Brain Development and Behavior as a Toxic Target of Dioxin and Other Environmental Chemicals

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First of all, I would like to tell Dr. Bernard Weiss's words. He apologizes to everyone for being absent, but it is a great pleasure that we have the opportunity to show our data. Slide please, thank you. Next slide, please.

Endocrine disrupters such as TCDDs or PCBs are obliquitous and persistent environmental circumstance contaminants, and powerful developmental and reproductive toxins. Their developmental effects, especially the effect of TCDDs have evoked intense public health concern because they accumulate in the food chain and are retained in body tissues for extended periods. Next please.

The half-life in humans of the most important congener, TCDD is in the range of 7 to 10 years. Next please.

We have learned our lessons from dioxin, the risk to adult of which conveys little information about the risk for the fetus, infant, and child. Functional effects are most pronounced when exposure occurs *in utero*.

So we focused on behavior of offspring exposed to TCDD *in utero* because behavior measures provide a diversity of endpoint for assessing the functional consequences of developmental neurotoxicants. During the critical period of brain development, even more perturbations in this complex changes of processes can permanently alter behavior. Next please. We decided to undertake a study of offspring exposed to various doses of TCDD on GD18 and GD8. These are the index of projects that have been done in our lab so far. Next please.

Rice and her colleagues reported that under some experimental situations, TCDD related compounds changed behaviors in monkeys and rats. Several neural behavioral studies have shown that prenatal TCDD exposure caused demasculinization of male offspring sexual behavior because abnormal behavior and morphology can result from exposure to endocrine disrupting toxicants by altering central nervous system structures during critical developmental stages. Next please.

In behavioral teratology, the most important matter is the developing nervous system and its susceptibility to toxic exposure. To examine the effect of another exposure of TCDD on animal behavior, we employed the experimental procedure named schedule controlled operant behavior.

This procedure was established by behavioral analysis, which is one field of experimental psychology. It focuses on behavior, which are revealed under certain experimental condition. Also, it focuses on behavioral modification before and after condition changes. Using this procedure, it is possible to understand or predict behavioral patterns. Some of these behavioral changes are often gender specific and may not become apparent until after puberty. Next please.

One comparing example of gender specific behavior is the daily amount of growth locomotor activity displayed by the female rat. In running-wheel experimental procedures, the rate of wheel running by adult female rats followed a four or five day period corresponding to the estrous cycle. It is believed that wheel running is a very sensitive index to examine the effect of chemicals. This is the wheel-running apparatus. It has lever here. Next please.

We exposed a single oral dose of 0, 20, 60, 180 μg/kg of TCDD to pregnant Holtzman rats on GD18. Their adult female offsprings were used in a wheel running procedure called incremental FR. This procedure was established in my lab. The FR values specify how many responses are required to subject for getting a reward.

For instance, under an FR5 schedule of reinforcement, after a five-time response, a subject gets a reward such as food pellet or drinking water for a specific time. In our procedure, if animals responded on a lever at certain times, they got brief opportunities to the running wheel.

Once they had begun responding on a FR1 schedule of reinforcement, FR requirement for lever pressing is increased at five-session intervals to values of FR2, 3, 5, 10, 20 and FR30. Such a progression allowed us to study the transition state performance that occurs in response to changes in experimental conditions.

Transition state performances are of particular interest because they reflect the ability of the subject to learn, adapt, and/or adjust to changing environmental circumstances. In this study, we also examined vaginal cytology after each behavior session to track the estrous cycle. Next please.

Under each of the FR values, prenatal TCDD exposure produced a significant dose-related reduction. This figure is the related rate of earned opportunities to run. It is expressed as percent of control group performance for the three prenatal exposure groups. The other variable also had the same behavioral tendencies. Estrous cycle does not affect the animals' wheel running response. Next please.

Because of the consistent dose-response relationship at all FR values, we used the behavioral data to calculate the benchmark doses based on the displacement from modeled zero dose performance of ED01 and ED10, as determined by a quadratic fit to the dose-response function. The mean ED10 benchmark dose for earned run opportunity was 10.13 μ g/kg. The corresponding ED01 was 0.98 μ g/kg. Next slide, please.

The mean ED10 for another variable, total wheel revolution, was calculated as 7.32 μ g/kg. The corresponding ED01 was 0.71 μ g/kg. These values should be viewed from the perspective of current human body burdens, which average value, based on TCDD toxic equivalents, has been calculated as 13 μ g/kg, and they are close to our value. Next please.

Now I will show another of our behavioral studies from GD8 exposure. We examined schedule control operant performance of male and female littermates. The experimental task here was lever pressing. This is the chamber box used for our experiment. If the rat presses the lever with specific number of times or time interval, they get a pellet as a reward from feeder. If the rat presses the lever, one pellet comes from here. Next please.

We used four dose groups, 2, 20, 60, 180 μ g/kg of TCDD. We measured the behaviors of both male and female offsprings under two different operant procedures. On postnatal day 90, the first operant procedure incremental FR schedule started. The FR value was increased every four sessions, in an ascending series of values ranging between 1 and 71. Then, multiple schedules were presented in successively alternating compounds.

In this study, a schedule named Differential Reinforcement of Low Rates, DRL 10 seconds, and FR11 schedule were combined in one multiple schedule. The DRL 10 second schedule requires a pause of at least 10 seconds between responses. Under the DRL schedule, therefore, low rates of responding yield high rates of pellet delivery.

In contrast, under the FR schedule, high rates of response yield a high rate of food delivery. A number of studies of operant behavior have reported that under FR schedule, normal male rats tend to emit higher response rates than females. Females, on the other hand, tend to respond more efficiently than males under the DRL schedule. Next please.

These figures show the mean response rate of the males and females during the FR component in multiple schedule. Behavioral tendencies in DRL component and in incremental schedule were similar to this figure. The left panel is male and the right is female. White dot is control and filled dot is exposed.

In both female and DRL components and also in incremental FR, TCDD evoked a sexually dimorphic response pattern for the male. All the three groups exposed to TCDD responded at lower rates

than control. In contrast, for females, all three TCDD exposed groups responded at higher rates than control.

For the multiple reinforcement schedule, the response rate differences between males and females were used to calculate the benchmark dose based on the related displacement from modeled zero dose performance of ED01 and ED10 as determined by a second order polynomial fit to the dose effective function. Next slide, please.

This is the benchmark dose for male/female different scores in FR and DRL performance. In FR rate of responding, the mean ED10 was 2.77 μ g/kg. ED01 was 0.27 μ g/kg. In DRL, ED10 was 2.97 μ g/kg, and ED01 was 0.30 μ g/kg. These values fall close to, but below, current estimates of human body burdens of 13 μ g/kg based on TCDD toxic equivalent.

Next, I would like to show some of our data on brain morphometry from both groups of animals exposed on GD18 and GD8. This study has been done to examine whether similar changes can be observed in offspring of rats prenatally exposed to TCDD. Next please.

Sexually dimorphic patterns of cortical lateralization are well known in both human and animal brains. Males tend to exhibit strong right hemisphere dominance compared to females while females typically exhibit not explicit lateralization patterns and a greater left hemisphere bias compared to males. Next please.

Many factors such as steroid hormones, stress, and environmental complexity may also affect cortical laterality in the normal brain. Factors such as environmental pollutants have not been studied extensively, so we decided to examine the effect of TCDD on cortical thickness in rat brains. Next please.

The wild life bird study indicates that exposure to TCDD and related compounds may be one of the factors in the development of brain asymmetry. Ovo chicken embryos exposed to low doses of TCDD demonstrated a dose-related increase in both frequency and severity of growth-brain asymmetry in developing chickens. Next please.

We examined control and 180 μ g/kg animal brains. The sampling date was 10 month old for GD18 and PND90 for GD8. Brains of one male and one female offspring per litter were removed. Three coronal sections were selected according to the atlas of Paxinos and Watson. These sections contained tissue corresponding to Craig's cortical region — these eight areas, one, two, three, four, five, six, seven, eight. These eight areas were measured by two observers. Next please.

This is a picture of the brains. We measured the thickness from here to here for three times in one area, and the difference between right and left hemispheres was calculated. Prenatal exposure to 180 μ g/kg of TCDD resulted in several changes in brain lateralization, in both GD18 and GD8. Next please.

This is the result of a male exposed on GD18. The right is control, and the left is exposed. Changes of direction of the bar between control and exposed like this one and this one mean a reversal of hemisphere dominance. As these data were calculated by right minus left, the right side from zero point, this side, is bigger than the left. On GD18 in males, six out of eight areas were reversed. The pattern of these changes suggested a process of brain demasculinization.

The female brain exhibits not explicit changes in cortical lateralization without specific direction. Next please.

This is the data of females exposed on GD8. Please take a look carefully. Control data is here, exposed data is here. GD8 exposed females exhibit reversals in the brain lateralization in most areas, seven out of eight areas. Male animals demonstrated reversals in the hemispheric dominance also, but less pronounced. Next please.

As a result of schedule controlled operant behavior, we obtained these conclusions. From wheel running, prenatal exposure to TCDD on GD18 significantly reduced operant responding in a dose

dependent manner, and the results from lever pressing showed sexually dimorphic response pattern. Next please.

In brain morphometry, prenatal TCDD exposure caused gender specific changes in rat cortical asymmetry. In GD18 exposure, the prenatal male TCDD exposure demonstrated reversal of cortical dominance in most of the analyzed areas. In GD8 exposure, both males and females demonstrated changes in cortical asymmetry with more visible reversal in females.

The difference between GD18 and GD8 can be derived from several conditional differences. One of them can be strain difference and others can be different periods of exposure and sampling. In summary, even very low doses of prenatal TCDD exposure affected animals' behavior and a brain asymmetry pattern. Thank you very much for your kind attention.

Q&A

Q: I'm an amateur when it comes to this field, so I hope you don't mind a beginner type question. My question has to do with certain chemical substances and the formation of the brain and nervous system. If we do not compare how the structure of the brain changes when conventional hormones derived from organisms are administered, we will not know if development of the brain is not affected by endocrine disrupters or if formation of the brain by the molecular structure of those chemicals is abnormal. How is this distinguished?

Hojo: We have just started a project for studying the morphology of the brain. We start by measuring the thickness and counting the number of cells of the cerebral cortex. If a series of consistent data is obtained, we'd also like to conduct a more detailed study by varying the number of days of exposure and date of sampling. We've just begun so we're still studying and making preparations. Thank you for pointing that out.

A: I'd like to add a little to that if I might. Dimorphism in the brain due to sex hormones has more or less been confirmed. It is more or less known that if sex hormones are administered during a certain period, the structure of the brain changes as such.

Q: Just as you have just said, as for methodology of experiments, in the case of certain behavioral deformations for example, like you just said, it appears as the effect of conventional sex hormones or as the effect of certain chemical substances, and whether that is an endocrine disrupting effect or due to a certain molecular structure is not known. We therefore have to sort out and discuss this. The media give attention to all such situations as being part of the endocrine disrupter problem, but I think science should distinguish between what is and what is not.

Hojo: Thank you very much.

Q: I thought that thickness of the cortex was the result of an irreversible phenomena that depends upon secretion of sex hormones when adulthood is reached. I am however interested in whether or not you measured concentration of the sex hormones in the body at the time you obtained that data, concentration of estrogen in particular. Could you tell me this?

Hojo: No, we did not measure estrogen concentration in particular.

Q: For example, so-called sexual dimorphism is said to have the irreversible effect of sex hormones in the embryo and infant stages of especially rats. There is a lot of literature about the cortex, and what I know is, I have heard that the sexual difference between male and female disappears after gonadectomy due to a difference in concentration of sex hormones after one reaches adulthood. I am not really checking out the literature on that field, so my knowledge may be distorted.

Hojo: Sorry, could you say that a little slower.

Q: Sorry. I read a book about sex differentiation of the brain, and it said there were several types of sexual dimorphism. With one type, the sex hormones from the embryo stage to the infant stage, especially in mammals, sex differentiation by testosterone produced by the testicles irreversibly masculinizes the brains of children. Experts in this field of course know this, but the fact that females will be masculinized if administered sex steroids in the embryo or lactation stages is important for the problem of environmental endocrine disrupters as well. As for sexual differentiation of the cortex, I have heard that, more than being irreversible, it depends upon the capacity of steroid hormones from the testicles or ovaries when adulthood is reached.

Hojo: I think so, but what was interesting about my experiment was the fact that I got the opposite pattern for deflection of the brain when the exposure was altered according to the exposure date, GD18 or GD8. If I surmise that it might have changed or might change after reaching adulthood, the opposite pattern for GD8 and GD18 — which is male and female and which has a distinct difference can no longer be explained. Not when adulthood is reached, but rather depending upon the date of exposure, an irreversible change may clearly have occurred before that. But this is not clearly known.

Q: Thank you very much. The next question is that you have identified the region from which the cortex was taken – a little above the hippocampus, perhaps the parietal lobe. Are you thinking about studying any other regions?

Hojo: Yes, the hypothalamus. I am thinking about the hippocampus and the SDN-POA region. We actually did this, but there was not much difference for the hippocampus. Concerning sexual dimorphism, because such dyeing was performed, we were unable to identify it. It still has not been done with the same slide.

Q: By the way, did you study POA of AVPVA?

Hojo: No, not yet.

Koibuchi: Sorry. We're just about out of time, so let's get questions from the floor.

Q: To what degree have you discussed the relationship with final estrogen receptors? I'd like to know if you have any data on this.

Hojo: Concerning the brain project, we have just started, and we are still thinking about what to do about it.

Q: I really hope you will arrange it together with ER.

Hojo: Thank you very much.

Q: One more thing please. My comment has to do with the problem of the cortex. The part at the very back of the brain you looked at, I believe, is called the visual cortex. I think androgen receptors are more involved in this region than estrogen receptors. I am extremely interested in looking into this as well as estrogen receptors.

Hojo: Thank you very much.

Koibuchi: OK, time is up, so we need to proceed to the next speaker.

Hojo: Thank you.

Koibuchi: Thank you very much, Dr. Hojo.