Seminar: Cognitive and Behavioral Neuroscience Seminar (603)
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Chairs: Herbert S. Terrace, Yaakov Stern

Speaker: Evan Balaban, CUNY: College of Staten Island

Topic: The new study of Instinct: Brain transplants for the study of species behavioral differences

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Abstract:

Heritable, biologically important differences in perception and motor behavior that do not result from exposure to different environments are well-documented among closely-related species of higher vertebrates, yet little is known about the developmental and evolutionary changes in nervous systems which produce them. These inborn biases are important for understanding cognitive development because they provide the scaffolding upon which learning builds complex perceptions, emotions, evaluations and reactions. Transplants of defined portions of tissue that will later become the central nervous system are carried out between the embryos of two bird species at early stages of development, and host embryos are allowed to hatch. The resulting animals, called chimeras, are used to identify interacting cell groups in the developing nervous system that make a decisive contribution to both the differentiation of behaviorally-important neural circuits, and to the evolution of species behavioral differences.

Dr. Balaban first described his general interests that serve to guide his work: He is very interested in things that come before behaviors with cognitive involvement and how the nervous system is set up in order to make these behaviors possible and what learning is needed to make these behaviors possible. How does this get done differently in different species that end up behaving very differently – that end up getting very different things from very different experiences, and what goes on at the level of developmental biology in brains to produce instinctive differences in behavior.

Dr. Balaban then gave a historical review: acoustic communication in birds – the first cross-fostering experiment was published in 1773. Young birds were reared with parents of different species and then examined to determine the effect that this had on the birds' subsequent singing behavior. Songs were then transcribed into musical notation and subsequent analyses revealed that that the development of song was determined by the song to which the young birds were exposed.

Dr. Balaban argued that Barrington's conclusions are at odds with most of the current experimental data. Dr. Balaban then explained that male swamp sparrow songs are culturally transmitted, and noted that there are slight differences in male swamp sparrow song across the different regions.

Dr. Balaban explained differences between swamp sparrows and song sparrows (two closely related species that co-occur across the entire species range): Swamp sparrow songs generally consist of a repetition of a small series of identical minimal acoustic units – and this pattern is repeated for about 2 seconds. Song sparrow songs are a bit more complicated. Song sparrow songs consist of a set of units similar to those of swamp sparrows, but their songs consist of many more parts – more sets of unique units. The songs are structurally very different.

Marler showed that the differences in song structure observed in Song and Swamp sparrows cannot be accounted for by exposure to different song models. When the birds are reared in isolation (and do not have exposure to other bird song), Song and Swamp sparrows still show many of the same structural song differences that are present in birds that have been exposed to their species-specific birdsong. Even when the birds are deafened and cannot hear their own song, the song loses some integrity, but there are still identifiable species-specific differences that occur across the two species. So, it appears that there are basic motor structure differences between the two species upon which additional learning is built.

While some of the characteristic features of normal birdsong are present in isolation-reared and deafened birds, normal song does not develop unless there is exposure to a song model. However, the source is also important. Balaban asserts that sparrows may be attentionally predisposed to selectively listen to their own species-specific song. Evidence: Sparrows presented with songs from both species end up learning songs specific to their own species. Both species are capable of producing the song of the other species; so limits in song production ability cannot account for the selective learning of species specific song.

Dr. Balaban conducted a cross-fostering experiment in which male and female Swamp sparrows were exposed to the birdsong of swamp sparrows from different regions (and with different songs). He found that males readily learned the song to which they were exposed. Females on the other hand, did not show preference for the song to which they were exposed, but rather showed preferences for songs typical of their native region.

Herb Terrace: Was the female data obtained with a recognition paradigm?

Balaban: It is a little more complicated than simple recognition in that it is a competition experiment. For males, two speakers are placed in the territory. One of the speakers plays one acoustic arrangement, and the other speaker plays the same acoustic units, but with a different arrangement. It is of interest then to see which of the two speakers competes for the bird's attention more successfully. For females: Isolated females would be exposed to male songs of both types. The behavioral measure was the frequency of sexual display in response to presentation of the male song types.

Herb Terrace: This kind of relates back to the structural differences?

Balaban: I have essentially taken the structural units and arranged them in two different temporal patterns. When these units are presented in a manner consistent with the population, then they are very attractive to a female. The same units arranged in the other temporal pattern become less attractive.

Dr. Balaban went on to describe his interest in work by some French embryologists studying cell migration in the nervous system who developed a technique in which neural tissue was transplanted across two species of birds prior to hatching (domestic chickens and Japanese quails). Dr. Balaban recognized this as a useful technique for addressing some of the questions in which he was interested. Specifically, he hypothesized that one could transplant different portions of the brain to identify which portions are responsible for species differences in behavior, and then using this as a starting point for further examining how these regions differ neurally during development and how they impact behavior.

Why chickens and quails? One reason is that their cells stain differently due to differences in DNA dispersion in the cell. This is useful because one can identify which cells have been transplanted after the fact (at the level of single cells).

Dr. Balaban then describes the process of how the surgery is conducted, and comments that given that surgery takes place before cell differentiation, that development continues normally. He also comments that brain structures in the chimera chicken do not show any gross structural abnormalities.

Jon Horvitz: This is of those [chickens] that survive?

Balaban: Yes, this is for those chickens that survive and behave normally – able to hatch themselves and feed themselves etc.

Jon Horvitz: Did you say what proportion of them that would be?

Balaban: About 80% of them hatch normally, and of those 80%, about 70% turn out to be "normal". Problem animals are relatively easy to identify in that they either do not hatch, or if they do hatch, they have severe problems that are consistent with brain damage. We do not use any of these animals in our studies. We only use animals that look normal.

When neural tissue from a quail brain is transplanted into a chicken, the forebrain develops into a size consistent with that of a normal quail – and considerably smaller than the normal size of a chicken. This disproves a major theory regarding skull development – Particularly, that the skull develops from the brain pushing up on it. This however, proves otherwise because while the brain in the chimera chicken is quail-sized, the skull itself is chicken-sized. The remainder of the space inside the chimera chicken skull is occupied by fluid.

Dr. Balaban then shifted the focus of his presentation to the major effects found in songbirds – particularly, motor production and effects on perception. Japanese quail and chickens have vocalizations that are quite different, and there are also different gestures that go along with these vocalizations. When chickens are injected with testosterone, they tend to develop a vocalization that is substantially different from the usual adult form of the vocalization. Quail on the other hand, show (very rapidly) a normal adult male form of the song after being injected with testosterone,

The motor topography that accompanies a rooster vocalization is as follows: The rooster sticks its head up and forward, levels the head, emits vocalization and then finally puts head back down. When a quail vocalizes, it moves its head up and forward and then vibrates its head rapidly up and down before putting the head back into its resting position.

Dr. Balaban and colleagues were able identify two of the major brain structures: One area identified was the back part of the mid-brain in which the animals demonstrated quail vocalizations with chicken head movements. A second region was located at the bottom part of the brain stem that conferred the quail head movement but left the structure of the vocalization intact. This seemed contrary to some theories which suggested that higher level regions of the brain were responsible for assembling these individual motor components into a sequence of movements.

The motor topography of the head movements varied depending upon where the transplants occurred. Transplants a little further forward in the brainstem resulted in transfer of the motor component from the end of the motor sequence, and transplants further back in the brainstem resulted in a transfer of the motor component from the beginning of the motor sequence. During these brainstem transplants, no changes in vocalization occurred.

Dr. Balaban noted that interesting results were obtained with his work examining motor predispositions, and that he further wanted to investigate the more controversial area of perceptual predispositions.

Dr. Balaban noted that the birds are generally able to feed themselves within hours of hatching, and in order to feed themselves, they must disperse. This poses some risk to the newly hatched chicks, and so when the male or female parent (guarding the chicks) emits a special call indicating danger, the chicks to run toward the parent. These calls are acoustically different in chickens and quail, and so Dr. Balaban concluded that this represented an excellent system for studying these phenomena.

The experiment: Birds were hatched in incubators, and although they could hear their own vocalizations, their vocalizations (in either species) did not acoustically resemble the vocalizations of the adult animal. Therefore, their own self-stimulation cannot be a means for developing species-specific preferences.

After the first day of hatching, the chicks were exposed to the adult call (quail and chicken) for 30 minutes. In each case, a stuffed rotating bird (quail or chicken) was emitting the vocalization. During the second day after hatching, the chicks were placed in a wire chamber. Chicks were then exposed to both quail and chicken vocalizations simultaneously – with chicken vocalizations being played on one side of the chamber, and quail vocalizations being played on the opposite side of the chamber. The dependent measure was which of the two vocalizations the chick approached.

While as a group chickens and quails tended to approach the side that contained their own species-type vocalization, there was quite a bit of variation that occurred at the individual level. There appear to be 3 “kinds” of animals (in both groups): 1) There are chickens and quails that have almost exclusive preferences for the maternal call of their own species, 2) there is another group of animals have a weaker preference, but the preference is still directed at the side playing their own species-type vocalization, 3) and finally, there is a small group of animals that showed no preferences at all.

Dr. Balaban was very interested in whether this auditory preference could be transplanted. He found that transplants from the front part of the mid-brain not only produced preferences similar to those of normal quail, but tended to produce preferences that were more extreme than those of normal quail. This area of the brain does not seem to be within the structures in any of the primary auditory pathways of birds. It is in the very rostral part of the midbrain or in the very back part of the thalamus. This suggests the possibility of a new auditory pathway that may exist in these animals.

Dr. Balaban continued to explain that both the motor behaviors and the perceptual predispositions he described earlier can be addressed by one of two possible models.

One possibility is a module or self-contained circuit which is somewhat analogous to a computer with several functional boards. In this case, a chicken board is removed and it is replaced with the quail board, but nothing else within the brain is altered. The quail “board” works autonomously with inputs and outputs that interact normally with the chicken brain. The second theory is that the early developmental transplants influence and interact with the developing nervous system to create global changes throughout the brain. That is, because the transplant occurs before neural circuitry develops, the transplanted cells may have large developmental effects on cells that differentiate later on with which the transplanted cells interact. Dr. Balaban was interested in determining which of the two models seem to apply to the effects of the transplant.

One method of making such a determination in young birds is to identify which parts of the brain respond to sound. Examining cell activity can be done by looking at immediate-early genes (products in cell which change gene expression). For example, an area of the brain with historically little activity that suddenly becomes active (for whatever reason). This increase in activity requires a concomitant increase in energy. The immediate-early genes up regulate the production of all sorts of enzymes and proteins that are responsible for processing energy molecules. In order for this to happen, gene expression needs to be changed, and the immediate-early genes are the molecules that do this. The activation of the immediate-early genes serves as a means of measuring increases in neural activity.

Two primary areas of activation seemed to be showing up using the described method: 1) regions that received either primary or secondary auditory projections, 2) regions that are thought to be involved in the modulation of emotional behavior associated with attention in birds. In terms of the staining patterns, there didn't seem to be any differences between birds that heard the maternal vocalization of an adult conspecific versus a maternal vocalization of the other species-type.

Jon Horvitz: In the previous slide you were showing us colored regions – those colored regions showing differences between…?

Evan Balaban: The colored areas simply tell you the types of brain areas you are dealing with. The brain areas that turn out to respond very well with this immediate-early genes were of two kinds: Either one that gets auditory input or ones that are involved in motivational...

Jon Horvitz: So you weren't subtracting the conspecific from the…?

Evan Balaban: Not in that slide. That is what I am doing right here. This is the subtraction.

Jon Horvitz: So that one is the one that showed activity…or whatever it was?

Evan Balaban: These are the areas that show…this is a gene called ZENK…. NGF1A…. and what I showed you were the levels of expression, but the interesting thing is in the species the relative level of expression of each nucleus is pretty much the same.

Jon Horvitz: So that was compared to some baseline levels?

Evan Balaban: That's compared to staining over white fiber tracts.

The staining reveals subtle differences across all of the nuclei in the direction of preferences for the conspecific vocalization. There are small differences in a lot of areas which suggests that the processing is occurring in a distributive fashion – but this differs depending on area of the brain and species. In chickens, the auditory areas don't really tell the difference between conspecific and heterospecific, but the modulatory ones do. In quail – it is the opposite pattern – the auditory areas strongly differentiate, but the modulatory ones don't. The question is whether this represents a developmental sequential difference or whether this represents a fundamental difference in how these brains dynamically operate.

This technique can also help answer developmental questions. Obviously, one cannot obtain behavioral data from the unhatched chicken, but given that their brains can tell the difference between the two types of stimuli, one can then ask when in development it first happens. So the design of the new experiment (that Dr. Balaban described) was identical to the earlier experiment except that eggs were placed in the chamber instead of hatched chickens.

Dr. Balaban found that there is sound-induced gene expression in the midbrain by E8, which is surprising given that no one suspected that the coupling of the midbrain and the brainstem would already be working at this young age. Around E13, expression of the genes begins to occur in the forebrain region as well.

Dr. Balaban concluded his talk by explaining that he currently uses specialized PET-imaging techniques with high spatial resolution to examine the birds' brains. He offered to explain it in detail after the talk to anybody who was interested.

QUESTIONS

Herb Terrace: Did you say how many genes were involved in this behavioral difference?

Balaban: Thousands. Early in my career I became convinced that any of these behaviors we are interested in are impacted by so many genes that going gene by gene would not teach us much about the organization of the developmental system – and that is why I focus on cells, because populations of cells are the level at which these differences exist. Once the cells are identified that are dominant in the developmental difference and we find how they are acting in the brain, we can then proceed to look at the genes that influence the cellular differences, but it is the cellular differences that are the level of explanation that will draw everything together.

The exciting thing is that there is no technique in biology so far that has been able to show people developmental interactions between cells that then contribute to biologically important behaviors. This is the one technique that can do that because by finding the sets of cells in the transplant that seem to be doing something developmentally important, putting that together with the imagining information and then be able to see dynamically how they are communicating with the other populations with whom they have connected in development – I think I could get a really good picture, both with how transplants work and how normal cells work.

Jon Horvitz: Do you know if the reason for having this diffuse change in many brain areas was because the transplant cells were somehow communicating with these distant receivers as opposed to the transplant cells migrating somewhere?

Evan Balaban: Well, because the cells are marked, we know exactly where they go. The second thing is why has the midbrain come up in both of these behaviors? We are always thinking of the forebrain being so important for everything and I have surveyed the entire brain here, and forebrain transplants don't transfer this behavioral difference. A chicken with a quail forebrain behaves like a perfectly good chicken. There are a couple of things that are different: 1) The time that they hatch, so there is some developmental signal that is given by the forebrain but if you look at the forms of most of the behaviors that they are doing, you don't see any big difference. Why the midbrain? Well, it turns out that the two areas of the brain where the neurons differentiate the earliest are the midbrain and the very bottom part of the hindbrain. The area of the brain that forms the first long tracts communicating with other areas of the brain is the midbrain. So, why does it keep turning up in these experiments? Because it is very well placed both in terms of evolution and development. If you want to make the biggest bang for your buck in terms of changing things all over the brain, that is where you are going to do it. These guys are differentiating earlier than everybody and they are connected widely.

Jon Horvitz: Then do you imagine them to be ascending catacholamine systems that are key?

Evan Balaban: Well this is what I think the work with the immediate-early genes suggests because those are a lot of the areas (unintelligible), so I wouldn't be surprised if the circuit that is going to come out in a few years that I am transplanting – If what I am transplanting is an interface between classical auditory system and neuromodulatory systems because one way of doing this kind of sound recognition is to have a bunch of inputs to the auditory areas of the forebrain both auditory and neuromodulatory and if you have different patterns of arriving input synchronously. This is a really good way to both in development and for function for brain systems to tell the difference between different (unintelligible). It wouldn't surprise me to find out that that is the crude basic diagram that we still want to know on a finer level how this think works and specifically what the kinds of developmental interactions are. For a long time people have thought that behaviors like the song birds' song learning which are quintessentially culturally transmitted behaviors – that these use different neural mechanisms than the chicken and quail calls, and I won't be surprised again if what this work shows us is that to produce this so-called hardwired behavior, you have to use all the same dynamic machinery that has to change constantly during development to change the organism – as you do for the learned behaviors, but on a molecular level, these kinds of behaviors are going to look very very similar. And the way this stuff is deployed over the lifetime of the individual…..

Truman Brown: Could you expand on why you think it is thousands of genes that are different?

Evan Balaban: How different are the two species in terms of total sequence differences?

Truman Brown: Yes.

Evan Balaban: Well, we don't know because all we know about are DNA from our hybridization results which are very very crude, but….

Truman Brown: It would take 10 million years to make a hybrid like that…..

Evan Balaban: Well, again the differences that are really important are under consideration here – for instance if you compare humans and chimps, it isn't that our gene products are so much different from theirs – by and large they are not. What is different are the sequences of their control regions that control when and where in development things are modulated. And so those are the kind of differences that are going to figure very largely in the behaviors that I am studying, and since we don't know very much yet about how much small and large change affect that, we can't make a very good guess about mapping actual numbers of base-pair differences onto differences in numbers of genes. It is just that people have tried to do genetics on these things before – the crowing – they have tried on different strains of quail, and in the domesticated chickens and quails, you can actually mate them. You can have offspring that are sterile, but you can use variation among different offspring to try and tell you how many genes are involved – and those distributions are incredibly continuous implicating a very large number of genes.

Truman Brown: Is that 50 or is it thousands?

Evan Balaban: For the exercise that I went through to get the thousands number is to sit down and think about the source of cellular differences that are involved here – and to think of the number of gene products that would impact on each of the cellular differences, and for any behavior that you would want to choose, you quickly get into large numbers. This does not mean that you can't have single genes that have large effects but if you look at any of these biochemical pathways, the number of genes that impacts on each pathway is very very large. These are organisms that probably have on the order of between 50,000 and 100,000 genes.

Truman Brown: Let me ask the question in a different way. Suppose you could go in and tweak genes. How many genes would you have to tweak to go from a chicken-like behavior to a quail-like behavior?

Evan Balaban: Are you talking about the minimum number you have to tweak, or are you talking about the mean number you have to tweak? There may be many different combinations of genes that you can tweak that you can go from chicken to quail – not that they are a one to one thing necessarily. I would still stick to my 1000 estimate because when I think about the numbers of brain areas and the kinds of differences in cells that fire differently and are wired differently etc. and then all the biochemistry that feeds into that. It is true that we could get a set of maybe 200 genes that will make its way between those two pathways, but that is not necessarily going to be (unintelligible) in each set, so by the time add all the sets together, it wouldn’t surprise me if it numbered around 1000.