Between new genetic discoveries and large randomized trials—neurological research in the era of systems medicine

Thomas Misgeld, Stefan F. Lichtenthaler & Martin Dichgans

The 2012 Eibsee meeting on 'Cellular Mechanisms of Neurodegeneration' addressed the need to integrate research on classical neurodegenerative mechanisms with investigations that relate to the immunological, glial and vascular sequels that accompany and often propagate neuronal injury. We report on the central topics that were addressed and discuss future directions towards establishing 'systems neurology' as a new integrated research field.

Large trials, great expectations

In the field of neurodegeneration research, the impressive pace of new discoveries related to the genetic causes of dementia and motor neuron loss contrasts with a growing number of large-scale trials that have failed to deliver the expected benefits for patients. Against this backdrop, 80 scientists met at Lake Eibsee in the Bavarian Alps at a meeting on the 'Cellular Mechanisms of Neurodegeneration', organized by Christian Haass (LMU Munich and the German Centre for Neurodegenerative Diseases Munich (DZNE-M), Germany). What truly set this meeting apart, in addition to the remarkable science presented, was the diversity of its participants.

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To understand the new complexity that researchers in neurodegenerative mechanisms face, it is worth turning to the highlight that ended the conference, the presentation by Kenneth S. Kosik (UC Santa Barbara, USA), who introduced the anti-A β antibody trial set to begin in the hinterland of Colombia in an attempt to relieve the plight of the large local families affected by a familial form of early-onset Alzheimer disease. Comparable trials—such as the dominantly inherited Alzheimer Network

(DIAN; www.dian-info.org) trial—are also underway to preventively treat Northern Americans and Europeans who have inherited similar mutations [1]. Beyond their immediate medical goals, these trials are a crucial test for the explanation that the proponents of the 'amyloid hypothesis' [2,3] have for one of the bigger disappointments of 2012: the failure of several major trials that used anti-A β antibody treatment in sporadic forms of Alzheimer disease and mild cognitive impairment [1]. The consensus among scientists, who maintain the idea that A β -aggregates are the main toxic influence in the brain of Alzheimer disease patients, is that treatment in the failed trials was simply started too late when irreversible structural damage is so advanced that stopping or even reversing aggregation will fail to improve cognitive function. Thus, trials as described above are shifting to treatment before the disease strikes. Moreover, whilst the previous clinical trials might have seemed to weaken the case of the pro-amyloid fraction, genetics came to the rescue with the discovery of a mutation in the amyloid precursor protein (APP) that protects Icelandic individuals from Alzheimer disease [4]. Still, this does not mean that the amyloid cascade necessarily offers suitable points of entry for small-molecule pharmacology, as emphasized at the Eibsee meeting by several presentations that use ever more sophisticated tools to explore the side-effects of interfering with the protease cascade that results in A β production.

Consider BACE-1, a long-favoured drug target due to its central role in A β generation. Jochen Herms (LMU Munich and DZNE-M, Germany) struck a cautionary note by demonstrating altered spine plasticity in mice treated with BACE inhibitors. Indeed, the range of BACE-1 substrates is much broader than previously anticipated, as revealed by new proteomics approaches such as those presented at the meeting by Stefan Lichtenthaler (DZNE-M and TU Munich, Germany; [5]). Whether reduced proteolysis of these alternative substrates will cause side-effects in pharmacotherapies targeted at BACE-1 in humans remains to be seen. However, loss of cleavage of one of these substrates, the cell adhesion protein CHL1, probably induces axon guidance defects in BACE1-deficient mouse brains [6].

However, there are more twists to the amyloid story emerging. Harald Steiner (LMU Munich and DZNE-M, Germany) presented data that showed the influence of membrane lipids on the activity of γ -secretase—another key protease of the APP-A β -cascade [7]. This might offer other targets for protease modulation, adding to the promise of γ -secretase modulation rather than outright blockade, which induces developmental side-effects.

But perhaps the most remarkable—and to some degree, most threatening—recent revision of the classical amyloid hypothesis

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is the rapidly spreading view of amyloid 'infectivity' [8,9]. Mathias Jucker (DZNE-Tübingen, Germany), presented a measured view on this emerging topic, stressing the potential of monitoring A β levels in body fluids of murine Alzheimer disease models to provide a mechanistic understanding of these biomarkers that are increasingly included in human studies. Indeed, the fact that aggregation of A β proteins—and other degeneration-related proteins—might spread in an almost 'prion-like' fashion [8] underscores the continued importance of a better understanding of the commonalities and diversities that exist between classical neurodegenerative pathways and the prion world.

The unifying principles of cell biology

The possibility that protein aggregates might spread along neuronal pathways is threatening, but also offers the potential for unconventional therapeutic interventions aimed more at the machinery of spread than aggregation itself. This insight points towards the need to better understand how toxic aggregates might capture the intrinsic cell biology of neurons, such as the mechanisms of axonal transport or synaptic release. Indeed, one of the unifying themes of the Eibsee meeting was that neurodegeneration research needs to integrate more closely with research into neuronal cell biology and physiology, as the toxic mechanisms that neuro-degenerative proteins might capture are broad and deeply embedded in the normal function of neural cells. This certainly applies to intraneuronal and transneuronal transport processes, but equally to the role of the cytoskeleton in defining neuritic stability and function, the contribution of mitochondria to neuronal survival and signalling, the role of catabolic mechanisms such as autophagy, and the way RNA processing is realized in complex cells such as neurons. Indeed, genetic studies, for which 2012 has been an extremely fruitful year [10], support this point. This is particularly true for fronto-temporal lobar degeneration (FTLD), which is one of the most common causes of dementia in younger people, and amyotrophic lateral sclerosis (ALS), a motor

neuron disease now seen to lie within a disease spectrum with FTLD [11]. In contrast to the genetics of familial Alzheimer disease, which neatly fall into a single proteolytic pathway, the genetic loci that cause FTLD and ALS still scatter widely among possible mechanisms, leaving the field equally confused and excited. Numerous new insights into the intricate cell biology of neurons that are linked to neurodegenerative mechanism were presented. Undoubtedly, several strands of evidence point to a possible unifying theme of disrupted RNA processing as a possible root cause of several forms of familial FTLD and ALS. Starting from this assumption, Magdalini Polymenidou (UC San Diego, USA) presented the results of a comprehensive analysis of changes in RNA processing in ALS. This analysis shows that FTLD- and ALS-related proteins such as TAR DNA-binding protein 43 (TDP-43) or fused in sarcoma (FUS) bind to broad populations of mRNAs, including some that encode gene products themselves implicated in neurodegeneration, suggesting the possibility of complex cross-regulation. Remarkably the transcript populations that are bound by the FTLD- and ALS-related proteins investigated here are largely non-overlapping, giving special significance to those overlapping transcripts that encode proteins essential for neuronal integrity, which the UC San Diego team identified [12]. Interestingly, the results of this comprehensive analysis converged in intriguing aspects with a more candidate-based approach presented by Dieter Edbauer (DZNE-M and LMU Munich, Germany)—both studies showing that FUS might be involved in processing of Tau mRNA, another well-known molecular player in both Alzheimer disease and FTLD [13].

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The fact that the microtubule-binding protein Tau remains a recurrent theme in the pathogenic narrative of neurodegenerative dementia keeps a focus on the role of the cytoskeleton in establishing and maintaining neuronal homeostasis and polarity, by providing mechanical stability and ensuring efficient transport. Obviously, past studies have supported this view and have documented early and profound defects

in axonal transport in neurodegenerative disease models in general [14], as well as in models of tauopathies in particular. This was underscored by Maria G. Spillantini (U. Cambridge, UK), who reported on a new cellular model of tauopathies based on long-term culture of adult dorsal root ganglionic neurons. However, reports have also stressed the subtleties, underscoring that reduced axonal transport alone might not immediately lead to axon loss [15], arguing for the need of a much deeper understanding of how intraneuronal transport and homeostasis works. Here, the data presented by Casper Hoogenraad (U. Utrecht, The Netherlands) showed how sophisticated *in vitro* assays dissect the specific contributions of motor–adaptor interactions to the many undercurrents of transport present in vertebrate neurons. For example, in the case of mitochondria, the mammalian duplication of the *Drosophila* adaptor protein Milton, which couples mitochondria to molecular motors, has been harnessed to generate specific isoforms that either shuttle mitochondria towards dendrites or into the axons—in this way immediately linking transport to polarity [16].

How crucial preserving the cytoskeleton can be was shown by Frank Bradke (DZNE-Bonn, Germany), who demonstrated that limited doses of microtubule-stabilizing drugs, such as taxol, can suffice to improve outcome in experimental models of spinal cord injury [17], which in turn can be assayed by *in vivo* imaging and new large-scale imaging strategies of neural circuitry [18]. Bradke's presentation made it clear that multiple effects probably contribute to the therapeutic effects seen in spinal cord injury—many of which might affect non-neuronal cells by inhibiting scar formation. Here again, a recurrent theme of the Eibsee meeting emerged: that understanding neurodegeneration requires a systemic approach that extends beyond the traditional boundaries set by classical definitions of research areas.

Translation meets systems medicine

The fact that fishing for the physiological role of neurodegeneration-related proteins can deflect research into unexpected directions was made clear by Bettina Schmid's (DZNE-M, Germany) presentation of the deletion phenotype of TDP-43 in zebrafish. Due to a partial genome duplication, zebrafish have two TDP-43-related genes, both of which the Schmid and Haass groups

deleted by zinc-finger-based genome editing. Remarkably, the double-deletion results in a profound vascular phenotype with vessels mis-patterning and accompanying muscle and motor axon pathology [19]. Comprehensive proteomics analysis in collaboration with Lichtenthaler—in this form a novelty in zebrafish—subsequently revealed mis-regulation of certain muscle proteins that could also be detected in the brains of a subpopulation of human FTLD patients, demonstrating the power of zebrafish-based ‘omics’ analysis to discover molecular phenotypes of potential disease relevance.

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Martin Dichgans (LMU Munich, Germany) presented follow-up studies on an identified risk locus for atherosclerotic stroke: histone deacetylase 9 (HDAC9; [20]). By using advanced proteomics tools, Dichgans’ group in collaboration with Matthias Mann (MPI of Biochemistry, Martinsried, Germany), could demonstrate that a genetic variant showing one of the strongest signals in previous genome-wide association studies disrupts a canonical binding site for a suppressor–enhancer complex and alters HDAC9 expression. HDACs in general, and HDAC9 in particular, are regulators of regulatory T lymphocytes, which can protect against atherosclerosis—suggesting that in this case, the vascular phenotype might emerge from immune cell dysregulation. These findings add to the growing evidence of an intricate relationship between vascular and inflammatory mechanisms in neurological disorders.

The full extent to which investigations into the pathogenesis of a neurological disease can take investigators away from their original starting point was impressively demonstrated by Hartmut Wekerle (MPI of Neurobiology, Martinsried, Germany), who discussed insights into the pathogenesis of neuroinflammatory diseases, such as multiple sclerosis. The migratory behaviour of peripherally activated immune cells in a rodent model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) and even the activation event in the spinal

cord can now be identified by using an elegant combination of *in vivo* two-photon imaging and large-scale histological analysis of immune cells tagged with fluorescent proteins [21]. Still, how could such T cells be primed in the first place in the spontaneous human disease? The fact that EAE has a clearly artificial mode of initiation—namely peripheral immunization against a central nervous system (CNS) antigen—has thus far largely prevented the use of this animal model to explore questions related to the initiation event of CNS-directed autoimmunity. Wekerle and colleagues have succeeded in generating a ‘spontaneous’ model of CNS autoimmunity in mice by genetically skewing the T-cell repertoire towards CNS auto-antigens in mice of a suitable genetic background. Remarkably, the incidence of this spontaneous relapsing–remitting condition depends on the animal’s gut ‘microbiome’. Mice brought up in pathogen-free conditions remain healthy, but develop a multiple sclerosis-like disease with high incidence once regular gut flora is introduced [22]. This allows ‘screening’ for the specific members of the gut flora that initiate disease, and this information can be used to explore whether this predicts abnormalities in the gut microbiome of patients with multiple sclerosis.

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A fascinating new look into the multiple facets of Alzheimer disease was provided by Michael Heneka (U. Bonn, Germany), who presented new data showing that the neuro-inflammatory component of Alzheimer disease involves activation of the NLRP3 inflammasome and of caspase 1. Chronic deposition of A β has long been known to stimulate persistent activation of microglial cells, which cluster in the vicinity of plaques and adopt a chronically activated phenotype. Cytokines, including interleukin (IL)-1 β impair microglial clearance functions, and increased IL-1 β levels have been implicated in the response to A β deposition. However, the exact role of neuroinflammation in promoting Alzheimer disease pathology remains a matter of debate. The findings presented at the Eibsee meeting provide

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compelling evidence for an A β -induced chronic inflammatory response with harmful consequences. When crossed with mice that carry familial Alzheimer disease mutations (APP/PS1), *Nlrp3*^{−/−} mice were found to exhibit reduced IL-1 β activation, enhanced A β clearance and marked protection from loss of spatial memory and other Alzheimer disease-related cognitive deficits [23]. NLRP3 deficiency preserved synaptic spines in pyramidal neurons and long-term potentiation in APP/PS1 mice. Microglial cells surrounding A β -plaques in APP/PS1 mice were found to phagocytose A β to a lesser extent than was observed in APP/PS1/*Nlrp3*^{−/−} mice. These findings add to evidence suggesting that impaired clearance of A β might be a crucial factor in sporadic Alzheimer disease—a view seconded at the meeting by data presented by Jens Pahnke (U. Magdeburg and DZNE-Magdeburg, Germany), who showed that loss of ABC transporters B1 and C1, which extrude A β from the CNS parenchyma, can lead to a substantial increase in A β -load [24]. These transporters not only are regulated by mitochondria [25], but also have a substantial impact on cerebral regeneration [26]. Thus, future investigations into toxic protein aggregation will have to consider production, as well as clearance, and hence the homeostatic contributions of the immune system and tissue barriers, such as the neurovascular unit or the choroid plexus.

The Eibsee Meeting left a lasting impression and an appreciation of what diverse fields—be it neuroinflammation, glio-vascular research or systems neurology—will contribute to the larger field of neurodegeneration, and how direct collaboration ‘across’ classical research field definitions can be fruitful. We believe that with ever more sophisticated studies of entire genomes derived from individual patients, the improved availability of matched patient-derived neuron-like cells, and refined proteomics and imaging techniques that span from single molecules to pathway signatures in patients, such interdisciplinary research will become more tangible. Indeed, we hope that a few

years down the line, the parallel interrogation of neurodegenerative, vascular and immunological disturbances not only in disease models, but also in human diseases, will become the rule, and not the exception. That such efforts then would lead to 'translatable' results that benefit patients and could overcome the widely perceived doldrums in translational activity in neurodegeneration is another hope; it is too early yet for a verdict. Our feeling is, however, that out of integration across different research areas in neurology, a new field might emerge that could be rightfully described as 'systems neurology'. The 2012 Eibsee Meeting felt—at least to us—as if it was a first, spirited step in this direction.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Thomas Misgeld, Stefan Lichtenthaler and Martin Dichgans are affiliated with the Munich Cluster for Systems Neurology, Munich, Germany, and the German Centre for Neurodegenerative Diseases, Munich, Germany.
Thomas Misgeld and Stefan Lichtenthaler are at Technische Universität München, Germany. Martin Dichgans is at the Institute for Stroke and Dementia Research, Ludwig-Maximilians-University, Munich, Germany.
E-mails: thomas.misgeld@lrz.tum.de; stefan.lichtenthaler@tum.de; martin.dichgans@med.uni-muenchen.de

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