GLENN SMITH: Good afternoon and welcome to CCATS' grand rounds for today. I'm Glenn Smith. I'm the associate director of education in CCATS. And it's my pleasure today to introduce the director of education in CCATS, Dr. David Warner, who is a professor of anesthesiology in the College of Medicine, received his medical degree at the Ohio State University and completed his anesthesia residency and fellowship training here in 1987.

He's had many institutional administrative roles. He currently directs the Office for Health Disparities Research, as well as the aforementioned director of education programs in CCATS. He also serves as director for the American Board of Anesthesiology and he's given over 60 invited presentations to national and international meetings. His research includes outcomes of anesthesia and tobacco control on surgical patients.

He taught me all about teachable moments in tobacco cessation research. He's mentored over 25 research fellows and published over 200 peer-reviewed manuscripts and authored 18 chapters. He practices pediatric anesthesiology. He married his high school sweetheart. He has three grown up kids, and I can verify that last night we got to meet his beautiful granddaughter, who appears to be the light of his life. So David.

DAVID O. WARNER: Thank you, Glenn. And, indeed, she is. You might even see her during the presentation today. Nothing to disclose, and here is what I hope you get out of the presentation today. I'd like to tell you a story today about translational research, a story that I hope will illustrate why what we do as translational scientists is important, that will illustrates some of the important features of translational research, and go over some of the challenges that we face as we try to translate research in various ways.

We start the story with rats. In 1999, there was a publication that came out that if you gave young animals NMDA antagonists, it would cause widespread apoptotic nerve degeneration in this developing brain-- not thought it would be a good thing. People started to wonder, because some of the anesthetic drugs that we use have some of these properties, whether other receptors might also be involved in this phenomenon.

And starting in about 2003, pretty much whatever they put in the rats-- GABA A agonists, ethanol, antieleptics, midazolam, and almost every anesthetic that we clinically used-- actually caused similar findings of apoptotic neurodegeneration, which is a concern. Because we want to know whether these drugs which we use every day when we sedate and anesthetize children have the same kind of effects in my granddaughter as they do in rats.

So I don't know if you've ever been to a surgical lounge before. This is a place where you rest between cases and you discuss very important matters of medical science and local politics and all the rest. We were sitting in the anesthesia lounge one day, me and some of my other pediatric anesthesiology colleagues, and we were talking about these animal studies.

And we knew-- because we take care of children and we put them to sleep, we wake them up every day, they go home and they're fine-- we knew that these animal studies, in fact, just couldn't apply to children. There's been plenty of other instances where, especially with anesthesia and the brain, findings have been reported in animals that didn't turn out to have any translational significance at all. And we knew that this was the case for these particular animal experiments, as well.

But we were thinking about, well, how actually could you prove that these animals experiments didn't have any relevance in children so that we as pediatric anesthesiologists wouldn't feel guilty for actually causing damage to the very children that we were trying to help? Well, we talked a little bit about the science and we talked a bit about what this neuraoapoptosis this was. And we talked that this, in fact, is a normal process. As your brain develops, you have to get rid of some neurons and synapses so that you could form others as you learn and develop, so it's not necessarily an abnormal process.

It is very much a normal process that has to be very precisely regulated in order for you to develop as you should. This is a process that occurs most intensely in the early part of life. And it turns out that it depends on what species you are when this occurs most intensely. In the small rodents, where these pre-clinical results of anesthetics were found, it happens to occur most frequently between about one day prenatal to 14 days postnatal. And as it turns out, that's also the period of greatest vulnerability to this anesthetic-induced acceleration of neuroapoptosis.

It turns out that you can pretty conveniently measure this process in the brains of animals by looking at one of the enzymes that's responsible for this normal cascade of neuroapoptosis, the caspases. So that if you take a brain of a mouse, in this case seven days old, and you give the mouse either saline or a dose of ketamine, which is one of the NMDA active agents that we use to provide sedation and anesthesia in children, you can see in this slide that compared with the saline exposed mouse on the left that the addition of ketamine caused a marked increase in the expression of this caspase, indicating a marked increase in the amount of neuroapoptosis.

As the studies progressed, they moved from rats into non-human primates. And this is a similar kind of experiment, this time with a rhesus monkey and this time with isoflurane, which is one of the class of volatile anesthetics that we use everyday in our clinical practice in children. Very much the same findings, so that if you take an infant rhesus monkey, give them a clinically relevant exposure to isoflurane, and you see a marked increase in markers of neuroapoptosis quantified in the right part of the slide.

This caused further concern. If you summarize all the pre-clinical data, and this is in a systematic review that was done late last year, we now have some answers, at least in the animals, about which drugs this happens with and at what doses; what the timing of exposure needs to be and how long animals need to be exposed; what some of the mechanisms might be. And remember that what I've shown you so far is just histological logical evidence.

Does this really translate into any differences in behavior in later life? Well, in terms of what drugs and what dose, it really happens with everything that we use-- all the different anesthetics that we use and other sedatives, such as midazolam. And as they've studied it more, the doses have gotten less and less so that it's definitely occurring within the range of clinically relevant doses that we use in children.

In the rodents and the neonatal primates, this happens if you do it in utero, this happens if you do it within the first two weeks of life. After this period of time, it doesn't happen. So there is definitely a window of vulnerability to exposure. Exposures as little as one hour in rodents caused these changes. And it looks like in the animals that multiple exposures are particularly injurious.

The mechanisms, I've mentioned the apoptosis. There are some other mechanisms associated with that that are being found now, including the suppression of synaptic signaling that may trigger apoptosis. Some changes in oligodendrocytes may also serve to trigger the apoptotic cascade. But it's not just apoptosis. As people have looked more closely, they've found evidence of neuroinflammation, they've found other evidence of impaired synaptogenesis and neurogenesis that may involve mitochondria or other neurotrophic factors.

So actually now, there's multiple potential mechanisms by which anesthetics can cause neural problems. Do these problems observed in histology translate into behavioral problems? The answer is yes. Most of the studies in animals that have looked at this find persistent cognitive impairment, especially in domains related to learning and memory. And there are some specific domains that have been noted in the primate study. So it's not just a global problem with neural function, but there are some very specific effects, again mainly related to learning and to memory.

However, there is really a lack across all the animal models in any consistency in the pattern of histologic injury. So it makes it difficult to really tie a particular pattern of injury noting the histology to specific behavioral problems. It's also apparent now that the effect of the exposure can be modified. Things like just giving the animals enriched environments after anesthetic exposure, for example, can ameliorate the injuries that have been seen, which is encouraging because it does suggest that if there is something that's going on here, it's something that we may be able to correct with the right intervention.

So thinking about whether this translates into humans, one of the issues is what is a comparable developmental stage between the rodents and the primates and the humans? And the answer is that we really don't know. I'm not a neural developmental biologist, but people who write about this argue a lot about what really is comparable. And to make a very long debate short, it's thought that the period, at least in primates, that seems to correspond to vulnerability to anesthetic agents would translate to about the first two or three years of life and the immediate prenatal period.

So it would suggest, if this is true, that there is a fairly good window of vulnerability where children may be injured. And here she is, isn't she cute? As of the time we were having that first lounge conversation, what we knew in humans was very little. We knew that if you looked at critically ill children, that if you looked at, for example, congenital heart disease children, they did have some evidence of impairment. If you looked at very sick neonates that had had necrotizing enterocolitis or ligation of a PDA they had some problems.

But they had lots of other problems beyond just needing anesthesia that could certainly explain some of these adverse neurodevelopmental outcomes. There was some short-term information about behavioral outcomes in the first days or weeks after surgery, but that really didn't have much applicability to this particular problem and there really weren't any long-term neurocognitive assessments. So as we were debating in the lounge, nobody really had any idea what was happening in children.

So here we are sleeping in the lounge. As we were continuing the conversation, I had recently been to a presentation by one of my colleagues, Dr. [INAUDIBLE] in epidemiology, who was presenting some findings that they had done looking at a cohort of children that were born in [INAUDIBLE] County identified through the Rochester Epidemiology Project where they were interested in what the actual incidence and outcomes of learning disabilities were in the population.

Learning disabilities are specific problems with understanding or using spoken or written language that manifest as you see here. The main thing to understand about learning disabilities is that it's defined by there being a discrepancy between your potential, as measured by things like intelligence quotient, and your actual performance, as measured by let's say a standardized individual achievement test. So if you are able to actually perform less than what you should be able to according to your intellectual ability, you have a learning disability.

And what she was saying was that they had actually done a lot of work here. They'd taken all children that were born in 1976 to '82 here in Rochester and were still here at school age, and they did an incredible amount of labor to review all public and private and homeschool records, records from all medical providers, from other centers, reading centers, dyslexia institute, and so forth to try to find which of these children developed learning disabilities prior to the age of 19. And they had very strict research criteria to be able to do this and they published a very nice series of papers in order to describe this problem in our local population.

And then we thought, hm, they've already done all this work. We also have through the Rochester Epidemiology Project all the birth records and all episodes of anesthesia and surgery for all these children. And it turns out that 593 of them had gotten anesthesia prior to age four. So because of all this work that my colleague had done, it was really a relatively simple thing to be able to determine if there's an association between exposure to anesthesia in surgery and learning disabilities.

And this is what we found, much to our chagrin. So what this graph shows is as a function of age the incidence of learning disability in this birth cohort according to whether you had no anesthesia exposure in the dashed line, one anesthesia exposure in the solid line, or multiple anesthesia exposures in the bolded line. And you can see if you look over the course of their school age life that having multiple exposures to anesthesia was associated with almost a doubling in the incidence of learning disabilities.

Not what we wanted to see. If you had just one exposure, there was no difference. That's good. To quantify this a bit, these are the hazard ratios for zero, one, two, or three or more anesthetics. No evidence that single exposures were associated with learning disabilities. But once you got to two or especially three or more, impressive hazard ratios. Not a lot of children here so it took a pretty big effect to be able to see the difference, but the difference was definitely there.

An association. Only an association because it's perfectly possible that just having anesthesia and surgery is a marker for other things that might be causative of having learning disabilities, right? If you need surgery, you're different. We do not do this for fun in children, we only do it when we have to do it. And these children often have other co-morbid conditions that might increase their risk for learning disabilities. For example, if you have multiple ear infections, you can't hear so well. You might need a myringotomy and the hearing loss may be responsible for some learning problems.

If you are needing lots of surgeries, if you're very sick, you may not go to school, you may have some other things going on in your life that also makes it more difficult for you to learn. And if you are going to the physician more often, it's more likely that you might get another diagnosis like a learning disability. So there's lots of different reasons why this association, occurs but this was really the first suggestion in any human population that there, in fact, was an association.

We went on and did some other analyses with the same data set to try to, for example, go down and say, well, maybe when is this vulnerable period? Let's just look at less than two years of age. Let's adjust for some co-morbidities. And we did some pretty sophisticated things to try to do that, to adjust for example, the burden of illness, to make sure that this just wasn't the sickest kids. We looked at other outcomes that we had to be able through the work that Dr. [INAUDIBLE] and colleagues had done.

They looked at, for example, the incidence of ADHD, as well. We also had data of group administered achievement tests. And what we found was consistently still that multiple, but not single, anesthetic exposures increased the frequency of learning disability and ADHD, hazard ratios of about two, and also tended to decrease achievement test scores. So all pretty consistent.

Other people started to look. The University of Iowa did a study of children that were anesthetized at their university hospital and had a single exposure to one of three different surgical procedures at less than a year of age. And they looked at group administered achievement tests as their outcome. If they looked across all 287 children that they examined, the means scores of these achievement tests were lower than the normative values.

If they took out the children that had obvious problems, so they had other types of developmental problems, they were left with 58 children. And their mean scores actually weren't different than the normative values, but there were a relatively high proportion that scored very lowly. Columbia University did some work using another type of data. These were large administrative data sets that they had available to them, Medicaid data in the city of New York.

They did some master designs using twin sibling pairs, they did some unmatched analyses. They looked at exposure to less than three either specifically for some procedures by [INAUDIBLE] or for all procedures and they looked at ICD-9 codes available in the administrative data set of developmental and neurobehavioral disorders. And they came up with relatively similar results that we did using a very different data set that they were able, in unmatched analysis, to show that the hazard ratios for single exposures were not impressive, but they were for multiple exposures.

They actually did a matched analysis for a single exposure during a hernia operation that suggested that maybe this was associated with some neurodevelopmental problems. Lots of different limitations with all the data sets that we are looking at. We won't go through what some of the specific limitations of this is, but consistent results. In Denmark they have a very nice system where they have complete ascertainment of all children in Denmark. They have medical records.

They were able to look at all children that had these two particular types of surgical procedures and they looked at group administered achievement tests across the whole country and didn't find any differences in scores in those achievement tests after they adjusted for several other co-factors. But the kids who were exposed had an increased rate of actually not even taking the test, which makes you wonder what's going on with those kids. In the Netherlands, they did at twin registry study-- they've got a large active twin registry study program-- and they looked, again, at exposures less than three years.

Their outcome was also group administered achievement tests. And they did find also some lower tours and more kind of the problems in exposed children. But if they limited it to co-twin pairs which were discordant for the anesthesia exposure, that is one twin got anesthesia and the other didn't, they didn't have very many so it didn't have much power but didn't see much difference. In Australia, there is a birth cohort where they have done longitudinal detailed psychometric analyses of kids that were born from '89 to '92 and they were also able to look at anesthesia exposure less than three years.

They looked at this wide range of neurodevelopmental tests and found that if you had any kind of exposure, single or multiple, that they had an increased risk of disabilities in language and in cognition, but not in academic achievement scores. So they scored just the same on your standardized tests, but if you looked in enough detail at these particular domains, you could see this association between exposure and outcome. And finally, a very recent study looked at a very small number of children that were exposed for longer periods of time at a very young age.

And they looked at some very specific domains of recognition memory and overall intelligence. They saw no difference if they compared children who did and did not get anesthesia exposure in IQ, overall intelligence, but they did see significant differences in these particular domains related to memory. And they were able to do some true translation here. They did comparable studies in an animal model and found the same result.

So there's lots of different studies that use lots of different methods, all of which, again, have their own limitations, but this is all still hanging together. Everybody that's looked has seen some sort of association, which still does not prove that this is causative but it continues to increase our worry. This is an area where it's very difficult to do prospective studies. You don't take children and say, we're going to randomize you to either get an anesthetic or not. You just can't do that.

But there are some people that are trying to do some prospective types of things. There's actually a randomized trial in infants undergoing herniorrhaphy that are randomized to either get a general anesthetic to go to sleep or to get a spinal anesthetic, a regional anesthetic technique, which is part of clinical practice in some areas. The supposition is that if you get a spinal, you're not exposed to as many anesthetics and sedation medications, and maybe if anesthesia is causing a problem you'll see a difference in some neurodevelopmental outcome.

So they're looking at IQ as their main outcome at age two and five. The study is ongoing, finished enrolling. We should have within the next year the two-year outcome for IQ. It will be very interesting to see what happens, although based on our data and on the data of others, because this is looking at a single exposure, they may not see anything, even if multiple exposures are associated with problems.

There's another observational study that's doing prospective detailed neurodevelopmental testing of sibling pairs-- so one sibling got anesthesia, the other one didn't to try to control for environmental influences and the like-- to try to see if they also can see any differences between the exposed and the unexposed sibling pair. We'll have those results within a couple of years, But still, in the meantime, we're left with these very striking results in rats, these very striking results in primates. But do they translate?

And this is not a theoretical concern. My granddaughter was very ill when she was three weeks old and needed a surgical procedure. I have real concerns about this. And I had a talk with my daughter about should she have this procedure or not? What do you tell your daughter? Is anesthesia harmful? How are we going to actually answer that question?

Well, looking at how you look at translational research, you're going to have to more definitively know what the phenotype of any injury is and what the mechanism or mechanisms of injury are in animals. And we're working through that, but we're not there yet. Lots of different mechanisms, still working on what the exact phenotype might be. But you have to nail that down in order to inform the human work.

We have to know what the actual phenotype of the injury, if there is an injury phenotype, is in children. Right now we have some hints that it may have something to do with learning or memory or language, but we still haven't had the kind of detailed testing that we need to truly identify what an anesthesia injury phenotype might be in children, if there is one at all. Somehow, you're going to have to link those two. You're going to have to show that what you're observing in the animal models is actually a model for what might be happening in children.

Ideally, you would try to measure the same domains in animals and children, although that can be a challenge. What's it mean when a rat can't negotiate a maze? What exactly does that translate into what a child might need to do in school? And then once you understand the mechanism, once you understand the phenotype, in the animals you try to find out what helps, either by developing other anesthetics or by some treatment that you can give prophylactically to children who are exposed to the anesthesia that works in the animals and that eventually you will be able to demonstrate works in the children.

So letting that guide how you're going to either change anesthetics in children or intervene to try to prevent this from happening if, in fact, it happens at all. So to try to establish all these links, there's actually some good scientific precedent. Sir Austin Bradford Hill was a very distinguished epidemiologist who was interested in causative relationships between exposures. He was more interested in environmental exposures and public health types of outcomes.

And in this lecture and his other work, he set down 10 different principles that you needed to satisfy in order to establish causality between an exposure and an outcome. And putting that in the framework that I've just given you here, several of them actually apply to this. And we won't have time to go through this in detail.

But in general, you want to show that any association that you have is strong; that it's consistent; that it's specific to the type of exposure that you're looking at and not many different kinds of exposure; that there's a plausible temporal gradient, so the outcome has to follow the exposure; and a biologic gradient, so the greater the intensity of the exposure, the greater the intensity of injury. Going to the right, it has to be that it's biologically plausible, that you understand enough about the basic science of how that agent is interacting with the organism that it makes some sort of scientific sense.

And then eventually, on the bottom right, you want to get to experiment, which in our case would be you show that if you use a different anesthetic in a child, it doesn't happen or if you use an amelioration technique in intervention, that that takes away the association between, in this case, anesthesia and any adverse neurodevelopmental outcomes. As our next step, we've started this study to try to contribute to some of the steps in that scheme that I showed you, Mayo Anesthesia Safety in Kids Study, or the MASK Study.

The main thing that we're trying to do in this study is to actually define the injury phenotype and to link phenotypes that have been observed in animals with children. So this is an observational study, it's population-based so that we avoid problems like referral bias, which are common when you're looking at surgical procedures and other such interventions in big academic medical centers. We're testing prospectively children in two age ranges with an assessment battery in multiple different domains to try to really nail down what is the exact relationship between exposure and a particular pattern of abnormalities in these domains?

The other thing that we are doing is that it turns out that you can do a test that the FDA uses to look for toxicity of different drugs in primates to get some idea of whether drugs might be also toxic in humans called the operant test battery. I'll show you a picture of it. It's largely a big mechanical video game that you can train monkeys to use to perform certain cognitive tasks that children can also use.

So we'll be able to do the same assessments in children that are being done in primate models to see if the kinds of injury patterns that are seen in primates can be translated into humans as well. So the basic design of the study is that we're taking a larger birth cohort. These are kids born in '94 to 2007 to moms that live in Olmstead County and that are still residents at age eight in the area so that we can get to them for testing.

We look at medical record information, we look at birth certificate information that we have through the Minnesota Department of Health to determine who satisfies these criteria and to gather co-variates that we will use in our analysis. One of the major problems in this whole area is the potential for confounding-- again, that children who need anesthesia are fundamentally different than those who don't. And you can't ever get totally away from that problem in an observational study like this.

But what we're going to try to do is to try to select the unexposed children that we test to be as alike to the exposed children as we possibly can. We're going to be using a propensity matching technique, generating a propensity score for the likelihood of needing anesthesia so that we can try to select control children to study who are as close as possible in every characteristic that we can measure to children who actually needed anesthesia so that we have a propensity matched set of children who have had no exposure, single exposures, or multiple exposures.

We're planning on bringing 1,000 children in total, numbers in each group as you see; studying them when they're either from eight to 12 or 15 to 19 to try to see if there's any progression of injury over this period or time or any differences in the manifestation of any injury within younger and older adolescents. And we're going to be performing this operant test battery, so this is the study that is done also in primates, and a whole range of different assessments that were chosen to try to cover as many domains as we could within a reasonable testing period.

This is the operant test battery. You can see the child is responding to some lights with lever presses, again something that monkeys can do. Actually, the monkeys can do it better than children for some of the tasks once they get trained. You'll see he's holding a little bucket. The monkeys get food pellets, the children get nickels. We were worried that nickels were not enough money to be a positive reinforcer. They love nickels. They just love nickels.

It takes about an hour for them to do the test. And again, it's very exciting because we can directly compare what we will see in the children with results. There's some sample results on the right there which show a training effect, which is what you have to do in the monkeys. But there are some specific-- this particular experiment was looking at ketamine exposure, and you can see that there was some impairment in the ability of these monkeys to learn this particular task. And we'll see if we can see the same pattern of results in the children or not.

Now, as you start to do this kind of work, you think, well, this is pretty simple, right? You just have a bunch of kids, and you invite him in and how hard could that be? Well everything's harder in translational research than you think it will be. For example, the rage of the moment is well, you should just go ahead and get blood samples from all these children, right, because who knows what you might want to do in the future with various forms of genomic testing or analysis and those sorts of things.

Well, do you really want to do that? Is that really going to contribute, or will that be a barrier to recruitment? You think about things like we're going to bring in children, many of whom are thought to be perfectly normal children. We're going to give them a whole bunch of tests. Some of those tests are probably going to be abnormal. Do you tell the children, do you tell the parents if they haven't had any suspicion that anything is wrong? How do you deal with, in other words, return of results when you're doing this kind of extensive of testing on a population for other than clinical purposes?

So we actually did a fairly extensive community engagement exercise before we actually started enrolling the patients. We went to the CCAT ethics and community engagement functions, and they helped us to do a series of focus groups and interviews to try to explore some of these issues. And it turns out the parents really didn't want us to obtain genetic information. They were really concerned about the future use of that information, especially because at the time that we would be collecting it, we wouldn't be able to say definitively what we were going to do with the information.

You get into issues like, for example, what happens when children who have material collected when they're minors turn into adults? Do you have to read consent them? What happens with that? So it turned out that it's actually a fairly big barrier to recruitment, and we decided not to do it because we didn't want to compromise the primary objective of the studies. There were a lot of discussions about return and results. What did parents want to know?

Parents wanted to know something, but not too much. They didn't always want to know only if they thought it was a problem. So we actually had to devise a procedure where the neuropsychologist who is working with us would review each one of the experimental studies, see if there was any concerning pattern of result, and then offer those parents a consultation. Not that we would do a full workup of any problems, but so that parents would have the opportunity to discuss any pattern of results that we obtained, which again is not a clinical assessment.

It was only a research assessment, but might indicate that more clinical assessments would be wise. I can tell you right now that unfortunately, we're finding a fairly high number of folks who have problems. And we've had quite a few parents that we've had come in and had these discussions. Some of them go on to have clinical evaluations. It's something that is actually going over very well with the parents and we hope is balancing the need that we have to responsibly deal with our research subjects and to let them know of anything that is concerning without necessarily causing any panic among the people that we're working with.

Think about just recruiting. I mean, we need to recruit about one child a day to a four-hour testing session. It's really tough to get these kids in at the right times. I mean, you can't just schedule them like you would a lab animal. They have school, they have other things. And it's actually very difficult to match the available children to the capacity and the timing of the testing facility that we have.

So we have to do all sorts of things to try to think about how we do our recruitment strategy because again, it's complicated. We've got kids that are born over a wide period of time that have to be tested within a specific window that's always moving as time goes on with a study. And so our great statistical team has worked out some very nice procedures to batch out the recruitment letters that we send out to try to get them time to try to match how many children say yes and want to sign up and how many children we can actually test and when they can be tested.

So it's a massive logistical problem that fortunately is going pretty well. But take a lot of thought to try to design. It really does take a village to do this kind of research, and that's really important about most forms of translational research. This came from our clinical concern as anesthesiologists. But as we put together the team to try to answer these kinds of questions, obviously epidemiology is important; obviously psychology is important; because we have to identify these children and we have to do a lot of work with our medical school records medical informatics becomes very important.

Rochester Epidemiology Project is absolutely key to be able to do this. You have to have some pretty sophisticated biostatistical support. We've engaged the toxicologists at the FDA as collaborators. They have this OTB machine and they have been very active collaborators in making that machinery available to us, getting it installed here in Rochester, and will be in the interpretation of the results as we get to that point. I've already talked about some of the ethics consultations that we took advantage of that were very important.

We have to have both clinical and research psychometrists involved that are actually administering the tests and obviously our pediatric colleagues are also very important. So just look at the range of expertise that need to be brought to bear to this pretty complicated problem. And this is very typical of most translational research projects. So what we hope to obtain from this MASK study is we're really trying to contribute to the circled area.

We're trying to better define what the phenotype of injury is in children and we're trying to link specifically phenotypes that have been observed in the monkeys with what might be observed in the children because that will help contribute. It won't definitively answer, but will help contribute to answering the crucial question of what we see in animals, does it apply to children or not? Many other groups are working on different pieces of this, especially the animal models.

We haven't gotten to the amelioration stage yet because we just don't know what to do, but there are some people that are actively working on that, at least in the animals. In the meantime, what do you tell parents, what do you tell surgeons? Because the surgeons need to know about this. It's not that this is definitive, it's not that we know that this is a problem. But the more we learn about it, the more concerned we are that it really may be a problem.

And it's probably something that should be taken into account when you're considering the risks and the benefits of any surgical procedure in a small child. Again, not that we just do anesthetics frivolously right now, but maybe there are some procedures that, for example, could be postponed until later in life, when children may not be as vulnerable. Or maybe there might be some diagnostic procedures that were fairly quick to do right now that we want to rethink in light of this potential risk.

We need to discuss with the patients. And what I tell parents right now is that most evidence suggests that single exposures are not associated with risk, that if you look at why most children need surgery, the benefit probably still far outweighs the risk. When my daughter was faced with a situation with my granddaughter, there was no question she needed this procedure. It was crucially important to her health.

And I had no hesitation with saying, she needs this, because it was clear. Really no question about what needed to be done. That's not as obvious in every case, and parents need to know that this may be something that you need to consider. Should it be a routine part of informed consent? That's a good question.

We have a concern, we don't have a definite risk yet. Should you routinely every child you put to sleep bring this up with the parents? I actually don't. I don't because it's not established enough for me to be able to routinely incorporate it into my informed consent discussion. Maybe I should, but I don't.

I certainly discuss this as it's brought up, and it's pretty frequently brought up now because the word is getting out about this and parents have concerns, and obviously we need to address those concerns as they come up. The FDA has put out a consensus statement as of December, it's being revised right now. But this is what it says right now. And we won't read all this, but it basically says, look, there are some potential concerns here and here's what we recommend providers to do that much is what I've just, said that you should discuss the risks and the benefits.

You should take into account what happens if you don't treat things, stay informed of new developments-- sure-- and recognize that yes, there are the majority of cases where we really do need to use these drugs. So this isn't terribly helpful, but this is where the government is right now in terms of recommendations specifically about this problem. So bottom line, we know now that anesthetics that are given to young animals can damage the brain. We know that many, but not all, studies in children show an association between multiple exposures to anesthesia and surgery and learning disabilities and ADHD.

Some also show associations between single exposures and these neurodevelopmental outcomes. Association does not equal cause, and I don't want you to forget that. Again, there are still many compounds that could be responsible for this association. But it could be the anesthesia, it could be surgery-- surgery itself causes a consistent inflammatory response that certainly could affect brain development, the conditions that require surgery themselves could affect development. Association does not equal cause.

So what do we need? More translational research. And this is not a simple one. Again, the ideal way scientifically to answer this would be to take a group of children and randomize them to anesthesia, and that's clearly not going to happen. We're going to have to do the best we can with Sir Hill's framework of piecing together the evidence. There's not going to be any single smoking gun, likely, study done, at least not anytime real soon.

Because for example, it's really difficult for us to construct a practical safe anesthetic based on the knowledge that we have from animal studies. You can do it, but it takes some pretty exotic drugs that we don't usually incorporate within our anesthetic practice. So based on what we know now, it would be very difficult to switch to anything that we think might be safe based on the results of the animal experiments.

So it's not going to be easy. It's going to take a lot of people generating a lot of different information and putting it all together in a thoughtful way to try to come up with as good an answer as we can for this particular crucially important problem. So lounge is done, we've got old bagels, we've got old newspapers. This is what it typically looks like at the end of the day. This all came from a conversation in the lounge, at least our involvement in it, where we knew something, we wanted to prove something, and we came to exactly the opposite conclusion and, in fact, provided most of the clinical evidence-- or at least the first clinical evidence that's out there-- about the potential association here.

So be careful what you ask about. You don't know where it's going to lead. You're going to develop lots of friends if you do translational research. And I just want to acknowledge all the friends, many of whom are here. But there are all the disciplines that I mentioned before are represented among this team, including folks from the FDA, as well. It would be impossible to do any of the work that we've done without contributions from each one of these folks.

And it's been fun, horrifying in a way, the question that we're examining. But it's really fun to work with a great team like this. And I think as you get more involved in translational research, there's lots of rewards. But this is one of the best rewards, the ability to come together and work with really bright people to answer a question of great significance. And of course, the most important thing about this project and translational research is your study coordinator.

This is Shonie, who makes everything run. She is an extraordinary individual. You think about mailing out 80 letters every two weeks and trying to schedule kids in this age to get to a particular place at a particular time and fill up 12 appointments a week. Think about that-- in addition to all the other regulatory and other things that she does for us. She is extraordinary and it's people like Shonie that actually make translational research work. So thanks to her and thanks to you for your attention.

[APPLAUSE]

SPEAKER 3: Are there any questions?

AUDIENCE: Thanks. That was a great talk. So have you looked at and compared the duration of anesthesia with these outcomes? It seems like it might be an important variable, as well.

DAVID O. WARNER: Yeah. There is a correlation, both in the animal experiments and in our observational work and others. It seems as though--

AUDIENCE: The reason I ask is because sometimes we have two procedures to do in a child and we say, oh, let's do them at the same time. But if that actually extends the total time, rather than two short surgeries, that might be an important distinction.

DAVID O. WARNER: It does seem, though, that if you are looking at an equivalent total amount of time that if you have two anesthetics, that is worse. That's suggested by the observational data and there's actually some animal experiments now. So it may be a multiple hit kind of phenomenon. So it's actually probably motivated us now to try to get as much done in a single anesthetic as we can because, again, it seems like for a given length as it's worse to have multiple anesthetics rather than just one longer one. That's a great question.

AUDIENCE: Thank you. This is wonderful work. May I say one thing is I heard you [INAUDIBLE] with twins. I learned one thing, that twins sometimes you need to think about monozygotic twin or dizyotic. I find one hormone, which is IGF-1 and that during growth of identical twins, monozygotic twins, they are almost identical in their growth, just like a straight line. I was so surprised to find that out. It's certainly very difficult to apply here. You can [INAUDIBLE] or somehow may consider. That's very, very powerful.

DAVID O. WARNER: No, it is. And the Netherlands study that I showed you were monozygotic twins.

AUDIENCE: Very good. I got the advantage from the Louisville, Kentucky. I already graduate from there. They have a twin study [INAUDIBLE] ask me to do that.

DAVID O. WARNER: Yeah. It's potentially very powerful.

AUDIENCE: Thank you. Thank you.

DAVID O. WARNER: Sure.

AUDIENCE: David, thank you for an excellent talk. Have you and your team considered different critical periods in terms of the phenotype? So have you considered that the phenotype may shift with age and exposure, and does the literature speak to that at all?

DAVID O. WARNER: There's not a lot of animal work that's really tried to do that. They've been more concerned about the vulnerable period in general, and they can't phenotype specifically enough to really look for very subtle differences. We haven't had enough data in our data or any of the observational studies to really look at smaller windows of exposure time.

We haven't had enough children. We may to some extent with this new data set, but it depends on how profound an effect it is. But it's certainly very possible. And in fact, you probably would predict that, that as you're going through different developmental stages, that different areas would become vulnerable at different times. Then you might see some later consequences. But again, we just haven't had the numbers that we've needed to be able to do that. Yeah, Michael.

AUDIENCE: So [INAUDIBLE]

DAVID O. WARNER: OK. So the question was, are there any studies associating anesthesia and Alzheimer's? So just like people have worried about babies' brains, people have worried about grandmas' brains. And so when Grandma goes to sleep, she wakes up and she's not quite the same. And certainly, there's a variety of different acute things that happen-- postoperative delirium, postoperative cognitive dysfunction-- So that yes, in general, the acute stress that accompanies surgery and anesthesia may not be good for Grandma's brain.

But in terms of whether it actually is associated with or causes Alzheimer's or any forms of dementia, there are some animal experiments that have been suggestive in terms of some of the markers of Alzheimer's disease. But we had the same lounge conversation a little later about, gee, is there an association between Alzheimer's disease and exposure to anesthesia? And so this actually was done several years ago with some of the Alzheimer's group patients here.

We updated it a couple of years ago. And there's no association. It's just not there. So that doesn't mean it can't be. We have the same observational study problems with this. It's just that if you're looking for a translational story to look at do the animal markers in Alzheimer's correspond to clinical problems compared with the child story? In the child's story, the animal and the human data hang together, they're all consistent.

At least so far, we haven't been able to find any corresponding problems in the human Alzheimer's data with the animal Alzheimer's data so it's less of a compelling translational story. It doesn't mean we shouldn't keep looking, but there just hasn't been any clinical evidence yet that exposure to anesthesia and surgeries is associated with an increased incidence of Alzheimer's or dementia.

AUDIENCE: Thank you. I heard from a nursing home, the nurses around there say that the senior citizens who come here for general anesthesia often come back totally different. [INAUDIBLE]. Is that true?

DAVID O. WARNER: They can be. Yeah. There's this entity called postoperative cognitive dysfunction where it's been noted that they do have cognitive changes for some period of time, usually in terms of months. But if you look at a larger population, within about six months, almost all of that is gone, any of those kinds of acute changes. So yes, it can happen, but it doesn't tend to be, unless there's some other complication that's occurred in the perioperative period, not a permanent or a long [INAUDIBLE].

AUDIENCE: They tend to say very positively that's happened. I wonder if the general anesthesia causes the problem or in that case the senior citizens should be more [INAUDIBLE] to use local anesthesia to do the job.

DAVID O. WARNER: Yeah. I mean, there's so many other things that happen around the perioperative time in the elderly with a surgical injury it's just it's difficult to know.

SPEAKER 3: OK. We have one more question here.

AUDIENCE: Hi. I just wanted to know when you're considering the exposure to anesthesia, does that include labor and delivery anesthesia that's been given to the mother?

DAVID O. WARNER: Oh, that's a great question. We actually did a study using the same cohort and we did not find any effect of using a labor epidural on neuraldevelopmental outcomes. So if you compare children who did and didn't get it, you didn't see any difference. Although interestingly, and this is maybe a problem with observational studies, for some reason, mothers who had Cesarean deliveries, if they had their Cesarean delivery with an epidural or a spinal, their children were actually less likely to develop learning disabilities, which is kind of strange.

Less likely not only than if they had general anesthesia, but less likely than if they had a vaginal delivery. Explain that one. And there actually may be some plausible explanations. But that would cause you to have to say that normal labor and delivery has adverse neurodevelopmental outcomes, which is probably not true, which makes me think that the observation that we saw is probably an artifact.

There's probably some compound in there that we didn't measure that's responsible for that association, which is a great cautionary tale for all this kind of observational data, which is really most useful for generating hypotheses, right? And the reason that our observational data is useful, or most useful, is that we have these animal experiments so that we can make this correspondence to help support one another. But the observational data by itself doesn't necessarily prove cause, neither does the animal data by itself prove that it happens in humans.

It's the two working together that work. So actually, thank you for asking that. That's a good example of some of the potential problems that we have with observational data.

SPEAKER 3: I think that's all the time we have, so thank you.

DAVID O. WARNER: Thank you.

[APPLAUSE]

[SIDE CONVERSATION]

[AUDIO OUT]

AUTOMATED COMPUTER VOICE: Your conference is scheduled to end in two minutes.

[AUDIO OUT]

AUTOMATED COMPUTER VOICE: Your conference is now over goodbye.

[AUDIO OUT]