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A Roundtable Discussion on Brain Connectivity

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Steven Laureys:

Welcome, everyone. It is an incredible honor to be here with such inspiring pioneers in the field of brain connectivity research. Today, our goal is to discuss the past and future directions of our field, focusing on both methodological challenges and clinical translation. As a neurologist, I have always been deeply interested in the clinical applications of our work. Since the late 1990s, I have been a strong advocate for the importance of connectivity studies, particularly in the context of patients with coma and chronic disorders of consciousness. Our research has highlighted how these patients suffer from disconnections (Laureys et al., 1999) and how they recover as they regain consciousness (Laureys et al., 2000).

I am especially grateful to Dr. Bharat Biswal, who co-founded this journal in 2011. Marc Raichle, who is with us today, published an influential paper in the very first issue titled "The Restless Brain" (Raichle, 2011). Marc, your pioneering work on resting state networks and the default mode network has been truly inspiring, enhancing our understanding of intrinsic brain activity. Since your seminal publication in 2011, what do you consider the most significant developments in our understanding of resting state networks? How has this knowledge advanced our comprehension of brain function and pathology?

Marc Raichle:

To tell that story, everybody here and many others have contributed over time. I guess I'd say it's interesting that there were hints about where this was going that go back many decades. Actually, a paper that always catches my attention was published in the 1950s by Nina Aladjalova (Aladjalova, 1957). It was a letter to Nature in which she talked about infraslow activity in the brain. When I read papers - anybody that cites Nina Aladjalova I know you thought about the history of this story.

There were other backgrounds historically in this. One of the iconic members of the neuroscience community at Washington University in St. Louis was George Bishop. He was the technical guy in the laboratory of two Nobel laureates as they were doing their work on the brain which led to their Nobel Prize- Erlanger and Gasser. He had this wonderful

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paper which he published in 1933, and what he was doing was to look at the response of the visual cortex when he stimulated the eye of a rabbit (Bishop, 1933). What he noticed was that the response in the visual cortex varied over time. It was response variability, which now there is a vast literature and it's related to the ongoing activity. First described by Nina Aladjalova, and then it was followed by Vernon Mountcastle, who was one of the great neuroscientists of our time at Johns Hopkins. A wonderful paper by Werner and Vernon Mountcastle (Werner and Mountcastle, 1963) again pointed out that the variability in cortical activity and responsivity to ongoing activity to evoke our task related activity. So this was all brewing in the background.

Then along came Seymour Mety and Lou Sokolof. Using Kety's technique where they measured - they stuck a needle into the femoral artery and another needle into the jugular bulb - and I've had this done to me - and what they asked their subjects to do was difficult mathematical calculations. The idea was that this would be provoking increased activity - and we of course know that that is a fact.

What was so interesting about this paper was that they saw absolutely no change in the overall metabolic rate of the brain, looking at the whole brain. It's surprising to me how little attention that particularly seminal paper received. It basically said that the ongoing activity of the brain and its cost was ever present. What we added to it - as I am saying now, talking to you - if I were in a scanner the change in my brain metabolism as a result of this is very, very small. But the importance of that of course laid the groundwork for this notion that there was a whale of a lot going on in the brain that was costing a lot of, if you will, energy as related to the rest of the body. We had no account of what in the devil that was all about.

Anyway, the story unfolded: there was this whole story about when we were doing measurements of various tasks and how things changed was, "wait a minute - what about the baseline level of activity?"
Can we assume that we have a baseline and that when we insert something else, some task that we have- what's called pure insertion. That is, we didn't change, nothing in the brain changed as a result of that. So, what we observe as a difference was exactly what

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took place. It was prompted by this challenge of, "What's the baseline here?" I just kind of casually started reversing subtractions. And, much to my surprise by God, there was stuff going down - as if something was going on prior to our engagement in the task as it was going down. There were all these different pieces out there kind of suggesting that there was something deeply important going on in the brain both from the neurophysiology and the energy and so forth that we were not accounting for.

So, then Bharat Biswal comes along and he notices that if you know where the motor hand area is and you just ask the question, "What is this noise? How does that relate to what the rest of the brain is doing?" By God, there was the entire motor system of the brain going along in a rhythmic sort of way. So being around and during all of this and reflecting on the background, I consider myself rather lucky to have timed my career to collide with all of this background information about what the brain might be really up to.

I think it's both incredibly interesting as we look at this large-scale integration of how the brain is operating based on resting state studies and also how that relates to the brain and the body relationships. Like the work of Peter Strick: the brain is talking to the stomach and the stomach is talking to the brain, surprisingly the hippocampus of all things. So, this large scale complex system approach, that Karl has been a real pioneer in thinking about all of this, is surfacing in a major, major way. What we're faced with is something that I think has immense importance from a therapeutic point of view: understanding how the brain works and how we might deal with it. It deals with the fact that we have among our networks in the brain this ongoing conversation, if you will, that can be unidirectional, can be reversed and all of that.

There was a paper recently - and I'm not here to advertise what I've been involved in - but it's a nice example of work that came out of Stanford University by one of my graduates, former graduate students, Anish Mitra. Stanford has developed a helmet that you can wear that produces transcranial magnetic fields that can be stereotactically targeted at particular areas of the brain. What they observed at first off was that people with treatment-resistant depression had a conversation between two areas in the prefrontal cortex and elsewhere that was reversed. It was going in the wrong direction. So the target

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was this area and the effect was to reverse that process and, lo and behold, these people were cured of their depression. This was published in PNAS a short time ago - but there are other examples of this. This of course brought memories of my time as a medical student on the psychiatry service where we witnessed electroconvulsive therapy, which is a crude thing that's still being used - and we still don't have a good understanding of what in fact is accomplished - but it is effective.

I was thinking if we can approach this in a much more sophisticated way, based on the ongoing activity of the brain and the conversations among these networks, this is really a revolution in how we would take the information we're getting about the ongoing activity of the brain and translate that into an understanding of disorders of the brain. Psychiatry is a bushel load of things of that sort. Anyway, it's really been a privilege to somehow or other have had a career that allowed me to witness all of this and all of this stuff that's ongoing. So that's kind of where I'm coming from.

Steven Laureys:

Incredible, very informative 10 minutes, Marc. Where we come from, the brain-body relationship you mentioned, and then the possibilities of neuromodulation in the field of neuropsychiatry. Thank you so much. I hope there will be time to come back to the many points you raised while now giving the mic to Karl, who also wrote in that very first issue of *Brain Connectivity*, the wonderful paper on functional effective connectivity (Friston 2011).

You are one of the masterminds of SPM free energy principle, developing dynamic causal modeling, helping us to understand brain connectivity and function. So, same question to you Karl: How do you see, with the years of experience behind you, where we're going in the field of the methodological challenges, modeling, and theoretical models in understanding the brain and applying it to help clinicians do a better job?

Karl Friston:

First of all, thank you so much for inviting me to participate. It is a great privilege to follow Marc. If I remember, my paper was the second paper (Friston 2011) after Marc's in the first issue of brain connectivity. I can't give the scholarly background that Marc did,

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because I am from the generation that followed him. But I do remember developing an understanding of the basic principles of functional brain architectures by listening to his generation. People like Vernon Mountcastle, Gerry Edelman, and their colleagues, Horace Barlow and Semir Zeki. There were two sets of questions that inherit from neuropsychology pertaining to functional localization and integration.

Having a view of the brain in terms of functionally segregated regions — and the cartography problem that ensues from that picture of the brain — was one perspective. But the other key perspective was brought to the table — in terms of understanding distributed processing — was the notion of functional integration. Again, something that Marc foregrounded in his review. Functional integration became really important from my perspective when considering how to make sense of brain scanning data from schizophrenia. The ensuing technical work — Steven referred to — like dynamic causal modeling its forerunners focused on functional integration and distributed processing. I remember Randy Macintosh looking at structural equation modeling and others earnestly studying Granger causality. We were asking the question: can we now move beyond cartography and start to understand the coupling or the connectivity among different areas that underwrite the integration of brain regions and how to they coordinate with each other? So, much of the development and the history — that I was involved with — was essentially providing a way of characterizing functional connectivity in terms of directed connections among different brain regions or sources. The short version of a very long story is you have to have a hypothesis or a model underneath your data. And, in brief, that is dynamic causal modeling, having a biophysically plausible model of the way in which neuronal processes influence each other and are influenced by each other.

Why is that relevant for schizophrenia or — that's my preoccupation — why is that relevant for neurology and psychiatry? It's become apparent that many neurological and psychiatric disorders can now be framed in terms of a pernicious kind of synaptopathy. When we're talking about the psychic disintegration in schizophrenia — in a Bleulerian sense, we're not talking about lesions to the organs of connection (white-matter tracts) — this is Wernicke's sejunction hypothesis that Danny Weinberger articulated beautifully. However, I think that was a wrong kind of picture of the failures of functional integration

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in neurology and psychiatry. It has transpired is that the failures in question are a subtler kind of disconnection; in the sense that it is primarily a failure of synaptic connectivity. In particular, the modulation of synaptic efficacy through neuromodulatory mechanisms that could range from classical neuromodulatory transmitter systems through to fast synchronous exchanges between pyramidal cells and inhibitory interneurons.

So that, to my mind, is the offering of brain connectivity – as a method or a principle that one can apply to functional integration of the brain. How would you apply brain connectivity? Well, it gives you an *in vivo* assay of synaptic integrity and its changes due to psychiatric illness or interventions, pharmacological, or TMS and the like. So, I imagine — and indeed fondly hope to see – an integration or a convergence of these noninvasive tools to get a handle on synaptic integration and synaptic efficacy, our understanding of the molecular biology of plasticity and its modulation, the way in which different brain states contextualize our sense-making, and all the kinds of inferences we rely on — and that fail in various psychiatric conditions. In short, I would see the future of brain connectivity as a tool to provide another window or perspective on the mechanistic approaches to understanding disorders in biological psychiatry, but also from a more cognitive and psychotherapeutic point of view, using advances in things like functional genomics, brain stimulation and the like.

And I suspect that Susan's going to speak to these applications after me. So, that's where I would see the future.

Steven Laureys:

Thanks so much and for making the bridge directly. Thank you for that, Karl. Now to Susan who published in 2012, the CONN toolbox, one of the most cited papers from the journal and the correlations in these neural networks (Whitfield-Gabrieli and Nieto-Castanon 2012). So, Susan - again - you with your background: how have you seen the field evolve in terms of understanding mental health disorders and the potential of brain connectivity biomarkers in both the diagnosis and the treatment of these challenging conditions? Again, big thanks for being here.

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Susan Whitfield-Gabrieli:

Thank you, Steven, so much. It is such an incredible honor to be here with these tremendous neuroscientists and psychiatrists and I would love to talk just a moment about the CONN toolbox.   I give full credit to Alfonso Nieto-Castonon, who's my wonderful colleague who developed that toolbox. We developed the toolbox in a time when people were questioning resting state networks in terms of their relevance in the context of these low-frequency fluctuations being contaminated with physiological aliasing.

When I met Alfonso, I wanted to do a different way of cleaning up the data, if you will. So, we ended up implementing the anatomical CompCor (aCompCor) method of noise reduction. And we were doing this at a time when Birn and Bandini (Birn et al., 2006) and Murphy and Bandini (Murphy et al., 2009) were highlighting really important issues. Instead of doing global signal regression, which mathematically mandate these anticorrelations, we decided to do this aCompCor method of noise reduction, which would allow, we thought, us to interpret these default mode network anticorrelations, which we're very interested in for a number of different reasons.

We do think in some ways they're a proxy or do correlate with cognitive performance - that there's significant decrease in many different psychiatric populations who have cognitive impairment. We even think that in some ways, as with our work together, they can form some approximation of consciousness. We've been very interested in that specific feature and that will kind of be a thread along the next part of the conversation.

In terms of the future of brain connectivity, I think the future of brain connectivity largely relies on the plasticity of these brain networks as both Marc and Karl were talking about and the possibility of using these networks as targets for precision network therapeutics. In our case, we've been using real time FMRI neurofeedback to show individuals - mostly patients with psychosis, anxiety, and depression - how to modulate their own individualized networks. This has been tremendously rewarding for the patients and for the researchers because it gives these patients agency, rather than being the recipient of a drug or a TMS or deep-brain stimulation or any other form of treatment that might be

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applied where they feel like they're just receiving a treatment. This is really an opportunity for them to be an actor in their own play.

It also allows us to have the ability to modulate our neurodynamics on a more network level with neurofeedback, which might be a more effective method of neuroregulation than neuromodulation involving a single region or anatomically unspecified pharmacological interventions. So we've been really excited about this precision network therapeutics and not only do we see mitigation of clinical symptoms and improvement in attention with real-time FMRI neurofeedback of these resting state networks, but in addition, I think future use of intrinsic network connectivity might aid precision psychiatry. That is, you could form perturbation indices, which would basically be the change of network connectivity pre- and post-perturbation. That perturbation can be anything - it could be real-time FMRI, neurofeedback, TMS or an SSRI - but the idea would be that you would take a resting state measurement before and then directly after that perturbation and look at the malleability, elasticity or flexibility if you will, to that particular perturbation. That may be the best predictor of treatment efficacy in the long run.

There's already been a paper showing that five hours after the administration of an SSRI, changes in brain connectivity can predict treatment efficacy in depression. I mean, that's just one paper, but the concept I think is brilliant and a beautiful way that we might be able to use resting state network connectivity. In addition, we're also really interested in using real time FMRI to trigger individuals when their resting state networks may be in a physiologically vulnerable state of being. So, you could imagine that if you use real-time FMRI to track the default mode network, you could trigger experience sampling where you would ask the person what they're experiencing. If you could continue to track the individuals' default network and trigger again and again and again until you get a series of experience sampling questions that would allow you then to go back in time and look at the connectivity matrices, the FMRI as well as physiology, that then might allow you to form a predictive model for that individual so that you could identify the individual network architecture that preceded a particular mental feature or clinical symptom with a

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goal of then being able to build these scalable predictive models that can trigger just in time adaptive interventions.

So, I see a great future in brain connectivity.

Steven Laureys:

Thank you. Thank you so much. So, Jennifer, I would like to hand the mic to you and, first of all, thank you because you've been very active as an editor within the journal for Paul. You focus on brain connectivity changes when we get older and how it relates to a number of diseases, cognitive decline, dementia. So, in that field, how do you see the study of brain connectivity as useful to deal with what obviously is a big, big challenge for the aging society?

Jennifer Whitwell:

Okay, well thank you for inviting me to speak today. I feel I'm here with giants, and I hope my contributions are helpful. I realize I haven't published in the journal yet either from everybody speaking - so I really should get on that! I joined the journal last year and thank you for commenting. I felt like I've been very slow in my responsibilities to the journal, so I'm glad you appreciate those contributions, I'm not as bad maybe as I think I am.

So, I really focus on clinical translational approaches and using neuroimaging. Connectivity is sort of a part of what I've been doing over the last 20 years really. I focus on, just to give you guys a background, a lot of different neurodegenerative diseases that includes Alzheimer's disease and particularly different clinical phenotypes of Alzheimer's diseases, but I also have focuses on movement disorders like progressive supernuclear palsy and also speech language disorders.

We've been doing a lot of work in patients with progressive apraxia speech - problems with their speech and their language. Of course some of those diseases overlap with each other and I've been using connectivity, both in resting state connectivity but also diffusion tensor imaging. I think another aspect of connectivity that perhaps we should cover in the

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journal (and is covered) is structural connectivity and how we can put structural and functional connectivity together. I've been using both kinds of techniques over the years.

Our work in Alzheimer's disease has really been focusing on looking at different phenotypes of Alzheimer's disease, not just the typical amnestic AD. We've been looking at how networks are broken down in these different phenotypes. There's a lot of similarities and differences depending on the clinical presentation of Alzheimer's disease with changes in, for example, the default mode network. Pretty common across all the variants of Alzheimer's disease, but each variant having their own specific networks that they're strongly targeting.

I think we need in the future to understand that a little better and why that's occurring. We've also looked at how connectivity is breaking down within those networks, but also between the networks. There's a lot of different moving parts and connections increasing and decreasing between different networks in these different diseases.

Progressive supranuclear palsy has been a really interesting disease for me. It's slightly simpler than Alzheimer's disease, but we've been able to show using tractography - white matter tractography - and also resting state, a real defined network of involvement in PSP that's centered around this dentatorubrothalamic tract starting from the cerebellar dentate - the tract goes up into the brainstem and then through up to the corte. Essentially you have this axis of involvement in PSP, and you can use these techniques to really illustrate that degeneration, both functionally and structurally, of that tract. Similar in the apraxia of speech patients, we see very focal patterns of disruptions in connectivity and that seems to relate to a lot of other aspects of the disease.

I think what I'm most interested in perhaps now and going forward is what connectivity can teach us about disease mechanisms in these different neurodegenerative diseases. There's a lot of different aspects to that.

So, one would be how is it governing disease spread? There's a lot of work out there looking at how connectivity relates to protein deposition in these diseases, and we've started to do some of that kind of work as well, and that connectivity is determining how the protein spreads through the brain and determines everything else in these patients.

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How does connectivity relate to perhaps vulnerability? Why do some variants of Alzheimer's disease target the visual network and others target the language network and how does connectivity play a role in that? So, determining spread and vulnerability and, really, why these patients are presenting with these different spectrum of clinical phenotypes.

I think the other interesting thing that we've been looking at and, for the future, is how functional connectivity is related to a lot of other different imaging modalities.

So, there's a lot of multimodal analysis we can start to do. We've shown that functional connectivity breakdowns are related to rates of atrophy in some of these patients. So, it determines how fast patients are therefore going to decline, which could have really important clinical outcomes. If you can predict with your connectivity at baseline what's going to happen to that patient - how fast they might decline, how their syndrome might spread - then that could be really useful clinically. Also, understanding how the functional connectivity is related to the structural connectivity - and that's been a little challenging to actually find where we think it's related. As the disease spreads through these networks - that the white matter tracts are going to degenerate as you spread - but finding connections between them and proving that this is all a network, the function, the structure and how that breaks down and determines spread, determines the clinical syndrome.

Proteins we can measure with PET - that's what we've been doing a lot of. We can measure tau, we can measure amyloid in the brain. We can look to see how - and we found good relationships between the tau and the functional connectivity supporting this idea of the functional connectivity determining spread.

I guess the last thing I would say is biomarkers as well, whether we can use functional connectivity. I think maybe we're a little further from that: can we use connectivity as some sort of individual level biomarker either to track change in patients,or to predict change in patients? And that has been a little bit more challenging. Connectivity can be pretty variable at the individual level, but I think there's still growth there that's needed to

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determine how best we can harness the connectivity to make individual predictions or a diagnosis.

So, I think overall maybe multimodal approaches are really interesting to me and looking at how connectivity is really contributing to disease mechanisms in all of these different neurodegenerative diseases we study. And of course, they're all very different and target different regions of the brain and connectivity is an important component of that.

I haven't done so much in aging, Steven, so you mentioned aging. It's really mainly disease. A lot of these diseases are diseases of aging, but not necessarily normal aging. I haven't done a lot of connectivity with the normal aging spectrum. It's really more these different diseases and how connectivity is related to all the other different aspects of disease we can measure on imaging.

Steven Laureys:

Thanks, and by the way, this will then be your first paper in *Brain Connectivity*. So thanks for bringing up the multimodal imaging challenges and that really makes the transition to Vince.

Vince, you've been publishing prolifically in that field - multimodal imaging data fusion - integrating the different imaging modalities to understand brain networks. So, my question to you would be, well, how do you see the challenges of multimodal integrations in brain connectivity research and where do we come from and where are we going again, if possible, with the clinical translation? Thanks, Vince.

Vince Calhoun:

Well, if I could, I'm going to back up a little bit and start with just when I got kind of interested in brain connectivity and functional connectivity. coming at this as an electrical engineer who ended up working in a psychiatry department, I kind of felt like I was an engineer who was analyzing the psychiatrist; so I was trying to figure out what they need, what sort of problems they need to solve, et cetera. Karl of course is the exception here, but basically trying to come up with approaches to answer the questions that were

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constantly being asked. One of the things that really got me into this - obviously I've done a lot of work with independent component analysis - was kind of thinking of the data as essentially a time series or a signal or an image - and then we're looking at patterns, we're looking at sources, things like that.

I still remember kind of discussing a lot of this stuff in the early days. In 2001 at Brighton, on the beach Christian Beckman and I were kind of batting some ideas back and forth about ICA early on. So of course, this kind of thing isn't new. Partial Least Squares you mentioned applied to PET data in '96 with Randy Macintosh's work and lots of multivariate type approaches have been applied. This is really something that we focused on for rest FMRI. I'm really trying to say: there's a lot in this data. We don't know what's going on necessarily, so let's use higher order statistics to try to identify, use this information to separate the signals. Then that ended up looking like brain networks. Obviously, there were a couple of early papers on ICA as well that showed that.

For me it was really about, well, how can we use that to do something? It was kind of initially hard to think about how we make any sort of inference from these? We're just getting these things out - what do we do with them? So that's what led to doing approaches that would provide some sort of inferential framework for data-driven approaches; and so that's kind of the principle behind the group ICA approach and other things, which is we want to make individual subject inferences but in a common framework somehow. I think this was, for me, really exciting. We've continued to use approaches like this going forward.

Then to your other question, we started bringing in multimodal data into this equation as well, which is, I think, was one of the first two papers in *Brain Connectivity* that we published in 2011 and 2012. One was like, what is a network? Define what a network is, right?

There's many different definitions for that. Is it a pattern that you see if you used a linear model and got a pattern, is that your network or are you really interested in how do you actually directly connect things to one another or couple things to one another?

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Then another one was looking at structural covariation, which we call - we did this with ICA - we called it source-based morphometry. Essentially if we look at covariation of gray matter voxels across subjects, you get out, if you have enough data, you get out patterns that look very much like resting networks. They're not exactly the same - they're not as specific. We might find one that represents two or three that we get in rest FMRI. That was really interesting to me, and it made me start to get more into the multimodal side of things and try to look at what can we learn with this information.

I think to Jennifer's point, having structural connectivity and functional connectivity, but also volumetrics genomic data, all sorts of information. We've got so much all of the clinical, the behavioral data, all of that should really be integrated so that we can learn from it. It's really pretty easy to show in simple examples that if you have two variables and there's some shared information between them, it can be kind of masked if you look just at one of the variables. If you put them together, just think of a PCA plot and, if you just draw the line this way, you start to see things separate. So, it's simple, in a sense - but then how do we do that in a way given all this data that can be noisy?

So again, kind of continuing the same framework, thinking about using higher order statistics, using multivariate approaches and trying to extract these patterns. All of this is kind of in service, in the back of my mind was: we really want to get at some - we want to study what's going on. We want to learn about either clinical conditions or developing brain or aging brain, et cetera. So that kind of led us to approaches where we kind of try to bring together data-driven approaches with priors. We want to sort of try to automate these approaches and try to come again to a prediction that we can make or a description of what's going on. What are the neuroimaging factors that are sort of linked to these kinds of questions? If we want to look at a response to medication, can we predict a medication response using resting networks in the context of, for example, an ICA model? You can do that quite well, so I think there's a lot of ongoing work.

I'm still very optimistic even though FMRI in general has struggled with really sort of killer app - clinical applications, so to speak. I think that that is a real concern, but I think there is

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some progress being made in various areas. Susan had mentioned some of the work she's doing as well, which is really important.

 I think it's this cycle of … we're trying to answer this particular question, but we're also trying to de-noise the data. We're trying to understand what are the signals and what are the features that are relevant. Then if we do it a different way, we get a slightly different answer - and which one's right? How do we kind of put all that together? So, I think we've gone around this circle, I think, a few times, at least in my career, and I have learned a lot.

So, I'm kind of very optimistic about that.

I think we're still early in this, but bringing together functional connectivity with models, trying to get at what are called foundational models - or can you learn everything? Can you learn all the relationships from the data and then ask a question that slices through it in a certain way. I don't think we're there yet. I think there's too much that we are still trying to learn about, depending on how you set up your model, what the output is. But I think this sort of, again, extending flexible modeling, multivariate modeling, deep neural networks, et cetera, is really going to help us, I think, move forward in this field. I think we also have, just as a warning, a lot of noise in the results that we're seeing right now that we have to filter through.

There's so many papers that come out and some of them, the way they're done, really makes a difference in terms of what the output gets. I don't want to say anything about anybody else's papers, but with my own papers, to go under the hood a little bit, we have this cycle where somebody will come up with a result and we'll talk about it. But it'll be like, I've got this great prediction and it's exciting and it's impactful, and then we'll ask some questions. Well, let's look at what it looks like. Can you show me a picture? Can you try to go back to the data?

Then we'll see this kind of random speckly ugly looking thing that doesn't relate to anything. Then we'll be," oh, actually there's a problem in the code here. We've got to fix this and this and this…" We go through this all the time and finally we get a result and we put pictures in our papers, right? Because we've worked so hard to get them. Of course, we have to validate these in independent data and ensure that it's not just a result that

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we've found by hacking through our data. But I see so many papers that don't have any pictures of anything brain-related in it that just makes me wonder - did they go through this process? If you go through this process, you're going to show what you found because it took you so much work to get there. So, it's sort of a note of caution. It's always been the case I think throughout history about modeling, modeling of data.

Those are just a few thoughts that I've had. I think, again, bringing together lots of data with flexible models and trying to sort of automate those as much as possible is really important.

One last thing I'll say is there's a lot of data now. We can get data - and that's great - but there's a lot of people using the same data. So I feel like we're going to have a little bit of a circle of bias that might self-perpetuate unless we're careful. I think Tom Nichol had said everybody should have a lifetime multiple comparison setting on their CV or something like that. So we've worked with enough data that ours - we would never find anything I think if we did that - but hopefully someone else then can.

Anyway, I think I've used up my time, so I'll go ahead and stop there.

Steven Laureys:

Thanks for bringing up a number of important points and the importance, of course, to go back and look at, actually, the data and the images. Next, Linda, we're going to talk about your work in neurological conditions, epilepsy, brain tumors. We're all here because we're fascinated by brain connectivity. How is that helping in these fields and when you see the future, how do you think this is going to go?

Linda Douw:

Great question and *bedankt* for asking me to be here as well. It's very exciting to exchange thoughts and maybe also a slight throwback because I guess I came to the field in a slightly different manner than some of you because I'm a clinical neuropsychologist. I was mainly intrigued by this problem that many people with brain disease had, especially people with brain tumors who have a very circumscript lesion in the brain, but their cognitive deficits

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were all over the place and also their quality of life and other types of symptoms that they had from the tumor were really difficult to understand - still are. That brought me to the field of complexity science and oscillations. I come more from a neurophysiological background and I was trained by Kees Stam who was one of the, I would say, godfathers of neurophysiological network science connectivity.

In 2006 I started my research into how does brain connectivity - mainly physiologically in the beginning - change as people have a brain tumor? That's where it started, very descriptive, although it made total sense to me that everything is related in the brain. So, in that sense, I found that the conceptual framework of graph theory was very useful to better understand that these focal lesions had widespread effects on patients or no effects at all - even if they were somewhere in the network. As we've progressed over the last almost 20 years, I continue to be amazed by how this network perspective helps me understand better what is contributing to human behavior.

Some of the main points I've learned about that - and that I take with me for the future perspective - will be, first of all, that the case-control studies that were very important for our field, and that have made very important contributions, we are gradually leaving behind. Rightfully so, because no patient was ever like an average of the healthy controls.

I think a lot of work also in recent years has shown that no one is the same and that individual variation is the very foundation of what makes us human and what makes our behavior differ. And by throwing out those variations, even in the healthy situation, I think we're sort of obscuring these effects that we need to focus on more. So some of the recent work I find really interesting in that space is on individual differences in functional connectivity and networks - like the very recent paper by the group of Caterina Gratton on boundary and ectopic variants of functional connectivity showing that almost everyone without a brain disorder has these islands of functional connectivity that are very different from the mean of the healthy controls and that relate to behavior (Dworetsky et al., 2024).

That's something that we also see in our clinical data. So, in the brain tumor patients, at first we thought, "Oh, patients have a brain tumor that impacts connectivity in the brain!" but more and more we're finding out that the brain tumor actually also may develop as a

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result of connectivity patterns, or at least there's an association between these two; and, moreover, that there's an interaction between brain connectivity and tumor growth. So, this is seminal work from Michelle Monje who did this in preclinical studies, but synaptic connectivity and activity determines whether a tumor grows slow or fast, which means that the pattern of connectivity in the brain directly impacts whether a tumor grows (Venkatesh et al, 2015). This is all the more complex as, of course, the connectivity also impacts or relates to how patients behave in terms of cognition and other types of functional behaviors.

In recent years we've been focusing on this basically multidimensional network of connectivity in all sorts of ways. On the one hand, we have the standard connectivity based on fMRI, MEG-EEG, structural connectivity, but I would also say networks of connectivity at the behavioral level. Symptoms rarely come in isolation. So, at the behavioral larger level of the individual, we also need to take into account that there's additional complexity that we're not taking into account when we simply correlate one behavior to a connectivity pattern.

On the other side: the cellular pattern. We're also doing - and of course the brain tumor population is a very good and unique population to do this - we're studying cellular principles or characteristics that could relate to these larger scale brain networks.

If I think about the future, in addition to all the things that were already mentioned, I would say computational modeling will be more and more important because of course we're all looking for this predictor that we can first do a measurement of brain network or brain connectivity and predict whether patients will respond to treatments, or target our TMS, or all those things. But I think something that could really help in this respect is to have a computational model that simulates what will happen after diverse interventions, perturbations, or disease progression. This will, I think, also help us to sort of trace back what we can't do now.

In my field in neurooncology, we can't of course measure brain networks before the tumor occurs, but what we do see is that if we look at the healthy brain networks, tumors tend to occur in regions that are highly connected in healthy people. So, my question would be:

can we back trace somehow through computational modeling and see whether we can better understand what is happening over the entire disease course before the diagnosis was made? Of course - especially as we try to intervene in these patients through different treatments - I think one thing that would be super important here also in light of the structure-function relationship that has been mentioned before will be to develop models that are adaptive so that they can generate function based on structure, but that the resulting function can also back impact the structure itself. Because this is of course how the brain works.

If we change our functional connectivity, the structure underlying it will also change. That's something that right now is very difficult to do in computational modeling - but having some grasp, or more grasp on that - I think will help us to virtually simulate both disease progression and interventions in all of these sorts of patients (which I guess would also help with a more general aspect of sample sizes). We have a lot of huge data sets that are in the healthy subjects or in larger disease or disorder populations, but that will be impossible for some of the more rare types of diseases. Having a virtual set of tools could really help in that. That would be my bet for the future.

Steven Laureys:

Thanks again. Wonderfully on time for Melanie. Last but not least, you and I were both fascinated by consciousness and neuroimaging. So, same question here - what are the challenges when it comes to that big question: how can we reduce our ignorance when it comes to better understanding our internal universe, thoughts, perceptions, emotions, and the neural code of consciousness through the study of brain connectivity? Melanie?

Melanie Boly:

I wanted to thank you, Steven, for inviting me. It's an honor for me to be here among so many pioneers and also dear friends like Steven and Karl, who have mentored me and continue to mentor and teach me over the years. I'm really happy to be here. Like Steven, I'm a neurologist. We started a while ago doing research on coma in Liege. I wanted to get back to that story where we started to look at some measures of level of consciousness

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using stimuli together. We were very influenced by, Marc Raichle and Bharat Biswal and all that emerging literature on resting state, and that idea that the brain is doing so much that intrinsic brain activity seems to be much larger than what actually is accounted for in the responses to stimuli - and then we thought maybe there's some consciousness there.

We started to look at that and, indeed, there seemed to be the case that connectivity was very decreased in coma or anesthesia or sleep. We continued that interest over the years. I also explored different techniques that looked not only at the amount of connectivity but really the structure of it -that combination like Karl was saying about integration and segregation together. We also saw that that was actually even a better predictor, this kind of combination of differentiation and integration in the brain, for being conscious and also the importance of feedback on connectivity. I did some DCM for EEG with Karl and Steven and we saw that, both in coma and around anesthesia - I'm getting new data on sleep now - it seems that that directional connectivity is very important, that feedback connection in the brain is very important for consciousness.

With Marcello Massimini and Giulio Tononi, we started to develop new tools. We're excited to see that we have a very accurate consciousness detector, like a measure for being conscious versus none that really works across different states like sleep, anesthesia, coma. It's a combination of transcranial magnetic stimulation with EEG. It's able to pick up at the timescale of neuron interactions, that differentiation and integration together. It really works a hundred percent of the time. When we did that in validation data sets, subjects can tell us if they're conscious or not.

Now, as a neurologist, I'm excited about this.B ecause we have that tool, we can now apply and go in the ICU with eyes on the structure, the organizational brain connectivity, intrinsic activity, to try to diagnose COVID consciousness, how patients are going to recover. We're also trying to understand what type of connectivity - long range, short range, thalamocortical - is linked to that complexity in the brain so that maybe we can find some new interventions to wake up patients in coma quicker and improve outcomes.

That's something where I'm really excited with the progress that I see coming from these past few years, where the field was emerging and our understanding of connectivity

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leading to these clinical applications. Another thing that was mentioned - and is very important to me to understand - is this link with plasticity - as Karl mentioned too across different scales - try to link the plasticity changes that actually start to be better understood at the micro level to the large scale networks. We see, for example, over the last, I would say, decade, there has been quite solid evidence in animals that plasticity at the synaptic level is heavily regulated by sleep versus awake. There was the work from Chiara Cirelli - and now another paper just appeared in *Nature* confirming this independently - that it looks like there is a net potentiation of about 20% of synaptic strengths when you're awake, decreasing during sleep. That's a massive change on the micro level that, for me, would be very interesting to link to this larger scale network. Also, what's going on in disease so that we can better understand, not only where but also the kind of manipulation, and the time of the day or the neuromodulation pattern that actually are best to induce these plastic changes for therapy.

In that context, too, having that better understanding of the multimodal level has been mentioned - the multimodal structural and functional MRI, for example. Incorporating this EEG - intracranial EEG - data that we have now in humans. You have single unit recordings in humans in a mechanistic model. It's really something that I feel is very appealing in finding the best interventions in terms of how exactly we should manipulate these networks and also find some personalized approaches.

Back to the dynamics of modeling or computational modeling, I think the way to go to try to bridge this case together is having these biophysically informed models like Carness has been developing, and more people are doing, to try to link the whole picture together.

Steven mentioned consciousness - we still remain convinced, right - even that there's a lot of this intrinsic brain activity and consciousness that aren't actually related. Most of what we are is also interacting with stimuli - but we are much more than that. There's a lot to understand about how we feel the experiences we have - as Susan was saying, try to push this experience-sampling approaches and link phenomenology to brain metrics. Not only is it relevant conceptually, but for patients with neurological disorders or psychiatric disorders, it's also one of the excitements in that, the better we understand brain

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organization and plasticity, the better we can try to map these kinds of intrinsic experiences we have to these networks we observe.

There has been a lot of emphasis over the years on the long-range connectivity and these resting state networks. It's also interesting to think about these more detailed local organization patterns, like what is picked up by retinotopic or other maps in the brain, or these kinds of layer-specific changes. I think there's a lot of richness of questions that can be approached that will have clinical relevance as well. At the end we try to not only improve function, but also patients' quality of life and how they feel in general.

So that's my excitement about seeing the little bit of history myself and learning from all of you and then so much to do to try to improve patient outcomes and make it a better world for patients with brain disorders.

Steven Laureys:

Thanks, Melanie.

Thanks again, everyone. We have 20 minutes left. Is there anything any of you want to come up with now - things that should be addressed, discussed? Please feel free to speak up.

Vince?

Vince Calhoun:

It's all done. We've covered it all.

Steven Laureys:

Then I would like to know, if I may, what are your biggest frustrations? What would you like to see happen? To deal with, obviously, the challenges in the field of understanding brain connectivity? Maybe we can learn from that and, through sharing your difficulties, maybe help the young scientists coming up with the solutions.

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So, question for everyone here: what are your biggest frustrations in the study of brain connectivity? Don't tell me you have none...

Vince Calhoun:

No pictures. That's one.

Steven Laureys:

You want more pictures, Vince?

Vince Calhoun:

Show me more. Yeah, yeah, I'll let others speak and then I can pipe in a little.

Steven Laureys:

I will start with mine. That is that I think we're slaves of technology and I don't have the machine I want. We have the sexy images from structural and functional MRI and PET imaging and MEG and EEG, high density TMS, whatever. Yet we don't capture the dynamics of those thousands of billions of synapses in a soup of neurotransmitters. It's to me very frustrating to look at indirect measures and trying to make sense of it, even if of course we want to go multimodal, and of course we're also linking to animal data – which is something that we didn't discuss much today. That's my frustration - hoping the engineers will build better machines - but I'm curious about yours.

Vince Calhoun:

I would agree with that. I really think we're still pretty far from what we want to study when we're dealing with imaging of living humans. Maybe brain implants will help? BCI type stuff - I don't know. Technology is continuing to evolve and there are some pretty rich MR Pulse sequences coming out that can kind of give you more information per unit time that you can then use advanced methods to try to pull out information from. It's still, again, very macro scale. I think I'll just say maybe one or two more, which is brain stimulation helps with this, but what can we say about the causal relationships? Can we

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even? You can obviously do an intervention and see a result from that, and that's probably the closest thing we have but there's so much else that we want to say given the data that we really can't. Right now, it's all associational, right? So, I think I'd love to see that continue to advance.

I think this cycle between embedded … like if I just think about dynamics, there's a lot of dynamics in the brain. We're still not really doing, I think, a complete job at modeling that. You can embed a dynamic model into your data. You can look at just dynamics at the very sort of macro scale - and there's a gulf in between those two. I think depending on what models you embed you get cool, but different, results. Maybe they're both right? Maybe we need to be fusing models instead in addition to fusing modalities? I think there's a lot of choices that we make and it's unclear yet what we're going to find in the end. Which is going to be: how do we optimize across all of those things?

Stephen Laureys:

Thank you.

Jennifer Whitwell:

I could maybe… one thing that I find frustrating and maybe I would like everyone's input, actually, is what to do about dealing with atrophy? So, you have these diseases, the brains are very atrophic, and then you look at connectivity and you find reduced connectivity or whatever. How do you figure out how much of that might be due to atrophy - and reviewers ask it all the time - or not? Or is it independent? Dealing with atrophy is just challenging and you could put volume in as covariates and things. I don't know if that really gets at it.

So, I'd be interested to hear everybody's thoughts on that: when the brain has shrunk so much, how do you make sure that what you're measuring is the connectivity versus you've just got less tissue left? It's a complicated issue I think, and difficult to deal with I find - but does everybody else have any thoughts on that? It might be an issue that's very specific to degenerative disease, obviously, because we have so much atrophy in the brain. But structural changes in general, because you could be looking at correlations between the

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two processes - or one could be driven by the other - and I do not know how to dissociate these possibilities.

Melanie Boly:

There may be some new data coming from, for example, patients with epilepsy or tumors. I believe these kind of slices analysis they can do in human tissue can actually provide some very detailed kind of ground truth about the kind of anatomical connectivity at the micro level you have in these areas. Most of the time these patients also have preoperative functional MRI, for example, an anatomical MRI. And given these two comparisons - for example, patients with epilepsy - they can have atrophy or not, they have widespread changes in signals in the whole brain, nearly. I think this kind of data combining the macro and micro scale can be very useful not only for epilepsy or tumors, but more broadly to understand what do signals mean and how exactly you can link them at these different scales.

Linda Douw:

That's interesting.

Melanie Boly:

Maybe not a solution to the problem you mentioned, but more turning it around: one of my frustrations sometimes is that we think that everything should be independent, which is paradoxical to the idea of connectivity. I fully agree that we should exclude artifacts and try to minimize them - make sure we are not measuring things a couple of times over in different ways. However, I think there's still some room to broaden our epistemic view of what we are doing instead of trying to think in causal or independent processes to think of it more - per definition - as something that is complex and interrelated all the time, in all directions. Sometimes it can be tempting to see connectivity as another way of just ascribing one behavior to one connection. To me it seems like that kind of thinking undermines the whole idea of brain connectivity. So, I would say, let's also expand on the idea that everything is interrelated and that we need a framework that allows us to put everything into it.

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Steven Laureys:

Thank you. Sue, dare I ask about your biggest frustration?

Susan Whitfield-Gabrieli:

I have many… many of them have already been discussed, and I'll just tap on a little bit more to what Vince and Melanie were referring to earlier. I think that one of my biggest frustrations is despite the tremendous progress that we've made, we still haven't moved the needle in mental health treatment, and that is my biggest frustration.

One of the things that Vince was talking about was moving from association to causal methods. I think that one way to do that is - although the intersubject variation is really interesting - if we could, in addition to looking at these large models, we could also switch to - not switch to - but a complimentary design would be much deeper phenotyping with the individual, have a large and multimodal end with an individual idiographic phenotyping so that you could get measures from the body and the mind and the brain. If you get all of those measures together and biologically trigger experience sampling and really get the experience - the full experience from the individual - then you can go back in time and make predictive, real causal associations between the portfolio features that you might be acquiring in real time to the subsequent mental feature or clinical symptom. In that way, you could potentially build an individualized, personalized therapy. So I'd like to see more deep phenotyping happening.

Steven Laureys

Thank you. Karl, are you willing to share what you see as the biggest obstacles and how can we solve them?

Karl Friston

I think my frustration is with hype-cycles. And, at the moment, it's a frustration with machine learning that I see it as a dangerous distraction of young talent and commitment to the scientific process in building generative models — digital twins, forward models, dynamic causal models, mechanistic models — that generate our data. The importance of

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generative models speaks to a couple of cross-cutting themes in this roundtable. Perhaps the most obvious one is the circular causality between structure and function: experience-dependent plasticity tells you immediately that synaptic function depends upon experience, but of course plasticity is manifest in terms of a structural change. But neuronal firing depends upon the connectivity. So, there's a circular causality here, which we are going to have to model to answer any of the questions that have been posed, whether it's about tumors or epilepsy, whether it's in terms of deep phenotyping or precision medicine.

To do that, one has to build mechanistic models that have this bi-directional coupling between structure and function. It's not easy to do that because there is an implicit separation of temporal scales. If you want a digital twin — to do *in silico* psychopharmacology or psychosurgery for epilepsy, for example, or simulated TMS safely in your personalized digital twin — you have to have a proper model of what's under the hood: what's generating all of these multimodal data. This points towards improved models that generate both EEG and FMRI data that — in a complementary way — constrain your estimates of the neurovascular coupling and the intrinsic and extrinsic connectivity — at different time scales — so that you can understand slow fluctuations (say dynamic functional connectivity) in terms of the past fluctuations or synchronization at a microcircuit level. All of these wonderful questions are only addressable if you take the care and the time — and skill yourself in terms of early training — to build explicit observation, forward or dynamic causal models of the complex system at hand.

At the moment, I'm very frustrated because everybody wants to do deep learning, which of course is unexplainable. Because this kind of modeling is unexplainable, there is no mechanistic insight. So, that's my little rant. That's my frustration.

Steven Laureys

Thanks for sharing that.

Marc, inspiring pioneer: share your wisdom and frustration and how can we transform that into something inspiring for the younger generation?

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Marc Raichle:

I think in various ways we've talked about the brain as an incredibly complex system, and I think the frustration that I experience in trying to come to grips with this is the multidisciplinary challenge that that represents. So that you can be in the world of endocrinology, cell biology, genetics, and so forth - and each one of these are complex and difficult to deal with - and I think the frustration is grappling with the complexities in many different areas, where people devote their entire life to working in those areas. My concern in all of this is that we try as best we can to integrate our thinking across these different disciplines - that we don't focus just on this panel or just on the genetics, and so forth - and appreciate that it is going to require adequate conversations among people of different disciplines to deal with this. Maybe this frustration is an age effect - I'm 87 years old now and I'm told repeatedly that my brain is likely degenerating already - and maybe part of the frustration is my decreasing ability to deal with this broad complexity. But I think conversations help immensely, and people that are devoting to cell biology or genetics or endocrinology or metabolism - you bring up metabolism and it's about energy, but it's far more complex than that - and how that is integrated into this.

My favorite cell is the VIP neuron and we talk a lot about this - this is Mike Stryker's big thing in arousal and how it plays an important role in this context. We simply forget about VIP, the protein that comes out the back door and goes over to the astrocyte and couples with norepinephrine to break down glycogen, as part of the process. To engage in the breadth of that set of ideas requires a diverse group of people sitting at a table and talking about this. So my frustration is the complexity of the problem and the necessity of having broadly-based discussions of where we're going with all this, because none of us have the ultimate tool that will give us all the answers we ever wanted. We need to work as a group of people of diverse backgrounds that can work together and compare the complexities of the things we all face.

Steven Laureys:

Thank you so much, Marc. Understanding brain connectivity needs connectivity between scientists.

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Marc Raichle:

Yes. That's a nice way of putting it. I like that.

Steven Laureys:

It was really wonderful to have you all here, even if it's virtual. Thank you so much for all your thoughtful contributions.

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