1 They've followed this migratory pathway as you might
2 expect.
3 However, in the exact same animal, if you look
4 at its opposite side, at the side ipsilateral to the
5 injury, both here and here, it's very dramatically
6 different. The cells are not out here. In fact, they're
7 back over here around the subventricular zone and, as you
8 can see over here, are starting migrate, or at least we
9 think, they're starting to at least lean into the
10 infarction cavity. If you actually look to see what is
11 going on in these non-neuronogenic regions, for instance,
12 in the cortex, in the striatum, in the hippocampus, and you
13 double label these lacZ positive cells for the mature
14 neuronal marker NeuN, as you can see in this double
15 labeling, they actually seem to have shifted and move up to
16 this non-neuronogenic area giving rise to neurons where one
17 would not expect.
18 Now, obviously, this is probably not enough to
19 cure the animal. However, it does suggest that there's a
20 developmental mechanism of shifted and altered migration to
21 areas of injury. Here is a lacZ positive cell, double-
22 stained for NeuN. If you do double staining for c-fos,
23 some of these neurons appear to at least be active in
24 concert with the other cells, the other neurons in those
25 particular regions.

1 So, this is my stem cell slide because I think
2 that some of these plasticity is actually programmed, or at
3 least the stem cell or what we'd like to call the stem cell
4 is the repository of some of this plasticity. The reason
5 I'm using this is because this way I don't have to define
6 what my vanilla ice cream is. You can fill in your blank.
7 I don't have to even say what they're going to give rise
8 to. But the idea of some immature cell that's in small
9 abundance that can be pulled out and expanded by the means
10 that you wish to choose, by epigenetic means or genetic
11 means, suggests that perhaps we can pull out and harness
12 this kind of biology.
13 Now, one of the issues we have to figure out is
14 how are we going to expand this cellular population. We've
15 heard about a number of different ways, and it's an open
16 issue. Are we going to use epigenetic growth stimulatory
17 signals? Are we going to try to intervene right in cell
18 cycle? Ideally we'd love some technique that either is
19 constituitively or self-regulated in terms of propagating
20 the cells or at least one that we can control. We've used
21 various models that have taught us a lot about stem cell
22 biology that take advantage of a lot of these.
23 One of the early models that we used was
24 actually a clone that was propagated or augmented by a
25 mutated Myc, which was actually constituitively self-

1 regulated. In other words, it simply kept the cells
2 propagating in a dish, but in the brain was constituitively
3 and automatically self-regulated. But it taught us a lot
4 about stem cell biology or what we should expect.
5 I'll be initially talking a lot about that
6 particular clone that seems to at least follow the
7 operational definition of a stem cell. It has also taught
8 us a lot about what we should be looking for if we want to
9 start anew looking at human stem cells or human neural stem
10 cells.
11 Now, what's interesting is that the biology at
12 least of the neural stem cells seems to emulate a lot of
13 the biology of stem cells from other organ systems. This
14 is some collaborative work that Richard Mulligan and Lou
15 Kunkel at our lab are doing in which we actually will take
16 these operationally defined clones of stem-like cells and
17 put them through a FACS sorter looking for what's called
18 the side population, what you've heard about before. It's
19 simply a population, actually defined over here, in which
20 Hoechst red and blue dye are excluded. It's a very small
21 population. It's a population to which hematopoietic cells
22 seem to migrate to that reconstitute bone marrow. Muscle
23 stem cells seem to migrate there.
24 Well, it's kind of interesting that cells that
25 fulfill the operational definition of a stem cell -- and

1 you've heard a lot about that before -- actually seem to
2 engorge that particular area and actually seem to amplify a
3 population that even from primary brain seems to migrate
4 there, or at least segregate there. So, it actually
5 engorges and amplifies a population in small abundance that
6 segregates to this particular region.
7 Now, a few points can be brought out here.
8 First of all, it's interesting that this clone of mouse
9 neural stem cells segregates to this area in exactly the
10 same way as cells from the human nervous system that
11 fulfill an operational definition of a stem cell also
12 migrate to that particular area and augment it.
13 One can also see that if one actually buys that
14 maybe this does recognize stem-like cells, it's certainly a
15 small population and one will need to figure out a way to
16 augment that, to expand it. Hematologists, obviously,
17 depend on expanding this population in vivo. We in the
18 nervous system are probably going to have to figure out a
19 way to expand that ex vivo if we're envisioning
20 transplantation paradigms.
21 Well, what do I mean by the operational
22 definition of a stem cell? Well, it should be self-
23 renewing and we can discuss what that's about.
24 It certainly should be multipotent in a dish
25 and also in vivo. One way of showing that the cells are

1 multipotent is to take this particular population that
2 encodes lacZ and put it into a developing mouse embryo, let
3 the animal go to adulthood, and see that the cells at
4 maturity have integrated throughout the neural axis, and we
5 know will respond to the particular temporal and anatomical
6 cues present at that particular time.
7 They actually go to both sides, but I'm showing
8 this particular section where they're segregated to one
9 side to bring out the point that except for the fact that
10 these areas are chimeric, the cytoarchitecture seems to be
11 identical. In other words, they seem to integrate in a
12 fairly seamless fashion and what one sees there are the
13 cells that you would expect based on your fundamental
14 knowledge of neurogenesis in the developing nervous system.
15 So, if one does a transplant to allow cells to get into the
16 embryonic cortex, they'll give rise to pyramidal neurons.
17 Here's a nonpyramidal neuron putting out a process onto a
18 host pyramidal neuron.
19 Outside of that window of neuronogenesis, the
20 exact same clones and the exact same region will no longer
21 in a normal adult cortex give rise to these type of
22 neurons, but will give rise to oligodendrocytes, for
23 instance, or to astrocytes putting out foot processes onto
24 blood vessels.
25 Now, one of the operational definitions of a

1 stem cell is this ability to do serial grafting. It's one
2 that hematologists do. Well, we've tried to at least
3 emulate that, also to show that the environment seems to
4 mold these cells. One can do, for instance, an embryonic
5 transplant and particularly go to the forebrain, pull out
6 cells, pull out a section, a section like that with
7 engrafted cells from the adult in the forebrain, can pull
8 them out, propagate them again, and then reimplant them at
9 another time in another region, for instance, now in the
10 newborn cerebellum, and in fact, find that those cells that
11 have been retrieved now will give rise to granule neurons
12 in the internal granular layer. So, again, part of the
13 operational definition of a stem cell to at least suggest
14 that maybe we have a model that can teach us a little bit.
15 Another major question is -- and this is
16 unanswered I think satisfactorily in our field. This
17 experiment that I'm mentioning is not going to answer it
18 either, but are these cells in any way functioning or are
19 they simply there anatomically or is there any hope that
20 they can start integrating into the circuitry. Well, we
21 really don't know because the experiments haven't really
22 been done well.
23 But one way of starting to get to that is to go
24 to systems that -- at least this is one particular system I
25 find very appealing, and that's a collaborative study that

1 we've done with Bill Schwartz at U Mass who is a circadian
2 rhythm biologist who focuses on this particular area, the
3 suprachiasmatic nucleus. Now, this is very appealing to us
4 because these are cells, circadian rhythm cells, that need
5 to respond to stimuli administered many way stations away,
6 light to the retina, for example. Yet, the cells here need
7 to work in a concerted effort back here in the
8 suprachiasmatic nucleus of the hypothalamus.
9 Well, we know that these donor clone of neural
10 stem cells can integrate into that region if you do a
11 transplant at the right time embryonically. These are
12 these Xgal positive cells intermixed there.
13 Now, Bill Field uses c-fos to talk about --
14 it's somewhat nonspecific, but it talked about appropriate
15 activations of the cells in response to a particular
16 stimuli. So, this particular area is stained for c-fos, so
17 it will be a brown nucleus. And then in an engrafted
18 animal -- and you can see the cells integrated in there.
19 Now the lights are turned on, so to speak, and there's an
20 up-regulation of c-fos activity. You can see a burst of
21 c-fos activity. And then a subpopulation of the donor
22 cells over there. It's a subpopulation that also expresses
23 vasopressin and a lot of the other appropriate cytochemical
24 markers. A subpopulation of those cells also starts
25 expressing c-fos appropriately and in the right percentage

1 as the host cells.

2 If you don't buy the light microscopic view,

3 this is confocal looking for c-fos among the host cells and

4 then double staining for beta-gal and c-fos activity.

5 Again, the population of the donor cells in response to

6 light, many synaptic connections away, will up-regulate

7 c-fos in the appropriate percentage.

8 So, what does this say? Well, who knows what

9 it says?

10 What is says, however, is that at least we have

11 a model that can teach us a little bit about what stem cell

12 biology would be if we buy certain operational definitions.

13 What it also says is, in going through this,

14 that whatever we're going to call them, there are cells

15 that we can pull out, that we can propagate that can go

16 into a brain, at least in a transplantation paradigm, might

17 emulate proper developmental mechanisms, but you can grow

18 them in a dish, put them back, and maybe have them become

19 appropriate neural cells. Certainly they can bring in

20 foreign genes with them that, even as was brought up

21 before, might be nothing more than glorified pumps, but

22 this is a kind of pump that may be useful. They can bring

23 in these genes and they can do this in not only a local

24 fashion, but in a widespread manner. That's actually

25 important, at least for a lot of the diseases that I as a

1 pediatrician deal with.
2 They may also -- and this is a big "may" -- be
3 able to repopulate. So, just the idea of their being able
4 to express therapeutic things throughout the brain we first
5 tested, as was mentioned before, just by looking at a mouse
6 mutant model of one of the lysosomal storage diseases. We
7 did this in collaboration with John Wolf down at Penn.
8 This particular mouse mutant was injected with these cells
9 into the ventricles, allowing them access to the
10 subventricular zone. The cells distributed themselves all
11 throughout the brain. So, there was extensive migration of
12 these cells, which is exactly what a model like this needs.
13 It needs the distribution of a missing gene all throughout
14 the brain because it's missing. This distribution of cells
15 corresponded to the distribution of therapeutic enzymes all
16 throughout the brain, almost like a bone marrow transplant
17 to the brain. Normally this mutant would have 0 levels,
18 and that resulted in the elimination of this lysosomal
19 storage, as you can see, in an untransplanted control
20 animal in the cortex, but there the lysosomal storage in
21 the host neurons and glia was virtually eliminated.
22 Well, this kind of paradigm of taking advantage
23 of this migration can be applied to a lot of diseases where
24 you need to replace not only genes throughout the brain,
25 but maybe even replace cells. So, some of the mouse

1 mutants that require that are the white matter mutants that
2 need dysfunctional oligodendrocytes replaced. So, for
3 instance, we're looking at a lot of these mutants that are
4 white matter degeneration mutants just to test that.
5 So, we've looked at the twitcher mouse, which
6 is a model of the leukodystrophies, the shiverer mouse,
7 which is a model of myelin basic protein degeneration. In
8 those models we get that same distribution of cells.
9 In a twitcher model, for example, they will
10 differentiate into oligodendrocytes that start myelinating
11 host axons, and under EM you can appreciate this a little
12 bit better. Here's a donor-derived oligodendrocyte
13 surrounded by 30 to 50 myelinated host axons. The kind of
14 myelin that one sees is actually fairly respectable. As
15 you can see over here, it has an appropriate thickness and
16 compaction. The Xgal precipitate indicates that it's
17 donor-derived.
18 In the shiverer mouse, this kind of
19 distribution of cells can even result in a decrement in
20 some of the symptomatic shiver.
21 So, taking advantage of this migratory
22 potential can be used for certain diseases that need this
23 perhaps. So, one can use this perhaps to distribute cells
24 and enzymes.
25 But there's another component to migration that

1 I think is actually very appealing and very intriguing.
2 That's the idea that maybe these cells can migrate to areas
3 of pathology, somewhat the way the initial experiments I
4 mentioned illustrated that maybe the brain tries to also
5 shift stereotypical migratory patterns to deposit cells
6 where it at least perceives maybe they're needed. Whether
7 it does it sufficiently is another argument, but can we
8 ride this crest?
9 What I wanted to do was just very briefly talk
10 about three different examples that somewhat demand
11 alterations in migration of these cells. That would be
12 infarcts. I'll get back to exactly the model with which I
13 started, which is a very dramatic acute injury of multiple
14 cell types, of selective cell death. What I'm going to
15 look at is motor neuron degeneration where just that cell
16 dies but the other cells are pretty much intact, and then
17 tumor, which is a slow, indolent kind of pathology, which
18 in and of itself is also a very migratory pathology.
19 So, just to remind you, this is the kind of
20 injury that we're going to impose, and we're going to
21 impose it on normal animals that simply have these reporter
22 cells intermixed throughout and see what the cells do.
23 Krucken Park in the lab has taken animals like that and put
24 them directly into the infarcted area or put them into the
25 opposite side. Some of you have seen these data before.

1 But what I want to bring out is this migratory
2 component. So, the injury is imposed over there. Cells
3 are placed over here. It doesn't really matter whether
4 they're implanted first and then the injury is imposed or
5 whether you impose the injury and then you do the
6 transplantation of these reporter cells. The phenomenon is
7 the same. In an intact animal, the cells are pretty much
8 restricted to this particular side of the brain.
9 However, a different story is witnessed when
10 there's damage done to this particular side. Now, one sees
11 that cells implanted over here will migrate across
12 commissures or the corpus callosum, throughout the
13 hemisphere, and move from their area of initial engraftment
14 to home in, so to speak, into this infarcted or to this
15 very damaged area. The kind of cells that you would see
16 there at higher power are what have the kind of profile
17 that we recognize as being somewhat suggestive of a
18 migratory cell with the leading process moving in the area
19 of movement and then a cell, for instance, here on the
20 corpus callosum, moving over here. When it actually does
21 get to the area of infarction, these cells under this EM
22 will be migrating right into this very necrotic area as if
23 there is a trophic response there.
24 Interestingly, if the cells are placed directly
25 into that area, they never move back in the opposite

1 direction. They always stay right there, which also
2 suggests what one might want to ask. But what happens when
3 the cells are directly placed in there?
4 Well, they seem to engraft very robustly into
5 this very infarcted area. The interesting thing would be,
6 well, what happens to these cells? They change their
7 migration. Do they change their differentiation? Now,
8 this is a time -- and if we look at the cortex -- neurons
9 should not be born anymore. Oligodendrocytes, maybe a low
10 level of oligodendroglial genesis, but yet, these are the
11 two cell types that take the biggest hit in this kind of
12 injury. Under EM, when we ask this question, we see
13 something that electromicroscopists would like to say is a
14 fortuitous EM section, one can see two donor-derived
15 oligodendrocytes sitting next to a big, large donor-derived
16 pyramidal neuron. And the apical dendrite of this
17 pyramidal neuron shown at higher power here -- here you can
18 see the Xgal precipitate in there -- is receiving synaptic
19 input from the host.
20 If one does immunocytochemistry not only to
21 confirm this but to help with quantification, this top
22 panel is anti-beta-gal antibody to recognize these donor
23 reporter cells. This is anti-cell type markers to clarify
24 what they are. This is under the merged image. One, in
25 fact, does see new neurons there, new oligodendrocytes, new

1 neurons as much as a millimeter away on the side of the
2 injury.
3 If one does the mathematics -- and I'm putting
4 this up more to make a qualitative point than a
5 quantitative point -- on the intact side, as you might
6 expect, one sees no neurons at all, low level of
7 oligodendrocytes. But now there seems to be a shift, and
8 now there seems to be new neuronogenesis in that particular
9 area. So, that as a developmental biologist is an
10 interesting observation, and it suggests that maybe some of
11 what we saw with the endogenous progenitors can be
12 augmented with these exogenous progenitors or cells.
13 Now, you can look at this and say, well, 5
14 percent. What's the big deal there? Well, 5 percent of
15 this large amount of cells may translate into tens of
16 thousands of neurons, and we really don't know how much it
17 takes to reconstruct circuitry.
18 However, the fact that you get this robust lacZ
19 expression suggests that perhaps one could actually
20 engineer these cells to over-express a trophic factor.
21 Maybe if they're already expressing a trophic factor,
22 perhaps they can over-express it and then respond to it.
23 In collaboration with Isack Fisher, these cells
24 have been actually engineered to over-express NT-3. It was
25 first established that the cells actually have a TRK C

1 receptor that appropriately becomes phosphorylated, that
2 then signals to a MAP kinase intracellular signaling,
3 suggesting that the cells should be able to respond in an
4 autocrine or paracrine fashion to this NT-3. In fact, they
5 do give rise to many neurons.

6 Then one can do the exact same transplant, get
7 the exact same kind of engraftment, find that these now
8 engineered cells, these beta-gal positive cells, continue
9 now to express NT-3 in vivo. If one looks to see what the
10 cells become, a beta-gal positive cell double-stained for
11 MAP-2.

12 But the purpose of this slide is mostly to
13 bring out the point that red fluorescence and green
14 fluorescence, when superimposed will give you yellow or
15 orange fluorescence because on the next slide, looking at
16 the penumbra of the infarction where beta-gal is recognized
17 in red, NeuN. The mature neuronal marker is recognized in
18 green. Certainly one can see plain green cells and plain
19 red cells, but now you see a fair number of orange and
20 yellow cells, recognizing new neurons added to this
21 penumbra as a result of this manipulation. In the MAP, one
22 can now see over 80 percent neurons in this penumbral area.

23 But it also suggests that maybe if we
24 understand some of the trophic factors we can use this
25 perhaps to push cells down lineages that they're already

1 pursuing but that are desirable to us. Also, it's an
2 interesting example of doing gene therapy and cell
3 replacement with the same cells simultaneously. The
4 neurons that one sees are exactly what you would expect,
5 donor-derived neurons that are cholinergic. One sees
6 glutamatergic and GABAergic.
7 So, this is a very dramatic kind of infarction,
8 acute injury of many different cell types.
9 What about a selective loss of a particular
10 kind of neurons? This particular model is a well-known
11 model in the ALS field in which if one first takes a
12 newborn mouse, ligates its sciatic nerve, and then by the
13 time the animal reaches maturity, these motor neurons have
14 degenerated by apoptotic mechanisms.
15 Now, again, John Flax and Chou Xan Lo and Ted
16 Tang in the lab have done a number of different kinds of
17 manipulations here, but I want to show you one that brings
18 out this idea of migration towards areas of injury.
19 They would take the cells and put it not in the
20 area where the ventral motor neurons have degenerated, but
21 actually up here in the dorsal horn. This is immediately
22 after transplant, just to show that they've hit their
23 target. In an intact spinal cord, the cells certainly
24 distribute themselves and engraft but pretty much
25 restricted to the dorsal part of the cord in a normal

1 animal.

2 However, in a mature animal in which the motor
3 neurons have been made to degenerate, particularly if you
4 go towards the point of active degeneration of these cells,
5 now something very different they've observed. That is
6 that the cells implanted up here have now migrated from the
7 dorsal part of the cord down to the ventral part of the
8 cord. They've done it at multiple levels throughout the
9 cord of this particular animal. So, there not only is
10 migration in this dorsal to ventral axis, but because these
11 are semi-serial sections of the cord of one animal, they've
12 done it also for about a millimeter in this rostral/caudal
13 dimension which is the extent of motor neuron degeneration
14 here.

15 If you look at the kinds of cells that are
16 there at higher power, the kind of cells that you see are
17 these large Xgal positive cells that certainly have the
18 morphology reminiscent of a motor neuron, similar to one of
19 the residual host motor neurons.

20 They stain for NeuN, indicating that these
21 donor-derived cells immunocytochemically are becoming
22 neurons. Here's a donor-derived NeuN positive cell sitting
23 next to a residual host NeuN positive neuron.

24 Here's a MAP-2 positive cell double-stained for
25 beta-gal sending its process out of the plane of focus.

1 So, it looks as if they're moving there and becoming
2 neurons, but it looks as if they may also be trying to
3 become spinal motor neurons. If one does an in situ
4 hybridization against ARIA, a neuregulin, called
5 acetylcholine receptor inducing activity necessary for
6 characterizing or determining that a cell is a spinal motor
7 neuron, if you do an in situ on this section and engraft an
8 axotomized section like that, you can see hybridization in
9 this particular axotomized engrafted animal and a
10 distribution that looks very similar to that of the
11 engrafted cells and pretty respectable compared to the
12 animal's own intact control, very different than an
13 axotomized untransplanted animal. The cells that seem to
14 be hybridizing to the ARIA probe are these big beta-gal
15 positive cells heavily overlaid by silver grains ARIA
16 hybridization at a level much higher than background.
17 Interestingly, the beta-gal positive cells,
18 which have stayed as small cells up in the dorsal horn,
19 don't hybridize better than background. And these kind of
20 cells express ChAT. They express ILa-2, suggesting that
21 the cells have migrated there and are now trying to become
22 perhaps motor neurons or at least responding to signals
23 that may recapitulate some developmental signals.
24 It was mentioned that perhaps some of the
25 efficacy of the cells is not only going to be whether they

1 can replace cells or not.

2 These are the exact same cells -- actually this
3 is a spinal cord slice done in collaboration with Jeff
4 Rothstein. The cells actually are placed out here of the
5 spinal cord slice and one can see that the neurofilament
6 fibers of the motor neurons are actually homing in right on
7 some factor, almost looking as if they're making a ventral
8 root, seeming to be expressed by this particular factor.

9 I want to end with one last model and that's
10 tumors, which is an indolent kind of slow growth that
11 requires migration. This is done by Karen Abuti in the
12 lab. She implanted cells directly into the adult cortex.
13 Now, one of the problems of treating brain tumors is the
14 fact that they migrate and they insinuate into normal
15 tissue. Karen wondered what would happen if she first took
16 the cells and implanted them directly into the tumor.

17 Here the tumor is seen in red. Here the tumor
18 is expressed to express green fluorescent protein so that
19 it will fluoresce green. The red are neural stem cells
20 implanted in there for lacZ. They're Xgal blue here.

21 The cells were implanted directly into the
22 tumor and within 48 hours they migrate out to the very
23 interface between tumor and normal tissue and come to a
24 halt right there except, as you can see over here at higher
25 power, where a tumor cell is infiltrating normal tissue.

1 There a neural stem cell seems to perhaps jump on top of it
2 and move in direct juxtaposition to the cell, continuing to
3 express its lacZ gene.
4 This is simply to show immunocytochemically
5 that the exact same thing is happening, the cell migrating
6 out, even in very virulent tumors, as there is rapid
7 migration of tumor cells into the tissue, being followed by
8 neural stem cells.
9 Now, how robust is this migratory capacity?
10 One would not necessarily want to treat tumors in this
11 particular fashion, but Karen established the tumor over
12 here but implanted the neural stem cells over here, and
13 then after about a week followed to see what those cells
14 were doing. She could see, looking at the corpus callosum,
15 that there were again cells moving from this area of
16 implantation -- this cell shown at higher power here, this
17 cell shown at higher power here -- classic migratory
18 profile moving towards the tumor cell. Then when she
19 actually looked at the tumor, she could see that it was
20 filled with blue cells. The only source had to be from
21 this particular implantation of neural stem cells over
22 there.
23 Now, this is the last point that I want to
24 bring out. This allows me to transition to whether this
25 biology is preserved in human neural stem cells. This is a

1 human neural stem cell grown in growth factors that has
2 been pulled out of a human fetus. It's now an established
3 clone that seems to emulate many of the qualities of mouse
4 neural stem cells.
5 Well, Karen took a cell like that and did the
6 exact same paradigm that I described before. Here's the
7 edge of a tumor over here. Here's actually the edge of a
8 human glioblastoma. You can see it's filled with brown
9 nuclei. These are human neural stem cells recognized by a
10 human-specific nuclear antibody, and it's completely
11 engorging the tumor cell. The interesting thing is that
12 the cells were implanted over here on the opposite side and
13 they migrated into there.
14 The other interesting aspect is that this
15 particular clone of human neural stem cells is engineered
16 now to over-express a gene that would allow us to test
17 bioactivity. It's now been engineered to express the gene
18 cytosine deaminase, which most of you know is a gene which
19 has been used in prodrug paradigms against brain tumors.
20 If one exposes this benign drug, 5-FC, to cytosine
21 deaminase, it converts it to the oncolytic drug 5-FU which
22 then will kill tumor cells.
23 The neural stem cells over-expressing this
24 particular gene now, first in a dish, are exposed to 5-FU
25 to see whether this gene is bioactive. Here they're

1 intermixed with tumor cells. One now overlays with 5-FC
2 and the cells were now converted to the oncolytic gene
3 product 5-FU and eliminates most of the tumor cells, as
4 quantitated over here.
5 If one now takes this and does the exact same
6 paradigm, taking the cells and putting it into the
7 established tumor, and then does exactly the same thing,
8 gives the animal 5-FU triggering the action, one can see in
9 this image analysis of camera lucida, here is the tumor,
10 our profiles of the tumor for image analysis, untreated,
11 large, big like that. If one now triggers this reaction
12 mediated by these human neural stem cells, one can see a
13 dramatic decrement in the size of the tumor, suggesting
14 that the gene is bioactive, quantitated at an 80 percent
15 decrement in the size of the tumor, suggesting in fact that
16 one does have a bioactive gene delivered by this particular
17 migratory cell.
18 I'm going to skip this except simply to say
19 that we see same type of phenomenon in motor neuron
20 degeneration, also in stroke.
21 And then simply to end with this last bit to
22 say can this biology be translated to models that are
23 closer to the human condition. This is some work that Curt
24 Freed in our lab did in which we would take these human
25 neural stem cells and now see whether at least their

1 biology will be recognized by a brain that's closer to that
2 of a human.
3 So, we've gone actually to fetal monkeys. This
4 is a lot earlier than we've done it, but under ultrasonic
5 guidance, taken these human neural stem cells, placed them
6 into the ventricles of fetal monkeys -- except the animal
7 is a lot older at this particular point -- let the animal
8 develop, particularly the cortex. The cells are labeled by
9 anti-BrdU, so one will see a black nucleus. The cells will
10 migrate out from the ventricular zone, and one can see,
11 recognizing the cells, migrating up radial glial fibers
12 quite appropriately. Those that jump off at the lower
13 lamina of the cortex, where neurogenesis has stopped, will
14 then differentiate into glial cells very appropriately.
15 Those that continue up this elevator, so to speak, and jump
16 off at the higher lamina where neurogenesis is ongoing will
17 now become neurons, intermixed right in the appropriate
18 lamina with the monkey's own cortical neurons. And that
19 these are neurons is indicated by double staining for NeuN,
20 doubling staining with BrdU for calbindin, but will also
21 give rise to oligodendrocytes and to astrocytes, suggesting
22 that they're responding to the appropriate developmental
23 cues.
24 Interestingly, a subpopulation that does not
25 migrate out to the cortex will hang as a quiescent

1 population around the ventricle, conceivably to become this
2 population that later on in life may be activated. So, the
3 hope would be that human neural stem cells, because they're
4 reflecting a biology, may be able to also recapitulate this
5 biology in the human brain perhaps as they're doing it in
6 non-human primate brain.

7 I think this is probably the touchstone of the
8 entire meeting that I think we have to question all of our
9 previous precepts, and that life's answers being questioned
10 over here, but I think by the end of the two-day session,
11 we're going to find out that all of our answers that we
12 thought we had are certainly being questioned as we explore
13 more of the stem cell biology.

14 Sorry to have gone over.

15 (Applause.)

16 DR. SALOMON: I think, just because of the time
17 constraints here, what I'd like to do is go on to the last
18 talk of the day, and then if there's some discussion, we
19 can have that after both the talks. So, I invite John
20 McDonald to come and talk about animal models of spinal
21 cord injury.

22 Comments? Go ahead.

23 DR. DRACHMAN: Evan, you're at Children's
24 Hospital. So is Judah Folkman. You surely must have
25 looked at VEGF, angiopoietin, basic FGF, and so forth to

1 see whether that's what's leading your cells around. Did
2 you?
3 DR. SNYDER: We're actually in the midst of
4 looking at all of that. As you would imagine, we're doing
5 a lot of chip technology.
6 The thing that I think is going to be clear is
7 that I think is going to be clear is that it's not going to
8 be one factor. It's probably going to be a cocktail of
9 factors not only some diffusible factors, not only the
10 factors that you mentioned, but also changes in
11 extracellular matrix. There's probably also going to be
12 alterations in not only attractants but also disinhibition
13 of repellants and things of that sort.
14 So, yes, we're starting to look at that. We're
15 trying to go at it, not in a fishing expedition kind of
16 way, though you could say, well, that's what chip
17 technology is. But those are certainly candidates.
18 One of the interesting things that we're seeing
19 that's a difference between acute and chronic injury, which
20 is where we see a lot of these phenomena going on, is
21 alterations in various cytokine receptors, alterations in
22 LIF receptors, various G proteins. So, some of the
23 candidates that seem to be very important in fundamental
24 stem cell biology also seem to be changed in these milieu
25 where we see these alterations in migration.

1 DR. SALOMON: John, you're on.
2 DR. McDONALD: So, what I'm going to focus on
3 today is really embryonic stem cells. What I'd like to do
4 is go over some of the models in spinal cord injury and
5 then use an example of a couple different models as an
6 approach to potential clinical trials.
7 So, we'll go through a single example and
8 really define what the problem is, what's doable, what's
9 doable in regeneration, what are we modeling. Appropriate
10 age of the models is incredibly important. What models are
11 available? What are ES cells? It's still very early days
12 and what to do to move forward.
13 So, this is the real problem with spinal cord
14 injury. You get breaks in the bony spine, compress the
15 soft spinal cord. The most common cause is motor vehicle
16 accidents. And you're left with an injury oftentimes that
17 looks like this where you get a cyst in the middle of the
18 cord. In fact, here we're looking at a cross section of
19 that same cord. In between the dotted line and the closed
20 circle line is the spared tissue. So, the spinal cord is
21 kind of built opposite of the brain, where the white matter
22 containing the long tracks in the brain and going to the
23 brain are on the outside. So, death typically occurs in
24 the middle. You are always left with a small rim or donut
25 of intact connections.

1 The key thing is that this is a person that has
2 run triathelons. So, you don't need to cure the spinal
3 cord to regain function. You just need to get minimal
4 anatomic improvement. The estimates are that you need less
5 than 10 percent of the functional connections in order to
6 walk.
7 This is really to point out that the choices
8 are really immense. When you look at the choices between
9 time of treatment after the injury, what cell type do you
10 use and what disease to choose. So, in this case we're
11 going to choose spinal cord injury, a subchronic treatment
12 at 9 days using ES cells at a very specific neuroprogenitor
13 stage and focus on myelination.
14 So, the thing is the critical balance in moving
15 forward with potential preclinical studies or any studies
16 in this. I think that you'll see the studies that we look
17 at have many of these features.
18 Of course, it's important that there's very few
19 alternative treatments, making it more reasonable to use
20 interventions such as cell transplantation. Also, the
21 potential risks of the treatment are reduced. I think as
22 I'll point out, transplanting oligodendrocytes really pose
23 less of a risk than transplantation of neurons. Neurons
24 have a much higher propensity to do abnormal things such as
25 pain, abnormal transmission, seizures.

1 Doability of treatment, of course, is very
2 important. And myelination I think fits this. That is,
3 after an injury and even in demyelination, many of the
4 circuits remain but are dysfunctional, and you simply need
5 to remyelinate. That's going to be an easier task than
6 recapitulate the circuits.
7 And then severity of disease. I think Evan
8 pointed this out very well, that it's going to be much
9 harder to fix a hole in the spinal cord than it is to
10 replace single, individual lost cells.
11 Then proof of principle. What do we know in
12 preclinical studies as far as mechanisms, anatomical and
13 behavioral effects and the appropriateness of those animal
14 models?
15 Then most importantly, we need to have
16 measurable and interpretable endpoints for a clinical
17 trial, both anatomical behavior and physiologic.
18 So, far as doability, of course, preventing the
19 injury is much easier than regeneration. In the spinal
20 cord in animals, there are multiple new treatments that
21 have been designed to be given immediately after the injury
22 that are able to prevent some of the secondary injury.
23 Despite substantial progress in the last 10 years on
24 regeneration, overall it's still very limited. In humans,
25 we only have one acute medical treatment,

1 methylprednisolone and no regenerative treatments.
2 So, if you really look at level of difficulty
3 -- this is my personal view of this as far as regeneration
4 strategies. I think if you look at some of the easiest
5 things, cell birth, such as glial cell birth, in many cases
6 we want to limit this, in the case of astrocytosis.
7 Remyelination and birth of oligodendrocytes are just
8 remyelination of surviving oligodendrocytes. Axonal
9 sprouting is doable, particularly in the local fashion.
10 Cellular expression of molecules through gene expression is
11 becoming very doable. Neuronal cell birth, as Rusty
12 pointed out, is becoming something that we're just at the
13 beginning of getting handles on.
14 The most difficult things, of course, are
15 axonal sprouting, particularly over long distances, with
16 appropriate reconnectivity.
17 So, in spinal cord injury, just to put things
18 in perspective, here are the different events. Trauma. Of
19 course, you want to reduce any compression that's occurring
20 immediately, then limit the secondary injury, limit
21 secondary complications, and that's the main focus of
22 rehabilitation, and then of course, interact with mobility
23 and environment, which again is ergonomics of
24 rehabilitation. Then what we want to focus on today is
25 just replacing lost cells, myelin and axonal connections.

1 So, the first important question is what should
2 we be modeling in spinal cord injury. There are just a
3 zillion different choices. It really depends on what
4 condition you want to model because there are many
5 different causes of spinal cord injury. The most common is
6 trauma which is mixed from vehicular accidents, violence,
7 falls, sports. But then there's a myriad number of medical
8 causes including demyelinating disorders, orthopedic
9 tumors, infectious degenerative disorders. So, we have to
10 decide on which one of those we want to treat, some of
11 which potentially are going to be easier than others. But
12 in most cases, most of those disorders are a one-time hit
13 and it's not a recurrent event.
14 Now, this is just a schematic that I put
15 together today just to really look at the age
16 appropriateness of models. I think Evan pointed this out
17 incredibly well, that if you transplant progenitor cells
18 earlier in development, they really migrate a much greater
19 distance and probably have a better chance of survival as
20 opposed to the same transplantation in the adult. Then as
21 Evan also showed, injury also supports additional length of
22 migration. So, it's very important to assess the
23 appropriate age for the model that you want to look at.
24 Then most importantly, it's a constantly
25 changing problem. For example, if you want to look at

1 spinal cord trauma, it's a very different problem if you
2 want to have an intervention that's to be instituted
3 immediately after the injury blocking, say, for example,
4 necrosis, or one a little bit later blocking, for example,
5 programmed cell death, or a regenerative type event. Even
6 the regenerative events are going to be dramatically
7 different depending on the timing, whether it's 1 week, 2,
8 weeks, 2 months. So, the problem you're facing is
9 constantly changing.
10 So, what models are out there right now that
11 people are focusing on? I think it's useful to divide them
12 up into these mixed cell death models where you get death
13 of everything, basically infarcts, neuronal, glial, and
14 axonal injury, and then more selective cell loss.
15 So, the weight drop contusion model is probably
16 one of the best held models for traumatic spinal cord
17 injury. You recapitulate the syrinx that occurs in the
18 middle of the spinal cord and many of the aspects of the
19 physiology as well.
20 Compression injury is a bit of an older type
21 model, but it's still being use.
22 Ischemic injury, replicating what happens with
23 triple A surgeries.
24 And then transections, both partial, complete,
25 and suction methods.

1 A lot of these have come out mainly because
2 these are very difficult models to care for the animals.
3 But then the more selective cell loss injury
4 models, such as just focal myelin loss, like Evan pointed
5 out with the myelin mutants, chemical demyelination, which
6 you heard about earlier today, and then focal neuronal
7 loss, including neuronal mutants and neurotoxins. These
8 can be subdivided into axon sparing and non-axon sparing.
9 That really changes the model and what you want to
10 accomplish.
11 So, what is a stem cell or an embryonic stem
12 cell? Well, basically it's the earliest form of stem cell
13 capable of making, in theory, every subsequent stem cell.
14 It's self-renewing. It's truly pluripotent. In most cases
15 for new ES cells, we proved this by producing teratomas in
16 SCID mice with cells from all three germ layers.
17 Probably one of the most important aspects of
18 an ES cell is you have the best proof of principle that
19 it's genetically normal. That is, you can produce a
20 chimeric animal, which you really can't do with almost any
21 other stem cell.
22 It's highly genetically manipulable. Of
23 course, this is one of the reasons for the transgenic error
24 that's occurred, and this is going to have a lot of
25 advantages. I think in the long run embryonic stem cells

1 are going to have most of its input as a tool rather than
2 as a therapy, although a therapy will be important as well.
3 And they have the same advantages as other stem
4 cells.
5 So, from the public's standpoint, I think these
6 types of videos are very useful. What is an ES cell?
7 Well, basically you just take an egg and a sperm and do in
8 vitro fertilization is one of the most common ways it is
9 done. It can also be done in an animal and then flush the
10 fallopian tubes. But if you let that grow to a blastocyst
11 stage -- and at this stage, it's still pre-implantation.
12 If it were in the animal, it would be in the fallopian tube
13 before it got to the uterus.
14 If you split that in half, it's the inner cell
15 mass that you're interested in. If you take out these
16 cells, these are embryonic stem cells. More typically it's
17 done through immunodissection.
18 This is very easy to take these cells and
19 culture them really indefinitely. So, it really provides
20 an unlimited source of cells. You begin the week with 1 of
21 these, you end the week with 256 flasks. So, already in
22 the last couple of years, there has been over 50 knock-ins
23 and knock-outs in ES cells that are frozen cell lines that
24 you can just obtain from other people around the world.
25 So, let's move on to transplantation. So, we

1 use a differentiation protocol worked out by David Gottlieb
2 at Washington University called the 4 minus/4 plus stage
3 embryoid body where you take the ES cells and you grow them
4 floating in a dish so they don't stick for the first 4
5 days, and then expose them in the last 4 days to retinoic
6 acid. Retinoic acid has been shown to be a very strong
7 stimulus to push them down and commit them to a neuroglial
8 lineage.
9 So, what is one of these 4 minus/4 plus EBs?
10 Well, if you cut these in half, you can see that there are
11 very few differentiated cells. The majority are nestin
12 positive or neural-restricted precursors.
13 If you take these and culture them at that
14 point, you can show that you can make, of course, neurons,
15 type 1 astrocytes, type 2 astrocytes, and oligodendrocytes.
16 Really now, applying just basic tissue culture
17 methodologies, you can obtain almost every type of culture.
18 This is a very typical mixed neuronal/astrocyte culture
19 where you see ganglia of neuronal cell bodies with axons
20 over astrocytes.
21 This is the model that we use for
22 transplantation. It's a contusion model injury where we
23 drop a 10 gram weight over a laminectomy site over the
24 exposed spinal cord. They have a moderate/severe injury
25 such that when the animal recovers over the next 2 or 3

1 weeks, it plateaus to the point where it's not able to bear
2 any weight on its hind limbs. It can move its legs, but
3 not in a coordinated fashion, and it can't place its paws.
4 So, it's one of the most sensitive points on the BBB Scale,
5 which is an open field assessment of hind limb function
6 following spinal cord injury.
7 In this study, this is the experimental time
8 line we used for the transplantation. We did the contusion
9 injury and then we waited 9 days. We waited 9 days for two
10 purposes. One, we wanted to wait till the inflammation
11 went down, and two, we wanted to get out past the period
12 where most cell death occurs. If you really want to look
13 at regeneration, you should make sure you exclude
14 potentially preventing cell death. Many regenerative
15 studies are done with co-transplantation. Then we assessed
16 behavior over this whole time period and then 2 and 5 weeks
17 later looked at the histology.
18 In these studies -- these were very early
19 studies -- we used multiple labels because no one label is
20 perfect. So, the cells were genetically labeled with lacZ
21 or GFP. We could also use species-specific antibodies. We
22 transplanted mouse male ES cells into female rats so we
23 could also use Y chromosome in situ hybridization. It
24 turns out that the mouse-specific antibodies were very
25 effective because you were able to see processes very well.

1 We always did triple labeling. It's not enough
2 just to see lacZ or GFP, but we want to see the appropriate
3 phenotype specificity, as well as a nucleus that's
4 appropriate for that cell type because it's very difficult.
5 There are so many macrophages that occur after any injury
6 that it impairs the assessment of this.
7 We moved on now to label these cells with
8 paramagnetic markers so that we can follow them with MRI,
9 and there are other cell tracking methods as well.
10 So, if we look 2 weeks after transplantation,
11 this is a longitudinal cord from one of those rats. This
12 is the formed cyst that occurs within 2 weeks. This is the
13 middle of the transplant. We actually transplanted a
14 million cells in 5 microliters right into the forming cyst.
15 2 weeks later we found that the cells would migrate up to a
16 centimeter down. In fact, cells would be a centimeter down
17 within 12 hours after transplantation, and they're not
18 doing this by migrating through the tissue. This syrinx
19 connects with the central canal, and they would walk down
20 the central canal in both directions, and then migrate out
21 across the radial glial.
22 We can quantitate this. I'm not going to go
23 through this in detail, but basically you can demonstrate
24 that there are all three lineages, oligodendrocytes,
25 astrocytes, and neurons, and they have the appropriate

1 phenotype. NeuN doesn't stain the nucleolus. It labels
2 the nucleus more than the cytoplasm. There's a nucleus
3 that fits in that, and that fits in this label for M2 which
4 is a mouse-specific antibody, larger astrocyte, larger
5 nucleus, co-labels with M2, which is a surface label
6 marker. There's an oligodendrocyte. APC is a cytoplasmic
7 label, and there's the nucleus that fits in.
8 What we were very surprised at, because what we
9 were hoping to do is just see cells survive and then use
10 those cells as genetic vectors, as Evan mentioned, was that
11 we did see a very consistent behavioral reproducible
12 improvement in function. For those of you who have worked
13 with the BBB Scale, you know that this is not a linear
14 scale, and this 2-point difference is a big functional
15 difference. 2 points down here between 6 and 4 or 6 and 8
16 means almost nothing. 2 points between 18 and 16 means
17 almost nothing. But I'll show you what this does.
18 We've repeated this and added an additional
19 control. We added another control where we looked at adult
20 mouse cortical cells and transplanted them to control for a
21 rat versus mouse immune response. Certain groups have
22 shown that pro-inflammatory cytokines can improve some
23 anatomical function after injury. But we saw the
24 consistent same 2-point difference.
25 What I'll show you now are little video

1 segments that are from animals that are exactly these
2 scores, so they're the average scores. It's not the best
3 or the worst or one group. It's exactly the average.
4 You'll see it's a dramatic difference.
5 This is a control animal that just received the
6 control media. You'll see he walks. He can't lift his
7 tail. He can move his hind limbs. He doesn't bear weight
8 and he can't place his paws.
9 This is the average transplant. You can see he
10 bears weight. You can see he lifts his butt off the ground
11 and his tail, and he can stand on his hind limbs. All the
12 animals would do this, so this is a very average response.
13 What we found in that study is that the
14 majority of the cells went on to become oligodendrocytes,
15 over 60 percent, and less than 10 percent were neurons.
16 In the next phase, we looked at how well can
17 these ES-derived oligodendrocytes myelinate in culture.
18 This is a culture that's derived from 4 minus/4 plus EBs
19 and were stained for O1 here which is a myelin product.
20 This is an oligodendrocyte with its multiple branches, and
21 it's overlaid over a phase image. The white arrows
22 indicate axons, and here this axon continues and then
23 becomes myelinated. Here's an axon. It then becomes
24 myelinated. You can actually follow each branch out. So,
25 you can see that a single oligodendrocyte will wrap

1 multiple axons or multiple segments of a single axon.
2 Now, of course, to prove that this is
3 myelination, you can do standard transmission electron
4 microscopy to demonstrate early wrapping.
5 This is an amazing amount of wrapping. This is
6 an average amount that we saw in the culture already
7 occurring by 9 days. So, not only did the cells have to
8 differentiate from a neural progenitor, but they had to
9 form both cell types and interact within 9 days. So, it's
10 occurring more rapidly than primary derived
11 oligodendrocytes.
12 We went on to assess this in a more focal
13 model. Rather than doing a contusion injury, now we said
14 let's just do a chemical demyelination and irradiate the
15 animals. So, here you primarily lose astrocytes and
16 oligodendrocytes and some axons, but you spare the majority
17 of axons.
18 So, we did the demyelination and then
19 transplanted 3 days later and then assessed anatomy 10 and
20 30 days later. It was a very similar transplantation
21 paradigm, although we've transplanted half as many cells.
22 What this really depicts is that basically the
23 majority of the cells that are transplanted turn into
24 oligodendrocytes, not astrocytes, not neurons.
25 So, what's shown here is a panel stained with

1 M2, which is the anti-mouse antibody. It just really
2 depicts the transplant site. These are three different
3 animals. This is a control that received just vehicle
4 medium, so it's a very good antibody. This is a small
5 growth of a transplant, a much larger growth of the
6 transplant. APC labels oligodendrocytes, and at this low
7 level you don't see APC, but you see it mainly in the
8 transplant site. But interestingly, the GFAP is almost
9 excluded from the transplant site. It was only on the
10 edge.

11 You can actually see at this early time point,
12 which is 10 days after transplantation, there's actually
13 increased nuclear density. If you were to look at these
14 same animals 30 days later, they would achieve close to a
15 normal nuclear density. So, cells would either die or
16 migrate.

17 We can immunolabel the myelin to demonstrate
18 that oligodendrocytes derived from ES cells myelinated.
19 You can see the immunoprecipitant. This is 30 days after
20 transplantation. It's a similar picture, now at a much
21 higher power, showing the immunoprecipitant in the multi
22 layers of myelin and yet next to axons that don't have
23 labels.

24 We then went on to develop a pretty simple
25 method for enriching for oligodendrocytes, really following

1 methods developed by McKay and Ian Duncan, but a little bit
2 different.
3 We took the 4 minus/4 plus EBs and ground them
4 up and then made oligospheres and grew them in a
5 specialized, defined media so that we could highly enrich
6 for oligodendrocytes. This procedure also enriches for
7 neurons. So, basically if you look at this, now the
8 majority of the cells are beyond nestin positive, and if
9 you culture these cells in oligosphere condition media, you
10 can get cultures that are over 92 percent oligodendrocyte
11 pure. But there are virtually no astrocytes under these
12 methods.
13 If we take those cells and then transplant,
14 like Evan discussed with the shiverer mouse, you can see
15 that they integrate. This is a CTO-labeled cell that lines
16 up with the other intrafascicular oligodendrocytes. This
17 is an animal that doesn't express any myelin basic protein.
18 So, if you use myelin basic protein and see here's the cell
19 body, here's the nucleus that fits in there, and you can
20 see its processes. It's a transplanted cell.
21 This is a sham transplant and a controlled ES
22 cell transplant. You would see myelination for a couple
23 millimeters, and at high power, you can actually see
24 individual axons with the myelin wrapping them in the
25 intrafascicular white matter.

1 At a much lower power, you can see that the
2 transplant rapidly falls off. So, we don't see nearly the
3 migration that you see in the immature animals. You
4 initially see a higher nuclear density and then it
5 decreases over time, in this case, to below normal.
6 You can use transmission electron microscopy to
7 demonstrate that there's over 10 layers of more highly
8 compact myelin achieved within a month in these animals, so
9 this has to come from an embryonic stem cell.
10 We've gone on now to utilize fluorescently
11 labeled cell lines, ones that are just on a CMV promoter.
12 This is 2 weeks after transplantation in the middle of the
13 transplant. Then we also have ones that are labeled
14 selectively for tau and then also for myelin basic protein
15 so that we can track individual cell types.
16 The other advantage is people like Austin Smith
17 have developed a genetic selection. So, they've linked a
18 resistance gene to the SOX-2 so that they can select for
19 neural restricted precursors just by adding an antibiotic.
20 He's been giving that out. So, that's a very effective
21 selection method to get purified cultures very rapidly and
22 cheaply.
23 So, just to follow up what we started with,
24 transplantation and myelination. I don't think this is
25 necessarily the best thing to move forward from a clinical

1 standpoint, but it's a good example of something that's
2 doable.
3 There are not very many alternatives for a
4 spinal cord injury. There are very few treatment options.
5 There are rehabilitation treatment options. There's
6 functional electrical stimulation, but otherwise not much
7 to offer in these people that are very debilitated and have
8 a long life span.
9 The potential risks for transplantation of
10 oligodendrocytes is much lower than neurons potentially.
11 The doability of treatment. Multiple
12 laboratories have shown that they can reconstitute
13 myelination around circuits.
14 The severity of the disease is important. It
15 might not be the best thing to start off with the most
16 severe injury. A more selective injury, transverse
17 myelitis, where you don't see an infarct might be a good
18 place to start.
19 Then proof of principle. I think that
20 myelination is one of the areas that has some of the best
21 anatomical behavioral correlates.
22 Then in humans, this is a very measurable and
23 interpretable endpoint. Just with MRI alone, now with the
24 higher magnets, you can follow myelination in the spinal
25 cord. Now with the paramagnetic molecules, you can really

1 follow it very easily. Of course, you can't use this
2 quantitatively. It really lights up the spinal cord, but
3 this is a very effective way of following these cells.
4 Then, of course, you can have both positive and negative
5 controls. You can see tracks that have been remyelinated
6 and tracks that are not myelinated, and you know exactly
7 the function of that track. You can use motor volt
8 potentials and somatosensory volt potentials. So, it's a
9 very well-outlined anatomical system to look for
10 physiologic correlates.
11 I'd just like to thank my collaborators at
12 Washington University and the people in my lab. If you
13 ever get out there, you can come visit us.
14 (Laughter.)
15 DR. McDONALD: Junior faculty move around a
16 lot. But this is where we're moving now. We're really
17 marrying the idea of having inpatient rehabilitation for
18 spinal cord with the basic science. The basic science for
19 spinal cord will all be on this fourth floor and all the
20 rehabilitation will be on the lower floors.
21 Anyway, thanks.
22 (Applause.)
23 DR. SALOMON: Well, we did it. Thank you very
24 much.
25 Well, guys, it's 10 after 6:00. We're 40

1 minutes late, not too bad given the fact that we went into
2 lunch 40 minutes late. So, we've stayed basically on track
3 I think.
4 We did get through a lot of material. I for
5 one feel privileged to have been allowed to hear all these
6 excellent talks. I certainly got a lot out of it. I had a
7 lot to get out of it.
8 Do we want to have any discussion at all at
9 this point? We're going to return to this whole thing in
10 depth tomorrow. I think the time is late. Everybody is a
11 little bit tired. So, I'm going to make an executive
12 decision. I hope that the last two speakers don't take it
13 personally. But I think that clearly these last two talks
14 and a couple that followed it all starting with Rusty with
15 the animal stuff is really going to be front and center
16 tomorrow as we talk about what kind of animal models are
17 appropriate for clinical trials. I think we'll get plenty
18 of time to discuss these.
19 So, again, I'd like to thank everybody for the
20 energy and the time all the speakers took to put these
21 talks together and for your attention.
22 See you tomorrow morning at 8:30.
23 (Whereupon, at 6:15 p.m., the committee was
24 recessed, to reconvene at 8:30 a.m., Friday, July 14,
25 2000.)