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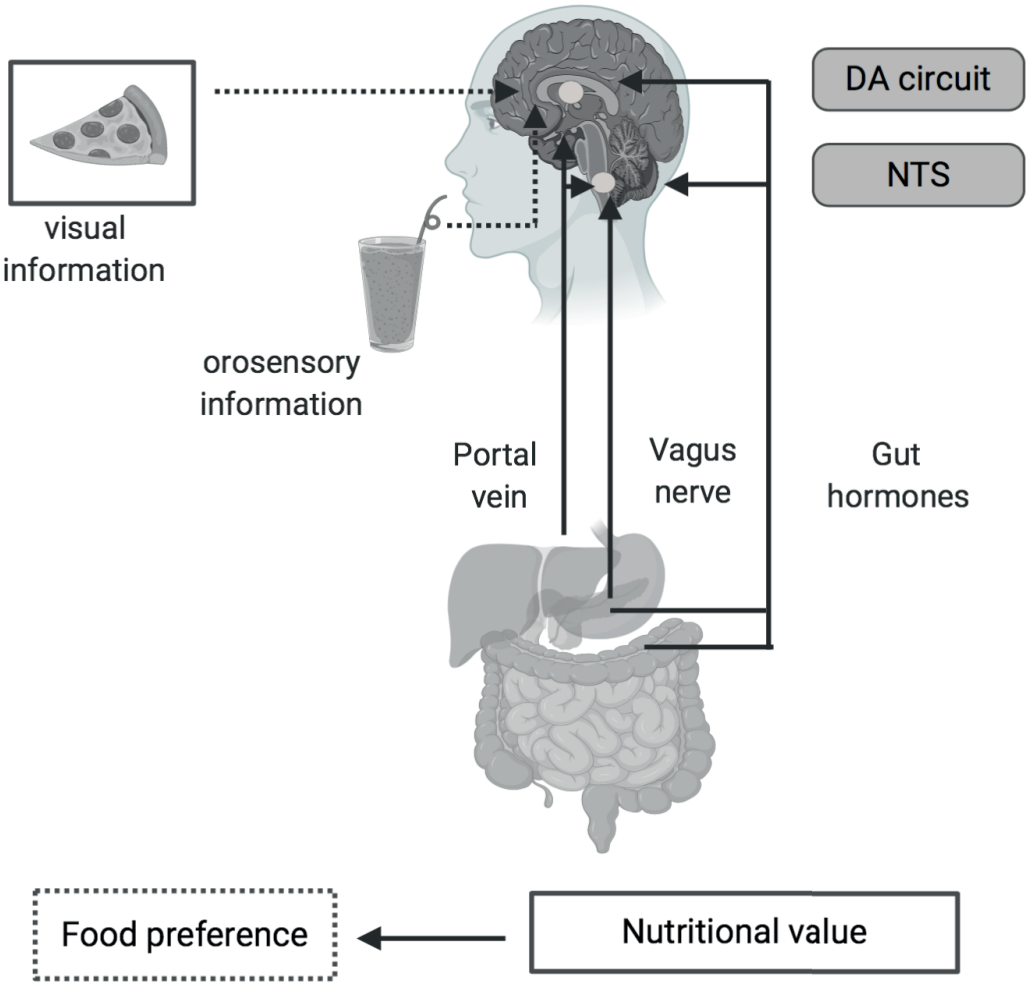

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HERAUSGEBER Neurowissenschaftliche Gesellschaft e.V. (NWG), Kontakt: Meino Alexandra Gibson, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Straße 10, 13092 Berlin, Tel.: +49 (0)30 9406 3336, gibson@mdc-berlin.de, www.nwg-info.de

CHEFREDAKTEURIN Petra Wahle, AG Entwicklungsneurobiologie, Fakultät für Biologie & Biotechnologie, Ruhr-Universität, ND 6/72, 44780 Bochum, wahle@neurobiologie.ruhr-uni-bochum.de

REDAKTION Susanne Hannig, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13092 Berlin (Germany), Tel.: +49 (0)30 9406 3336, susanne.hannig@mdc-berlin.de

JOURNAL MANAGER Torsten Krüger, De Gruyter, Genthiner Straße 13, 10785 Berlin, Germany. Tel.: +49 (0)30 260 05-173, Fax: +49 (0)30 260 05-250, E-Mail: Neuroforum.Editorial@degruyter.com

ANZEIGENVERANTWORTLICHE top-ad Bernd Beutel, Schlossergäßchen 10, 69469 Weinheim, Tel.: +49 (0)6201 290 92-0, Fax +49 (0)6201 290 92-20 20, disposition@top-ad-online.de

COVER ILLUSTRATION Food preference is modulated by bodily signals. Visual and orosensory aspects of food items stimulate the neural dopamine circuit (DA circuit) to establish food preference as well as prediction of caloric content. The preference is updated by the nutritional value sensed in the body and transmitted to the brain through different pathways; carbohydrate metabolism is linked to brain dopamine through the portal vein but the exact pathway is unknown, lipid-dependent dopamine signalling is mediated by the vagus nerve, possibly through activation of PPARα receptors expressed on enterocytes. The vagus nerve sends afferents to the nucleus of the solitary tract (NTS), that synapse to dopaminergic downstream areas. In addition, neural dopamine pathways are directly modulated by gut hormones that are released during fat and glucose ingestion. Hence, neural dopamine mediates the reward value of food and thereby shapes food preferences. Cover figure provided by Sharmili Edwin Thanarajah and Marc Tittgemeyer (nf-2019-0020, in this issue).

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Review Article

Sharmili Edwin Thanarajah* and Marc Tittgemeyer

Food reward and gut-brain signalling

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Abstract: The increasing availability of ultra-processed, energy dense food is contributing to the spread of the obesity pandemic, which is a serious health threat in today's world. One possible cause for this association arises from the fact that the brain is wired to derive pleasure from eating. Specifically, food intake activates reward pathways involving dopamine receptor signalling. The reinforcing value of specific food items results from the interplay between taste and nutritional properties. Increasing evidence suggests that nutritional value is sensed in the gut by chemoreceptors in the intestinal tract and the hepatic portal vein, and conveyed to the brain through neuronal and endocrine pathways to guide food selection behaviour. Ultra-processed food is designed to potentiate the reward response through a combination of high fat and high sugar, therebye seeming highly appetizing. There is increasing evidence that overconsumption of processed food distorts normal reward signalling, leading to compulsive eating behaviour and obesity. Hence, it is essential to understand food reward and gut-brain signalling to find an effective strategy to combat the obesity pandemic.

Keywords: dopamine, gut-brain axis, obesity, processed food, reward

Zusammenfassung: Zur Sicherstellung eines ausgeglichenen Energiehaushalts des Körpers wirkt Essen als primärer Belohnungsreiz. Daher haben Nahrungsmittel einen starken Einfluss auf das Belohnungssystem im Gehirn. Wenn wir essen, wird im Gehirn der Botenstoff Dopamin frei gesetzt. Wie belohnend wir Lebensmittel finden hängt dabei sowohl vom Geschmack als auch vom Nährwert ab. Jüngste Forschungsergebnisse belegen, dass unser Magen-Darm-Trakt im engen Austausch mit dem Gehirn steht und Informationen über den Nährwert an das Gehirn übermittelt. Auf diese Weise kontrollieren Signale aus dem Magen-Darm-Trakt unser Verlangen nach Essen. Industriell verarbeitete Lebensmittel sind so konzipiert, dass sie besonders appetitanregend wirken; außerdem zeichnen sie sich durch einen hohen Kaloriengehalt aus. Fertiggerichte veranlassen Menschen damit offenbar, mehr zu essen als sie benötigen. Die zugrundeliegenden Mechanismen hierfür sind bislang noch nicht hinreichend verstanden. Allerdings ist davon auszugehen, dass hierbei die Vermittlung sensorischer Informationen zwischen Magen-Darm-Trakt und Gehirn eine tragende Rolle spielt. Aktuellen Studienergebnissen zu Folge kommt es bei übermäßigem Verzehr von Fertigprodukten zu anhaltenden Veränderungen im Belohnungssystem. Diese begünstigen ein impulsives Essverhalten und können dadurch zu Übergewicht führen. Das Verständnis dieser Prozesse ist daher grundlegend, um eine wirksame Strategie zur Bekämpfung der Adipositas-Pandemie zu entwickeln.

Schlüsselwörter: Adipositas, Dopamin, Belohnungssignal, Prozessierte Nahrung, Darm-Gehirn-Achse

Introduction

Obesity is a global epidemic. Excess body fat accumulation (Body Mass Index (BMI) of above 25 is considered overweight, and BMI of above 30 is considered obese) is a key risk factor for a range of chronic noncommunicable diseases, including metabolic syndrome, diabetes, cancer, and cardiovascular and neurodegenerative disorders (World Health Organization, 2000). The increasing prevalence of obesity in children and adults over the past few decades suggests that environmental changes are driving this trend. To assess variation in weight between individuals, factors influencing both energy loss and gain need to be considered; evolutionary pressures favouring metabolic efficiency and storage, as well as increasing variability in energy expenditure across populations might be one aspect (Prentice et al., 1991), whilst increased food intake and changing eating habits may be the other (Swinburn et al., 2009).

Additionally, increasing evidence suggests that obesity is predominantly a neurobehavioural problem. Food is a

***Corresponding author: Sharmili Edwin Thanarajah**, Max Planck Institute for Metabolism Research, Gleueler Strasse 50, 50931 Cologne, Germany, e-mail: sharmili.edwin@sf.mpg.de
Marc Tittgemeyer, Max Planck Institute for Metabolism Research, Gleueler Strasse 50, 50931 Cologne, Germany, e-mail: marc.tittgemeyer@sf.mpg.de

basic requirement for survival. Our brain is wired to desire food and experience pleasure (reward) from eating. Thus, food is considered to be a primary reward: newborns and a variety of primates show a hedonic facial response to the pleasant taste of sucrose (Steiner et al., 2001). Recent findings indicate that the reinforcing value of food results from the interplay between its pleasant taste (orosensory value) and caloric content (nutritional value). Nutritional value is sensed in the gut and communicated to the brain through neuronal and hormonal pathways (Kim et al., 2018; Liang and Krashes, 2017). Based on this information the evaluation of taste and the desire for specific food items is updated. Processed food items, such as burgers and cakes, are perceived as exceptionally rewarding, possibly due to their impact on the gut-brain axis. This may lead to overconsumption and obesity (Hall et al., 2019). In fact, there is an ongoing debate as to whether excess desire for processed food and overeating is comparable to addiction behaviour (DiFeliceantonio and Small, 2019; Hoebel, 1985; Johnson and Kenny, 2010). Hence, understanding food reinforcement is critical to revealing the mechanisms underlying overeating and combatting the obesity epidemic.

Food intake and reward circuitry

The observation of dopamine release during active feeding in studies of rodents revealed the essential role of the brain's dopaminergic system in eating behaviour (Palmiter, 2007; Taber and Fibiger, 1997). Neural dopaminergic pathways are critical for reward processing and reinforcement learning (Schultz, 2016). Mice genetically engineered to be dopamine deficient starve to death unless they are supplemented with dopamine (Szczypka et al., 2001; Zhou and Palmiter, 1995).

Two features of food have been revealed to elicit dopaminergic release and signalling: pleasant taste and nutritional composition (Araujo et al., 2011). The perception of the sweet taste of sucrose in the oral cavity induces dopamine release in mice and promotes sucrose intake (Schneider, 1989). Conversely, the administration of dopamine-antagonists reduce dopamine release and attenuate the preference for sweet tasting nutrients (Smith, 2004). To isolate the effects of orosensory stimuli on dopamine, Hajnal et al. (2004) implanted an intra-gastric catheter in rats to prevent a sucrose solution from reaching the gut and inducing metabolic effects. Indeed, orosensory stimulation alone revealed concentration-dependent dopamine release. Later, de Araujo et al. (2008) demonstrated that mice genetically engineered to lack taste receptor signalling showed dopamine efflux and developed sugar preference, indicating a taste-independent mechanism. Accordingly, a direct nutrient infusion into the stomachs of mice was able to elicit dorsostriatal dopamine release (Ferreira et al., 2012). These findings suggest that gut derived sensory signals – often referred to as "post-ingestive signals" – are also linked to the neural dopamine system.

Now, the leading theory is that post-ingestive signals communicate nutritional value to the central nervous system and thus update food preferences. For example, mice learn to establish preferences for flavours presented in parallel with intragastric caloric infusions compared to flavours without a caloric association (Sclafani and Ackroff, 2012). This form of learning, called "flavour-nutrient conditioning", highlights the fact that a preference for specific food items is established if certain taste cues are followed by metabolic effects indicating high nutritional value (Araujo et al., 2011).

It is still unclear how these post-ingestive signals are conveyed to the brain. Afferents of the vagal nerve transmit information on nutritional composition and gastric dilatation to the hindbrain (Schwartz et al., 2000). Using optogenetic stimulation, Han et al. (2018) activated vagal afferents and induced neural dopamine release and reward behaviour. More specifically, Tellez et al. (2013) suggested that a mechanism involving fatty acid amides and peroxisome-proliferator activated receptor alpha (PPARα) expressed on enterocytes builds the physiological link between fat consumption and vagal nerve activation. To this end, PPARα antagonism and knockout abolished dopamine release following a high-fat diet. Intriguingly, vagus-dependent dopamine release differs across macronutrients. Vagotomy impairs lipid- and amino-acid-dependent dopamine release, while the carbohydrate dependent signal remains unimpaired (Qu et al., 2019; Ritter and Taylor, 1990). This implies that carbohydrates are sensed differently. Indeed, novel data suggest that carbohydrate-dependent dopamine signalling is transmitted via the mesenteric portal system (Zhang et al., 2018). The exact molecular mechanisms relevant for nutrient sensing through several chemoreceptors in the gut and hepatic portal vein system are currently under intense scrutiny (see Sclafani and Ackroff, 2012 for a review). Besides the vagus nerve and the portal vein system, gastrointestinal hormones, such as insulin, glucagon-like peptide 1 (GLP1) and ghrelin, are considered to be critical components of the gut-brain axis and modulate food-dependent dopamine release (Dickson et al., 2012; Skibicka et al., 2012; Stouffer et al., 2015).

Food reward in humans

Research on human eating behaviour faces three major challenges, namely: differential presentation of food cues to the oral cavity and the gastrointestinal tract, direct assessment of neurotransmission in the brain, and experimental modulation of gut-brain mediators, such as the vagus nerve and gastrointestinal hormones. There is increasing evidence of similarities in the reinforcement mechanisms operating in human eating behaviour to those previously reported in studies of rodents. In humans, food intake is associated with activity in dopaminergic target areas and subjective pleasure reported after eating correlates with regional activity highlighted by functional magnetic resonance imaging (fMRI) (Small et al., 2003).

In a recent study, we were able to present the first evidence of orosensory and post-ingestive dopamine release in humans (Thanarajah et al., 2019). We provided participants with a palatable milkshake whilst they were lying in an fMRI scanner. To directly assess dopamine release we performed [^{11}C] raclopride positron emission tomography (PET) and applied a novel analysis method (Lippert et al., 2019). Interestingly, we identified two distinct windows of neural dopamine release. The pleasant taste of the milkshake immediately elicited dopamine release in primarily orosensory pathways, including the nucleus of the solitary tract, thalamus and the insular and frontal cortex. At a delay of 15 to 20 minutes, there was a second dopamine release in another circuit relevant for reward perception, cue-learning and goal-directed behavior and involving the caudate nucleus, prefrontal cortex, amygdala and anterior insula. These findings clearly extend previous rodent work and fMRI reports in humans. Interestingly, both orosensory and post-ingestive dopamine release were related to the subjective desire to eat. Specifically, our findings indicated that immediate dopamine release related to the desire to eat may suppress post-ingestive signalling in the putamen. These findings strongly support the role of the brain's dopamine system as a nutritional sensor that modulates food intake by updating its value as a reward based on metabolic outcome.

This is further supported by data on flavor-nutrient association learning tasks in humans (Araujo et al., 2013; Yeomans et al., 2008).

In parallel to previous rodent work, de Araujo et al. conceptualized an fMRI study (de Araujo et al., 2013) in which participants were first introduced to beverages with different flavours corresponding to either a low calorific value or no calorific value in training trials, before being presented with the same flavours without any added calories whilst in an fMRI scanner. The flavour that was pre-

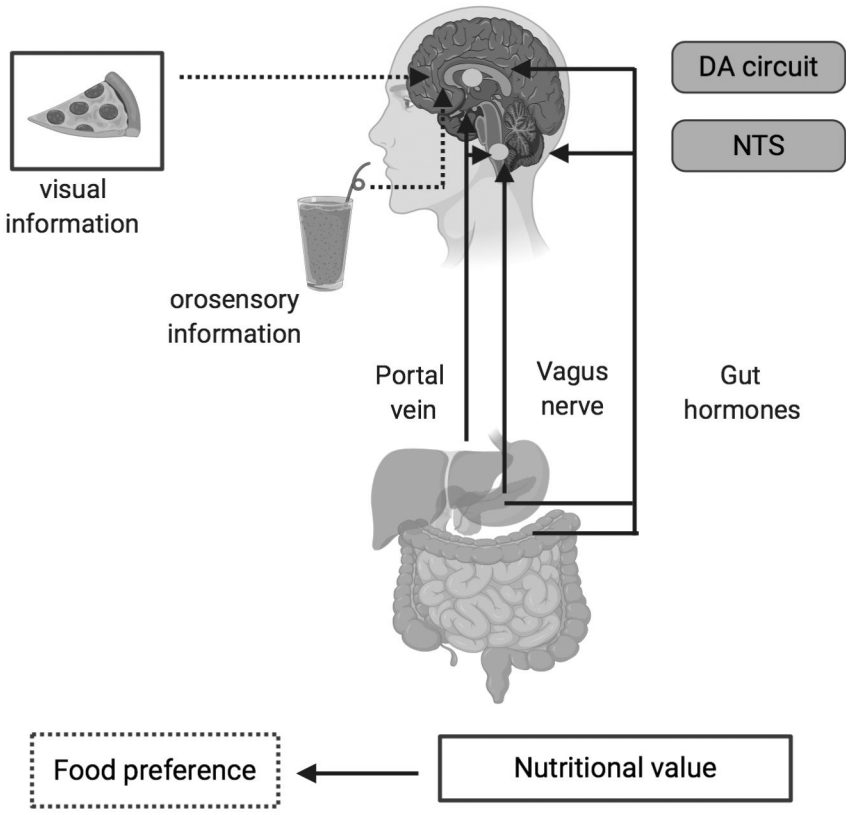

Fig. 1: Food preference is modulated by bodily signals. Visual and orosensory aspects of food items stimulate the neural dopamine circuit (DA circuit) to establish food preference as well as prediction of caloric content. The preference is updated by the nutritional value sensed in the body and transmitted to the brain through different pathways; carbohydrate metabolism is linked to brain dopamine through the portal vein but the exact pathway is unknown, lipid-dependent dopamine signalling is mediated by the vagus nerve, possibly through activation of PPARα receptors expressed on enterocytes. The vagus nerve sends afferents to the nucleus of the solitary tract (NTS), that synapse to dopaminergic downstream areas. In addition, neural dopamine pathways are directly modulated by gut hormones that are released during fat and glucose ingestion. Hence, neural dopamine mediates the reward value of food and thereby shapes food preferences.

dictive of calories was associated with activation in reward areas. Neural activation observed via fMRI correlated with the rise in blood glucose level observed in the test run. This finding suggests a direct link between neural dopamine and peripheral metabolism.

This has behavioural consequences: foods with flavours that have been learned to be high in calories are preferred and consumed more than those with flavours associated with low calories (Yeomans et al., 2008). Interestingly, the reinforcing effect of food is independent of conscious perception. In other words, the actual energy density and not our conscious belief about the calorie content, determine the activation of reward networks (DiFeliceantonio et al., 2018; Tang et al., 2014). Applying an auction task, DiFeliceantonio et al. (2018) tested willingness to pay for different food items that were rich in carbohydrates, fat or both. Participants were more willing to pay more for food that contained both fat and carbohydrates than either macronutrient alone. This was associated with higher activity in the reward network.

In humans, the mechanisms underlying gut-brain communication related to the regulation of food intake await elucidation. Recent studies provide evidence for effects of gastrointestinal hormones, such as insulin and GLP1, on brain reward pathways and food intake regulation following intranasal and intravenous application (Bloemendaal et al., 2014; Tiedemann et al., 2017). However, investigating vagus nerve signalling in humans remains a challenge. Transcutaneous stimulation systems (Frangos 2015; Warren et al., 2019) may provide useful tools in this context and should be considered in future research. Moreover, a growing body of literature suggests the relevance of enteric microbiota in gut-brain interactions through immune, neuronal and endocrine signalling mechanisms (Cryan et al., 2019). In the context of food processing, gut microbiota are directly involved due to their role in metabolizing nutrients and synthesizing vitamins. On the other hand, microbiotic composition itself is highly modulated by our daily diet. Early correlative studies suggest that obesity as well as neuropsychiatric disorders are associated with dysbiosis of gut microbiota, yet a mechanistic understanding of these links is yet to be uncovered (Cryan and Dinan, 2012; Cryan et al., 2019).

Ultra-processed food and food-induced obesity

Modern diets increasingly consist of easily available, cheap, ultra-processed food that is overly appetizing and higher in caloric density than natural products. This may introduce a discrepancy between expected caloric value, based on sensory perception, and the actual caloric load. Particularly, we are seeing a shift towards higher ratios of cheaper fats and carbohydrates that replace dietary proteins, amongst other nutritional components. As described previously, this high-fat and high-sugar combination influences food reinforcement and is associated with an increased reward response (DiFeliceantonio et al., 2018). This may be a major reason why cakes, burgers and fries seem irresistible, leading to overconsumption of these foodstuffs and, subsequently, excess body weight gain (Volkow and Wise, 2005). Another hypothesis put forward to explain increased intake of processed food is the "protein leverage hypothesis", which suggests that we overeat processed food to keep our protein intake constant (Gosby et al., 2014; Raubenheimer et al., 2005). However, this theory is hotly debated (Fürnsinn, 2015) and we need future research to disentangle the differential effects of macronutrients on brain reward functioning.

Another problem of ultra-processed food, and in particular modern beverages, is the addition of low-caloric sweeteners to increase palatability. Despite the general belief that non-nutritive sweeteners are healthy substitutes for sugar, these sweeteners irritate the nutrition-sensing system by introducing a mismatch between sweetness and caloric content (Pepino, 2015). In fact, the use of sweeteners is related to increased appetite, hunger and food consumption in both animals and humans (Lavin et al.; Rogers et al.; Tordoff et al.). Moreover, the majority of observational studies report an association between consumption of sweeteners and the development of obesity and metabolic syndrome in both children and adults (Fowler et al., 2008; Lutsey et al., 2008; Stellman et al., 1988). The mechanisms underlying this association are subject to current research. Early rodent work using classical conditioning suggested that sweeteners may weaken cephalic responses to sweet tastes by introducing the mismatch between taste and caloric load (Swithers et al., 2013). In line with this, recent data provides evidence that oral infusions of sweeteners evoke the same orofacial response in rats as sucrose, indicating pleasure, but the neural dopamine response is attenuated after flavour-nutrient conditioning (McCutcheon et al., 2012). Similar observations were made in human fMRI data; Veldhuizen

et al. (2017) demonstrated that reward activation is different for beverages matched on calorie content and sweetness, compared to beverages where nutritional value and sweetness are not related. Hence, neural dopamine release is altered by artificial sweeteners, but how this is linked to gut-brain signalling and overconsumption is still unclear.

There is an ongoing debate whether highly palatable ultra-processed food has drug-like characteristics (Fletcher and Kenny, 2018). Similar to drug addiction, the repeated stimulation of reward circuits by palatable food may lead to habit formation and learned preferences through neurobiological adaptations (Volkow and Wise, 2005). In rodents with extended access to highly palatable food the development of obesity was accompanied by an elevated reward threshold and reduced D2-receptor availability (Johnson and Kenny, 2010). Confirming the causal link, the knockdown of D2-receptors in rats accelerated weight gain and compulsive eating behaviour. Van de Giessen et al. (2013) demonstrated that the D2-receptor system is specifically compromised by the fat ratio of high energy diets; in contrast to high energy diets with low fat ratios, diets with high fat ratios decreased D2-receptor availability. This is highlighted by recent evidence that a high fat diet compromises fat-dependent dopamine release by suppressing gut lipid messengers (Tellez et al., 2013). Supplementation of lipid messengers restored dopamine release mediated by the vagus nerve.

Another hypothesis is that chronic exposure to a high fat diet activates inflammatory processes involving Toll-like receptors (Sun et al., 2017). In line with rodent studies, human PET-imaging revealed reduced D2-receptor availability in obese participants correlating with increasing BMI (Wang et al., 2001). In overweight subjects, the response to palatable milkshake was diminished, indicating an impaired reward response with increasing body weight (Stice et al., 2008). Hence, there is an ongoing debate as to whether diet-induced obesity is related to hypofunctioning reward circuitry that leads to overeating as a compensatory mechanism. However, the mechanisms by which our modern diet induces neurobehavioural adaptations and how these are modulated by gut-brain interactions are still poorly understood and require further research.

Conclusion

Current research in the field of obesity over the last decade has revolutionized our view on gut-brain interactions and food intake behaviour. Food selection is no more regarded as a purely conscious process, but involves several metabolic and central nervous system mechanisms that are highly dependent on one another. Understanding gut-brain signalling will drive future research in this field and potentially reveal new treatment avenues to combat the obesity pandemic.

References

Araujo, I. E. de, Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., Nicolelis, M.A.L., and Simon, S. A. (2008). Food Reward in the Absence of Taste Receptor Signaling. Neuron. *57*, 930–941.

Araujo, I. E. de, Ren, X., and Ferreira, J.G. (2011). Metabolic Sensing in Brain Dopamine Systems. Results Probl. Cell Differ. *52*, 69–86.

Araujo, I. E. de, Lin, T., Veldhuizen, M.G., and Small, D.M. (2013). Metabolic Regulation of Brain Response to Food Cues. Curr. Biol. *23*, 878–883.

Bloemendaal, L. van, IJzerman, R.G., Ten Kulve, J.S., Barkhof, F., Konrad, R.J., Drent, M.L., Veltman, D.J., and Diamant, M. (2014). GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. Diabetes. *63*, 4186–4196.

Cryan, J.F., and Dinan, T.G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. *13*, 701–712.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, … Dinan T.G. (2019). The Microbiota-Gut-Brain Axis. Physiol. Rev. *99*, 1877–2013.

Dickson, S. L., Shirazi, R.H., Hansson, C., Bergquist, F., Nissbrandt, H., and Skibicka, K.P. (2012). The Glucagon-Like Peptide 1 (GLP-1) Analogue, Exendin-4, Decreases the Rewarding Value of Food: A New Role for Mesolimbic GLP-1 Receptors. J. Neurosci. *32*, 4812–4820.

DiFeliceantonio, A.G., and Small, D.M. (2019). Dopamine and diet-induced obesity. Nat. Neurosci. *22*, 1–2.

DiFeliceantonio, A.G., Coppin, G., Rigoux, L., Edwin Thanarajah, S., Dagher, A., Tittgemeyer, M., and Small, D.M. (2018). Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward. Cell Metab. *28*, 33–44.e3.

Edwin Thanarajah, S., Iglesias, S., Kuzmanovic, B., Rigoux, L., Stephan, K.E., Brüning, J.C., and Tittgemeyer, M. (2019). Modulation of midbrain neurocircuitry by intranasal insulin. NeuroImage. *194*, 120–127.

Ferreira, J.G., Tellez, L.A., Ren, X., Yeckel, C.W., and Araujo, I. E. de (2012). Regulation of fat intake in the absence of flavour signalling. J. Physiol. *590*, 953–972.

Fletcher, P.C., and Kenny, P.J. (2018). Food addiction: a valid concept? Neuropsychopharmacology. *43*, 2506–2513.

Fowler, S.P., Williams, K., Resendez, R.G., Hunt, K.J., Hazuda, H.P., and Stern, M.P. (2008). Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain. Obesity. *16*, 1894–1900.

Fürnsinn, C. (2015). Die moderne Adipositasepidemie vor dem Hintergrund physiologischer Regulation und biologischer Evolution. J. Klin. Endokrinol. Stoffwechsel. *8*, 101–105.

Giessen, E. van de, Fleur, S.E. la, Eggels, L., Bruin, K. de, Brink, W. van den, and Booij, J. (2013). High fat/carbohydrate ratio but

not total energy intake induces lower striatal dopamine D2/3 receptor availability in diet-induced obesity. Int. J. Obes. *37*, 754–757.

Gosby, A.K., Conigrave, A. D., Raubenheimer, D., and Simpson, S.J. (2014). Protein leverage and energy intake. Obes. Rev. *15*, 183–191.

Hajnal, A., Smith, G.P., and Norgren, R. (2004). Oral sucrose stimulation increases accumbens dopamine in the rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. *286*, R31–R37.

Hall, K.D., Ayuketah, A., Brychta, R., Cai, H., Cassimatis, T., Chen, K.Y., … Zhou, M. (2019). Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. Cell Metab. *30*, 67–77.e3.

Han, W., Tellez, L.A., Perkins, M.H., Perez, I.O., Qu, T., Ferreira, J., … de Araujo, I. E. (2018). A Neural Circuit for Gut-Induced Reward. Cell *175*, 665–678.e23.

Hoebel, B.G. (1985). Brain neurotransmitters in food and drug reward. Am. J. Clin. Nutr. *42*, 1133–1150.

Johnson, P.M., and Kenny, P.J. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat. Neurosci. *13*, 635–641.

Kim, K.-S., Seeley, R.J., and Sandoval, D.A. (2018). Signalling from the periphery to the brain that regulates energy homeostasis. Nat. Rev. Neurosci. *19*, 185–196.

Lavin, J., French, S., and Read, N.W. (1997). The effect of sucrose-and aspartame-sweetened drinks on energy intake, hunger and food choice of female, moderately restrained eaters. Int. J. Obes. Relat. Metab. Disord. *21*, 37–42.

Liang, J., and Krashes, M.J. (2017). AgRP Accountants Compute Caloric Cost. Cell Rep. *21*, 2647–2648.

Lippert, R.N., Cremer, A.L., Edwin Thanarajah, S., Korn, C., Jahans-Price, T., Burgeno, L.M., … Backes, H. (2019). Time-dependent assessment of stimulus-evoked regional dopamine release. Nat. Commun. *10*, 336.

Lutsey, P.L., Steffen, L.M., and Stevens, J. (2008). Dietary intake and development of the metabolic syndrome: The atherosclerosis risk in communities study. Circulation. *117*, 754–761.

McCutcheon, J.E., Beeler, J.A., and Roitman, M.F. (2012). Sucrose-predictive cues evoke greater phasic dopamine release than saccharin-predictive cues. Synapse. *66*, 346–351.

Palmiter, R.D. (2007). Is dopamine a physiologically relevant mediator of feeding behavior? Trends Neurosci. *30*, 375–381.

Pepino, M.Y. (2015). Metabolic effects of non-nutritive sweeteners. Physiol. Behav. *152*, 450–455.

Prentice, A.M., Goldberg, G.R., Jebb, S. A., Black, A.E., Murgatroyd, P.R., and Diaz, E.O. (1991). Physiological responses to slimming. Proc. Nutr. Soc. *50*, 441–458.

Qu, T., Han, W., Niu, J., Tong, J., and Araujo, I. E. de (2019). On the roles of the Duodenum and the Vagus nerve in learned nutrient preferences. Appetite. *139*, 145–151.

Ritter, S., and Taylor, J.S. (1990). Vagal sensory neurons are required for lipoprivic but not glucoprivic feeding in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. *258*, R1395–R1401.

Rogers, P.J., and Blundell, J.E. (1989). Separating the actions of sweetness and calories: effects of saccharin and carbohydrates on hunger and food intake in human subjects. Physiol. Behav. *45*, 1093–9.

Schneider, L.H. (1989). Orosensory Self-Stimulation by Sucrose Involves Brain Dopaminergic Mechanisms. Ann. N. Y. Acad. Sci. *575*, 307–320.

Schultz, W. (2016). Dopamine reward prediction-error signalling: a two-component response. Nat. Rev. Neurosci. *17*, 183–195.

Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., and Baskin, D.G. (2000). Central nervous system control of food intake. Nature. *404*, 661–671.

Sclafani, A., and Ackroff, K. (2012). Role of gut nutrient sensing in stimulating appetite and conditioning food preferences. Am. J. Physiol. Regul. Integr. Comp. Physiol. *302*, R1119–R1133.

Simpson, S.J., and Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. Obes. Rev. *6*, 133–42.

Skibicka, K.P., Hansson, C., Egecioglu, E., and Dickson, S. L. (2012). Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. Addict. Biol. *17*, 95–107.

Small, D.M., Jones-Gotman, M., and Dagher, A. (2003). Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. NeuroImage. *19*, 1709–1715.

Smith, G.P. (2004). Accumbens dopamine mediates the rewarding effect of orosensory stimulation by sucrose. Appetite. *43*, 11–13.

Steiner, J.E., Glaser, D., Hawilo, M. E., and Berridge, K.C. (2001). Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. Neurosci. Biobehav. Rev. *25*, 53–74.

Stellman, S. D., and Garfinkel, L. (1988). Patterns of artificial sweetener use and weight change in an American Cancer Society prospective study. Appetite. *11*, 85–91.

Stice, E., Spoor, S., Bohon, C., and Small, D.M. (2008). Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science. *322*, 449–452.

Stouffer, M.A., Woods, C.A., Patel, J.C., Lee, C.R., Witkovsky, P., Bao, L., … Rice, M. E. (2015). Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. Nat. Commun. *6*, 8543.

Sun, X., Luquet, S., and Small, D.M. (2017). DRD2: Bridging the Genome and Ingestive Behavior. Trends Cognit. Sci. *21*, 372–384.

Swinburn, B., Sacks, G., and Ravussin, E. (2009). Increased food energy supply is more than sufficient to explain the US epidemic of obesity. Am. J. Clin. Nutr. *90*, 1453–1456.

Swithers, S.E., Sample, C.H., and Davidson, T.L. (2013). Adverse effects of high-intensity sweeteners on energy intake and weight control in male and obesity-prone female rats. Behav. Neurosci. *127*, 262–274.

Szczypka, M.S., Kwok, K., Brot, M.D., Marck, B.T., Matsumoto, A.M., Donahue, B.A., and Palmiter, R.D. (2001). Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron. *30*, 819–828.

Taber, M.T., and Fibiger, H.C. (1997). Activation of the mesocortical dopamine system by feeding: lack of a selective response to stress. Neuroscience. *77*, 295–298.

Tang, D.W., Fellows, L.K., and Dagher, A. (2014). Behavioral and neural valuation of foods is driven by implicit knowledge of caloric content. Psychol. Sci. *25*, 2168–2176.

Tellez, L.A., Medina, S., Han, W., Ferreira, J.G., Licona-Limon, P., Ren, X., … Araujo, I. E. de (2013). A Gut Lipid Messenger Links Excess Dietary Fat to Dopamine Deficiency. Science. *341*, 800–802.

Thanarajah, S.E., Backes, H., DiFeliceantonio, A.G., Albus, K., Cremer, A.L., Hanssen, R., … Tittgemeyer, M. (2019). Food Intake Recruits Orosensory and Post-ingestive Dopaminergic Circuits to Affect Eating Desire in Humans. Cell Metab. *29*, 695–706.e4.

Tiedemann, L.J., Schmid, S.M., Hettel, J., Giesen, K., Francke, P., Büchel, C., and Brassen, S. (2017). Central insulin modulates food valuation via mesolimbic pathways. Nat. Commun. *8*, 16052.

Tordoff, M.G., and Alleva, M.A. (1990). Oral stimulation with aspartame increases hunger. Physiol. Behav. *47*, 555–559.

Veldhuizen, M.G., Babbs, R.K., Patel, B., Fobbs, W., Kroemer, N.B., Garcia, E., Yeomans, M.R., and Small, D.M. (2017). Integration of Sweet Taste and Metabolism Determines Carbohydrate Reward. Curr. Biol. *27*, 2476–2485.e6.

Volkow, N.D., and Wise, R.A. (2005). How can drug addiction help us understand obesity? Nat. Neurosci. *8*, 555–560.

Wang, G.J., Volkow, N.D., Logan, J., Pappas, N.R., Wong, C.T., Zhu, W., Netusil, N., and Fowler, J.S. (2001). Brain dopamine and obesity. Lancet. *357*, 354–357.

Warren, C.M., Tona, K.D., Ouwerkerk, L., Paridon, J. van, Poletiek, F., Steenbergen, H. van, Bosch, J.A., and Nieuwenhuis, S. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. Brain Stimul. *12*, 635–642.

World Health Organization. (2000). Obesity: preventing and managing the global epidemic. Report of a WHO Consultation (WHO Technical Report Series 894).

Yeomans, M.R., Leitch, M., Gould, N.J., and Mobini, S. (2008). Differential hedonic, sensory and behavioral changes associated with flavor–nutrient and flavor–flavor learning. Physiol. Behav. *93*, 798–806.

Zhang, L., Han, W., Lin, C., Li, F., and Araujo, I. E. de (2018). Sugar Metabolism Regulates Flavor Preferences and Portal Glucose Sensing. Front. Integr. Neurosci. *12*, 57.

Zhou, Q.Y., and Palmiter, R.D. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell. *83*, 1197–1209.

Bionotes

Sharmili Edwin Thanarajah
Max Planck Institute for Metabolism Research,
Gleueler Strasse 50,
50931 Cologne, Germany
e-mail: sharmili.edwin@sf.mpg.de

Sharmili Edwin Thanarajah studied Medicine at the Justus Liebig University in Giessen. She obtained her medical doctorate at the Max-Planck-Institute for Brain Research in Frankfurt under the supervision of Prof. Dr. Wolf Singer and PD Dr. Peter Uhlhaas on structural brain connectivity changes in Schizophrenia. Dr. Edwin Thanarajah performed her neurology training at the University Hospital of Cologne (Prof. Dr. Gereon Fink) and specialized in movement disorders. As a post-doc at the Max-Planck Institute for Metabolism Research, her main research interest is on understanding how signals from the periphery interact with brain reward pathways to modulate human behaviour. In particular, her research focuses on gut-brain signalling mediated by hormonal pathways and vagal transmission.

Marc Tittgemeyer
Max Planck Institute for Metabolism Research,
Gleueler Strasse 50,
50931 Cologne, Germany
e-mail: marc.tittgemeyer@sf.mpg.de

Marc Tittgemeyer obtained his PhD at the Physics faculty of the Technical University in Karlsruhe. Thereafter, at the Max-Planck-Institute for Human Cognitive and Brain Research in Leipzig he was introduced to the field of Neuroscience. He later moved to Cologne to head a research group associated with the Department of Neurology at the Max-Planck-Institute for Neurological Research. After the Institute's reorientation to become the Max-Planck-Institute for Metabolism Research in 2010, he became an independent group leader (Translational Neurocircuitry group). Dr. Tittgemeyer's research concerns the question how our organism integrates information about the internal state with environmental cues. He is especially interested in understanding how the brain senses the needs of the body – such as the need for food – and then generates specific behavioural responses that restore physiologic homeostasis.

Review Article

Astrid Rollenhagen and Joachim H. R. Lübke*

Synapses: Multitasking Global Players in the Brain

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Abstract: Synapses are key elements in the communication between neurons in any given network of the normal adult, developmental and pathologically altered brain. Synapses are composed of nearly the same structural subelements: a presynaptic terminal containing mitochondria with an ultrastructurally visible density at the pre- and postsynaptic apposition zone. The presynaptic density is composed of a cocktail of various synaptic proteins involved in the binding, priming and docking of synaptic vesicles inducing synaptic transmission. Individual presynaptic terminals (synaptic boutons) contain a couple of hundred up to thousands of synaptic vesicles. The pre- and postsynaptic densities are separated by a synaptic cleft. The postsynaptic density, also containing various synaptic proteins and more importantly various neurotransmitter receptors and their subunits specifically composed and arranged at individual synaptic complexes, reside at the target structures of the presynaptic boutons that could be somata, dendrites, spines or initial segments of axons.

Beside the importance of the network in which synapses are integrated, their individual structural composition critically determines the dynamic properties within a given connection or the computations of the entire network, in particular, the number, size and shape of the active zone, the structural equivalent to a functional neurotransmitter release site, together with the size and organization of the three functionally defined pools of synaptic vesicles, namely the readily releasable, the recycling and the resting pool, are important structural subelements governing the 'behavior' of synaptic complexes within a given network such as the cortical column.

In the late last century, neuroscientists started to generate quantitative 3D-models of synaptic boutons and their target structures that is one possible way to correlate structure with function, thus allowing reliable predictions about their function. The re-introduction of electron microscopy (EM) as an important tool achieved by modern high-end, high-resolution transmission-EM, focused ion beam scanning-EM, CRYO-EM and EM-tomography have enormously improved our knowledge about the synaptic organization of the brain not only in various animal species, but also allowed new insights in the 'microcosms' of the human brain in health and disease.

Keywords: synaptic organization, neocortex, electron microscopy, quantitative 3D-volume reconstruction, 3D-models of synaptic boutons

Zusammenfassung: Synapsen sind Schlüsselelemente der Kommunikation zwischen Neuronen in jedem beliebigen Netzwerk des normal adulten, sich entwickelnden, bzw. krankhaft veränderten Gehirns. Synapsen sind nahezu aus den gleichen strukturellen Subelementen aufgebaut: einem präsynaptischen Element, welches Mitochondrien und eine ultrastrukturell sichtbare Proteinverdichtung der Membran mit einem Cocktail verschiedener synaptischer Proteine enthält, welche für die Bindung, das „Priming" und das Andocken synaptischer Vesikel verantwortlich sind. Das präsynaptische Terminal (synaptischer Bouton) kann einige hundert bis zu einigen tausend synaptische Vesikel enthalten. Die präsynaptische Seite ist durch den synaptischen Spalt von der postsynaptischen Dichte der Zielstruktur getrennt, die entweder Somata, Dendriten, dendritische „Spines" oder Axoninitialsegmente darstellen. Die postsynaptische Dichte enthält wiederum spezifische synaptische Proteine, aber noch wichtiger verschiedene Neurotransmitter-Rezeptoren und deren Untereinheiten, die je nach Synapsentyp individuell komponiert und arrangiert sind.

Neben dem Netzwerk, in welches Synapsen integriert sind, kommt deren Aufbau, d. h. der Anzahl, Verteilung und dem Aufbau aktiver Zonen (strukturelles Äquivalent

***Corresponding author: Joachim H. R. Lübke**, Institute of Neuroscience and Medicine INM-10, Research Centre Jülich GmbH, Leo-Brandt Str., 52425 Jülich, Germany; Department of Psychiatry, Psychotherapy and Psychosomatics, Medical Faculty/RWTH University Hospital Aachen, Pauwelstr. 30, 52074 Aachen, Germany; JARA Translational Brain Medicine, Jülich/Aachen, Germany, e-mail: j.luebke@fz-juelich.de

Astrid Rollenhagen, Institute of Neuroscience and Medicine INM-10, Research Centre Jülich GmbH, Leo-Brandt Str., 52425 Jülich, Germany, e-mail: a.rollenhagen@fz-juelich.de

zur funktionellen Neurotransmitter-Freisetzungsstelle) und den drei funktionell definierten „Pools“ synaptischer Vesikel, dem sog. „Readily Releasable“, dem „Recycling“ und dem Reservepool, eine entscheidende Rolle für die Funktion einzelner synaptischer Verbindungen innerhalb eines gegebenen und des gesamten Netzwerks, wie zum Beispiel der kortikalen Kolumne, zu.

Zum Ende des letzten Jahrhunderts wurde begonnen quantitative 3D-Modelle synaptischer Boutons und deren Zielstrukturen zu generieren, welches eine Möglichkeit darstellt korrelierte Struktur/Funktions-Beziehungen herzustellen. In anderen Worten: erlaubt die strukturelle Komposition von Synapsen verlässliche Voraussagen zu ihrer Funktion?

Die „Wiederentdeckung“ der Elektronmikroskopie (EM) als ein wichtiges Instrument hat mittels hochmoderner, hochauflösender Transmission-EM, der Einführung der „Focused Ion Beam Scanning-EM“ Technologie, die Etablierung von CRYO-EM sowie EM-Tomographie zu einem enormen Erkenntnisgewinn der synaptischen Organisation in verschiedenen Tiermodellen, aber auch zu neuen Erkenntnissen im „Mikrokosmos“ des gesunden und erkrankten menschlichen Gehirns geführt.

Schlüsselwörter: Synaptische Organisation, Neocortex, Elektronenmikroskopie, Quantitative 3D-Volumenrekonstruktion, 3D-Modelle synaptischer Boutons

Historical background

For centuries, the belief that the structure of the brain and its elements, namely neurons, their dendrites, axons and synapses, and non-neuronal astro- and oligodendrocytes, reflect its function had been a ‘driving force’ for investigations and the source of major discoveries in neuroscience. One of the most important ones was, beside the definition of the neuron, the introduction of the term ‘Synapse’ originally termed more than 100 years ago by Charles Sherrington, a well-known electrophysiologist at that time. Ramon y Cajal, one of the most influential neuroanatomists and the founder of the neuronal doctrine, later adopted this term. The word ‘Synapse’ comes from the Greek synapsis (συνάψις), meaning ‘conjunction’, and from συνάπτειν (συν ‘together’) and ἅπτειν (‘to fasten’). This early fundamental discovery and description is even more intriguing because both Sherrington and Ramon y Cajal suggested a direct connection between neurons via these structures, but without ever seeing them.

The introduction of EM and its further development leading nowadays to high-end, fine-scale transmission electron microscopy (TEM), focused ion beam scanning electron microscopy (FIB-SEM), CRYO-EM and EM tomography combined, for example with high-pressure freezing (see Studer et al. 2014; Imig et al. 2014) extended the structural investigations from the light microscopic visible neuron to the subcellular and even the molecular level of the synapse, the elementary building block of any neural networks in the brain. It has to be mentioned thought, that CRYO-EM is more typically suited for the analysis of the structure of proteins at high resolution, rather than subcellular structures.

In addition, modern light microscopic techniques, like Stimulated Emission Depletion (STED) and direct stochastic optical reconstruction microscopy (dSTORM) aimed to visualize synaptic structures at the nanoscopic level, however focused more on the abundance of various synaptic proteins at active zones. Furthermore, the introduction of CRYO-correlative light- and electron microscopy (CRYO-CLEM) allows both fluorescence microscopy as well as three dimensional (3D) CRYO-EM tomography to reveal the ultrastructure of significant target molecules with specific cellular functions at high temporal and spatial resolution (Plitzko et al. 2009). Since then, a wealth of information was obtained that deepened our understanding of synaptic structures, in the developmental and adult brain in health and disease based on studies undertaken in various animal species, including rodents, higher mammals, non-human primates and even humans.

Although synapses had been looked at from different viewpoints, summarized in meanwhile thousands of original publications, reviews and numerous textbooks, a detailed, comprehensive and quantitative knowledge about their morphology is still limited to a relative small number of CNS synapses in different brain regions (Calyx of Held: Rowland et al. 2000; Sätzler et al. 2002; Wimmer et al. 2006; Cochlear bushy cell synapses: Nicol and Walmsley 2002; Climbing fiber synapses: Xu-Friedman et al. 2001; Cerebellar mossy fiber: Xu-Friedman and Regehr 2003; Hippocampal Mossy Fiber Bouton: Chicurel and Harris 1992; Rollenhagen et al. 2007; Synapses in the dentate gyrus: Marrone et al. 2005; Area CA1 synapses: Sorra and Harris 1993; Harris and Sultan 1995; Spacek and Harris 1998; Schikorski and Stevens 1997, 2001; Ribbon synapses in the retina and cochlear: Sikora et al. 2005; Moser et al. 2006; Michanski et al. 2019; Olfactory cortical synapses: Schikorski and Stevens 1999). Such detailed descriptions, however, are required to understand and link structural and functional components of the signal cascades underlying synaptic transmission and plasticity.

An important first step towards an improved understanding of synaptic function were simultaneous patch-

clamp recordings from a glutamatergic giant synapse, the so-called Calyx of Held terminating on the principal neurons in the medial nucleus of the trapezoid body in the auditory brainstem by Sakmann, Neher and co-workers (for example see Borst and Sakmann 1996, 1998, 1999; Takahashi et al. 1996; Schneggenburger et al. 1999, Schneggenburger and Neher 2000; for review see also Schneggenburger et al. 2002). However, it turned out that the Calyx of Held is rather the exception than the rule with respect to its synaptic properties perfectly adapted to audition. Hence the investigation of the Calyx of Held synapse strongly suggested that synapses are 'unique' entities, in both structural and functional terms.

The second, more central synapse, where paired recording became possible was the mossy fiber bouton-CA3 pyramidal cell synapse in the hippocampus, a synapse involved in learning and memory processes (Geiger and Jonas 2000; Bischofberger and Jonas 2002; Hallermann et al. 2003; Engel and Jonas 2005; Alle and Geiger 2006). The work on this synapse strongly supported and extended the view that synapses are unique in their structural and functional properties. Thus, the dream to create a general 'model synapse' for the brain was over.

Nevertheless, the simultaneous recordings from two different CNS synapses and their target structures made it possible for the first time to measure transmitter release under defined internal and external ionic and membrane potential conditions. In addition, the size and time course of action potential evoked Ca^{2+} influx (Borst and Sakmann 1996, 1998; Bischofberger and Jonas 2002), the occupancy of the putative Ca^{2+} sensor driving vesicle fusion (Bollmann et al. 2000; Schneggenburger and Neher 2000), the equilibration of intracellular Ca^{2+} with the endogenous Ca^{2+} buffer, and the eventual Ca^{2+}-clearance (Helmchen et al. 1997) can be accurately measured. Furthermore, the latency, size and time course of evoked quantal and multiquantal EPSCs (Borst and Sakmann 1996; Silver et al. 2003; Molnar et al. 2016; Holderith et al. 2016; Seeman et al. 2018; Rollenhagen et al. 2018; Vaden et al. 2019; reviewed by Neher 2015; Chamberland and Toth 2016) can be determined. However, there are still steps in the signal cascades that at present can only be simulated (Yamada and Zucker 1992; Bertram et al. 1999; Meinrenken et al. 2002, 2003; Freche et al. 2011). This includes the site, time- and space-dependent build-up and collapse of Ca^{2+}-domains around the pore of Ca^{2+} channels at a synaptic contact and the buffered diffusion and the subsequent interaction of free Ca^{2+} with the Ca^{2+} sensor.

Thus, realistic values of the geometry of synaptic boutons, including the number, size and shape of active zones, and the three functionally defined pools of synaptic vesicles (Rizzoli and Betz 2005), namely the readily releasable (RRP), the recycling (RP) and resting pool are essential for constraining realistic geometrical models of synaptic structures.

On the postsynaptic side, the time course and amplitude of spontaneous and evoked excitatory postsynaptic potentials (EPSCs) were used to infer the characteristics of quantal release (Henze et al. 1997; Silver et al. 2003; Biro et al. 2005; Szabadics et al. 2006; Saviane and Silver 2006; Rollenhagen et al. 2018; Vaden et al. 2019). This interference requires simulations of the transient increase of the glutamate concentration in the synaptic cleft, reversible binding of glutamate to appropriate glutamate receptors and eventual uptake and diffusion of glutamate out of the cleft (Freche et al. 2011). To a large extent, these processes are governed by the geometry of the synaptic cleft and the shape and size of pre- and postsynaptic densities. These parameters can only be estimated from 3D-reconstructions of synaptic structures.

This review will focus on recent findings on the detailed quantitative structural description of the most common type of synaptic bouton in the CNS: excitatory synaptic boutons in different layers of the rodent, non-human primate and human neocortex. Their small size and the great diversity of neurons and synaptic boutons in different layers of a cortical column made this investigation a challenging and difficult task. Such detailed morphological descriptions are useful to directly correlate structure with function of synapses and may therefore explain their different and specific functional performance and computational properties within the network in which they are integrated. On the other hand, such quantitative data provide the basis for numerical and/or MonteCarlo simulations of various synaptic parameters that are still only partially accessible for experiment, at least in the human brain.

Methodological considerations

One possible way to describe synaptic boutons and their target structures in such great detail are either 3D-volume reconstruction based on serial ultrathin sections using TEM (Fig. 1A) or FIB-SEM (Fig. 1B). With the second approach, serial digital EM images were obtained by constant milling a defined area of the sample containing the area of interest by a gallium ion laser beam and subsequent imaging of the block surface (block-face imaging).

From the resulting z-stacks of EM images quantitative 3D-models of synaptic boutons and their prospective target structures can then be generated using different commer-

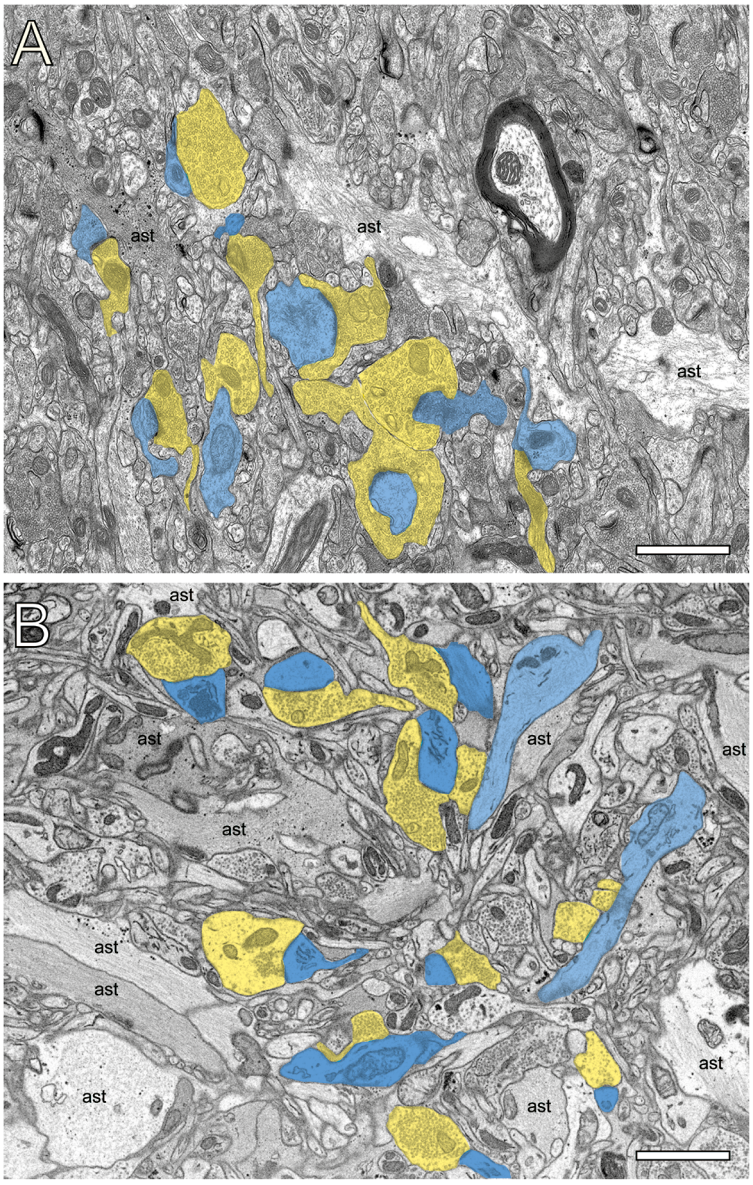

Fig. 1: Comparison of the ultrastructure between TEM and FIB-SEM
A, Low power electron micrograph of the neuropil in the lower part of layer 1 in the human temporal lobe neocortex as visualized with TEM. Note that even at this relatively low EM magnification several synaptic complexes between synaptic boutons and either dendritic shafts or spines, are clearly identifiable.
B, Low power electron micrograph of the neuropil in layer 4 of the human temporal lobe neocortex taken with FIB-SEM. Scale bar in A and B 1 µm.
In both electron micrographs, synaptic boutons are given in transparent yellow and postsynaptic structures in transparent blue. Several thick astrocytic (ast) processes are clearly visible.
Note the differences in the appearance of active zones and synaptic vesicles due to the use of a different EM protocol required for SEM-FIB.

cially or self-made reconstruction software tools running on high-performance computer systems. Both, TEM and FIB-SEM have advantages, but also disadvantages. Serial sectioning and subsequent TEM examination of ultrathin sections within a series is a very labor-intensive and thus time-consuming process with a comparable low throughput of tissue samples. Secondly, in ultrathin sections, the tilting of the electron beam restricts the area of interest, and during the cutting and imaging process, malformations or distortions or the complete loss of the tissue sample can be a limiting factor. However, the major advantage of using serial ultrathin sections and TEM imaging is their very high quality at high resolution that is required for the detailed analysis of important structural subelements such as the number, size and shape of active zones and the organization and size of the three functionally defined pools of synaptic vesicles (Figs. 1A, 2C, D, 3A-E).

In contrast, FIB-SEM (Fig. 1B), a relatively new, modern EM technology, allows a much higher throughput of tissue samples because the time and labor-intensive step of serial ultrathin sectioning is no longer required. Secondly, a larger area of interest ~50 by 50 µm can be obtained compared to TEM where the area of interest is limited to ~10–20 by 10–20 µm. Finally, since the surface of the block is milled and polished rather no malformations or distortions are expected thus no or minor alignment processing of adjacent images is required. The major disadvantage, however, are limitations in the resolution of

active zones and synaptic vesicles which appear structurally different due to the use of a different EM embedding protocol required for FIB-SEM (Fig. 1B; Movie 1).

In the future, the combination of both TEM and FIB-SEM will be the method of choice to address specific questions and further unravel the 'microcosms' of the brain, for example in describing the 'connectomics' and synaptic organization of various layers, nuclei and brain regions.

Synaptic boutons in the neocortex of rodents and non-human primates

Meanwhile numerous publications described structural and functional aspects of synaptic transmission and plasticity in different layers mainly in the rodent neocortex using paired or multiple recordings and subsequent morphological analysis of synaptically coupled pairs filled with biocytin or fluorescent dyes during recording (reviewed by Lübke and Feldmeyer 2007; Feldmeyer 2012; Feldmeyer et al. 2013; Qi et al. 2015; Radnikow and Feldmeyer 2018). It has been demonstrated that, beside similarities huge differences exist between intralaminar (synaptic connections within a given layer) and translaminar (synaptic connections across layers) excitatory-excitatory, excitatory-inhibitory and inhibitory-inhibitory synaptic connections with respect to synaptic efficacy, strength, release probability, short-term plasticity and contribution of various neurotransmitter receptors and their subunits, for example different glutamate and GABA receptors. However, these studies aimed to correlate structural with functional properties of a given synaptic connection rather than on the structural composition of individual synaptic contacts.

In contrast, only a few coherent and quantitative structural studies exist for synaptic boutons in the rodent neocortex (Rollenhagen et al. 2015, 2018; Dufour et al. 2016; Bopp et al. 2017; Hsu et al. 2017; Rodriguez-Moreno et al. 2018) and non-human primate neocortex (Anderson and Martin 2006, 2009; Freese and Amaral 2006; Hsu et al. 2017). These studies have demonstrated, for example, layer, region and gender specific differences in the density of synaptic boutons (Alonso-Nanclares et al. 2008). Most strikingly, synaptic boutons beside layer and area-specific differences (see also Rollenhagen 2015, 2018; Bopp et al. 2017; Hsu et al. 2017), differ substantially not only in their shape and size, but even more importantly in the number, size and shape of active zones and in the organization and size of the three pools of synaptic vesicles summarized in Table 1. Interestingly, some structural parameters such as bouton size, pre- and postsynaptic density surface area, content of mitochondria, and synaptic vesicles pools are in some cortical synapses well correlated but in others, no or only a weak correlation between several structural subelements are found (Rollenhagen 2015, 2018; Dufour et al. 2016; Hsu et al. 2017; Bopp et al. 2017; Rodriguez-Moreno et al. 2018). The most striking difference at cortical synaptic boutons is the total pool, and the three functionally defined pools of synaptic vesicles, namely the RRP, the RP and resting pools. Is has to be noted that a structural correlate for the functionally defined pools is not identifiable at the EM level due to the 'randomly' distribution of synaptic vesicles within the synaptic bouton. However, an attempt was made to sort synaptic vesicles with respect to their distance from the presynaptic density by a perimeter analysis. This approach allows the identification of synaptic vesicles belonging to one of the functionally defined pools (for criteria see Rizzoli and Betz 2005) and match nearly perfectly with functional estimations of the RRP (Hallermann et al. 2003) and RP (Rollenhagen et al. 2018). However, it is for example, still controversially discussed whether only so-called 'docked' vesicles identified at the ultrastructural level (Figs. 3F, 4C inset) already fused at the presynaptic density represent the RRP or also vesicles very close (10–20 nm from the active zone) belong to the RRP (Rollenhagen et al. 2015, 2018; Yakoubi et al. 2019a, b). The same holds true for the RP, how is it defined, and how many vesicles it contains at which distance from the presynaptic density is largely unknown for most of the CNS synapses (but see Rollenhagen et al. 2018). For the role and importance of the resting pool of synaptic vesicles that is thought not to be recruited under 'normal' physiological conditions, rather no information is available. However, it has been shown that vesicles from the resting pools can be transferred to the RP and RRP under the control of mitochondria (Verstreken et al. 2005). As a consequence, further experiments using a combination of labeling synaptic vesicles, for example with SM1–43, and high-resolution STED or two-photon laser microcopy using *in vitro* acute brain slices or neuronal cell cultures can address such questions (Verstreken et al. 2008) in more detail.

Table 1: Comparison of structural parameters of synaptic boutons in different regions or layers of the rodent, non-human primate and human neocortex

Species (Strain)	**Mouse** (C57/BL6)*		**Mouse** (C57/BL6)**	**Mouse** (C57/BL6)***		**Rat** (Wistar)		**Monkey** (Macaca mulatta)***		**Human**	
Region	L4_M1	L4_S1	L4_S1	L2–3_V1	L2–3_FC	L4_S1	L5_S1	L2–3_V1	L2–3_LPFC	L4_TL	L5_TL
Sex	♂	♂	♂	—	♀ / ♂	♀ / ♂	♀ / ♂	♀ / ♂	♀ / ♂	♀ / ♂	♀ / ♂
Age	—	—	60–65 days	2–14 month		90–120 days		5–20 years		20–63 years	
Synaptic boutons											
Density†	16.3 ± 2.7	31.7 ± 4.9	—	0.93 ± 0.08	1.05 ± 0.13	—	—	0.52 ± 0.06	0.48 ± 0.04	2.37x10^6	—
Surface area (µm^2)	—	—	4.67 ± 2.20	—	—	3.03 ± 0.71	8.19 ± 2.84	—	—	2.50 ± 1.78	6.09 ± 0.85
Volume (µm^3)	0.169$^+$ / 0.067$^-$	0.306$^+$ / 0.070$^-$	0.46 ± 0.27	0.10 ± 0.01	0.08 ± 0.03	0.20 ± 0.07	0.38 ± 0.23	0.20 ± 0.04	0.30 ± 0.01	0.16 ± 0.16	0.63 ± 0.17
Mitochondria											
Volume (µm^3)	0.034$^+$ / 0.00$^-$	0.061$^+$ / 0.008$^-$	0.09 ± 0.06	—	—	0.05 ± 0.02	0.07 ± 0.05	—	—	0.03 ± 0.04	0.12 ± 0.10
% of the total volume	20.12$^+$ / 0.000$^-$	19.93$^+$ / 11.43$^-$	23.03	—	—	20.29	15.09	—	—	13.11	12.04
Active zones											
Number per bouton	1.3$^+$ / 1.1$^-$	2.1$^+$ / 1.2$^-$	1.6	—	—	1.06 ± 0.06	1.12 ± 0.09	—	—	1–3	1–2
PreAZ surface area (µm^2)	—	—	—	—	—	0.18 ± 0.06	0.29 ± 0.19	—	—	0.13 ± 0.07	0.23 ± 0.05
PSD surface area (µm^2)	0.064$^+$ / 0.56$^-$	0.042$^+$ / 0.039$^-$	0.21 ± 0.11	0.08 ± 0.01	0.07 ± 0.02	0.18 ± 0.06	0.31 ± 0.21	0.08 ± 0.01	0.11 ± 0.01	0.13 ± 0.07	0.28 ± 0.11
Cleft width (nm)											
Lateral	—	—	—	—	—	17.22 ± 1.50	15.52 ± 0.39	—	—	14.11 ± 0.69	17.24 ± 2.21
Central	—	—	—	—	—	30.22 ± 1.42	31.32 ± 1.81	—	—	16.47 ± 1.85	19.05 ± 2.72
Synaptic vesicles											
Total number	4846$^+$ / 4861$^-$	5032$^+$ / 5233$^-$	740 ± 285	—	—	561.00 ± 108.00	811.47 ± 272.25	337± 23	555 ± 48	1820.64 ± 980.34	1518.52 ± 303.18
Diameter (nm)	—	—	—	—	—	29.85 ± 4.63	33.75 ± 4.55	~35	~35	19.80 ± 5.63	36.69 ± 1.71
Volume (µm^3)	—	—	—	—	—	0.01 ± 0.00	0.02 ± 0.02	—	—	0.01 ± 0.01	—
Pool size of synaptic vesicles											
Putative RRP at p10 nm	—	—	—	—	—	1.97 ± 2.57	3.89 ± 3.35	—	—	20.20 ± 18.58	5.42 ± 4.09
Putative RRP at p20 nm	—	—	—	—	—	6.30 ± 6.40	11.55 ± 4.16	—	—	48.59 ± 39.02	15.21 ± 9.09
Putative RP 60–200 nm	—	—	—	—	—	130.16 ± 20.79	162.83 ± 56.37	—	—	382.10 ± 248.23	181.86 ± 24.20
Putative resting pool >200nm	—	—	—	—	—	408.84 ± 100.04	599 ± 212.21	—	—	1251.82 ± 471.17	1264.05 ± 269.91

The various synaptic parameters were taken from *Bopp et al. 2017 † values are taken from VGluT2-labeled boutons (+) or unlabeled VGluT2 boutons (–) density in µm^2; **Rodriguez-Moreno et al. 2018; ***Hsu et al. 2017; Medalla and Luebke 2015 † density per neuron; Rat Barrel Cortex: L4 and L5, Rollenhagen et al. 2015, 2018; Human TL: L4 and L5, Yakoubi et al. 2019a, b † density in mm^3.
Abbreviations: L, layer; TL, temporal lobe; M1, primary motor; S1, primary sensory; V1, primary visual; FC, frontal cortex; LPFC, lateral prefrontal cortex;
p (perimeter) 10 nm and p20 nm is the distance of synaptic vesicles from the active zone

Very special entities: Synaptic boutons in humans?

One of the major question in synaptic neuroscience is whether results obtained in experimental animals can be transferred one-to-one to the human brain. Research on the human brain was for a long-time restricted to postmortem brains. However, for fine-scale, high-resolution EM it turned out that tissue samples from postmortem brains are not suitable because the time window between the removal and fixation of the brain is far too long to guarantee an excellent preservation of the ultrastructure, a pre-requisite for EM investigations at the cellular and subcellular level. To overcome this problem access, non-epileptic tissue from epilepsy- or brain tumor surgery became the method of choice. Here, care was taken that the tissue samples were selected far away from the epileptic focusas monitored by magnetic resonance imaging and electrophysiology and may thus be regarded as non-affected (non-epileptic) as also demonstrated by other studies using the same experimental approach (Alonso-Nanclares et al. 2008; Navarrete et al. 2013; Mohan et al. 2015; Molnar et al. 2016; Seeman et al. 2018). After its removal, tissue sample can be either immediately immersion-fixed or even prepared for acute brain slice preparations. Meanwhile several studies have studied structural (for example: Alonso-Nanclares et al. 2008; Blazquez-Llorca et al. 2013; Morales et al. 2014; Liu and Schumann 2014, Yakoubi et al. 2019a, b) and functional aspects (for example: Holderith et al. 2016; Molnar et al. 2016; Seeman et al. 2018) of synaptic transmission and plasticity in humans. However, coherent and comprehensive studies about the synaptic organization of the human brain, in particular quantitative 3D-models of synaptic boutons in humans are still very rare (but see Yakoubi et al. 2019a, b).

Using non-affected neocortical access tissue taken from epilepsy surgery, we have started to study the layer-specific synaptic organization of the temporal lobe neocortex (TLN), a typical example of a six-layered granular associational neocortex (Fig. 2–4B, C). The growing interest in the TLN is motivated by its importance in high-order brain functions as audition, vision, memory, language processing, and various multimodal associations. Moreover, the TLN is also involved in several neurological diseases most importantly as the area of origin and onset of TL epilepsy (TLE). TLE is the most common form of refractory epilepsy characterized by recurrent, unprovoked focal seizures that may, with progressing disease, also spread to other areas of the brain. Taken together, the TLN represents an important region in the normal and pathologically altered brain in humans.

So far, the synaptic organization of layer 4, the receiving input layer of signals from the sensory periphery thus representing the first station of intracortical information processing and layer 5 the major output layer was quantitatively analyzed in the TLN (Yakoubi et al. 2019a, b). The final goal of our investigations is to describe the synaptic organization of a cortical column, the elementary building block of the neocortex also in humans, exemplified for the TLN.

Synaptic boutons in the human TLN have an average size of ~2.5 to 6 μm^2 and are, beside similarities, strikingly different in some structural parameters from their counterparts in experimental animals (Table 1). Like in rodents and non-human primates so-called *en passant* (Fig. 2B; 4A) and endterminal synaptic boutons (Fig. 2C) contact either dendritic shafts (Figs. 2D; 3A, 4B), but the vast majority (~90 %) of excitatory synaptic boutons was established on dendritic spines of different types including stubby (Figs. 2D, 3A, B), mushroom (Figs. 3C, 4A, C), filopodial and elongated spines which is different to various animal species. Secondly, the majority of spines (~90 %) contained a so-called spine apparatus (Figs. 2D, 3A, B), a derivate of the endoplasmic reticulum, responsible for spine motility and stabilization of the synaptic complex during single or repetitive high-frequency stimulation. Thus, it was hypothesized that spines containing a spine apparatus partially contribute in modulating short-term synaptic plasticity (for example Holtmaat et al. 2006; for review see Knott and Holtmaat 2008). Interestingly, so-called dendro-dendritic synapses, regarded as a feature of the developmental brain, occur more frequently in the human TLN when compared to the neocortex in experimental animals. In addition, so-called clathrin-coated pits were frequently observed in synaptic boutons, some of which are located near the active zone (Fig. 2C). Clathrin-coated vesicles selectively sort cargo at the cell membrane, trans-Golgi network, and endosomal compartments for multiple membrane traffic pathways, for example exo- and endocytosis. A subpopulation is used in synaptic vesicle formation at the active zone.

Finally, also astrocytes receive direct synaptic input (Fig. 3D), although infrequently, supporting their involvement in synaptic transmission and plasticity (Min and Nevian 2012). Astrocytes have long been thought to act as nutrition suppliers and providing a stabilizing corset for neurons in the brain. However, it is now well established that astrocytes also play an important role in synaptic function, acting not only as physical barriers to glutamate diffusion, but also mediate transmitter uptake by

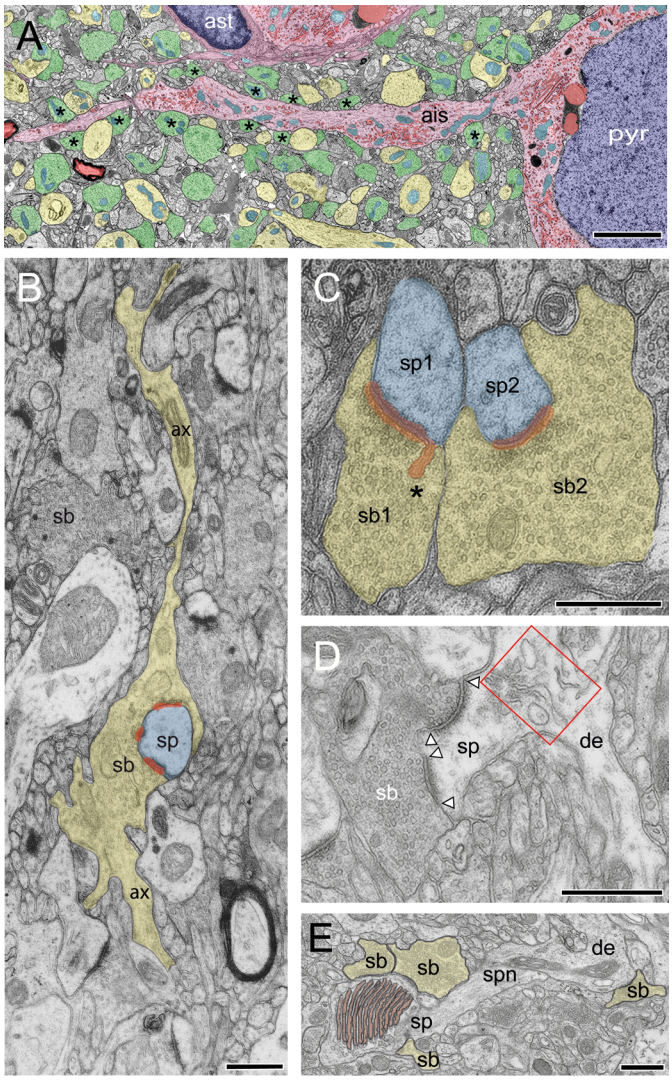

Fig. 2: Synaptic organization in different layers of the human TLN

A, Low power TEM micrograph of the neuropil in layer 2/3. For better visualization, some structures are highlighted in different colors. Transparent magenta: a pyramidal cell (pyr) and an astrocyte (ast) close to another adjacent pyramidal cell. The nuclei are given in transparent blue. Numerous dendritic profiles of different shape and size in the neuropil are highlighted in transparent yellow and synaptic boutons in green. Mitochondria in all structures are given in transparent blue. Note the axon initial segment (ais) originating at the base of one pyramidal cell soma. The ais is innervated by several synaptic boutons (marked by asterisks). Scale bar 5 µm.

B, *En passant* axon (ax) giving rise to a synaptic bouton (sb, transparent yellow) innervating a dendritic spine (sp, transparent blue) in layer 2/3. Active zones are outlined in red. Scale bar 2 µm.

C, Two endterminal boutons (sb1, sb2) establishing synaptic contacts with two neighboring spines (sp1, sp2) in layer 4. Note the different shape and size of the boutons and the active zones (red contours) in both synaptic complexes that nearly covers the entire pre- and postsynaptic apposition zone. A so-called coated pit (asterisk) is located close to the active zone. Scale bar 0.2 µm.

D, Representative example of a large stubby spine (sp) emerging from a dendrite (de) in layer 1 innervated by a large synaptic bouton (sb) with two active zones (marked by arrowheads) full of synaptic vesicles. Note also the prominent spine apparatus (framed area). Scale bar 0.5 µm.

E, Large mushroom spine (sp) with a long spine neck (spn) emerging from a small dendrite (de) in layer 1. Note the disk-like shape of the prominent spine apparatus (red contours) that covers nearly the entire volume of the spine head. The spine head is innervated by three synaptic boutons (sb) and a fourth establishes a synaptic contact at the base of the spine neck. Scale bar 0.5 µm.

glutamate transporters (Min and Nevian 2012; for review see Allen 2014; Dallerac et al. 2018). A striking common feature in both the human and the animal neocortex is the tight ensheathment of synaptic complexes with astrocytic processes forming the 'tripartite' synaptic complex (Fig. 3A, C), in contrast to MFBs and calyx of Held synapses, where astrocytic processes were never located close to individual active zones (Rollenhagen et al. 2007; Müller et al. 2009). This may explain the occurrence of glutamate spillover, synaptic cross talk and the switch from asynchronous to synchronous release upon repetitive stimulation as shown for the MFB (Hallermann et al. 2003) and Calyx of Held synapses (reviewed by von Gersdorff and Borst 2002). Astrocytes can actively take-up excessive or 'spilled' neurotransmitter when close to the synaptic cleft; hence they modulate the temporal and spatial neurotransmitter concentration thus controlling the induction, maintenance and termination of synaptic transmission but also modulate short-term synaptic plasticity in the neocortex.

The most striking difference between synaptic boutons in the human, non-human primate and rodent neocortex is, however, the shape and size of the active zones and that of the three functionally defined pools of synaptic vesicles, namely the RRP, RP and resting pool. Although small in size synaptic excitatory synaptic boutons in layer 4 and layer 5 of the TLN contain active zones that were on average 2-fold larger in size (~0.2 – 0.25 µm^2 in surface area) when compared with their counterparts of comparable size in other brain regions in rodents or non-human primates (Table 1), or even much larger CNS synapses such as the cerebellar and hippocampal mossy fiber bouton and the Calyx of Held endterminal. In numerous synaptic boutons in the human TLN, the active zones covered most of the pre- and postsynaptic apposition zone (Figs. 2 C, 3A, B) hence enlarging the presynaptic 'docking' zone

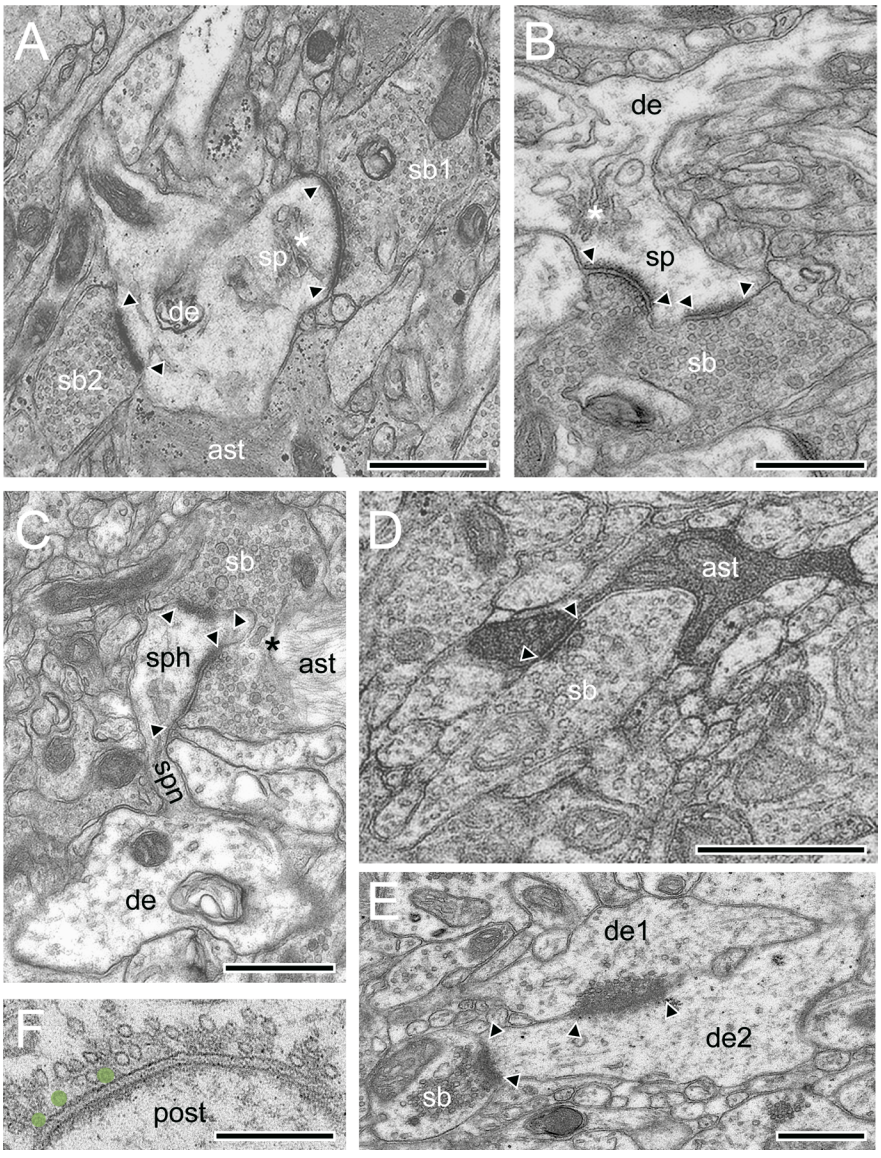

Fig. 3: Innervation pattern of synaptic boutons in different layers of the human TLN

A, Two opposite synaptic complexes (sb1-sp) and (sb2-de) in layer 1 one of which (sb1) establishes a glutamatergic synapse with a stubby spine (sp) identified by the shape and appearance of the active zone (arrowheads) and the size and more roundish shape of the synaptic vesicles. Sb2 is also glutamatergic as identified by the appearance of the active zone (arrowheads) but directly terminating on the dendritic shaft. Note the large astrocytic finger (ast) close to the dendrite and synaptic boutons and the spine apparatus in the stubby spine. Scale Bar 0.5 µm

B, A large stubby spine (sp) in layer 2/3 innervated by a synaptic bouton (sb) with two separated active zones (arrowheads). The spine apparatus is marked by an asterisk. Scale bar 0.5 µm

C, Typical example of a large mushroom spine in layer 2/3 with a thick spine head (sph) and a smaller but thick spine neck (spn) receiving input by a large synaptic bouton (sb). Note the two active zones (arrowheads) one directly on top of the spine head and the other located aside. The relatively large active zone (arrowheads) covers the entire pre- and postsynaptic apposition zone. de: dendrite; ast: astrocytic profile. Scale bar 0.5 µm.

D, Astrocytic process (ast) identified by its opaque appearance and the vesicles containing gliotransmitter contacted by a synaptic bouton (sb) in layer 5. This type of contact is rarely found. Scale bar 0.5 µm.

E, Dendro-dendritic synapse (de1, de2) where de1 serves as the presynaptic element identified by the cluster of synaptic vesicles at the active zone (arrowheads) in layer 6. In addition, de2 receives a synaptic bouton (sb) with a non-perforated active zone marked by arrowheads. Scale bar 0.25 µm.

F, High-magnification of a large non-perforated active zone in layer 6 showing three 'docked' vesicles (highlighted in transparent green) among the population of synaptic vesicles close to the presynaptic density contacting a dendritic spine at the postsynaptic (post) element. Scale bar 0.25 µm.

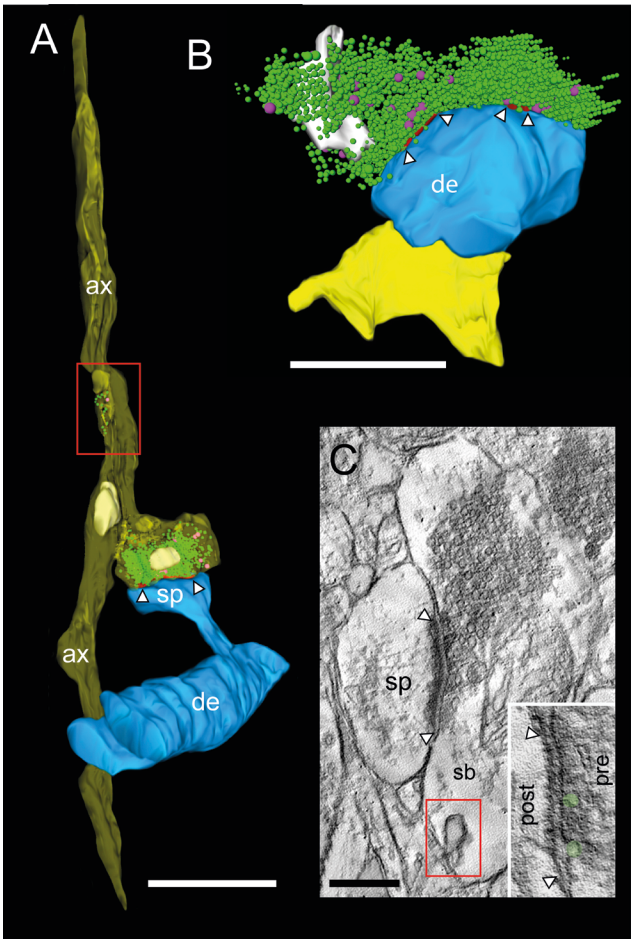

Fig. 4: 3D-volume reconstructions of synaptic boutons and their target structures and electron microscopic tomography in the rodent and human temporal lobe neocortex
A, *En passant* axon (ax, transparent gold) followed over long-distance in consecutive electron micrographs establishing a synaptic contact on a dendritic spine (sp) of a postsynaptic dendritic segment (de, blue) in rodent layer 5. The active zone is marked by arrowheads. Note the association of mitochondria (white) with the pool of synaptic vesicles (green dots). Note the presence of synaptic (green dots) vesicles and dense-core vesicles (magenta dots) within the *en passant* axon (framed area). Scale bar 1 µm.
B, Two synaptic boutons terminating opposite to each other onto a small caliber dendrite (de) in layer 5. In one bouton, the envelope of the terminal is omitted to better visualize the distribution of synaptic (green dots) and dense-core (magenta) vesicles. The mitochondrion is given in white and the two active zones are marked by arrowheads. Scale bar 1 µm.
C, Synaptic bouton (sb) terminating on a dendritic spine (sp) as visualized with electron microscopic tomography. A coated pit fused with the bouton membrane is shown in the framed area. Inset: High-power magnification of the active zone (arrowheads) with two 'docked' vesicles highlighted in transparent green. Pre: presynaptic; post: postsynaptic. Scale bar 0.25 µm.

for synaptic vesicles. In addition, more synaptic boutons contain not only a single but up to three active zones (Figs. 2B, 3A-C). Numerous of the active zones were perforated at the pre-, post- or both synaptic densities. Even more striking these boutons containing a total pool of synaptic vesicles (average 1500–1800 synaptic vesicles) that was 2–3-fold larger to that reported in the rodent and non-human primate neocortex suggesting also comparable large RRPs (Fig. 3F), RPs and resting pools. Indeed, the RRPs are by 3–5-fold, the RPs by 2-fold and the resting pools by 2-fold larger than in rodent and non-human primate neocortex. It has been recently shown that the size of the RRP dynamically regulates multivesicular release in mice (Vaden et al. 2019). Thus these large pools suggest reliable synaptic transmission even at high-frequency stimulation; hence a rapid depletion of the RRP and RP could be prevented by replenishment of synaptic vesicles from a large resting pool. It has to be noted though that like in non-human primates and rodents, a huge variability exists in the structural composition between individual synaptic boutons in humans, in particular the size of RRP, RP and resting pool that may partially contribute in modulating synaptic plasticity.

Taken together, the structural composition of both the presynaptic terminal and the spine as the main target structure suggests high synaptic efficacy and reliability of synaptic transmission but also in the induction, regulation and termination of short-term plasticity at synaptic boutons in the human brain.

This structural heterogeneity was confirmed by a recent structural/functional investigation about synaptic connections in the human TLN using paired recordings (Seeman et al. 2018). This study demonstrated that synaptic connections in the TLN are indeed highly reliable and strong as indicated by large excitatory postsynaptic potential (EPSP) amplitudes when compared to mouse neocortex, but also show layer-specific differences and in modulating short-term plasticity.

Perspectives

In summary, excitatory synaptic boutons in the brain represent 'unique entities' in rodents, non-human primates and even more in humans. Their individual composition with marked structural differences strongly suggest that they are perfectly adapted to the 'job' they have to fulfill in different neural networks of the brain in which they are embedded. However, there are still a lot of questions remaining that have to be addressed in the future. For

example, and most importantly how and when synapses are generated during the development of the human brain and do they undergo the same selective layer-specific pruning or elimination as shown in experimental animals? How and when do they undergo severe structural and functional changes during neurological disorders like schizophrenia, autism and neurodegenerative diseases like Morbus Alzheimer and Morbus Parkinson? How age-, strain-, sex, left/right-, and subregion-specific differences would influence their structural composition? How the three functionally defined pools of synaptic vesicles become differentially recruited during high-frequency brain activity, during different biological rhythms or behavior still remains rather unclear. At the molecular level, how are the numerous pre- and postsynaptic proteins, neurotransmitters and their subunits involved in the induction, maintenance and termination of synaptic transmission and plasticity arranged at the active zone? What is about their density and possible co-localization at individual synaptic complexes? To date we are still far away in our understanding of these fascinating structures.

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Abbreviations in alphabetic order

Ca^{2+}	calcium
CA3	hippocampal subregion CA3
CNS	central nervous system
CRYO-CLEM	CRYO-correlative light- and electron microscopy
3D	three-dimensional
dSTORM	stochastic optical reconstruction microscopy
EM	electron microscopy
EPSCs	excitatory postsynaptic currents
EPSPs	excitatory postsynaptic potentials
FIB-SEM	focused ion beam scanning electron microscopy
RRP	readily releasable pool of synaptic vesicles
RP	recycling pool of synaptic vesicles
STED	stimulated emission depletion microscopy
TEM	transmission electron microscopy
TLE	temporal lobe epilepsy
TLN	temporal lobe neocortex

References

Alle, H., and Geiger, J.R. (2006). Combined analog and action potential coding in hippocampal mossy fibers. Science *311*, 1290–1293.

Allen, N.J. (2014). Astrocyte regulation of synaptic behavior. Ann Rev Cell Dev Biol *30*, 439–463.

Alonso-Nanclares, L., Gonzalez-Soriano, J., Rodriguez, J.R., and DeFelipe, J. (2008). Gender differences in human cortical synaptic density. Proc Natl Acad Sci USA *105*, 14615–14619.

Anderson, J.C., and Martin, K.A.C. (2006). Synaptic connections from cortical areas V1 and V2 in macaque monkey. J Comp Neurol *495*, 709–721.

Anderson, J.C., and Martin, K.A.C. (2009). The synaptic connections between cortical area V4 to V2 in macaque monkey. J Neurosci *29*, 11283–11293.

Bertram, R., Smith, G.D., and Sherman, A. (1999). Modeling study of the effects of overlapping Ca^{2+} microdomains on neurotransmitter release. Biophys J *76*, 735–750.

Biro, A.A., Holderith, N.B., and Nusser, Z. (2005). Quantal size is independent of the release probability at hippocampal excitatory synapses. J Neurosci *25*, 223–232.

Bischofberger, J., and Jonas, P. (2002). TwoB or not twoB: differential transmission at glutamatergic mossy fiber-interneuron synapses in the hippocampus. Trends Neurosci *25*, 600–603.

Blazquez-Llorca, L., Merchan-Perez, A., Rodriguez, J.R., Gascon, J., and DeFelipe, J. (2013). FIB/SEM technology and Alzheimer's disease: three-dimensional analysis of human cortical synapses. J Alzheimers Dis *34*, 995–1013

Bollmann, J.H., Sakmann, B., and Borst, J.G. (2000). Calcium sensitivity of glutamate release in the calyx-type terminal. Science *289*, 953–957.

Bopp, R., Holler-Rickauer, S., Martin, K.A., and Schuhknecht, G.F. (2017). An Ultrastructural Study of the Thalamic Input to Layer 4 of Primary Motor and Primary Somatosensory Cortex in the Mouse. J Neurosci *37*, 2435–2448.

Borst, J.G., and Sakmann, B. (1996). Calcium influx and transmitter release in a fast CNS synapse. Nature *383*, 431–434.

Borst, J.G., and Sakmann, B. (1998). Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. J Physiol *506*, 143–157.

Borst, J.G., and Sakmann, B. (1999). Depletion of calcium in the synaptic cleft of a calyx-type synapse in the rat brainstem. J Physiol *521*, 123–133.

Chamberland, S., and Toth, K. (2016). Functionally heterogeneous synaptic vesicle pools support diverse synaptic signalling. J Physiol *594*, 825–835.

Chicurel, M.E., and Harris, K.M. (1992). Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. J Comp Neurol *325*, 169–182.

Dallerac, G., Zapata, J., and Rouach, N. (2018). Versatile control of synaptic circuits by astrocytes: where, when and how? Nat Rev Neurosci *19*, 729–743.

Dufour, A., Rollenhagen, A., Sätzler, K., and Lübke, J.H.R. (2016). Development of Synaptic Boutons in Layer 4 of the Barrel Field of the Rat Somatosensory Cortex: A Quantitative Analysis. Cereb Cortex *26*, 838–854.

Engel, D., and Jonas, P. (2005). Presynaptic action potential amplification by voltage-gated Na^+ channels in hippocampal mossy fiber boutons. Neuron *45*, 405–417.

Feldmeyer, D. (2012). Excitatory neuronal connectivity in the barrel cortex. Front Neuroanat *6*, 24 doi: 10.3389/fnana.2012.00024. eCollection 2012.

Feldmeyer, D., Brecht, M., Helmchen, F., Petersen, C.C., Poulet, J.F., Staiger, J.F., Luhmann, H.J., and Schwarz, C. (2013). Barrel cortex function. Prog Neurobiol *103*, 3–27.

Freche, D., Pannasch, U., Rouach, N., and Holcman, D. (2011) Synapse geometry and receptor dynamics modulate synaptic strength. PLoS One 6, e25122. Doi 10.137/journal.pone.0025122.

Freese, J.L., and Amaral, D.G. (2006) The synaptic organization of projections from the amygdala to visual cortical areas TE and V1 in the macaque monkey. J Comp Neurol *496,* 655–667.

Geiger, J.R., and Jonas, P. (2000). Dynamic control of presynaptic $Ca(^{2+})$ inflow by fast-inactivating $K(^+)$ channels in hippocampal mossy fiber boutons. Neuron *28*, 927–939.

Hallermann, S., Pawlu, C., Jonas, P., and Heckmann, M. (2003). A large pool of releasable vesicles in a cortical glutamatergic synapse. Proc Natl Acad Sci USA *100*, 8975–8980.

Harris, K.M., and Sultan, P. (1995). Variation in the number, location and size of synaptic vesicles provides an anatomical basis for the nonuniform probability of release at hippocampal CA1 synapses. Neuropharmacology *34*, 1387–1395.

Helmchen, F., Borst, J.G., and Sakmann, B. (1997). Calcium dynamics associated with a single action potential in a CNS presynaptic terminal. Biophys J *72*, 1458–1471.

Henze, D.A., Card, J.P., Barrionuevo, G., and Ben-Ari, Y. (1997). Large amplitude miniature excitatory postsynaptic currents in hippocampal CA3 pyramidal neurons are of mossy fiber origin. J Neurophysiol. *77*, 1075–1086.

Holderith, N., Lorincz, A., Katona, G., Rozsa, B., Kulik, A., Watanabe, M., and Nusser, Z. (2016). Release probability of hippocampal glutamatergic terminals scales with the size of the active zone. Nat Neurosci *19*, 172.

Holtmaat, A., Wilbrecht, L., Knott, G.W., Welker, E., and Svoboda, K. (2006). Experience-dependent and cell-type-specific spine growth in the neocortex. Nature *441*, 979–983.

Hsu, A., Luebke, J.I., and Medalla, M. (2017). Comparative ultra-structural features of excitatory synapses in the visual and frontal cortices of the adult mouse and monkey. J Comp Neurol *525*,2175–2191.

Imig, C., Min, S.W., Krinner, S., Arancillo, M., Rosenmund, C., Südhof, T.C., Rhee, J., Brose, N., and Cooper, B.H. (2014). The morphological and molecular nature of synaptic vesicle priming at presynaptic active zones. Neuron *84*, 416–431.

Knott, G., and Holtmaat, A. (2008). Dendritic spine plasticity--current understanding from in vivo studies. Brain Res Rev. *58*; 282–289. doi: 10.1016/j.brainresrev.01.002.

Liu, X.B., and Schumann, C.M. (2014). Optimization of electron microscopy for human brains with long-term fixation and fixed-frozen sections. Acta Neuropathol Commun *2*, 42.

Lübke, J., and Feldmeyer, D. (2007). Excitatory signal flow and connectivity in a cortical column: focus on barrel cortex. Brain Struct Funct *212*, 3–17.

Marrone, D.F., LeBoutillier, J.C., and Petit, T.L. (2005). Ultrastructural correlates of vesicular docking in the rat dentate gyrus. Neurosci Lett *378*, 92–97.

Medalla, M., and Luebke, J.I. (2015). Diversity of glutamatergic synaptic strength in lateral prefrontal versus primary visual cortices in the rhesus monkey. J Neurosci *35*, 112–127. doi: 10.1523/JNEUROSCI.3426-14.2015.

Meinrenken, C.J., Borst, J.G., and Sakmann, B. (2002). Calcium secretion coupling at calyx of Held governed by nonuniform channel-vesicle topography. J Neurosci *22*, 1648–1667.

Meinrenken, C.J., Borst, J.G., and Sakmann, B. (2003). Local routes revisited: the space and time dependence of the Ca^{2+} signal for phasic transmitter release at the rat calyx of Held. J Physiol *547*, 665–689.

Michanski, S., Smaluch, K., Steyer, A.M., Chakrabarti, R., Setz, C., Oestreicher, D., Fischer, C., Mobius, W., Moser, T., Vogl, C., *et al.* (2019). Mapping developmental maturation of inner hair cell ribbon synapses in the apical mouse cochlea. Proc Natl Acad Sci USA *116*, 6415–6424.

Min,R.,and Nevian,T. (2012) Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat Neurosci *15*, 746–753.

Mohan, H., Verhoog, M.B., Doreswamy, K.K., Eyal, G., Aardse, R., Lodder, B.N., Goriounova, N.A., Asamoah, B., AB, B.B., Groot, C., *et al.* (2015). Dendritic and Axonal Architecture of Individual Pyramidal Neurons across Layers of Adult Human Neocortex. Cereb Cortex *25*, 4839–4853.

Molnar, G., Rozsa, M., Baka, J., Holderith, N., Barzo, P., Nusser, Z., and Tamas, G. (2016). Human pyramidal to interneuron synapses are mediated by multi-vesicular release and multiple docked vesicles. eLIFE *5*.

Morales, J., Benavides-Piccione, R., Dar, M., Fernaud, I., Rodríguez, A., Anton-Sanchez, L., Bielza, C., Larrañaga, P., DeFelipe, J., and Yuste, R. (2014). Random positions of dendritic spines in human cerebral cortex. J Neurosci. *34*, 10078–10084. doi: 10.1523/JNEUROSCI.1085-14.2014.

Moser, T., Brandt, A., and Lysakowski, A. (2006). Hair cell ribbon synapses. Cell Tissue Res *326*, 347–359.

Müller, J., Reyes-Haro, D., Pivneva, T., Nolte, C., Schaette, R., Lübke, J., and Kettenmann, H. (2009). The principal neurons of the medial nucleus of the trapezoid body and NG2(+) glial cells receive coordinated excitatory synaptic input. J Gen Physiol *134*, 115–127.

Navarrete, M., Perea, G., Maglio, L., Pastor, J., Garcia de Sola, R., and Araque, A. (2013). Astrocyte calcium signal and gliotransmission in human brain tissue. Cereb Cortex *23*, 1240–1246.

Neher, E. (2015). Merits and Limitations of Vesicle Pool Models in View of Heterogeneous Populations of Synaptic Vesicles. Neuron *87*, 1131–1142.

Nicol, M.J., and Walmsley, B. (2002). Ultrastructural basis of synaptic transmission between endbulbs of Held and bushy cells in the rat cochlear nucleus. J Physiol *539*, 713–723.

Plitzko, JM, Rigort, A, and Leis, A. (2009) Correlative cryo-light microscopy and cryo-electron tomography: from cellular territories to molecular landscapes. Curr Opin Biotechnol *20*, 83–89.

Qi, G., Radnikow, G., and Feldmeyer, D. (2015). Electrophysiological and morphological characterization of neuronal microcircuits in acute brain slices using paired patch-clamp recordings. J Vis Exp, 52358.

Radnikow, G., and Feldmeyer, D. (2018). Layer- and Cell Type-Specific Modulation of Excitatory Neuronal Activity in the Neocortex. Front Neuroanat *12*, 1.

Rizzoli, S. O., and Betz, W.J. (2005). Synaptic vesicle pools. Nat Rev Neurosci. *6*:57–69.

Rodriguez-Moreno, J., Rollenhagen, A., Arlandis, J., Santuy, A., Merchan-Perez, A., DeFelipe, J., Lübke, J.H.R., and Clasca, F. (2018). Quantitative 3D Ultrastructure of Thalamocortical Synapses from the "Lemniscal" Ventral Posteromedial Nucleus in Mouse Barrel Cortex. Cereb Cortex *28*, 3159–3175.

Rollenhagen, A., Sätzler, K., Rodriguez, E.P., Jonas, P., Frotscher, M., and Lübke, J.H.R. (2007). Structural determinants of transmission at large hippocampal mossy fiber synapses. J Neurosci *27*, 10434–10444.

Rollenhagen, A., Klook, K., Sätzler, K., Qi, G., Anstötz, M., Feldmeyer, D., and Lübke, J.H.R. (2015). Structural determinants underlying the high efficacy of synaptic transmission and plasticity at synaptic boutons in layer 4 of the adult rat 'barrel cortex'. Brain Struct Funct *220*, 3185–3209.

Rollenhagen, A., Ohana, O., Sätzler, K., Hilgetag, C.C., Kuhl, D., and Lübke, J.H.R. (2018). Structural Properties of Synaptic Transmission and Temporal Dynamics at Excitatory Layer 5B Synapses in the Adult Rat Somatosensory Cortex. Front Synaptic Neurosci *10*, 24.

Rowland, K.C., Irby, N.K., and Spirou, G.A. (2000). Specialized synapse-associated structures within the calyx of Held. J Neurosci *20*, 9135–9144.

Sätzler, K., Söhl, L.F., Bollmann, J.H., Borst, J.G., Frotscher, M., Sakmann, B., and Lübke, J.H.R. (2002). Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body. J Neurosci *22*, 10567–10579.

Saviane, C., and Silver, R.A. (2006). Fast vesicle reloading and a large pool sustain high bandwidth transmission at a central synapse. Nature *439*, 983–987.

Schikorski, T., and Stevens, C.F. (1997). Quantitative ultrastructural analysis of hippocampal excitatory synapses. J Neurosci *17*, 5858–5867.

Schikorski, T., and Stevens, C.F. (1999). Quantitative fine-structural analysis of olfactory cortical synapses. Proc Natl Acad Sci USA *96*, 4107–4112.

Schikorski, T., and Stevens C.F. (2001). Morphological correlates of functionally defined synaptic vesicle populations. Nat Neurosci. *4*, 391–395.

Schneggenburger, R., Meyer, A.C., and Neher, E. (1999). Released fraction and total size of a pool of immediately available transmitter quanta at a calyx synapse. Neuron *23*, 399–409.

Schneggenburger, R., and Neher, E. (2000). Intracellular calcium dependence of transmitter release rates at a fast central synapse. Nature *406*, 889–893.

Schneggenburger, R., Sakaba, T., and Neher, E. (2002). Vesicle pools and short-term synaptic depression: lessons from a large synapse. Trends Neurosci *25*, 206–212.

Seeman, S.C., Campagnola, L., Davoudian, P.A., Hoggarth, A., Hage, T.A., Bosma-Moody, A., Baker, C.A., Lee, J.H., Mihalas, S., Teeter, C., *et al.* (2018). Sparse recurrent excitatory connectivity in the microcircuit of the adult mouse and human cortex. eLIFE *7*.

Sikora, M.A., Gottesman, J., and Miller, R.F. (2005). A computational model of the ribbon synapse. J Neurosci Methods *145*, 47–61.

Silver, R.A., Lübke, J., Sakmann, B., and Feldmeyer, D. (2003). High-probability uniquantal transmission at excitatory synapses in barrel cortex. Science *302*, 1981–1984.

Sorra, K.E., and Harris, K.M. (1993). Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. J Neurosci *13*, 3736–3748.

Spacek, J., and Harris, K.M. (1998). Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus. J Comp Neurol *393*, 58–68.

Studer, D., Zhao, S., Chai, X., Jonas, P., Graber, W., Nestel, S., and Frotscher, M. (2014). Capture of activity-induced ultrastructural changes at synapses by high-pressure freezing of brain tissue. Nat Protoc *9*, 1480–1495.

Szabadics, J., Varga, C., Molnar, G., Olah, S., Barzo, P., and Tamas, G. (2006). Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. Science *311*, 233–235.

Takahashi, T., Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., and Onodera, K. (1996). Presynaptic calcium current modulation by a metabotropic glutamate receptor. Science *274*, 594–597.

Vaden, J.H., Banumurthy, G., Gusarevich, E.S., Overstreet-Wadiche, L., and Wadiche, J.I. (2019). The readily-releasable pool dynamically regulates multivesicular release. eLIFE *8*.

Verstreken, P., Ly, C.V., Venken, K.J., Koh, T.W., Zhou, Y., and Bellen, H.J. (2005). Synaptic mitochondria are critical for mobilization of reserve pool vesicles at Drosophila neuromuscular junctions. Neuron *47*, 365–378.

Verstreken, P., Ohyama, T., and Bellen, H.J. (2008). FM 1–43 labeling of synaptic vesicle pools at the Drosophila neuromuscular junction. Methods Mol Biol.*440*, 349–69. doi: 10.1007/978-1-59745-178-926.

von Gersdorff, H., and Borst, J.G. (2002). Short-term plasticity at the calyx of Held. Nat Rev Neurosci *3*, 53–64.

Wimmer, V.C., Horstmann, H., Groh, A., and Kuner, T. (2006). Donut-like topology of synaptic vesicles with a central cluster of mitochondria wrapped into membrane protrusions: a novel structure-function module of the adult calyx of Held. J Neurosci *26*, 109–116.

Xu-Friedman, M.A., Harris, K.M., and Regehr, W.G. (2001). Three-dimensional comparison of ultrastructural characteristics at depressing and facilitating synapses onto cerebellar Purkinje cells. J Neurosci *21*, 6666–6672.

Xu-Friedman, M.A., and Regehr, W.G. (2003). Ultrastructural contributions to desensitization at cerebellar mossy fiber to granule cell synapses. J Neurosci *23*, 2182–2192.

Yakoubi, R., Rollenhagen, A., von Lehe, M., Shao, Y., Sätzler, K., and Lübke, J.H.R. (2019a). Quantitative Three-Dimensional Reconstructions of Excitatory Synaptic Boutons in Layer 5 of the Adult Human Temporal Lobe Neocortex: A Fine-Scale Electron Microscopic Analysis. Cereb Cortex. *29*, 2797–2814, doi: 10.1093/cercor/bhy146.

Yakoubi, R., Rollenhagen, A., von Lehe, M., Miller D., Walkenfort, B., Hasenberg, M., Sätzler, K., and Lübke, JHR. (2019b). Ultrastructural heterogeneity of human layer 4 excitatory synaptic boutons in the adult temporal lobe neocortex. Program No. 037.01. 2019 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2019. Online.

Yamada, W.M., and Zucker, R.S. (1992). Time course of transmitter release calculated from simulations of a calcium diffusion model. Biophys J *61*, 671–682.

Bionotes

Joachim H. R. Lübke
Institute of Neuroscience and Medicine
INM-10, Research Centre Jülich GmbH
Leo-Brandt Str., 52425 Jülich
Phone: 02461/612288
e-mail: j.luebke@fz-juelich.de
Orcid number: 0000-0002-4086-3199

Born 11.04.1956 in Recklinghausen, Germany; 1974–1977: Education as a biological technician; 1981–1982: 'Begabten-Abitur' at the Abend-gymnasium Göttingen; 1982–1987: Study of Biology, Georg-August Universität Göttingen; 1987–1991: Diploma and doctoral thesis, Max-Planck-Institut für Biophysikalische Chemie, Abt. Neurobiologie (Prof. Dr. O.-D. Creutzfeldt); 1991–1993: Postdoctoral Fellow (Scolarship of the Royal Society of Science), Dept. of Human Anatomy (Prof. Dr. Ray Guillery, University of Oxford; 1993–1995: 'Von Helmholtz Stipendiat' of the BMBF, Anatomisches Institut (Prof. Dr. Michael Frotscher), Albert-Ludwigs Universität Freiburg; 1996: Wolfgang-Bargmann Preis, Anatomische Gesellschaft; 1996: Fachanatom, Anatomische Gesellschaft; 1999: Habilitation in Anatomy and Neuroanatomy, Albert-Ludwigs Universität Freiburg; 2000: Appointment as a C2 Lecturer, Albert-Ludwigs Universität Freiburg; 2003: Tenured-track appointment and Group leader position 'Cellular Neurobiology', Institute of Medicine (Prof. Dr. Karl Zilles, Research Centre Jülich GmbH; 2005: APL-Professor in Anatomy and Neuroanatomy, Heinrich-Heine Universität Düsseldorf; Since 2008: Professor W2, Medical Faculty, University Hospital/RWTH Aachen. Current Position: W2-Proffesor and group leader 'Structure of Synapses', Institute of Neuroscience and Medicine INM-10, Research Centre Jülich GmbH.

Astrid Rollenhagen
Institute of Neuroscience and Medicine
INM-10, Research Centre Jülich GmbH
Leo-Brandt Str., 52425 Jülich, Germany
e-mail: a.rollenhagen@fz-juelich.de

Born 06.11.1959 in Bielefeld, Germany; 1976–1978: Education as a doctor's assistant; 1980–1982 'Abitur' at the Westfalen Kolleg Bielefeld; 1982–1987: Study of Biology, Universität Bielefeld; 1987–1996: Diploma and doctoral thesis, Universität Bielefeld, Lehrstuhl Verhaltensforschung, AG Prof. Dr. H.-J. Bischof; 1996–1997: Scientific Assistant, Universität Bielefeld, Lehrstuhl Verhaltensforschung, AG Prof. Dr. H.-J. Bischof; 1998–2002: Universität Hamburg, Center for Molecular Neurobiology Hamburg, Abt. Prof. Dr. M. Schachner; 2002–2004: Albert-Ludwigs-Universität Freiburg, Dept. of Anatomy (Prof. Dr. M. Frotscher, AG PD Dr. J.H.R. Lübke); Since 2004: Senior Post-Doc, Group: 'Structure of Synapses' (Prof. Dr. J.H.R. Lübke), INM-10, Research Centre Jülich GmbH.

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Movie 1: Representative example of a z-stack of 100 consecutive images through layer 1b of the human temporal neocortex taken with a FIB-SEM. Note the rapid change in the organization of the neuropil. (Collaboration with Dr. Mike Hasenberg and his team at the IMCES Electron Microscopy Unit (EMU), Medical Research Centre, University Hospital Essen).

Movie 2: Representative example of a shaft synapse in layer 4 of the human temporal lobe neocortex as revealed by EM tomography. Note the occurrence of three mitochondria closely associated with the pool of synaptic vesicles in the presynaptic terminal and the rapid change in the shape and size of the active zone. Scale bar 0.2 µm. (Collaboration with Dr. Mike Hasenberg and his team at the IMCES Electron Microscopy Unit (EMU), Medical Research Centre, University Hospital Essen).

Review Article

Theresa Pohlkamp*

Apolipoprotein E: Cholesterol metabolism and Alzheimer's pathology

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Abstract: Age is the greatest risk factor for Alzheimer's disease (AD). Today, due to an increase in global life expectancy, AD-related deaths are ranked as the sixth most common cause of death. The allele isoform ε4 of apolipoprotein E (ApoE4) is the most important genetic risk factor for AD. Three ApoE isoforms are common in humans: ApoE2, ApoE3, and ApoE4. ApoE3 is the most frequent isoform and considered neutral with regards to AD, whereas the isoform ApoE2 is protective. Thus it is important to understand how ApoE isoforms affect amyloid-β (Aβ) and tau toxicity, the key drivers of AD pathology. Aβ and tau accumulate to form the hallmarks of AD, plaques and neurofibrillary tangles, respectively. ApoE, primarily expressed by astrocytes, is the major lipid transporter in the brain. In this review I summarize some important historic and scientific aspects of our progress in understanding the role of the cholesterol transporter ApoE in the brain, and how the isoform ApoE4 contributes to AD pathology.

Keywords: amyloid-β plaques, apolipoprotein receptor 2/ApoER2/LRP8, endosomal vesicle transport, hyperphosphorylated tau

Zusammenfassung: Je älter man wird, desto größer ist die Chance an Alzheimer Demenz (AD) zu erkranken. Aufgrund steigender Lebenserwartung ist AD heute eine der häufigsten Todesursachen weltweit. Die Apolipoprotein E (ApoE) Allelvariante ε4 ist der stärkste genetische AD-Risikofaktor. Der Fetttransporter ApoE existiert in drei Allelvarianten: ApoE2, ApoE3 und ApoE4. Die häufigste Form ApoE3 wird im Zusammenhang mit AD als neutral betrachtet, während ApoE2 schützend wirkt. Daher ist es wichtig zu verstehen, wie die verschiedenen ApoE-Varianten zu der Toxizität von Amyloid-β (Aβ) und Tau beitragen. Aβ und Tau akkumulieren in Plaques bzw. bilden intraneuronale Fibrillen, die zusammen die pathologischen Hauptmerkmale von AD darstellen. Überwiegend von Astrozyten produziert, ist ApoE der wichtigste Lipidtransporter im Gehirn. In diesem Review-Artikel erläutere ich den wissenschaftlichen Fortschritt zum Verständnis der Funktion des Cholesterintransporters ApoE im Gehirn und welche Rolle ApoE4 in der AD-Pathologie spielt.

Schlüsselwörter: Amyloid-β Plaques, Apolipoprotein Rezeptor 2/Apoer2/Lrp8, endosomaler Vesikel Transport, hyperphosphoryliertes Tau

Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease associated with profound memory loss and cognitive dysfunction. More than a century after Alois Alzheimer described the first case of AD, according to *Alzheimer's Disease International,* the disease now afflicts 50 million people worldwide. Since age is the greatest risk factor for AD, increasing life expectancy makes a dramatic contribution to these demographics. It was only in the late 1970 s, when Robert Katzman defined AD as one of the world's greatest killers, that AD was recognized as an epidemiological disease (Katzman, 1976). Two different types of AD exist; the early onset (EOAD) and the late onset (LOAD) forms. By definition, patients below the age of 65 when diagnosed suffer from EOAD, and patients who develop symptoms after 65 years of age have LOAD. Katzman observed that the general decline in cognition and the progression of neurodegeneration followed a similar pattern in LOAD and EAOD. The more aggressive form of EOAD is rare, accounting for only 1–5 % of all AD cases, and is caused by de novo or familial genetic mutations. Affected genes encode the amyloid-β (Aβ) precursor protein (APP) or APP processing proteins, each of which trigger enhanced production of the Aβ-peptide that forms neurotoxic oligomers and ultimately aggre-

***Corresponding author: Theresa Pohlkamp**, Department of Molecular Genetics, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8570, USA; Center for Translational Neurodegeneration Research, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8570, USA, e-mail: Theresa.Pohlkamp@UTSouthwestern.edu

gates in extracellular deposits, called plaques. In LOAD, mechanisms involved in reduced Aβ clearance, rather than overproduction of Aβ, are believed to be a major contributor to Aβ-toxicity and plaque deposition (Wildsmith et al., 2013). Importantly, the vast majority of AD cases are defined as LOAD and the most prevalent genetic risk factor for these cases is apolipoprotein E (ApoE) isoform ε4 (E4); 45–65 % of AD patients are E4 positive (Farrer et al., 1997). Each E4 allele decreases the age of AD-onset by approximately five years (Roses, 1994). In the past two decades, research into the role of ApoE in AD increased exponentially, driven especially by numerous failures of Aβ-targeting clinical trials (Panza et al., 2019). A complete understanding of the pathological mechanism of E4 in AD will provide an urgently needed alternative research strategy for the discovery of druggable targets.

In the following sections I introduce the three major molecular players in AD: amyloid-β, microtubule-binding protein tau, and ApoE. I then highlight some important mechanisms by which E4 contributes to AD.

Amyloid-β and hyperphosphorylated tau

In the late 19th century, the German physician Alois Alzheimer was confronted with the 51-year old patient Auguste D. who suffered from profound memory loss, confusion, and irritability. Today, Auguste D. would have been diagnosed with EOAD. Following her death in 1906 at the age of 56, Alois Alzheimer examined her brain. Besides neuronal cell death and massive loss of neuronal tissue, he observed (1) abnormal deposits around neurons, which are today known to be plaque depositions of accumulated Aβ, and (2) fibrillary tangles inside neuronal cell bodies, caused by hyperphosphorylation and accumulation of tau. To this day, these occurrences are still known as the major pathological hallmarks of AD.

Amyloid-β, the major component of extracellular plaques, is a proteolytic fragment of the transmembrane protein APP. Aβ is highly prone to self-assembly and forms soluble oligomers and fibers, ultimately accumulating in solid extracellular deposits. It wasn't until the end of the 1990 s that scientists discovered that the soluble Aβ-oligomers, rather than monomers or plaques, are neurotoxic (Arriagada et al., 1992). The famous "Nun study" demonstrated that even huge amounts of plaque deposits do not necessarily cause cognitive decline (Snowdon et al., 1997). Today it is considered that plaques entrap toxic material to protect the brain, explaining the failure of plaque-targeting drugs in clinical studies – solubilized plaques release toxic material.

Tau is a microtubule-binding and stabilizing protein primarily expressed in neurons. Hyperphosphorylation of tau results in microtubule-dissociation, translocation to the cell body and dendrites, and aggregation into neurofibrillary tangles (Wischik et al., 1996). Seeds of accumulated tau can be transmitted from one neuron to another, comparable to an infection. Braak and colleagues described tau seeding originating in the entorhinal cortex, the main interface between hippocampus and cortex. From there, seeding proceeds along axons of the perforant pathway to the hippocampus, a region critical for memory formation. In the final stages, tau seeds reach the neocortex, where long-term memories are stored (Braak et al., 1993). The spread of tau deposition matches the brain networks responsible for the cognitive functions that decline in AD. For instance, mild cognitive impairment is associated with neuronal death in the entorhinal cortex.

How do Aβ and tau act together? Soluble forms of Aβ accumulate into plaques and tau into tangles. George Bloom described Aβ as the trigger and tau as the bullet (Bloom, 2014). More specifically, upstream Aβ triggers the conversion of tau from a normal to a toxic state, which then enhances Aβ toxicity in a feedback loop that accelerates AD pathology. Due to the self-propagation of soluble Aβ and tau species, the disease spreads through the brain in a prion-like fashion. AD pathology is thought to start at least 20 years before symptoms arise.

ApoE-isoforms and lipid transport in the brain and periphery

Apolipoproteins transport lipids, such as cholesterol and fats, which make up the major components of the cell membrane, and deliver their cargo to cells by ligand-induced receptor endocytosis. For cellular uptake, ApoE binds to members of the low-density lipoprotein receptor (LDLR) related protein (LRP) family. ApoE is expressed in several tissues – liver hepatocytes are the main peripheral source, with the majority of ApoE in the brain being secreted by astrocytes. ApoE is the main apolipoprotein in the nervous system, where cholesterol plays an important role in membrane fluidity, vesicle formation, synaptogenesis, and repair. The human brain contains 25 % of the body's total cholesterol, which it must produce locally

due to the difficulty cholesterol molecules face in crossing the blood-brain barrier (BBB). Besides AD, ApoE plays a role in cardiovascular diseases. In fact, ApoE was first described in the 1970 s as an arginine-rich, blood-cholesterol clearing protein. The different ApoE isoforms were discovered by separation of serum proteins derived from hyperlipidemia patients on a pH gradient via isoelectric focusing. The numbering of these isoforms refers to their separation based on their isoelectric point (IEP), which describes the pH at which the charge of the protein is neutral (Ordovas et al., 1987; Shore and Shore, 1969). In the general population, E2, E3, and E4 are the major alleles and have approximate allele frequencies of 8 %, 78 %, and 14 %, respectively. After the discovery of cholesterol lowering statins, ApoE-research stagnated. In the 1990 s, ApoE gained new popularity following its detection in plaques in the brains of AD patients, and after the discovery that E4 dramatically increases the risk for AD, whereas E2 decreases the risk (Nagy et al., 1995; Strittmatter et al., 1993).

Evolutionarily, E4 is the oldest isoform and carries arginines at the amino acid positions 112 (Arg-112) and 158 (Arg-158). The most common allele, E3, evolved about 200,000 years ago via an arginine to cysteine substitution at position 112 (Arg112Cys). The youngest isoform, E2, evolved from E3 about 80,000 years ago via an Arg-158Cys substitution (Huebbe and Rimbach, 2017). The two polymorphisms alter the molecular structure, lipidation, receptor binding, degradation, and toxicity of the protein. Overall, ApoE contributes to coronary artery disease, myocardial infarction, and AD in the same isoform-specific stepwise pattern – from highest to lowest contribution: E4 > E3 > E2. Until now, E4 has been shown to be the greatest genetic risk factor for AD and the ApoE gene ranks fifth among the most studied human genes (Dolgin, 2017). Interestingly, Alois Alzheimer himself described "adipose inclusions", indicating a defect in lipid metabolism. To date, E4 has been described as contributing to AD in a multitude of different ways, including through peripheral and central pathways. In the following section I will focus on a selection of mechanisms by which E4 contributes to AD pathology.

Cholesterol metabolism and Aβ-clearance

In the periphery, ApoE is present in "good" high-density lipoprotein cholesterol (HDL), which is capable of removing lipids for degradation, but not in "bad" low-density lipoproteins (LDL). In contrast to E2 and E3, E4 is poorly lipidated, which leads to different HDL/LDL ratios in people according to their ApoE genotype (Bennet et al., 2007). Peripheral circulating HDL particles are capable of traversing the BBB via ApoA-1 mediated transcytosis, thus contributing to Aβ clearance (Dal Magro et al., 2019). Importantly, the capacity of ApoE isoforms to bind to Aβ in the brain correlates with their lipidation efficiency in forming HDL-like particles: E2 > E3 > E4 (Strittmatter et al., 1993). Studies on Aβ-overproducing AD mouse models suggest that the E4-genotype and ApoE deficiency promote Aβ pathology to a comparable extent (Bell et al., 2012; Liu et al., 2015). Overexpression of the primary ApoE lipidator, ABCA1, increased Aβ-clearance in an AD mouse model (Wahrle et al., 2008), suggesting ApoE lipidation as a potential drug target. AD-linked single nucleotide polymorphisms have been discovered in several genes encoding various apolipoproteins and their numerous receptors. Thus, apolipoprotein metabolism became a new focus in understanding the various mechanisms of Aβ clearance via microglia, astrocytes, and neurons in the brain, as well as endothelial cells and pericytes at the BBB (Pohlkamp et al., 2017). Whereas LDLR plays an important role in Aβ clearance from the brain across the BBB (Castellano et al., 2012), the function of LRP1 in Aβ metabolism seems to be more complicated and partially conflicting (for a review, see Shinohara et al., 2017).

Microglia are the resident immune cells in the brain and provide the most important mechanism for Aβ degradation. ApoE modulates their inflammatory response in an isoform-specific manner. Specific types of activated microglia are found around plaque deposits in AD brains. Microglia express the receptor TREM2 on their surface, which represents the second greatest genetic risk factor for LOAD, after ApoE. Interestingly, ApoE binds to TREM2 (Atagi et al., 2015). This interaction is potentially involved in a process that puts microglia in a state in which they phagocytose Aβ-particles (Shi and Holtzman, 2018). ApoE was also described as a checkpoint inhibitor of unresolvable inflammation in response to Aβ plaques (Yin et al., 2019). However, the precise mechanism – describing, for example, how E4 would alter this microglial response – is not understood. Recently it has been found that ApoE isoforms differentially regulate the transcriptome of brain cells, particularly those of microglia and astrocytes, with consequences for the expression of genes regulating inflammation and lipid metabolism (TCW et al., 2019). This and other recent studies stress that ApoE-isoform-specific functions in cholesterol metabolism are involved in AD pathology.

ApoE4 accelerates tauopathy

Stressed neurons express ApoE, and the E4 isoform in particular undergoes enhanced proteolysis to neurotoxic fragments that stimulate tau hyperphosphorylation under these conditions (Brecht et al., 2004). Mutations in tau leading to hyperphosphorylation cause Frontotemporal Dementia (FTD) with tauopathy. In a tau-mutant FTD mouse model, ApoE-deficiency had a protective effect, whereas the E4-genotype accelerated neurodegeneration, neuroinflammation, and tau propagation (Shi et al., 2017). In agreement with this, in human FTD patients with tau mutations, the E4-genotype decreased the age of disease onset (Koriath et al., 2019). Additionally, tau pathology is strongly associated with chronic inflammatory processes, particularly activation of microglia involving ApoE and TREM2 (Keren-Shaul et al., 2017; Krasemann et al., 2017; Shi and Holtzman, 2018). However, research into the effect of ApoE on tauopathy is at an early stage.

ApoE4 causes an endosomal traffic jam in neurons

The different amounts of positively charged arginines in ApoE isoforms affect their net charge, and thereby their IEP follows the order E2 (5.9) < E3 (6.2) < E4 (6.4). At its IEP, a protein is uncharged, becomes hydrophobic, and self-assembles. After cellular uptake, ApoE enters an intravesicular (endosomal) sorting machinery in which the endosomal lumen undergoes gradual acidification. Luminal pH is critical for endosomal function. ApoE binds to its receptor via the interaction of domains that are oppositely charged. In early endosomes, increasing amounts of protons intervene in the binding of ligand to receptor at pH 6.4, causing dissociation, which is required for re-expression of the receptor at the surface and ligand re-secretion (Van der Horst et al., 2009). Further acidification in late endosomes and lysosomes assists in the sorting and degradation of biomolecules. ApoE containing endosomes have the propensity to convert into recycling endosomes that stay at the periphery (Heeren et al., 2006) and do not experience further acidification. Notably, E4 has the most basic IEP (6.4) of the three isoforms. Recent data indicate that the congruence of the IEP of E4 and the early endosomal pH causes E4 to accumulate, leading to its intracellular entrapment, along with its receptor (ApoER2/LRP8) and glutamate receptors relevant for synaptic function and plasticity. Endosomal acidification attenuates E4 mediated defects in synaptic plasticity, thus endosomal pH provides a novel drug target (Xian et al., 2018).

ApoE4 and HSV-1 as partners in crime

Recently, the link between the very prevalent herpes simplex virus 1 (HSV-1) infection and AD has become one of huge interest. As early as 1995 it was reported that E4-frequency is increased not only among AD patients but also HSV-1 infected individuals suffering from cold sores. Moreover, the combination of HSV-1 infection and the E4-genotype has been suggested to cause AD, whereas either of these features alone has not (Lin et al., 1996). More recent research indicates that E4 facilitates HSV-1 endocytosis and infection in the brain to a higher degree than E3 does (Burgos et al., 2006). Interestingly, cell membrane cholesterol plays a key role in HSV-1 entry, infection, replication, and cell-to-cell spread (Wudiri and Nicola, 2017).

Besides that, heparan sulfate proteoglycans (HPSGs), polysaccharides that decorate cell surface and secretion proteins, serve as receptors for HSV-1 particles. Moreover, HSPGs are receptors for ApoE, and recombinant ApoE fragments have been used to reduce viral infection, including that caused by HSV-1 (Dobson et al., 2006; Tudorache et al., 2017). HSPGs also bind Aβ and tau, and are enriched in plaques and neurofibrillary tangles (Holmes et al., 2013; Zhang et al., 2014). Recent findings credit Aβ with potent antimicrobial properties as it may entrap pathogens like HSV-1 in plaques (Eimer et al., 2018). HSPGs have also been implicated in the propagation of tau species from neuron to neuron (Katsinelos et al., 2018). Antiviral drugs for herpes have been shown to reduce Aβ aggregation and tau hyperphosphorylation *in vitro*. In line with this, treatment of humans with the respective drugs is associated with a decrease in the incidence of AD (Qin and Li, 2019).

Taken together, the AD risk factor ApoE4 is linked to the accumulation of both Aβ and tau, the major pathological hallmarks of AD, as well as inflammatory responses in neurodegeneration. Numerous findings indicate that the basic function of ApoE as a lipid transporter is responsible for this. Accordingly, E4 lipidation and improving its trafficking through the endosomal system attenuates AD-relevant impairments. Thus, improving the main functions of E4 is currently the focus of different drug development strategies for AD prevention and treatment in E4 carriers.

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References

Arriagada, P.V., Growdon, J.H., Hedley-Whyte, E.T., and Hyman, B.T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology *42*, 631–639.

Atagi, Y., Liu, C.C., Painter, M.M., Chen, X.F., Verbeeck, C., Zheng, H., Li, X., Rademakers, R., et al. (2015). Apolipoprotein E Is a Ligand for Triggering Receptor Expressed on Myeloid Cells 2 (TREM2). J. Biol. Chem. *290*, 26043–26050.

Bell, R.D., Winkler, E.A., Singh, I., Sagare, A.P., Deane, R., Wu, Z., Holtzman, D.M., Betsholtz, C., et al. (2012). Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature *485*, 512–516.

Bennet, A.M., Di Angelantonio, E., Ye, Z., Wensley, F., Dahlin, A., Ahlbom, A., Keavney, B., Collins, R., et al. (2007). Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA *298*, 1300–1311.

Bloom, G.S. (2014). Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA, Neurology *71*, 505–508.

Braak, H., Braak, E., and Bohl, J. (1993). Staging of Alzheimer-related cortical destruction. Eur. Neurol. *33*, 403–408.

Brecht, W.J., Harris, F.M., Chang, S., Tesseur, I., Yu, G.Q., Xu, Q., Dee Fish, J., Wyss-Coray, T., et al. (2004). Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J. Neurosci. *24*, 2527–2534.

Burgos, J.S., Ramirez, C., Sastre, I., and Valdivieso, F. (2006). Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. J. Virol. *80*, 5383–5387.

Castellano, J.M., Deane, R., Gottesdiener, A.J., Verghese, P.B., Stewart, F.R., West, T., Paoletti, A.C., Kasper, T.R., et al. (2012). Low-density lipoprotein receptor overexpression enhances the rate of brain-to-blood Abeta clearance in a mouse model of beta-amyloidosis. Proc. Natl. Acad. Sci. U. S. A. *109*, 15502–15507.

Dal Magro, R., Simonelli, S., Cox, A., Formicola, B., Corti, R., Cassina, V., Nardo, L., Mantegazza, F., et al. (2019). The Extent of Human Apolipoprotein A-I Lipidation Strongly Affects the beta-Amyloid Efflux Across the Blood-Brain Barrier in vitro. Front. Neurosci. *13*, 419.

Dobson, C.B., Sales, S. D., Hoggard, P., Wozniak, M.A., and Crutcher, K.A. (2006). The receptor-binding region of human apolipoprotein E has direct anti-infective activity. J. Infect. *193*, 442–450.

Dolgin, E. (2017). The most popular genes in the human genome. Nature *551*, 427–431.

Eimer, W.A., Vijaya Kumar, D.K., Navalpur Shanmugam, N.K., Rodriguez, A.S., Mitchell, T., Washicosky, K.J., Gyorgy, B., Breakefield, et al. (2018). Alzheimer's Disease-Associated beta-Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. Neuron *99*, 56–63 e53.

Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA *278*, 1349–1356.

Heeren, J., Beisiegel, U., and Grewal, T. (2006). Apolipoprotein E recycling: implications for dyslipidemia and atherosclerosis. Arterioscler., Thromb., Vasc. Biol. *26*, 442–448.

Holmes, B.B., DeVos, S. L., Kfoury, N., Li, M., Jacks, R., Yanamandra, K., Ouidja, M.O., Brodsky, F.M., et al. (2013). Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. Proc. Natl. Acad. Sci. U. S. A. *110*, E3138–3147.

Huebbe, P., and Rimbach, G. (2017). Evolution of human apolipoprotein E (APOE) isoforms: Gene structure, protein function and interaction with dietary factors. Ageing Res. Rev. *37*, 146–161.

Katsinelos, T., Zeitler, M., Dimou, E., Karakatsani, A., Muller, H.M., Nachman, E., Steringer, J.P., Ruiz de Almodovar, C., et al. (2018). Unconventional Secretion Mediates the Trans-cellular Spreading of Tau. Cell rep. *23*, 2039–2055.

Katzman, R. (1976). Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. Arch. Neurol. *33*, 217–218.

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., David, E., Baruch, K., et al. (2017). A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell *169*, 1276–1290 e1217.

Koriath, C., Lashley, T., Taylor, W., Druyeh, R., Dimitriadis, A., Denning, N., Williams, J., Warren, J.D., et al. (2019). ApoE4 lowers age at onset in patients with frontotemporal dementia and tauopathy independent of amyloid-beta copathology. Alzheimer's Dementia *11*, 277–280.

Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., Beckers, L., O'Loughlin, E., et al. (2017). The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity *47*, 566–581 e569.

Lin, W.R., Shang, D., and Itzhaki, R.F. (1996). Neurotropic viruses and Alzheimer disease. Interaction of herpes simplex type 1 virus and apolipoprotein E in the etiology of the disease. Mol. Chem. Neuropathol. *28*, 135–141.

Liu, D.S., Pan, X.D., Zhang, J., Shen, H., Collins, N.C., Cole, A.M., Koster, K.P., Ben Aissa, M., et al. (2015). APOE4 enhances age-dependent decline in cognitive function by down-regulating an NMDA receptor pathway in EFAD-Tg mice. Mol. Neurodegener. *10*, 7.

Nagy, Z., Esiri, M.M., Jobst, K.A., Johnston, C., Litchfield, S., Sim, E., and Smith, A. D. (1995). Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease. Neuroscience *69*, 757–761.

Ordovas, J.M., Litwack-Klein, L., Wilson, P.W., Schaefer, M.M., and Schaefer, E.J. (1987). Apolipoprotein E isoform phenotyping methodology and population frequency with identification of apoE1 and apoE5 isoforms. J. Lipid Res. *28*, 371–380.

Panza, F., Lozupone, M., Logroscino, G., and Imbimbo, B.P. (2019). A critical appraisal of amyloid-beta-targeting therapies for Alzheimer disease. Nat. Rev. Neurol. *15*, 73–88.

Pohlkamp, T., Wasser, C.R., and Herz, J. (2017). Functional roles of the interaction of APP and lipoprotein receptors. Front. Mol. Neurosci. *10*, 54.

Qin, Q., and Li, Y. (2019). Herpesviral infections and antimicrobial protection for Alzheimer's disease: Implications for prevention and treatment. J. Med. Virol. *91*, 1368–1377.

Roses, A. D. (1994). Apolipoprotein E affects the rate of Alzheimer disease expression: beta-amyloid burden is a secondary consequence dependent on APOE genotype and duration of disease. J. Neuropathol. Exp. Neurol. *53*, 429–437.

Shi, Y., and Holtzman, D.M. (2018). Interplay between innate immunity and Alzheimer disease: APOE and TREM2 in the spotlight. Nat. Rev. Immunol. *18*, 759–772.

Shi, Y., Yamada, K., Liddelow, S. A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S., et al. (2017). ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature *549*, 523–527.

Shinohara, M., Tachibana, M., Kanekiyo, T., and Bu, G. (2017). Role of LRP1 in the pathogenesis of Alzheimer's disease: evidence from clinical and preclinical studies. J. Lipid Res. *58*, 1267–1281.

Shore, B., and Shore, V. (1969). Isolation and characterization of polypeptides of human serum lipoproteins. Biochemistry *8*, 4510–4516.

Snowdon, D.A., Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A., and Markesbery, W.R. (1997). Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. J. Am. Med. Assoc. *277*, 813–817.

Strittmatter, W.J., Weisgraber, K.H., Huang, D.Y., Dong, L.M., Salvesen, G.S., Pericak-Vance, M., Schmechel, D., Saunders, A.M., et al. (1993). Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc. Natl. Acad. Sci. USA *90*, 8098–8102.

TCW, J., Liang, S. A., Qian, L., Pipalia, N.H., Chao, M.J., Bertelsen, S.E., Kapoor, M., Marcora, E., et al. (2019). Cholesterol and matrisome pathways dysregulated in human APOE E4 glia. CELL-D-19-02156.

Tudorache, I.F., Trusca, V.G., and Gafencu, A.V. (2017). Apolipoprotein E – A Multifunctional Protein with Implications in Various Pathologies as a Result of Its Structural Features. Comput. Struct. Biotechnol. J. *15*, 359–365.

Van der Horst, D.J., Roosendaal, S. D., and Rodenburg, K.W. (2009). Circulatory lipid transport: lipoprotein assembly and function from an evolutionary perspective. Mol. Cell. Biochem. *326*, 105–119.

Wahrle, S.E., Jiang, H., Parsadanian, M., Kim, J., Li, A., Knoten, A., Jain, S., Hirsch-Reinshagen, V., et al. (2008). Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease. J. Clin. Invest. *118*, 671–682.

Wildsmith, K.R., Holley, M., Savage, J.C., Skerrett, R., and Landreth, G.E. (2013). Evidence for impaired amyloid beta clearance in Alzheimer's disease. Alzheimer's Res. Ther. *5*, 33.

Wischik, C.M., Edwards, P.C., Lai, R.Y., Roth, M., and Harrington, C.R. (1996). Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. Proc. Natl. Acad. Sci. USA *93*, 11213–11218.

Wudiri, G.A., and Nicola, A.V. (2017). Cellular cholesterol facilitates the postentry replication cycle of herpes simplex virus 1. J. Virol. *91*.

Xian, X., Pohlkamp, T., Durakoglugil, M.S., Wong, C.H., Beck, J.K., Lane-Donovan, C., Plattner, F., and Herz, J. (2018). Reversal of ApoE4-induced recycling block as a novel prevention approach for Alzheimer's disease. eLife *7*.

Yin, C., Ackermann, S., Ma, Z., Mohanta, S.K., Zhang, C., Li, Y., Nietzsche, S., Westermann, M., et al. (2019). ApoE attenuates unresolvable inflammation by complex formation with activated C1q. Nat. Med. *25*, 496–506.

Zhang, G.L., Zhang, X., Wang, X.M., and Li, J.P. (2014). Towards understanding the roles of heparan sulfate proteoglycans in Alzheimer's disease. BioMed Res. Int. 516028.

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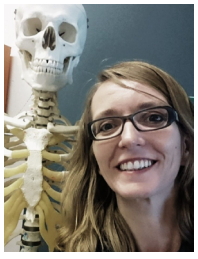

Theresa Pohlkamp
Department of Molecular Genetics & Center for Translational Neurodegeneration Research
e-mail: Theresa.Pohlkamp@UTSouthwestern.edu

Theresa Pohlkamp is currently an instructor at UT Southwestern in Dallas, Texas. She received her Diploma in Biology (2007) in Münster, Germany. She completed her PhD in Neuroscience (2012) in Freiburg, studying the neuromodulator Reelin at the Department of Anatomy. In 2013 she started her postdoc on Alzheimer's Disease in the Joachim Herz lab at UT Southwestern, where her main project focuses on endosomal trafficking of ApoE, Reelin, and lipoprotein receptors.

Review Article

Anita Annahazi* and Michael Schemann

The enteric nervous system: "A little brain in the gut"

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Abstract: The gut's own autonomous nervous system, the enteric nervous system (ENS), has fascinated scientists for more than 100 years. It functions, in the true sense of the word, autonomously, by performing complex tasks and controlling vital functions independently of extrinsic inputs. At the same time, the ENS is bombarded with signals from other cells in the gut wall and lumen and has to integrate all of these inputs. We describe the main functions of the ENS under physiological conditions and give a few examples of its role in gut diseases. The ENS has received increasing attention recently as scientists outside the field of Neurogastroenterology realize its important role in the pathogenesis of Parkinson's, autism and multiple sclerosis.

Keywords: Hirschsprung's disease, irritable bowel syndrome, motility, myenteric plexus, submucous plexus

Zusammenfassung: Darmfunktionen werden durch das autonom agierende enterische Nervensystem (ENS) reguliert. Es kontrolliert vitale Funktionen des Darms unabhängig von extrinsischen Einflüssen. Das ENS muss eine Fülle von Signalen anderer Zellen in der Darmwand oder Faktoren im Darmlumen integrieren. In diesem Artikel beschreiben wir die wesentlichen Funktionen des ENS und erläutern Beispiele aus der klinischen Neurogastroenterologie. Darüber hinaus eröffnen sich neue Aspekte für das Verständnis systemischer neurologischer Erkrankungen wie Parkinson, Autismus oder Multipler Sklerose, bei denen die Rolle des Darms und des ENS immer offensichtlicher wird.

Schlüsselwörter: Morbus Hirschsprung, Reizdarmsyndrom, Motilität, Peristaltischer Reflex, Enterisches Nervensystem

***Corresponding author: Anita Annahazi**, Human Biology, Technical University of Munich, Liesel-Beckmann Strasse 4, 85354 Freising, Germany, e-mail: anita.annahazi@tum.de
Michael Schemann, Human Biology, Technical University of Munich, Liesel-Beckmann Strasse 4, 85354 Freising-Weihenstephan, e-mail: schemann@wzw.tum.de

Introduction

The gastrointestinal tract (GI) fulfils complex tasks that are essential for survival. Apart from mechanical and chemical digestion of food, transit of luminal content, and absorption of nutrients, it functions as an important immune organ by recognizing and fighting luminal pathogens. These complicated processes are regulated by a unique autonomic network of neurons called the enteric nervous system (ENS). Although the ENS communicates with the central nervous system (CNS) as well as sympathetic and parasympathetic nerves, it operates independently. This is why an isolated intestinal segment separated from all external input behaves as if it would do inside the body. It is because of such abilities that the ENS is often referred to as a "second brain". The *Hydra* provides evolutionary evidence that the ENS was present before a CNS (Furness and Stebbing, 2018). This animal has a net-like nervous system located in the wall of the gut tube, which does not form brain-like aggregations. This nervous system contains sensory, motor and interneurons, is related to the circular and longitudinal layers of the body wall of the *Hydra*, and controls movements needed for digestion: peristalsis, mixing and expulsion. Consequently, it must be considered as an ENS-equivalent, while no CNS is present in this species (Shimizu et al., 2004). It is therefore fair to assume that the brain is an encephalized ENS, with the latter being considered as the "first brain" to appear during evolution. The objectives of this review are to present an overview of the structure and function of the mammalian "little brain", and to describe some of the important pathologies caused by its dysfunction.

Anatomy of the ENS

There are around 200–600 million nerve cells in the mammalian ENS which are organized, along with glial cells, into interconnected ganglia. The ENS extends from the esophagus to the anal sphincter and has branches to the liver, gall bladder, biliary tract and pancreas. The ganglia

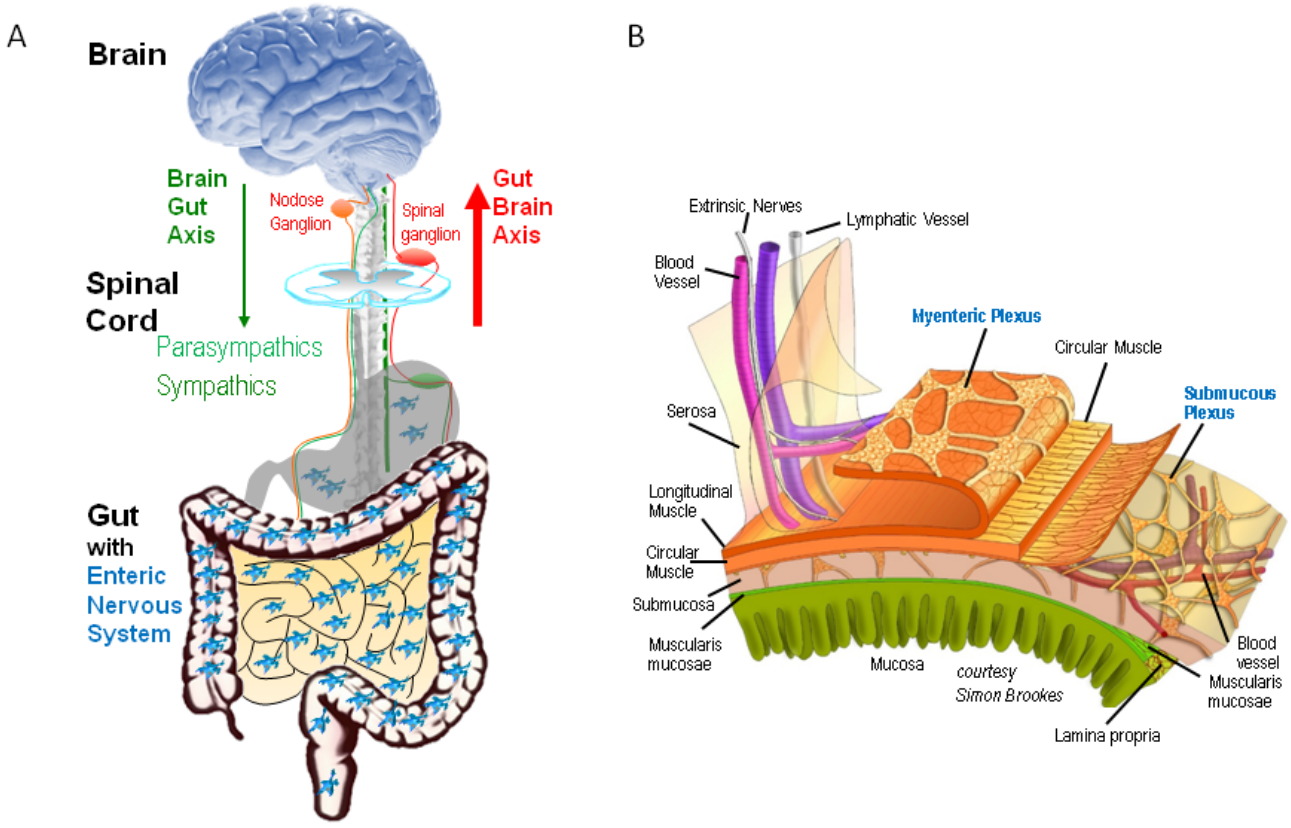

Fig. 1: Functional anatomy of gut-brain communication and the enteric nervous system (ENS).
Panel A is a simplified scheme to demonstrate control of gut functions at different levels. The most relevant is the ENS, which controls gut functions independent of extrinsic inputs. Nevertheless, the gut is connected to the brain via nerves which function as the gut-brain axis – sensory neurons with cell bodies in dorsal root ganglia or in parasympathetic relay ganglia, e. g. nodose ganglion, and efferent nerves of the parasympathetic and sympathetic nervous systems. Note that there is no region in the brain exclusively dedicated to gut functions.
Panel B illustrates the myenteric and submucous plexus layers of the ENS in the gut wall.
The figure was provided by Prof. Simon Brookes, Flinders University, Adelaide, Australia.

form neuronal networks – the so-called plexuses – that are interconnected (Figure 1). The myenteric plexus lies between the longitudinal and circular layers and controls muscle activity. The submucous plexuses are located beneath the mucosa and control epithelial functions. Both plexuses modulate blood flow and the activity of the enteric immune system. The ENS develops mainly from vagal neural crest cells, which migrate into and along the bowel over 5 gestational weeks in humans (Stamp, 2017). A small number of neurons, however, originate from sacral neural crest cells and Schwann cell precursors.

Central connections

Although the ENS is able to function autonomously, connections between the ENS and CNS exist and are referred to as the gut-brain axis (Furness and Stebbing, 2018; Schemann and Grundy, 1992; Mayer, 2011; see Figure 1). The gut’s extrinsic nerve supply carries efferent as well as afferent nerves and uses the ENS as an interface. This explains why parasympathetic nerves support motility and secretion during the digestive period, whereas sympathetic nerves inhibit those functions through presynaptic inhibition at synapses within the ENS (Furness et al., 2014). The vast majority of nerves travelling with vagal and spinal trunks are afferents, which, apart from transmitting sensory inputs from the GI tract to the CNS, have collateral branches to blood vessels and enteric ganglia, providing local axon reflexes. Further connection occurs through intestinofugal neurons in the ENS, which project to the sympathetic postganglionic neurons within prevertebral ganglia and even to the trachea, gall bladder and pancreas (Furness and Stebbing, 2018). Furthermore, the brain also provides input to the ENS via hormonal path-

ways, such as the hypothalamic-pituitary-adrenal (HPA) and sympatho-adrenal axes, and descending monoaminergic pathways (Mayer, 2011). A significant part of signaling from the gut to the brain is performed by enteroendocrine cells (EEC). They are located in the gut wall and represent the largest endocrine organ in the body, serving to detect mechanical (e. g. shear forces) as well as chemical stimuli, including nutrients, microbial products or toxins. Communication to the CNS is via blood or sensory nerves travelling with the sympathetic or parasympathetic trunks (Latorre et al., 2016). There are more than 20 types of EECs, producing different endocrine and paracrine mediators, which participate in the regulation of GI motility and secretion, pancreatic enzyme and bile secretion, and the regulation of food intake.

Function of the ENS

Despite the connections between the CNS and the ENS, the vital functions of the GI tract almost completely depend on the ENS, while the CNS monitors gut activity and may, at most, modulate it (Furness et al., 2014). The total depletion of the ENS, which occurs in cases of the congenital lack of enteric ganglia in a colonic segment in Hirschsprung's disease (detailed later) is lethal, despite supply by extrinsic nerves remaining. The most prominent function of the myenteric plexus is the regulation of the motility of the circular and longitudinal muscles. The submucous plexus mainly controls ion and water secretion, absorption of ions, vitamins and nutrients, as well as release of endocrine and paracrine mediators.

The ENS contains sensory neurons, interneurons and motor neurons, but these are often multifunctional and fulfil various tasks (Furness et al., 2014; Kugler et al., 2015; Smith et al., 2007). Sensory neurons are able to detect mechanical and chemical stimuli. Mechanosensitive ENS neurons respond to tensile and compressive stress rather than to shear stress (Mazzuoli-Weber and Schemann, 2015). The chemosensitive ENS neurons express receptors for amino acids, fatty acids, glucose, pH, osmolarity, temperature, odorants and tastes (Blackshaw et al., 2007; Neunlist et al., 2001; Bertrand et al., 1997). Some glucose-sensitive enteric neurons show behavior reminiscent of glucosensitive and glucoresponsive neurons in the hypothalamus (Liu et al., 1999).

Studies investigating the presence of neurotransmitters, neuropeptides and neuromodulators revealed that identical types occur in the CNS and ENS. It is assumed that the complexity of transmitter and receptor expression is similar, if not identical, between the ENS and CNS (Furness, 2006). For example, unlike in the CNS, glycine acts as an excitatory transmitter in the adult ENS (Neunlist et al., 2001). Some of the signaling molecules released by the ENS are primary transmitters and others serve modulatory roles. In Table 1 we list only those that are considered primary transmitters as they are central to the initiation and maintenance of reflex activated muscle or epithelial activity. Sensory neurons innervate motor neurons directly or via inhibitory or excitatory interneurons. Excitatory or inhibitory motor neurons project to the various muscle layers or epithelium to affect motility or secretion, respectively. Motor neurons also innervate enteric endocrine cells and lymphoid aggregations in the GI tract (Furness et al., 2014).

We recently reviewed the evidence that the ENS is able to perform higher functions. Although a rigid scientific proof or disproof is still required, it seems that the ENS is able to learn, memorize and forget (Schemann et al., 2019). All the electrophysiological and molecular proxies for habituation, facilitation and conditioned learning exist in the ENS. If this proves to be correct, it may revolutionize the way we interpret plasticity in the ENS and pathogenesis of gut diseases (Schemann et al., 2019).

The peristaltic reflex

Motility of the GI organs is indispensable for survival (Huizinga and Lammers, 2009). The basis for all motility patterns is the peristaltic reflex, which was described as early as the end of the 19th century (Bayliss and Starling, 1899; Lüderitz, 1890; see Figure 2). Lüderitz provided a comprehensive description of peristalsis as the basis for proximal to distal movement of content for the first time in anesthetized animals. He noticed that mechanical distension induced a response which consisted of a contraction proximal to the distension and muscle inhibition distal to the distension, and suggested that the ENS triggers this reflex. Bayliss and Starling confirmed the validity of this hypothesis by observing the peristaltic reflex even when all connections to the CNS were interrupted (Figure 2).

Today we know that the first step in this process is the activation of sensory neurons in the ENS by mechanical and/or chemical stimuli. Activation builds up in a sensory interneuronal network and eventually activates excitatory motor neurons projecting up the gut and inhibitory motor neurons projecting down the gut. This results in the release of acetylcholine proximal to the stimulus and

Tab. 1: Summary of the functions of some of the main transmitters in the ENS.

Transmitter	Released from	Target (Receptor)	Function
Acetylcholine (ACh)	Muscle motor neuron Secretomotor neuron Sensory neuron / Interneuron Parasympathetic nerves	Muscle (muscarinic) Epithelial secretion (muscarinic) Enteric neurons (nicotinic) Enteric neurons (nicotinic) Enteric neurons (nicotinic)	Promotility Prosecretory Enhance nerve activity Enhance nerve activity Enhance transmitter release from enteric nerves
Substance P	Muscle motor neuron Sensory neuron / Interneuron	Muscle (NK-2) Enteric neurons (NK-1,2,3)	Promotility Enhance nerve activity
ATP, β-nicotinamide adenine dinucleotide	Muscle motor neuron Interneuron	Muscle ($P2Y_1$) Interneuron, Motor neuron (P2X)	Antimotility Enhance nerve activity
Nitric Oxide	Motoneuron Secretomotor neuron Interneuron	Muscle (increased cGMP) Epithelial secretion Enteric neurons (presynaptic inhibition of non-cholinergic non adrenergic transmitters)	Antimotility Prosecretory Inhibit nerve activity (slow EPSP)
Vasoactive Intestinal Peptide	Muscle motor neuron Secretomotor neuron	Muscle Epithelial secretion (VPAC2)	Antimotility Prosecretory
Neuropeptide Y	Interneuron Secretomotor neuron	Presynaptic inhibition of ACh release Epithelial secretion	Inhibit nerve activity (fast EPSP) Antisecretory
Enkephaline Endorphin	Interneuron Muscle motor neuron Secretomotor neuron	Presynaptic inhibition of ACh release (μ,δ,κ) Muscle Epithelial secretion	Inhibit nerve activity (fast EPSP) Antimotility Antisecretory
Serotonin (5-HT)	Sensory neuron / Interneuron	Presynaptic potentiation of ACh release ($5\text{-}HT_4$) Enteric motor and interneurons ($5\text{-}HT_3$) Excitatory motor neuron ($5\text{-}HT_{1p}$) Inhibitory motor neuron ($5\text{-}HT_{1p}$)	Enhance nerve activity (fast EPSP) Enhance nerve activity Enhance peristalsis Enhance peristalsis
Noradrenaline	Sympathetic nerves	Enteric interneurons, presynaptic inhibition of ACh release (α_2)	Inhibition of enteric neurons

nitric oxide (NO), adenosine triphosphate (ATP) and/or vasoactive intestinal peptide (VIP) distal to the stimulus. The axonal projections of these motor neurons are rather short, spanning about 1cm, which means that the peristaltic reflex has to be sequentially activated in cases where fast transit is appropriate (Figure 3). In contrast, sequential activation is halted if transit needs to be slowed down during the digestive period. Thus, the peristaltic reflex is not an all-or-nothing response, but instead is highly modulated. Interneurons with descending projections are required to initiate sequential activation. Inhibition of interneuronal synapses, the presence of synapses between interneurons and motor neurons, or a decrease in the sensitivity of sensory neurons are all means to halt sequential activation (Figure 3).

The actual motility pattern is modified by additional factors, such as the activity of pacemaker cells (Interstitial cells of Cajal), hormones, immune mediators, and sympathetic and parasympathetic inputs. This in turn creates the complex gut movements of propulsion, segmentation and storage which permit the digestion and absorption of nutrients (Mazzuoli-Weber and Schemann, 2015; Huizinga and Lammers, 2009).

Pathologies of the ENS

Diseases linked to disorders of the gut are diverse in their symptoms and severity. They can range from bothersome, but not life threatening, disturbances of motility, such as in irritable bowel syndrome (IBS), to the potentially fatal motility impairments seen in Hirschsprung's disease. There are numerous diseases in which the ENS is expected to play a role (Table 2). Strikingly, it turns out that the ENS is involved in the pathogenesis of diseases not commonly

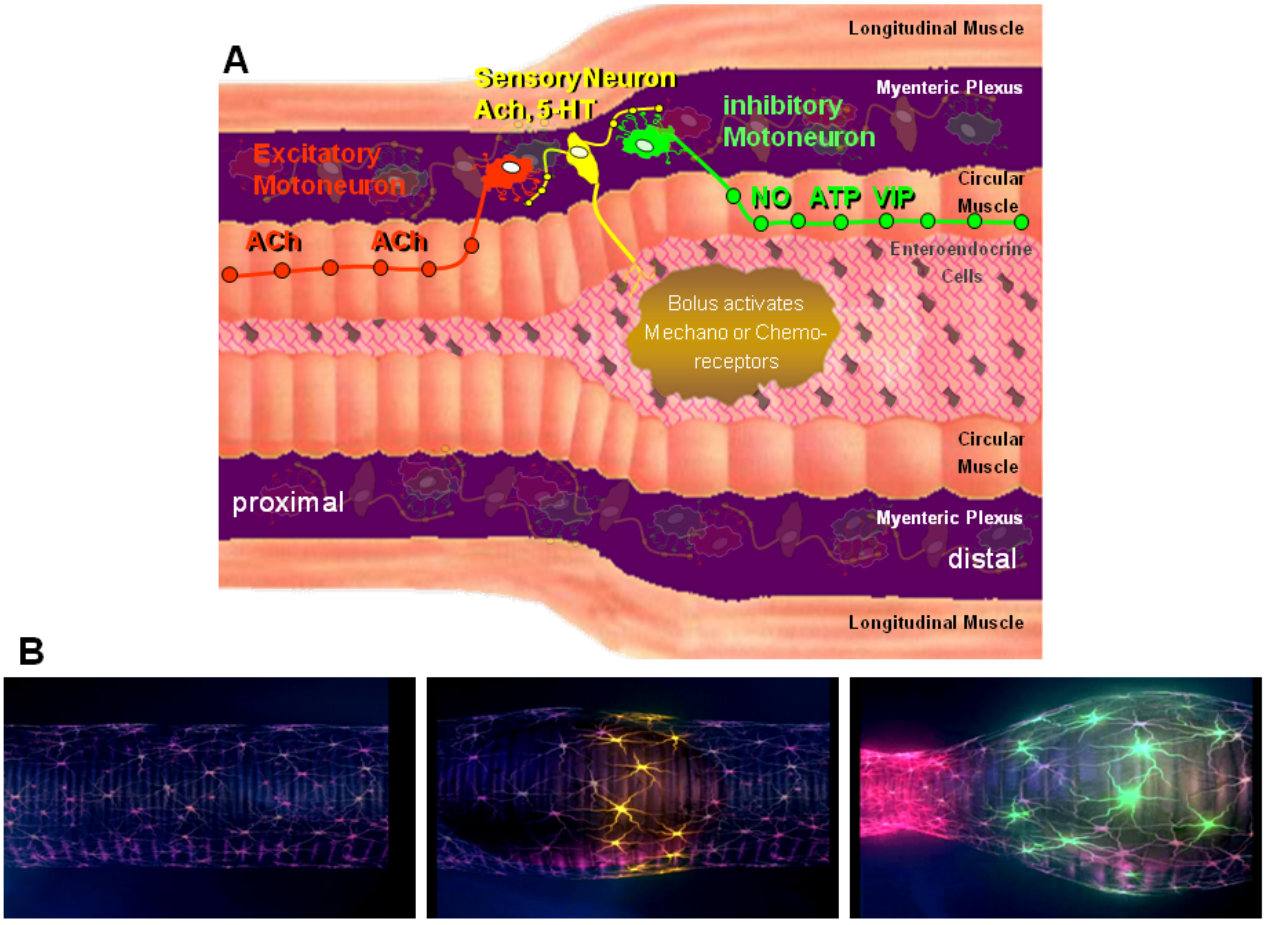

Figure 2: Circuits in the ENS activate the peristaltic reflex.
Panel A shows a schematic drawing of the intestinal wall with the different layers. Circuits in the myenteric plexus responsible for initiating the peristaltic reflex are highlighted. Initially, sensory neurons in the ENS are activated by mechanical or chemical stimuli. They then activate excitatory and inhibitory muscle motor neurons with polarized projection patterns. The inhibitory motor neurons project down the gut and release nitric oxide, ATP and vasoactive intestinal peptide, whereas the excitatory motor neurons project up the gut to release acetylcholine. The projection length for both is about 1cm, maximum. This circuit guarantees that a bolus triggers a proximal contraction and a distal inhibition of the muscle, allowing the content to be pushed in an anal direction. For simplicity, interneurons are not shown (see Figure 3).
Panel B shows the spatiotemporally coordinated activity within the circuit. It starts with quiescence (left). As soon as the bolus enters the region, mechanosensitive neurons (middle; yellow) start to fire actions potentials. This will eventually lead to activation of excitatory (right; red) and inhibitory motor neurons (right; green).

linked to the gut, such as multiple sclerosis, autism, Parkinson's disease and cancer. A common feature of functional gut disorders is that therapy is, all in all, unsatisfying, and causal therapies are particularly lacking. As the discussion of all ENS pathologies is far beyond the scope of this review, some diseases have been selected to provide a glimpse into the diverse pathomechanisms.

One of the most devastating gut motility disorders is Hirschsprung's disease, also called congenital megacolon or intestinal aganglionosis, and first described by Harald Hirschsprung in 1888 (Hirschsprung, 1888; Sergi, 2015). The underlying histology was discovered shortly after the initial description and consists of sparse or lacking ganglia in the colonic ENS but a normal ganglionic network in the ileum (Tittel, 1901). It is now known that the disease is caused by the disruption of normal neural crest cell migration or development (Butler Tjaden and Trainor, 2013). The resulting aganglionosis may be limited to narrow segments of the gut or extend to the entire colon. The lack of ganglia causes tonic contraction of the affected segment, resulting in a bowel obstruction, and typically appearing immediately after birth. The contraction is the result of continuous uncontrolled release of acetylcholine from extrinsic parasympathetic nerves and

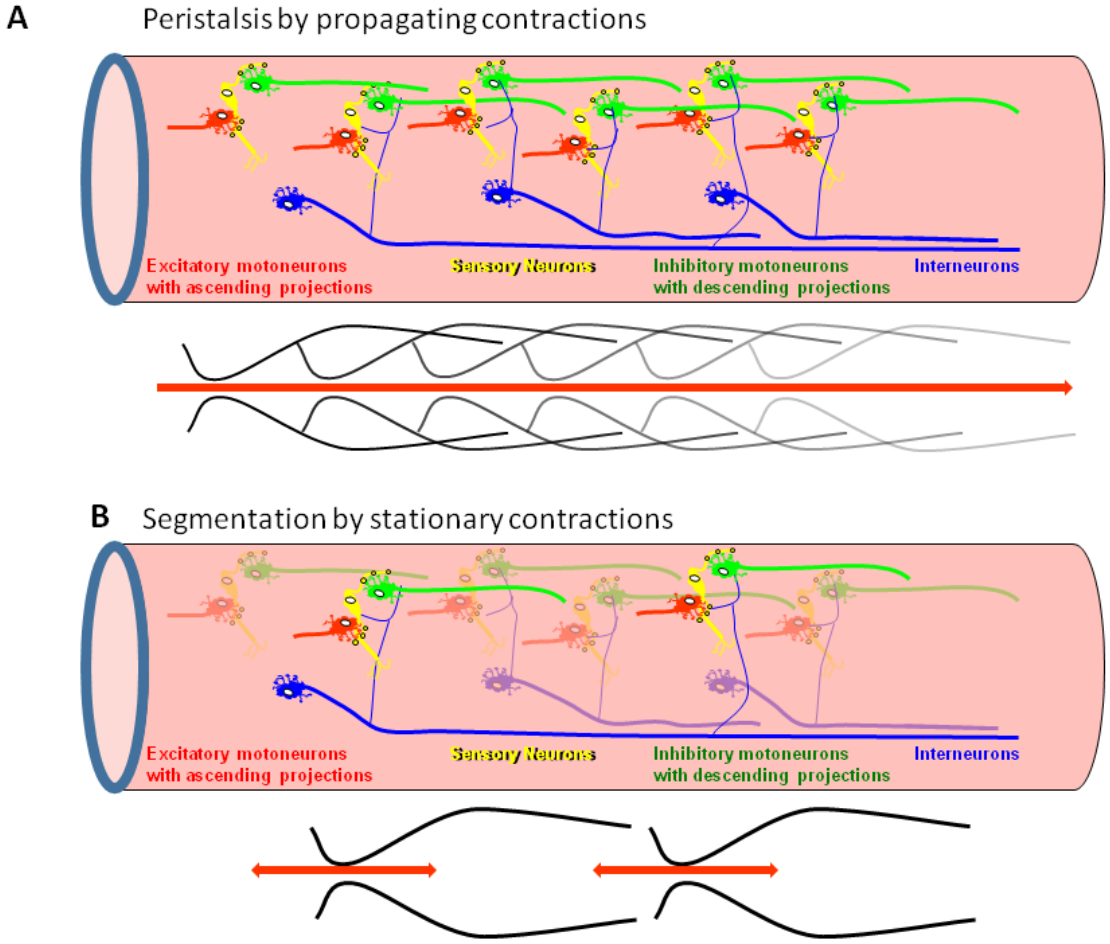

Figure 3: A model to explain how spatiotemporally coordinated peristaltic reflex circuits in the ENS lead to peristalsis.
Panel A shows a chain of peristaltic reflex circuits consisting of sensory neurons synapsing onto excitatory and inhibitory muscle motor neurons. The circuits are connected via excitatory interneurons which sensitize the peristaltic reflex circuits, thus allowing peristalsis and movement of intestinal content over longer distances.
Panel B shows some interneurons failing to sensitize consecutive peristaltic reflex circuits. This will prevent the spread of activation along the circuits. The result is disrupted propagation of peristaltic reflexes causing stationary contractions.

the lack of coordinated release of inhibitory transmitters of the ENS. The newborn thus fails to pass meconium in the first 24 hours. The routine treatment in Hirschsprung's disease is the removal of the aganglionic segment, but as a late outcome of this life-saving procedure patients must deal with bowel disorders that result in reduced bowel-related quality of life during adulthood (Gustafson et al., 2019).

Recently, some promising results of experiments with ENS stem cell transplantation using optogenetic techniques have been published (Wang, 2018). Optogenetics uses a genetically implanted light sensitive channel to detect or control the activity of a specific cell type (Deisseroth et al., 2006). In a mouse model, transplanted enteric neural cells expressing the light-sensitive ion channel, channelrhodopsin, formed a ganglionated network and upon activation by light stimulus induced inhibitory or excitatory electrical events in the circular muscle (Stamp et al., 2017). Furthermore, enteric neuronal precursor cells isolated with magnetic immunoselection and transplanted into the colons of Piebald mice (a model of Hirschsprung's disease with a reduced number of colonic neurons and an aganglionic distal colonic segment) or nNOS$^{-/-}$ mice successfully improved colonic contractility and relaxation, respectively (Ro et al., 2006; Anitha et al., 2008). In a study aiming to evaluate the feasibility of autologous transplantation in ENS disorders, neural crest progenitors were isolated from neonatal rats and transplanted into the chemically denervated distal colon of other rats (Pan et al., 2011). The transplanted cells successfully colonized the gut and reversed motility impairments induced by denervation.

Tab. 2: Diseases associated with pathologies of the ENS or the gut-brain axis.

"Classical" gut diseases	"Non-classical" gut diseases
Achalasia	Alzheimer's Disease
Amyloidosis	Autism spectrum disorder
Chronic intestinal pseudoobstruction	Multiple sclerosis
Diabetes	Paraneoplastic disorder
Inflammatory Bowel Disease	Parkinson's Disease
Functional gastrointestinal diseases; e. g. irritable bowel syndrome, functional dyspepsia	Transmissible spongiform encephalopathy
	Viral or bacterial Infection
Hirschsprung's Disease	
Ileus	
Intoxication	
Food allergy or intolerance	
Mast cell mediator syndrome	
Slow transit constipation	
Tumor development (colon cancer)	

Irritable bowel syndrome is a chronic functional GI disorder with an outstandingly high global prevalence of 11.2 % (Enck et al., 2016). Although the disease is not life threatening, with its distressing symptoms of abdominal pain, bloating and altered bowel habits, as well as limited, mainly symptomatic, therapy, it has devastating consequences for quality of life. Patients are classified according to their stool patterns as IBS-D (IBS with predominant diarrhea), IBS-C (IBS with predominant constipation) or IBS-M (IBS with mixed stool pattern) (Drossman, 2016). IBS is a multifactorial disease with numerous putative pathologies, including ENS disturbances and altered signaling along the gut-brain axis (Enck et al., 2016).

Physical or psychological stress, with its ability to disturb the hypothalamic-pituitary-adrenal axis, is a major player in IBS development and relapse (Moloney et al., 2015; Jahng and Kim, 2016). Stress induces histological and functional changes in the ENS (Li et al., 2016b). An increased number of mast cells in the lamina propria, a known phenomenon in IBS patients, has also been demonstrated in IBS models (Traini et al., 2016; O'Sullivan et al., 2000). It was shown that mast cells of IBS-D patients release more mediators, such as histamine or mast cell tryptase, that are capable of neuronal activation, and the number of mast cells in close proximity to nerves significantly correlated with severity and frequency of abdominal pain and/or discomfort in IBS patients (Barbara et al., 2004). In line with these findings, biopsy samples from the colon or rectum of IBS patients released proteases with 2 to 3 fold higher activity than biopsies from controls, and these biopsy supernatants with elevated protease activity caused hyperalgesia and allodynia in response to colorectal distension after intracolonic injection in mice (Cenac et al., 2007). It has been demonstrated that biopsy supernatants of IBS patients are able to activate enteric neurons due to their protease, histamine and serotonin content (Buhner et al., 2009; Buhner et al., 2012). Furthermore, biopsy supernatants of patients who showed hypersensitivity to rectal distension produced significantly stronger activation in submucous and dorsal root ganglion neurons (Buhner et al., 2014). Interestingly, submucous neurons in biopsies obtained from IBS patients responded significantly less strongly to a cocktail of compounds mimicking IBS biopsy supernatants (a mixture of serotonin, histamine, tryptase, and tumor necrosis factor alpha (TNF-α)) than neurons in biopsies from healthy controls, suggesting desensitization caused by the constant release of these mediators (Ostertag et al., 2015). Similarly to the increased protease activity in mucosal biopsy supernatants, IBS-D patients show elevated fecal serine-protease activity compared to healthy controls or IBS-C patients, which may be a factor triggering epithelial barrier dysfunction and visceral hypersensitivity (Róka et al., 2007; Gecse et al., 2008). An increased cysteine-protease activity has been found in fecal samples of a subgroup of IBS-C patients, which may play a role in the disruption of the intestinal barrier and visceral hypersensitivity (Annaházi et al., 2013). These findings offer new therapeutic options in IBS that target mast cell activation and degranulation, and mast cell products and receptors, some of which have already shown benefits in clinical trials (Zhang et al., 2016).

Structural changes in the ENS in IBS have led to suggestions by some authors that this may be an autoimmune phenomenon (Wood et al., 2012; Fan et al., 2018). Anti-enteric neuronal antibodies were found in the sera of a higher percentage of IBS patients than controls, and such sera induced apoptosis in guinea pig myenteric neurons (Wood et al., 2012; Fan et al., 2018). The authors suggest

that a subgroup of patients with high anti-enteric neuronal antibody titer may benefit from antibody-depleting therapies (Fan et al., 2018).

In recent years, growing evidence supports the theory that the ENS, and in particular enteric neuropathies, are important players in several diseases that were previously considered to primarily affect the CNS, with ENS disorders representing risk factors in many cases. It is well known that bovine spongiform encephalopathy and kuru have their origin in the gut. Namely, the ingestion of infectious amyloids, so-called prions, transfers the disease by homologous seeding – in other words, by oligomers of the misfolded protein promoting the aggregation of that same protein.

Interestingly, it has been suggested that a similar pathogenesis, cross-seeding, may account for Parkinson's disease (PD). In this case, a specific misfolded protein induces the polymerization of a different protein (for a review, see Friedland and Chapman, 2017; Chapelet et al., 2019). In the brain, alpha-synuclein aggregates consisting of misfolded proteins are indicative of PD, but are also found in the myenteric plexus of these patients during autopsy. Furthermore, most PD patients suffer from GI symptoms besides neurological deficits. These observations led to Braak and colleagues' hypothesis that the disease may start in the stomach (part of the ENS) with aggregation of alpha-synuclein due to an environmental pathogen (Braak et al., 2006). The pathological process may reach the brain through the vagus nerve. Although this hypothesis is disputed (Lionnet et al., 2018), it has been shown that vagotomy indeed decreases the risk of PD (Svensson et al., 2015). Several bacterial species, for example *E. coli, Staphylococcus, Streptococcus* and *Salmonella*, produce extracellular amyloids that are capable of cross-seeding (Friedland and Chapman, 2017). Aged rats exposed to amyloid producing *E. coli* developed alpha-synuclein deposits both in their gut and brain, accompanied by microgliosis and astrogliosis (Chen et al., 2016). This concept is supported by the fact that PD patients exhibit changes in their gut microbiota not seen in healthy individuals (Chapelet et al., 2019). Furthermore, in animal studies, the gut microbiota has been shown to play a role in the development of neurological symptoms (Choi et al., 2018; Sampson et al., 2016). The involvement of the GI tract is also supported by the fact that certain major susceptibility genes for inflammatory bowel diseases are over-represented (Bialecka et al., 2007; Hui et al., 2018) in PD patients, and gut inflammation (Devos et al., 2013) and impaired intestinal barrier function (Clairembault et al., 2015; Davies et al., 1996) may also be present in at least a subset of PD patients. Today, it is widely accepted that in some PD patients the disease may start in the gut and be clinically evident as severe constipation before the CNS-triggered symptoms occur.

Another disorder of the CNS that affects the ENS is multiple sclerosis (MS). GI problems are very common among MS patients, yet the pathogenesis is distinct from that of PD. In a cohort of 218 patients, almost two thirds suffered from GI symptoms (Levinthal et al., 2013). Some of these symptoms, such as dysphagia or defecation problems, can be explained by the underlying musculoskeletal dysfunction, others, like nausea and vomiting, may be side effects of medical therapy. Nevertheless, a surprisingly high rate of patients show symptoms corresponding to functional gastrointestinal disorders, such as functional dyspepsia, functional constipation or IBS. GI pathologies were previously thought to be primarily explained by spinal cord lesions causing dysfunction of the autonomous nervous system. However, recently it has been suggested that the autoimmune process, which attacks the CNS, directly affects the ENS (Wunsch et al., 2017). In a mouse model of MS called experimental autoimmune encephalomyelitis (EAE), macrophages and T- and B-lymphocytes were observed in the myenteric plexus of the gut, even in the preclinical state. The invasion of immune cells was accompanied by a degeneration of the myenteric plexus, which preceded the degeneration of the spinal cord. GI transit time was significantly decreased and muscular cholinergic signaling and NO release were reduced in this MS model. The majority of mice with EAE had ENS-reactive autoantibodies, while antibodies against a component of the ENS could be detected in 10 of 33 human MS patients. These results were corroborated by another study showing accelerated gastric emptying and reduced colonic motility in EAE mice, accompanied by immunoreactivity against the ENS in sera (Spear et al., 2018).

A further CNS disorder that has recently been shown to simultaneously affect the ENS is autism spectrum disorder (ASD). GI symptoms in patients with ASD are very common, and they correlate strongly with disease severity (Adams et al., 2011). The GI tract is an important source of serotonin (5-HT), which, besides other functions, plays a role in GI motility. Almost one third of ASD patients have hyperserotonaemia, leading to a link between 5-HT and the GI symptoms of autism patients being suspected (for a review see: Israelyan and Margolis, 2019). Some gain-of-function mutations of the gene encoding the serotonin reuptake transporter, SERT, are associated with ASD, and a murine model with one of these mutations, the "SERT Ala56 mouse", shows brain and behavioral anomalies typical of ASD. Interestingly, these mice also present with abnormalities of the ENS, such as a low neuronal count,

slow intestinal motility and reduced frequency and speed of colonic peristaltic contractions (Margolis et al., 2016). Similarly to in other CNS disorders, altered microbial flora has been observed in ASD patients and is suspected to contribute to symptom development – probiotic treatments have therefore been suggested (Hsiao et al., 2013). Furthermore, it has been hypothesized that increased intestinal permeability, gut inflammation and motility impairments may be explained by enteric glial cell dysfunction (Grubišić and Parpura, 2015).

The role of the gut-brain axis, which includes the ENS, in carcinogenesis is underestimated and vastly understudied. For example, colon tumor development in the Apc(Min/+) mouse model is inhibited after vagotomy, but not by sympathetic denervation (Liu et al., 2015). Last but not least, the ENS is the target of paraneoplastic neurological autoimmunity often associated with small cell lung cancer. Autoantibodies against the Hu-antigen, which are released by the tumor, attack enteric neurons as the vast majority of them express Hu proteins. Chronic exposure of enteric neurons to anti-Hu containing patient serum causes neuronal damage. Even more fascinating is the finding that acute application of the patient serum for only a few hundreds of milliseconds evokes immediate spike discharge involving interaction between anti-HuD and nicotinic receptors on enteric neurons (Li et al., 2016a).

Summary and outlook

Neurogastroenterology, which interested only a small group of experts in the past, has been gradually coming into the spotlight and gaining visibility among both medical/scientific audiences and the lay public. Apart from known structural and functional disorders of the ENS, microbiome-gut-brain axis disturbances are now linked to a constantly growing range of pathologies, including Parkinson's disease, autism spectrum disorder and multiple sclerosis. A better understanding of the physiology of the enteric nervous system and the pathogenesis of ENS disorders gained over recent years may lead to the development of new therapies.

References

Adams, J.B., Johansen, L.J., Powell, L.D., Quig, D., and Rubin, R.A. (2011). Gastrointestinal flora and gastrointestinal status in children with autism – comparisons to typical children and correlation with autism severity. BMC Gastroenterol. *11*, 22.

Anitha, M., Joseph, I., Ding, X., Torre, E.R., Sawchuk, M.A., Mwangi, S., … Srinivasan, S. (2008). Characterization of Fetal and Postnatal Enteric Neuronal Cell Lines With Improvement in Intestinal Neural Function. Gastroenterology *134*, 1424–1435.

Annaházi, A., Ferrier, L., Bézirard, V., Lévêque, M., Eutamène, H., Ait-Belgnaoui, A., … Bueno, L. (2013). Luminal Cysteine-Proteases Degrade Colonic Tight Junction Structure and Are Responsible for Abdominal Pain in Constipation-Predominant IBS. Am. J. Gastroenterol. *108*, 1322–1331.

Barbara, G., Stanghellini, V., De Giorgio, R., Cremon, C., Cottrell, G.S., Santini, … Corinaldesi, R. (2004). Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology *126*, 693–702.

Bayliss, W.M., and Starling, E.H. (1899). The movements and innervation of the small intestine. J. Physiol. *24*, 99–143.

Bertrand, P.P., Kunze, W.A., Bornstein, J.C., Furness, J.B., and Smith, M.L. (1997). Analysis of the responses of myenteric neurons in the small intestine to chemical stimulation of the mucosa. Am. J. Physiol.: Gastrointest. Liver Physiol. *273*, G422–G435.

Bialecka, M., Kurzawski, M., Klodowska-Duda, G., Opala, G., Juzwiak, S., Kurzawski, G., Tan, E.-K., and Drozdzik, M. (2007). CARD15 variants in patients with sporadic Parkinson's disease. Neurosci. Res. *57*, 473–476.

Blackshaw, L.A., Brookes, S.J.H., Grundy, D., and Schemann, M. (2007). Sensory transmission in the gastrointestinal tract. Neurogastroenterol. Motil. *19*, 1–19.

Braak, H., de Vos, R.A.I., Bohl, J., and Del Tredici, K. (2006). Gastric α-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci. Lett. *396*, 67–72.

Buhner, S., Li, Q., Vignali, S., Barbara, G., De Giorgio, R., Stanghellini, V. … Schemann, M. (2009). Activation of Human Enteric Neurons by Supernatants of Colonic Biopsy Specimens From Patients With Irritable Bowel Syndrome. Gastroenterology *137*, 1425–1434.

Buhner, S., Li, Q., Berger, T., Vignali, S., Barbara, G., De Giorgio, R., Stanghellini, V., and Schemann, M. (2012). Submucous rather than myenteric neurons are activated by mucosal biopsy supernatants from irritable bowel syndrome patients: Enteric plexuses and IBS supernatants. Neurogastroenterol. Motil. *24*, 1134-e572.

Buhner, S., Braak, B., Li, Q., Kugler, E.M., Klooker, T., Wouters, M., … Schemann, M. (2014). Neuronal activation by mucosal biopsy supernatants from irritable bowel syndrome patients is linked to visceral sensitivity: Neuronal activation in irritable bowel syndrome. Exp. Physiol. *99*, 1299–1311.

Butler Tjaden, N.E., and Trainor, P.A. (2013). The developmental etiology and pathogenesis of Hirschsprung disease. Transl. Res. *162*, 1–15.

Cenac, N., Andrews, C.N., Holzhausen, M., Chapman, K., Cottrell, G., Andrade-Gordon, P., … Vergnole, N. (2007). Role for protease activity in visceral pain in irritable bowel syndrome. J. Clin. Invest. *117*, 636–647.

Chapelet, G., Leclair-Visonneau, L., Clairembault, T., Neunlist, M., and Derkinderen, P. (2019). Can the gut be the missing piece in uncovering PD pathogenesis? Parkinsonism Relat. Disord. *59*, 26–31.

Chen, S.G., Stribinskis, V., Rane, M.J., Demuth, D.R., Gozal, E., Roberts, A.M., … Friedland, R.P. (2016). Exposure to

the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. Sci. Rep. *6*, 34477.

Choi, J.G., Kim, N., Ju, I.G., Eo, H., Lim, S.-M., Jang, S.-E., Kim, D.-H., and Oh, M.S. (2018). Oral administration of Proteus mirabilis damages dopaminergic neurons and motor functions in mice. Sci. Rep. *8*, 1275.

Clairembault, T., Leclair-Visonneau, L., Coron, E., Bourreille, A., Le Dily, S., Vavasseur, F., … Derkinderen, P. (2015). Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol. Commun. *3*, 12.

Davies, K.N., King, D., Billington, D., and Barrett, J.A. (1996). Intestinal permeability and orocaecal transit time in elderly patients with Parkinson's disease. Postgrad. Med. J. *72*, 164–167.

Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A., and Schnitzer, M.J. (2006). Next-Generation Optical Technologies for Illuminating Genetically Targeted Brain Circuits. J. Neurosci. *26*, 10380–10386.

Devos, D., Lebouvier, T., Lardeux, B., Biraud, M., Rouaud, T., Pouclet, … Derkinderen, P. (2013). Colonic inflammation in Parkinson's disease. Neurobiol. Dis. *50*, 42–48.

Drossman, D.A. (2016). Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. Gastroenterology *150*, 1262–1279.e2.

Enck, P., Aziz, Q., Barbara, G., Farmer, A. D., Fukudo, S., Mayer, E.A., … Spiller, R.C. (2016). Irritable bowel syndrome. Nat. Rev. Dis. Primers *2*, 16014.

Fan, W., Fei, G., Li, X., Wang, X., Hu, C., Xin, H., … Fang, X. (2018). Sera with anti-enteric neuronal antibodies from patients with irritable bowel syndrome promote apoptosis in myenteric neurons of guinea pigs and human SH-Sy5Y cells. Neurogastroenterol. Motil. *30*, e13457.

Friedland, R.P., and Chapman, M.R. (2017). The role of microbial amyloid in neurodegeneration. PLoS Pathog. *13*, e1006654.

Furness, J.B. (2006). The enteric nervous system (Malden, Mass: Blackwell Pub).

Furness, J.B., and Stebbing, M.J. (2018). The first brain: Species comparisons and evolutionary implications for the enteric and central nervous systems. Neurogastroenterol. Motil. *30*, e13234.

Furness, J.B., Callaghan, B.P., Rivera, L.R., and Cho, H.-J. (2014). The Enteric Nervous System and Gastrointestinal Innervation: Integrated Local and Central Control. In Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease. M. Lyte and J.F. Cryan, eds. (New York, NY: Springer New York), pp. 39–71.

Gecse, K., Roka, R., Ferrier, L., Leveque, M., Eutamene, H., Cartier, C., … Bueno, L. (2008). Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. Gut *57*, 591–599.

Grubišić, V., and Parpura, V. (2015). The second brain in autism spectrum disorder: could connexin 43 expressed in enteric glial cells play a role? Front. Cell. Neurosci. *9*, 242.

Gustafson, E., Larsson, T., and Danielson, J. (2019). Controlled outcome of Hirschsprung's disease beyond adolescence: a single center experience. Pediatr. Surg. Int. *35*, 181–185.

Hirschsprung, H. (1888). Stuhlträgheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons. In Jahrbuch Für Kinderheilkunde Und Physische Erziehung, 27: pp. 1–7.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., … Mazmanian S.K. (2013). Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders. Cell *155*, 1451–1463.

Hui, K.Y., Fernandez-Hernandez, H., Hu, J., Schaffner, A., Pankratz, N., Hsu, N.-Y., … Peter, I. (2018). Functional variants in the *LRRK2* gene confer shared effects on risk for Crohn's disease and Parkinson's disease. Sci. Transl. Med. *10*, eaai7795.

Huizinga, J.D., and Lammers, W.J.E.P. (2009). Gut peristalsis is governed by a multitude of cooperating mechanisms. Am. J. Physiol. Gastrointest. Liver Physiol. *296*, G1–G8.

Israelyan, N., and Margolis, K.G. (2019). Reprint of: Serotonin as a link between the gut-brain-microbiome axis in autism spectrum disorders. Pharmacol. Res. *140*, 115–120.

Jahng, J., and Kim, Y.S. (2016). Irritable Bowel Syndrome: Is It Really a Functional Disorder? A New Perspective on Alteration of Enteric Nervous System. Neurogastroenterol. Motil. *22*, 163–165.

Kugler, E.M., Michel, K., Zeller, F., Demir, I. E., Ceyhan, G.O., Schemann, M., and Mazzuoli-Weber, G. (2015). Mechanical stress activates neurites and somata of myenteric neurons. Front. Cell. Neurosci. *9*, 342.

Latorre, R., Sternini, C., De Giorgio, R., and Greenwood-Van Meerveld, B. (2016). Enteroendocrine cells: a review of their role in brain-gut communication. Neurogastroenterol. Motil. *28*, 620–630.

Levinthal, D.J., Rahman, A., Nusrat, S., O'Leary, M., Heyman, R., and Bielefeldt, K. (2013). Adding to the Burden: Gastrointestinal Symptoms and Syndromes in Multiple Sclerosis. Mult. Scler. Int. *2013*, 1–9.

Li, Q., Michel, K., Annahazi, A., Demir, I. E., Ceyhan, G.O., Zeller, F., … Schemann, M. (2016a). Anti-Hu antibodies activate enteric and sensory neurons. Sci. Rep. *6*, 38216.

Li, S., Fei, G., Fang, X., Yang, X., Sun, X., Qian, J., Wood, J.D., and Ke, M. (2016b). Changes in Enteric Neurons of Small Intestine in a Rat Model of Irritable Bowel Syndrome with Diarrhea. Neurogastroenterol. Motil. *22*, 310–320.

Lionnet, A., Leclair-Visonneau, L., Neunlist, M., Murayama, S., Takao, M., Adler, C.H., Derkinderen, P., and Beach, T.G. (2018). Does Parkinson's disease start in the gut? Acta Neuropathol. *135*, 1–12.

Liu, M., Seino, S., and Kirchgessner, A.L. (1999). Identification and characterization of glucoresponsive neurons in the enteric nervous system. J. Neurosci. *19*, 10305–10317.

Liu, V., Dietrich, A., Kasparek, M.S., Benhaqi, P., Schneider, M.R., Schemann, M., Seeliger, H., and Kreis, M. E. (2015). Extrinsic intestinal denervation modulates tumor development in the small intestine of ApcMin/+ mice. J. Exp. Clin. Cancer Res. *34*, 39.

Lüderitz, C. (1890). Experimentelle Untersuchungen über die Entstehung der Darmperistaltik. Archiv f. pathol. Anat. *122*, 1–28.

Margolis, K.G., Li, Z., Stevanovic, K., Saurman, V., Israelyan, N., Anderson, G.M., … Gershon, M.D. (2016). Serotonin transporter variant drives preventable gastrointestinal abnormalities in development and function. J. Clin. Invest. *126*, 2221–2235.

Mayer, E.A. (2011). Gut feelings: the emerging biology of gut–brain communication. Nat. Rev. Neurosci. *12*, 453–466.

Mazzuoli-Weber, G., and Schemann, M. (2015). Mechanosensitivity in the enteric nervous system. Front. Cell. Neurosci. *9*, 408.

Moloney, R.D., O'Mahony, S.M., Dinan, T.G., and Cryan, J.F. (2015). Stress-Induced Visceral Pain: Toward Animal Models of Irritable-Bowel Syndrome and Associated Comorbidities. Front. Psychiatry *6*, 15.

Neunlist, M., Michel, K., Reiche, D., Dobreva, G., Huber, K., and Schemann, M. (2001). Glycine activates myenteric neurones in adult guinea-pigs. J. Physiol. *536*, 727–739.

Ostertag, D., Buhner, S., Michel, K., Pehl, C., Kurjak, M., Götzberger, M., … Schemann, M. (2015). Reduced Responses of Submucous Neurons from Irritable Bowel Syndrome Patients to a Cocktail Containing Histamine, Serotonin, TNFα, and Tryptase (IBS-Cocktail). Front. in Neurosci. *9*, 465.

O'Sullivan, M., Clayton, N., Breslin, N.P., Harman, I., Bountra, C., McLaren, A., and O'Morain, C.A. (2000). Increased mast cells in the irritable bowel syndrome. Neurogastroenterol. Motil. *12*, 449–457.

Pan, W.K., Zheng, B.J., Gao, Y., Qin, H., and Liu, Y. (2011). Transplantation of Neonatal Gut Neural Crest Progenitors Reconstructs Ganglionic Function in Benzalkonium Chloride-Treated Homogenic Rat Colon. J. Surg. Res. *167*, e221–e230.

Ro, S., Hwang, S.J., Muto, M., Jewett, W.K., and Spencer, N.J. (2006). Anatomic modifications in the enteric nervous system of piebald mice and physiological consequences to colonic motor activity. Am. J. Physiol. Gastrointest. Liver Physiol. *290*, G710–718.

Róka, R., Rosztóczy, A., Leveque, M., Izbéki, F., Nagy, F., Molnár, T., … Bueno, L. (2007). A Pilot Study of Fecal Serine-Protease Activity: A Pathophysiologic Factor in Diarrhea-Predominant Irritable Bowel Syndrome. Clin. Gastroenterol. Hepatol. *5*, 550–555.

Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., … Mazmanian, S.K. (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell *167*, 1469–1480.e12.

Schemann, M., and Grundy, D. (1992). Electrophysiological identification of vagally innervated enteric neurons in guinea pig stomach. Am. J. Physiol. Gastrointest. Liver Physiol. *263*, G709–G718.

Schemann, M., Frieling, T., and Enck, P. (2019). To learn, to remember, to forget—How smart is the gut? Acta Physiol. e13296.

Sergi, C. (2015). Hirschsprung's disease: Historical notes and pathological diagnosis on the occasion of the 100th anniversary of Dr. Harald Hirschsprung's death. World J. Clin. Pediatr. *4*, 120.

Shimizu, H., Koizumi, O., and Fujisawa, T. (2004). Three digestive movements in Hydra regulated by the diffuse nerve net in the body column. J. Comp. Physiol. A *190*.

Smith, T.K., Spencer, N.J., Hennig, G.W., and Dickson, E.J. (2007). Recent advances in enteric neurobiology: mechanosensitive interneurons. Neurogastroenterol. Motil. *19*, 869–878.

Spear, E.T., Holt, E.A., Joyce, E.J., Haag, M.M., Mawe, S.M., Hennig, G.W., Lavoie, B., Applebee, A.M., Teuscher, C., and Mawe, G.M. (2018). Altered gastrointestinal motility involving autoantibodies in the experimental autoimmune encephalomyelitis model of multiple sclerosis. Neurogastroenterol. Motil. *30*, e13349.

Stamp, L.A. (2017). Cell therapy for GI motility disorders: comparison of cell sources and proposed steps for treating Hirschsprung disease. Am. J. Physiol. Gastrointest. Liver Physiol. *312*, G348–G354.

Stamp, L.A., Gwynne, R.M., Foong, J.P.P., Lomax, A.E., Hao, M.M., Kaplan, D.I., … Young, H.M. (2017). Optogenetic Demonstration of Functional Innervation of Mouse Colon by Neurons Derived From Transplanted Neural Cells. Gastroenterology *152*, 1407–1418.

Svensson, E., Horváth-Puhó, E., Thomsen, R.W., Djurhuus, J.C., Pedersen, L., Borghammer, P., and Sørensen, H.T. (2015). Vagotomy and subsequent risk of Parkinson's disease: Vagotomy and Risk of PD. Ann. Neurol. *78*, 522–529.

Tittel, K. (1901). Ueber eine angeborene Missbildung des Dickdarmes. Wien. klin. Wchnschr. 903–907.

Traini, C., Evangelista, S., Girod, V., Faussone-Pellegrini, M.S., and Vannucchi, M.G. (2016). Changes of excitatory and inhibitory neurotransmitters in the colon of rats underwent to the wrap partial restraint stress. Neurogastroenterol. Motil. *28*, 1172–1185.

Wang, W. (2018). Optogenetic manipulation of ENS – The brain in the gut. Life Sci. *192*, 18–25.

Wood, J.D., Liu, S., Drossman, D.A., Ringel, Y., and Whitehead, W.E. (2012). Anti-Enteric Neuronal Antibodies and the Irritable Bowel Syndrome. Neurogastroenterol. Motil. *18*, 78–85.

Wunsch, M., Jabari, S., Voussen, B., Enders, M., Srinivasan, S., Cossais, F., … Kuerten, S. (2017). The enteric nervous system is a potential autoimmune target in multiple sclerosis. Acta Neuropathol. *134*, 281–295.

Zhang, L., Song, J., and Hou, X. (2016). Mast Cells and Irritable Bowel Syndrome: From the Bench to the Bedside. Neurogastroenterol. Motil. *22*, 181–192.

Legend to Movie

The movie shows the spike pattern in enteric neurons related to muscle movement.

The top image shows a myenteric ganglion labeled with a voltage sensitive dye. Individual neurons are seen as black ring-like structures because the dye incorporates into their outer membrane. The trace at the bottom shows the spike discharge in one of the neurons, which is indicated by the grey frame. The red dot moving over the trace corresponds to the false color-coded activity level in the movie (red is the peak of the spike). Each spike is coded as a red color. The recording period is several seconds and the spike burst corresponds to the frequency of intestinal contractions. The activity within the ganglion very likely reflects activity in muscle motor neurons.

Supplementary Material: The online version of this article offers a movie as supplementary material (https://doi.org/10.1515/nf-2019-0027).

Bionotes

Anita Annahazi
Technical University of Munich
Human Biology
Liesel-Beckmann Strasse 4
85354 Freising, Germany
e-mail: anita.annahazi@tum.de

Anita Annahazi studied Medicine at the University of Szeged, Hungary, where she received her MD for her thesis completed in the Cerebral Circulation Research Group of Professor Ferenc Bari. In the following years, while specializing in internal medicine at the First Department of Medicine, she joined the Gastrointestinal Motility Team. After her PhD project on the pathogenesis of irritable bowel syndrome and inflammatory bowel disease, under the supervision of Professor Tibor Wittmann and Dr Richard Roka, she pursued the development of noninvasive diagnostic approaches for intestinal diseases. As a post-doctoral fellow she spent two and a half years in the laboratory of Dr Lionel Bueno in the Neurogastroenterology and Nutrition Unit of INRA in Toulouse, France. Since 2015 she has been working at the Chair of Professor Michael Schemann at the Technical University of Munich, Germany. Currently she focuses on the role of the enteric nervous system in different gastrointestinal pathologies.

Professor Dr. Michael Schemann
Technical University of Munich
Human Biology
Liesel-Beckmann Strasse 4
85354 Freising-Weihenstephan
e-mail: schemann@wzw.tum.de

Michael Schemann studied Agricultural Biology at the University of Hohenheim, Germany where he also received his Dr. rer. nat. with a project on the relation between intestinal motility and gastrointestinal transit under the supervision of Hans-Jörg Ehrlein. His interest in the enteric nervous system started during his post-doctoral research with Jackie Wood at Ohio State University, Columbus, Ohio, USA. Shortly after moving back to the University of Hohenheim he moved to the Max-Planck Institute for Physiological and Clinical Research in Bad Nauheim with a Heisenberg Fellowship. Before he moved to his current position as Full Professor for Human Biology at the Technical University of Munich, he was Professor at the Institute of Physiology at the University of Veterinary Medicine in Hannover. His research interests lie in the field of translational Neurogastroenterology and focus on nerve mediated gut function under physiological and pathological conditions.

Review Article

Friederike Zunke*

The function of lysosomes and their role in Parkinson's disease

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Abstract: Lysosomes are cellular organelles that are important for the degradation and recycling of various biomolecules. Specialized lysosomal membrane proteins, as well as soluble enzymes, are important for the efficient turn-over of lysosomal substrates. A deficiency in the degradative capacity of lysosomes leads to severe pathologies referred to as lysosomal storage disorders. There is increasing evidence for the importance of lysosomal function in neurodegenerative disorders, including Parkinson's disease. One reason for this might be the vulnerability of neuronal cells. Since neurons do not undergo further cell division, non-degraded substrates accumulate in aging cells, causing a buildup of toxicity. Recent genomic screenings identified a number of lysosome-associated genes as potential risk factors for Parkinson's disease, which are discussed in this review. Moreover, it is outlined how targeting lysosomal function might help in developing novel therapeutic strategies.

Keywords: α-synuclein, lysosomes, lysosomal enzymes, lysosomal storage disorders (LSD), Parkinson's disease

Zusammenfassung: Lysosomen sind membranumschlossene Zellorganelle, in denen lösliche Enzyme für den Abbau sowie das Recycling intrazellulärer als auch extrazellulärer Biomoleküle sorgen. Kommt es dagegen zu einer unvollständigen Degradation hat das schwerwiegende pathologische Konsequenzen und führt zu sogenannten lysosomalen Speichererkrankungen. Forschungsergebnisse der letzten Jahre deuten auf einen Zusammenhang zwischen lysosomaler Dysfunktion und dem Krankheitsverlauf neurodegenerativen Erkrankungen hin – so wie zum Beispiel beim Morbus Parkinson. Eine mögliche Erklärung hierfür ist, dass neuronale Zellen keine Zellteilung mehr durchlaufen und sich so über die Zeit lysosomale Substrate anhäufen. Interessanterweise zeigen genetische Untersuchungen von Parkinson Patienten eine Anhäufung lysosomaler Gene, welche als Risikofaktoren für die Erkrankung beschrieben und in diesem Review behandelt werden. Des Weiteren wird diskutiert, welche Rolle Lysosomen bei der Entwicklung neuartiger Therapien zur Behandlung der Parkinson Erkrankung spielen können.

Schlüsselwörter: Lysosomen, Lysosomale Speichererkrankungen, lysosomale Enzyme, Morbus Parkinson, α-Synuclein

Introduction

Recent studies have shown that lysosomal dysfunction is not only linked to rare lysosomal storage disorders, but also to age-dependent neurodegenerative diseases, such as Parkinson's disease (Fraldi et al., 2016). Whereas most cases of Parkinson's disease are idiopathic (~90 %), meaning they do not have a clear heritable genetic link, the remaining ~10 % of Parkinson's disease cases are familial and show Mendelian inheritance of affected genetic variants (Kalia and Lang, 2015). In order to understand the underlying molecular disease pathways, single genetic variants are of particular value for scientific studies. Recent genetic meta-analyses of Parkinson's patients (both familial and idiopathic) reveal enrichment in genetic risk loci related to lysosomal homeostasis (Chang et al., 2017; Robak et al., 2017). Hence, the majority of Parkinson's disease-associated genes are linked to lysosomal protein trafficking or lysosomal function (Klein and Mazzulli, 2018). The following review will give an overview of the composition and function of lysosomes in general, their role in neurodegeneration with focus on Parkinson's disease, and discuss potential new treatment strategies targeting lysosomal proteins or pathways.

***Corresponding author: Friederike Zunke**, Christian-Albrechts-Universität zu Kiel, Fachbereich Biochemie, Rudolf-Höber Strasse 1, 24118 Kiel, Germany, e-mail: fzunke@biochem.uni-kiel.de

The composition and function of lysosomes

Lysosomes were first described in the 1950 s by Christian DeDuve, and are membrane-surrounded cellular organelles ubiquitously found within the cytosol of mammalian cells (DeDuve et al., 1955). Lysosomes contain specialized membrane proteins, as well as luminal hydrolytic enzymes, that can break down many kinds of biomolecules, including intracellular and extracellular substrates. The importance of lysosomal function is highlighted by a number of diseases called lysosomal storage disorders (LSDs), that result from defects in soluble lysosomal proteins and proteins of the lysosomal membrane (Eskelinen et al., 2003).

Importantly, it has been realized that lysosomes are not only degradative organelles, but also play a pivotal role in cell metabolism. Hence, they are involved in processes of cell signaling, repair of the plasma membrane, defense against pathogens, cholesterol metabolism, cell death, and energy metabolism (Schulze and Sandhoff, 2011; Settembre et al., 2013). Lysosomes emerge from the fusion of endosomes, which are specialized vesicles with an acidic pH. The pH within the lysosomes reaches as low as pH 4.5–5.0, and is mainly mediated by the ATP-dependent vacuolar(v)-type H^+ ATPase (Ohkuma et al., 1982) (Figure 1).

Lysosomal enzymes reach lysosomes via the secretory pathway, which means that they are synthesized in the endoplasmic reticulum (ER) as membrane-integrated or soluble glycoproteins and pass through the Golgi apparatus. Since the majority of lysosomal proteins are transported to lysosomes by the mannose-6-phosphate (M6P)-dependent pathway, proteins are tagged with an M6P recognition marker. This terminal residue is then recognized by M6P-receptors, which mediate protein delivery to lysosomes via clathrin coated vesicles (Kornfeld, 1992). It must be noted that M6P-independent lysosomal transport mechanisms also exist. Moreover, some lysosomal proteins can escape the lysosomal targeting pathways. They can be transported to the plasma membrane or outside the cell, where they are taken up and transported back to lysosomes by a process called endocytosis (Janvier and Bonifacino, 2005).

To date, 60 different lysosomal enzymes have been found to be responsible for the degradation of various substrates (Settembre et al., 2013). Depending on their degradation products, lysosomal enzymes are classified as proteases, nucleases, glucosidases, phosphatases, polysaccharide hydrolyzing enzymes or lipid degrading enzymes (Bainton, 1981). All require an acidic environment for optimal enzymatic function (De Duve and Beaufay, 1959).

The lysosomal membrane contains highly glycosylated membrane proteins, which contribute to the stability of the membrane, as well as the luminal glycocalyx (Schwake et al., 2013). Most abundant in the lysosomal membrane, and thus important for its integrity, are the lysosomal associated membrane proteins type-1 and -2 (LAMP-1, LAMP-2), and the lysosomal integral membrane proteins type-1 and -2 (LIMP-1, LIMP-2/SCARB2). Interestingly, the LIMP-2 protein has not only been shown to be important for the integrity of the lysosomal membrane, but also for the M6P-independent lysosomal transport of the lysosomal enzyme β-glucocerebrosidase (GCase) (Blanz et al., 2015; Reczek et al., 2007; Zunke et al., 2016).

Furthermore, various channel proteins and small molecule transporters, like the transmembrane protein 175 (TMEM175), a potassium channel, or the sialic acid transporter, Sialin, are major integral components of the lysosomal membrane (Eskelinen et al., 2003). Additionally, transporter proteins, like the lysosomal cholesterol transporter Niemann-Pick Type C1 (NPC1), are found in the lysosomal membrane and play an important role in cellular cholesterol homeostasis (Infante et al., 2008) (Figure 1).

Lysosomal dysfunction and neurodegenerative diseases

Genetic variants within genes encoding for lysosomal proteins resulting in less or non-functional protein (loss-of-function mutation) lead to lysosomal dysfunction and buildup of lysosomal substrates. This disease family is called lysosomal storage disorders (LSDs) and comprises over 70 distinct diseases, which in total have an incidence of ~1:7,000 live births (Gieselmann, 1995). The majority of LSDs are autosomal recessive and are caused by severe loss-of-function mutations within lysosomal trafficking components, lysosomal enzymes or membrane proteins (Platt et al., 2018). For instance, the most common LSD – Gaucher disease – is caused by mutations within the lysosomal enzyme GCase (gene name: *GBA1*). The resulting enzymatic dysfunction leads to the lysosomal accumulation of the non-degraded substrate glucosylceramide (a glycolipid), causing an enlargement of organs (organomegaly), anemia and bone malformation as well as, in some cases, neurological dysfunction (Pastores, 1997).

In a group of LSDs called neuronal ceroid lipofuscinosis (NCL), a lipopigment (lipofuscin) aggregates within lysosomal structures, resulting in a progressive loss of

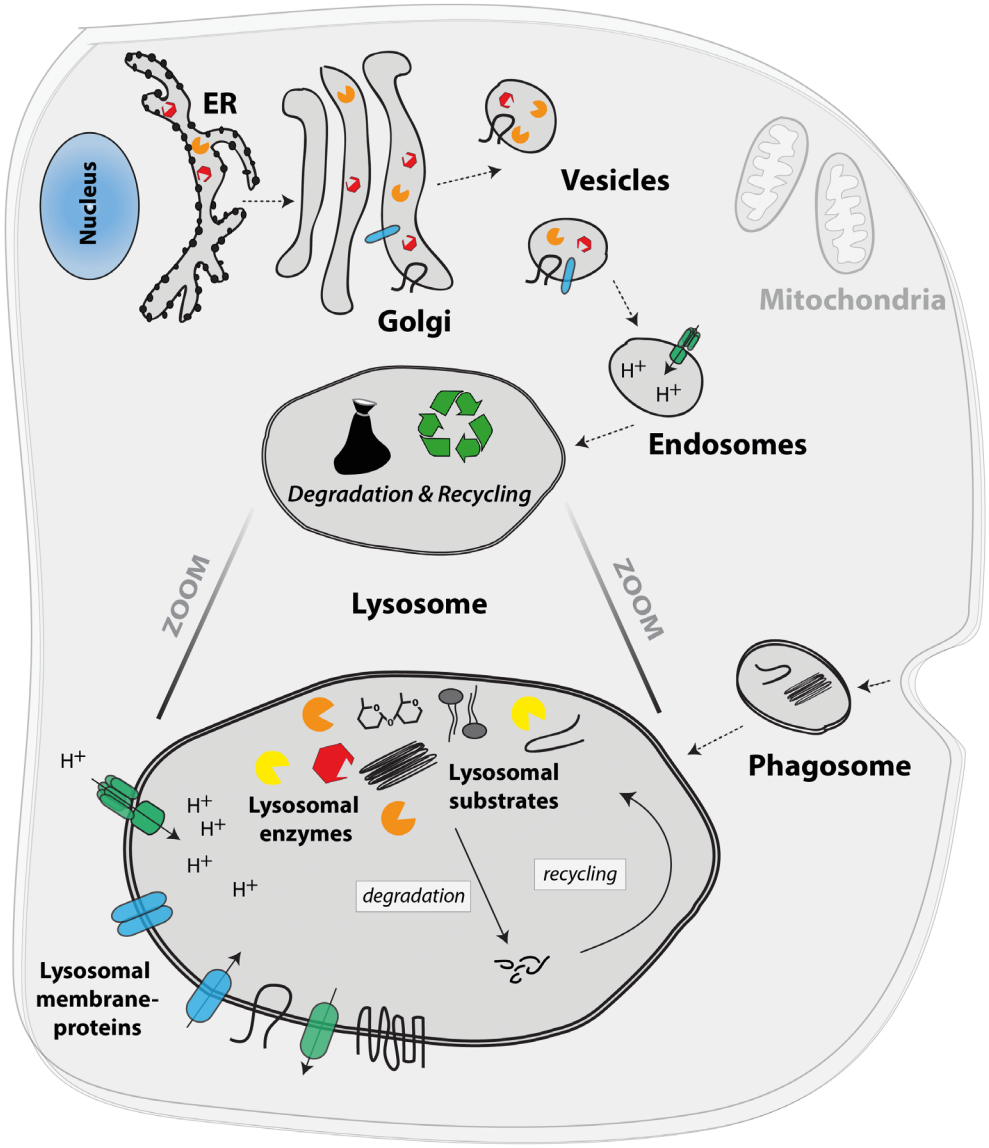

Fig. 1: The eukaryotic cell and its organelles, highlighting the lysosome and its components.
The nucleus contains the majority of the cell's genetic material and is the location of the DNA transcription process (DNA → RNA). Further protein translation (RNA → protein) is mediated at free ribosomes within the cytosol (not shown) or at ribosomes located at membranes of the endoplasmic reticulum (ER; indicated as black dots on ER membrane). The Golgi apparatus (Golgi) is important for further protein modification and sorting. Vesicles originating from the Golgi compartment can fuse with intracellular organelles, like the lysosome, or with the cell membrane. Mitochondria are the 'powerhouse' of the cell, generating energy by performing cellular respiration and producing ATP. The lysosome is the cell's degradative organelle and arises from the fusion of Golgi-derived vesicles with endosomes, as indicated by dotted arrows. Lysosomes are not only responsible for degradation and recycling, but are also involved in pathogen defense, cell death, and energy metabolism. Their acidic pH (pH 4.5–5.0) is mainly maintained by the vacuolar-type H^+ ATPase, which pumps H^+ ions via the lysosomal membrane. A total of around 50 specialized lysosomal membrane proteins (blue and green molecules) are not only important for the maintenance and stability of the organelle, but also for the formation of the lysosomal glycocalyx at the inside of lysosomes, lysosomal protein transport, and the transport and exchange of biomolecules through the lysosomal membrane (mediated by a variety of channel and transporter proteins). Lysosomal substrates comprise a variety of intracellular as well as extracellular biomolecules including proteins, peptides, lipids, nucleic acids and carbohydrates. Substrates enter the lysosome via macro-, micro- and chaperone-mediated autophagy, as well as by fusion of lysosomes with phagosomes, which comprise extracellular material (indicated by dotted arrows). Around 60 different lysosomal enzymes (yellow, orange and red symbols) are responsible for the breakdown and recycling of specific substrates (shown in black and gray within the lysosome). These enzymes require an acidic pH and include proteases, nucleases, glucosidases, phosphatases, polysaccharide hydrolyzing enzymes, and lipid degrading enzymes.

motor and cognitive abilities. NCLs are classified into ten different types caused by mutations within lysosomal enzymes – such as cathepsin D – but also membrane proteins (Bennett and Rakheja, 2013).

Interestingly, neurodegeneration is observed in nearly all LSDs, which emphasizes the importance of lysosomal degradation within neuronal cell homeostasis (Fraldi et al., 2016). There is increasing evidence for the contribution of lysosomal dysfunction in age-related neurodegenerative diseases, such as Huntington's, Alzheimer's and Parkinson's diseases (Klein and Mazzulli, 2018; Pitcairn et al., 2019). The efficient degradation of lysosomal substrates (proteins, lipids, carbohydrates, etc.) is essential for neuronal survival, since neurons are particularly sensitive to alterations in the lysosomal degradation pathway. This is probably due to their post-mitotic state, since non-degraded lysosomal substrates will not be diluted by cell division, but accumulate over time until they reach critical concentrations. Studies have shown that abnormal protein accumulation can cause neurotoxicity and induce neuronal cell death (Hara et al., 2006; Komatsu et al., 2006). Since our brain cannot regenerate this cell type, the progressive loss of neurons results in neuropathology. Interestingly, a decrease in lysosomal activity could also be measured in postmortem brain samples derived from Parkinson's disease patients (Dehay et al., 2010).

Parkinson's disease is a progressive neurodegenerative disorder that affects about 1–2% of the population above the age of 65. Parkinson's disease symptoms comprise movement abnormalities, including tremor, bradykinesia (slowness of movement), and muscle rigidity (Kalia and Lang, 2015). During the course of the disease, non-motor symptoms may also arise, consisting of dementia, behavioral and digestive problems, as well as depression (Chaudhuri et al., 2006). These clinical manifestations result from the specific loss of neurons that produce the neurotransmitter dopamine. These so-called dopaminergic neurons are located within the substantia nigra, an area of the midbrain (Davie, 2008). Moreover, a histological hallmark of Parkinson's disease is the intracellular aggregation of the cytosolic protein α-synuclein, which is a major component of Lewy body inclusions found in post-mortem brains of Parkinson's disease patients (Baba et al., 1998; Braak and Del Tredici, 2017; Xia et al., 2008). It has been shown that intracellular α-synuclein aggregation leads to pathological manifestations and conveys cell toxicity (Lashuel and Lansbury, 2006; Lashuel et al., 2002). Importantly, accumulation of α-synuclein is a common feature observed in many LSDs (Shachar et al., 2011), further emphasizing the strong interplay between lysosomal function and α-synuclein aggregation.

The effect of lysosomal dysfunction on α-synuclein aggregation

α-Synuclein is a small cytosolic protein consisting of 140 amino acids encoded by the *SNCA* gene, and is (under physiological conditions) mainly found in the pre-synaptic terminals of neurons, where it is involved in synaptic neurotransmitter release and synaptic plasticity (George, 2002; Sulzer and Edwards, 2019). Recent studies further illuminate the relationship between lysosomal dysfunction and α-synuclein aggregation. Physiological α-synuclein can be degraded within lysosomes, where lysosomal cathepsins (CTSD and CTSB) are most likely involved in its degradation (Cuervo et al., 2004; Cullen et al., 2009; McGlinchey and Lee, 2015). Since α-synuclein is a very abundant and aggregation-prone protein, even subtle elevations in its concentration within the cell may drive the protein into pathological aggregates (Giasson et al., 1999). Hence, impairments in the lysosomal system have been shown to affect α-synuclein levels and aggregation. Moreover, multiple studies have demonstrated that metabolites like glycolipids that accumulate under lysosomal impairment can specifically interact with α-synuclein and further induce its aggregation (Mazzulli et al., 2011; Suzuki et al., 2015; Taguchi et al., 2017) (Figure 2). This process is thought to be mediated through a toxic structural conversion of α-synuclein oligomers resulting in amyloid fibril formation (Zunke et al., 2018) (Figure 2). Likewise, intra-lysosomal cholesterol may be an important modulator in Parkinson's disease, as it has been shown to directly induce α-synuclein fibrilization (Bosco et al., 2006). Taken together, molecular data indicate that lysosomal storage of certain metabolites influences α-synuclein structure and aggregation capacity. This gives rise to the selective relationship between lysosomal dysfunction and α-synuclein aggregation, similar to that observed in Parkinson's disease.

Lysosomal risk factors in Parkinson's disease

Over the years, numerous molecular, clinical and genetic studies have emphasized a central role for lysosomal pathways and proteins in the pathogenesis of Parkinson's disease (Kalia and Lang, 2015; Klein and Mazzulli, 2018; Mazzulli et al., 2011; Pitcairn et al., 2019). Importantly, genetic meta-analyses of Parkinson's disease patients

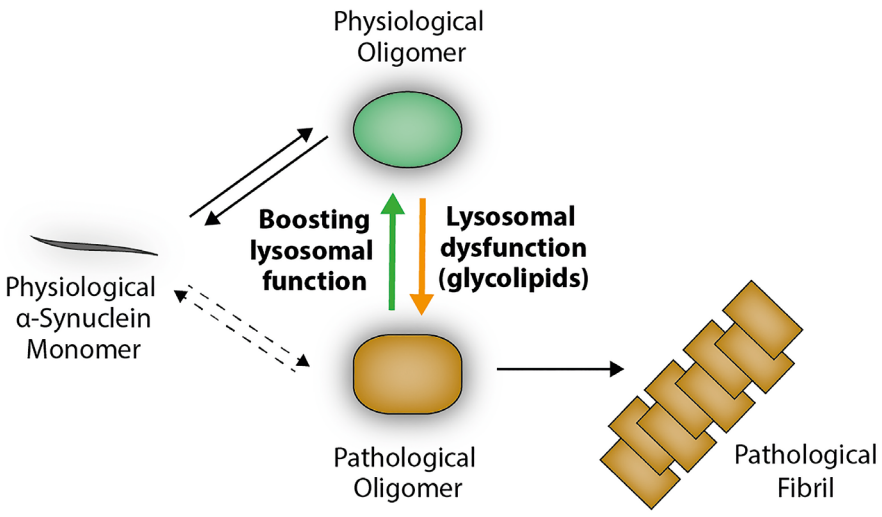

Fig. 2: Schematic overview of α-synuclein aggregation.
Under physiological conditions, α-synuclein mainly exists as a monomer (gray), but can also form physiological oligomers (green) within the cytosol. Lysosomal dysfunction and aggregation of substrates like the GCase-substrate glucosylceramide (a glycolipid) have been shown to interfere with α-synuclein within lysosomes. This interaction of the protein with lysosomal glycolipids results in a structural change in the protein oligomer (orange). These pathological oligomers (orange) have been shown to be neurotoxic and induce pathological fibril formation (orange fibril), which is enhanced by the acidic pH within lysosomes. Interestingly, boosting lysosomal function or decreasing glycolipid levels has been shown to reverse pathological oligomers (orange) back to their physiological state (green). This molecular mechanism is utilized in substrate reduction therapy or by application of small compounds activating substrate-degrading enzymes (e. g. GCase) (modified from Riederer et al., 2019; Zunke et al., 2018).

reveal enrichment in genetic risk loci related to lysosomal function (Chang et al., 2017; Robak et al., 2017; Sidransky et al., 2009). Among these genetic risk factors are lysosomal hydrolases, such as GCase (*GBA1*), galactosylceramidase (*GALC*), sphingomyelin phosphodiesterase 1 (*SMPD1*), N-acylsphingosine Amidohydrolase 1 (*ASAH1*), and N-acetyl-glucosaminidase (NAGLU), as well as the two cathepsins B and D (CTSB, CTSD), which have been shown to degrade α-synuclein (Table 1). All enzymes degrade different lysosomal substrates comprised of various lipids (glycolipids, sphingomyelin, sphingosine) and proteins. Additionally, lysosomal membrane proteins have been added to the list of Parkinson's disease risk genes, including the lysosomal integral membrane protein type-2 (*LIMP-2*), which is the lysosomal transport receptor of GCase, the transmembrane protein 175 (*TMEM175*) and the lysosome-associated membrane protein 3 (*LAMP3*). Furthermore, lysosomal transporter and channel proteins have been identified as risk genes and comprise cation-transporting ATPase 13A2 (*ATP13A2*), the proton pump ATPase H+ transporting V0 subunit A1 (*ATP6V0A1*), which is important for the regulation of lysosomal pH, the sialic acid exporter Sialin (*SLC17A5*), and the cholesterol transporter Niemann-Pick C protein (*NPC1*) (Table 1). Moreover, it is no surprise that two vacuolar protein sorting-associated proteins (VPS35/13C), that play an important role in vesicle-mediated protein trafficking, as well as two ras-related proteins (Rab7L1/39) crucial for vesicle fusion, are found on the list of lysosome-associated risk factors for Parkinson's disease.

Of the lysosomal risk genes, *GBA1* (GCase) has the highest prevalence and thus has been most intensively studied to date. The general mechanistic understanding is that slowed lysosomal digestion of GCase-substrates, including the glycolipid glucosylceramide, interferes with α-synuclein and accelerates its accumulation (Mazzulli et al., 2011; Zunke et al., 2018) (Figure 2). Data from genome studies indicate that mutations in *GBA1* are linked to a ~20-fold increase in the risk of developing Parkinson's disease (Beavan and Schapira, 2013). Additionally, genetic studies show that 5–10 % of Parkinson's disease patients carry mutations in *GBA1* (Lesage et al., 2011; McNeill et al., 2012; Sidransky et al., 2009).

For most other identified genetic risk factors shown in Table 1 there is still a lack of knowledge about their contribution to molecular disease mechanisms and disease progression. Future studies, for instance in induced-pluripotent stem cell (iPSC) derived patient neurons (Zunke and Mazzulli, 2019), will shed light on the impact of some of these pathology-associated genes. This will help further our understanding of Parkinson's disease pathology and give rise to novel therapeutic approaches.

Tab. 1: Lysosomal genes associated with Parkinson's disease.

Gene name	Protein name	Function	Reference
Lysosomal enzymes			
GBA1	β-Glucocerebrosidase (GCase)	Degradation of lysosomal sphingolipids, mainly glucosylceramide	(Chang et al., 2017; Nalls et al., 2014; Robak et al., 2017; Sidransky et al., 2009)
GALC	Galactocerebrosidase	Degradation of lysosomal sphingolipids, mainly galactosylceramide	(Chang et al., 2017)
SMPD1	Sphingomyelin phosphodiesterase 1	Degradation of sphingomyelin	(Gan-Or et al., 2015; Gan-Or et al., 2013; Robak et al., 2017)
ASAH1	N-Acylsphingosine Amidohydrolase 1	Degradation of ceramide into sphingosine and free fatty acid	(Robak et al., 2017)
CTSB	Cathepsin B	Cysteine protease, involved in degradation of various substrates including α-synuclein	(Chang et al., 2017)
CTSD	Cathepsin D	Aspartyl protease, involved in degradation of various substrates including α-synuclein	(Robak et al., 2017)
NAGLU	N-Acetylglucosaminidase	Hydrolysis of terminal non-reducing N-acetyl-D-glucosamine	(Winder-Rhodes et al., 2012)
Lysosomal membrane proteins			
TMEM175	Transmembrane protein 175	Potassium channel	(Chang et al., 2017; Nalls et al., 2014)
ATP13A2	Cation-transporting ATPase 13A2	Cation transporter	(Ramirez et al., 2006)
ATP6V0A1	ATPase H^+ transporting V0 subunit A1	Component of the V-ATPase, acidification of lysosomes	(Chang et al., 2017)
LAMP3	Lysosome-associated membrane protein 3	Type I transmembrane glycoprotein, lysosomal glycocalyx	(Pihlstrom et al., 2013; Simon-Sanchez et al., 2009)
SCARB2	Lysosomal integral membrane protein type-2 (LIMP2)	Type III transmembrane glycoprotein, lysosomal glycocalyx, lysosomal transport of GCase	(Hopfner et al., 2011)
SLC17A5	Sialin	H^+-coupled sialic acid exporter	(Robak et al., 2017)
NPC1	Niemann-Pick C1 protein	Lysosomal cholesterol transporter	(Kluenemann et al., 2013)
Proteins involved in lysosomal trafficking/autophagy			
VPS35	Vacuolar protein sorting-associated protein 35	Trafficking machinery, retromer complex component	(Vilarino-Guell et al., 2011; Zimprich et al., 2011)
VPS13C	Vacuolar protein sorting-associated protein 13C	Trafficking machinery, mitophagy, mitochondrial depolarization	(Lesage et al., 2015)
Rab7L1	Ras-related protein Rab-7L1	Retrograde trafficking pathway for recycling proteins	(MacLeod et al., 2013; Nalls et al., 2014)
Rab39B	Ras-related protein Rab-39B	Trafficking machinery, autophagy	(Lesage et al., 2015; Mata et al., 2015)
GAK	Cyclin G-associated kinase	Involved in the uncoating of clathrin-coated vesicles	(Dumitriu et al., 2011)
KAT8	Lysine acetyltransferase 8	Modulator of autophagic flux	(Chang et al., 2017)

Therapeutic approaches targeting lysosomes

As highlighted above, dysfunction in lysosomal pathways not only causes LSDs, but also plays a central role in neurodegenerative disorders, like Parkinson's disease. A number of treatment strategies aiming to boost lysosomal function have been identified or are under development for LSDs, and may also provide novel opportunities for treating Parkinson's disease. Considering the importance of cellular degradation in Parkinson's disease etiology, strategies to enhance lysosomal function involve: (a) enzyme replacement to substitute dysfunctional enzymes; (b) chaperones to stabilize unstable or misfolded enzymes in order to increase their lysosomal activity; (c) small molecules that act as direct allosteric activators on lysosomal enzymes; (d) substrate reduction therapies in order to decrease lysosomal load; (e) cell therapies to replace injured cells; (f) gene therapy (Klein and Futerman, 2013).

The most common treatment strategy for certain LSDs is the administration of functional recombinant enzymes by repetitive infusions (enzyme replacement therapy) (Beck, 2018). Although this treatment has been shown to be effective in patients, enzyme replacement therapy has several disadvantages. First of all, the production of enzymes is very expensive and, secondly, recombinant enzymes are usually large proteins and thus difficult to transport across the blood-brain barrier. Hence, most enzyme replacement therapies are able to improve non-neuronopathic, but not neurological pathologies. Compared to enzyme replacement therapy, small activators of lysosomal enzymes have the potential to cross the blood-brain barrier and thus are able to treat neurological symptoms. Various small activators of lysosomal enzymes, including chaperones for GCase, are under development or already approved as drugs for the treatment of LSDs (Beck, 2018).

Interestingly, some of the compounds applied for LSDs are now tested for their effectiveness in neurodegenerative disorders, including Parkinson's disease. Recent studies utilizing small activators/chaperones of the lysosomal enzyme GCase have been shown to improve Parkinson's disease pathology by reducing α-synuclein aggregation in iPSC-derived neurons of Parkinson's patients (Mazzulli et al., 2016), as well as in a transgenic Parkinson's disease mouse model (Migdalska-Richards et al., 2016). The effect of GCase activators is due to increased enzymatic turnover and a reduction in glucosylceramide, which has been shown to interfere with α-synuclein, resulting in neurotoxic oligomeric conformers (Zunke et al., 2018) (Figure 2: conversion from physiological (green) to pathological (orange) oligomer). Hence, a reduction in the glycolipid decreases the amount of toxic α-synuclein forms and thus α-synuclein-induced pathology (Figure 2: reversal of toxic form (orange) to physiological form (green)).

A similar reduction in α-synuclein aggregation could be observed after substrate reduction therapy. A small blood-brain-barrier-permeable molecule reducing the synthesis of the GCase-substrate glucosylceramide has been shown to reduce α-synuclein levels in Parkinson's iPSC neurons (Zunke et al., 2018), as well as to improve cognitive symptoms in Parkinson's disease mice (Sardi et al., 2017). Although substrate reduction is already in clinical use for some LSDs (e. g. Gaucher disease), this treatment option is still under investigation in clinical phases for Parkinson's disease patients.

Likewise, therapies aiming to lower levels of other lysosomal molecules that might interfere with protein aggregation have shown to be beneficial for neurodegenerative disorders. Accordingly, reducing cholesterol levels in a mouse model of Parkinson's disease also revealed a decrease in α-synuclein aggregation (Bar-On et al., 2006).

In summary, targeting lysosomal pathways in Parkinson's disease pathology provides new possibilities to treat this progressive neurological disorder, for which no curative treatment strategy currently exists. The molecular and genetic evidence reviewed here will help us to further understand cellular disease mechanisms and direct future drug development.

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References

Baba, M., Nakajo, S., Tu, P.H., Tomita, T., Nakaya, K., Lee, V.M., Trojanowski, J.Q., and Iwatsubo, T. (1998). Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am. J. Pathol. *152*, 879–884.

Bainton, D.F. (1981). The discovery of lysosomes. J. Cell Biol. *91*, 66s–76 s.

Bar-On, P., Rockenstein, E., Adame, A., Ho, G., Hashimoto, M., and Masliah, E. (2006). Effects of the cholesterol-lowering compound methyl-β-cyclodextrin in models of α-synucleinopathy. J. Neurochem. *98*, 1032–1045.

Beavan, M.S., and Schapira, A.H. (2013). Glucocerebrosidase mutations and the pathogenesis of Parkinson disease. Ann. Med. *45*, 511–521.

Beck, M. (2018). Treatment strategies for lysosomal storage disorders. Dev. Med. Child Neurol. *60*, 13–18.

Bennett, M.J., and Rakheja, D. (2013). The neuronal ceroid-lipofuscinoses. Dev. Disabil. Res. Rev. *17*, 254–259.

Blanz, J., Zunke, F., Markmann, S., Damme, M., Braulke, T., Saftig, P., and Schwake, M. (2015). Mannose 6-phosphate-independent Lysosomal Sorting of LIMP-2. Traffic *16*, 1127–1136.

Bosco, D.A., Fowler, D.M., Zhang, Q., Nieva, J., Powers, E.T., Wentworth, P., Jr., Lerner, R.A., and Kelly, J.W. (2006). Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate α-synuclein fibrilization. Nat. Chem. Biol. *2*, 249–253.

Braak, H., and Del Tredici, K. (2017). Neuropathological staging of brain pathology in sporadic Parkinson's disease: separating the wheat from the chaff. J. Parkinsons Dis. *7*, S71-S85.

Chang, D., Nalls, M.A., Hallgrimsdottir, I.B., Hunkapiller, J., van der Brug, M., Cai, F., International Parkinson's Disease Genomics Consortium, 23andMe Research Team, et al. (2017). A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat. Genet. *49*, 1511–1516.

Chaudhuri, K.R., Healy, D.G., Schapira, A.H., and National Institute for Clinical Excellence (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol. *5*, 235–245.

Cuervo, A.M., Stefanis, L., Fredenburg, R., Lansbury, P.T., and Sulzer, D. (2004). Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science *305*, 1292–1295.

Cullen, V., Lindfors, M., Ng, J., Paetau, A., Swinton, E., Kolodziej, P., Boston, H., Saftig, P., et al. (2009). Cathepsin D expression level affects α-synuclein processing, aggregation, and toxicity *in vivo*. Mol. Brain *2*, 5.

Davie, C.A. (2008). A review of Parkinson's disease. Br. Med. Bull. *86*, 109–127.

De Duve, C., and Beaufay, H. (1959). Tissue fractionation studies. 10. Influence of ischaemia on the state of some bound enzymes in rat liver. Biochem. J. *73*, 610–616.

De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J. *60*, 604–617.

Dehay, B., Bove, J., Rodriguez-Muela, N., Perier, C., Recasens, A., Boya, P., and Vila, M. (2010). Pathogenic lysosomal depletion in Parkinson's disease. J. Neurosci. *30*, 12535–12544.

Eskelinen, E.L., Tanaka, Y., and Saftig, P. (2003). At the acidic edge: emerging functions for lysosomal membrane proteins. Trends Cell Biol. *13*, 137–145.

Fraldi, A., Klein, A.D., Medina, D.L., and Settembre, C. (2016). Brain disorders due to lysosomal dysfunction. Annu. Rev. Neurosci. *39*, 277–295.

George, J.M. (2002). The synucleins. Genome Biol. *3*, REVIEWS3002.

Giasson, B.I., Uryu, K., Trojanowski, J.Q., and Lee, V.M. (1999). Mutant and wild type human α-synucleins assemble into elongated filaments with distinct morphologies *in vitro*. J. Biol. Chem. *274*, 7619–7622.

Gieselmann, V. (1995). Lysosomal storage diseases. Biochim. Biophys. Acta *1270*, 103–136.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature *441*, 885–889.

Infante, R.E., Radhakrishnan, A., Abi-Mosleh, L., Kinch, L.N., Wang, M.L., Grishin, N.V., Goldstein, J.L., and Brown, M.S. (2008). Purified NPC1 protein: II. Localization of sterol binding to a 240-amino acid soluble luminal loop. J. Biol. Chem. *283*, 1064–1075.

Janvier, K., and Bonifacino, J.S. (2005). Role of the endocytic machinery in the sorting of lysosome-associated membrane proteins. Mol. Biol. Cell *16*, 4231–4242.

Kalia, L.V., and Lang, A.E. (2015). Parkinson's disease. Lancet *386*, 896–912.

Klein, A.D., and Futerman, A.H. (2013). Lysosomal storage disorders: old diseases, present and future challenges. Pediatr. Endocrinol. Rev. *11* (Suppl. 1), 59–63.

Klein, A.D., and Mazzulli, J.R. (2018). Is Parkinson's disease a lysosomal disorder? Brain *141*, 2255–2262.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature *441*, 880–884.

Kornfeld, S. (1992). Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. Annu. Rev. Biochem. *61*, 307–330.

Lashuel, H.A., and Lansbury, P.T., Jr. (2006). Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? Q. Rev. Biophys. *39*, 167–201.

Lashuel, H.A., Petre, B.M., Wall, J., Simon, M., Nowak, R.J., Walz, T., and Lansbury, P.T., Jr. (2002). Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J. Mol. Biol. *322*, 1089–1102.

Lesage, S., Anheim, M., Condroyer, C., Pollak, P., Durif, F., Dupuits, C., Viallet, F., Lohmann, et al. (2011). Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. Hum. Mol. Genet. *20*, 202–210.

Mazzulli, J.R., Xu, Y.H., Sun, Y., Knight, A.L., McLean, P.J., Caldwell, G.A., Sidransky, E., Grabowski, G.A., et al. (2011). Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell *146*, 37–52.

Mazzulli, J.R., Zunke, F., Tsunemi, T., Toker, N.J., Jeon, S., Burbulla, L.F., Patnaik, S., Sidransky, et al. (2016). Activation of β-Glucocerebrosidase Reduces Pathological α-Synuclein and Restores Lysosomal Function in Parkinson's Patient Midbrain Neurons. J. Neurosci. *36*, 7693–7706.

McGlinchey, R.P., and Lee, J.C. (2015). Cysteine cathepsins are essential in lysosomal degradation of α-synuclein. Proc. Natl. Acad. Sci. U.S.A. *112*, 9322–9327.

McNeill, A., Duran, R., Hughes, D.A., Mehta, A., and Schapira, A.H. (2012). A clinical and family history study of Parkinson's disease in heterozygous glucocerebrosidase mutation carriers. J. Neurol. Neurosurg. Psychiatry *83*, 853–854.

Migdalska-Richards, A., Daly, L., Bezard, E., and Schapira, A.H. (2016). Ambroxol effects in glucocerebrosidase and α-synuclein transgenic mice. Ann. Neurol. *80*, 766–775.

Ohkuma, S., Moriyama, Y., and Takano, T. (1982). Identification and characterization of a proton pump on lysosomes by fluorescein-isothiocyanate-dextran fluorescence. Proc. Natl. Acad. Sci. USA *79*, 2758–2762.

Pastores, G.M. (1997). Gaucher's Disease. Pathological features. Baillieres Clin. Haematol. *10*, 739–749.

Pitcairn, C., Wani, W.Y., and Mazzulli, J.R. (2019). Dysregulation of the autophagic-lysosomal pathway in Gaucher and Parkinson's disease. Neurobiol. Dis. *122*, 72–82.

Platt, F.M., d'Azzo, A., Davidson, B.L., Neufeld, E.F., and Tifft, C.J. (2018). Lysosomal storage diseases. Nat. Rev. Dis. Primers *4*, 27.

Reczek, D., Schwake, M., Schroder, J., Hughes, H., Blanz, J., Jin, X., Brondyk, W., Van, P.S., et al. (2007). LIMP-2 is a receptor for lysosomal mannose-6-phosphate-independent targeting of beta-glucocerebrosidase. Cell *131*, 770–783.

Riederer, P., Berg, D., Casadei, N., Cheng, F., Classen, J., Dresel, C., Jost, W., Kruger, R., et al. (2019). α-Synuclein in Parkinson's disease: causal or bystander? J. Neural Transm. (Vienna) *126*, 815–840.

Robak, L.A., Jansen, I. E., van Rooij, J., Uitterlinden, A.G., Kraaij, R., Jankovic, J., International Parkinson's Disease Genomics Consortium, Heutink, P., et al. (2017). Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain *140*, 3191–3203.

Sardi, S.P., Viel, C., Clarke, J., Treleaven, C.M., Richards, A.M., Park, H., Olszewski, M.A., Dodge, J.C., et al. (2017). Glucosylceramide synthase inhibition alleviates aberrations in synucleinopathy models. Proc. Natl. Acad. Sci. U.S.A. *114*, 2699–2704.

Schulze, H., and Sandhoff, K. (2011). Lysosomal lipid storage diseases. Cold Spring Harb. Perspect. Biol. *3*.

Schwake, M., Schroder, B., and Saftig, P. (2013). Lysosomal membrane proteins and their central role in physiology. Traffic *14*, 739–748.

Settembre, C., Fraldi, A., Medina, D.L., and Ballabio, A. (2013). Signals from the lysosome: a control centre for cellular clearance and energy metabolism. Nat. Rev. Mol. Cell Biol. *14*, 283–296.

Shachar, T., Lo Bianco, C., Recchia, A., Wiessner, C., Raas-Rothschild, A., and Futerman, A.H. (2011). Lysosomal storage disorders and Parkinson's disease: Gaucher disease and beyond. Mov. Disord. *26*, 1593–1604.

Sidransky, E., Nalls, M.A., Aasly, J.O., Aharon-Peretz, J., Annesi, G., Barbosa, E.R., Bar-Shira, A., Berg, D., et al. (2009). Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N. Engl. J. Med. *361*, 1651–1661.

Sulzer, D., and Edwards, R.H. (2019). The physiological role of α-synuclein and its relationship to Parkinson's Disease. J. Neurochem. *150*, 475–486.

Suzuki, M., Fujikake, N., Takeuchi, T., Kohyama-Koganeya, A., Nakajima, K., Hirabayashi, Y., Wada, K., and Nagai, Y. (2015). Glucocerebrosidase deficiency accelerates the accumulation of proteinase K-resistant α-synuclein and aggravates neurodegeneration in a Drosophila model of Parkinson's disease. Hum. Mol. Genet. *24*, 6675–6686.

Taguchi, Y.V., Liu, J., Ruan, J., Pacheco, J., Zhang, X., Abbasi, J., Keutzer, J., Mistry, P.K., et al. (2017). Glucosylsphingosine promotes α-synuclein pathology in mutant GBA-associated Parkinson's disease. J. Neurosci. *37*, 9617–9631.

Xia, Q., Liao, L., Cheng, D., Duong, D.M., Gearing, M., Lah, J.J., Levey, A.I., and Peng, J. (2008). Proteomic identification of novel proteins associated with Lewy bodies. Front. Biosci. *13*, 3850–3856.

Zunke, F., Andresen, L., Wesseler, S., Groth, J., Arnold, P., Rothaug, M., Mazzulli, J.R., Krainc, D., et al. (2016). Characterization of the complex formed by beta-glucocerebrosidase and the lysosomal integral membrane protein type-2. Proc. Natl. Acad. Sci. USA *113*, 3791–3796.

Zunke, F., and Mazzulli, J.R. (2019). Modeling neuronopathic storage diseases with patient-derived culture systems. Neurobiol. Dis. *127*, 147–162.

Zunke, F., Moise, A.C., Belur, N.R., Gelyana, E., Stojkovska, I., Dzaferbegovic, H., Toker, N.J., Jeon, S., et al. (2018). Reversible conformational conversion of α-synuclein into toxic assemblies by glucosylceramide. Neuron *97*, 92–107 e110.

Bionote

Friederike Zunke
Christian-Albrechts-Universität zu Kiel,
Fachbereich Biochemie
Rudolf-Höber Strasse 1, 24118 Kiel, Germany
e-mail: fzunke@biochem.uni-kiel.de
orcid id: 0000-0002-0408-6388

Friederike Zunke studied Biochemistry (B.Sc.) and Biomedical Research (M.Res.) at the University of Kiel and St. George's University of London, respectively. She obtained her PhD in Biochemistry in Prof. Paul Saftig's group at the University of Kiel in 2015, and moved to Chicago to perform postdoctoral studies in Prof. Joseph Mazzulli's laboratory. Since the end of 2016 she is independent junior group leader at the University of Kiel working on the molecular role of lysosomal function and protein aggregation pathways in Parkinson's disease.

Rezension

Konrad Lehmann: Das schöpferische Gehirn
Auf der Suche nach der Kreativität – eine Fahndung in sieben Tagen

besprochen von **Anja Hoffmann**, Bayer AG, Translational Medicine, Muellerstrasse 178, 13342 Berlin, Germany

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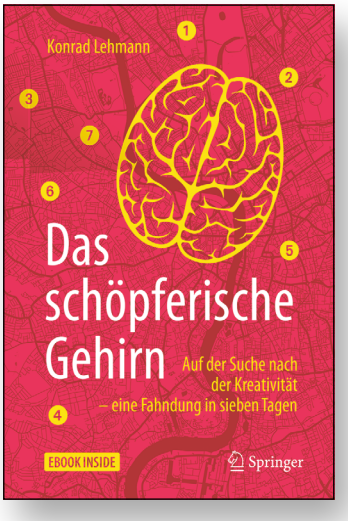

Das leere Blatt, das sich partout nicht füllen will. Eine wissenschaftliche Fragestellung, über die man lange nachgrübelt. Eine Alltagssituation, für die man eine pfiffige Lösung braucht. Wer kennt sie nicht: die Suche nach dem Geistesblitz, der Eingebung, durch die sich ein Problem in Luft auflöst. Oder aber die Freude an der neuen Gestaltung des Gartens, das Basteln in Keller und Garage, die Idee, die alten Gegenständen einen neuen Sinn gibt. Die verschiedenen Facetten der Kreativität sind uns allen gut bekannt. Und jeder von uns hat sich schon gewünscht, dass uns diese Geistesgabe auf Abruf zur Verfügung stehen möge. Tut sie häufig aber leider nicht. Warum ist das so?

In „Das schöpferische Gehirn – Auf der Suche nach der Kreativität – eine Fahndung in sieben Tagen" begibt sich Konrad Lehmann auf Spurensuche. Eingebettet in eine kleine Kriminalgeschichte mit italienischen Anklängen nähert sich der Autor dem Thema von sechs unterschiedlichen Seiten, um diese im siebten Kapitel zusammenzufügen. Da geht es zunächst um die Frage, wie sich Kreativität definiert, ob es verschiedene Formen gibt und wie sie gemessen werden kann. In „eine(r) kurze(n) Führung durch das Gehirn" wird der Schauplatz umrissen. Um die kreative Persönlichkeit zu erfassen, werden die Grundlagen der Persönlichkeitspsychologie und der Neurochemie betrachtet, Intelligenz und psychiatrische Erkrankungen werden als Vergleich herangezogen und Studien zum Zusammenhang von „Genie und Wahn" vorgestellt. Als notwendige Grundlage wird der Schöpfungsdrang oder Gestaltungswillen als intrinsische Motivation beschrieben. Lehmann erläutert die Funktionsweise des „neuronale(n) Motor(s)", den Zusammenhang zwischen Kreativität und Stimmung sowie die Verbindung zum Flow. Er schildert Untersuchungen zu Ideenreichtum, Improvisation und Erkenntnis und beschreibt die dabei aktiven Netzwerke und ihre Eigenschaften. Des Weiteren erklärt er, was „das Gehirn (tut), wenn es nichts tut", was für einen Einfluss das Nichtstun, Schlaf und Drogen auf das Entstehen von Ideen haben und welche Rolle das „Default Mode Network" spielt. Im 7. Kapitel, ergo am 7. Tag (die Schöpfungsgeschichte lässt grüßen), ergibt sich aus diesen Bausteinen das Gesamtbild, in dem die verschiedenen Elemente der Kreativität sichtbar werden: „Kreativität, so scheint es, ist nicht monolithisch, sondern ein Gebäude aus raffiniert ineinander verschränkten Steinen". Am Ende hat man als Leser zum einen die unterschiedlichen Grundlagen für Kreativität kennengelernt: Es erschließt sich, dass unterschiedliche Denkprozesse wie konvergentes und divergentes Denken zu unterschiedlichen Zeitpunkten in einem kreativen Prozess notwendig sind, dass ausgeprägtes Wissen eine notwendige Basis für die Generierung von neuen Ideen darstellt, dass aber ebenso das Aufbrechen von eingespielten Denkmustern ein unverzichtbares Element bildet. Zum anderen werden für den Leser auch die entsprechenden neurobiologischen Prozesse ersichtlich, die in verschiedenen Hirnarealen ablaufen und somit Kreativität als ein „Wechselspiel von Erzeugung und Auslese" ermöglichen.

Konrad Lehmann, Verhaltensforscher und Neurobiologe, derzeit tätig an der Friedrich-Schiller-Universität Jena, gelingt es auf unterhaltsame und verständliche Art, den Leser schrittweise an diese komplexe Materie heranzuführen. Der Sprachstil ist lebendig. Man merkt dem Autor seine Begeisterung für dieses Thema an. Die zahlreichen Verweise auf Beispiele aus der Kunst- und Kulturgeschichte illustrieren die Theorie auf abwechslungsreiche Weise. Die Episoden um Commissario Prefrontale und seinen Assistenten lockern den Text auf und fassen im Stil einer Tagesrevue am Ende eines jeden Kapitels die Hauptaspekte noch einmal zusammen. Eine kleine Auswahl von schlichten, schwarz-weißen Abbildungen erläutert wesentliche Informationen, und jedes Kapitel hat ein ausführliches Verzeichnis mit Quellenangaben.

Nun ist dieses Buch nicht das erste Buch im deutschen Sprachraum, das versucht, die noch relative junge Neurowissenschaft der Kreativität für eine breitere Leserschaft zu erschließen. Bereits 2014 und 2015 sind zwei sehr lesenswerte Bücher von Jonah Lehrer und Bas Kast zu diesem Thema erschienen (siehe unten). Lehmanns Herangehensweise unterscheidet sich – abgesehen von der Rahmenhandlung – dahingehend, dass er sich etwas stärker auf die neurobiologischen Grundlagen fokussiert. Das mag für einen Neueinsteiger in dieses Thema, der kein Neurowissenschaftler ist, vielleicht herausfordernder sein, dafür aber auch sehr lohnend, weil es einem die Augen für Zusammenhänge öffnet. Wer eine simple Gebrauchsanweisung mit Ratschlägen à la „Wie Sie die Kreativität Ihres Gehirnes steigern" sucht, liegt mit diesem Buch falsch. Wer aber ein tieferes Verständnis für den kreativen Prozess entwickeln will, der wird Freude an „Das schöpferische Gehirn" haben und über dieses Verständnis dann auch Schlussfolgerungen für den Umgang mit seinen eigenen kreativen Ressourcen ziehen können.

Konrad Lehmann
Das schöpferische Gehirn
Auf der Suche nach der Kreativität – eine Fahndung in sieben Tagen
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Zum Weiterlesen und -hören

Lehrer, Jonah (Verlag C.H.Beck oHG, München 2014, engl. Originalausgabe 2012): „ Imagine! Wie das kreative Gehirn funktioniert"
In zwei großen Teilen, „Allein" und „Gemeinsam" beschreibt Jonah Lehrer kreative Entwicklungen bei Einzelnen und in Gruppen anhand zahlreicher interessanter Fallbeispiele aus Forschung, Wirtschaft, Sport und Kultur. Er stellt dabei insbesondere den Einfluss eines Teams, des sozialen Umfelds und der Umgebung dar und zieht interessante historische Vergleiche.

Bas Kast (S. Fischer Verlag GmbH, Frankfurt am Main 2015): „Und plötzlich macht es KLICK!: Das Handwerk der Kreativität oder wie die guten Ideen in den Kopf kommen")
Bas Kast nähert sich der Thematik auf ähnliche Art und Weise. Auch hier bilden der persönliche Austausch mit Forschern und Fallgeschichten den Ausgangspunkt der Darstellung. Interessante Ergänzung sind die Beschreibungen von Untersuchungen, bei denen sich der Autor selber als Versuchsteilnehmer zur Verfügung gestellt hat und so aus erster Hand Erfahrungen beitragen kann.

F.A.Z. Hörbuch „Hirnforschung 7 – Das Geheimnis der Kreativität" (2015)
Auf der Doppel-CD werden Artikel aus der F.A.Z. und der Frankfurter Allgemeinen Sonntagszeitung aus den Jahren 2006 bis 2013 zusammengefasst. Der Schwerpunkt liegt auf Erörterungen zum Thema „Kunst und Neurowissenschaften". Es gibt z. B. einen Beitrag von Wolf Singer zu der Frage, was Kunst und Neurowissenschaften voneinander lernen können und Informationen zum Forschungsbereich der Neuroästhetik.

Obituary

Wolfgang J. Streit* and Manuel B. Graeber

Prof. Dr. med. Georg W. Kreutzberg

(2.9.1932 – 20.3.2019)

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Georg W. Kreutzberg was born on September 2, 1932 as the middle child of three siblings. His street-wise mother lovingly guided the family through the chaos of World War II. Through his father, a surgeon, he soon became familiar with the world of medicine, and decided at a young age that he was going to be a scientist one day. His scientific journey began. Due to frequent excursions to the nearby Rhine valley, young Georg developed an interest in Rhine stones and minerals that could be found there which gave rise to his early interest in chemistry, but he also used a microscope, which he shared with his one and a half-year older brother. The secondary school Georg Kreutzberg attended, the Ahrweiler Gymnasium, left a long-lasting impression on him because it provided a rich academic atmosphere. Quite exceptional at the time and also for German schools today, the majority of its teachers held a doctorate and some were still engaged in academic activities while teaching at the school.

Following his Abitur (final high school exam) in 1951, Georg Kreutzberg pursued his medical studies in Bonn and Freiburg im Breisgau (Germany) but also gave in to "Wanderlust" that led him to study at the Universities of Innsbruck and Vienna (Austria). In addition, he engaged in biochemical studies at the University of Bonn during his semester vacations. Georg Kreutzberg passed the State Examination in Medicine at the University of Freiburg in 1957. According to the medical doctor-training scheme at the time, this examination was followed by internships, which Georg Kreutzberg spent at clinics of the Universities of Bonn and Freiburg (1957–1959). In 1960, he obtained his general medical license. In 1961, the University of Freiburg awarded the Dr. med. degree to Georg Kreutzberg for a thesis entitled, "Studies on the metabolism of tryptophan in various diseases of the nervous system" which he had undertaken in their Psychiatry Department. Georg Kreutzberg had a long-standing interest in chemistry and developed a special interest in physiological chemistry (biochemistry), which was relatively new at the time. According to his own words, he was considered sort of a "mooncalf" by his peers because he pursued neurochemistry in a Psychiatry Department that was very much influenced by the philosophy of Martin Heidegger and whose patient records would even reflect his literary style. Heidegger still filled large lecture halls at the University of Freiburg, and Georg Kreutzberg attended Heidegger's lectures with great interest.

However, Georg Kreutzberg's fascination by the biological basis of brain diseases persisted and took him to Bonn where the first Chair of Neuropathology in Germany had been created for Professor Gerd Peters. Thus, starting in 1960, Georg Kreutzberg received 5 years of training in basic neuropathology under Gerd Peters, first at the Brain Research Institute and Department of Neuropathology at the University of Bonn (1960) and then as research assis-

***Corresponding author: Wolfgang J. Streit**, Department of Neuroscience, University of Florida College of Medicine, PO Box 100244, Gainesville, FL 32610, USA. e-mail: pschorr@ufl.edu
Manuel B. Graeber, Brain and Mind Centre, University of Sydney, Camperdown, NSW 2050, Australia, e-mail: manuel@graeber.net

tant in neuropathology (1961–1964) at the Max-Planck-Institute of Psychiatry (Deutsche Forschungsanstalt für Psychiatrie) in Munich where he had moved together with Peters.

A postdoctoral fellowship followed (1964–1965) in the newly established Department of Psychology at the Massachusetts Institute of Technology (MIT) in Cambridge, MA, USA, which was being set up by Hans-Lukas Teuber (1916–1977), a pre-World War II German emigrant. The great professionalism of "Luk" Teuber's operation, which featured regular departmental conferences that were attended by scientists such as David Hubel and Torsten Wiesel, was most inspiring for Georg Kreutzberg. The scientific purpose of his stay was to learn autoradiography on nervous tissue and life-long friendships with Joe Altman and Walle Nauta ensued. The techniques learned proved crucial for Georg Kreutzberg's later description of dendritic transport and axotomy-induced microglial proliferation.

Georg Kreutzberg returned from MIT to the Max-Planck-Institute of Psychiatry in Munich as a research associate (1965–1967) before serving as Guest Investigator at Rockefeller University in New York by invitation of Paul Weiss (1968), the discoverer of axonal transport. A key publication on blockage of intra- axonal enzyme transport by colchicine soon followed. Georg Kreutzberg was appointed Chief of Section for Experimental Neuropathology at the Max-Planck-Institute of Psychiatry, Munich, in 1969. It became the Department of Neuromorphology and was relocated in 1984 together with the Theoretical Institute of the Max-Planck-Institute of Psychiatry; the latter was renamed MPI of Neurobiology in 1998. With Georg Kreutzberg's retirement and the concurrent closure of his Department in 2000, the tradition of the famous Munich school of neuropathology ended in Germany.

In 1989, Georg Kreutzberg became Director of the Max Planck Institute of Psychiatry, as it was known then, in Martinsried, Germany, where he led both the Institute and the Department of Neuromorphology for eleven years. As Director, his main concern was the well-being of his co-workers and employees. He took a genuine interest not only in their work but also in their lives and quickly became a role model for many. His approach towards his employees was not bossy or top-down, but rather interactive, engaging, and nurturing, and this was particularly beneficial for the many younger scientists who were just starting their careers under his tutelage. His motto was "We need courage, luck, and patience", which he had chalked on the laboratory door of Dietmute Bühringer, one of the superbly skilful technicians working in the department (Graeber et al., 2012). He took pride in the fact that many of his coworkers went on to leading positions in research and clinical practice. While Georg was an excellent teacher and respected leader with unquestionable integrity, he was also a "regular guy". Not infrequently, he joined his postdocs, students, and others for a quick beer after work, which usually amounted to an hour or two of pub time, where he enjoyed good conversation over a few beers and a tasty Bavarian snack. He was not the kind of person to engage solely in shoptalk, but in fact commonly digressed into all sorts of other subjects, in particular, historical matters of various kinds. The study of the history of neuroscience became one of his favorite past times in his retirement.

Georg Kreutzberg will be missed by many of his trainees, including the authors of this orbituary, who spent some of their most productive years as postdocs in his department. The experience of working with "Georgie" (as they sometimes referred to him amongst themselves) shaped their scientific careers and quite possibly their lives. He was a powerful influence and role model because of who he was and how he conducted himself. May he rest in peace.

References

M.B. Graeber, R.B. Banati, A. Flugel, W.J. Streit, W. Tetzlaff, Courage, luck and patience: in celebration of the 80th birthday of Georg W. Kreutzberg, Acta neuropathologica, 124 (2012) 593–598.

Nachrichten aus der Gesellschaft

https://doi.org/10.1515/nf-2019-0036

EU-Projektförderung 2020 Neurowissenschaften

Die European Research Area Networks (ERA-NETs) und das EU Joint Programme Neurodegenerative Disease Research (JPND) laden Projektvorschläge mit Einreichungsfrist im Frühjahr 2020 ein. Beide Programme sind EU finanziert. Für folgende Themenbereiche können Anträge gestellt werden.

1. Sensorische Störungen
Förderung von multinationalen und translationalen Projekten zur Erforschung sensorischer Störungen
Budget: ca. 11 Mio Euro
Einreichungsfrist: 8. Januar – 10. März 2020
https://www.neuron-eranet.eu/en/921.php

2. Ethische, rechtliche und soziale Aspekte der Neurowissenchaften (ELSA)
Förderung von multinationalen und translationalen Projekten, die die ethischen, rechtlichen und sozialen Aspekte der Neurowissenchaften untersuchen
Budget: ca. 3 Mio Euro
Einreichungsfrist: 8. Januar – 28. April 2020
https://www.neuron-eranet.eu/en/918.php

3. Entwicklung neuer Technologien im Bereich Neurodegenerative Erkrankungen
Förderung von multinationalen Projekten zum Thema „Novel imaging and brain stimulation methods and technologies related to neurodegenerative diseases“ durch das EU Joint Programme Neurodegenerative Disease Reserch (JPND)
Einreichungsfrist: Januar – März 2020
https://www.neurodegenerationresearch.eu/2019/12/pre-call-announcement-novel-imaging-and-brain-stimulation-methods-and-technologies-related-to-neurodegenerative-diseases/

Ergebnis der DFG-Fachkollegienwahl 2019

Im Herbst 2019 führte die DFG die Fachkollegienwahl für die Amtsperiode 2020 – 2023 durch. Die Neurowissenschaftliche Gesellschaft war in 12 Fächern vorschlagsberechtigt, nämlich im Fach Neurowissenschaften (206-01 bis 206-11) und im Fach Grundlagen der Biologie und Medizin (201-07).

In diesen Fächern kandidierten insgesamt 106 Personen, davon hatte die NWG 74 Personen vorgeschlagen, meistens in Abstimmung mit anderen vorschlagsberechtigten Gesellschaften oder Institutionen. Die DFG hatte aus diesen Vorschlägen 69 Personen auf die Kandidatenliste übernommen.

Ende November 2019 gab die DFG das vorläufige Wahlergebnis bekannt. In die 12 Fachkollegien, für die die NWG Vorschläge einreichen konnte, wurden insgesamt 37 Personen gewählt, darunter 19 NWG-Mitglieder mit einem Frauen-/Männeranteil von 42 % zu 58 %. Die NWG-Mitglieder konzentrieren sich vor allem in den grundlagenwissenschaftlich orientierten Fachkollegien 206-01 bis 206-4, d. h. Entwicklungsneurobiologie, Molekulare Biologie und Physiologie von Nerven- und Gliazellen, Experimentelle und Theoretische Netzwerk-Neurowissenschaften sowie Kognitive, Systemische und Verhaltensneurobiologie. In diesen vier Fachkollegien sind alle neu gewählten Personen NWG-Mitglieder. In den klinisch orientierten Humanneurowissenschaften hingegen sind keine NWG-Mitglieder vertreten. Weitere 11 Personen, die die NWG zwar unterstützend vorgeschlagen hatte, die aber keine NWG-Mitglieder sind, wurden ebenfalls gewählt.

Außerdem wurden fünf NWG-Mitglieder in Fachkollegien gewählt, in denen die NWG nicht vorschlagsberechtigt war. Damit sind in allen Fachkollegien insgesamt 24

NWG-Mitglieder vertreten, zwei weniger als in der Amtsperiode 2015–2019.

Wir gratulieren den gewählten NWG-Mitgliedern und danken allen Kandidaten für Ihre Mitarbeit.

Fachkollegium (Zahl in Klammern: Anzahl aller neu gewählten Personen; * in diesen Fachkollegien war die NWG nicht vorschlagsberechtigt)	**Gewählte NWG-Mitglieder**
110-02 **Biologische Psychologie und Kognitive Neurowissenschaften***	Christian **Thiel** (Oldenburg)
201-03 **Zellbiologie***	Paul **Saftig** (Kiel)
203-05 **Biochemie und Physiologie der Tiere***	Thomas **Roeder** (Kiel)
205-29 **Hals-Nasen-Ohrenheilkunde***	Tobias **Moser** (Göttingen)
205-33 **Anatomie***	Ingo **Bechmann** (Leipzig)
206-01 **Entwicklungsneurobiologie** (2)	Amparo **Acker-Palmer** (Frankfurt)
	Michael **Wegner** (Erlangen)
206-02 **Molekulare Biologie und Physiologie von Nerven- und Gliazellen** (4)	Tobias **Böckers** (Ulm)
	Daniela **Dietrich** (Magdeburg)
	Angelika **Lampert** (Aachen)
	Christine **Rose** (Düsseldorf)
206-03 **Experimentelle und theoretische Netzwerk-Neurowissenschaften** (4)	Christian **Alzheimer** (Erlangen)
	Andreas **Engel** (Hamburg)
	Eckhard **Friauf** (Kaiserslautern)
	Sonja **Grün** (Jülich)
206-04 **Kognitive, systemische und Verhaltensneurobiologie** (4)	Marlene **Bartos** (Freiburg)
	Jan **Benda** (Tübingen)
	Frank **Bremmer** (Marburg)
	Charlotte **Förster** (Würzburg)
206-05 **Experimentelle Modelle zum Verständnis von Erkrankungen des Nervensystem** (3)	Rüdiger **Köhling** (Rostock)
206-06 **Molekulare und zelluläre Neurologie und Neuropathologie** (3)	Joachim **Weis** (Aachen)
	Guido **Reifenberger** (Düsseldorf)
206-07 **Klinische Neurologie, Neurochirurgie und Neuroradiologie** (4)	Otto Wilhelm **Witte** (Jena)
	Ghazaleh **Tabatabai** (Tübingen)

Fehlende Mitgliederadressen

Von folgenden Mitgliedern fehlen uns die korrekten Kontaktdaten:

Carus-Cadavieco Dr., Marta (bisher: Berlin)
Engelhard, Christina (bisher: Freiburg)
Grohmann Dr., Marcus (bisher: Victoria, Australien)
Hardt Dr., Martin (bisher: Giessen)
Heck, Sebastian (bisher: Mainz)
Ott Dr., Torben (bisher: Tübingen)
von Staden, Dr. Sabine (bisher: Konstanz)
Winkelmann, Aline (bisher: Berlin)

Für Hinweise an die entsprechenden Mitglieder bzw. uns sind wir dankbar.

Dank an die Neuroforum-Gutachter im Jahr 2019

Für ihr Engagement in den Reviewprozessen im Jahr 2019 möchten wir folgenden Kolleginnen und Kollegen herzlich danken:

Büschges, Ansgar
Doyle, Kristian P.
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Ludolph, Albert
Luhmann, Heiko
Mallot, Hanspeter
Manahan-Vaughan, Denise
Münte, Thomas
Rose, Christine
Rotter, Stefan
Schwarting, Rainer
Steinhäuser, Christian
Stengl, Monika
Thiel, Christiane
Vogel, Tanja
Wegener, Christian

Ergebnis der Umfrage zur Sektionszugehörigkeit

Die Einrichtung der neuen, zehnten Sektion „junge NWG“ (jNWG) brachte eine Revision der Sektionszugehörigkeit mit sich. Jedes Mitglied darf den eigenen Interessensgebieten entsprechend für maximal zwei Sektionen optieren, was bei der Beantragung der Mitgliedschaft geschieht. Durch die Gründung der neuen Sektion jNWG und auch angesichts dessen, dass sich das Interessenspektrum seit Eintritt in die NWG verändert haben könnte, sollte nun diese Zuordnung nun von jedem Mitglied neu getroffen werden.

Auf die Umfrage, die vom 17. November bis 16. Dezember 2019 lief, haben 182 Mitglieder reagiert, davon 103 Studenten und 79 Vollmitglieder. 48 haben sich für die jNWG entschieden, womit diese neue Sektion naturgemäß noch die kleinste ist. Es ist aber zu erwarten, dass in Zukunft durch Neueintritte von jungen Mitgliedern die neue Sektion weiter gestärkt wird.

Neueintritte

Folgende Kolleginnen und Kollegen dürfen wir als Mitglieder der Neurowissenschaftlichen Gesellschaft begrüßen:

Bahar Aksan (Heidelberg)
Afsaneh Asgari-Taei (Tehran, Iran)
Mihaela Anca Corbu (Köln)
Johanna Daubner (Bonn)
Zahra Fatahivanani (Tehran, Iran)
Daria Guseva, PD Dr. (Stuttgart)
Alexander Hodapp (Heidelberg)
Saereh Hosseindoost (Tehran, Iran)
Shole Jamali (Tehran, Iran)
Moritz Küchler (Greifswald)
Venissa Machado, Dr. (Göttingen)
Christa Maurer, Dr. (Heidelberg)
Fahimeh Parsaei (Tabriz, Iran)
Laura Plantera (Greifswald)
Safura Pournajaf (Tehran, Iran)
Viola Priesemann, Dr. (Göttingen)
Yasaman Razavi (Tehran, Iran)
Sahar Seifzadeh (Tabriz, Iran)
Zhou Wu (Bonn)

Der Mitgliedsstand zum 15. Dezember 2019 beträgt 2.282 Mitglieder.

Ausblick

Finja Grospietsch
Neuromyths as a Problem and Object of University Instruction

Sven Mueller
Neuroscience in Transgender People: An Update

Steffen Harzsch
Exploring crustacean brain diversity: sensory systems of black smoker shrimps

Lisa Marshall
Manipulating neural activity toward sleep-dependent memory consolidation

Manfred Radmacher
Atomic Force Microscopy for Cell Mechanics in Diseases

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NEUROWISSENSCHAFTLICHE GESELLSCHAFT
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- Beitrittserklärung -

Hiermit erkläre ich meinen Beitritt zur Neurowissenschaftlichen Gesellschaft e.V. (NWG).

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Datum/Unterschrift des Mitglieds

Datum/Unterschrift des Mitglieds

Bitte senden Sie Ihren Antrag an die Geschäftsstelle der NWG:

Stefanie Korthals
Neurowissenschaftliche Gesellschaft e.V.
MDC
Robert-Rössle-Str. 10
13092 Berlin

Email: korthals@mdc-berlin.de
Tel.: +49 30 9406 3127

Ich optiere für folgende 2 Sektionen:

- ☐ Computational Neuroscience
- ☐ Entwicklungsneurobiologie/Neurogenetik
- ☐ junge NWG (jNWG)
- ☐ Klinische Neurowisschenschaften
- ☐ Kognitive Neurowissenschaften
- ☐ Molekulare Neurobiologie
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie

Ich bin Student ☐ ja ☐ nein
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