Priya Balasubra...:	00:00	Welcome everyone. I'm Priya Balasubramanian, and I am Associate Director of Research at Citizens United for Research in Epilepsy, or CURE. Thank you all for joining us today.
Priya Balasubra...:	00:12	So today we bring you our first virtual seminar as part of our Frontiers in Epilepsy Research Seminar Series. This program is generously funded by the Nussenbaum-Vogelstein Family, and it aims to help 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.
Priya Balasubra...:	00:36	Unfortunately, due to the social distancing guidelines, we've been unable to provide this interaction live at academic institutions around the world. So until conditions allow us to come back together in person, CURE will present these seminars virtually.
Priya Balasubra...:	00:51	Today's seminar is titled: What's New at the Epilepsy Therapy Screening Program, or ETSP? The ETSP is a preclinical screening program, funded by the NINDS. It provides researchers the opportunity to screen potential therapeutic agents in established rodent seizure models.
Priya Balasubra...:	01:12	Since its establishment, the ETSP has played a role in the development of several FDA approved epilepsy drugs. Today's seminar will broadly discuss the scope of the ETSP, and also talk about new models and assays available at the University of Utah, which houses the ETSP.
Priya Balasubra...:	01:33	Cure has been proud to be a leader in the epilepsy research community for over 20 years, funding over 240 projects spanning 15 countries. We have three different funding mechanisms currently, and our key research priority areas include viral and pediatric epilepsies, treatment-resistant epilepsies, sleep and epilepsy, and sudden unexpected death in epilepsy, or SUDEP. All of our grant applications progress through a letter of intent phase and then a full proposal review by scientific reviewers, as well as members of our community who are touched by epilepsy.
Priya Balasubra...:	02:13	The Catalyst Award is our newest award, and it is intended to fund translational research that aims to advance new therapies into clinical application. Our CURE Epilepsy award is open to established investigators, whereas our Taking Flight Award is intended to support junior researchers who have at least three

		years of postdoctoral experience, but have yet to obtain significant funding.
Priya Balasubra...:	02:38	Look out for the call for letters of intent for the CURE Epilepsy and Taking Flight Awards. That call will be coming out in late November.
Priya Balasubra...:	02:50	Today's presenter is Dr. Cameron Metcalf, who is Research Assistant Professor in Pharmacology and Toxicology at the University of Utah. He is also co-investigator and the Associate Director of the ETSP Contract Site. Dr. Metcalf's research interests include the evaluation and advancement of novel therapies for the treatment of epilepsy and pain, as well as the pathophysiology of SUDEP.
Priya Balasubra...:	03:12	But before Dr. Metcalf begins, I'd like to encourage everyone to ask questions. You may submit your questions at any time during the presentation by typing them into our Q&A tab, located on the bottom of your Zoom panel, and click "Send." We'll do our best to get through as many of the questions as we can.
Priya Balasubra...:	03:32	And I also want to mention that today's virtual seminar, as well as all of our future seminars, will be recorded and are available on the CURE website.
Priya Balasubra...:	03:41	With that, I'll turn it over to Dr. Metcalf. Thank you.
Dr. Cameron Met...:	03:46	Thank you so much, and I'd like to thank CURE for this opportunity to be able to discuss the work that we've performed at the University of Utah. And it's my privilege to have colleagues at CURE that are supportive and enthusiastic about the work that we're doing.
Dr. Cameron Met...:	04:05	So let me first add to the introduction ... and thank you again for that ... by stating that my role as the Associate Director is at the Contract Site for the ETSP. So the ETSP is housed at NINDS, and I'll speak about that just a little bit more. They're the contract holder, whereas we are the contract site. So the testing that I'll describe is generally performed at the contract site, with some exceptions. But all of our activities are performed under the direction of the ETSP.
Dr. Cameron Met...:	04:44	These are my disclosures. None of these have any major bearing on the discussion today, with the exception of my role as a co-investigator and Associate Director of the contract site.

Dr. Cameron Met...: 04:57 For any of you that are familiar with the history of this program, it was formerly known as the Anticonvulsant Screening Program, or the ASP. And also historically our program has been identified as the Anticonvulsant Drug Development Program, or ADD Program. However, that nomenclature has recently changed, and I'll get into that in just a little bit. So going forward, I'll refer broadly to the program as the ETSP, or Epilepsy Therapy Screening Program.

Dr. Cameron Met...: 05:25 So this contractual agreement has been in place for many years with the University of Utah College of Pharmacy being the contract site since its inception. The former PIs of this program include Ewart Swinyard, Hal Wolf, Steve White, and our current principal investigator is Dr. Karen Wilcox.

Dr. Cameron Met...: 05:47 Our goal is really to encourage and facilitate discovery and development of new antiseizure drugs. And to that point, I'd like to just add to what was said earlier in the introduction, is that we have had the privilege of contributing to the discovery and development of several antiseizure drugs that have entered the market since the inception of our program. And those are shown here with the red arrows. So our roles have varied from compound to compound, but in the general sense, we feel very fortunate to have played a part in the development of several of these, and most recently including cannabidiol and cenobamate.

Dr. Cameron Met...: 06:32 For program participants ... and what I mean by that is for individuals, either at small biotech companies, small pharma, large pharmaceuticals, academic organizations, and others that approach the ETSP and are then brought into the program as program participants ... the testing that is performed at the contract site is free of charge to NINDS participants. And this program has therefore been a great benefit to the epilepsy research community over the years because of this taxpayer-funded benefit.

Dr. Cameron Met...: 07:09 We at the contract site are blinded to compounds, and to supplier, and to compound structures, and all of our testing is performed in a blinded manner. And so, for those that enter into the program as a program participant, there are open discussions between the participants and with our colleagues at NINDS. But when the compounds come to us at the contract site, we only receive them as a compound number with formulation instructions and dose range. But we're blinded, as I mentioned, to who is supplying the compound. We often find

out later through public disclosures that compounds may have advanced through our program, but we're obviously not aware of that as we're testing those.

Dr. Cameron Met...: 07:54 The goal of the studies that I'll describe here is really to allow program participants to have an idea about preclinical efficacy and tolerability for their respective test compounds. One of the main themes of that work, that I'll touch on at several points, is to demonstrate that over time, as needs within the epilepsy research field advance and change, we are continually working along with the ETSP to try to change our overall testing scheme to provide the best possible screening program that fits the needs of the epilepsy research community. And so that includes development and implementation of new animal models from time to time.

Dr. Cameron Met...: 08:46 And for any that are on the call, if you're interested in participating as a program participant, meaning that you have a compound or compounds that you would be interested in having screened, please reach out to our colleague, Dr. Brian Klein, who is currently the Director of the ETSP at NINDS, and his contact information is listed here. Again, I can't encourage you enough to be able to reach out to Dr. Klein and approach the ETSP, and potentially be able to participate and send compounds our way.

Dr. Cameron Met...: 09:20 So as I mentioned before, our program has been around for quite some time. However, there are several new directions that we've gone in, and so I'd like to just clarify: what is it that's new? Well, the original goal of the program from several years ago was to screen novel antiseizure drugs. We've recently changed our focus, and this is in cooperation with the ETSP and also through the recommendations of leaders in the field, including working groups at NIH and other organizations. And the goal here is to really help to identify and design a therapeutic strategy to meet or direct our efforts toward unmet medical needs within the field of epilepsy research.

Dr. Cameron Met...: 10:10 And so, most recently, in 2016, we received a five year contract, and that was through a competitive renewal application program, and during that time, or prior to that time, the ETSP had undergone its internal review and changes, and rebranded itself from the previous name to the Epilepsy Therapy Screening Program. And again, this is in keeping with the goal of being able to identify and design testing strategies to address unmet medical needs.

Dr. Cameron Met...: 10:43 And so our major areas of interest, again based on those recommendations from leaders in the field, has been to target our efforts towards pharmacoresistant epilepsy, toward special populations including genetic and acquired epilepsies, and also toward epilepsy prevention, and disease modification. And so that's what I'll be talking about today.

Dr. Cameron Met...: 11:05 We are continually addressing these needs within the field to the best of our ability, and that includes bringing on new models as appropriate, and developing and adapting those in an optimized way for screening.

Dr. Cameron Met...: 11:19 We've also brought a new direction to our program to try to help implement a more rigorous quality control and data retention program. And that includes the implementation of case report forms and the incorporation of common data elements. And so, for any of you that are familiar with some of the goals of the ILAE and AES Task Force, in recent years that includes the incorporation of common data elements, and we're working to do that, and to have that be part of our data package provided to program participants.

Dr. Cameron Met...: 11:58 So as I mentioned, what I'll be talking about is how our workflows have been redirected toward unmet medical needs within the epilepsy community, and part of that work has really been directed not only by the ETSP but also by our external consultant board. And these are leaders within the field that have provided feedback to NINDS and to us at the contract site, on testing strategies and how to best implement various animal models, again in trying to meet the goals of the ETSP.

Dr. Cameron Met...: 12:30 So I'm going to describe to you three major performance areas. Performance area one is geared toward pharmacoresistant epilepsy, and while I won't be able to speak to you about everything within that performance area, what I'd like to point out is that by and large, the work is done at the contract site, with a few exceptions. One is we utilize the intrahippocampal kainic acid model. And that model work is performed with our collaborator, SynapCell, in Grenoble, France, whose PI is Corinne Roucard. And we've had this relationship with SynapCell for many years, and they've performed tests for several compounds in their model.

Dr. Cameron Met...: 13:11 We also have a subaward with our former PI of our program, Steve White, at the University of Washington. His group is helping to perform replication studies for some of our animal

models that I'll describe today. The goal there is to provide program participants with data for a select group of tests, wherein data were obtained at a separate lab, although under similar conditions, to try to demonstrate reproducibility for selected tests and under select conditions.

Dr. Cameron Met...: 13:48 We also are working to develop a model of intra-amygdala kainic acid as a model of mesial temporal lobe epilepsy. And while I won't be talking about that today in depth, this is a model that's been around for quite some time, and we are working and considering use of this model in several different potential areas, again, as a means of looking at using this model for pharmacoresistant epilepsy, and also in performance area three, that I'll speak a little bit further about.

Dr. Cameron Met...: 14:23 I want to briefly just give you a sense of what the testing scheme is for performance area one. As we have worked to gear our testing scheme toward pharmacoresistant epilepsy, we have developed the flow chart shown here. Briefly I'll go over this, but the main point here is that we are seeking to provide program participants with a sense of dose range, efficacy, and tolerability for compounds as they advance through this program.

Dr. Cameron Met...: 14:59 So I'll just briefly go over this by stating first that in our identification phase, what that largely consists of is screening in what I'll refer to as acute seizure models, and what I mean by that is single administration of a compound, generally to naïve animals, as shown here in the 6 Hz and MES seizure models in both mouse and rat. Using these models helps us to understand dose range and tolerability that can then be informed for later assays and performing the program.

Dr. Cameron Met...: 15:32 We also perform a number of behavioral tolerability assays that include the mouse rotarod test, and also locomotor assay. And we've recently brought on board a modified functional observation battery for an Irwin screen that's commonly used by CROs and in pharma as a means of neurological tolerability assessment.

Dr. Cameron Met...: 15:54 We've also included the corneal kindled mouse model. Earlier on, during the identification phase, this has come as a recommendation that was given several years ago within our program to help to identify compounds that don't fit the mold, if you will, of some of the acute seizure models but still have demonstrated antiseizure efficacy, and the prime example of that is levetiracetam.

Dr. Cameron Met...:	16:20	Following the identification phase, we refer to the next phase as differentiation. And what I mean by that is that we're using models that are more etiologically relevant. They're often considered as chronic seizure models, and the examples I show here are the intrahippocampal kainic model that's utilized at SynapCell and also the lamotrigine-resistant amygdala kindled rat, and I'll speak about that briefly in just a few minutes. And also the post-kainate status epilepticus-induced spontaneously seizing rat that we use for compounds that demonstrate reasonable efficacy and tolerability in assays, and compound supplies allowed for this assay. We can perform this test in rats, using either IP, intraperitoneal administration, or a Drug and Food Administration over subchronic administration for five to six days.
Dr. Cameron Met...:	17:21	So if a compound is tested in this overall flow scheme, hopefully what we're providing is a spectrum of activity across several animal models. We don't anticipate that all compounds, for example, need to work in all models, but what we would like to provide program participants with is a sense of which models, or which conditions rather, a compound is working, and hopefully that is informative for participants as they're considering a development strategy.
Dr. Cameron Met...:	17:59	Differentiation: the testing scheme there is really intended to bring on more etiologically relevant seizure models, such as the spontaneously seizing rat, so that in contrast to many of the models used historically, where testing was performed in drug and seizure naïve animals, we're now testing novel compounds in the context of a diseased brain in the mammal model of epilepsy.
Dr. Cameron Met...:	18:28	We also employ both acute ... that is to say single administration of a compound ... or subchronic administration, which is five to seven days, in some cases longer, drug administration. Again, that helps to inform about efficacy and tolerability under these various conditions.
Dr. Cameron Met...:	18:48	We're using two species, both mouse and rat, and we can use various administrations: intraperitoneal, subcutaneous, oral ... and that also includes administration of drug-in-food, and I'll speak about that just briefly.
Dr. Cameron Met...:	19:05	I want to tell you first about the lamotrigine-resistant kindled rat model, and some of the things that we've learned as we've brought this model on board in recent years. So first, for those

of you that may not be familiar with the kindled model, the way this is used is an electrical or chemical stimulation is provided at low or subthreshold doses or currents. As that stimulation is provided repeatedly ... which is to say several days in a row ... eventually that subthreshold stimulation produces behavioral seizures. And in the case of amygdala kindling, what we do is implant an electrode in the amygdala, and initiate the kindling process with a subthreshold stimulation which, over the course of several days consecutively, begins to elicit behavioral seizures that we then quantify and record. And when an animal shows multiple generalized seizures over consecutive stimulations, that animal is considered kindled.

Dr. Cameron Met...: 20:11 It was observed in this model several years ago that administration of antiseizure drugs such as lamotrigine ... but other drugs like phenytoin and phenobarbital have been used ... when those are administered at a low dose during the kindling process, that they do not impair the kindling process in and of itself, which is to say that animals can still achieve a full kindled state. What happens though, is that these animals then are resistant to a later challenge of that antiseizure drug. And so, the purpose of this model is to really generate a cohort of animals that can be tested that have been exposed to antiseizure drugs ... in this case lamotrigine ... and have had several seizures, and therefore this is a model that we refer to as a model of pharmacoresistance.

Dr. Cameron Met...: 21:07 What we have observed as we've tested several established antiseizure drugs ... that are abbreviated here, and I'll try to point these out as we go through ... that several of the drugs, such as phenytoin, ethosuximide, lamotrigine, rufinamide, topiramate, and levetiracetam ... are given at doses up to a maximum tolerated dose, and are ineffective in reducing seizures in this model.

Dr. Cameron Met...: 21:31 Second, we also observed that when we look at tolerability in naïve animals, which is to say animals that haven't been kindled and haven't been tested in this model, we get a sense of what the dose range is, where we begin to see, for example, motor impairment. From that we can obtain a median tolerated dose or a median toxic dose, which is referred to as a TD50.

Dr. Cameron Met...: 21:57 However, when we move to observing for tolerability in the amygdala-kindled rat model, what we find is that in many cases, the doses that are used or the drugs that are used, are better tolerated. And it may come as no surprise that the examples that I'm showing here are sodium channel blockers such as

carbamazepine, lacosamide, phenytoin, and lamotrigine that are better tolerated in these animals that had been exposed to repeated administrations of lamotrigine.

Dr. Cameron Met...: 22:31 However, that's not always the case. What we observed with rufinamide is that while this drug is very well tolerated in naïve animals after single administration, we observe a marked difference in its tolerability, and saw that we couldn't go above a dose of 40 milligram per kilogram. So not only was it ineffective, but the tolerability had shifted, relative to what naïve animals experience.

Dr. Cameron Met...: 23:00 So let me just summarize by saying that we use this model as a late-stage testing screen for compounds that have advanced through the program. And we use it to help to identify compounds or differentiate compounds that work in this model of pharmacoresistance, and that can be really informative for participants that are working to develop compounds within this testing scheme. It's also very useful to us as we go into our latest stage or final stage testing, which is the spontaneously seizing post-kainate rat.

Dr. Cameron Met...: 23:39 And so briefly, I'll just describe the animal model to you for those that are unfamiliar. But following administration of the chemoconvulsant kainate in rats, animals will experience a period of status epilepticus. And for animals surviving past that initial period of status following kainate treatment, they'll go through what I'll refer to very broadly as a period of epileptogenesis. And what I mean by that is that we know during this period that there are a number of biochemical changes, for example, and neurophysiologic changes that are happening in the brain that is broadly defined as epileptogenesis.

Dr. Cameron Met...: 24:23 However, we wait for a period of about 10 weeks, and we do this because while animals do have seizures during this epileptogenesis period, they tend to, in a very broad sense, come to a local leveling off period, where they reach somewhat of a stable number of seizures across a cohort of animals after about 10 weeks. And so, we'll plant a radio telemeters in rats that have advanced through this period, and these radio telemetry units allow us to, in a minimally invasive way, monitor by video EEG behavioral seizure activity. And so when these animals are implanted, they'll go through an evaluation period for several weeks, during which time we'll quantify those seizures that are occurring. And then, much like with the clinical

trial, we use as an inclusion criteria, animals that are having a sufficient number of spontaneous seizures.

Dr. Cameron Met...: 25:31 So once we identify and enroll animals in those studies, the way that a study is performed is after a baseline evaluation of seizures over the course of about a week, we will enroll animals in a randomized cross-over design that includes both drug and vehicle treatment, during which time animals will receive five days of drug treatment or vehicle, and then be crossed over to the opposite group.

Dr. Cameron Met...: 25:58 And so, the type of data that we see in this model is shown here in the example of phenobarbital. So phenobarbital was given to animals over a five day period in a cross-over design at a dose of 30 milligram per kilogram by intraperitoneal injection once a day.

Dr. Cameron Met...: 26:18 And let me briefly just orient you to the type of data that we see from this model. So first on the far left, there's a series of 12 animals, and that's our cohort size for an antiseizure drug screen in this model. And on the far left is the baseline period that I referred to that, takes place over the course of about a week. And what we see during this period of time is that the animals that are enrolled in the study have previously demonstrated themselves as having spontaneous seizures. So generalized seizures are shown in diamonds here, whereas emerged a few instances of it here, focal seizures are shown here with the dots.

Dr. Cameron Met...: 26:59 And what we see, and we've seen this consistently across many cohorts, is that although we are seeing spontaneous seizures in this model, there is a great deal of variability, both within animals and between animals. And what I mean by that is in the case of this fourth animal here, you can see a seizure cluster that is occurring early on, but then no seizures afterward. Whereas other animals that are shown down here, A9, 10, and 11, have more sporadic, somewhat more consistent seizures. And then we have other animals, such as A12, that although they've previously had seizures, they don't have any seizures during the baseline period. In any case, these animals are enrolled into a study and they receive either drug treatment, which is shown here in red, or vehicle treatment, shown here in blue. And then again, as I mentioned, they cross over after a week in washout period and continue.

Dr. Cameron Met...: 27:56 So what this allows us to do is to quantify seizure burden, which is the sum of all of the Racine seizure scores that we observe,

and also look at the number of animals that experience full seizure freedom, meaning no behavioral seizures observed during the treatment period.

Dr. Cameron Met...: 28:17 We've evaluated several anti-seizure drugs in this model, and what we observe is that there are several compounds that can reduce seizure burden. But there are also many compounds, such as those shown here at the bottom ... valproic acid, lamotrigine, phenytoin, ethosuximide .... at the doses and the dose frequency administered are unable to significantly reduce seizure burden.

Dr. Cameron Met...: 28:41 The other thing that we observe is we also use, in very similar way to what's used in clinical trials, is trying to assess animals that are seizure-free after the treatment has been given over the course of five days. And we see that while there are several drugs ... phenobarbital is an example ... that can produce seizure freedom in the majority of animals significantly different than vehicle treatment does, we haven't yet tested any drugs that are able to produce seizure freedom in all animals.

Dr. Cameron Met...: 29:15 And so what this tells us is that this model is useful as an animal model of pharmacoresistance, and that we still have a ways to go in being able to identify drugs that can ideally produce full seizure freedom over the course of treatment as I've outlined previously.

Dr. Cameron Met...: 29:34 So moving on to performance area two; performance area two is specifically geared toward special populations of epilepsy, including genetic and acquired epilepsy syndromes. And this is a good opportunity for me to say that we acknowledge a lot of the work that is going on, both within NINDS and also with CURE and foundations such as the Dravet Foundation. And we work with the ETSP to try to plan our activities or our streaming programs so as to minimally overlap with other areas. And so the models that I'll describe here include the TMEV viral-induced epilepsy model, and also a screening model, a model of Dravet Syndrome that's in development. I won't discuss it here, but we also are looking at the pilocarpine-induced status rat model as a model of benzodiazepine refractory status epilepticus. And so, these are three ways that we can help to identify compounds that are potentially effective in special populations of epilepsy.

Dr. Cameron Met...: 30:49 Many of you may be familiar with the TMEV model. It is also referred to as the Theiler's Murine Encephalomyelitis Virus Model, which I'll refer to as TMEV. And this is a model of viral

acquired epilepsy. It's been observed in several studies, including many that came from our colleagues at the University of Utah, including our Director Karen Wilcox, that infection with TMEV, which is to say, intracortical administration of the virus, produces acute seizures, and that generally those seizures resolve after a brief period of time, but many of the animals go on to develop spontaneous seizures later on.

Dr. Cameron Met...: 31:36 The way that we're utilizing this model is to administer a TMEV, followed by about a week of observation and handling of the animals. And what we see is that animals exposed to TMEV have behavioral handling-induced seizures. And that really has allowed us to utilize this model as a screen for compounds with potential activity, and particularly compounds with potential anti-inflammatory activity. I don't have time to go through it today, but this model has been demonstrated to have a very strong inflammatory component, and so we have not only looked at antiseizure drugs but also anti-inflammatory drugs in this model.

Dr. Cameron Met...: 32:27 Primarily what we're doing is utilizing the occurrence of behavioral handling-induced seizures during the acute seizure infection period and administering the drug subchronically, which is to say once or twice a day prior to handling induced-seizures, to look at the antiseizure effect during this acute period.

Dr. Cameron Met...: 32:48 Having said that, although we're not currently using it in the program, we also know that many of the animals will go on to develop spontaneous seizures after the acute infection period. And that's something that has been largely described by Karen Wilcox, but others in the field have used this model and have shown that spontaneous seizures occur following TMEV infection. But again, the way that we're using this model right now is focused primarily on the acute post-infection period.

Dr. Cameron Met...: 33:24 In addition, we're also considering use of a mouse model of Dravet Syndrome. I won't have time to go into it in great detail. There are many different opportunities to study Dravet Syndrome using various models. Mouse models of Dravet Syndrome have been used to study the pathophysiology morbidity and mortality, and antiseizure pharmacology of Dravet Syndrome. What we're using is a little bit different from some of the other animal models that have been tested or published for Dravet Syndrome. Our animal model consists of mice that are heterozygous for this A1783V mutation. And it's a little bit different than other models, for example, that use a

knockout of the gene entirely. We've found, in bringing this model on board, that these animals have both hyperthermia-induced seizures and also spontaneous seizures.

Dr. Cameron Met...: 34:30 What I'm showing you here, if I can direct your attention to the black lines here in the middle, is that after we administer a vehicle such as methylcellulose and expose the animals to increase in core temperature by use of a heat lamp, that as we increase body temperature, all of the animals will go on to eventually have a seizure. And we use a top level threshold of 42.5 Celsius. And so, we can use that as a metric to compare drug administration against. If we treat with the sodium channel blocker, such as carbamazepine, we see that the threshold for hyperthermia-induced seizures goes down, and so the animals will have seizures at a lower relative temperature. That may not come as a surprise to many of you that are familiar with Dravet Syndrome and the warning against using sodium channel blockers, as they may worsen symptoms of this disorder.

Dr. Cameron Met...: 35:31 By contrast, we also can use clobazam, which is used clinically in Dravet Syndrome and we see that at a dose of 10 milligrams per kilogram clobazam, we can increase the threshold for hyperthermia-induced seizures. And so this gives us a good means of comparison, these two drugs, from which other drugs can be screened against, for example.

Dr. Cameron Met...: 35:53 We also looked over time across several different testing runs, and that's why these are shown here as multiple lines and why they're purple, black, or green. We performed the same tests four separate weeks in these animals to show that hyperthermia-induced seizures are consistent over time, and that the drug response is consistent over time.

Dr. Cameron Met...: 36:15 So one way that we can utilize this model is by following acute administration of a test compound and asking the question, "Does it reduce or prevent hyperthermia-induced seizures?"

Dr. Cameron Met...: 36:27 Another way that we can look at these animals has been spearheaded by a very talented postdoctoral fellow in our lab, Chelsea Pernici. What she has shown is that ... excuse me, I'm going to try to just get this video to play ... animals heterozygous for this mutation can go on to have spontaneous seizures.

Dr. Cameron Met...: 36:50 Looks like it won't play, but I can describe that briefly as that we're using ... oh, thank you very much ... tethered EEG. Animals are freely behaving, and what we see is that they go on

to have spontaneous seizures that generally consist of forelimb clonus, as shown here, rearing and falling, and this is very consistent with many of our other models looking at mouse video EEG behavior.

Dr. Cameron Met...: 37:25 Another approach that we can take is to take advantage of the fact these animals are having spontaneous seizures and then for example, apply a drug subchronically and ask the question as to whether it reduces these spontaneous seizures, similar in a manner to the way that I described the post-kainate rat model.

Dr. Cameron Met...: 37:49 Let's see if I can advance to the next slide now. Thank you very much.

Dr. Cameron Met...: 37:57 As I mentioned, we know that mice that are heterozygous for this mutation will all have hyperthermia-induced seizures, and that clobazam will elevate the seizure threshold and conversely that carbamazepine will lower the temperature at which seizures occur.

Dr. Cameron Met...: 38:16 We've also seen that cannabidiol and fenfluramine and other drugs do not perform well against hyperthermia-induced seizures, and so this is something that we are continuing to look at. And I think in many ways it's beneficial to know what the spectrum of activity in this animal model is, with respect to hyperthermia-induced seizures.

Dr. Cameron Met...: 38:36 And we have ongoing studies where we're looking at different drugs and different routes of administration, including drug-in-food, and asking the question of what the difference in pharmacology is for spontaneous seizures versus hyperthermia-induced seizures? Again, this is a model under consideration, and as we continue to work on this we will be working with the ETSP and potentially including this in the overall testing scheme for the program.

Dr. Cameron Met...: 39:04 Finally, in the last few minutes, I just want to say for performance area three, I don't have data to share with you today, but we're very privileged to work with our collaborator at the University of Washington, whose PI is Steve White. And we work with them, they're using the rat kainate model to perform antiepileptogenesis studies. What I mentioned before is, in the post-kainate spontaneously seizing rat model, whereas we're administering drugs to animals that are already seizing, in this paradigm, drugs are administered much earlier and we ask the question, "Does early drug administration prevent or modify the

progression of epilepsy and spontaneous seizures in this model?"

Dr. Cameron Met...: 39:53 As I alluded to before, we're also looking at the intra-amygdala kainic acid mouse model as a means of looking at disease modification. You might be able to imagine that if we were to administer a drug early on following kainic acid, we could then ask the question about spontaneous seizures that occur later on.

Dr. Cameron Met...: 40:13 I'd also like to point out that under the direction of my colleague, Peter West, we have been able to bring on board a very robust mouse video EEG monitoring system, wherein that we can monitor anywhere from 48 to almost 100 animals at any given point in time, and in the models that I've described, either the intra-amygdala kainate model or the Dravet model. And so this really allows us to be able to expand what our capabilities are as we are continuing to adapt to the needs of the ETSP.

Dr. Cameron Met...: 40:48 In summary, I would just like to point out that if we compare the models that we are either using now, or that we are considering using in the future, these are very different than the models that have been used historically within the program, and I've only been able to speak with you about a couple of these today. And so I think this highlights the changes that continue to occur within the ETSP and the contract site, and how we are trying to respond to what the needs are within the epilepsy research community.

Dr. Cameron Met...: 41:20 So how do we know if we're successful? Well, what we've seen thus far is as drugs come through our program, in so much as we've had a contribution to the development of those drugs, when they get into the clinic, we can have an understanding by comparing what it is that we saw with how those drugs eventually performed in the clinical setting.

Dr. Cameron Met...: 41:44 When we look at pharmacoresistant epilepsy, we are still burdened with a third of patients that are not responsive to current therapies. That number, as many of you may know, hasn't changed in recent years. And so I think as development efforts continue and hopefully as we're identifying compounds with novel mechanisms of action and new therapies, that we can begin to identify and implement compounds that begin to work at that one-third of patients that really need new therapies.

Dr. Cameron Met...:	42:22	Similarly, within special populations of epilepsy, I think this is an area that will continue to be of emerging importance, and that our contribution is only one of many as we're continuing to work in this area.
Dr. Cameron Met...:	42:38	Finally, while we don't have any approved compounds within the epilepsy research field for prevention or modification of epilepsy, I think recent work, such as a meeting that was held a few years ago that was geared toward antiepileptogenesis and disease modification strategies, may help us to collectively come together to come up with a strategy to help to identify compounds that could work in this area.
Dr. Cameron Met...:	43:06	And let me finish just by pointing out that we are very privileged to work with many individuals in this area. I've already spoken about this just briefly, but I'd like to reiterate the role of our Director Karen Wilcox and her leadership, my colleagues Peter West and Misty Smith, and our many lab specialists, analysts and other individuals without whom this work wouldn't be possible.
Dr. Cameron Met...:	43:32	We also are very grateful for our relationship with the NINDS and the ETSP, and as I mentioned before, please reach out to my colleague, Brian Klein, for any potential program participants that are interested in sending their compounds through the ETSP. And I did mention our subcontractors, but I'd like to point them out as well: SynapCell under Corinne Roucard, and the University of Washington under Steve White.
Dr. Cameron Met...:	43:58	I want to thank you all again for listening to this presentation, and please ask any questions, and please feel free to approach us for any questions after this presentation. And thank you again to CURE for inviting me.
Priya Balasubra...:	44:18	Thank you so much, Cameron. That was an excellent overview of the new models at the ETSP. I think we have time for a few questions, and I just want to remind everyone if you have questions, please submit them through the Q&A tab, which is located at the bottom of your Zoom panel, and then click "Send," and we'll try our best to get through as many of them as we can.
Priya Balasubra...:	44:42	I can start with one question that came in. They'd like to know in the intrahippocampal model, what areas do you record the EEG from?

Dr. Cameron Met...:	44:54	So I would refer you to our colleagues at SynapCell for greater detail; they published their pharmacology and their methodology recently. But these recordings are deaf electrodes in the hippocampus. And since I don't have any direct involvement with the actual performance of that model, I can't say more than that. But I can refer you to their publications.
Priya Balasubra...:	45:24	Thank you. And then another question is: in your testing scheme that you showed at the beginning of your presentation, is it a step-wise process to go from the identification to the differentiation step? Or how do you determine when a compound can move into the differentiation?
Dr. Cameron Met...:	45:46	Sure. Thank you for the question. So one thing that I'll say is that the decisions to advance compounds from one part of the testing scheme to another ... we're not always privy to those, and so I would defer to my colleagues at NINDS for specific examples.
Dr. Cameron Met...:	46:04	What I can say generally is that the scheme is designed for it to be step-wise, meaning that as a compound advances through the identification phase, it would be a candidate to move toward the differentiation phase. What you might also envision is that while there are many compounds that could initially be tested in identification, not all those compounds have been advanced.
Dr. Cameron Met...:	46:27	And so, there are some benchmarks and some go/ no-go decisions if you will, that help the ETSP in advancing compounds. There's a lot of different factors that can come into play, and not all participants enter the program in equivalent ways. Some have to enter and exit for various reasons. Others may be limited in what their desires are to obtain information from the program. So I think it really varies, but in a very broad sense, it is intended to be step-wise.
Priya Balasubra...:	47:04	Thank you. I see a couple more questions coming in. One is: which amygdala nucleus is injected with kainic acid?
Dr. Cameron Met...:	47:15	The model was designed around injection of the basolateral amygdala with kainic acid.
Dr. Cameron Met...:	47:22	And I should mention, this is also a good opportunity to highlight that we do have an upcoming manuscript that's being prepared that will describe some of our methodologies.

Priya Balasubra...:	47:35	Thank you. There's one more question here which has to do with: do you have any preliminary results in the intra-amygdala kainic acid model in mice, in terms of the seizure frequency and progression of the spontaneous seizures?
Dr. Cameron Met...:	47:51	Yeah, great question, and again I would refer you to a paper that we will have forthcoming. We've also presented these at AES in the past.
Dr. Cameron Met...:	48:03	What I can say very broadly is that we do see variable seizure frequency similar to what I described for the rat kainate model. In a very general sense, we see on average about one seizure per day, but that really can vary quite dramatically. We can have animals that have a very high seizure burden, and we can have other animals that don't have as many seizures, or seizures at all. And so we're trying to find the best way to optimize our use of this model, and that's going to be something that we're going to continue to look at going forward.
Priya Balasubra...:	48:42	When you do the tolerability toxicity assays, are you only working with models to look at motor activity, or are there other models that you look at for other types of toxicity?
Dr. Cameron Met...:	48:56	Yeah, great question. This is something that we've thought a lot about recently. Historically, the models that we used to look at tolerability were really geared toward motor activity, and so that would include the rotarod assay in mice. We used a modified open field observational assay in rat. And we also used an open field automated locomotor open field assay.
Dr. Cameron Met...:	49:21	However, in recent years, we've also brought on board a modified Irwin test. And while this isn't as comprehensive as many you may be familiar with, it continues to be largely geared toward motor activity, but it also does allow us opportunity to look at other potential means of toxicity, such as autonomic, audio-visual and others. And so, this is helping us, but for those of you that work with rodent behavior, there's only so much we can do, I think, to look at tolerability and be predictive of what happens in the clinical setting. But we have recently expanded our abilities to look at tolerability, particularly in rats.
Priya Balasubra...:	50:08	Wonderful. I see one more question coming in. They would like to know what mice strain do you use for the acute seizure, the 6 Hz instead of epilepticus, because different strains can have drastic changes with regards to the severity of this?

Dr. Cameron Met...:	50:27	Wonderful question, thank you, and this is also something we think a lot about. It does vary by model, so for 6 Hz and MES we use the CF-1 mouse strain from Charles River. For the corneal kindling assay, we have used the CF-1 animal in the past, however recently we've gone to the Charles River C57 black 6 model of mouse. For the TMEV assay, and for intra-amygdala kainate, we use C57s from Jackson. And so it does vary, but most directly to answer your question, we use the CF-1 mouse for our acute seizure assays.
Dr. Cameron Met...:	51:10	But I do take the point that there are notable differences, not only in seizures but also in seizure pharmacology.
Priya Balasubra...:	51:22	Thank you, and I think that is it with the questions.
Priya Balasubra...:	51:27	I'd like to thank you again, Cameron, for your time. And I'd like to say a special thanks to the Nussenbaum-Vogelstein family for their generous support of the Frontiers in Epilepsy Research Seminar Series. And I'd like to say thank you to all of you in the audience today for participating and asking questions.
Priya Balasubra...:	51:49	If you'd like more information about hosting a seminar series at your institution, or if you'd like to apply for one of our CURE grants, you can visit the for researchers page at our website, cureepilepsy.org or feel free to write to us at research@cureepilepsy.org.
Priya Balasubra...:	52:08	We have two more seminars coming up in this series, on October 21st and November 4th, so please make sure to register for those. I thank you all again, and stay safe.