful or be allowed by clinical trial committees to treat humans. But despite this problem these results with the chemotrode did prove the principle by showing that SCAP was effective in the mutant brain. So then the problem became how can we get the protein into the human brain without using a chemotrode.”

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