

**CURE Epilepsy Webinar**  
**Serotonergic Mechanisms of Seizure Induced Central Apnea – Seminar**  
**(Transcript)**

Priya Balasubramanian:	00:01	<p>Hi everyone. I'm Priya Balasubramanian, and I am Associate Director of Research at CURE Epilepsy. I want to thank you all for joining us today. Today, we bring you our first virtual seminar of 2022. This is part of our Frontiers in Research Seminar Series. Today's seminar is entitled Serotonergic Mechanisms of Seizure-Induced Central Apnea. This seminar will be presented by Dr. George Richerson from the University of Iowa, who will talk about the role of serotonergic neurons as carbon dioxide sensors, and present evidence that seizures inhibit these neurons in a mouse model of Dravet syndrome. Our Frontiers and Research Seminar Series is generously supported by the Nussenbaum-Vogelstein Family. The seminar series that helps to educate and expose researchers, clinicians, and students to exciting epilepsy research, and also provide opportunities for young investigators to interact with leaders in the field. Our next virtual seminar is on March 10th on Clinical and Research Advances in Non-Epileptic Seizures and will be presented by Dr. Curt LaFrance from Brown University. CURE Epilepsy has been proud to be a leader in the epilepsy research community for almost 25 years. We've funded over 270 projects spanning 17 countries.</p>
Priya Balasubramanian:	01:27	<p>We currently have three different funding mechanisms and our key research priority areas include the acquired in pediatric epilepsy, treatment-resistant epilepsy, sleeping epilepsy and SUDEP. All of our grant applications go through a letter of intent phase and a full proposal review phase. They are reviewed by both scientific reviewers, as well as members of our community who are touched by epilepsy. The Catalyst Award is intended to fund translational research that aims to advance new therapies into clinical application. The RFA for this award will be announced in May, so you can keep an eye out for that. Both the CURE Epilepsy Award and Taking Flight Award for this year are underway. The CURE Epilepsy Award is open to established investigators, whereas the Taking Flight Award is a training grant. That's intended to support junior researchers who have at least three years of postdoc experience but have yet to attain significant funding. So the next cycle for these awards will be in 2023.</p>
Priya Balasubramanian:	02:33	<p>As I mentioned, our presenter today is Dr. George Richerson. He is a professor and Roy J. Carver Chair in Neuroscience at the University of Iowa. Dr. Richerson is a very well-known leader in this field of SUDEP research. His lab is interested in understanding the mechanisms of central respiratory chemoreception and role of serotonergic neurons. Dr. Richerson</p>

collaborated with Dr. Rup Sainju also at the University of Iowa on a study that was funded in part by CURE Epilepsy. They measured the hypercapnic ventilatory response in adults with drug-resistant epilepsy, who were admitted to the epilepsy monitoring unit. Interestingly, they found that patients who had a low hypercapnic ventilatory response or HCVR, were more likely to have breathing abnormalities and a longer period of high carbon dioxide levels in the blood after a generalized convulsive seizure. So we're really happy to have funded this research and looking forward to hearing more about it.

- Priya Balasubramanian: 03:37 Our other research in Dr. Richerson's Lab includes understanding how GABA is released from neurons and glia. Before he begins, I'd like to encourage everyone to ask questions and you can do this at any time during the presentation, by typing them into the Q&A tab located at the bottom of your Zoom panel and just click send. We'll do our best to get through as many of the questions as we can. Finally, I want to mention that this virtual seminar, as well as all of our future seminars, are recorded and are available on our website. With that, I will turn it over to you, George. Thank you.
- Dr. George Richerson: 04:19 All right. Great. Thank you very much. Thank you to CURE Epilepsy for asking me to give this talk and it will start with some background data, including already published data that we've done and then finish with some very new data that we think is very exciting, that builds on that. Thank you all for coming and listening. So, learning objectives are that many cases in SUDEP are due to central apnea. I think this is pretty well established, but I want to go over the data just to be sure that everybody's clear on that. Serotonin neurons are sensors of CO<sub>2</sub> and pH and control breathing in response to a rise in CO<sub>2</sub>. SUDEP is linked to a dysfunction of serotonergic neurons and impaired CO<sub>2</sub> chemoreception, but the reason for this link is not clear. The mechanistic link is not clear. I'm going to show you data at the end, which illustrates seizures cause transient inhibition of the ventilatory response to CO<sub>2</sub>, and that this is due to a serotonergic mechanism. I have nothing to disclose.
- Dr. George Richerson: 05:50 Just to be sure everybody's on the same page in terms of what SUDEP is. This is the definition of sudden unexpected death of a patient with epilepsy, not due to trauma, drowning or status epilepticus and an autopsy does not reveal any other clear, cause. There's been a lot of discussion about how common this is. It really depends upon the group of patients you're talking about. And with people with refractory epilepsy, the incidence is around six for a thousand patients per year. I've heard people

even in the field say, this is rare. I would say to you, that if you were told that you had a chance of dying of 6% for 10 years, that you'd be pretty appalled. That you'd be pretty worried. So even though it may be rare at any one minute of the day, the accumulated risk is actually substantial. I would not describe that as rare.

Dr. George Richerson: 07:09 So it's a big problem and it's important to try to understand why it happens. For a long time SUDEP was thought to be due to cardiac arrest it'd just basically be a sudden cardiac death in a person with epilepsy. But over the recent years, it's become clear that it's much more heterogeneous and that a large number of cases seem to be a result of respiratory arrests, central apnea. In fact, Hughlings Jackson, back in 1899, published a paper saying that patients after generalized seizures would stop breathing and turn blue. This has been replicated multiple times since then. This is a paper by Lisa Bateman and Masud Seyal showing that this is a benign seizure. It's a focal unaware seizure in which the patient dropped the O2 saturation to 30% during the seizure. So, it's pretty substantial. And that happens with both generalized seizures and partial seizures.

Dr. George Richerson: 08:43 But I think that a lot of people thought that was a phenomenon that occurred, but was not usually fatal until the Mortemus study, which really changed the view of the field. Where they reported the deaths of 11 patients with SUDEP in epilepsy monitoring units, while they recorded the activity of breathing cardiac activity and other EEG and other measurements. I have taken advantage of simplifying this figure, which shows a lot of different cardiac and respiratory patterns to just show you the terminal apnea and terminal asystole. So here you can see that terminal apnea always precedes terminal asystole in the nine cases of patients where they have the complete data. So, in these examples, respiratory arrest was the primary cause of death.

Dr. George Richerson: 09:52 We wanted to study this in more detail. It's very hard to study that in humans, in terms of the mechanistic cause of death. So, we built actually using chemo talented biomedical engineering student in the lab designed and built a mouse, epilepsy monitoring unit, which allows all these different measurements for 24 hours a day for as long as it takes for an animal to die spontaneously from a seizure. We initially began by studying Dravet syndrome. Mice with mutation of SCN1A, and this was an example of a spontaneous death that occurred where ... I don't know, Priya, can you see my mouse?

Priya Balasubramanian:	10:47	Yes.
Dr. George Richerson:	10:48	Okay. So, you can see that a seizure occurred here and in the middle of the seizure, breathing just stopped and never came back. And the EKG slowly, continued for a number of minutes until it finally stopped after this trace here. We expanded the time base of this recording, and you can see that at time one before the seizure breathing was normal, EKG was normal and this normal EEG. During the seizure, there was an increase in the frequency of breathing. EKG was pretty normal and the EEG shows the seizure. Then after the seizure is over, you can see that breathing is completely stopped. It's flat. EEG is now flat and the EKG is slow and it continues to get slower and slower with time and decreases in amplitude.
Dr. George Richerson:	11:57	There was the belief that this was a result of an increase in vagal output, causing parasympathetic slowing of the heart rate. But in this paper, we showed that that was not the case. And that bradycardia was due to a direct effect of hypoxia that resulted from the apnea that occurred. It could be prevented by giving the animals atropine, but that could be replicated by extremely low levels of atropine given in the central nervous system and not the peripheral. It was due to the prevention of the apnea that occurred, which makes sense because most chronic receptors are involved in respiratory rhythm generation.
Dr. George Richerson:	12:52	So this result has been replicated in a number of different mouse models, including one of a mutation SCN8A, a 5-HTR2C knockout, and then other mice with audiogenic or maximal electric shock seizures. So, it's a quite common cause of death in mouse models and as I told you in humans. So everybody focuses on apnea, but it shouldn't be assumed that postictal respiratory dysfunction only takes the form of frank apnea. We've seen a lot of abnormal respiratory rhythm in mice, after seizures, as well as patients, particularly those with Dravet syndrome.
Dr. George Richerson:	13:51	We also saw a phenomenon in a patient with Dravet syndrome, that was a nine-year-old girl, we recorded her EEG versus [inaudible 00:14:06] and EKG, as well as a transcutaneous CO2. Before at baseline, she had normal breathing, normal EKG, normal CO2, and then she had a flurry of three seizures over about a 10 minute period. During that time she had a couple of periods of apnea, but EKG continued on normally. Then almost 30 minutes later, she had her last episode of apnea. None of these were very long and EKG was normal, but at about 60 minutes after the seizure, breathing was a normal frequency of

breathing. But the amplitude was about half normal and as a result, she was hypoventilating with a CO<sub>2</sub> level that was about twice normal.

- Dr. George Richerson: 15:10 So this was not really apnea at this point, but this continued for several hours after the seizure and indicates that the brain stem is not responding appropriately to the rise in CO<sub>2</sub>. This patient several years later actually died from SUDEP. This made us wonder whether or not this problem with handling of CO<sub>2</sub> levels might be a biomarker for high risk for SUDEP, and made us become more interested in studying why this might occur. So Seizures can cause postictal hypoventilation. This hypoventilation may be a biomarker for SUDEP risk. And the question becomes, how could seizures induce a prolonged increase in CO<sub>2</sub>. To answer this question, you really need to understand how the respiratory rhythm is generated and modulated. It's produced by a group of neurons in the brain stem mostly in the medulla, including the pre-Botzinger complex that generate rhythmic respiratory activity that drives motor neurons that cause inspiration and expiration of the lungs.
- Dr. George Richerson: 16:35 This respiratory CPG receives ton of drive or input stimulatory input from two main sources. One is wakefulness drive from the cortex and the other is chemoreceptor drive from central respiratory chemoreceptors that respond to CO<sub>2</sub> and peripheral chemoreceptors mostly in the clotted body that respond to an increase in CO<sub>2</sub> and a decrease in O<sub>2</sub>. You can measure the elements of the respiratory network using what's called pulmonary function tests. Actually, I'm sorry. I wanted to just point out that one subset of central respiratory chemoreceptors are serotonin neurons that respond to CO<sub>2</sub>, then you can measure these elements using pulmonary function tests. I was fortunate to enlist the help of my now partners, Brian Gehlbach, who's a pulmonary critical care physician at Iowa and Rup Sainju, who's an epilepsy doctor at Iowa, and they've perfected this technique in epilepsy patients with help of Harold Winnike and Deidre Dragon.
- Dr. George Richerson: 17:52 This is the kind of data that you can get with this technique, where as you raise CO<sub>2</sub> below, what's called the ventilatory recruitment threshold. You don't really have much sensitivity or much dependence on the level of CO<sub>2</sub>. This VRT can range anywhere from 40 to 50, depending upon the conditions. Once you get above this level, now you get a linear response in proportion to the CO<sub>2</sub> levels. The slope of this response is a measurement of the sensitivity of the chemoreceptors. This

activity, or the drive that occurs in this lower part of the curve is primarily wakefulness drive coming from cortical inputs. This is coming from chemoreceptors, both peripheral and central. Our interest was to understand the relationship between the slope of this chemoreceptor response and the risk of respiratory arrest after a seizure.

Dr. George Richerson: 19:16 So Brian and Rup measured the slope of the H [inaudible 00:19:24] in a group of patients that were epilepsy patients in the epilepsy monitoring unit, and found that there was a wide range of chemosensitivity. This is an example of a patient with a median response. And this patient here had almost no response to CO<sub>2</sub>. So as CO<sub>2</sub>, and this is above the VRT or ventilatory recruitment threshold, there was really no response as CO<sub>2</sub> increased. At the time we saw this, we were concerned, but there was no evidence that this was dangerous. And many people that are walking around seemingly normal, have this kind of a shallow response and don't die, but also might not have epilepsy. But this patient actually ended up dying from SUDEP Plus 11 months after this test was performed. This is the histogram of the size of the response of the patients that we did. You can see this patient here had one of the lowest responses to CO<sub>2</sub>. So this interictal CO<sub>2</sub>, we thought also, might be a biomarker for high risk for SUDEP.

Dr. George Richerson: 20:59 So taking together with the data that we got from the nine-year-old Dravet syndrome girl. We wanted to know in a larger group of patients, whether or not there was any relationship between the interictal CO<sub>2</sub>, hypercapnic ventilatory response, and the change in the hypercapnic ventilatory response after a seizure and any other measure of the respiratory dysfunction after seizures, such as apneas. So we looked at a group of patients and measured the HCVR slope before a seizure, and then after a seizure. And in this particular patient, we found that there was a drop in the sensitivity, CO<sub>2</sub> sensitivity after the seizure, after a generalized seizure. And this lasted, this was three hours after the seizure was over and there was still blunting of that response. This is a group of seven patients with nine seizures showing that in all but one case, in all but one seizure there was a decrease in the hypercapnic ventilatory response after the seizure. This was true with focal seizures and with generalized convulsive seizures, there were four generalized seizures and five focal seizures.

Dr. George Richerson: 22:35 So at this point, based on the data that I've shown you, we came up with a hypothesis that postictal depression of CO<sub>2</sub> chemosensitivity is due to seizure-induced dysfunction of the

serotonin system. And we sought out to test this hypothesis. Now, to explain why we think serotonin was involved. I go back to literature on the evidence that serotonin neurons are sensors of CO<sub>2</sub> that are important for controlling CO<sub>2</sub> and pH homeostasis. This paper gives a review of a lot of the early data that helps support this hypothesis. Some of that includes the finding that serotonin neurons are very closely associated with large arteries in the brain stem, including the basilar artery, and its penetrating branches.

Dr. George Richerson: 23:40 So showing in green and yellow here are serotonin neurons, and they're surrounding the basilar artery in the midline of ventral medulla, and also close to penetrating arteries in the midline of the medulla. This is a perineal tract here on either side in the basilar artery. At this location these neurons would be very able to faithfully monitor the levels of CO<sub>2</sub> shortly after the blood leaves, the heart and the lungs. So kind of an ideal location, similar to the carotid bodies for the peripheral chemoreceptors. These neurons are also highly sensitive to changes in CO<sub>2</sub>. And this occurs as a result of changes in intracellular pH in these neurons. And there's an average tripling of firing rate of these neurons in response to a drop in pH from seven four to 7.2.

Dr. George Richerson: 24:46 There's also evidence in animal models for a role of serotonin in SUDEP, including the fact that the first genetic mouse model of SUDEP was the 5-HT<sub>2C</sub> knockout mouse that spontaneously died from seizures. And a variety of data showing that drugs that enhance in serotonin system can reduce spontaneous and evoked seizures in mice. There's also clinical data indicating that serotonin acts as an anti-convulsant to decrease seizures. So some anticonvulsant drugs are known to increase serotonin and fenfluramine was recently approved for both drug-resistant epilepsy. And there's now some circumstantial really evidence that fenfluramine may reduce SUDEP. So, Working with Hal Blumenfeld, we found that in rats, when seizures are induced by hippocampal stimulation, seizures are induced. When the seizures are induced, there's depression of breathing and inhibition of firing of serotonin neurons.

Dr. George Richerson: 26:30 So the serotonin neurons receive input from descending pathways, from the cortex during seizures to cause them to be inhibited. So, if this is true, then you need to ask the question, what happens if serotonin neurons are inhibited, just in general? We were able to answer this question through studies that we had done in the past in which serotonin neuron ... And we actually used two different approaches to answer this question. One was to genetically delete serotonin neurons

during development, using an approach based on the knowledge that there's a transcription factor called Pet1. Pet1 is expressed only in serotonin neurons and in all serotonin neurons. Lmx1b is another transcription factor, that's found in a number of neurons that's required for the development of serotonin neurons. So, mating these two groups of mice led to the deletion of Lmx1b and all serotonin neurons that as a result serotonin neurons did not develop. That's shown here using in situ hybridization for the serotonin transporter in which there are essentially no serotonin neurons in the brain.

Dr. George Richerson: 28:09 When we did this, Matt Hodges, a post-doc in the lab showed that the hypercapnic ventilatory response in these mice was decreased to about half of what the normal wild type mice response was. If you take a wild type mouse and you basically put them in a refrigerator and measure their core body temperature wild type mice do pretty well by maintaining body temperature. However, mice that have no serotonin neurons really can't control their body temperature. We show that this was because of a lack of brown fat metabolism and inability to shiver properly. So, the motor output pathways for generating heat are abnormal. All of these mice, even though they dropped their body temperature, really precipitously were able to be brought back to life by warming them up and they survived. But there's clearly a severe problem with thermal regulation. So decreased HCVR and impaired thermal regulation are the two of the big problems in these mice with no serotonin neurons.

Dr. George Richerson: 29:29 We then working with Susan Dymecki and Russell Ray showed that if you express the DREADD receptor that's inhibitory in neurons that express the serotonin transporter, SLC6A4 and then you give them the designer drug, CNO, Clozapine N-oxide. What you find is that the response to CO2 drops to about half of normal, and this does not occur in the control littermates. In addition, if you inject CNO into these mice and they're just in their home cage at room temperature, they are unable to maintain their body temperature. So again, impaired HCVR and impaired thermal regulation with chronic loss of serotonin neurons or with acute inhibition of serotonin neurons. So guess I already told you this, these are two of the things that you expect to see this when serotonin neurons are inhibited. Then based on the data that I showed you working with Hal Blumenfeld and rats, we would expect that seizures if they inhibit serotonin neurons may mimic the effect of impairing serotonergic neuron function on the HCVR and on thermoregulation.



- Dr. George Richerson: 31:20 So Frida Teran, an MD PhD student in the lab performed a series of experiments that examined this hypothesis. She started by taking Dravet syndrome mice and wild type litter mates and exposing them to CO<sub>2</sub> from 0% to 7% and found that they really had a response, a ventilatory response to CO<sub>2</sub>, that was no different from each other. And the slope of these two groups of mice was plotted here in this graph, and you can see they're basically the same. She then heated mice up to a temperature that induced seizures, generalized seizures, in the Dravet syndrome mice, and to an equivalent temperature in the wild type mice. And then repeated the CO<sub>2</sub> response and found that the Dravet syndrome mice had a drop in their hypercapnic ventilatory response that was not seen in the wild type mice. The slope is plotted over here, and you can see that the Dravet syndrome mice had a drop in their slope.
- Dr. George Richerson: 32:39 So that's consistent with what you'd expect of serotonin neurons that are inhibited. We found that there were two groups ... Well, there were Dravet syndrome mice that had a large inhibition of the CO<sub>2</sub> response and other Dravet syndrome mice that had a small or no response to the seizures. This is a mouse that had a large response. You can see the force, the seizure occurred raising the CO<sub>2</sub> from 0% to 7% causes a large increase in ventilation that's normal. After the seizure, there was really no response and that's very abnormal. But there are other mice in this case that showed that they had a response to an increase in CO<sub>2</sub> and after a seizure, they really didn't change that response. We don't know what the difference between these are, but this is obviously a million-dollar question is what are the biomarkers that could predict, which of these two groups of mice ... Was different about these two groups of mice?
- Dr. George Richerson: 34:00 We plotted the response of 11 mice that had this kind of response showing a big drop in HCVR and 12 mice that had this kind of response, a mild response that had very little drop in the slope of the HCVR. Curiously when we plotted the temperature change for these two, they correlated with their response, their HCVR response. The wild type mice had really a small, but not that significant of a drop in their body temperature after heating them up, the mice that had a mild response to the HCVR had a drop in their body temperature. But those that had a severe response to the HCVR had a much larger drop in their body temperature, again, consistent with this being due to serotonin neurons, being inhibited transiently after seizures.

- Dr. George Richerson: 35:11 If you plot the slope of the VE versus the body temperature, nadir, you can see that it correlates. That ones with the biggest drop in the HCVR had the largest drop in body temperature and vice versa. Now, I don't want to take too long to explain this, but what we did was use parochlorophenylalanine which is an inhibitor of tryptophan hydroxylase and causes prevention of formation of serotonin from tryptophan. And when we did that compared to vehicle, there was a drop in the baseline response to the hypercapnic ventilatory response. Then when we induced a seizure, the drop in the HCVR that occurred with the vehicle-treated mice was partially occluded by the PCPA in the other group of mice, the PCPA treated mice.
- Dr. George Richerson: 36:29 Those that were treated with PCPA had a much larger drop in body temperature than those that were treated with vehicle. Again, suggesting this was a serotonin mechanism. So, then we use fenfluramine, which is a drug that has a number of serotonin agonist actions. What that did was, fenfluramine reversed the baseline drop in ventilation and improved the slope of the HCVR. So [inaudible 00:37:23] asked the question, what kind of mice these are? These are Dravet syndrome mice, and they have a background of mixture of C57 Black 6 and C3H and they're all at an age between, I believe 25- and 30-day postnatal days.
- Dr. George Richerson: 37:52 And fenfluramine also prevented the drop or reduced the drop in body temperature induced by the seizures. So in summary seizures, we found inhibit the firing of serotonin neurons. They can inhibit the ventilatory response to hypercapnia and impair thermoregulation. The risk of SUDEP may be increased by this impaired interictal CO2 chemosensitivity, or by seizure-induced chemoreceptor dysfunction. So we think that either one, either just the baseline interictal CO2 response or the seizure-induced inhibition can both increase the risk of SUDEP. These data provide a mechanistic link between breathing serotonin and SUDEP to explain that link. Targeting serotonergic chemoreception may help to reduce SUDEP risk.
- Dr. George Richerson: 39:06 I don't yet know what the best agent would be. I know somebody will ask that question, but we don't know yet what the best approach will be, but enhancing this pathway should improve survival. So, I want to thank our funding agencies, including the Center for SUDEP Research of the INDS. Also, I can't read the note, but NIH also supported this work through an RO1 through the KO8 and Diversity Supplements, and I also want to thank CURE Epilepsy for a SUDEP Award to Dr. Sainju

and the Tross Epilepsy Research Fund. So with that Priya, do you want to take over?

- Priya Balasubramanian: 40:18 Yeah. Thank you so much that was a fascinating talk. And we have time for some questions, and there's a couple of questions that have come in. So, before I get started, I just want to let the audience know, if you have questions, please send them in through the Q&A tab and we'll try to get through as many as we can. So, the first question that came in was asking whether you recommend that patients undergo pulmonary function testing to indicate whether or not they may be predisposed to SUDEP. And if yes, is this only available at the University of Iowa or elsewhere.
- Dr. George Richerson: 40:55 So this procedure takes about two minutes or maybe three minutes. It's quite straightforward. The equipment that you need to perform, it is not that expensive, and usually, it's pretty standard in most hospitals. So, the question is what tests should be done to be able ... One advantage is that if you have a pulmonary technician who does this routinely, then it makes a big difference. If you have different people doing the test in different subjects, there can be variability. So, finding somebody who's devoted to this can help.
- Dr. George Richerson: 41:45 I guess the first question that I think of is, should it be that you want to look at the interictal HCVR or the change in the HCVR induced by a seizure? The interictal HCVR we've been testing in patients in the clinic and our plan is repeating every six months to see how stable it is and how reliable it is as a measure of the constitutional ability of patients to have a response to CO<sub>2</sub>. That's a lot easier to do than trying to bring patients into the epilepsy monitoring unit, measure the preictal and the postictal HCVR and see how much it changes. So, if the interictal can work and we can get it as an outpatient, then that would be a very good screening tool.
- Dr. George Richerson: 42:49 I am an advocate for if you are bringing a patient in for epilepsy monitoring already, then measuring respiratory parameters is I think really should be just routine standard operating procedure. And that those measurements should include things like end-tidal CO<sub>2</sub>, or transcutaneous CO<sub>2</sub> before, during, and after seizures, chest wall movement with respiratory MPS polysomnography and O<sub>2</sub> saturation. O<sub>2</sub> saturation is probably the easiest thing to do, and not all epilepsy monitoring units measure that but I think it should be just a standard thing that everybody does.

Priya Balasubramanian:	43:45	Great. So along those lines, what are limitations to developing HCVR as a biomarker for SUDEP?
Dr. George Richerson:	43:55	So what is going to require is enough measurements from enough patients to determine what the relationship is between the slope of the HCVR or the change in the slope after a seizure and the risk of dying from SUDEP. And that will take time and a fairly large number of patients. That's where we're headed with trying to measure the interictal HCVR. You could use surrogates, but we don't know how good those surrogates are. Things like how much do patients have apnea after seizures with nonfatal apnea or hypoventilation with a rise in CO <sub>2</sub> ? And those would take more effort, but also, they need to be verified as being representatives of the risk of SUDEP.
Dr. George Richerson:	44:59	We don't know the answer to that yet. But once those studies are done, then we can answer that question of whether or not the risk of SUDEP is greater in people that have impaired CO <sub>2</sub> handling. There's two parts of the respiratory network. One is the response to CO <sub>2</sub> and O <sub>2</sub> to some degree and the baseline rhythm generation. I think that the CO <sub>2</sub> handling is a very important part of this, but there may be also rhythm generation problems in some groups of patients.
Priya Balasubramanian:	45:46	Thank you. So, Dan Moki has a [inaudible 00:45:49] question see if that information is enough.
Dr. George Richerson:	46:01	You froze, I don't don't know if it's in your end ...
Priya Balasubramanian:	46:01	The question is, do you think inhibitory inputs to directly, namely central amygdala neurons are somehow resistant to [SCMONE 00:46:09] deficiency?
Dr. George Richerson:	46:15	Yeah, that's a good question. I think that's still at the stage of hypothesis, we don't have any data that addresses that one way or the other. But I think ... The amygdala, we do think and as Dan points out, rightly that the amygdala plays a big role in this. We don't know for sure that it's GABA neurons in them, maybe a little bit, that's what everybody would suppose since they're playing a big role in the output pathways.
Priya Balasubramanian:	46:55	Another question was you mentioned that there is some clinical evidence that fenfluramine might reduce SUDEP. Is there any indications from clinical trials that this might be the case, from the fenfluramine trials?

- Dr. George Richerson: 47:11 No, the data wasn't randomized and prospective, it was based on retrospective analysis of patients that had been on fenfluramine. And there was a fairly large difference in the number of patients that died from SUDEP with more in the control and placebo than in the fenfluramine group. So that needs to be done. There needs to be a trial to look at that. It's proof of Dravet syndrome, which is a epilepsy that has a high risk of SUDEP. So that would be a good population to study. It has a number of serotonin mechanisms that may be involved, different receptors and also reversal of the serotonin transporter and release of serotonin, nonrestricted release of serotonin.
- Priya Balasubramanian: 48:22 Thank you. Again, if there are more questions [inaudible 00:48:27]. Do we know anything about circulating serotonin levels in seizures and breathing abnormalities?
- Dr. George Richerson: 48:46 So there is evidence that we published that the postictal serotonin levels are greater in those individuals that have less apnea after a seizure. The issue is whether or not that blood serotonin has access to the brain or in some other way affects breathing or survival. We generally think of serotonin in the blood has not been able to get into the brain because the blood-brain barrier is impermeable to serotonin. But there is some evidence that during seizures, this is usually thought of as during status epilepticus, that during seizures, serotonin can cross the blood-brain barrier and may have an effect. The other may be a more realistic possibility is that there's something that's occurring in the metabolic pathways that generate serotonin that is common to both the blood and to the brain. So, whatever is causing the increase in serotonin in the blood is mimicked by something that's occurring in the brain and has an effect on serotonin levels.
- Dr. George Richerson: 50:16 So, and it's very hard to measure brain serotonin in the compartment that matters. So in animals, you can measure whole brain or regional total serotonin levels, but that is a combination of intercellular serotonin, the intracellular serotonin and extracellular serotonin, but it's really only extracellular serotonin that we care about. That has a biologically relevant effect on the excitability of the network. And you can theoretically measure that with microdialysis, but that is hard to do in the brain stem. It also disrupts the extracellular space. So, you're not likely to be getting the levels that are there under normal conditions. In other words, it's hard to know the answer to that question.

Priya Balasubramanian: 51:27 Very interesting. So, there are no more questions. I'd like to take this opportunity to say thank you to you again, George, for your time, and for the really wonderful talk today. I'd also like to say thank you to the Nussenbaum-Vogelstein family for their generous support of this Frontiers in Epilepsy Research Seminar series. Of course, thank you to everyone in the audience for being here and asking questions. So, if you would like more information about hosting a seminar at your institution, or about our grants, you can visit our website and visit the four researchers tab of our website. You can find more information there, or you can feel free to reach out to us. Our email is [research@cureepilepsy.org](mailto:research@cureepilepsy.org). As I mentioned previously, please keep an eye out for our next virtual seminar, which is on March 10th and will be presented by Dr. Curt LaFrance. Thank you all for joining us and stay safe.