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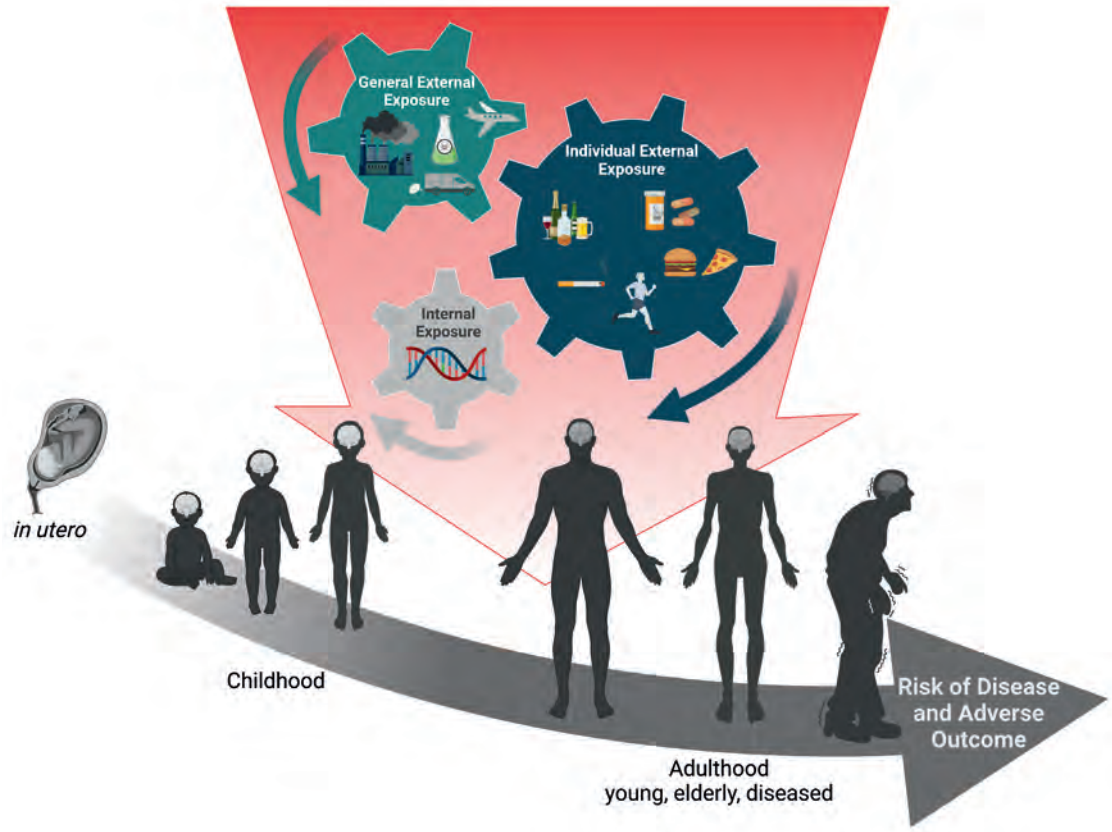

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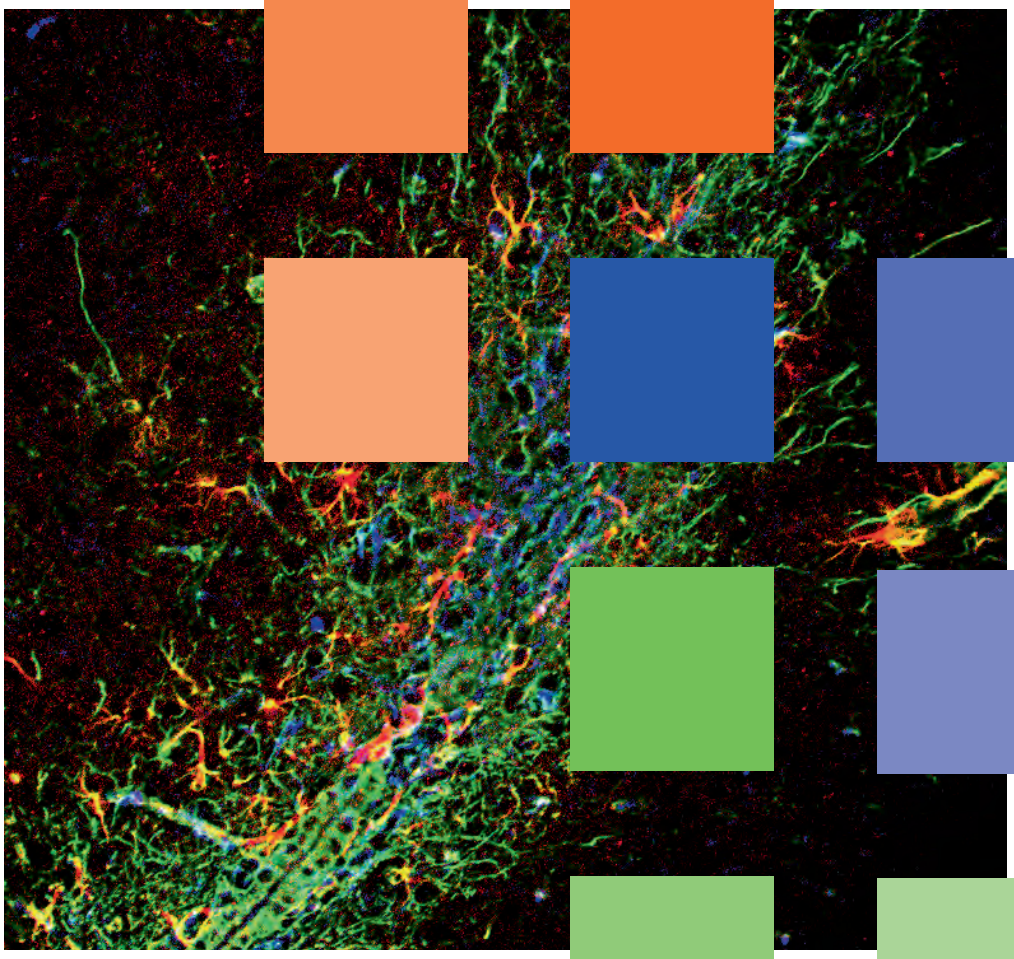

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COVER ILLUSTRATION Overview of different human real-life exposures impacting the central nervous system (CNS). Created with BioRender.com. Cover illustration provided by J. Tigges, T. Schikowski and E. Fritsche, Environmental exposures impact the nervous system in a life stage-specific manner, (nf-2021-0021, pp. 201–212 in this issue).

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Review article

Raquel Suárez-Grimalt and Davide Raccuglia*

The neural architecture of sleep regulation – insights from *Drosophila*

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Abstract: The neural mechanisms that balance waking and sleep to ensure adequate sleep quality in mammals are highly complex, often eluding functional insight. In the last two decades, researchers made impressive progress in studying the less complex brain of the invertebrate model organism *Drosophila melanogaster*, which has led to a deeper understanding of the neural principles of sleep regulation. Here, we will review these findings to illustrate that neural networks require sleep to undergo synaptic reorganization that allows for the incorporation of experiences made during the waking hours. Sleep need, therefore, can arise as a consequence of sensory processing, often signalized by neural networks as they synchronize their electrical patterns to generate slow-wave activity. The slow-wave activity provides the neurophysiological basis to establish a sensory gate that suppresses sensory processing to provide a resting phase which promotes synaptic rescaling and clearance of metabolites from the brain. Moreover, we demonstrate how neural networks for homeostatic and circadian sleep regulation interact to consolidate sleep into a specific daily period. We particularly highlight that the basic functions and physiological principles of sleep are highly conserved throughout the phylogenetic spectrum, allowing us to identify the functional components and neural interactions that construct the neural architecture of sleep regulation.

Keywords: *Drosophila*; neural oscillations; sensory gating; slow-wave sleep.

Zusammenfassung: Die neuronalen Mechanismen, die das Gleichgewicht zwischen Wachheit und Schlaf etablieren, um eine angemessene Schlafqualität in Säugetieren zu gewährleisten, sind hochkomplex und entziehen sich oft funktioneller Einsicht. Mit Hilfe des weniger komplexen Gehirns des wirbellosen Modellorganismus *Drosophila melanogaster* haben Forscher in den letzten zwei Jahrzehnten beeindruckende Fortschritte gemacht und ein tieferes Verständnis der neuronalen Prinzipien der Schlafregulation erlangt. In dieser Arbeit werden wir diese Erkenntnisse zusammenfassen, um zu veranschaulichen, dass neuronale Netzwerke Schlaf benötigen, um synaptische Verknüpfungen zu reorganisieren und somit die am Tag gemachten Erfahrungen zu verarbeiten. Schlafbedürfnis kann also als Folge sensorischer Verarbeitung entstehen, was in neuronalen Netzwerken häufig zur Synchronisierung elektrischer Muster führt und Slow-Wave-Aktivität erzeugt. Slow-Wave-Aktivität liefert die neurophysiologische Grundlage für die Etablierung eines sensorischen Tores, welches die sensorische Verarbeitung unterdrückt und somit eine Ruhephase schafft, die die Reorganisation synaptischer Verknüpfungen und den Abtransport von Stoffwechselprodukten aus dem Gehirn fördert. Darüber hinaus zeigen wir, wie neuronale Netzwerke für die homöostatische und zirkadiane Schlafregulation interagieren, um zu gewährleisten, dass ein Organismus immer innerhalb einer festen Periode schläft. In dieser Arbeit heben wir besonders hervor, dass die grundlegenden Funktionen und physiologischen Prinzipien des Schlafs über das gesamte phylogenetische Spektrum hinweg konserviert sind, was uns ermöglicht, die funktionellen Komponenten und neuronalen Interaktionen zu identifizieren, die die neuronale Architektur der Schlafregulation bilden.

Schlüsselwörter: Oszillationen; Slow-Wave Schlaf; *Drosophila*; sensory gating.

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Introduction

For billions of years, the Earth's rotation has been creating a daily biphasic light and dark cycle. Neural networks across the phylogenetic spectrum evolved to anticipate

active foraging and hunting in one phase while the other phase became specialized for sleeping and recuperative processes. The proper balance between waking and sleep is extremely important for our overall health and cognitive functions, as not being able to fall asleep and waking up in the middle of the night are hallmarks of poor sleep quality, which has been linked to excessive inflammatory processes, hypertension, diabetes, depression, and deficits in attention and learning (Alhola and Polo-Kantola, 2007; Mullington et al., 2021).

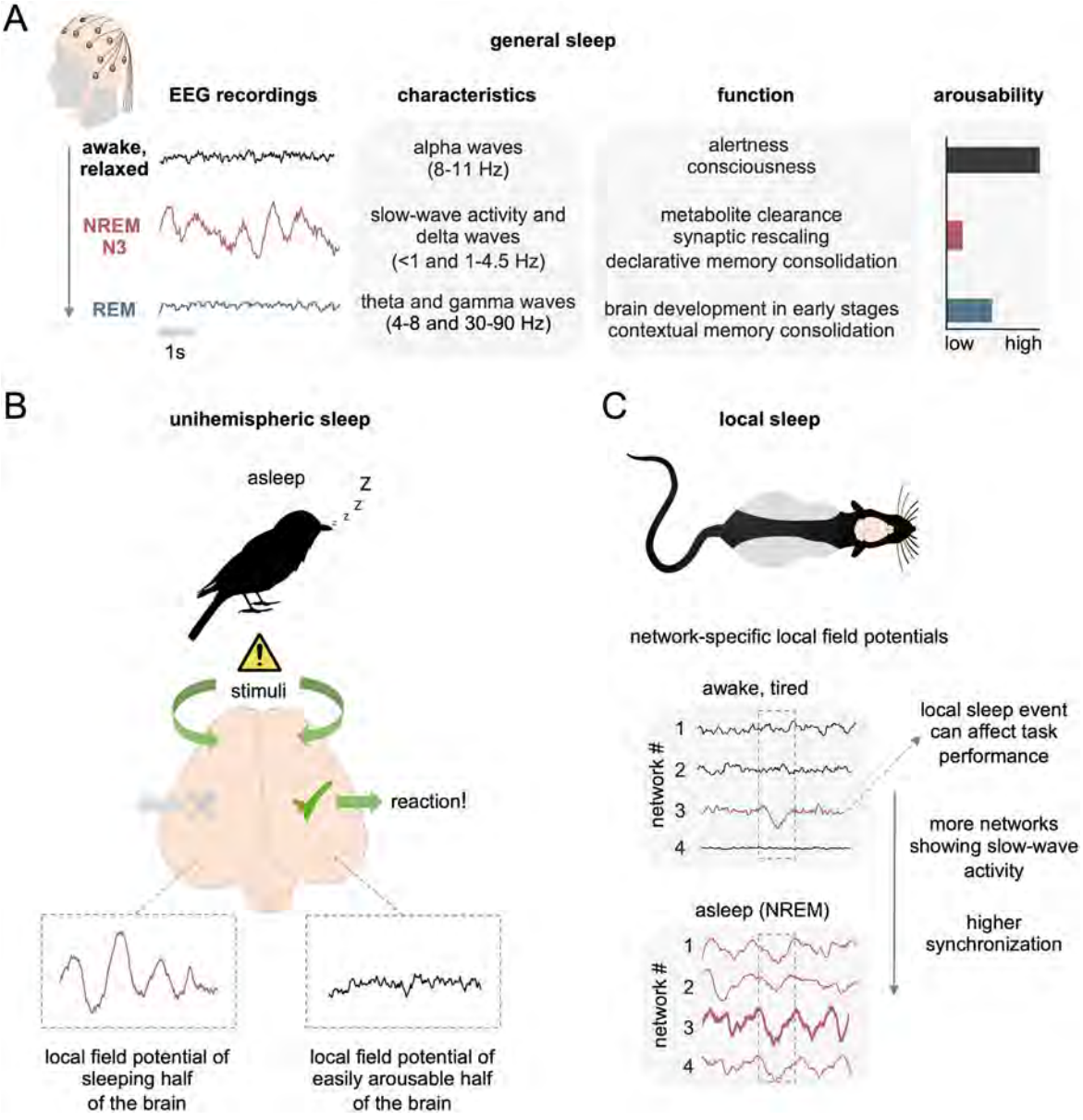

Figure 1: Oscillating circuitries during sleep and wake in vertebrates.
(A) Human sleep stages differ in their electrophysiological signatures, biological functions, and levels of reactivity to environmental stimuli. During NREM sleep (stage N3 in particular), slow-wave activity (SWA), resulting from large-scale synchronizations of cortical areas, reduces the functional connectivity within the brain, resulting in the low arousability characteristic for deep sleep. **(B)** In contrast to deep sleep in humans, some birds and marine mammals exhibit unihemispheric sleep, during which one half of the brain generates SWA while the other half remains more easily arousable, allowing the sleeping animal to maintain a certain level of alertness. **(C)** In sleep-deprived but awake rats, specific networks that were intensely engaged in a task can display sleep-like periods of synchronized inactivity, which reduces the excitability of this network, leading to task-specific impairments (network 3). During subsequent NREM sleep, the same network exhibits stronger slow-wave sleep, demonstrating that synchronized SWA is a use-dependent mechanism to promote sleep and synaptic plasticity. All depicted electrophysiological traces are representative. Animal images in **C** adapted with permission from SciDraw.io.

In this review, we will shed light on the conserved neural mechanisms that make us sleepy and address how neural networks in our brain keep us asleep during the night. Some answers to these questions can be found in the neural activity patterns our brains exhibit during our sleep. The major sleep phases to be distinguished are called REM (Rapid Eye Movement) and NREM (non-REM) sleep (Figure 1A) (Aserinsky and Kleitman, 1953). REM sleep, during which we experience most of our dreams, is characterized by high-frequency activity that is similar to the waking state (Figure 1A). In contrast, deep sleep occurring during NREM sleep is characterized by a particularly high arousal threshold and slow-wave activity (SWA, up to 4.5 Hz) resulting from cortical neurons synchronizing their electrical patterns (Figure 1A) (Bonnet et al., 1978; Buzsaki and Draguhn, 2004). During SWA, cortical neurons alternate between a depolarized state ("upstate") during which they may fire action potentials and a hyperpolarized state ("downstate") during which neurons are silent and less likely to be excited. This so-called slow-wave sleep has been shown to be immensely important for memory consolidation (Klinzing et al., 2019) and the clearance of metabolites (Fultz et al., 2019), which would otherwise accumulate and increase the susceptibility for neurodegenerative diseases. While both REM and NREM sleep are capable of cognitively isolating us from our environment, the neural representations of sensory stimuli such as tones remain surprisingly intact within the primary sensory cortices (Andrillon and Kouider, 2020). However, during slow-wave sleep these neural representations fail to engage higher-level cortical areas, reducing the functional connectivity within the cortex and establishing the low arousability that characterizes deep sleep (Tononi and Massimini, 2008).

While this provides a clue why poor sleep quality has a negative impact on our health, it should be noted that the degree of unconsciousness that accompanies deep sleep can make an organism vulnerable to predatory attacks. Therefore, mechanisms must exist for specific sensory signatures to awaken us even from our deepest slumber. How neural networks can differentiate sensory stimuli and mediate awakening from deep sleep is very complex and far from understood. However, looking across the animal kingdom, we find that in all mammals, reptiles as well as birds, SWA is linked to deeper sleep, demonstrating that a deep sleep phase must have an immense evolutionary advantage that outweighs the risks of being eaten during your sleep (Joiner, 2016).

How did SWA evolve to become such a crucial factor for deep sleep and the associated recuperative processes? At a neurophysiological level, the question arises, how SWA reduces the functional connectivity to promote deep sleep? We here follow the hypothesis that the occurrence of SWA is more ancient than anticipated. Moreover, we argue that with the advent of optogenetic tools we have reached a level of precision that allows us to look at simpler, evolutionarily distant brains, to probe for fundamental processes regulating sleep and wakefulness.

Sleep as an inherent property of neural networks

We mostly think of our brains as either fully awake or fully asleep. However, the sight of a sleepwalking child should challenge this view and ask whether different neural networks within the same brain can be simultaneously "awake" and "asleep" (Singh et al., 2018). In fact, recent evidence indicates that specific neural networks can exhibit a sleep-like state independent of the rest of the brain, a phenomenon that has been termed "local sleep" (Krueger et al., 2019). Cognitive impairments in sleep-deprived humans have been linked to local synchronizations of SWA, indicating a role for SWA in shutting down sensory processing and signaling sleep need to undergo synaptic plasticity (Quercia et al., 2018).

The idea that SWA is a network-specific marker of sleep need becomes particularly clear when looking at marine mammals and birds, in which only one brain hemisphere at a time exhibits slow-wave sleep while the other hemisphere is more easily arousable and ready to process sensory information (Figure 1B) (Mascetti, 2016). Depriving specifically one hemisphere of slow-wave sleep leads to unilateral sleep rebound with increased SWA (Mascetti, 2016), demonstrating that sleep need is in fact not necessarily experienced by the whole brain, but by specific neural networks.

Experiments in sleep-deprived humans and rats have shown that the local occurrence of a sleep-like state in an awake individual is highly task-specific and therefore correlates with the intensity and complexity of sensory processing (Krueger et al., 2019; Quercia et al., 2018; Vyazovskiy et al., 2011). Sleep-deprived but awake rats exhibit synchronized silent periods in specific networks strongly involved in the task, while "unused" cortical networks remain "awake" and arousable (Figure 1C) (Rector et al., 2009; Rector et al., 2005). Moreover, cortical networks that are intensely engaged in sensory processing when awake exhibit stronger SWA during subsequent NREM sleep (Figure 1C) (Huber et al., 2006; Kattler et al., 1994; Mascetti et al., 2013; Vyazovskiy et al., 2011). The observation that specific neural networks exhibit use-dependent homeostatic regulation of a sleep-like state indicates that slow-wave sleep might be an inherent property of neural networks (Krueger et al., 2008). In fact, even isolated cortical slices retain the ability to generate synchronized SWA (Sanchez-Vives, 2020).

SWA associated with sleep-regulation has also been observed in crustacea and zebrafish, animals that are evolutionarily older than reptiles (Leung et al., 2019; Ramon et al., 2004). We have recently shown that even in the vinegar fly *Drosophila melanogaster* (colloquially referred to as fruit flies), synchronized SWA in specific sleep-regulating neurons does not only mediate sleep need but also facilitates consolidated sleep phases and decreases arousability during the night (Raccuglia et al., 2019). Therefore, network synchronizations generating SWA might have evolved as an optimized strategy to facilitate a quiescent state by suppressing sensory processing and simultaneously promoting repair mechanisms and synaptic plasticity.

In the next chapters, we will highlight the basic principles and functional analogies revealed by sleep research in *Drosophila*. Due to its simpler brain, we can aim at identifying the essential neural components and interactions that provide the neural architecture of sleep regulation.

A vinegar nightcap for some fly folks

Recent years have witnessed rapid progress in our understanding of the circuit and molecular mechanisms underlying sleep control in *Drosophila*. Over the decades, research in *Drosophila* has created unprecedented genetic accessibility as well as a huge and easily usable neural toolbox. The much simpler fly brain (~200,000 neurons) and the functional analogies in the neural networks involved in sleep offer the opportunity to understand the neural architecture of sleep regulation. In 2017, the Nobel Prize in Physiology was awarded to the *Drosophila* researchers that made significant contributions to understanding the molecular clockwork of circadian rhythms (Hardin et al., 1990; Zehring et al., 1984). Intriguingly, the transcriptional and translational feedback loops that represent the mechanistic basis for generating circadian rhythms at the cellular level are found in all animals and plants. The neuronal regulation of circadian rhythms and sleep are inevitably interwoven with each other as they

likely coevolved, ensuring that sleep always occurs during the same phase of the day.

Sleep in *Drosophila* is primarily defined as a state of prolonged inactivity. Fruit flies are mostly active during dusk and dawn, having an extended period of rest in the middle of the day and exhibiting deeper sleep throughout the night (Shaw et al., 2000). Importantly, sleep in fruit flies underlies homeostatic regulation as sleep loss leads to a subsequent compensatory increase in sleep duration and facilitation of uninterrupted sleep phases (Shaw et al., 2000). Similar to human sleep, sleep in *Drosophila* is characterized by an overall decrease in brain activity and has been shown to be a dynamic process consisting of deeper and lighter sleep stages (van Alphen et al., 2013). Interestingly, the transition from waking to sleep has been associated with an increase in oscillatory activity (7–10 Hz) in the central brain (Yap et al., 2017). While flies can survive long stretches of sleep deprivation (Geissmann et al., 2019), sleep is critical for normal cognitive functions. As in humans, sleep deprivation leads to deficits in selective visual attention (Kirszenblat et al., 2018) and memory performance (Donlea, 2019) as well as to the accumulation of metabolites in the brain (van Alphen et al., 2021), demonstrating that functions of sleep are evolutionarily conserved. Moreover, recent evidence has shown that sleep deprivation leads to a build-up of reactive oxygen species (ROS) in the guts of mice and flies, which eventually can lead to the organism's premature demise (Vaccaro et al., 2020).

Sleep research in *Drosophila* has shown how various neural networks with different functions affect sleep behavior. For example, neural networks involved in mediating hunger and sexual arousal reduce sleep (Beckwith et al., 2017; Keene et al., 2010) while neural networks processing visual information and social cues have been shown to be sleep-promoting (Ganguly-Fitzgerald et al., 2006; Kirszenblat et al., 2019). The mushroom bodies, a higher-order brain network crucially involved in olfactory memory in *Drosophila*, have also been implicated in sleep regulation (Donlea, 2019). Demonstrating how complex sleep regulation is, some mushroom body neurons have been shown to be wake-promoting while others seem sleep-promoting (Sitaraman et al., 2015).

A special focus has however been on the networks of the central complex, where the integration of navigation, locomotion control, and sleep regulation demonstrates how tightly interwoven sensory processing and sleep need are (Pfeiffer and Homberg, 2014; Strauss and Heisenberg, 1993). Indeed, it is within the central complex that we find an oscillatory activity that is tightly linked to sleep need

and sleep quality (Guo et al., 2018; Raccuglia et al., 2019; Yap et al., 2017). In the following chapters, we will therefore dig deeper into the neural interactions that provide circadian and homeostatic sleep regulation in the central complex of *Drosophila*.

Sensory gates for homeostatic sleep regulation

Homeostatic sleep regulation refers to the process that sleep depth, as well as our subjective tiredness, depends on the amount of time spent in wakefulness (Borbely, 1982). A crucial factor of homeostatic sleep regulation in humans is the activity-dependent build-up of adenosine in our brains which activates sleep-promoting neurons, eventually making us feel sleepy (Lazarus et al., 2019). To combat sleepiness we consume caffeine, which antagonistically blocks adenosine receptors and increases levels of wake-promoting dopamine (Jagannath et al., 2021; Lazarus et al., 2019). In fruit flies, caffeine promotes wakefulness by directly activating dopaminergic neurons (Nall et al., 2016), impressively demonstrating that humans and flies must share ancient neural mechanisms for homeostatic sleep regulation.

Of particular interest for homeostatic sleep regulation in *Drosophila* is a neural circuitry within the central complex in which R5 ring neurons are recurrently connected to sleep-promoting dorsal fan-shaped-body neurons (dFSB) via the so-called helicon cells (Figure 2) (Donlea et al., 2018). The dFSB is often referred to as "master sleep switch" because its optogenetic activation has been tightly linked to reducing arousability (Troup et al., 2018) and inducing sleep (Donlea et al., 2009; Pimentel et al., 2016). During the course of a day, activity-dependent build-up of ROS in dFSB neurons increases their excitability (Kempf et al., 2019), leading to increased inhibition of the helicon cells during the night (Donlea et al., 2018). Helicon cells process light cues to induce locomotion and their inhibition, therefore, reduces the flow of visual information and suppresses locomotion (Donlea et al., 2018). Here, R5 ring neurons, which have reciprocal synaptic connections to the helicon cells (Figure 2), provide another layer of homeostatic sleep regulation that is directly linked to processing complex visual stimuli and orientation behavior (see next chapter for more details).

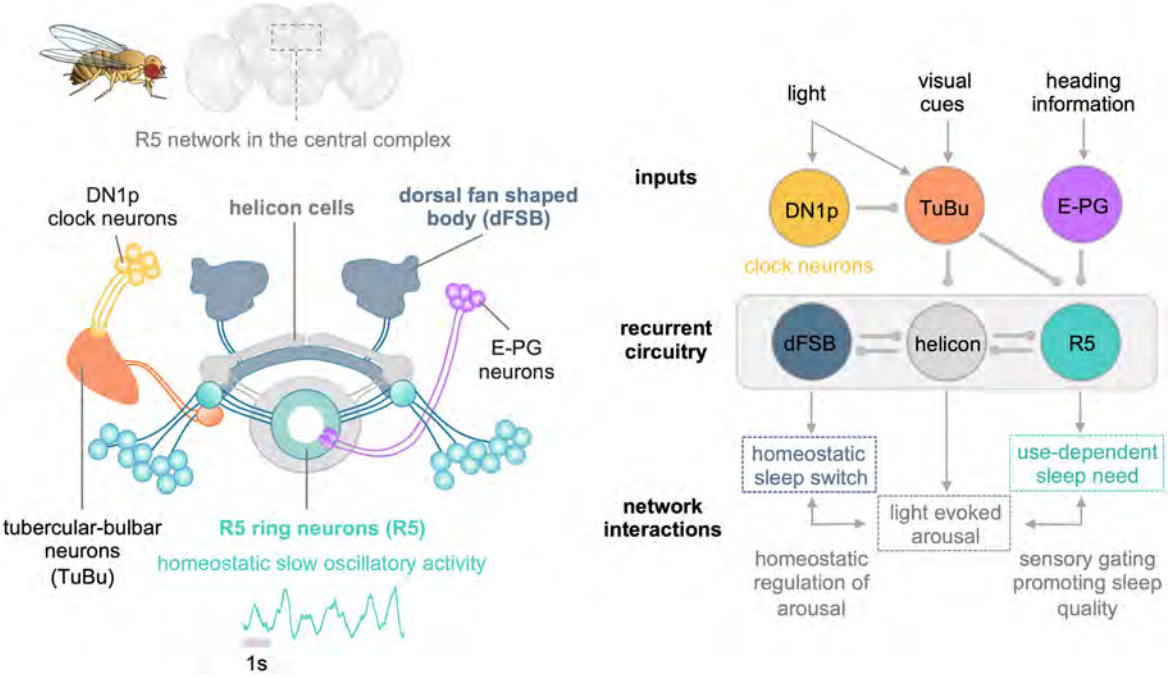

Figure 2: Network interactions mediating homeostatic and circadian sleep regulation in the central complex of *Drosophila*.
At the center of this neural architecture is an interconnected circuitry composed of the dFSB, helicon cells, and R5 neurons. Daily ROS build-up in dFSB increases excitability to mediate homeostatic sleep need. This sleep need is modulated via circadian input from DN1p clock neurons to visually sensitive TuBu neurons, which themselves mediate visual information to helicon cells and R5 neurons. Interactions between dFSB and helicon cells modulate visually evoked arousal dependent on homeostatic sleep need. R5 neurons, which also receive heading information from E-PG neurons, provide the neural basis for navigation during the day. At night, R5 neurons generate synchronized slow-wave activity to establish a sensory gate, reducing arousability and promoting synaptic plasticity. Depicted SWA is a representative trace. Fly and brain are adapted with permission from SciDraw.io.

Interestingly, R5 neurons display intriguing functional and physiological analogies to the mammalian thalamus, which often is pictured as "the gate to our consciousness" (McCormick and Bal, 1994). Since almost all sensory information must pass through the thalamus before reaching cortical areas, the thalamus can filter sensory information, essentially acting as a sensory gate that regulates sleep/wake transitions (Gent et al., 2018). As sleep need increases, thalamic reticular neurons switch from tonic firing to bursting and promote network synchronization that generates SWA and facilitates the transition to sleep (Llinas and Steriade, 2006). Similarly, with increasing sleep need, R5 neurons start bursting and synchronize their electrical patterns, generating NMDA receptor-dependent compound SWA (Liu et al., 2016; Raccuglia et al., 2019). Perturbing NMDA receptor functioning abolishes SWA in R5 neurons, which leads to sleep fragmentation and increases arousability at night (Raccuglia et al., 2019).

Thus, similar to the thalamus or the local sleep phenomenon, network-specific slow-wave synchronizations in *Drosophila* can establish a gate at the level of neural networks that suppresses sensory processing and mediates sleep need (Figure 2). More widespread synchronizations within the central complex could lock neural populations into a neurophysiological state that is directly linked to the time spent awake and therefore to the intensity of sleep need. Moreover, reciprocal interactions between the dFSB and R5 neurons could integrate sleep need to be mediated by the daily build-up of ROS with sleep need that comes with processing complex visual stimuli necessary for orientation behavior (Figure 2) (see next chapter).

Importantly, sleep need and subjective tiredness also need to be regulated by other internal drives such as hunger and sexual arousal, which generally are wake-promoting. The neural representation of such internal states could modulate the neural interactions within the recurrent circuitry, potentially inhibiting network synchronization to temporarily overwrite homeostatic sleep need. Taken together, the functional units represented by specific networks in *Drosophila* and their interactions illustrate how basic neurophysiological principles and properties establish sensory gates to adequately balance wake and sleep.

Balancing sensory processing and synaptic rescaling

As explained before, generating SWA might be an inherent property of neural networks which directly links sensory processing to network-autonomous homeostatic regulation of sleep need (Krueger et al., 2008). Accordingly, *Drosophila* ring neurons capable of generating SWA are most likely also involved in processing visual cues (Seelig and Jayaraman, 2013) and orientation behavior (Kim et al., 2017; Turner-Evans et al., 2017). Visual information is mediated to R5 neurons and helicon cells via tubercular-bulbar (TuBu) neurons (Figure 2) (Omoto et al., 2017), which are sensitive to polarized light (Hardcastle et al., 2021) and thus enable flies to use the sun as a navigational cue even when it is hidden behind clouds. Considering the dual role of navigation and sleep regulation inherent to R5 neurons, it raises the question of how these seemingly different functions can be integrated and properly regulated at the level of neural networks (Flores-Valle et al., 2021).

Homeostatic sleep need in mammals and flies is generated by an accumulation of byproducts of neural activity, which often correlates with the amount and complexity of sensory information. Therefore, engaging in new or complex tasks requiring more elaborate neural processing should have a more profound effect on sleep need than engaging in familiar or simple tasks. In fact, the daytime visual load has been shown to increase slow-wave sleep in humans (Horne and Walmsley, 1976) and time spent sleeping in flies (Kirszenblat et al., 2019). Moreover, as in flies, there is a peculiar link between SWA and navigation in mammals. The mammalian hippocampus is not only the center of our spatial memories but is also crucially involved in the consolidation of declarative memories, which, among other things, entail our personal experiences (Klinzing et al., 2019). The consolidation of our declarative memories, as well as spatial memories, crucially depends on the power of SWA during deep sleep, which promotes synaptic reorganization to implement experiences made during the waking hours (Marshall et al., 2006).

In *Drosophila*, spatial learning depending on ring neurons has been shown to deteriorate in sleep-deprived flies (Melnattur et al., 2020; Neuser et al., 2008). Analogous to what has been demonstrated in cortical columns in mammals, spatial information could "accumulate" in ring neurons to the point where they switch from processing sensory information to mediating sleep need which might require R5 neurons to start bursting and synchronize their electrical patterns (Liu et al., 2016; Raccuglia et al., 2019). In fact, prolonged optogenetic activation of visually sensitive TuBu neurons leads to synchronized SWA in R5 neurons and dramatically increases sleep (Guo et al., 2018; Lamaze et al., 2018; Raccuglia et al., 2019), illustrating the link between visual processing, network-synchronization and sleep need.

According to Hebbian principles, synchronized activity can promote synaptic rescaling and thus provide the mechanistic basis for memory consolidation during sleep (Bocchio et al., 2017). Indeed, modulating the molecular machinery at the presynaptic zones of R5 neurons affects sleep behavior as well as associative olfactory memory (Huang et al., 2020). Moreover, sleep provides the necessary rest to not only promote synaptic plasticity but also clear out metabolites which potentially induced sleep need in the first place. The necessary alleviation of sleep need might have evolved into an elaborate neural clearing that is linked to SWA during deep sleep in humans (Fultz et al., 2019).

The link between sleep and spatial navigation in *Drosophila* illustrates how sensory information can be used to generate homeostatic sleep need and promote synaptic plasticity. While it remains elusive how specific sensory signatures can break up a sensory gate to awaken an organism, our knowledge about these circuits might one day provide an answer to this question.

Integration of homeostatic and circadian sleep regulation

Circadian sleep regulation refers to the process that we live and sleep in a ~24 h cycle that is driven by our internal molecular clocks and determined by alternating light and dark phases (Borbely, 1982). As our molecular clocks are not precisely running on a 24 h cycle, so-called clock neurons need light input to constantly reset the molecular clocks (Dunlap, 1999). Therefore, light is not only essential for processing visual information but also light per se is needed to constantly fine-tune the activity patterns of clock neurons to maintain stable physiological periodicity and adjust to seasonal changes of the light/dark cycle.

In mammalian brains, the suprachiasmatic nucleus (SCN) is considered the master circadian pacemaker since it receives direct light input from the retina and informs all other neural circuits whether it is light or dark outside the skull (Hastings et al., 2018). While the SCN activates wake-promoting neurons during the day, the onset of darkness leads to the production of melatonin, which facilitates the activation of sleep-promoting neurons in diurnal animals and also acts as an antioxidant to eliminate ROS accumulated during the day (Aulinas, 2000).

In *Drosophila*, various clock neurons receive light information from other neural networks to regulate circadian behaviors (Helfrich-Förster, 2005). Most interestingly, specific dorso-posterior clock neurons (DN1p) provide circadian information to the TuBu neurons, illustrating two important aspects of circadian regulation (Figure 2) (Guo et al., 2018). Firstly, circadian time influences sensory processing. This is for example important for migrating monarch butterflies to adjust their sun-compass to seasonal changes (Heinze and Reppert, 2011; Pfeiffer and Homberg, 2007). Secondly, the circadian time could determine when and to what extent sensory processing leads to the accumulation of sleep need or subjective tiredness, illustrating that circadian and homeostatic sleep regulation have co-evolved and are thus inexorably linked to each other. In fact, optogenetic activation of DN1p clock neurons leads to oscillatory activity in ring neurons, demonstrating the circadian influence of generating sleep need at the level of neural networks (Guo et al., 2018).

Circadian input to visually sensitive TuBu neurons might promote sensory processing during the day while facilitating the switch of R5 neurons from tonic firing to bursting in the night. Moreover, light information mediated by TuBu neurons might "charge" the circuitry and mediate sleep need that correlates with the amount and intensity of light perceived (Donlea et al., 2018; Mazzotta et al., 2020). Interestingly, TuBu neurons provide parallel synaptic input to helicon cells and R5 neurons (Hulse et al., 2020). While R5 neurons might use this visual information for navigation, light information in helicon cells might be decisive whether locomotion is induced or not. Interactions between the helicon cells and R5 neurons could therefore integrate use-dependent sleep need, the presence or absence of light, and the circadian time. These interactions in *Drosophila* are representative of more complex interactions in mammals that have ultimately co-evolved to ensure that sleep occurs during a predetermined and consolidated phase of the day. While deep sleep makes an animal potentially vulnerable to predatory attacks, the ability to promote synaptic plasticity across widely distributed neural networks as well as perform a brain-wide clearance of metabolites could have improved cognitive abilities and therefore brought about an immense evolutionary advantage.

Abbreviations

dFSB	dorsal fan shaped body
DN1p	dorso-posterior clock neurons
EEG	electroencephalogramm
E-PG	ellipsoid body-protocerebral bridge-gall neurons
LFP	local field potential
N-REM	non-rapid eye movement
REM	rapid eye movement

ROS	reactive oxygen species
SCN	suprachiasmatic nucleus
SWA	slow-wave activity
TUBU	tubercular-bulbar neurons

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References

Alhola, P. and Polo-Kantola, P. (2007). Sleep deprivation: Impact on cognitive performance. Neuropsychiatric Dis. Treat. *3*, 553–567.

Andrillon, T. and Kouider, S. (2020). The vigilant sleeper: Neural mechanisms of sensory (de)coupling during sleep. Curr. Opin. Physiol. *15*, 47–59.

Aserinsky, E. and Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science *118*, 273–274.

Aulinas, A. (2000). Physiology of the Pineal Gland and Melatonin. Endotext (South Dartmouth (MA): MDText.com, Inc.).

Beckwith, E.J., Geissmann, Q., French, A.S., and Gilestro, G.F. (2017). Regulation of sleep homeostasis by sexual arousal. Elife *6*, e27445.

Bocchio, M., Nabavi, S., and Capogna, M. (2017). Synaptic plasticity, engrams, and network oscillations in amygdala circuits for storage and retrieval of emotional memories. Neuron *94*, 731–743.

Bonnet, M.H., Johnson, L.C., and Webb, W.B. (1978). The reliability of arousal threshold during sleep. Psychophysiology *15*, 412–416.

Borbely, A.A. (1982). A two process model of sleep regulation. Hum. Neurobiol. *1*, 195–204.

Buzsaki, G. and Draguhn, A. (2004). Neuronal oscillations in cortical networks. Science *304*, 1926–1929.

Donlea, J.M. (2019). Roles for sleep in memory: Insights from the fly. Curr. Opin. Neurobiol. *54*, 120–126.

Donlea, J.M., Pimentel, D., Talbot, C.B., Kempf, A., Omoto, J.J., Hartenstein, V., and Miesenbock, G. (2018). Recurrent circuitry for balancing sleep need and sleep. Neuron *97*, 378–389 e374.

Donlea, J.M., Ramanan, N., and Shaw, P.J. (2009). Use-dependent plasticity in clock neurons regulates sleep need in *Drosophila*. Science *324*, 105–108.

Dunlap, J.C. (1999). Molecular bases for circadian clocks. Cell *96*, 271–290.

Flores-Valle, A., Gonçalves, P.J., and Seelig, J.D. (2021). Integration of sleep homeostasis and navigation in Drosophila. PLoS Comput Biol *17*, e1009088.

Fultz, N.E., Bonmassar, G., Setsompop, K., Stickgold, R.A., Rosen, B.R., Polimeni, J.R., and Lewis, L.D. (2019). Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. Science *366*, 628–631.

Ganguly-Fitzgerald, I., Donlea, J., and Shaw, P.J. (2006). Waking experience affects sleep need in *Drosophila*. Science *313*, 1775–1781.

Geissmann, Q., Beckwith, E.J., and Gilestro, G.F. (2019). Most sleep does not serve a vital function: Evidence from *Drosophila melanogaster*. Sci. Adv. *5*, eaau9253.

Gent, T.C., Bandarabadi, M., Herrera, C.G., and Adamantidis, A.R. (2018). Thalamic dual control of sleep and wakefulness. Nat. Neurosci. *21*, 974–984.

Guo, F., Holla, M., Diaz, M.M., and Rosbash, M. (2018). A circadian output circuit controls sleep-wake arousal in *Drosophila*. Neuron *100*, 624–635 e624.

Hardcastle, B.J., Omoto, J.J., Kandimalla, P., Nguyen, B.M., Keles, M.F., Boyd, N.K., Hartenstein, V., and Frye, M.A. (2021). A visual pathway for skylight polarization processing in *Drosophila*. Elife *10*, e63225.

Hardin, P.E., Hall, J.C., and Rosbash, M. (1990). Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. Nature *343*, 536–540.

Hastings, M.H., Maywood, E.S., and Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. Nat. Rev. Neurosci. *19*, 453–469.

Heinze, S. and Reppert, S.M. (2011). Sun compass integration of skylight cues in migratory monarch butterflies. Neuron *69*, 345–358.

Helfrich-Förster, C. (2005). Neurobiology of the fruit fly's circadian clock. Gene Brain Behav. *4*, 65–76.

Horne, J.A. and Walmsley, B. (1976). Daytime visual load and the effects upon human sleep. Psychophysiology *13*, 115–120.

Huang, S., Piao, C., Beuschel, C.B., Gotz, T., and Sigrist, S.J. (2020). Presynaptic active zone plasticity encodes sleep need in *Drosophila*. Curr. Biol. *30*, 1077–1091.e5.

Huber, R., Ghilardi, M.F., Massimini, M., Ferrarelli, F., Riedner, B.A., Peterson, M.J., and Tononi, G. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat. Neurosci. *9*, 1169–1176.

Hulse, B.K., Haberkern, H., Franconville, R., Turner-Evans, D.B., Takemura, S., Wolff, T., Noorman, M., Dreher, M., Dan, C., Parekh, R., et al. (2020). A connectome of the *Drosophila* central complex reveals network motifs suitable for flexible navigation and context-dependent action selection. bioRxiv, 2020.2012.2008.413955.

Jagannath, A., Varga, N., Dallmann, R., Rando, G., Gosselin, P., Ebrahimjee, F., Taylor, L., Mosneagu, D., Stefaniak, J., Walsh, S., et al. (2021). Adenosine integrates light and sleep signalling for the regulation of circadian timing in mice. Nat. Commun. *12*, 2113.

Joiner, W.J. (2016). Unraveling the evolutionary determinants of sleep. Curr. Biol. *26*, R1073–R1087.

Kattler, H., Dijk, D.J., and Borbely, A.A. (1994). Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J. Sleep Res. *3*, 159–164.

Keene, A.C., Duboue, E.R., McDonald, D.M., Dus, M., Suh, G.S., Waddell, S., and Blau, J. (2010). Clock and cycle limit starvation-induced sleep loss in *Drosophila*. Curr. Biol. *20*, 1209–1215.

Kempf, A., Song, S.M., Talbot, C.B., and Miesenbock, G. (2019). A potassium channel beta-subunit couples mitochondrial electron transport to sleep. Nature *568*, 230–234.

Kim, S.S., Rouault, H., Druckmann, S., and Jayaraman, V. (2017). Ring attractor dynamics in the *Drosophila* central brain. Science *356*, 849–853.

Kirszenblat, L., Ertekin, D., Goodsell, J., Zhou, Y., Shaw, P.J., and van Swinderen, B. (2018). Sleep regulates visual selective attention in *Drosophila*. J. Exp. Biol. *221*, https://doi.org/10.1242/jeb.191429.

Kirszenblat, L., Yaun, R., and van Swinderen, B. (2019). Visual experience drives sleep need in *Drosophila*. Sleep *42*, https://doi.org/10.1093/sleep/zsz102.

Klinzing, J.G., Niethard, N., and Born, J. (2019). Mechanisms of systems memory consolidation during sleep. Nat. Neurosci. *22*, 1598–1610.

Krueger, J.M., Nguyen, J.T., Dykstra-Aiello, C.J., and Taishi, P. (2019). Local sleep. Sleep Med. Rev. *43*, 14–21.

Krueger, J.M., Rector, D.M., Roy, S., Van Dongen, H.P., Belenky, G., and Panksepp, J. (2008). Sleep as a fundamental property of neuronal assemblies. Nat. Rev. Neurosci. *9*, 910–919.

Lamaze, A., Kratschmer, P., Chen, K.F., Lowe, S., and Jepson, J.E.C. (2018). A wake-promoting circadian output circuit in *Drosophila*. Curr. Biol. *28*, 3098–3105.e3.

Lazarus, M., Oishi, Y., Bjorness, T.E., and Greene, R.W. (2019). Gating and the need for sleep: Dissociable effects of adenosine A1 and A2A receptors. Front. Neurosci. *13*, 740.

Leung, L.C., Wang, G.X., Madelaine, R., Skariah, G., Kawakami, K., Deisseroth, K., Urban, A.E., and Mourrain, P. (2019). Neural signatures of sleep in zebrafish. Nature *571*, 198–204.

Liu, S., Liu, Q., Tabuchi, M., and Wu, M.N. (2016). Sleep drive is encoded by neural plastic changes in a dedicated circuit. Cell *165*, 1347–1360.

Llinas, R.R. and Steriade, M. (2006). Bursting of thalamic neurons and states of vigilance. J. Neurophysiol. *95*, 3297–3308.

Marshall, L., Helgadottir, H., Molle, M., and Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. Nature *444*, 610–613.

Mascetti, G.G. (2016). Unihemispheric sleep and asymmetrical sleep: Behavioral, neurophysiological, and functional perspectives. Nat. Sci. Sleep *8*, 221–238.

Mascetti, L., Muto, V., Matarazzo, L., Foret, A., Ziegler, E., Albouy, G., Sterpenich, V., Schmidt, C., Degueldre, C., Leclercq, Y., et al. (2013). The impact of visual perceptual learning on sleep and local slow-wave initiation. J. Neurosci. *33*, 3323–3331.

Mazzotta, G.M., Damulewicz, M., and Cusumano, P. (2020). Better sleep at night: How light influences sleep in *Drosophila*. Front. Physiol. *11*, 997.

McCormick, D.A. and Bal, T. (1994). Sensory gating mechanisms of the thalamus. Curr. Opin. Neurobiol. *4*, 550–556.

Melnattur, K., Kirszenblat, L., Morgan, E., Militchin, V., Sakran, B., English, D., Patel, R., Chan, D., van Swinderen, B., and Shaw, P.J. (2020). A conserved role for sleep in supporting spatial learning in *Drosophila*. Sleep *44*, https://doi.org/10.1093/sleep/zsaa197.

Mullington, J.M., Cunningham, T.J., Haack, M., and Yang, H. (2021). Causes and consequences of chronic sleep deficiency and the role of orexin. Front. Neurol. Neurosci. *45*, 128–138.

Nall, A.H., Shakhmantsir, I., Cichewicz, K., Birman, S., Hirsh, J., and Sehgal, A. (2016). Caffeine promotes wakefulness via dopamine signaling in *Drosophila*. Sci. Rep. *6*, 20938.

Neuser, K., Triphan, T., Mronz, M., Poeck, B., and Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. Nature *453*, 1244–1247.

Omoto, J.J., Keles, M.F., Nguyen, B.M., Bolanos, C., Lovick, J.K., Frye, M.A., and Hartenstein, V. (2017). Visual input to the *Drosophila* central complex by developmentally and functionally distinct neuronal populations. Curr. Biol. *27*, 1098–1110.

Pfeiffer, K. and Homberg, U. (2007). Coding of azimuthal directions via time-compensated combination of celestial compass cues. Curr. Biol. *17*, 960–965.

Pfeiffer, K. and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. Annu. Rev. Entomol. *59*, 165–184.

Pimentel, D., Donlea, J.M., Talbot, C.B., Song, S.M., Thurston, A.J.F., and Miesenbock, G. (2016). Operation of a homeostatic sleep switch. Nature *536*, 333–337.

Quercia, A., Zappasodi, F., Committeri, G., and Ferrara, M. (2018). Local use-dependent sleep in wakefulness links performance errors to learning. Front. Hum. Neurosci. *12*, 122.

Raccuglia, D., Huang, S., Ender, A., Heim, M.M., Laber, D., Suarez-Grimalt, R., Liotta, A., Sigrist, S.J., Geiger, J.R.P., and Owald, D. (2019). Network-specific synchronization of electrical slow-wave oscillations regulates sleep drive in *Drosophila*. Curr. Biol. *29*, 3611–3621.e3.

Ramon, F., Hernandez-Falcon, J., Nguyen, B., and Bullock, T.H. (2004). Slow wave sleep in crayfish. Proc. Natl. Acad. Sci. U. S. A. *101*, 11857–11861.

Rector, D.M., Schei, J.L., Van Dongen, H.P., Belenky, G., and Krueger, J.M. (2009). Physiological markers of local sleep. Eur. J. Neurosci. *29*, 1771–1778.

Rector, D.M., Topchiy, I.A., Carter, K.M., and Rojas, M.J. (2005). Local functional state differences between rat cortical columns. Brain Res. *1047*, 45–55.

Sanchez-Vives, M.V. (2020). Origin and dynamics of cortical slow oscillations. Curr. Opin. Physiol. *15*, 217–223.

Seelig, J.D. and Jayaraman, V. (2013). Feature detection and orientation tuning in the *Drosophila* central complex. Nature *503*, 262–266.

Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. Science *287*, 1834–1837.

Singh, S., Kaur, H., Singh, S., and Khawaja, I. (2018). Parasomnias: A comprehensive review. Cureus *10*, e3807.

Sitaraman, D., Aso, Y., Jin, X., Chen, N., Felix, M., Rubin, G.M., and Nitabach, M.N. (2015). Propagation of homeostatic sleep signals by segregated synaptic microcircuits of the *Drosophila* mushroom body. Curr. Biol. *25*, 2915–2927.

Strauss, R. and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. J. Neurosci. *13*, 1852–1861.

Tononi, G. and Massimini, M. (2008). Why does consciousness fade in early sleep? Ann. N. Y. Acad. Sci. *1129*, 330–334.

Troup, M., Yap, M.H., Rohrscheib, C., Grabowska, M.J., Ertekin, D., Randeniya, R., Kottler, B., Larkin, A., Munro, K., Shaw, P.J., et al.

(2018). Acute control of the sleep switch in *Drosophila* reveals a role for gap junctions in regulating behavioral responsiveness. Elife *7*, e37105.

Turner-Evans, D., Wegener, S., Rouault, H., Franconville, R., Wolff, T., Seelig, J.D., Druckmann, S., and Jayaraman, V. (2017). Angular velocity integration in a fly heading circuit. Elife *6*, e23496.

Vaccaro, A., Kaplan Dor, Y., Nambara, K., Pollina, E.A., Lin, C., Greenberg, M.E., and Rogulja, D. (2020). Sleep loss can cause death through accumulation of reactive oxygen species in the gut. Cell *181*, 1307–1328.e15.

van Alphen, B., Semenza, E.R., Yap, M., van Swinderen, B., and Allada, R. (2021). A deep sleep stage in *Drosophila* with a functional role in waste clearance. Sci. Adv. *7*, https://doi.org/10.1126/sciadv.abc2999.

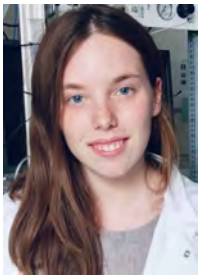
van Alphen, B., Yap, M.H., Kirszenblat, L., Kottler, B., and van Swinderen, B. (2013). A dynamic deep sleep stage in *Drosophila*. J. Neurosci. *33*, 6917–6927.

Vyazovskiy, V.V., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., and Tononi, G. (2011). Local sleep in awake rats. Nature *472*, 443–447.

Yap, M.H.W., Grabowska, M.J., Rohrscheib, C., Jeans, R., Troup, M., Paulk, A.C., van Alphen, B., Shaw, P.J., and van Swinderen, B. (2017). Oscillatory brain activity in spontaneous and induced sleep stages in flies. Nat. Commun. *8*, 1815.

Zehring, W.A., Wheeler, D.A., Reddy, P., Konopka, R.J., Kyriacou, C.P., Rosbash, M., and Hall, J.C. (1984). P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster*. Cell *39*, 369–376.

Bionotes



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Raquel Suárez-Grimalt studied biology at the University of A Coruña and received a master's degree in neuroscience from the Autonomous University of Barcelona. During a research stay in Andrew Lin's lab at the University of Sheffield, she became passionate about the neuronal networks responsible for complex behaviors in fruit flies. For her PhD, she joined the Emmy Noether research group of David Owald in Berlin, where she is now studying neural circuitries balancing wake and sleep in flies by using optogenetics and animal tracking. During this time, she received a PhD fellowship from the Einstein Center for Neurosciences Berlin. Raquel is planning on finishing her PhD end of 2021 and continuing her research career as a postdoc.

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Review article

Julia Tigges, Tamara Schikowski and Ellen Fritsche*

Environmental exposures impact the nervous system in a life stage-specific manner

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Abstract: Exposure to environmental pollutants like chemicals or air pollution is major health concern for the human population. Especially the nervous system is a sensitive target for environmental toxins with exposures leading to life stage-dependent neurotoxicity. Developmental and adult neurotoxicity are characterized by specific adverse outcomes ranging from neurodevelopmental disorders to neurodegenerative diseases like Alzheimer's and Parkinson's disease. The risk assessment process for human health protection is currently undergoing a paradigm change toward new approach methods that allow mechanism-based toxicity assessment. As a flagship project, an *in vitro* battery of test methods for developmental neurotoxicity evaluation is currently supported by the Organization for Economic Cooperation and Development (OECD). A plethora of stem cell-based methods including brain spheres and organoids are currently further developed to achieve time- and cost-saving tools for linking MoA-based hazards to adverse health effects observed in humans.

Keywords: brain organoids; developmental neurotoxicity; epidemiology; neurosphere; risk assessment.

Zusammenfassung: Die Exposition gegenüber Umweltschadstoffen wie Chemikalien oder Luftverschmutzung führt zu gesundheitlichen Problemen in der menschlichen Bevölkerung. Insbesondere das Nervensystem ist ein empfindliches Ziel für Umweltschadstoffe, wobei die Exposition zu Lebensphasen-abhängiger Neurotoxizität führt. Die Neurotoxizität während der frühkindlichen Entwicklung und im Erwachsenenalter ist durch spezifische negative Folgen gekennzeichnet, die von Störungen der Hirnentwicklung bis hin zu neurodegenerativen Erkrankungen wie der Alzheimer'schen und Parkinson'schen Erkrankung reichen. Bei der Risikobewertung zum Schutz der menschlichen Gesundheit vollzieht sich derzeit ein Paradigmenwechsel hin zu neuen Methoden, die eine auf Mechanismen basierte Toxizitätsbewertung ermöglichen. Als Vorzeigeprojekt wird derzeit von der OECD eine In-vitro-Batterie von Testmethoden für die Erfassung der Entwicklungsneurotoxizität unterstützt. Eine Vielzahl von Stammzell-basierten Methoden, darunter Gehirn Sphäroide und Organoide, werden derzeit weiterentwickelt, um zeit-und kostensparende Instrumente für die Verknüpfung des auf Mechanismen basierten Gefährdungspotentials von Umweltschadstoffen mit beim Menschen beobachteten Erkrankungen zu schaffen.

Schlüsselwörter: Entwicklungsneurotoxizität; Gehirn Organoide; Gehirn Sphäroide; Epidemiologie; Risikobewertung.

Introduction

During our lifespan, from the embryo to the aged human, our nervous system is crucial for body homeostasis. Besides cognitive brain function, nerve cells are involved in a plethora of sensory, motor, and visceral processes allowing functioning, orientation, and survival in the environment. When the neuronal function is disturbed due to neurotoxicity, consequences are neurochemical, anatomical, or physiological changes which might cause behavioral adversities and can be immediate or delayed, as well as transient or persistent (EPA, 1996). While neuropathological effects are structural changes in the nervous system, neurochemical, neurophysiological, or behavioral changes imply functional effects and include adverse changes in somatic/autonomic, sensory, motor, and cognitive functions (EPA, 1996; WHO, 2004).

Neurotoxicity caused by environmental pollutants is strongly life stage-specific (Figure 1). This is due to the high plasticity of the developing and the very low regenerative capacity of the adult brain (Rice and Barone, 2000; Rodier,

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1995). In addition, genetic interindividual variability determining responses to chemical stressors might alter the predisposition to neurologic diseases (Zeise et al., 2013). While a plethora of neurotoxic mechanisms is already well understood (Chang, 1995; Fritsche et al., 2021a), there are still large gaps in comprehension of how environmental exposure to a large variety of noxae contributes to neurological adversities ranging from neurodevelopmental disorders to neurodegenerative diseases. The next paragraphs will sum up a current understanding of life stage-specific, environmentally induced neurotoxicity and introduce novel methods that will facilitate studying causal relationships between environmental pollutants and adverse outcomes relating to the nervous system.

Developmental neurotoxicity

Neurotoxicity during the developmental period, known as developmental neurotoxicity (DNT) has been defined 'as potentially functional and morphological hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation' (EPA, 1996), yet due to the protracted nature of nervous system development, this can encompass toxic insults *in utero* through early adolescence (Magby and Richardson, 2018). Hence, developmentally neurotoxic chemicals have the potential to interfere with the normal development of the nervous system, which, if perturbed without compensation, may lead to adverse effects on the normal development of nervous system structures and/or functions (EPA, 1998; Mileson and Ferenc 2001). From the perspective of environmental health, attention has recently been focusing on DNT as the effects of environmental contaminants on the development of the human brain are considered a public health concern (Bal-Price et al., 2018; Sachana et al., 2019). Poisoning disasters with e.g. Mercury, lead or polychlorinated biphenyls revealed that chemicals can interfere with developmental processes of the human brain (Grandjean and Landrigan, 2006). However, not only poisoning accidents with high chemical exposure raise concerns for children's brain development but also low dose exposure especially to a mixture of compounds is thought to contribute to the increasing incidences of neurodevelopmental disorders currently observed (Bennett et al., 2016; Grandjean & Landrigan, 2014). One consequence of impaired brain development is a loss in cognitive function measured by the intelligence quotient (IQ). A reduction in mean IQ of a population by only five points, e.g. owed to lead background exposure, leads to a decrease in the number of gifted and simultaneously an increase of less gifted by 57% each (Schmidt, 2013). Hence, there is an individual as well as the societal impact of neurodevelopmental toxicity.

According to the World Health Organization (WHO), 5% of the world's children under 15 years of age have some type of moderate to severe neurophysical or cognitive-developmental disability (WHO, 2008). In industrialized countries like the United States, one out of six children between 3 and 17 years of age suffers from developmental disabilities (Boyle et al., 2011; Grandjean and Landrigan, 2006). These include attention deficit hyperactivity disorder, intellectual disability, hearing or vision loss, cerebral palsy, autism, seizures, stuttering or stammering, and learning disorders (Boyle et al., 2011). In most cases, the etiology of such disorders is largely unknown, yet in many cases, multifactorial mechanisms involving genetic, as well as environmental factors, are proposed (De Felice et al., 2016). Strong causative links between chemicals and specific neurodevelopmental health outcomes are proposed, but still have to be established (De Felice et al., 2016; Grandjean and Landrigan, 2006). Specifically, so far only a very limited number of human DNT compounds related to the environment have been identified. These include lead, methylmercury, polychlorinated biphenyls, arsenic, toluene, manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and the polybrominated diphenyl ethers (Grandjean and Landrigan, 2014).

The largest concern, therefore, is currently the lack of data on DNT for most of the environmental pollutants that humans, including pregnant women and young children, are exposed to (Egeghy et al., 2016; Ring et al., 2017). Of the approximately 100.000 chemicals in our everyday environment (Hartung et al., 2011) only 100–140 have been tested for their adverse effects on the developing brain so far (Makris et al., 2009; Paparella et al., 2020). The reason for this data gap lies in the current regulatory DNT testing. Environmental Protection Agency of the US (EPA) or Organization for Economic Co-operation and Development (OECD) test guidelines (OECD, 2007; US EPA, 1998) use *in vivo* rodent models that require high costs and very long times. Therefore, they are not suited for screening large numbers of chemicals (Crofton et al., 2011). Currently, the international consensus is that alternative strategies are needed to fill the knowledge gap on the DNT potential of thousands of chemicals in our environment to protect children's health. Hence, there is currently a paradigm shift for DNT evaluation that moves from solely testing for DNT *in vivo* in animals to DNT *in vitro* evaluation followed

by *in vivo* testing when necessary. This procedure will allow testing of large numbers of chemicals thereby reducing the data gap on a plethora of compounds and is thus strongly supported by the different stakeholders, academia, regulators, and industry (Behl et al., 2019; Fritsche et al., 2018; Sachana et al., 2019). To finally implement these actions into practice, the OECD provides a forum to develop internationally harmonized guidance on the use and interpretation of the DNT IVB by preparing an OECD guidance document on its application and interpretation of data (Sachana et al., 2021).

Alternative strategies for DNT evaluation

Due to the demand for testing environmental hazards for their potential to be developmentally neurotoxic, a variety of *in vitro* test methods have been set up for DNT evaluation (Bal-Price et al., 2018) following defined criteria (Crofton et al., 2011). These *in vitro* tests form a DNT *in vitro* battery (DNT IVB) that covers a variety of neurodevelopmental processes that are fundamental for brain development (Bal-Price et al., 2015, 2018; Fritsche et al., 2018; Masjosthusmann et al., 2020). Such include neural progenitor cell (NPC) proliferation, NPC differentiation into neurons and oligodendrocytes, neurite outgrowth, migration of neural crest cells, radial glia, neurons, and oligodendrocytes as well as neuronal network formation (Bal-Price et al., 2018; Masjosthusmann et al., 2020). These test methods of the DNT IVB are primarily based on human stem/progenitor cells and mainly utilize high content imaging analyses (HCA) for endpoint evaluation. This allows medium-throughput testing of compounds (Masjosthusmann et al., 2020). Examples of such images for endpoint evaluation are shown in Figure 2(A–E). Here, primary human NPC growing as neurospheres were plated onto laminin-coated surfaces for inducing NPC migration and differentiation. From these differentiated neurospheres, a large variety of endpoints can be assessed simultaneously due to multiple immunocytochemical stainings (Baumann et al., 2016; Nimtz et al., 2020) and a self-made software using artificial intelligence that was created in close collaboration with Axel Mosig (Ruhr-University Bochum) (Förster et al., unpublished data; Schmuck et al., 2017). Furthermore, hiPSC-derived neurons, neural crest cells (NCC), and LUHMES cells for assessing compound effects on neuronal network formation on MEAs, NCC migration, and neurite outgrowth of central and peripheral neurons complete the current battery (Figure 2(F–I)). As science evolves, also the DNT IVB will be expanded to cover the most crucial key processes in neurodevelopment (Masjosthusmann et al., 2020). However, results from the current DNT IVB have already been used for hazard assessment by the European Food Safety Authority (EFSA). Here an adverse outcome pathway (AOP)-informed Integrated Approach to Testing and Assessment (IATA) was applied for the first time for DNT hazard assessment. In this case study, the pesticide deltamethrin was reported to impact oligodendrocyte differentiation and neuronal network formation at nanomolar concentrations, an order of magnitude, which corresponds to fetal brain concentrations producing adverse effects *in vivo* (EFSA PPR Panel, 2021).

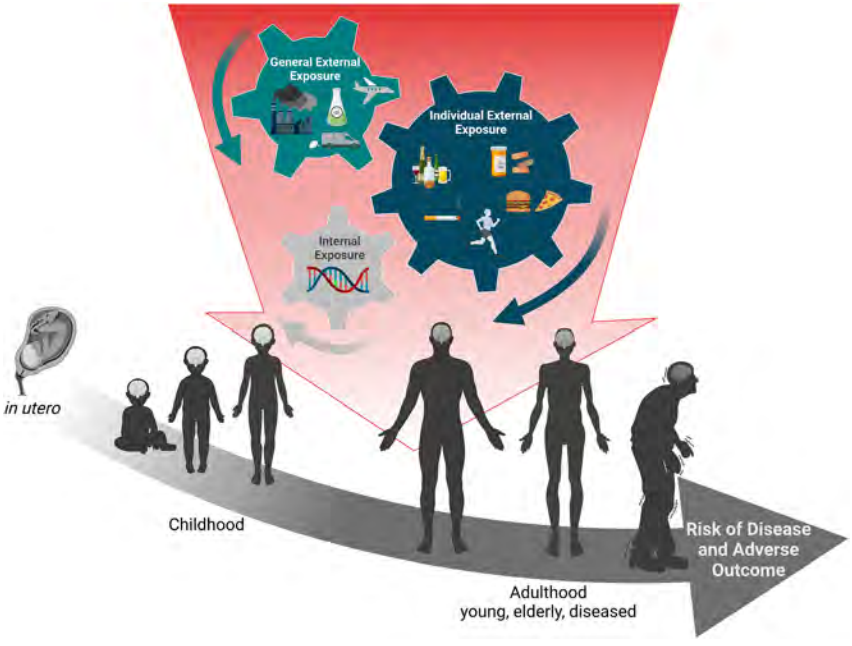

Figure 1: Overview of different human real-life exposures impacting the central nervous system (CNS). Exposures increase the risk of disease leading to an adverse outcome in a life stage-dependent manner. Exposure scenarios are divided into general external exposure (e.g. exhaust particles from industrial plants, motor vehicles/aircraft, and chemicals in the environment), specific external exposure (e.g. dietary factors, physical activity, drugs, alcohol, or tobacco consumption), and internal exposure due to e.g. inter-individual differences in gene expression concerning metabolism or resilience and hormonal signaling. Created with BioRender.com.

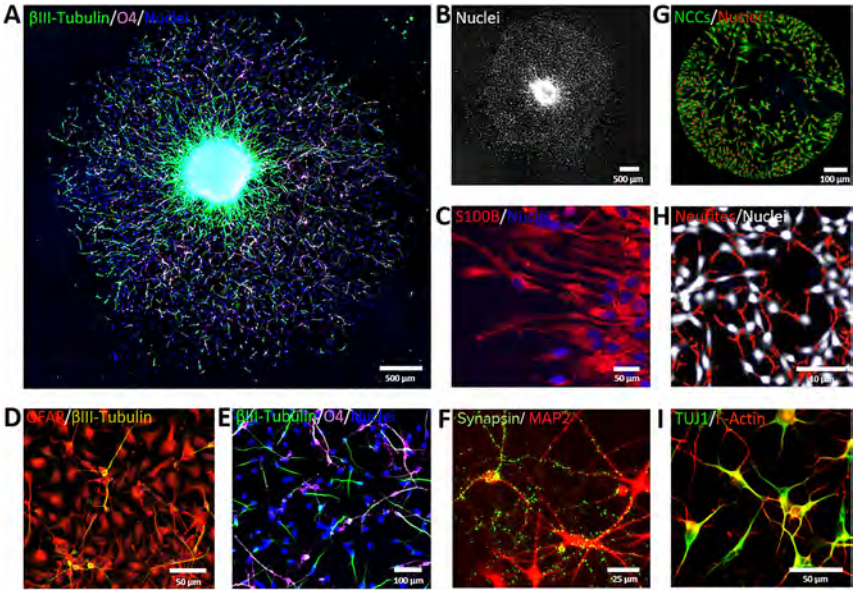

Figure 2: The current DNT IVB as described in Masjosthusmann et al., 2020. Due to the high developmental dynamic of human neurospheres (Mashosthusmann et al. 2018a), cell type composition differs due to differentiation time point (A–E). A: A representative image of a 5 days differentiated primary human neurosphere immunocytochemically stained with anti-βIII-Tubulin (neurons, green), anti-O4 (oligodendrocytes, pink), and Hoechst (nuclei, blue). Image was taken with high content imaging using the Cellomics ArrayScan. B: Binary representative image of 5 days differentiated primary human neurosphere. Hoechst + nuclei are shown in white and are used to measure migration distances from the sphere core to the edges of the migration area via a self-made software algorithm (Schmuck et al. 2017). C: Representative image of S100β + radial glia cells (red) of 24 h differentiated primary human neurosphere. D: Representative image of GFAP + astrocytes (red) and βIII-Tubulin + neurons (yellow) in the migration area of 5 days differentiated primary human neurosphere. E: Representative image of βIII-Tubulin + neurons (green) and O4+ oligodendrocytes (pink) in the migration area of 5 days differentiated primary human neurosphere. F: SynFire® cells consisting of hiPSC-derived neurons and human primary astrocytes (SynFire® Co-Culture Kit (MEA), NeuCyte, USA), which are plated on multi electrode arrays (MEAs) to assess the effects of compounds on the electrical activity of the cells. G: Representative images of neural crest cells (NCC) labeled in green (calcein-AM), nuclei are shown in red. These cells are used in the cMINC (UKN2) assay for measuring NCC migration (Nyffeler et al., 2017). H: Representative image of 24 h differentiated LUHMES cells (neurites marked in red, nuclei in white). These cells are used in the NeuriTox (UKN4) assay to evaluate CNS neurite outgrowth (Krug et al., 2013a; Stiegler et al., 2011). I: Representative image of hiPSC-derived sensory neurons (neurites marked in red, nuclei in white). These cells are used in the PeriTox (UKN5) assay to evaluate peripheral nervous system neurite outgrowth (Hoelting et al., 2016). Assays presented in A-F and G-I were developed at the IUF and at the University of Konstanz, Germany, respectively.

In addition to the utilization for screening, we have been studying modes-of-action (MoA) of a variety of environmental contaminants in the human neurospheres (Figure 2(A–E)). For example, phenomics and transcriptome analyses of neurospheres exposed to flame retardants during differentiation revealed that different substances of this compound class affected oligodendrocyte development by distinct MoA, including generation of oxidative stress and disruption of thyroid hormone signaling (Klose et al., 2021). Also, the food supplement epigallocatechin gallate inhibits the adhesion and migration of human NPC by interfering with cellular integrin-laminin binding (Barenys et al., 2017). Such studies describe the applicability domains of *in vitro* assays by understanding their power of detecting distinct MoA. As stem cell-based assays will be the basis for toxicity testing in the 21st century (Fritsche et al., 2021b), it is important to understand their potential and even more importantly their weaknesses.

Adult neurotoxicity

When adults are exposed to environmental pollutants, effects are largely different from developmental exposure because the processes that take place in the developing brain, like e.g. proliferation of NPC or neurogenesis, are found only as a fraction in the adult brain (Eriksson et al., 1998; Spalding et al., 2013). Hence, adult neurotoxicity plays by different rules than DNT primarily disrupting or even killing neurons or the surrounding glial cells, influencing the transmission and processing of signals in the brain and other parts of the nervous system (Fritsche and

Hogberg, 2020; Masjosthusmann et al., 2018b). Adult neurotoxicity can be acute as in poisoning incidents, yet currently, more and more attention is given toward long-term effects of low-dose exposure to environmental pollutants. Here, especially the aged brain is of relevance as brain cells show signs of compromised bioenergetics, impaired adaptive neuroplasticity and resilience, aberrant neuronal network activity, dysregulation of neuronal Ca^{2+} homeostasis, the accrual of oxidatively modified molecules and organelles like mitochondria, as well as inflammation. These render the aged brain more susceptible to Alzheimer's (AD) and Parkinson's diseases (PD) as well as stroke (Mattson et al., 2018). A special emphasis should be placed on mitochondrial dysfunction, which is involved in the pathologies of neurodegenerative disorders (Rey et al., 2022). This per se higher vulnerability of aged brain cells for disease compared to younger tissue also increases their sensitivity toward pollutant exposure and hence for environmental contribution to neurodegenerative diseases.

Neurodegenerative diseases are a heterogeneous group of disorders that are characterized by the progressive degeneration of the structure and function of the central nervous system or the peripheral nervous system with unknown etiology. These conditions, which are characterized by cognitive and functional decline, include amongst others mild cognitive impairment, dementia, AD, PD, and amyotrophic lateral sclerosis. The onset of neurodegenerative diseases can occur at any stage throughout life but the risk of being affected increases as a function of age, particularly after the third decade of life. It is predicted that this prevalence will continue to grow with changing age demographics toward an aged population, the growth of the global population, and increasing exposure to environmental toxins (Dugger et al., 2017; Feigin et al., 2020). A recent workshop report of the US-National Academies of Sciences, Engineering, and Medicine (2020) also clearly points out that a complex combination of genetic and environmental factors is thought to contribute to the pathogenesis of neurodegenerative disorders.

Alzheimer's disease

Exposure to air pollution has been linked to a variety of health outcomes, including cardiovascular and respiratory disease, however, in recent years the evidence has accumulated that air pollution exposure is associated with impaired cognitive function (Ailshire et al., 2017; Maragakis et al., 2006), accelerated cognitive decline (Schikowski et al., 2015; Tzivian et al., 2015), AD and all-cause dementia (Calderon-Garciduenas et al., 2019; Chen et al., 2017; Lelieveld et al., 2015; Peters et al., 2019; Shi, 2020; Tham and Schikowski, 2021). Exposure to particulate matter (PM) from air pollution was associated with accelerated loss of both gray and white matter as well as with a decline in episodic memory (Casanova et al., 2016; Younan et al., 2020). It is even suggested that half of the individual AD risk may be environmental (Gatz et al., 2006). The two fractions of PM predominantly implicated in neurodegenerative effects have sizes <2.5 μm ($PM_{2.5}$) and <0.1 μm (ultrafine particles; UFP) and are mostly derived from tailpipe and brake emissions from motor vehicles, aircraft, and marine vessels (Karagulian et al., 2015). Size matters as different sizes of PM from air pollution induce variable effects on different aspects of episodic memory. $PM_{2.5}$, for example, seems to be more strongly associated with the encoding aspect of episodic memory compared to retrieval and long-term recall aspects, suggesting that it may be impacting brain regions associated with learning new material versus long-term recall (Petkus et al., 2020).

Air pollution triggers oxidative stress and inflammation (Arias-Pérez et al., 2020; Costa et al., 2020), both contributing to the accumulation of hallmark neuropathologies associated with AD, including amyloid β and tau tangles, brain atrophy, cognitive decline, and eventually dementia (Berr et al., 2000; Calderon-Garciduenas et al., 2019; Cervellati et al., 2014; Costa et al., 2020; Levesque et al., 2011). Lately, signs of tau pathology, i.e. hyperphosphorylated tau (*P*-Tau), were even found in infants of Mexico City. These were detected at portals of entry of particulate material, the lower medulla, and the olfactory bulb (Calderon-Garciduenas et al., 2019). Also, *P*-Tau neurites were found in the dorsal motor nucleus of the vagus nerve and spinal trigeminal nucleus in the lower medulla sections of these infants. The nasal pathway via the olfactory bulb is also a potential portal for the direct transport of particles to the brainstem. In adults, the olfactory bulb has been found to be loaded with particulate matter and impaired olfactory function is a precursor of Alzheimer's disease (Sun et al., 2012). However, the exposure to particulate matter strongly depends on geographic location and the source of particulate matter. Urbanized areas with high exposures to the combustion-derived particulate matter have more detrimental effects on the brains of young people. These data support the notion that direct particle effects in the brain might contribute to AD pathologies.

In addition, there are more systemic approaches for explaining the pathophysiology of AD. For example, the same risk factors associated with cardiovascular and respiratory diseases are possible mediators for the association between air pollutants and neurological disorders (Hüls

et al., 2018; Saito et al., 2016). Global cortical Aβ deposition has also been associated with different cardiovascular risk factors such as diabetes and hypertension (Gottesman et al., 2017). As the health effects of PM on the cardiovascular system have been well described (Al-Kindi et al., 2020), one can envision the cardiovascular system as a possible link between particle exposure and AD. More research is clearly needed that enlightens the adverse outcome pathway(s) for air pollution exposure and the development of AD.

Gene-environment interactions also seem to play a role in environmentally induced AD. Higher concentrations of air pollution are associated with more rapid cognitive decline, particularly in carriers of the APOE4 allele, which is the strongest genetic risk factor for AD (Kulick et al., 2020). Animal studies supported this interplay between particle exposure, APOE4 genotype, and pathognomonic signs of AD (Cacciottolo et al., 2020; Haghani et al., 2020).

Parkinson's disease

Environmental exposure also increases the risk for PD. Recently, a meta-analysis found an association between pesticide exposure in general or paraquat specifically and the development of PD (Ntzani et al., 2013). Combined exposure, e.g. of the two pesticides paraquat and maneb, was associated with an approximately 75% increase in the risk of developing PD (Costello et al., 2009). These epidemiological observations were further mechanistically substantiated by the EFSA linking exposure toward the pesticides rotenone and paraquat to the adverse outcome. Mitochondrial dysfunction, impaired proteostasis, and degeneration of dopaminergic neurons of the nigrostriatal pathway were identified as key events in the adverse outcome pathways (Ockleford et al., 2017; Terron et al., 2018). Similar to AD, gene-environment interaction seems to increase the risk for developing PD. Carriers of a PON1 mutant genotype, that codes for a slow metabolizer phenotype, exerted a higher risk of PD when exposed to organophosphate pesticides compared to a nonexposed group (Lee et al., 2013; Narayan et al., 2013). Here, the genotype itself is not only a risk factor but also needs pesticide exposure for its disease contribution.

Besides pesticides, there is ample evidence that people highly exposed to traffic have an increased risk of PD (National Academy of Science, 2020). The risk for developing PD upon exposure to traffic pollutants is also elevated by a certain genetic predisposition. Carriers of a mutant interleukin-1β gene, which increases inflammatory responses in the brain, have an increased risk for developing PD when highly exposed to traffic pollution (Lee et al., 2016).

Alternative strategies for studying neurotoxicity

Neurotoxicity studies are generally performed in iterative tiered approaches in animals (Legradi et al., 2018). Besides the high resources that they require (time, money, animals), translation of results from animals to humans is often insufficient, e.g. leading to high attrition rates in the central nervous system (CNS) drug development (Fritsche and Hogberg, 2020). In addition, disease models truly representing human CNS disease are sparse. Therefore, there has been an effort to establish human-based *in vitro* models for studying the adverse effects of environmental pollutants on human physiology (Masjosthusmann et al., 2018b). While two-dimensional (2D) cultures using human cells have been relatively common, research of the last years focussed on generating three-dimensional (3D) cell systems based on human stem cells, i.e. human embryonic or lately mainly human-induced pluripotent stem cells (hiPSC; Fritsche et al., 2020; Pașca, 2018). If one wants to grow such 3D CNS models, one has at least two different options. Producing neural spheroids, also known as brain spheres, which are 3D aggregate cultures of stem cell-based NPC; or manufacturing 3D brain organoids. Brain spheres have the advantage that they contain the crucial cell types of the brain, i.e. neurons, astrocytes, and oligodendrocytes and can be produced in large numbers in a uniform manner (Pamies et al., 2017). It is even possible to add microglia to such systems (Abreu et al., 2018), a cell type transporting neuroprotection and neuroinflammation, which is therefore highly relevant for human disease (Harry, 2021). Despite their highly relevant cell type composition and dimensionality, they lack anatomical structures of the brain (Figure 3(A)). However, 3D brain spheres reach a degree of maturation that allows measuring neuronal activity, e.g. on microelectrode arrays (Figure 3(B)), and thus allows testing of compounds in a highly relevant, functional assay. In contrast, brain organoids contain much more complexity than brain spheroids as they form anatomical structures like cortices (Lancaster et al., 2013) and protocols are available for also including microglia (Bodnar et al., 2021; Xu et al., 2021). Due to their increased complexity and long culture periods, they render suitable for disease modeling and have so far been used e.g. generating AD (Cordella et al., 2022), PD (Kim et al., 2021), stroke (Song et al., 2021), as well as models for other

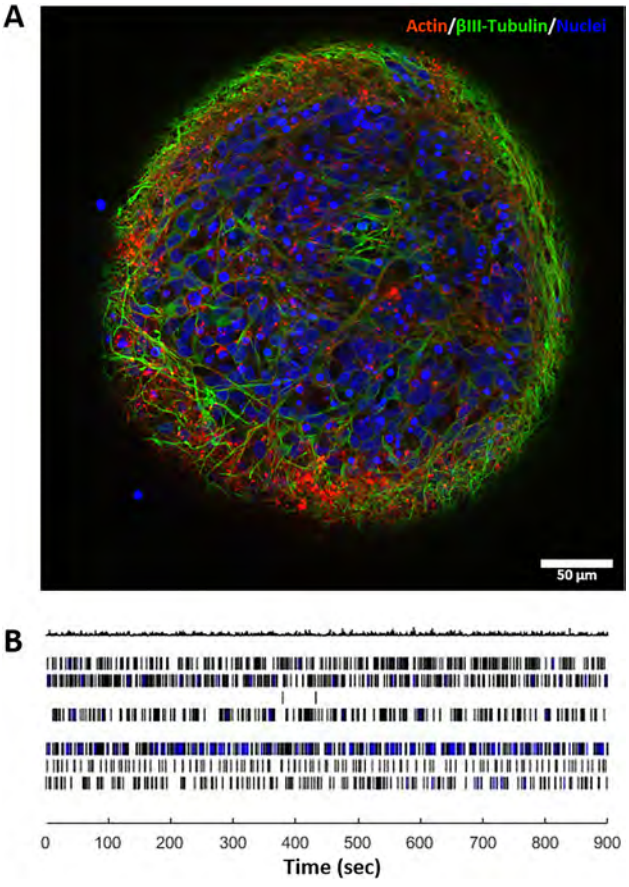

Figure 3: Example of a 3D *in vitro* model and one of its readouts for neurotoxicity evaluation. A: Brain sphere differentiated in 3D from hiPSC as described in Kapr et al., 2021. B: Neuronal activity of such a brain sphere measured after growing for 21 days on a multi electrode array (MEA). A representative spike raster plot indicates black bars as spikes and blue bars as bursts, which represent neuronal network maturity. Rows represent individual electrodes.

psychiatric disorders (Unterholzner et al., 2021). Due to rapid advances in the biotechnological and stem cell fields, brain organoids combined with genome editing and possibly 3D bioprinting are likely to shape toxicity testing and disease modeling in the future (Fritsche et al., 2020).

Conclusions

Neurotoxicity induced by environmental pollutants across different human life stages from the embryo to the elderly is of high societal relevance as diseases associated with neurodevelopmental as well as neurodegenerative disorders place heavy burdens on individuals, families, and society. Hence, knowledge of the hazards of pollutants that impact mental health is crucial for the protection of the brain. Novel technologies are already in place and are currently further developed to achieve time- and cost-saving tools for linking MoA-based hazards to adverse health effects observed in humans.

Owed to the multiple exposures of humans to a plethora of substances in our every-day-environment, e.g. pesticides, flame retardants, air pollution, and potential disease-modifying factors like socioeconomic status, physical fitness, psychological health, and subjective feeling of happiness, it is rather difficult to causally link background exposure to a single chemical with a disease. Therefore, the adverse outcome pathway concept as exemplified for pesticide exposure linked to parkinsonian motor deficits (Ockleford et al., 2017) will strongly support and is a novel way forward for unraveling hazards and risks of pollutant exposure for mental health in the future.

Emerging issues like challenging viral infections or climate change and their direct and indirect adverse effects on the brain are challenges that lie ahead of us to tackle. Interdisciplinary research embracing researchers from the fields of chemistry, medicine, cell biology, toxicology, biotechnology, biostatistics, bioinformatics, and epidemiology will be needed for future brain protection.

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References

Abreu, C.M., Gama, L., Krasemann, S., Chesnut, M., Odwin-Dacosta, S., Hogberg, H.T., Hartung, T., and Pamies, D. (2018). Microglia increase inflammatory responses in iPSC-derived human brainSpheres. Front. Microbiol. *9*, 2766.

Ailshire, J., Karraker, A., and Clarke, P. (2017). Neighborhood social stressors, fine particulate matter air pollution, and cognitive function among older U.S. adults. Soc. Sci. & Med. *172*, 56–63.

Al-Kindi, S.G., Brook, R.D., Biswal, S., and Rajagopalan, S. (2020). Environmental determinants of cardiovascular disease: lessons learned from air pollution. Nat. Rev. Cardiol. *17*, 656–672.

Arias-Pérez, R.D., Taborda, N.A., Gómez, D.M., Narvaez, J.F., Porras, J., and Hernandez, J.C. (2020). Inflammatory effects of particulate matter air pollution. Environ. Sci. Pollut. Res. Int. *27*, 42390–42404.

Bal-Price, A., Crofton, K.M., Leist, M., Allen, S., Arand, M., Buetler, T., Delrue, N., FitzGerald, R.E., Hartung, T., Heinonen, T., et al. (2015). International STakeholder NETwork (ISTNET): creating a developmental neurotoxicity (DNT) testing road map for regulatory purposes. Arch. Toxicol. *89*, 269–287.

Bal-Price, A., Pistollato, F., Sachana, M., Bopp, S.K., Munn, S., and Worth, A. (2018). Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using in vitro methods. Toxicol. Appl. Pharmacol. *354*, 7–18.

Barenys, M., Gassmann, K., Baksmeier, C., Heinz, S., Reverte, I., Schmuck, M., Temme, T., Bendt, F., Zschauer, T.-C., Rockel, T.D., et al. (2017). Epigallocatechin gallate (EGCG) inhibits adhesion and migration of neural progenitor cells in vitro. Arch. Toxicol. *91*, 827–837.

Baumann, J., Gassmann, K., Masjosthusmann, S., DeBoer, D., Bendt, F., Giersiefer, S., and Fritsche, E. (2016). Comparative human and rat neurospheres reveal species differences in chemical effects on neurodevelopmental key events. Arch. Toxicol. *90*, 1415–1427.

Behl, M., Ryan, K., Hsieh, J.H., Parham, F., Shapiro, A.J., Collins, B.J., Sipes, N.S., Birnbaum, L.S., Bucher, J.R., Foster, P.M.D., et al. (2019). Screening for developmental neurotoxicity at the national toxicology program: the future is here. Toxicol. Sci. *167*, 6–14.

Bennett, D., Bellinger, D.C., Birnbaum, L.S., Bradman, A., Chen, A., Cory-Slechta, D.A., Engel, S.M., Fallin, M.D., Halladay, A., Hauser, R., et al. (2016). Project TENDR: targeting environmental neuro-developmental risks the TENDR consensus statement. Environ. Health Perspect. *124*, A118–122.

Berr, C., Balansard, B., Arnaud, J., Roussel, A.M., and Alperovitch, A. (2000). Cognitive decline is associated with systemic oxidative stress: the EVA study. Etude du Vieillissement Arteriel. J. Am. Geriatr. Soc. *48*, 1285–1291.

Bodnar, B., Zhang, Y., Liu, J., Lin, Y., Wang, P., Wei, Z., Saribas, S., Zhu, Y., Li, F., Wang, X., et al. (2021). Novel scalable and simplified system to generate microglia-containing cerebral organoids from human induced pluripotent stem cells. Front. Cell. Neurosci. *15*, 682272.

Boyle, C.A., Boulet, S., Schieve, L.A., Cohen, R.A., Blumberg, S.J., Yeargin-Allsopp, M., Visser, S., and Kogan, M.D. (2011). Trends in the prevalence of developmental disabilities in US children, 1997-2008. Pediatrics *127*, 1034–1042.

Cacciottolo, M., Morgan, T.E., Saffari, A.A., Shirmohammadi, F., Forman, H.J., Sioutas, C., and Finch, C.E. (2020). Traffic-related air pollutants (TRAP-PM) promote neuronal amyloidogenesis through oxidative damage to lipid rafts. Free Radical Biol. Med. *147*, 242–251.

Calderón-Garcidueñas, L., Mukherjee, P.S., Kulesza, R.J., Torres-Jardón, R., Hernández-Luna, J., Ávila-Cervantes, R., Macías-Escobedo, E., González-González, O., González-Maciel, A., García-Hernández, K., et al. (2019). Mild cognitive impairment and dementia involving multiple cognitive domains in Mexican urbanites. J. Alzheim. Dis. *68*, 1113–1123.

Casanova, R., Varma, S., Simpson, B., Kim, M., An, Y., Saldana, S., Riveros, C., Moscato, P., Griswold, M., Sonntag, D., et al. (2016). Blood metabolite markers of preclinical Alzheimer's disease in two longitudinally followed cohorts of older individuals. Alzheim. Dement. *12*, 815–22.

Cervellati, C., Romani, A., Seripa, D., Cremonini, E., Bosi, C., Magon, S., Bergamini, C.M., Valacchi, G., Pilotto, A., and Zuliani, G. (2014). Systemic oxidative stress and conversion to dementia of elderly patients with mild cognitive impairment. BioMed Res. Int. *2014*, 309507.

Chang, L (1995). Handbook of Neurotoxicology. Chang L.W. and Dyer R.S., eds. (Taylor & Francis Group).

Chen, H., Kwong, J.C., Copes, R., Tu, K., Villeneuve, P.J., van Donkelaar, A., Hystad, P., Martin, R.V., Murray, B.J., Jessiman, B., et al. (2017). Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: a population-based cohort study. Lancet *389*, 718–726.

Cordella, F., Brighi, C., Soloperto, A., and Di Angelantonio, S. (2022). Stem cell-based 3D brain organoids for mimicking, investigating, and challenging Alzheimer's diseases. Neural Regen. Res. *17*, 330–332.

Costa, L.G., Cole, T.B., Dao, K., Chang, Y.C., Coburn, J., and Garrick, J.M. (2020). Effects of air pollution on the nervous system and its possible role in neurodevelopmental and neurodegenerative disorders. Pharmacol. Ther. *210*, 107523.

Costello, S., Cockburn, M., Bronstein, J., Zhang, X., and Ritz, B. (20092009). Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am. J. Epidemiol. *169*, 919–26.

Crofton, K.M., Mundy, W.R., Lein, P.J., Bal-Price, A., Coecke, S., Seiler, A.E., Knaut, H., Buzanska, L., and Goldberg, A. (2011). Developmental neurotoxicity testing: recommendations for developing alternative methods for the screening and prioritization of chemicals. ALTEX *28*, 9–15.

De Felice, A., Greco, A., Calamandrei, G., and Minghetti, L. (2016). Prenatal exposure to the organophosphate insecticide chlorpyrifos enhances brain oxidative stress and prostaglandin E2 synthesis in a mouse model of idiopathic autism. J. Neuroinflammation *13*, 149.

Dugger, B.N., and Dickson, D.W. (2017). Pathology of neurodegenerative diseases. Cold Spring Harb. Perspect. Biol. *9*, a028035.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Hernández-Jerez, A., Adriaanse, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Focks, A., Marinovich, M., Millet, M., et al. (2021). Scientific opinion on the development of integrated approaches to testing and assessment (IATA) case studies on developmental neurotoxicity (DNT) risk assessment. EFSA J. *19*, 6599.

Egeghy, P.P., Sheldon, L.S., Isaacs, K.K., Özkaynak, H., Goldsmith, M.-R., Wambaugh, J.F., Judson, R.S., and Buckley, T.J. (2016). Computational exposure science: an emerging discipline to support 21st-century risk assessment. Environ. Health Perspect. *124*, 697–702.

EPA, U.S. (1996). Health Effects Test Guidelines OPPTS 870.6300 Developmental Neurotoxicity Study. (Environmental Protection Agency (EPA)), Available at: https://nepis.epa.gov/Exe/ZyNET.exe/P100G6UI.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1995+Thru+1999&Docs=&Query=&Time=&EndTime=&

SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C95thru99%5CTxt%5C00000033%5CP100G6UI.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL.

EPA, U.S. (1998). Health Effects Guidelines OPPTS 870.6300 Developmental Neurotoxicity Study. Office of Prevention Pesticides and Toxic Substances (Environmental Protection Agency (EPA)), Available at: https://nepis.epa.gov/Exe/ZyNET.exe/P100IRWO.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1995+Thru+1999&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C95thru99%5CTxt%5C00000034%5CP100IRWO.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the adult human hippocampus. Nat. Med. *4*, 1313–7.

Feigin, V.L., Vos, T., Nichols, E., Owolabi, M.O., Carroll, W.M., Dichgans, M., Deuschl, G., Parmar, P., Brainin, M., and Murray, C. (2020). The global burden of neurological disorders: translating evidence into policy. Lancet Neurol. *19*, 255–265.

Fritsche, E., and Hogberg, H.T. (2020). A brainer on neurotoxicity. Front. Toxicol. *2*, 3.

Fritsche, E., Grandjean, P., Crofton, K.M., Aschner, M., Goldberg, A., Heinonen, T., Hessel, E.V.S., Hogberg, H.T., Bennekou, S.H., Lein, P.J., et al. (2018). Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes. Toxicol. Appl. Pharmacol. *354*, 3–6.

Fritsche, E., Tigges, J., Hartmann, J., Kapr, J., Serafini, M.M., and Viviani, B. (2021a). Neural in vitro models for studying substances acting on the central nervous system. Handb. Exp. Pharmacol. *265*, 111–141.

Fritsche, E., Haarmann-Stemmann, T., Kapr, J., Galanjuk, S., Hartmann, J., Mertens, P.R., Kämpfer, A.A.M., Schins, R.P.F., Tigges, J., and Koch, K. (2021b). Stem cells for next level toxicity testing in the 21st century. Small *17*, 2006252.

Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A., and Pedersen, N.L. (2006). Role of genes and environments for explaining Alzheimer disease. Arch. Gen. Psychiatry *63*, 168–74.

Gottesman, R.F., Schneider, A.L., Zhou, Y., Coresh, J., Green, E., Gupta, N., Knopman, D.S., Mintz, A., Rahmim, A., Sharrett, A.R., et al. (2017). Association between midlife vascular risk factors and estimated brain amyloid deposition. J. Am. Med. Assoc. *317*, 1443–1450.

Grandjean, P., and Landrigan, P.J. (2006). Developmental neurotoxicity of industrial chemicals. Lancet *368*, 2167–2178.

Grandjean, P., and Landrigan, P.J. (2014). Neurobehavioural effects of developmental toxicity. Lancet Neurol. *13*, 330–338.

Haghani, A., Cacciottolo, M., Doty, K.R., D'Agostino, C., Thorwald, M., Safi, N., Levine, M.E., Sioutas, C., Town, T.C., Forman, H.J., Zhang, H., Morgan, T.E., and Finch, C.E. (2020). Mouse brain transcriptome responses to inhaled nanoparticulate matter differed by sex and APOE in Nrf2-Nfkb interactions. ELife *9*, https://doi.org/10.7554/elife.54822.

Harry, G.J. (2021). Microglia in neurodegenerative events-an initiator or a significant other? Int. J. Mol. Sci. *22*, 5818.

Hartung, T., and McBride, M. (2011). Food for thought … on mapping the human toxome. ALTEX *28*, 83–93.

Hoelting, L, Klima, S, Karreman, C, Grinberg, M, Meisig, J, Henry, M, Rotseteyn, T, Rahnenführer, N, Blüthgen, N, and Sachinidis, A, et al. (2016). Stem cell-derived immature human dorsal root ganglia neurons to identify peripheral neurotoxicants. Stem Cells Transl. Med. *5*, 476–87.

Hüls, A., Vierkötter, A., Sugiri, D., Abramson, M.J., Ranft, U., Krämer, U., and Schikowski, T. (2018). The role of air pollution and lung function in cognitive impairment. Eur. Respir. J. *51*, 1701963.

Kapr, J., Petersilie, L., Distler, T., Lauria, I., Bendt, F., Sauter, C.M., Boccaccini, A.R., Rose, C.R., and Fritsche, E. (2021). Human induced pluripotent stem cell-derived neural progenitor cells produce distinct neural 3D in vitro models depending on Alginate/Gellan Gum/Laminin hydrogel blend properties. Adv. Healthc. Mater. *10*: e2100131.

Karagulian, F., Belis, C.A., Dora, C.F.C., Prüss-Ustün, A.M., Bonjour, S., Adair-Rohani, H., and Amann, M. (2015). Contributions to cities' ambient particulate matter (PM): a systematic review of local source contributions at global level. Atmos. Environ. *120*, 475–483.

Kim, S.W., Woo, H.J., Kim, E.H., Kim, H.S., Suh, H.N., Kim, S.H., Song, J.J., Wulansari, N., Kang, M., and Choi, S.Y., et al. (2021). Neural stem cells derived from human midbrain organoids as a stable source for treating Parkinson's disease: midbrain organoid-NSCs (Og-NSC) as a stable source for PD treatment. Progress in neurobiology *204*, 102086.

Klose, J., Tigges, J., Masjosthusmann, S., Schmuck, K., Bendt, F., Hübenthal, U., Petzsch, P., Köhrer, K., Koch, K., and Fritsche, E. (2021). BBPA targets converging key events of human oligodendrocyte development resulting in two novel AOPs. ALTEX-Altern. Anim. Exp. *38*, 215–234.

Krug, A.K., Balmer, N.V., Matt, F., Schönenberger, F., Merhof, D., and Leist, M. (2013). Evaluation of a human neurite growth assay as specific screen for developmental neurotoxicants. Arch. Toxicol. *87*, 2215–31.

Kulick, E.R., Elkind, M.S.V., Boehme, A.K., Joyce, N.R., Schupf, N., Kaufman, J.D., Mayeux, R., Manly, J.J., and Wellenius, G.A. (2020). Long-term exposure to ambient air pollution, APOE-ε4 status, and cognitive decline in a cohort of older adults in northern Manhattan. Environ. Int. *136*, 105440.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373–379.

Lee, P.C., Rhodes, S.L., Sinsheimer, J.S., Bronstein, J., and Ritz, B. (2013). Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. Environ. Int. *56*, 42–47.

Lee, P.C., Raaschou-Nielsen, O., Lill, C.M., Bertram, L., Sinsheimer, J.S., Hansen, J., and Ritz, B. (2016). Gene-environment interactions linking air pollution and inflammation in Parkinson's disease. Environ. Res. *151*, 713–720.

Legradi, J.B., Di Paolo, C., Kraak, M., van der Geest, H.G., Schymanski, E.L., Williams, A.J., Dingemans, M., Massei, R., Brack, W., Cousin, X., et al. (2018). An ecotoxicological view on neurotoxicity assessment. Environ. Sci. Eur. *30*, 46.

Lelieveld, J., Evans, J.S., Fnais, M., Giannadaki, D., and Pozzer, A. (2015). The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature *525*, 367–371.

Levesque, S., Surace, M.J., McDonald, J., and Block, M.L. (2011). Air pollution & the brain: subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. J. Neuroinflammation *8*, 105.

Magby, J., and Richardson, J. (2018). 5.17 – developmental neurotoxicology☆. Comprehensive Toxicology. McQueen, C.A., ed., 3rd ed. (Elsevier), pp. 250–256.

Makris, S.L., Raffaele, K., Allen, S., Bowers, W.J., Hass, U., Alleva, E., Calamandrei, G., Sheets, L., Amcoff, P., Delrue, N., and Crofton, K.M. (2009). A retrospective performance assessment of the developmental neurotoxicity study in support of OECD test guideline 426. Environ. Health Perspect. *117*, 17–25.

Maragakis, N.J., and Rothstein, J.D. (2006). Mechanisms of disease: astrocytes in neurodegenerative disease. Nat. Clin. Pract. Neurol. *2*, 679–689.

Masjosthusmann, S., Becker, D., Petzuch, B., Klose, J., Siebert, C., Deenen, R., Barenys, M., Baumann, J., Dach, K., Tigges, J., et al. (2018a). A transcriptome comparison of time-matched developing human, mouse and rat neural progenitor cells reveals human uniqueness. Toxicol. Appl. Pharmacol. *354*, 40–55.

Masjosthusmann, S., Barenys, M., El-Gamal, M., Geerts, L., Gerosa, L., Gorreja, A., Kühne, B., Marchetti, N., Tigges, J., Viviani, B., et al. (2018b). Literature review and appraisal on alternative neurotoxicity testing methods. EFSA Support. Publ. *15*, 1410E.

Masjosthusmann, S., Blum, J., Bartmann, K., Dolde, X., Holzer, A.-K., Stürzl, L.-C., Keßel, E.H., Förster, N., Dönmez, A., Klose, J., et al. (2020). Establishment of an a priori protocol for the implementation and interpretation of an in-vitro testing battery for the assessment of developmental neurotoxicity. EFSA Support. Publ. *17*, 1938E.

Mattson, M.P., and Arumugam, T.V. (2018). Hallmarks of brain aging: adaptive and pathological modification by metabolic states. Cell Metabol. *27*, 1176–1199.

Mileson, B.E., and Ferenc, S.A. (2001). Methods to identify and characterize developmental neurotoxicity for human health risk assessment: overview. Environ. Health Perspect. *109*, 77–78.

Narayan, S., Liew, Z., Paul, K., Lee, P.C., Sinsheimer, J.S., Bronstein, J.M., and Ritz, B. (2013). Household organophosphorus pesticide use and Parkinson's disease. Int. J. Epidemiol. *42*, 1476–1485.

National Academies of Sciences, Engineering, and MedicineDivision on Earth and Life StudiesHealth and Medicine DivisionBoard on Environmental Studies and ToxicologyBoard on Health Sciences PolicyForum on Neuroscience and Nervous System Disorders, Stroud, C, Posey Norris, SM, and Bain, L, (Eds.) (2020). Environmental Neuroscience: Advancing the Understanding of How Chemical Exposures Impact Brain Health and Disease: Proceedings of a Workshop. (Washington (DC): National Academies Press), Available at: https://www.ncbi.nlm.nih.gov/books/NBK566967/#.

Nimtz, L., Hartmann, J., Tigges, J., Masjosthusmann, S., Schmuck, M., Keßel, E., Theiss, S., Köhrer, K., Petzsch, P., Adjaye, J., et al. (2020). Characterization and application of electrically active neuronal networks established from human induced pluripotent stem cell-derived neural progenitor cells for neurotoxicity evaluation. Stem Cell Res. *45*, 101761.

Ntzani, E.E., Chondrogiorgi, M., Ntritsos, G., Evangelou, E., and Tzoulaki, I. (2013). Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA Support. Publ. *10*, EN-497.

Nyffeler, J, Dolde, X, Krebs, A, Pinto-Gil, K, Pastor, M, Behl, M, Waldmann, T, and Leist, M (2017). Combination of multiple neural crest migration assays to identify environmental toxicants from a proof-of-concept chemical library. Arch Toxicol *91* 3613–32.

Ockleford, C., Adriaanse, P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A.F., Bennekou, S.H., Klein, M., Kuhl, T., et al. (2017). Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA J. *15*, 4690.

OECD (2007). OECD Guidelines for the Testing Of Chemicals. Section 4: Health Effects. Test No. 426: Developmental Neurotoxicity Study (Organization for Economic Co-operation and Development (OECD)), Available at: https://www.oecd.org/chemicalsafety/testing/37622194.pdf.

Pamies, D., Barreras, P., Block, K., Makri, G., Kumar, A., Wiersma, D., Smirnova, L., Zang, C., Bressler, J., Christian, K.M., et al. (2017). A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. ALTEX *34*, 362–376.

Paparella, M., Bennekou, S.H., and Bal-Price, A. (2020). An analysis of the limitations and uncertainties of in vivo developmental neurotoxicity testing and assessment to identify the potential for alternative approaches. Reprod. Toxicol. *96*, 327–336.

Pașca, S.P. (2018). The rise of three-dimensional human brain cultures. Nature *553*, 437–445.

Peters, R., Ee, N., Peters, J., Booth, A., Mudway, I., and Anstey, K.J. (2019). Air pollution and dementia: a systematic review. J Alzheim. Dis. *70*, S145–S163.

Petkus, A.J., Younan, D., Widaman, K., Gatz, M., Manson, J.E., Wang, X., Serre, M., Vizuete, W., Chui, H., Espeland, M.A., et al. (2020). Exposure to fine particulate matter and temporal dynamics of episodic memory and depressive symptoms in older women. Environ. Int. *135*, 105196.

Rey, F., Ottolenghi, S., Zuccotti, G.V., Samaja, M., and Carelli, S. (2022). Mitochondrial dysfunctions in neurodegenerative diseases: role in disease pathogenesis, strategies for analysis and therapeutic prospects. Neural Regen. Res. *17*, 754–758.

Rice, D., and Barone, S., Jr. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. *108*, 511–533.

Ring, C.L., Pearce, R.G., Setzer, R.W., Wetmore, B.A., and Wambaugh, J.F. (2017). Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability. Environ. Int. *106*, 105–118.

Rodier, P.M. (1995). Developing brain as a target of toxicity. Environ. Health Perspect. *103*, 73–76.

Sachana, M., Bal-Price, A., Crofton, K.M., Bennekou, S.H., Shafer, T.J., Behl, M., and Terron, A. (2019). International regulatory and scientific effort for improved developmental neurotoxicity testing. Toxicol. Sci. *167*, 45–57.

Sachana, Magdalini, Shafer, Timothy, J, and Terron, Andrea (2021). Toward a Better Testing Paradigm for Developmental Neurotoxicity: OECD Efforts and Regulatory Considerations. Biology (Basel) *1086*.

Saito, S., and Ihara, M. (2016). Interaction between cerebrovascular disease and Alzheimer pathology. Curr. Opin. Psychiatr. *29*, 168–173.

Schikowski, T., Vossoughi, M., Vierkötter, A., Schulte, T., Teichert, T., Sugiri, D., Fehsel, K., Tzivian, L., Bae, I.S., Ranft, U., et al. (2015). Association of air pollution with cognitive functions and its modification by APOE gene variants in elderly women. Environ. Res. *142*, 10–16.

Schmidt, C.W. (2013). Beyond uncertainty factors: protecting the tails of the bell curve. Environ. Health Perspect. *121*, A26–29.

Schmuck, M.R., Temme, T., Dach, K., de Boer, D., Barenys, M., Bendt, F., Mosig, A., and Fritsche, E. (2017). Omnisphero: a high-content image analysis (HCA) approach for phenotypic developmental neurotoxicity (DNT) screenings of organoid neurosphere cultures in vitro. Arch. Toxicol. *91*, 2017–2028.

Shi, L., Wu, X., Danesh Yazdi, M., Braun, D., Abu Awad, Y., Wei, Y., Liu, P., Di, Q., Wang, Y., Schwartz, J., et al. (2020). Long-term effects of PM2·5 on neurological disorders in the American medicare population: a longitudinal cohort study. Lancet Planet. Health *4*, e557–565.

Song, G., Zhao, M., Chen, H., Zhou, X., Lenahan, C., Ou, Y., and He, Y. (2021). The application of brain organoid technology in stroke research: challenges and prospects. Front. Cell. Neurosci. *15*, 646921.

Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Boström, E., Westerlund, I., Vial, C., Buchholz, B.A., et al. (2013). Dynamics of hippocampal neurogenesis in adult humans. Cell *153*, 1219–1227.

Stiegler, N.V., Krug, A.K., Matt, F., and Leist, M. (2011). Assessment of chemical-induced impairment of human neurite outgrowth by multiparametric live cell imaging in high-density cultures. Toxicol Sci. *121*, 73–87.

Sun, G.H., Raji, C.A., Maceachern, M.P., and Burke, J.F. (2012). Olfactory identification testing as a predictor of the development of Alzheimer's dementia: a systematic review. Laryngoscope *122*, 1455–1462.

Terron, A., Bal-Price, A., Paini, A., Monnet-Tschudi, F., Bennekou, S.H., EFSA WG EPI1 Members, Leist, M., and Schildknecht, S. (2018). An adverse outcome pathway for parkinsonian motor deficits associated with mitochondrial complex I inhibition. Arch. Toxicol. *92*, 41–82.

Tham, R., and Schikowski, T. (2021). The role of traffic-related air pollution on neurodegenerative diseases in older people: an epidemiological perspective. J Alzheim. Dis. *79*, 949–959.

Tzivian, L., Winkler, A., Dlugaj, M., Schikowski, T., Vossoughi, M., Fuks, K., Weinmayr, G., and Hoffmann, B. (2015). Effect of long-term outdoor air pollution and noise on cognitive and psychological functions in adults. Int. J. Hyg Environ. Health *218*, 1–11.

Unterholzner, J., Millischer, V., Wotawa, C., Sawa, A., and Lanzenberger, R. (2021). Making sense of patient-derived iPSCs, transdifferentiated neurons, olfactory neuronal cells, and cerebral organoids as models for psychiatric disorders. Int. J. Neuropsychopharmacol, https://doi.org/10.1093/ijnp/pyab037 (Epub ahead of print).

WHO (2004). IPCS Risk Assessment Terinology (World Health Organisation (WHO)), Available at: http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf.

WHO (2008). The Global Burden of Disease: 2004 Update. WHO Press 1–160.

Xu, R., Boreland, A.J., Li, X., Erickson, C., Jin, M., Atkins, C., Pang, Z.P., Daniels, B.P., and Jiang, P. (2021). Developing human pluripotent stem cell-based cerebral organoids with a controllable microglia ratio for modeling brain development and pathology. Stem Cell. Rep. *16*, 1923–1937.

Younan, D., Petkus, A.J., Widaman, K.F., Wang, X., Casanova, R., Espeland, M.A., Gatz, M., Henderson, V.W., Manson, J.E., Rapp, S.R., et al. (2020). Particulate matter and episodic memory decline mediated by early neuroanatomic biomarkers of Alzheimer's disease. Brain *143*, 289–302.

Zeise, L., Bois, F.Y., Chiu, W.A., Hattis, D., Rusyn, I., and Guyton, K.Z. (2013). Addressing human variability in next-generation human health risk assessments of environmental chemicals. Environ. Health Perspect. *121*, 23–31.

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Review article

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Recent developments and future perspectives on neuroelectronic devices

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Abstract: Neuroscientific discoveries and the development of recording and stimulation tools are deeply connected. Over the past decades, the progress in seamlessly integrating such tools in the form of neuroelectronic devices has been tremendous. Here, we review recent advances and key aspects of this goal. Firstly, we illustrate improvements with respect to the coupling between cells/tissue and recording/stimulation electrodes. Thereafter, we cover attempts to mitigate the foreign body response by reducing the devices' invasiveness. We follow up with a description of specialized electronic hardware aimed at the needs of bioelectronic applications. Lastly, we outline how additional modalities such as optical techniques or ultrasound could in the future be integrated into neuroelectronic implants.

Keywords: electrical recording; electrical stimulation; implants; microfabrication; neuroelectronics.

Zusammenfassung: Neurowissenschaftliche Entdeckungen und die Entwicklung von Ableitungs- und Stimulationsmethoden sind stark verknüpft. Im Laufe der letzten Jahrzehnte hat sich ein immenser Fortschritt im Hinblick auf die nahtlose Integration solcher Methoden in Form von neuroelektronischen Schnittstellen ergeben. In diesem Artikel geben wir einen Überblick über aktuelle Entwicklungen in diesem Feld. Wir beleuchten zuerst Verbesserungen der Kopplung zwischen Zellen/Gewebe und Ableitungs- bzw. Stimulationselektroden. Danach betrachten wir Ansätze zur Vermeidung von Fremdkörperreaktionen durch eine reduzierte Invasivität der Schnittstellen. Anschließend beschreiben wir spezialisierte elektronische Hardware für bioelektronische Anwendungen. Zuletzt zeigen wir auf, wie neue Modalitäten z.B. durch optische Techniken oder Ultraschall zukünftig in neuroelektronische Implantate integriert werden könnten.

Schlüsselwörter: Elektrische Ableitung; elektrische Stimulation; Implantate; Mikrofabrikation; Neuroelektronik.

Introduction

Since its very beginning, neuroscience has been deeply intertwined with methods and technologies of measuring or controlling the electrical activity in biological tissue. Ultimately, this intricate connection can be traced back to the founding experiments of Luigi Galvani on frog legs (Galvani, 1791). In the 1950s, the development of tungsten microelectrodes enabled recordings from single neurons in living animals (Hubel, 1957). Continuing the push toward higher resolution, Neher and Sakmann introduced the patch-clamp technique in the 1970s, which allowed characterizing individual ion channels (Neher and Sakmann, 1976). These developments have reshaped neuroscience since the middle of the 20th century triggering an ever-accelerating technological development. In particular over the past decades, new microfabrication approaches, as well as dedicated efforts on merging biology and artificial probes, have led to a cornucopia of new tools. Probes to interrogate the nervous systems are constantly improving in terms of signal quality, number of channels, and seamlessness of their integration (Won et al., 2018). At the same time, the accompanying electronics are becoming smaller and require less energy (van Dongen and Serdijn, 2016) while additional modalities such as optical or chemical stimulation are added.

This review aims to provide an overview of recent developments as well as the core challenges met when integrating artificial devices with the nervous system or biological systems in general. While the main focus is on technologies to be used in *in vivo* applications, a few examples for *in vitro* applications are discussed as well to demonstrate specific approaches in this domain. In doing so, we do not aim for a complete historical overview.

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Instead, we hope to provide the reader with an insight into the central aspects and most recent advances in the field of bio- and neuroelectronics. Overall, the review is structured in four sections that mirror the key aspects toward which efforts have been directed over the past years (compare Figure 1). First, we will illustrate improvements in the coupling between the electrode and the tissue. Thereafter, approaches to reduce the devices' invasiveness for better long-term applicability will be described. Next, we will outline technological progress with regards to detection principles and the underlying electronics of the devices. The last section will shortly highlight novel approaches to include other-than-electrical principles (e.g. optical or fluidic technologies) into neuroelectronic devices before we conclude the review.

Electrode–tissue coupling

The vast majority of techniques are concerned with recording or eliciting electrical signals, which is rooted in the critical role that electrical activity plays in the nervous system. In this context, the interface between an electrode and the cells or tissue of interest takes a central place. The properties of this interface are dominated by several factors. In particular, the interface impedance contributes to the attenuation of signals that are supposed to be recorded

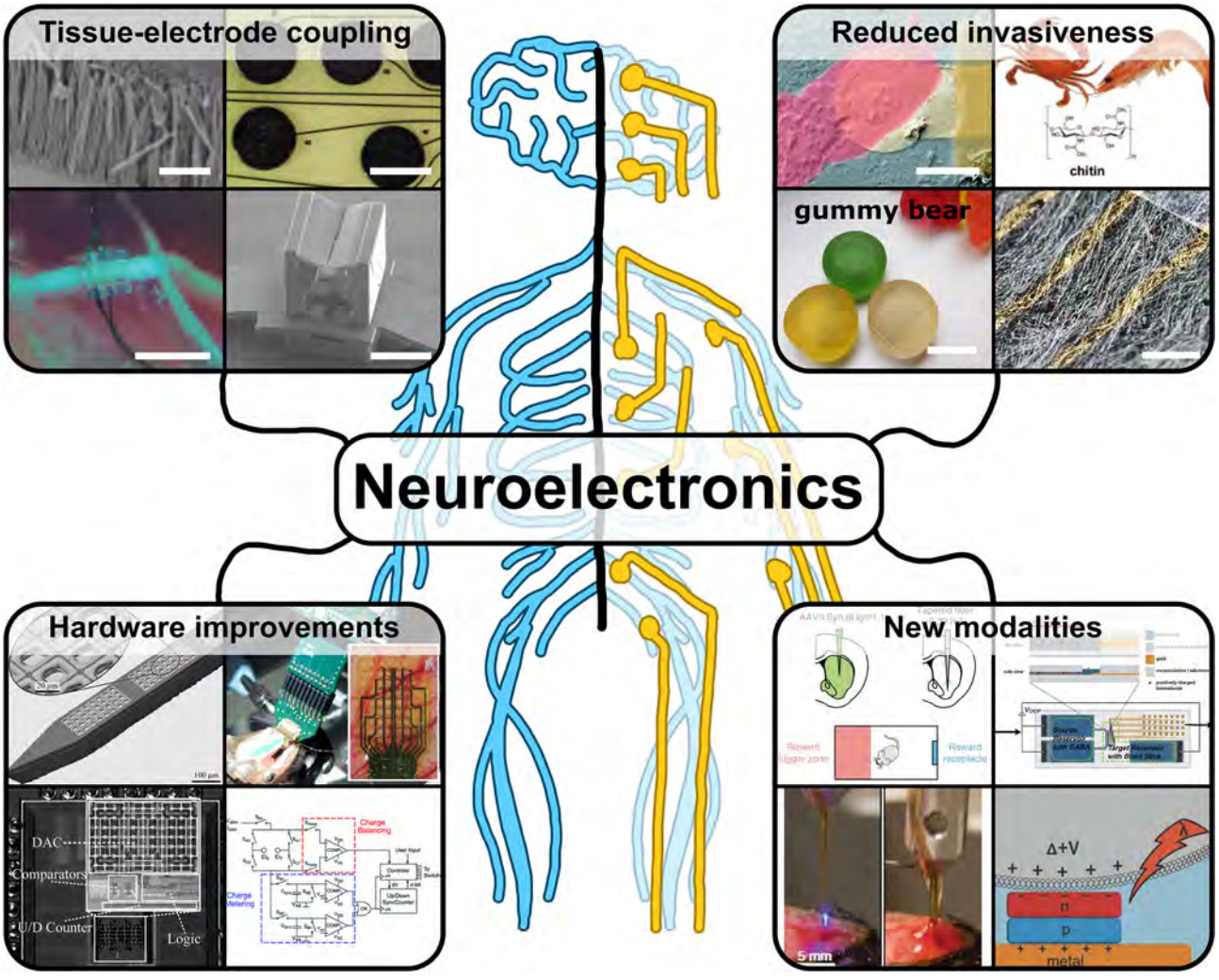

Figure 1: Schematic illustrating the four sectors in which neuroelectronic devices have advanced over the past years. Improvements in tissue–electrode coupling (top left; scale bars correspond to 2, 250, 500, and 200 µm, top-left to bottom-right, respectively) yield better signal transduction. A reduction in the devices' invasiveness (top right; scale bars correspond to 25 µm, approx. 10 mm, and 200 µm, top-left to bottom-right, respectively) mitigates the foreign body response. Specialized hardware (bottom left) delivers higher sensitivity and reduced power and spatial requirements for the accompanying electronics. The introduction of new modalities for recording and stimulation (bottom right) expands the neuroelectronic toolset. Details and copyright information for all four subpanels can be found in Figures 2, 3, 4, and 5.

by the electrode or likewise to be elicited in the tissue. At the same time, the impedance is a crucial factor in the voltage noise observed when recording with the electrode. On a general level, a lower impedance will mean better signal transduction and lower noise. Another factor is the so-called seal or junction resistance. This resistance represents undesired electrical pathways between the electrode and the ground. Increasing the seal resistance by tightening the tissue–electrode connection will generally result in improved signal fidelity.

Modifying the interface impedance

In the simplest case, the interface impedance can be approximated as an *RC* element. As such, the majority of interface modifications target either an increase in capacitance (e.g. by increasing the effective interface area) or a decrease in the interface resistance (e.g. by facilitating Faradaic charge transfer). A typical example is the functionalization of electrodes with platinum black. Since improvements in the adhesion of platinum black were made in the late 1980s (Marrese, 1987), it has been a predominant method to decrease the interface impedance by roughening the surface. Even despite known cytotoxic side effects (Schuettler et al., 2005), improvements in platinum black coating continue to pose a viable alternative for impedance reduction (Boehler et al., 2015; compare Figure 2a and b). Similar to the deposition of platinum black, electroplated gold was shown to be able to decrease the interface impedance by increased surface roughness (Brüggemann et al., 2011; Cui and Martin, 2003a). Aside from modifications to the surface geometry, chemical modifications have also been shown to bear great benefit in optimizing the interface for signal transduction and noise reduction (Bettinger, 2018; Ferro and Melosh, 2018). Among these, the application of conductive polymers takes a special place. The charge transport in such materials is often a mixture of electron/hole and ionic mechanisms. Applied to the electrodes of a neuroelectronic device, they can greatly improve the interface properties (Inal et al., 2018; Liang et al., 2021). Consequently, conductive polymers have found numerous applications in bioelectronic settings (Bettucci et al., 2021) with a continuously increasing toolset for structuring them on the microscale (Zhang and Travas-Sejdic, 2021).

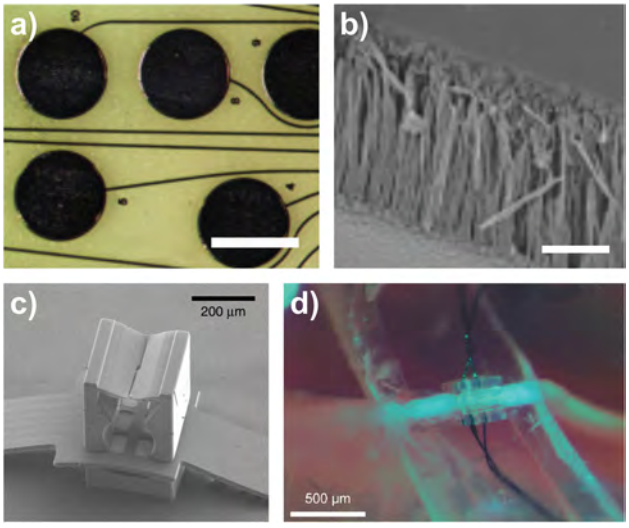

Figure 2: Improvements in tissue–electrode coupling. **a)** and **b)** Platinum nanograss functionalized electrodes and cross-sectional electron micrograph, respectively (Scale bars correspond to 200 and 2 µm, respectively; adapted from Boehler et al., Copyright (2015), with permission from Elsevier). **c)** and **d)** Microfabricated clips can be used to ensure a tight seal between peripheral nerves and recording/stimulation electrodes (**c** adapted from Otchy et al., 2020, **d** adapted from Lissandrello et al., 2017, © IOP Publishing. Reproduced with permission. All rights reserved).

While a number of conductive polymers have been applied in modifying bioelectronic interfaces, poly(3,4--ethylenedioxythiophene) (PEDOT), in particular when doped with polystyrene sulfonate (PSS), is considered by many to be the gold standard in this context (Liang et al., 2021). Already early on, the fuzzy nature of electrodeposited PEDOT:PSS films was not only shown to be beneficial for the electrode impedance but also for cell growth *in vitro* (Cui and Martin, 2003b). In addition to its benefits in a recording context, PEDOT was also shown to be an excellent choice for modifying stimulation electrodes. Similar to recording scenarios, the low interfacial impedance is beneficial for signal transduction across the interface. In this context, it was found that PEDOT-modified electrodes exhibit excellent charge storage capacities (Wilks et al., 2009). This property describes the amount of charge that can be applied through an electrode before undesired electrochemical reactions take place. As such, an increased charge storage capacity helps mitigate the increased current density requirements resulting from electrode miniaturization. Despite its long-standing history in this field, PEDOT:PSS has not lost its relevance as demonstrated by its recent application in conjunction with conductive hydrogels (Ferlauto et al., 2018) or in the realization of stretchable peripheral nerve interfaces (Decataldo et al., 2019).

Naturally, both approaches of impedance reduction can be combined, i.e. the application of conductive polymers can be tuned in order to increase their surface area to ultimately lower the overall impedance. Specifically, conductive polymers can be deposited in ways that render them porous (Yang and Martin, 2004a, b) or in the form of nanotubes that

offer an increased surface area due to their low dimensionality (Abidian and Martin, 2008; Abidian et al., 2009). Such nanotube-decorated electrodes have been shown to also promote improved adhesion of cells to the electrode (Abidian et al., 2010), an effect that was also observed when coating electrodes with blends of conductive polymers and biomolecules (Cui et al., 2001; Kim et al., 2007).

Tightening the tissue–electrode connection

As illustrated by these last examples, the degree of contact between the tissue and the electrode plays a similarly crucial role when probing biological tissue. Conceptually, a tighter coupling to the target tissue results in a higher seal or junction resistance and thereby more efficient transduction of signals both when recording or stimulating. Consequently, an interface as described above with cells closely interacting with the electrode's pores or nanostructures is expected to improve the electrode performance. However, the modification of the interface does not have to be performed before the addition of the cells. Instead, a tight interface can also be created by electropolymerizing PEDOT directly in the intercellular space both in cell culture studies and in living tissue explants (Richardsony-Burns et al., 2007a, b). Effectively, such approaches try to integrate the electrode directly into the target tissue's natural structure. Vice versa, a variety of methods use structural motives that the cells spontaneously engulf, i.e. they result in the tissue integrating more tightly with the electrode. Since the nanotechnology boom of the 2000s a large toolset to generate structures similar in size to those naturally encountered by cells is available. Numerous examples of how such structures can be used to create a tight tissue–electrode connection have been presented ranging from nanoparticles over nanopillars to nanocones and nanomushrooms (Angle et al., 2015; Spira and Hai, 2013). The majority of these techniques aim at tightening the contact between the electrode and the cell body. As such, they are to a certain degree focussed on applications in the central nervous system (CNS). While a number of these approaches will also bear advantage in applications involving the peripheral nervous system (PNS), there are also dedicated techniques that capitalize on properties specific to the PNS. One such property is given by the cylindrical nature of most structures found in the PNS. Exploiting this symmetry to create a more intricate tissue–electrode seal, microfabricated clip systems that embed electrodes for recording and stimulation have recently been presented (Lissandrello et al., 2017; Otchy et al., 2020; see Figure 2c and d). Other approaches make use of, for instance, adhesive hydrogel patches that wrap around the electrode and the nerve (Forssell et al., 2019; Horn et al., 2021; Ong et al., 2018).

Reduced invasiveness

The previous section highlighted the crucial importance of a tight coupling between the neuroelectronic device and the tissue of interest. In most cases, however, this coupling is required to last over days, if not months, in order to conduct biologically or clinically meaningful studies. Such timescales open up a new challenge as they allow activation of the body's defense mechanisms – the 'foreign body response' (FBR). Two of the main drivers of the FBR are suspected to be adsorption-related conformation changes in proteins (Hu et al., 2001) and a mechanical mismatch between the implanted materials and the target tissue (Moshayedi et al., 2014). The result is a cascade of events that attacks and encapsulates the implant leading to device failure (Barrese et al., 2013; Xie et al., 2014) or loss of neurons in the implant's vicinity (Biran et al., 2005). To counteract the FBR, neuroprobes are required to smoothly integrate into the soft, wet, and constantly moving environment of the body (Renz et al., 2018). The following section will thus illustrate strategies to mitigate FBR-related decreases in probe performance. In doing so, we will describe approaches that use modifications of the probe surface, soft materials to reduce mechanical mismatch, and overall reduced size to cause as little trauma in the target tissue as possible.

Surface modification

As described above, the probe surface can have a strong influence on the degree of FBR observed upon device implantation. Thus, modification of the device surface is an attractive candidate for effective mitigation strategies. In this context, not only a variety of both chemical but also structural approaches allow tuning of the cell–surface interaction (Stevens and George, 2005). Due to their soft and biologically inspired nature, hydrogels lend themselves to this application (Kim et al., 2010). Recent developments even yielded photostructurable gelatin systems that could be integrated into standard fabrication processes (Kang et al., 2020). While beneficial for cell adhesion, such coatings increase the electrode–cell distance and thereby lower the seal resistance. To mitigate this aspect, conducting polymers can be electropolymerized inside of the hydrogel. The thereby obtained structure of the conducting polymers

even decreases the electrode impedance potentially making up for the loss in seal resistance (Kim et al., 2004). Besides hydrogels, a number of bio-derived materials such as silk, collagen, chitin, or cellulose have already been demonstrated as viable substrates for bioelectronic interfaces (Pradhan et al., 2020; see Figure 3b). Increasingly, progress in the processability of these materials is blurring the line between classical microfabrication and bio-derived materials (Ju et al., 2020; compare Figure 3a). An approach that renders this line even less clear is based on so-called biohybrid interfaces. Early examples of this approach demonstrated that stem cell-seeded implants show lower neuron loss and glial encapsulation immediately after implantation (Purcell et al., 2009). Since then, the use of cells to relay or amplify artificial electronic signals has been realized in numerous studies *in vitro* and *in vivo* targeting both the PNS and the CNS (Rochford et al., 2019).

Addressing the mechanical mismatch

As highlighted by Moshayedi et al. (2014), the mechanical properties of neuroelectronic devices are another important factor in how well they fare in long-term applications. In this context, two strategies are commonly applied. The first employs materials that exhibit low Young's moduli, i.e. show mechanical properties closer to those of biological tissue than classic silicon or metal materials. Here, bio-friendly or bio-derived substrate materials have been demonstrated (compare Figure 3c; Adly et al., 2018; Maiolo et al., 2019). A common problem, however, is posed by the fact that metals usually suffer from a strong dependence of their conductivity on the applied strain, i.e. bending or stretching of the device results in a strong increase in the resistance. To mitigate this aspect, mesh-like conductor networks made from e.g. metal nanowires or carbon nanotubes can be applied (Lienemann et al., 2021; Terkan et al., 2020; Tybrandt et al., 2018). Alternatively, the microstructure of gold films can be engineered so that they remain conductive even when exposed to notable strain rates (Lacour et al., 2003, 2006). Both approaches have been successfully implemented in the recording and stimulation of nerve activity (Lienemann et al., 2021; Minev et al., 2015). Another approach is represented by using elastic conductive materials (Giagka and Serdijn, 2018; Jeong et al., 2015; Rogers et al., 2010; Sunwoo et al., 2020), such as composites of PEDOT:PSS and polyurethane (Cuttaz et al., 2019) or liquid metal conductors (Dong et al., 2021). Recent advances in this context cover rapid prototyping of soft bioelectronic implants (Afanasenkau et al., 2020) and ultrasoft mesh structures that seem to pose no hindrance to the mechanical activity of cardiac cells (Lee et al., 2018; see Figure 3d).

The second strategy is based on the fact that absolute mechanical stiffness is not only dependent on a material's bulk properties but also on its size and geometry. Similar to how both fingernails and hair consist of keratin, thinning down a macroscopically "stiff" material can result in highly pliable structures. As device thicknesses in the range of only a few micrometers are not uncommon in this area, materials that are sufficiently robust to allow handling and insertion are needed. Two of the most common examples in this category are polyimide (Borda et al., 2020; Kireev et al., 2019; Sperry et al., 2018) and parylene (Khodagholy et al., 2011, 2013, 2015), both of which offer additional benefits in terms of chemical inertness and biocompatibility. To increase the number of channels while maintaining a small form factor, multilayered and multiplexed devices have been proposed (Leccardi et al., 2019; Viventi et al., 2011). Overall, technological progress in this context has achieved a stage where even signals of individual cells can be detected (Almasri et al., 2020; Khodagholy et al., 2015), and transparent devices allowing parallel optogenetic modulation (Lee et al., 2017) have become possible. In terms of overall technology readiness, NeuraLink has recently presented a

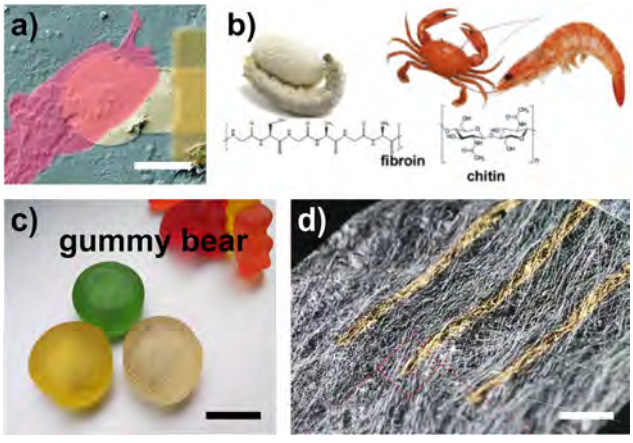

Figure 3: Reducing the invasiveness of neuroelectronic devices using novel materials. **a)** Scanning electron micrograph of a cardiomyocyte-like cell (pink) on an electrode (light yellow) insulated with photostructurable silk fibroin (dark yellow; scale bar corresponds to 25 µm; adapted from Ju et al., 2020). **b)** Sources and structures of silk fibroin and chitin (left and right, respectively; adapted from Pradhan et al., 2020). **c)** Electrode arrays printed on soft gelatin from gummy bears (scale bar correspond to approx. 10 mm; adapted from Adly et al., 2018). **d)** Ultrasoft polyurethane meshes with gold electrodes to record cardiac activity without disturbing mechanical activity (scale bar corresponds to 200 µm; adapted by permission from Lee et al., © Springer Nature 2018).

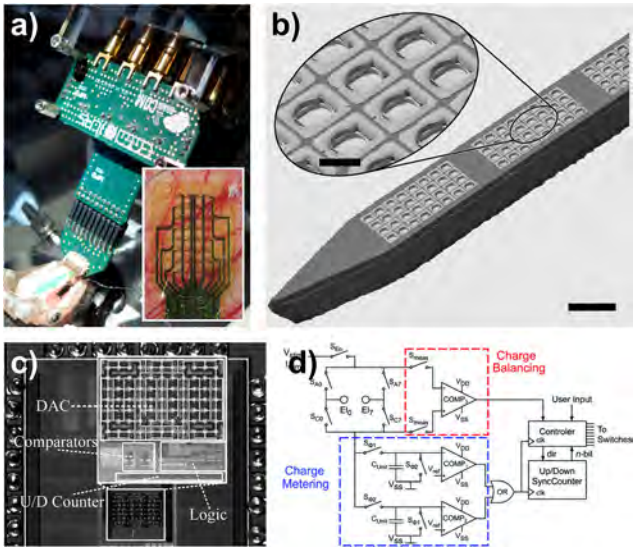

Figure 4: Specialized hardware for neuroelectronic devices. **a)** A graphene-based transistor array is used to record neuronal activity from the surface of the brain (adapted with permission from Garcia-Cortadella et al., © 2020 American Chemical Society). **b)** Complementary metal-oxide-semiconductor fabrication can be used to fabricate implantable electrode arrays with dense electrode spacing (scale bars in the main image and the inlay correspond to 100 and 20 µm, respectively; © 2018 IEEE. Adapted, with permission, from Sayed Herbawi et al., 2018). **c)** A specialized analog to digital converter consuming as little as 0.5 µW (© 2013 IEEE. Adapted, with permission, from Li et al., 2013). **d)** Circuit diagram for safe (i.e. charge-balanced) and power-efficient stimulation hardware (© 2020 IEEE. Adapted, with permission, from Guan et al., 2020).

platform that performs robotic implantation of and wireless recordings from thousands of channels in living animals (Musk and Neuralink, 2019). In particular, recordings from pigs and proof-of-concept brain-computer interface scenarios using monkeys have been demonstrated. Lastly, apart from thin probes, the importance of probe geometry in reducing glial scaring was shown early on (Seymour and Kipke, 2007). Since then, this approach has been heavily explored to yield shuttle techniques that only leave thin implants within the tissue (Williamson et al., 2015), injectable micron-scale meshes (Zhou et al., 2017), carbon fiber arrays (Jiman et al., 2020), and highly parallelized microwire bundles coupled to complementary metal-oxide semiconductor (CMOS) chips (Kollo et al., 2020; Obaid et al., 2020).

Hardware developments

So far, we have covered the progress neuroelectronic devices have made in increasing the efficiency of signal transduction as well as preventing the body from rejecting them. However, before such signals can be interpreted by a researcher they have to be both amplified and digitized. Here, several aspects of importance will be illustrated in the following section. Firstly, principles of local amplification to prevent long leads from introducing noise will be described. Subsequently, we will briefly dive into specialized electronics that aim to keep both spatial and energy requirements at a minimum. Lastly, wireless approaches to minimize constraints during e.g. behavioral studies will be introduced.

Local amplification

Despite the aforementioned advances in improving transduction, biological signals remain low in amplitude in the majority of scenarios. At the same time, they commonly have to be routed over significant lengths before they can be captured by recording electronics. For the most part, this combination results in challenging signal-to-noise ratios at the point of digitization. To address this challenge, different approaches aim to amplify the signal *before* routing – and thereby before the introduction of noise. A prominent approach to achieve such a local amplification is to use field-effect transistors (FETs) as recording elements. Bioelectronic applications of FETs have been presented not only on the basis of both PEDOT:PSS (Leleux et al., 2015) but also two-dimensional materials such as graphene (compare Figure 4a; Garcia-Cortadella et al., 2020; Schaefer et al., 2020). Alternatively, it was shown that it is even possible to directly integrate the necessary amplification circuitry within the recording device (Frey et al., 2009). The fabrication complexity needed to accomplish this is currently only manageable with CMOS fabrication. At the same time, however, CMOS allows ultra-dense electrode arrays that enable tracking action potentials down to a subcellular resolution (Bakkum et al., 2013; Lewandowska et al., 2015). More recently, CMOS fabricated shank electrodes that bring a similar electrode density to *in vivo* applications have been presented (Boi et al., 2020; Sayed Herbawi et al., 2018; see Figure 4b).

Specialized electronics

Despite the sizeable challenges of detecting biological signals, the electronic backend needed to digitize and record the data often represents a major bottleneck. This can have a number of reasons starting from the sheer amount of data to be handled to size or energy constraints when considering wireless, implantable systems. Driven by these constraints

and the increasing demand in terms of the functionality of neuroelectronic devices, the development of specialized hardware for bioelectronic recordings is a vivid field of research. In particular, the combined demands of recording, stimulation, and ideally pre-processing of the signals are of central interest (Liu et al., 2020; Rincón Montes et al., 2019). Especially when considering non-tethered, mobile applications, power consumption is a crucial factor. Here, digitization elements requiring only µW of power (Li et al., 2013; see Figure 4d), low-energy signal processing elements (Haddad and Serdijn, 2009; Hiseni et al., 2009), or efficient stimulation circuitry (compare Figure 4c; van Dongen and Serdijn, 2016; Guan et al., 2020; Kolovou Kouri et al., 2021) have been proposed. In particular for the latter, development has reached a point where even syringe-injectable stimulation hardware is within reach (Li et al., 2015).

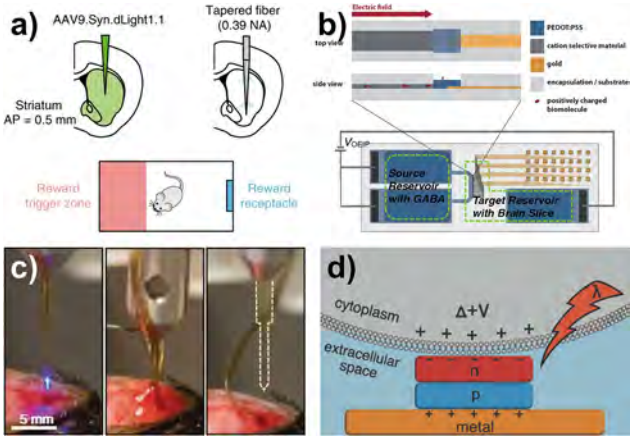

Figure 5: New modalities to broaden the neuroelectronic toolset. **a)** Tapered fibers can be used in depth-resolved photometric recordings (adpated by permission from Pisano et al., 2019). **b)** Schematic of a bioelectronic neural pixel, a device that can record electrically and deliver chemical stimuli in the same position (Jonsson et al., 2016). **c)** Image sequence of the insertion of an injectable, cellular scale optoelectronic device (From Kim et al., 2013. Reprinted with permission from AAAS). **d)** Schematic of direct photocapacitive stimulation. Shining light onto the stimulation site results in polarization of the photocapacitor, which in turn depolarizes adjacent cells (Rand et al., 2018).

New recording/stimulation modalities

Up to this point, the main focus of most applications we discussed revolved around purely electrical recording and stimulation. While electrical signals are – particularly with respect to the nervous system – of unquestionable importance, a range of other physical phenomena can be recorded from biological systems or used to elicit biological signals (Rivnay et al., 2017). Examples of such multimodal recordings include, for instance, the detection of strain and/or temperature in biological tissue (Xu et al., 2014; Yokota et al., 2015). Apart from the measurement of such physical quantities within the tissue, chemical signals belong to the body's own communication system. Here, protocols that counteract the common problem of electrode fouling have recently been presented to allow long-term monitoring of biologically relevant electroactive species *in vivo* (Weltin et al., 2019). In order to deliver chemical stimuli, passive release from the aforementioned conductive polymer nanotubes was investigated (Abidian and Martin, 2009). In applications that require a more discrete delivery of chemicals, electrophoretic (Proctor et al., 2019; Simon et al., 2009), electrochemical (Boehler et al., 2017), or microfluidic (Guo et al., 2021) delivery were shown to be viable options. In order to directly measure the tissue response to the stimulus, even chemical stimulation and electrical sensing with the very same electrode were demonstrated (Jonsson et al., 2016; compare Figure 5b).

The spread of optogenetics over the past two decades has demonstrated how versatile of a tool optical light can be. Naturally, implantable optoelectronic devices that allow coupling between the techniques described above and the potential of optogenetics are thus of great interest (Pisanello, 2019; Pisanello et al., 2016). But apart from delivering stimuli, optical tools can also be used to record photometric data in freely behaving animals (Pisano et al., 2019; refer to Figure 5a). Similar to electrical devices, optical technology was also advanced to reach wireless (Park et al., 2015; Samineni et al., 2017) or injectable (Kim et al., 2013; see Figure 5c) levels. Even without genetic modification, the use of photocapacitive or photofaradaic (i.e. materials that convert light into electricity) allows harnessing the tether-free nature of optical stimulation (Ghezzi et al., 2011; Paltrinieri et al., 2021; Rand et al., 2018; compare Figure 5d). Similarly, even ultrasonic or magnetoelectric signal transduction has been discussed (Seo et al., 2016; Singer et al., 2020).

Conclusions

Over the past 20 years, the key barriers for efficient multimodal recording and seamless integration of neuroelectronics devices have been identified and put under scrutinous investigation. Although some of these barriers have proven tougher than expected, immense progress was made to the point where clinical applications of more

sophisticated bioelectric approaches are within reach. Minev et al., for instance, were able to use soft neuroelectronic implants to restore locomotion after paralyzing injury in rats (Minev et al., 2015). They were able to demonstrate that their thin, soft implant not only limits inflammation but also results in a more natural gait after recovery. Similarly, the improved device-tissue coupling offered by hydrogel-supported electrode arrays allows fiber-selective stimulation of the vagus nerve in rats while presumably limiting compression (Forssell et al., 2019; Horn et al., 2021). In the CNS, organic electrochemical transistor arrays can record dopamine levels in different positions over time. Such recordings recently revealed a complex cross-talk between mesolimbic and nigrostriatal pathways in the rat brain (Xie et al., 2020).

We believe that in particular the concerted efforts from different fields such as electronic engineering, biochemistry, material science, and microfabrication ensure steady progress toward further blurring the line between technology and biology. Once this line is passed, neuroelectronic devices will deliver on their promise for many applications in prosthetic, diagnostic, and therapeutic applications.

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References

Abidian, M.R. and Martin, D.C. (2008). Experimental and theoretical characterization of implantable neural microelectrodes modified with conducting polymer nanotubes. Biomaterials *29*, 1273–1283.

Abidian, M.R. and Martin, D.C. (2009). Multifunctional nanobiomaterials for neural interfaces. Adv. Funct. Mater. *19*, 573–585.

Abidian, M.R., Ludwig, K.A., Marzullo, T.C., Martin, D.C., and Kipke, D.R. (2009). Interfacing conducting polymer nanotubes with the central nervous system: chronic neural recording using poly(3,4-ethylenedioxythiophene) nanotubes. Adv. Mater. *21*, 3764–3770.

Abidian, M.R., Corey, J.M., Kipke, D.R., and Martin, D.C. (2010). Conducting-polymer nanotubes improve electrical properties, mechanical adhesion, neural attachment, and neurite outgrowth of neural electrodes. Small *6*, 421–429.

Adly, N., Weidlich, S., Seyock, S., Brings, F., Yakushenko, A., Offenhäusser, A., and Wolfrum, B. (2018). Printed microelectrode arrays on soft materials: from PDMS to hydrogels. NPJ Flex. Electron. *2*, 15.

Afanasenkau, D., Kalinina, D., Lyakhovetskii, V., Tondera, C., Gorsky, O., Moosavi, S., Pavlova, N., Merkulyeva, N., Kalueff, A.V., Minev, I.R., et al. (2020). Rapid prototyping of soft bioelectronic implants for use as neuromuscular interfaces. Nat. Biomed. Eng. *4*, 1010–1022.

Almasri, R.M., AlChamaa, W., Tehrani-Bagha, A.R., and Khraiche, M.L. (2020). Highly flexible single-unit resolution all printed neural interface on a bioresorbable backbone. ACS Appl. Bio Mater. *3*, 7040–7051.

Angle, M.R., Cui, B., and Melosh, N.A. (2015). Nanotechnology and neurophysiology. Curr. Opin. Neurobiol. *32*, 132–140.

Bakkum, D.J., Frey, U., Radivojevic, M., Russell, T.L., Müller, J., Fiscella, M., Takahashi, H., and Hierlemann, A. (2013). Tracking axonal action potential propagation on a high-density microelectrode array across hundreds of sites. Nat. Commun. *4*, 2181.

Barrese, J.C., Rao, N., Paroo, K., Triebwasser, C., Vargas-Irwin, C., Franquemont, L., and Donoghue, J.P. (2013). Failure mode analysis of silicon-based intracortical microelectrode arrays in non-human primates. J. Neural Eng. *10*, 066014.

Bettinger, C.J. (2018). Recent advances in materials and flexible electronics for peripheral nerve interfaces. Bioelectron. Med. *4*, 6.

Bettucci, O., Matrone, G.M., and Santoro, F. (2021). Conductive polymer-based bioelectronic platforms toward sustainable and biointegrated devices: a journey from skin to brain across human body interfaces. Adv. Mater. Technol. 2100293, https://doi.org/10.1002/admt.202100293.

Biran, R., Martin, D.C., and Tresco, P.A. (2005). Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays. Exp. Neurol. *195*, 115–126.

Boehler, C., Stieglitz, T., and Asplund, M. (2015). Nanostructured platinum grass enables superior impedance reduction for neural microelectrodes. Biomaterials *67*, 346–353.

Boehler, C., Kleber, C., Martini, N., Xie, Y., Dryg, I., Stieglitz, T., Hofmann, U.G., and Asplund, M. (2017). Actively controlled release of dexamethasone from neural microelectrodes in a chronic *in vivo* study. Biomaterials *129*, 176–187.

Boi, F., Perentos, N., Lecomte, A., Schwesig, G., Zordan, S., Sirota, A., Berdondini, L., and Angotzi, G.N. (2020). Multi-shanks SiNAPS Active Pixel Sensor CMOS probe: 1024 simultaneously recording channels for high-density intracortical brain mapping. BioRxiv 749911.

Borda, E., Ferlauto, L., Schleuniger, J., Mustaccio, A., Lütolf, F., Lücke, A., Fricke, S., Marjanović, N., and Ghezzi, D. (2020). All-printed electrocorticography array for *in vivo* neural recordings. Adv. Eng. Mater. *22*, 1901403.

Brüggemann, D., Wolfrum, B., Maybeck, V., Mourzina, Y., Jansen, M., and Offenhäusser, A. (2011). Nanostructured gold microelectrodes for extracellular recording from electrogenic cells. Nanotechnology *22*, 265104.

Cui, X. and Martin, D.C. (2003a). Fuzzy gold electrodes for lowering impedance and improving adhesion with electrodeposited conducting polymer films. Sens. Actuators Phys. *103*, 384–394.

Cui, X. and Martin, D.C. (2003b). Electrochemical deposition and characterization of poly(3,4-ethylenedioxythiophene) on neural microelectrode arrays. Sens. Actuators B Chem. *89*, 92–102.

Cui, X., Lee, V.A., Raphael, Y., Wiler, J.A., Hetke, J.F., Anderson, D.J., and Martin, D.C. (2001). Surface modification of neural recording electrodes with conducting polymer/biomolecule blends. J. Biomed. Mater. Res. *56*, 261–272.

Cuttaz, E., Goding, J., Vallejo-Giraldo, C., Aregueta-Robles, U., Lovell, N., Ghezzi, D., and A. Green, R. (2019). Conductive elastomer composites for fully polymeric, flexible bioelectronics. Biomater. Sci. *7*, 1372–1385.

Decataldo, F., Cramer, T., Martelli, D., Gualandi, I., Korim, W.S., Yao, S.T., Tessarolo, M., Murgia, M., Scavetta, E., Amici, R., et al. (2019). Stretchable low impedance electrodes for bioelectronic recording from small peripheral nerves. Sci. Rep. *9*, 10598.

Dong, R., Liu, X., Cheng, S., Tang, L., Chen, M., Zhong, L., Chen, Z., Liu, S., and Jiang, X. (2021). Highly stretchable metal-polymer conductor electrode array for electrophysiology. Adv. Healthc. Mater. *10*, 2000641.

van Dongen, M. and Serdijn, W. (2016). Introduction. Design of Efficient and Safe Neural Stimulators: A Multidisciplinary Approach. M. van Dongen and W. Serdijn, eds. (Cham: Springer International Publishing), pp. 1–8.

Ferlauto, L., D'Angelo, A.N., Vagni, P., Airaghi Leccardi, M.J.I., Mor, F.M., Cuttaz, E.A., Heuschkel, M.O., Stoppini, L., and Ghezzi, D. (2018). Development and characterization of PEDOT:PSS/alginate soft microelectrodes for application in neuroprosthetics. Front. Neurosci. *12*, 648.

Ferro, M.D. and Melosh, N.A. (2018). Electronic and ionic materials for neurointerfaces. Adv. Funct. Mater. *28*, 1704335.

Forssell, M., Sciullo, M., Mou, C., Sun, F., Simpson, T.W., Xiao, G., Fisher, L.E., Bettinger, C., Horn, C.C., and Fedder, G.K. (2019). Compliant adhesive cuff electrode for selective stimulation in rat vagus nerve. 2019 IEEE SENSORS, pp. 1–4.

Frey, U., Egert, U., Heer, F., Hafizovic, S., and Hierlemann, A. (2009). Microelectronic system for high-resolution mapping of extracellular electric fields applied to brain slices. Biosens. Bioelectron. *24*, 2191–2198.

Galvani, L. (1791). De viribus electricitatis in motu musculari commentarius. Bologna Accad. Delle Sci.

Garcia-Cortadella, R., Schäfer, N., Cisneros-Fernandez, J., Ré, L., Illa, X., Schwesig, G., Moya, A., Santiago, S., Guirado, G., Villa, R., et al. (2020). Switchless multiplexing of graphene active sensor arrays for brain mapping. Nano Lett. *20*, 3528–3537.

Ghezzi, D., Antognazza, M.R., Dal Maschio, M., Lanzarini, E., Benfenati, F., and Lanzani, G. (2011). A hybrid bioorganic interface for neuronal photoactivation. Nat. Commun. *2*, 166.

Giagka, V. and Serdijn, W.A. (2018). Realizing flexible bioelectronic medicines for accessing the peripheral nerves – technology considerations. Bioelectron. Med. *4*, 8.

Guan, R., Zufiria, P.G., Giagka, V., and Serdijn, W.A. (2020). Circuit design considerations for power-efficient and safe implantable electrical neurostimulators. IEEE 11th Latin American Symposium on Circuits Systems (LASCAS), 2020, pp. 1–4.

Guo, J., Yu, Y., Cai, L., Wang, Y., Shi, K., Shang, L., Pan, J., and Zhao, Y. (2021). Microfluidics for flexible electronics. Mater. Today *44*, 105–135.

Haddad, S.A.P. and Serdijn, W.A. (2009). Ultra Low-Power Biomedical Signal Processing: An Analog Wavelet Filter Approach for Pacemakers (Springer Netherlands: Heidelberg).

Hiseni, S., Sawigun, C., Ngamkham, W., and Serdijn, W.A. (2009). A compact, nano-power CMOS action potential detector. IEEE Biomedical Circuits and Systems Conference, 2009, pp. 97–100.

Horn, C.C., Forssell, M., Sciullo, M., Harms, J.E., Fulton, S., Mou, C., Sun, F., Simpson, T.W., Xiao, G., Fisher, L.E., et al. (2021). Hydrogel-based electrodes for selective cervical vagus nerve stimulation. J. Neural Eng. *18*, 055008.

Hu, W.-J., Eaton, J.W., Ugarova, T.P., and Tang, L. (2001). Molecular basis of biomaterial-mediated foreign body reactions. Blood *98*, 1231–1238.

Hubel, D.H. (1957). Tungsten microelectrode for recording from single units. Science *125*, 549–550.

Inal, S., Rivnay, J., Suiu, A.-O., Malliaras, G.G., and McCulloch, I. (2018). Conjugated polymers in bioelectronics. Acc. Chem. Res. *51*, 1368–1376.

Jeong, J.-W., Shin, G., Park, S.I., Yu, K.J., Xu, L., and Rogers, J.A. (2015). Soft materials in neuroengineering for hard problems in neuroscience. Neuron *86*, 175–186.

Jiman, A.A., Ratze, D.C., Welle, E.J., Patel, P.R., Richie, J.M., Bottorff, E.C., Seymour, J.P., Chestek, C.A., and Bruns, T.M. (2020). Multi-channel intraneural vagus nerve recordings with a novel high-density carbon fiber microelectrode array. Sci. Rep. *10*, 15501.

Jonsson, A., Inal, S., Uguz, I., Williamson, A.J., Kergoat, L., Rivnay, J., Khodagholy, D., Berggren, M., Bernard, C., Malliaras, G.G., et al. (2016). Bioelectronic neural pixel: chemical stimulation and electrical sensing at the same site. Proc. Natl. Acad. Sci. *113*, 9440–9445.

Ju, J., Hu, N., Cairns, D.M., Liu, H., and Timko, B.P. (2020). Photo-cross-linkable, insulating silk fibroin for bioelectronics with enhanced cell affinity. Proc. Natl. Acad. Sci. *117*, 15482–15489.

Kang, P.-L., Lin, Y.-H., Settu, K., Yen, C.-S., Yeh, C.-Y., Liu, J.-T., Chen, C.-J., and Chang, S.-J. (2020). A facile fabrication of biodegradable and biocompatible cross-linked gelatin as screen printing substrates. Polymers *12*, 1186.

Khodagholy, D., Doublet, T., Gurfinkel, M., Quilichini, P., Ismailova, E., Leleux, P., Herve, T., Sanaur, S., Bernard, C., and Malliaras, G.G. (2011). Highly conformable conducting polymer electrodes for *in vivo* recordings. Adv. Mater. *23*, H268–H272.

Khodagholy, D., Doublet, T., Quilichini, P., Gurfinkel, M., Leleux, P., Ghestem, A., Ismailova, E., Hervé, T., Sanaur, S., Bernard, C., et al. (2013). *In vivo* recordings of brain activity using organic transistors. Nat. Commun. *4*, 1575.

Khodagholy, D., Gelinas, J.N., Thesen, T., Doyle, W., Devinsky, O., Malliaras, G.G., and Buzsáki, G. (2015). NeuroGrid: recording action potentials from the surface of the brain. Nat. Neurosci. *18*, 310–315.

Kim, D.-H., Abidian, M., and Martin, D.C. (2004). Conducting polymers grown in hydrogel scaffolds coated on neural prosthetic devices. J. Biomed. Mater. Res. A *71A*, 577–585.

Kim, D.-H., Richardson-Burns, S.M., Hendricks, J.L., Sequera, C., and Martin, D.C. (2007). Effect of immobilized nerve growth factor on conductive polymers: electrical properties and cellular response. Adv. Funct. Mater. *17*, 79–86.

Kim, D.-H., Wiler, J.A., Anderson, D.J., Kipke, D.R., and Martin, D.C. (2010). Conducting polymers on hydrogel-coated neural electrode provide sensitive neural recordings in auditory cortex. Acta Biomater *6*, 57–62.

Kim, T., McCall, J.G., Jung, Y.H., Huang, X., Siuda, E.R., Li, Y., Song, J., Song, Y.M., Pao, H.A., Kim, R.-H., et al. (2013). Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. Science *340*, 211–216.

Kireev, D., Rincón Montes, V., Stevanovic, J., Srikantharajah, K., and Offenhäusser, A. (2019). N3-MEA probes: scooping neuronal networks. Front. Neurosci. *13*, 320.

Kollo, M., Racz, R., Hanna, M.-E., Obaid, A., Angle, M.R., Wray, W., Kong, Y., Müller, J., Hierlemann, A., and Melosh, N.A., et al. (2020). CHIME: CMOS-hosted *in vivo* microelectrodes for massively scalable neuronal recordings. Front. Neurosci. *14*, 834.

Lacour, S.P., Wagner, S., Huang, Z., and Suo, Z. (2003). Stretchable gold conductors on elastomeric substrates. Appl. Phys. Lett. *82*, 2404–2406.

Kolovou Kouri, K., Soloukey, S., Harhangi, B., Serdijn, W.A., and Giagka, V. (2021). Development of Dorsal Root Ganglion (DRG) Multichannel Stimulator Prototype for use in Early Clinical Trials. Abstract from 8th Dutch Biomedical Engineering Conference (BME) 2021, Netherlands.

Lacour, S.P., Chan, D., Wagner, S., Li, T., and Suo, Z. (2006). Mechanisms of reversible stretchability of thin metal films on elastomeric substrates. Appl. Phys. Lett. *88*, 204103.

Leccardi, M.J.I.A., Vagni, P., and Ghezzi, D. (2019). Multilayer 3D electrodes for neural implants. J. Neural Eng. *16*, 026013.

Lee, W., Kim, D., Matsuhisa, N., Nagase, M., Sekino, M., Malliaras, G.G., Yokota, T., and Someya, T. (2017). Transparent, conformable, active multielectrode array using organic electrochemical transistors. Proc. Natl. Acad. Sci. *114*, 10554–10559.

Lee, S., Sasaki, D., Kim, D., Mori, M., Yokota, T., Lee, H., Park, S., Fukuda, K., Sekino, M., and Matsuura, K., et al. (2018). Ultrasoft electronics to monitor dynamically pulsing cardiomyocytes. Nat. Nanotechnol. *1*, 156–160.

Leleux, P., Rivnay, J., Lonjaret, T., Badier, J.-M., Bénar, C., Hervé, T., Chauvel, P., and Malliaras, G.G. (2015). Organic electrochemical transistors for clinical applications. Adv. Healthc. Mater. *4*, 142–147.

Lewandowska, M.K., Bakkum, D.J., Rompani, S.B., and Hierlemann, A. (2015). Recording large extracellular spikes in microchannels along many axonal sites from individual neurons. PLoS ONE *10*, 1–24.

Li, X., Serdijn, W.A., Zheng, W., Tian, Y., and Zhang, B. (2015). The injectable neurostimulator: an emerging therapeutic device. Trends Biotechnol. *33*, 388–394.

Li, Y., Zhao, D., and Serdijn, W.A. (2013). A sub-microwatt asynchronous level-crossing ADC for biomedical applications. IEEE Trans. Biomed. Circuits Syst. *7*, 149–157.

Liang, Y., Offenhäusser, A., Ingebrandt, S., and Mayer, D. (2021). PEDOT:PSS-based bioelectronic devices for recording and modulation of electrophysiological and biochemical cell signals. Adv. Healthc. Mater. *10*, 2100061.

Lienemann, S., Zötterman, J., Farnebo, S., and Tybrandt, K. (2021). Stretchable gold nanowire-based cuff electrodes for low-voltage peripheral nerve stimulation. J. Neural Eng. *18*, 045007.

Lissandrello, C.A., Gillis, W.F., Shen, J., Pearre, B.W., Vitale, F., Pasquali, M., Holinski, B.J., Chew, D.J., White, A.E., and Gardner, T.J. (2017). A micro-scale printable nanoclip for electrical stimulation and recording in small nerves. J. Neural Eng. *14*, 036006.

Liu, Y., Urso, A., Martins da Ponte, R., Costa, T., Valente, V., Giagka, V., Serdijn, W.A., Constandinou, T.G., and Denison, T. (2020). Bidirectional bioelectronic interfaces: system design and circuit implications. IEEE Solid-State Circuits Mag. *12*, 30–46.

Maiolo, L., Polese, D., and Convertino, A. (2019). The rise of flexible electronics in neuroscience, from materials selection to *in vitro* and *in vivo* applications. Adv. Phys. X *4*, 1664319.

Marrese, C.A. (1987). Preparation of strongly adherent platinum black coatings. Anal. Chem. *59*, 217–218.

Minev, I.R., Musienko, P., Hirsch, A., Barraud, Q., Wenger, N., Moraud, E.M., Gandar, J., Capogrosso, M., Milekovic, T., Asboth, L., et al. (2015). Electronic dura mater for long-term multimodal neural interfaces. Science *347*, 159–163.

Moshayedi, P., Ng, G., Kwok, J.C.F., Yeo, G.S.H., Bryant, C.E., Fawcett, J.W., Franze, K., and Guck, J. (2014). The relationship between glial cell mechanosensitivity and foreign body reactions in the central nervous system. Biomaterials *35*, 3919–3925.

Musk, E. and Neuralink (2019). An integrated brain-machine interface platform with thousands of channels. BioRxiv 703801.

Neher, E. and Sakmann, B. (1976). Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature *260*, 799–802.

Obaid, A., Hanna, M.-E., Wu, Y.-W., Kollo, M., Racz, R., Angle, M.R., Müller, J., Brackbill, N., Wray, W., Franke, F., et al. (2020). Massively parallel microwire arrays integrated with CMOS chips for neural recording. Sci. Adv. *6*, eaay2789.

Ong, X.C., Huang, W.-C., Kwon, I.S., Gopinath, C., Wu, H., Fisher, L.E., Gaunt, R.A., Bettinger, C.J., and Fedder, G.K. (2018). Ultra-compliant peripheral nerve cuff electrode with hydrogel adhesion. 2018 IEEE Micro Electro Mechanical Systems (MEMS), pp. 376–379.

Otchy, T.M., Michas, C., Lee, B., Gopalan, K., Nerurkar, V., Gleick, J., Semu, D., Darkwa, L., Holinski, B.J., Chew, D.J., et al. (2020). Printable microscale interfaces for long-term peripheral nerve mapping and precision control. Nat. Commun. *11*, 4191.

Paltrinieri, T., Bondi, L., Đerek, V., Fraboni, B., Głowacki, E.D., and Cramer, T. (2021). Understanding photocapacitive and photofaradaic processes in organic semiconductor photoelectrodes for optobioelectronics. Adv. Funct. Mater. *31*, 2010116.

Park, S.I., Brenner, D.S., Shin, G., Morgan, C.D., Copits, B.A., Chung, H.U., Pullen, M.Y., Noh, K.N., Davidson, S., Oh, S.J., et al. (2015). Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. Nat. Biotechnol. *33*, 1280–1286.

Pisanello, F. (2019). Implantable micro and nanophotonic devices: toward a new generation of neural interfaces. Microelectron. Eng. *215*, 110979.

Pisanello, F., Sileo, L., and De Vittorio, M. (2016). Micro- and nanotechnologies for optical neural interfaces. Front. Neurosci. *10*, https://doi.org/10.3389/fnins.2016.00070.

Pisano, F., Pisanello, M., Lee, S.J., Lee, J., Maglie, E., Balena, A., Sileo, L., Spagnolo, B., Bianco, M., Hyun, M., et al. (2019). Depth-resolved fiber photometry with a single tapered optical fiber implant. Nat. Methods *16*, 1185–1192.

Pradhan, S., Brooks, A.K., and Yadavalli, V.K. (2020). Nature-derived materials for the fabrication of functional biodevices. Mater. Today Bio *7*, 100065.

Proctor, C.M., Uguz, I., Slezia, A., Curto, V., Inal, S., Williamson, A., and Malliaras, G.G. (2019). An electrocorticography device with an integrated microfluidic ion pump for simultaneous neural recording and electrophoretic drug delivery *in vivo*. Adv. Biosyst. *3*, 1800270.

Purcell, E.K., Seymour, J.P., Yandamuri, S., and Kipke, D.R. (2009). *In vivo* evaluation of a neural stem cell-seeded prosthesis. J. Neural Eng. *6*, 026005.

Rand, D., Jakešová, M., Lubin, G., Vėbraitė, I., David-Pur, M., Đerek, V., Cramer, T., Sariciftci, N.S., Hanein, Y., and Głowacki, E.D. (2018). Direct electrical neurostimulation with organic pigment photocapacitors. Adv. Mater. *30*, 1707292.

Renz, A.F., Reichmuth, A.M., Stauffer, F., Thompson-Steckel, G., and Vörös, J. (2018). A guide towards long-term functional electrodes interfacing neuronal tissue. J. Neural Eng. *15*, 061001.

Richardson-Burns, S.M., Hendricks, J.L., Foster, B., Povlich, L.K., Kim, D.-H., and Martin, D.C. (2007a). Polymerization of the conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) around living neural cells. Biomaterials *28*, 1539–1552.

Richardson-Burns, S.M., Hendricks, J.L., and Martin, D.C. (2007b). Electrochemical polymerization of conducting polymers in living neural tissue. J. Neural Eng. *4*, L6–L13.

Rincón Montes, V., Gehlen, J., Lück, S., Mokwa, W., Müller, F., Walter, P., and Offenhäusser, A. (2019). Toward a bidirectional communication between retinal cells and a prosthetic device – a proof of concept. Front. Neurosci. *13*, 367.

Rivnay, J., Wang, H., Fenno, L., Deisseroth, K., and Malliaras, G.G. (2017). Next-generation probes, particles, and proteins for neural interfacing. Sci. Adv. *3*, e1601649.

Rochford, A.E., Carnicer-Lombarte, A., Curto, V.F., Malliaras, G.G., and Barone, D.G. (2019). When bio meets technology: biohybrid neural interfaces. Adv. Mater. *32*, 1903182.

Rogers, J.A., Someya, T., and Huang, Y. (2010). Materials and mechanics for stretchable electronics. Science *327*, 1603–1607.

Samineni, V.K., Yoon, J., Crawford, K.E., Jeong, Y.R., McKenzie, K.C., Shin, G., Xie, Z., Sundaram, S.S., Li, Y., Yang, M.Y., et al. (2017). Fully implantable, battery-free wireless optoelectronic devices for spinal optogenetics. PAIN *158*, 2108–2116.

Sayed Herbawi, A., Christ, O., Kiessner, L., Mottaghi, S., Hofmann, U.G., Paul, O., and Ruther, P. (2018). CMOS neural probe with 1600 close-packed recording sites and 32 analog output channels. J. Microelectromechanical Syst. *27*, 1023–1034.

Schaefer, N., Garcia-Cortadella, R., Martínez-Aguilar, J., Schwesig, G., Illa, X., Lara, A.M., Santiago, S., Hébert, C., Guirado, G., Villa, R., et al. (2020). Multiplexed neural sensor array of graphene solution-gated field-effect transistors. 2D Mater. *7*, 025046.

Schuettler, M., Doerge, T., Wien, S.L., Becker, S., Staiger, A., Hanauer, M., Kammer, S., Stieglitz, T. (2005). Cytotoxicity of platinum black. 10th Annual Conference of the International Functional Electrical Stimulation Society: IFESS 2005. Proceedings, pp. 343–348.

Seo, D., Neely, R.M., Shen, K., Singhal, U., Alon, E., Rabaey, J.M., Carmena, J.M., and Maharbiz, M.M. (2016). Wireless recording in the peripheral nervous system with ultrasonic neural dust. Neuron *91*, 529–539.

Seymour, J.P. and Kipke, D.R. (2007). Neural probe design for reduced tissue encapsulation in CNS. Biomaterials *28*, 3594–3607.

Simon, D.T., Kurup, S., Larsson, K.C., Hori, R., Tybrandt, K., Goiny, M., Jager, E.W.H., Berggren, M., Canlon, B., and Richter-Dahlfors, A. (2009). Organic electronics for precise delivery of neurotransmitters to modulate mammalian sensory function. Nat. Mater. *8*, 742–746.

Singer, A., Dutta, S., Lewis, E., Chen, Z., Chen, J.C., Verma, N., Avants, B., Feldman, A.K., O'Malley, J., Beierlein, M., et al. (2020). Magnetoelectric materials for miniature, wireless neural stimulation at therapeutic frequencies. Neuron *107*, 631–643.e5.

Sperry, Z.J., Na, K., Parizi, S.S., Chiel, H.J., Seymour, J., Yoon, E., and Bruns, T.M. (2018). Flexible microelectrode array for interfacing with the surface of neural ganglia. J. Neural Eng. *15*, 036027.

Spira, M.E. and Hai, A. (2013). Multi-electrode array technologies for neuroscience and cardiology. Nat. Nanotechnol. *8*, 83–94.

Stevens, M.M. and George, J.H. (2005). Exploring and engineering the cell surface interface. Science *310*, 1135–1138.

Sunwoo, S.-H., Han, S.I., Joo, H., Cha, G.D., Kim, D., Choi, S.H., Hyeon, T., and Kim, D.-H. (2020). Advances in soft bioelectronics for brain research and clinical neuroengineering. Matter *3*, 1923–1947.

Terkan, K., Zurita, F., Jamal Khalaf, T., Rinklin, P., Teshima, T., Kohl, T., and Wolfrum, B. (2020). Soft peripheral nerve interface made from carbon nanotubes embedded in silicone. APL Mater. *8*, 101111.

Tybrandt, K., Khodagholy, D., Dielacher, B., Stauffer, F., Renz, A.F., Buzsáki, G., and Vörös, J. (2018). High-density stretchable electrode grids for chronic neural recording. Adv. Mater. *30*, 1706520.

Viventi, J., Kim, D.-H., Vigeland, L., Frechette, E.S., Blanco, J.A., Kim, Y.-S., Avrin, A.E., Tiruvadi, V.R., Hwang, S.-W., Vanleer, A.C., et al. (2011). Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity *in vivo*. Nat. Neurosci. *14*, 1599–1605.

Weltin, A., Ganatra, D., König, K., Joseph, K., Hofmann, U.G., Urban, G.A., and Kieninger, J. (2019). New life for old wires: electrochemical sensor method for neural implants. J. Neural Eng. *17*, 016007.

Wilks, S.J., Richardson-Burn, S.M., Hendricks, J.L., Martin, D., and Otto, K.J. (2009). Poly(3,4-ethylene dioxythiophene) (PEDOT) as a micro-neural interface material for electrostimulation. Front. Neuroeng. *2*, 7.

Williamson, A., Ferro, M., Leleux, P., Ismailova, E., Kaszas, A., Doublet, T., Quilichini, P., Rivnay, J., Rózsa, B., Katona, G., et al. (2015). Localized neuron stimulation with organic electrochemical transistors on delaminating depth probes. Adv. Mater. *27*, 4405–4410.

Won, S.M., Song, E., Zhao, J., Li, J., Rivnay, J., and Rogers, J.A. (2018). Recent advances in materials, devices, and systems for neural interfaces. Adv. Mater. *30*, 1800534.

Xie, K., Wang, N., Lin, X., Wang, Z., Zhao, X., Fang, P., Yue, H., Kim, J., Luo, J., Cui, S., et al. (2020). Organic electrochemical transistor arrays for real-time mapping of evoked neurotransmitter release *in vivo*. ELife *9*, e50345.

Xie, Y., Martini, N., Hassler, C., Kirch, R.D., Stieglitz, T., Seifert, A., and Hofmann, U.G. (2014). *In vivo* monitoring of glial scar proliferation on chronically implanted neural electrodes by fiber optical coherence tomography. Front. Neuroeng. *7*, 34.

Xu, L., Gutbrod, S.R., Bonifas, A.P., Su, Y., Sulkin, M.S., Lu, N., Chung, H.-J., Jang, K.-I., Liu, Z., Ying, M., et al. (2014). 3D multifunctional integumentary membranes for spatiotemporal cardiac measurements and stimulation across the entire epicardium. Nat. Commun. *5*, 3329.

Yang, J. and Martin, D.C. (2004a). Microporous conducting polymers on neural microelectrode arrays: I. Electrochemical deposition. Sens. Actuators B Chem. *101*, 133–142.

Yang, J. and Martin, D.C. (2004b). Microporous conducting polymers on neural microelectrode arrays: II. Physical characterization. Sens. Actuators Phys. *113*, 204–211.

Yokota, T., Inoue, Y., Terakawa, Y., Reeder, J., Kaltenbrunner, M., Ware, T., Yang, K., Mabuchi, K., Murakawa, T., Sekino, M., et al. (2015). Ultraflexible, large-area, physiological temperature sensors for multipoint measurements. Proc. Natl. Acad. Sci. *112*, 14533–14538.

Zhang, P. and Travas-Sejdic, J. (2021). Fabrication of conducting polymer microelectrodes and microstructures for bioelectronics. J. Mater. Chem. C *9*, 9730–9760.

Zhou, T., Hong, G., Fu, T.-M., Yang, X., Schuhmann, T.G., Viveros, R.D., and Lieber, C.M. (2017). Syringe-injectable mesh electronics integrate seamlessly with minimal chronic immune response in the brain. Proc. Natl. Acad. Sci. *114*, 5894–5899.

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Review article

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Neuroscience meets cancer: networks and neuronal input to brain tumors

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Abstract: The nervous system with its complex organizational features and functions is well-known for its impressive ability to process information and drive countless biological processes. It has come to the surprise of many that the nervous system can also be intimately involved in an unwelcome area of human life: the initiation and progression of cancer. For brain tumors, the parallels to neurodevelopment and nervous system function can be found on multiple levels. First, cancer cells of incurable gliomas interconnect with long cellular extensions to a large communicating multicellular network. Second, indirect and direct neuronal input can generate, activate, and control brain tumor growth. Third, it is becoming increasingly clear that those features not only drive brain tumor progression but also the notorious resistance of these tumors against standard antitumor therapies. Remarkably, these recent insights have already generated novel ideas for better antitumor therapies.

Keywords: cancer neuroscience; neuron-tumor synapses; tumor cell networks; glioblastoma; brain metastases.

Zusammenfassung: Das Nervensystem ist gut bekannt für seine bemerkenswerte Fähigkeit zur Informationsverarbeitung und zur Steuerung unzähliger biologischer Vorgänge. Zum Erstaunen vieler ist das Nervensystem aber offenbar auch sehr intensiv an einem weniger erwünschten Prozess beteiligt: der Initiierung und dem Fortschreiten von Krebserkrankungen. Bei Hirntumoren können die Parallelen zur neuronalen Entwicklung und neuralen Funktion auf vielen Ebenen gefunden werden. Zunächst bilden Tumorzellen von unheilbaren Gliomen mittels sehr langer Membrantunnel Verbindungen zu anderen Tumorzellen aus, die zusammen ein kommunizierendes Netzwerk bilden. Nervenzellen stimulieren diese Netzwerke, und neuronale Aktivität kann Hirntumore generieren und im Wachstum antreiben, indirekt und direkt. Diese Verbindungen zum Nervensystem auf vielen Ebenen begünstigen auch die Therapieresistenz. Die hier vorgestellten jüngsten Erkenntnisse führten bereits zu neuen Ansätzen für Anti-Tumor-Therapien.

Schlüsselwörter: Cancer Neuroscience; Neuron-Tumor-Synapsen; Tumorzellnetzwerke; Glioblastom; Hirnmetastasen.

Cancer is not a disease of isolated cells that have just gone mad. It is a disease where multiple malignant and nonmalignant cells collaborate to drive cancer initiation and progression. That makes tumors to “organs”, where cancer cells interact with a complex microenvironment that is crucial for tumor growth (Hanahan and Weinberg, 2011). Remarkably, this also applies to the nervous system. Cancer can appear anywhere in the body – and together the central (CNS) and peripheral nervous systems (PNS) cover our entire organism, controlling its myriad functions. However, it has only recently been discovered that close and reciprocal interactions of the CNS and PNS on one side with cancer cells on the other can govern tumor initiation and growth. Multiple neuron-tumor interactions have been described so far. They can be of an unexpectedly direct nature involving neuron-tumor synaptic connections (see below) or consist of paracrine (Venkatesh et al., 2015, 2017) or systemic (including immunological and hormonal) interactions (Monje et al., 2020). Finally, anticancer therapies can also influence the nervous system in many, mostly unwanted ways.

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Together this has led to the generation of a new research field, "Cancer Neuroscience" (Faulkner et al., 2019; Jung et al., 2020; Monje et al., 2020; Venkataramani and Winkler, 2021). Of note, it has become increasingly clear that cancer cells themselves can harbor multiple neurodevelopmental (including stem cell-like) and other neuronal features which are an important feature of successful tumor growth, making science at the crossroads of Neuroscience and Cancer an exciting and rewarding endeavor with the potential to cross-inform both research areas alike (Jung et al., 2019). In this short review, we will provide an overview of this emerging field of science, with a focus on brain tumors. The neuronal features of cancer covered here extend our understanding of diseases that are among the most difficult to treat today – and at the same time inspire completely novel ideas on how to treat them better in the future (Dolgin, 2020). The ultimate aim is to develop a new area of "Neuroscience-instructed Cancer Therapy" (Venkataramani and Winkler, 2021). On the other hand, basic discoveries that are made in Cancer Neuroscience might help neuroscientists to better understand the development and functionality of the normal nervous system.

Malignant cellular networks in the brain and other tumors

In 2015, we published a discovery that changed the way we think about brain tumors, inspiring a new field of basic and translational research. As it is often the case in science, the development of new technologies was instrumental for this step forward; here, it was the combination of intravital two-photon microscopy, a technology that allows imaging the live mouse brain until a depth of many hundreds of microns, with a chronic cranial window technology, and implantation of patient-derived brain tumor cells that were strictly kept under nondifferentiating, floating, "stem-like" conditions outside the body. We focused on tumor cells from glioblastomas, particularly aggressive and incurable brain tumors that develop in the brain and colonize it rapidly, making glioblastoma a whole-brain disease from early on. Thus, the first question we asked was: how does the tumor achieve that? When following those glioblastoma cells – that were genetically modified to express various fluorescent proteins making them easily detectable – over months in the same brain region, it became apparent that tumor cells rapidly extended and retracted very long cell processes in a brain-scanning behavior, which fostered brain invasion and colonization by new tumor cells (Osswald et al., 2015). These membrane tubes can reach hundreds of microns, even millimeters (if not centimeters) in length, and can be found throughout the brain in mouse and human samples even in the macroscopically nonaffected brain hemisphere. We called these new cellular processes "Tumor Microtubes", or TMs, and demonstrated that they are highly reminiscent of neurites, neuronal cell processes that are formed during neurodevelopment, plasticity and repair in the normal brain, and that are the basis for the generation of axons and dendrites, the crucial cell-cell connections that our nervous system depends on. Remarkably, glioblastoma cells used their TMs to connect with each other to a large multicellular network, too (Figure 1). In this functional syncytium, single tumor cells were able to communicate with each other via intercellular calcium waves and were able to reach a high level of cellular resilience by improved homeostasis, e.g. with respect to intracellular calcium concentrations.

Figure 2 summarizes the current knowledge of the basic functions of these malignant brain tumor networks. The main molecular drivers known so far include: the neuronal growth-associated protein 43 (GAP-43) that drives neurite formation in neurons, and likewise the generation and extension of TMs in glioblastoma cells, is important for all known biological functions of TMs: invasion, proliferation with the distribution of tumor cell nuclei throughout the brain, interconnection, and stable

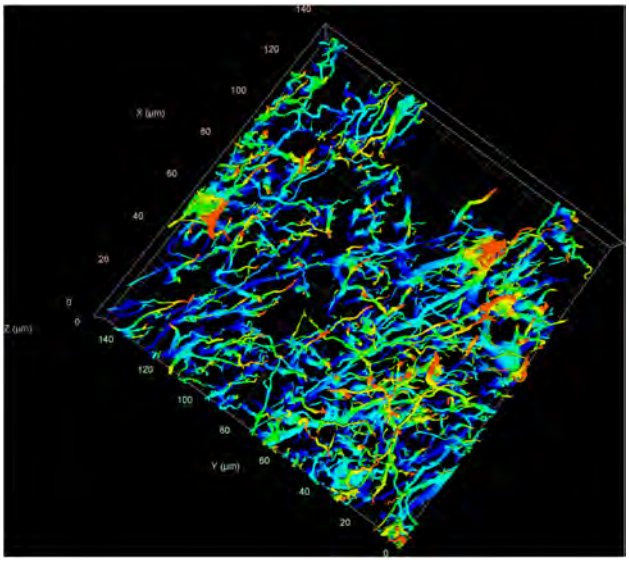

Figure 1: Many tumor cells of incurable brain tumors (here, glioblastoma cells) are connected with long membrane protrusions with each other. This generates a multicellular malignant network. Shown here is a 3D rendering of a confocal microscopy imaging stack from resected material of a human glioblastoma patient stained with an antibody against nestin.
Image: Varun Venkataramani.

network formation; the neurodevelopmental protein Tweety-homologue 1 (Ttyh1) which has been shown to be crucial for TM invasion and TM-dependent tumor proliferation only (Jung et al., 2017); and finally the gap junction protein connexin 43 (Cx43) which is not only important for normal brain astrocyte communication but also for the communication in brain tumor cell networks. It is located in and at TMs, forms connexons that may assemble to form intercellular channels that allow the exchange of smaller molecules between tumor cells (Osswald et al., 2015). The tumor cell network itself might also be involved in brain scanning and brain invasion (Gritsenko et al., 2020).

Resilience in tumor networks, and how to break it

Integration into TM-connected tumor cell networks can be very beneficial for the entire tumor organism; the first indications for this increasing body of knowledge were already published in 2015. When a laser beam was used to kill one single glioblastoma cell that was network-integrated, the network reacted, and perfectly replaced the killed comrade within 60 h. When a tumor cell that was not part of the malignant network (about 40–60% of all

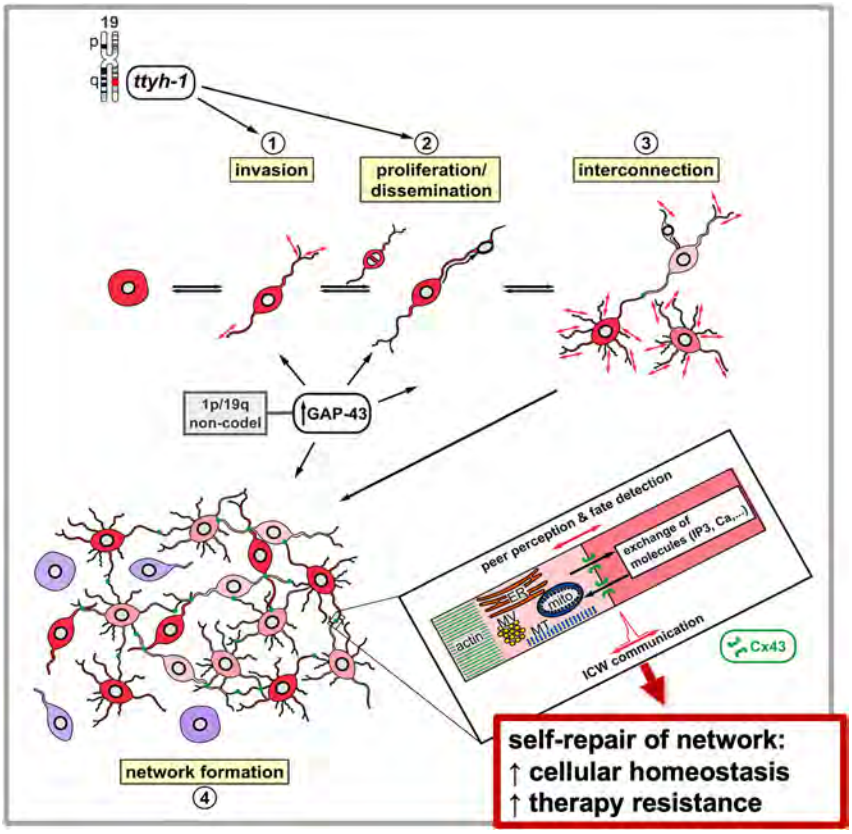

Figure 2: Glioblastoma cells with highly heterogeneous cell morphologies can be identified in the glioblastoma network as shown by the different colored cells in the glioma network. Connected cells are shown in pink/red, unconnected cells in purple. These subpopulations can be differentiated on their basis to form long membrane protrusions, or tumor microtubes (TMs). TMs are crucial cellular factors of brain tumor invasion, proliferation, brain colonization, and network formation. The network formation of glioblastoma cells connected to each other via gap junctions formed by connexin 43 (Cx43) has been shown to form a functional network characterized by intercellular calcium waves (ICW). This network contributes to the self-repair of the network and therapeutic resistance. It was shown that the ablation of single cells could be substituted by other cells of the network in a coordinated manner. Thus, the network is characterized by peer perception and fate detection of the members of the network. Furthermore, it is characterized by increased cellular homeostasis, potentially mediated by the exchange of molecules via the gap junctions, as compared to tumor-unconnected glioblastoma cells contributing to therapeutic resistance. GAP-43 and Ttyh1 are key molecular drivers of TM formation and shown to drive glioblastoma progression. These molecular factors are also drivers for the formation of neuronal cell processes during development and depend on an intact 1p/19q chromosome status. It could be seen that the intact 1p/19q chromosome status is associated with a worse clinical outcome for patients further connecting TM formation with clinical relevance for the human disease.
ICW, intercellular calcium wave; ER, endoplasmatic reticulum; MV, microvesicles; MT, microtubules; mito, mitochondrium; Cx43, the gap junction protein connexin 43. Modified from: Osswald et al., Nature 2015.

tumor cells in the regions studied) was killed, this tumor self-repair response was only rarely observed (Osswald et al., 2015). This was in line with the study of more than 100 samples from human glioma types where increasing TM and TM network formation was seen with increasing malignancy: lowest in low-grade oligodendrogliomas, which are associated with patient survival for decades, and highest in glioblastomas, where patients usually die within 1–2 years. Most importantly for clinical translation, the genetic reduction of GAP-43, the crucial neurodevelopmental factor that drives TM formation and function, not only deprived glioblastoma cells of the ability to form functional tumor networks but also made radiotherapy much more effective, by and large eradicating experimental tumors from the mouse brain, which is typically not possible with radiotherapy alone (Osswald et al., 2015). Consistent with the concept of tumor cell resilience by network integration, radiotherapy, and also chemotherapy (Weil et al., 2017) preferentially killed the unconnected glioblastoma cells, leaving the tumor cell networks largely intact. Even more, when surgical tumor resection was applied, the brain tumor network detected damage to itself, and answered with an overshooting self-repair response, such as that after single-cell ablation as described above. This can explain why so many glioma patients experience tumor recurrences directly at the resection cavity.

Taken together, integration into multicellular, highly functional, communicating, and resilient networks is not only a feature of the nervous system but also of incurable, most aggressive brain tumors. The tumor cell network is increasingly seen as the highly resistant backbone of the disease that resists the detrimental effects of radiochemotherapy, and that can apparently detect damage to itself, and execute highly complex self-repair responses. Future decoding of the complex intercellular calcium communication patterns that appear even hierarchical, with some tumor cells taking the lead and others following (Osswald et al., 2015), will tell us more about the organization of the network, and might ultimately even allow us to understand what the tumor cells are talking with each other. It is remarkable that these paradigmatic basic discoveries have already led to new ideas for better antitumor treatments in glioblastoma. Recent studies of Schneider et al. demonstrate how the TM networks can be pharmacologically silenced with the gap junction inhibitor meclofenamate (MFA) (Schneider et al., 2021a,b). Importantly, these studies also show a clear road for the introduction of TM-network targeting therapies into clinical concepts that aim to overcome the notorious treatment resistance of glioblastomas. MFA is an FDA-approved drug (for its NSAID actions against pain, fever, and inflammation), and is considerably well-tolerated by patients. Thus, MFA is currently tested in the German MecMeth trial (EudraCT2021-000708-39) in recurrent glioblastoma in combination with chemotherapy in patients. Other concepts of how to disconnect tumor cells for improved antitumor activities are currently pushed forward, too, partly already in drug development pipelines (Dolgin, 2020; Osswald et al., 2016). Last but not least, since cells of many other cancers can connect with each other via ultra-thin membrane tubes, too, we might even reach a situation in the future where this field of research in Neuro-Oncology can inspire other oncologists and cancer researchers to vigorously test disconnecting strategies (Winkler and Wick, 2018).

Neuron-tumor cell synapses activate brain tumors

Neurons of the normal brain constantly communicate with each other in a highly ordered, meaningful manner, using various classes of neurotransmitters and their receptors to fine-tune their network communication. Together this makes our neurological functions and is the foundation of our personality. The point-of-contact between single neuronal processes is called synapses, and synapse formation is a finely tuned process involving pre- and postsynaptic partners. It seems that synapses are something very stable; however, during brain development, and probably also in adult life, oligodendrocyte precursor cells (OPCs), a highly proliferative cell population in the brain and a likely cell of origin for various brain tumor types, form new synapses with more mature neurons, which seems to influence their biological behavior in a dynamic way (Bergles et al., 2000).

Remarkably, these “noncanonical synaptic contacts” between neurons and OPCs are not limited to normal nervous system functionality. Interestingly, excitatory synapses that use glutamate as neurotransmitters are also formed between neurons and cancer cells of various particularly aggressive, incurable brain tumor types, including glioblastoma, astrocytoma, and diffuse midline gliomas. This work of three different research groups was published in two articles in 2019, with striking overlaps and cross-validations in both of them (Venkataramani et al., 2019; Venkatesh et al., 2019). In short, presynaptic neuronal activity, both, physiological and pathological (as seen in epilepsy), leads to activation of glutamatergic neuron-glioma synapses (Figure 3). The AMPARs expressed by glioma cells are closer to those expressed during neurodevelopment than in the adult brain: they are calcium-permeable. Synaptic activation generates calcium waves which travel through the

glioma cell networks. All in all, some TMs of this network appear to have dendrite-like functions, whilst those connecting a tumor cell with others in the malignant network conduct the input of the neuronal stimulation to wider areas of the cancer cell network. Finally, this leads to increased invasion and proliferation of tumor cells, which means an accelerated tumor progression. In other words: neuronal activity drives tumor growth, not only via the indirect paracrine mechanisms described before (Venkatesh et al., 2015, 2017) but also via direct synaptic contacts between neurons (always pre-synaptic) and brain tumor cells (always post-synaptic). Great efforts were undertaken to prove the existence of these highly functional neuron-tumor synapses in resected material from human incurable brain tumors (Venkataramani et al., 2019; Venkatesh et al., 2019).

The existence of this direct and harmful neuron-cancer communication channel is highly uncomforting. At the same time, its discovery generated new ideas on how to better treat brain tumor types that are hitherto incurable. The approved antiepileptic drug perampanel, an AMPAR inhibitor with good anti-epileptic effectivity in glioma patients (Coppola et al., 2020), showed marked antitumor activity in various preclinical glioma models (Venkataramani et al., 2019; Venkatesh et al., 2019), as predicted by the discovery of glutamatergic neuron-glioma synapses. Currently, several international trial concepts aim to test perampanel in recurrent glioblastoma. Finally, neuron-tumor synapses can also be of another, rather an indirect type: tumor cells of brain-metastatic breast cancer cells can wrap around existing neuro-neuron synapses (like done by astrocytes in the normal brain), and receive growth-stimulatory glutamate from the synaptic cleft, mediated by NMDA (and not AMPA) receptors (Zeng et al., 2019). Since similar "pseudo-tripartite" synapses can also be found in gliomas (Venkataramani et al., 2019), it remains to be seen how many more functional neuron-cancer synaptic contacts will be identified in the future, and whether they can be the target for novel anticancer therapies, too (Venkataramani et al., 2021).

Summary and outlook

It is becoming increasingly clear that brain and other cancers can hijack neuronal stimulation and neurodevelopmental mechanisms to grow. That is concerning, but the important improvement of our understanding of key functions of the tumor organ allows for new ideas on how to better treat various cancer types in the future. A recent paper even found stimulation of the optic nerve by light-induced stimulation of retinal ganglion cells a key

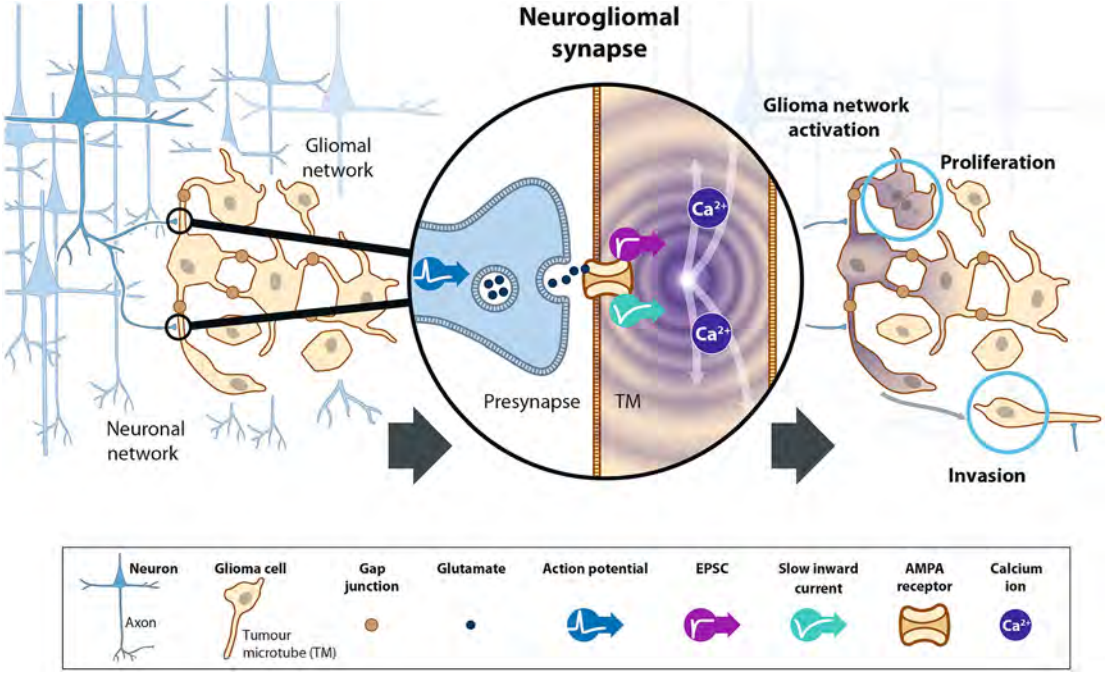

Figure 3: Bona-fide glutamatergic synapses bearing AMPAR between unidirectional presynaptic neurons and postsynaptic brain tumor (glioblastoma) cells. Synaptic stimulation by neuronal activity activates the tumor cell network, which drives brain tumor progression. These contacts are ultrastructurally comparable to physiological synapses formed in the healthy brain. Two types of electrophysiological responses can be recorded from glioblastoma cells that receive neuronal input in the form of short excitatory, postsynaptic currents, and slow inward currents. Both current types can induce calcium transients in the glioma network. Neuronal input can subsequently increase the proliferation and invasion of glioblastoma cells.
From: Venkataramani et al., Nature 2019.

factor for the *initiation* of optic nerve tumors in a tumor predisposition syndrome (Pan et al., 2021; Venkataramani and Winkler, 2021), confirming earlier reports for similar neuronal tumor-generation mechanisms outside the brain (Mauffrey et al., 2019; Saloman et al., 2016).

Neuronal activity can cause cancer, and also stimulate the growth of existing tumors. While we are still struggling to digest this information, it will be exciting to witness the development of the field in the next years – and to learn whether Neuroscience-instructed Cancer Therapy will emerge as one additional treatment pillar in Oncology not only for brain tumors but also for various tumors that are challenging to treat today. Technological hurdles need to be mastered, among them the mapping and mechanistic studies of nerve-tumor interactions outside the brain, and also with respect to the complexity of nerve-tumor interactions and the changes the tumor exerts to the normal nervous system (Monje et al., 2020). In the end, it is likely that the discoveries arising from this research will similarly cross-inform neuroscience and neuromedicine on one side, and cancer therapy and oncology on the other. In other words, it should be worth the effort.

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Conflict of interest statement: F.W. reports the patent (WO2017020982A1) "Agents for use in the treatment of glioma". F.W. is co-founder of DC Europa Ltd (a company trading under the name Divide & Conquer) that is developing new medicines for the treatment of glioma. Divide & Conquer also provides research funding to F.W.'s lab under a research collaboration agreement. All other authors declare no competing interests.

References

Bergles, D.E., Roberts, J.D., Somogyi, P., and Jahr, C.E. (2000). Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature *405*, 187–191.

Coppola, A., Zarabla, A., Maialetti, A., Villani, V., Koudriavtseva, T., Russo, E., Nozzolillo, A., Sueri, C., Belcastro, V., Balestrini, S., et al. (2020). Perampanel confirms to be effective and well-tolerated as an add-on treatment in patients with brain tumor-related epilepsy (PERADET study). Front. Neurol. *11*, 592.

Dolgin, E. (2020). Cancer-neuronal crosstalk and the startups working to silence it. Nat. Biotechnol. *38*, 115–117.

Faulkner, S., Jobling, P., March, B., Jiang, C.C., and Hondermarck, H. (2019). Tumor neurobiology and the war of nerves in cancer. Canc. Discov. *9*, 702–710.

Gritsenko, P.G., Atlasy, N., Dieteren, C.E.J., Navis, A.C., Venhuizen, J.H., Veelken, C., Schubert, D., Acker-Palmer, A., Westerman, B.A., Wurdinger, T., et al. (2020). p120-catenin-dependent collective brain infiltration by glioma cell networks. Nat. Cell Biol. *22*, 97–107.

Hanahan, D. and Weinberg, R.A. (2011). Hallmarks of cancer: The next generation. Cell *144*, 646–674.

Jung, E., Alfonso, J., Monyer, H., Wick, W., and Winkler, F. (2020). Neuronal signatures in cancer. Int. J. Canc. *147*, 3281–3291.

Jung, E., Alfonso, J., Osswald, M., Monyer, H., Wick, W., and Winkler, F. (2019). Emerging intersections between neuroscience and glioma biology. Nat. Neurosci. *22*, 1951–1960.

Jung, E., Osswald, M., Blaes, J., Wiestler, B., Sahm, F., Schmenger, T., Solecki, G., Deumelandt, K., Kurz, F.T., Xie, R., et al. (2017). Tweety-homolog 1 drives brain colonization of gliomas. J. Neurosci. *37*, 6837–6850.

Mauffrey, P., Tchitchek, N., Barroca, V., Bemelmans, A., Firlej, V., Allory, Y., Romeo, P.H., and Magnon, C. (2019). Progenitors from the central nervous system drive neurogenesis in cancer. Nature *569*, 672–678.

Monje, M., Borniger, J.C., D'Silva, N.J., Deneen, B., Dirks, P.B., Fattahi, F., Frenette, P.S., Garzia, L., Gutmann, D.H., Hanahan, D., et al. (2020). Roadmap for the emerging field of cancer neuroscience. Cell *181*, 219–222.

Osswald, M., Jung, E., Sahm, F., Solecki, G., Venkataramani, V., Blaes, J., Weil, S., Horstmann, H., Wiestler, B., Syed, M., et al. (2015). Brain tumour cells interconnect to a functional and resistant network. Nature *528*, 93–98.

Osswald, M., Solecki, G., Wick, W., and Winkler, F. (2016). A malignant cellular network in gliomas: Potential clinical implications. Neuro Oncol. *18*, 479–485.

Pan, Y., Hysinger, J.D., Barron, T., Schindler, N.F., Cobb, O., Guo, X., Yalçın, B., Anastasaki, C., Mulinyawe, S.B., Ponnuswami, A., et al. (2021). NF1 mutation drives neuronal activity-dependent initiation of optic glioma. Nature *594*, 277–282.

Saloman, J.L., Albers, K.M., Li, D., Hartman, D.J., Crawford, H.C., Muha, E.A., Rhim, A.D., and Davis, B.M. (2016). Ablation of sensory neurons in a genetic model of pancreatic ductal adenocarcinoma slows initiation and progression of cancer. Proc. Natl. Acad. Sci. U. S. A. *113*, 3078–3083.

Schneider, M., Potthoff, A.L., Evert, B.O., Dicks, M., Ehrentraut, D., Dolf, A., Schmidt, E.N.C., Schafer, N., Borger, V., Pietsch, T., et al. (2021a). Inhibition of intercellular cytosolic traffic via gap junctions reinforces lomustine-induced toxicity in glioblastoma independent of MGMT promoter methylation status. Pharmaceuticals *14*, 195–208.

Schneider, M., Vollmer, L., Potthoff, A.L., Ravi, V.M., Evert, B.O., Rahman, M.A., Sarowar, S., Kueckelhaus, J., Will, P., Zurhorst, D., et al. (2021b). Meclofenamate causes loss of cellular tethering and decoupling of functional networks in glioblastoma. Neuro Oncol., https://doi.org/10.1093/neuonc/noab092 (Epub ahead of print).

Venkataramani, V., Tanev, D.I., Kuner, T., Wick, W., and Winkler, F. (2021). Synaptic input to brain tumors: Clinical implications. Neuro Oncol. *23*, 23–33.

Venkataramani, V., Tanev, D.I., Strahle, C., Studier-Fischer, A., Fankhauser, L., Kessler, T., Korber, C., Kardorff, M., Ratliff, M., Xie, R., et al. (2019). Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature *573*, 532–538.

Venkataramani, V. and Winkler, F. (2021). Activation of retinal neurons triggers tumour formation in cancer-prone mice. Nature *594*, 179–180.

Venkatesh, H.S., Johung, T.B., Caretti, V., Noll, A., Tang, Y., Nagaraja, S., Gibson, E.M., Mount, C.W., Polepalli, J., Mitra, S.S., et al. (2015). Neuronal activity promotes glioma growth through neuroligin-3 secretion. Cell *161*, 803–816.

Venkatesh, H.S., Morishita, W., Geraghty, A.C., Silverbush, D., Gillespie, S.M., Arzt, M., Tam, L.T., Espenel, C., Ponnuswami, A., Ni, L., et al. (2019). Electrical and synaptic integration of glioma into neural circuits. Nature *573*, 539–545.

Venkatesh, H.S., Tam, L.T., Woo, P.J., Lennon, J., Nagaraja, S., Gillespie, S.M., Ni, J., Duveau, D.Y., Morris, P.J., Zhao, J.J., et al. (2017). Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. Nature *549*, 533–537.

Weil, S., Osswald, M., Solecki, G., Grosch, J., Jung, E., Lemke, D., Ratliff, M., Hanggi, D., Wick, W., and Winkler, F. (2017). Tumor microtubes convey resistance to surgical lesions and chemotherapy in gliomas. Neuro Oncol. *19*, 1316–1326.

Winkler, F. and Wick, W. (2018). Harmful networks in the brain and beyond. Science *359*, 1100–1101.

Zeng, Q., Michael, I.P., Zhang, P., Saghafinia, S., Knott, G., Jiao, W., McCabe, B.D., Galvan, J.A., Robinson, H.P.C., Zlobec, I., et al. (2019). Synaptic proximity enables NMDAR signalling to promote brain metastasis. Nature *573*, 526–531.

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Presentation of scientific institutions

Matthias Brand*, Rudolf Stark and Tim Klucken

DFG-Research Unit (FOR) 2974 “Affective and cognitive mechanisms of specific Internet-use disorders (ACSID)”

https://doi.org/10.1515/nf-2021-0024

In Mai 2021 the transregional Research Unit FOR 2974 entitled “Affective and cognitive mechanisms of specific Internet-use disorders (ACSID)”, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), has started work. Eleven principal investigators (PI) from nine different sites in Germany will investigate mechanisms potentially involved in the development and maintenance of specific Internet-use disorders. The Research Unit will be funded for up to six years (first funding period is three years). The 11 principal investigators are: Matthias Brand, Duisburg-Essen (Speaker), Martin Diers, Bochum, Tim Klucken, Siegen, Christian Montag, Ulm, Astrid Müller, Hannover, Hans-Jürgen Rumpf, Lübeck, Rudolf Stark, Giessen, Sabine Steins-Löber, Bamberg, Elisa Wegmann, Duisburg-Essen, Oliver Wolf, Bochum, Klaus Wölfling, Mainz. The group represents various disciplines necessary for studying neurobiological and psychological processes that may explain mental disorders, such as biological, cognitive, clinical, and personality psychology, neuropsychology, psychotherapy, and medicine, which will guarantee a high level of interdisciplinarity within the Research Unit.

The Research Unit focuses on the investigation of the fundamental affective and cognitive mechanisms, which are involved in the development and the maintenance of predominantly online addictive behaviors, namely pathological gaming, pornography use, buying-shopping, and social-networks use. The overall goal of the Research Unit is to contribute to a better understanding of the common and differential psychological and neurobiological processes underlying different types of specific Internet-use disorders. Gaming, pornography use, buying-shopping, social-networks use are usually unproblematic for most users and represent every-day behaviors. However, in some people the voluntary control over these behaviors may become diminished, the behaviors are given increasing priority in daily life, and the behaviors are continued or even escalated despite the occurrence of negative consequences resulting in significant impairment in personal, family, social, educational, occupational or other important areas of functioning and/or marked distress (see diagnostic characteristics of gaming disorder in the 11th revision of the International Classification of Diseases, ICD-11) (World-Health-Organization, 2019). The ICD-11 provides the category “disorders due to addictive behaviors” which includes gaming disorder as an own entity and which might be the appropriate diagnostic classification for the other specific Internet-use disorders as well (Brand et al., 2020).

The overall hypotheses are based on the theoretical framework of the Interaction of Person-Affect-Cognition-Execution (I-PACE) model (Brand et al., 2019), which integrates contemporary models of addiction research (e.g., Berridge and Robinson, 2016; Blum et al., 2012; Dong and Potenza, 2014; Everitt and Robbins, 2016; Goldstein and Volkow, 2011; Koob, 2015). In short, this model incorporates affective, cognitive, and executive processes to explain alterations in reinforcement and gratification mechanisms, which significantly contribute to the etiology of specific Internet-use disorders. Moreover, the I-PACE model allows to develop clear (neurobiological and psychological) hypotheses for the underlying processes and their alterations in the course of the development and maintenance of behavioral addictions.

The Research Unit will investigate various affective and cognitive mechanisms in different clinical samples. Clinical

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Rudolf Stark, Department of Psychotherapy and Systems Neuroscience, Justus Liebig University of Giessen, Giessen, Germany; Bender Institute of Neuroimaging, Justus Liebig University of Giessen, Giessen, Germany; and Center of Mind, Brain and Behavior, Universities of Marburg and Giessen, Giessen, Germany
Tim Klucken, Clinical Psychology and Psychotherapy, University of Siegen, Siegen, Germany

samples of patients with gaming disorder, patients with pathological use of pornography, patients with buying-shopping disorder, and patients with pathological social-networks use will be contrasted with respective samples with subclinical problems (problematic/risky use) and those with unproblematic use. Further comparison groups are patients with substance-use disorders (nicotine, alcohol) and (offline) gambling disorder. This approach will allow us to study the transition from unproblematic behaviors to risky behaviors, and finally from risky behaviors to pathological behaviors and across different types of addictive behaviors. A more comprehensive overview about the theoretical foundation, the challenges and goals of the Research Unit as well as the synergies across the research projects can be found in a current article, which has recently been published in Addiction Biology (Brand et al., 2021). Figure 1 summarizes the different research projects of the Research Unit. In the following, the seven projects are described briefly with a specific focus on two projects, which will use fMRI.

Central to the overall aims of the Research Unit is the Research Project led by **PI Brand**. All participants from the specific other projects of the Research Unit will be examined with a core battery, which includes diagnostic instruments and questionnaires related to Internet use, well-established experimental addiction-related paradigms, standard neuropsychological tasks, personality assessment, addiction-related questionnaires, and biological markers. Additionally, all participants will take part in a 14-day ambulatory assessment to investigate personal use in their natural environments, followed by a six-month follow-up measurement to identify variables potentially predicting the course of the disorder within this period. The collected data will allow us to test the overall hypotheses about mechanisms, including predictors, mediators, and moderators, derived from the I-PACE model. Another project (**RP Steins-Loeber/Müller**) will address aspects of appetitive conditioning and habitual responses using a Pavlovian-to-Instrumental-Transfer (PIT)-paradigm in combination with stress induction. The groups of interest in this project are participants with risky use of games and risky buying-shopping, which will be compared with non-problematic users of games and individuals with non-problematic buying-shopping behaviors. The project by **PI Müller, PI Wegmann, and PI Wolf** will examine cue-reactivity and implicit cognitions in buying-shopping disorder and social-networks-use disorder. They will also investigate effects of acute stress on these affective and cognitive processes. The **RP by PI Wölfling and PI Steins-Löber** will study implicit associations and cue-induced diminished impulse control in samples with

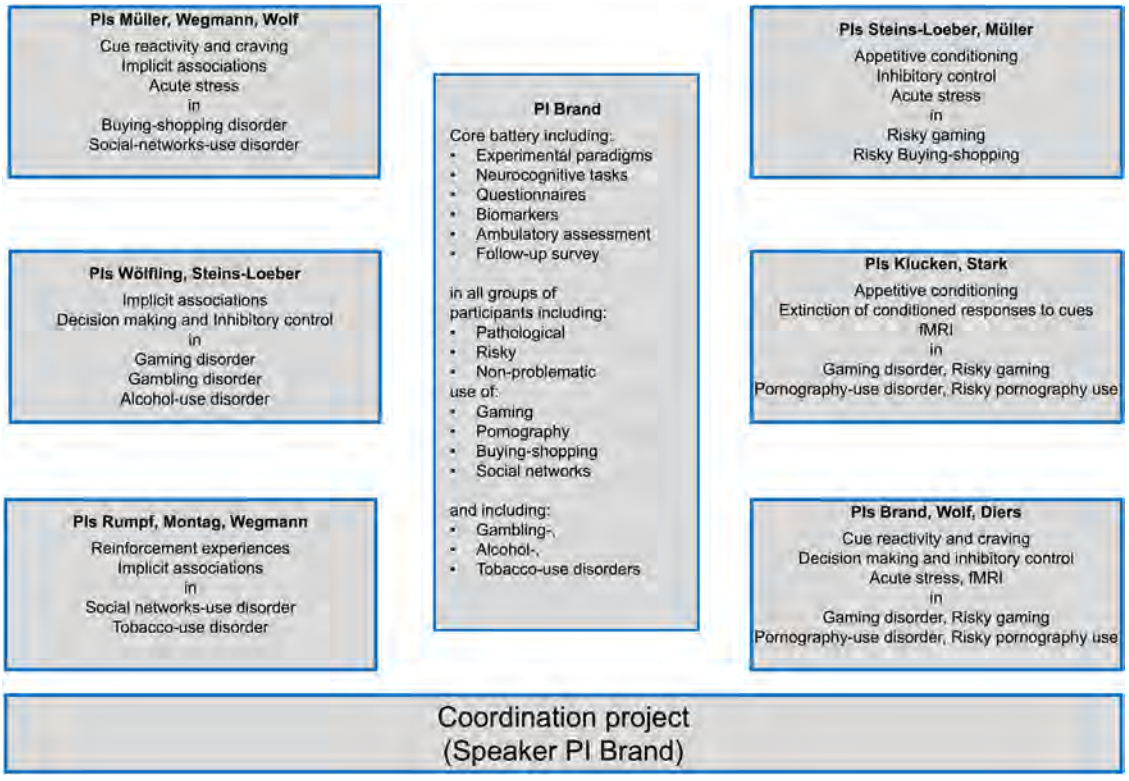

Figure 1: Summary of the research projects of the Research Unit FOR 2974 ACSID.

individuals with gaming disorder, (offline) gambling disorder, and alcohol-use disorder. A further project (**RP Rumpf/Wegmann/Montag**) will investigate reinforcement experiences in participants with pathological social-networks use and participants with tobacco-use disorder. This project will also include samples of individuals with risky use and non-problematic use of social networks and tobacco as comparison groups.

Neural correlates of specific Internet-use disorders will be exemplified by two fMRI projects. Both projects will investigate users of games and pornography with either unproblematic, risky, or pathological use. **PI Klucken and PI Stark** will focus on altered appetitive conditioning and extinction processes in patients with pornography-use disorder and gaming disorder (and the aforementioned comparison groups). During appetitive conditioning, a formerly neutral stimulus turns into a salient cue that announces a monetary reward or the addiction-related behavior. During extinction, this (new) salient cue will lose its significance by unpairing with the reward. Therefore, the participants have to learn, re-learn, and change contingency expectations between the stimuli and the rewards during the experiment. The project investigates whether individuals with a pornography-use disorder or gaming disorder (and the risky groups related to these disorders) show facilitated conditioning and/or deficits in extinction processes. Consistent with the proposed theoretical framework, a multifactorial approach will be conducted to measure the learning and re-learning processes on different response levels, i.e. subjective/cognitive level (e.g., stimulus evaluation), psychophysiological level (e.g., skin conductance responses), and neural level (e.g., amygdala and/or striatal reactivity). On the neural level, we expect differences in specific regions of interest (ventral striatum, amygdala, ventromedial prefrontal cortex) depending on whether the groups respond to their specific cues (e.g., pornography versus gaming) and whether they are classified as risky or pathological users of pornography or games, respectively.

The other fMRI project (**RP Brand/Wolf/Diers**) aims at investigating behavioral and neural correlates of cue reactivity and craving in gaming disorder compared to pornography-use disorder (also including groups of risky use and unproblematic use as comparisons). Effects of acute stress on cue reactivity and craving as well as interactions between cue reactivity and craving, acute stress, and measures of executive functions and inhibitory control will also be examined. The study includes a two-day assessment: On the first day, different behavioral experiments related to cue reactivity, and craving as well as decision making will be conducted after a stress induction as compared to a non-stressful placebo condition. At day 2, an fMRI study with a focus on cue reactivity will take place. The stimuli that will be used to induce cue reactivity during the fMRI investigation are either distal or proximal cues related to gaming and pornography use. Using this paradigm, we aim at testing whether a transition from risky to pathological behaviors is accompanied by a shift from ventral to dorsal striatum as neural correlate of cue processing and whether the neural responses to proximal and distal addiction-related cues differ for individuals with risky versus pathological use of games or pornography, respectively.

We hope that the Research Unit will contribute to a better and more detailed understanding of the underlying neural, cognitive, and affective processes of behavioral addictions, which may inspire future research. We also hope that our findings will pave the way for the development of new treatment options of such disorders by identifying crucial underlying processes and variables, which may be targeted more specifically in future treatment approaches.

Homepage of the Research Unit FOR 2974 ACSID: https://www.uni-due.de/for2974/.

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Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Berridge, K.C. and Robinson, T.E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. Am. Psychol. *71*, 670–679.

Blum, K., Gardner, E., Oscar-Berman, M., and Gold, M. (2012). "Liking" and "wanting" linked to Reward Deficiency Syndrome (RDS): Hypothesizing differential responsivity in brain reward circuitry. Curr. Pharmaceut. Des. *18*, 113–138.

Brand, M., Müller, A., Stark, R., Steins-Loeber, S., Klucken, T., Montag, C., Diers, M., Wolf, O.T., Rumpf, H.-J., Wölfling, K., et al. (2021). Addiction Research Unit: Affective and cognitive mechanisms of specific Internet-use disorders. Addiction Biol., e13087.

Brand, M., Rumpf, H.-J., Demetrovics, Z., Müller, A., Stark, R., King, D.L., Goudriaan, A.E., Mann, K., Trotzke, P., Fineberg, N.A., et al. (2020). Which conditions should be considered as disorders in the International Classification of Diseases (ICD-11) designation of "other

specified disorders due to addictive behaviors"? J. Behav. Addict. (Epub ahead of print).

Brand, M., Wegmann, E., Stark, R., Müller, A., Wölfling, K., Robbins, T.W., and Potenza, M.N. (2019). The Interaction of Person-Affect-Cognition-Execution (I-PACE) model for addictive behaviors: Update, generalization to addictive behaviors beyond Internet-use disorders, and specification of the process character of addictive behaviors. Neurosci. Biobehav. Rev. *104*, 1–10.

Dong, G. and Potenza, M.N. (2014). A cognitive-behavioral model of Internet gaming disorder: Theoretical underpinnings and clinical implications. J. Psychiatr. Res. *58*, 7–11.

Everitt, B.J. and Robbins, T.W. (2016). Drug addiction: Updating actions to habits to compulsions ten years on. Annu. Rev. Psychol. *67*, 23–50.

Goldstein, R.Z. and Volkow, N.D. (2011). Dysfunction of the prefrontal cortex in addiction: Neuroimaging findings and clinical implications. Nat. Rev. Neurosci. *12*, 652–669.

Koob, G.F. (2015). The dark side of emotion: The addiction perspective. Eur. J. Pharmacol. *753*, 73–87.

World-Health-Organization (2019). ICD-11 for Mortality and Morbidity Statistics (World Health Organization: Geneva). Available from: https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/1448597234.

Nachrichten aus der Gesellschaft

https://doi.org/10.1515/nf-2021-0027

Methodenkursprogramm der NWG 2022

Nachdem in diesem und im letzten Jahr die meisten Kurse Corona-bedingt nicht stattfinden konnten, hoffen wir, dass im kommenden Jahr das Methodenkursprogramm der NWG wieder ohne Einschränkungen durchgeführt werden kann. NWG-Mitglieder können kostenlos oder zu einem reduzierten Beitrag an diesen Veranstaltungen teilnehmen. Die Anmeldung erfolgt über die jeweils angegebene Kontaktadresse.

March 7–9, 2022: Comparative Anatomy and Pathology of the Rodent and Human Brain
Registration deadline: February 27, 2022
Venue: Section Clinical Neuroanatomy, Neurology, Center for Biomedical Research (ZBF), Helmholtzstr. 8/1, 89081 Ulm
Topics: The goal of the course is to compare pathologic alterations found in experimental rodent models of neurodegenerative diseases with the pathology in the human brain. Participants are encouraged to bring histological sections from their own experiments and rodent models to the course for discussion (2 weeks notice required). Overview of the anatomy of the rodent and human brain and spinal cord; hands-on-lab sessions for introduction into neuroanatomical techniques to study the human brain; pathological neuroanatomy of neurodegenerative disorders including but not limited to Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.
Organization and registration: Prof. Dr. Deniz Yilmazer-Hanke, phone (office): +49 (0)731 500 63157, phone (lab): +49 (0)731/500 63158, email: deniz.yilmazer-hanke@uni-ulm.de

March 10–11, 2022: Pathoanatomy of the Human Central Nervous System
Registration deadline: February 27, 2022
Venue: Section Clinical Neuroanatomy, Neurology, Center for Biomedical Research (ZBF), Helmholtzstr. 8/1, 89081 Ulm
Topics: Introduction to neuroanatomical techniques to study the neuroanatomy of the human brain including hands-on laboratory sessions; pathological anatomy, histology and histopathology of the human brain and spinal cord in neurodegenerative diseases; staging of pathological changes in Alzheimer's and Parkinson's Disease and Amyotrophic Lateral Sclerosis.
Organization and registration: Prof. Dr. Deniz Yilmazer-Hanke, phone (office): +49 (0)731 500 63157, phone (lab): +49 (0)731/500 63158, email: deniz.yilmazer-hanke@uni-ulm.de

March 17–18, 2022: Behavioral Testing in Rodents: from Cognition, Motor Function, Emotion, Anxiety to Pain
Registration deadline: March 4, 2022
Venue: Interdisciplinary Neurobehavioral Core INBC, University of Heidelberg INF 515; 69120 Heidelberg
Topics: Behavioral testing in rodents: from cognition, motor function, emotion, anxiety to pain. A hands-on course.
Organization and registration: Dr. Claudia Pitzer, Tel.: 06221 1858504, email: claudia.pitzer@pharma.uni-heidelberg.de, http://www.medizinische-fakultaet-hd.uni-heidelberg.de/Home.111344.0.html

July 25–29, 2022: Transcranial Brain Stimulation in Research and Clinic: Best Practice
Venue: 1) Universität Mainz, Langenbeckstr. 1, Gebäude. 308c, 55131 Mainz and Neuroimaging Center (NIC) der Universitätsmedizin der Johannes Gutenberg Universität (July 25 – 27, 2022; bus transfer from Mainz to Göttingen will be provided on July 27)
2) Klinik für Neurologie, Universitätsmedizin Göttingen, Robert-Koch-Str. 40, 37075 Göttingen (July 27 - 29, 2022)
Registration deadline: July 1, 2022
Topics: transcranial magnetic-, direct current-, alternating current, random noise, and ultrasound stimulation, combination of brain stimulation and neuroimaging (EEG, fMRI), theoretical background of the stimulation, animal models, modelling of current flow in the brain, research and clinical applications; neuronavigation, neuronal oscillations, cognition, ethical aspects of transcranial stimulation.
Organisation and registration: apl. Prof. Andrea Antal, Tel.: 0551 398461, Fax: 0551 398126, email: AAntal@gwdg.de; Prof. Til Ole Bergmann, Tel.: 06131-17-7720, Fax: 06131-17-8346, email: tobergmann@uni-mainz.de

September 26–30, 2022: Imaging Techniques in Neuroscience
Venue: Leibniz Institute for Neurobiology (LIN), Brenneckestraße 6, 39118 Magdeburg
Registration deadline: July 31, 2022
Topics: Hands-on introduction into advanced imaging techniques to study neuronal function: super-resolution STED, lightsheet microscopy (including clearing methods), 2photon microscopy, calcium imaging, FLIM/FRET, label-free metabolic imaging (NADH/FAD), image analysis.
Organisation: Leibniz Institute for Neurobiology, Combinatorial NeuroImaging Core Facility (CNI)
Registration: Torsten Stöter, Combinatorial NeuroImaging Core Facility (CNI), Leibniz Institute for Neurobiology, Tel.: 0391 6263 92171, email: cni-reg@lin-magdeburg.de

October 2022: Tübingen Systems Neuroscience Symposium 2022
Venue: MEG-Zentrum der Universität Tübingen, Otfried-Müller-Straße 47, 72072 Tübingen
Registration deadline: April 30, 2022
Topics: The 2022 Tübingen Systems Neuroscience Symposium brings together leading international researchers in the field of systems neuroscience. Topics range from neurophysiological testing in animals to functional imaging in humans (MEG, EEG, fMRI). One focus of the symposium is the presentation of state of the art methods. The talks target students and researchers with profound previous knowledge.
Organisation and registration: Prof. Dr. Christoph Braun, Tel: 07071 29 87706, Fax: 07071 29 5706, email: christoph.braun@uni-tuebingen.de

NEU auf dasGehirn.info

Im September 2021 hat sich das Portal um den Themenschwerpunkt **Sport** gekümmert, wofür drei Artikel entstanden

Allround-Wundermittel fürs Gehirn: Klar, Sport ist gesund. Aber nicht nur für den Körper, er hält auch Geist und Gehirn fit – auf vielfältige und auch überraschende Weise.

Wer tanzt, bleibt jung: Bewegung schützt das Gehirn in vielfältiger Weise – über Botenstoffe, Hormone sowie Gefäß- und Nervenzellwachstum. Sogar Herz und Leber senden bei sportlicher Betätigung eine gute Botschaft ans Oberstübchen.

Sport: Rundum-glücklich-Paket für Körper und Geist: Dass Sport gesund ist, wissen wir alle. Trotzdem kriegen wir den Hintern oft nicht hoch. Vielleicht hilft es, sich nochmals klarzumachen, welche vielfältigen positiven Effekte regelmäßige Bewegung hat – nicht zuletzt auf das Gehirn.

Das Schwerpunktthema **Struktur und Funktion neuronaler Netzwerke**, eine Themenpartnerschaft mit dem SFB 870, wurde um ein Videointerview ergänzt:

Struktur und Funktion: Regeneration: Der größte Teil unseres Körpers heilt, indem er neue Zellen bildet: Haut, Knochen, Blut … Nur das Gehirn muss mit dem leben, was es hat – es kann sich nicht regenerieren. Doch auch hier lässt sich die Stammzellbildung anregen. Über diese neuen Erkenntnisse sprechen wir mit den Profs. Benedikt Grothe und Magdalena Götz.

In dem Format **Frage an das Gehirn** beantworten Experten regelmäßig Fragen unserer Leser. Zuletzt ging es um die folgenden Fragen:

Wie kann ich mich besser konzentrieren? – Beim Arbeiten schweife ich öfter mal ab. Was kann ich dagegen tun?

Kann man Ängstlichkeit im Gehirn erkennen? – Lässt sich aus der Hirnstruktur oder anhand von biologischen Markern erkennen, ob ein Mensch ängstlich oder mutig ist?

Machen Walnüsse schlau? – Walnüsse gelten als besonders gute Nahrung fürs Gehirn. Ist da was dran?

In der Rubrik **Neues aus der Wissenschaft** macht dasGehirn.info im September 2021 auf die folgenden Pressemeldungen aus den Instituten aufmerksam:

Warum uns mit dem Alter Wörter schlechter einfallen | Max-Planck-Institut für Kognitions- und Neurowissenschaften (02.09.2021)

Wie das motorische System im Gehirn beim Fremdsprachenlernen helfen kann | Technische Universität Dresden (09.09.2021)

Wie Düfte eine Bedeutung bekommen | Ruhr-Universität Bochum (16.09.2021)

Lokale Lieferketten in Neuronen: Wer kommt an die Ware? | Max-Planck-Institut für Hirnforschung (20.09.2021)

Möchten Sie eine Pressemeldung an **dasGehirn.info** weitergeben oder Ihr Institut vorstellen, wenden Sie sich bitte an Arvid Leyh (email: a.leyh@dasgehirn.info).

Neueintritte

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

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Umberto Calleri (Düsseldorf)
Felix Fiederling, Dr. (New York, USA)
Johanna Hallenberger (Düsseldorf)
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Anna Josefine Torner (Hildesheim)
Linda Weiss, Dr. (Bochum)
Dieter Willbold, Prof. Dr. (Jülich)
Emile Wogram, Dr. med (Cambridge, USA)
Alfred Yamoah (Aachen)

Der Mitgliedsstand zum 30. September 2021 beträgt 2.062 Mitglieder.

We thank all colleagues who reviewed Neuroforum articles in 2021. We really appreciate your cooperation

Brandner, Sebastian
Deller, Thomas
Distler, Claudia
Engelhardt, Maren
Helfrich-Foerster, Charlotte
Luhmann, Heiko
Steinhäuser, Christian
Wahle, Petra
Wegener, Christian

Ausblick

Jutta Schöpfer
Is the natural distribution of Lithium in brain tissue a hint for essentiality?

Linda Weiß
The neurobiology of phenotypic plasticity in the light of human stressors

Katrin Franke
What the eye tells the brain: Retinal feature extraction

Julijana Gjorgjieva
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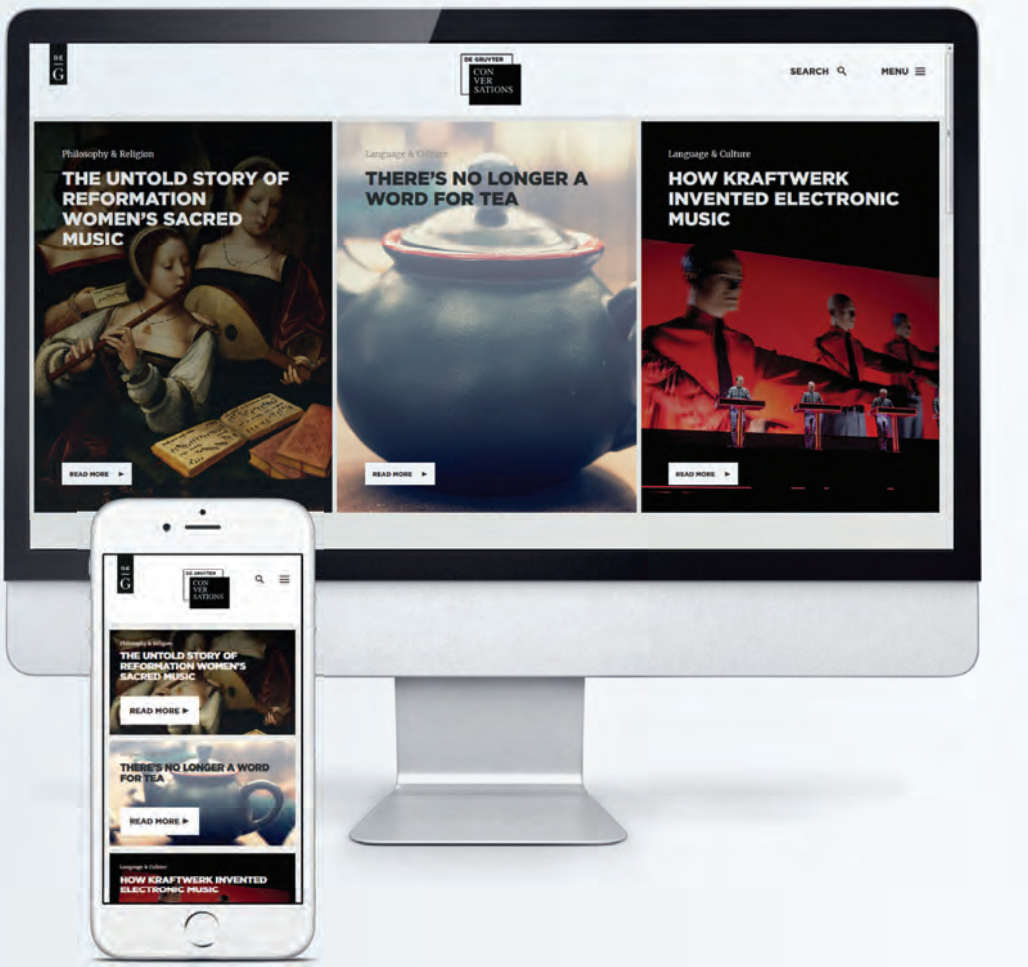

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Kontakt: Prof. Dr. Stefan Kins
Telefon: 0631 2052106/2107
E-Mail: l.hanke@biologie.uni-kl.de

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Telefon: 0631 2052106/2107
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24. NOVEMBER 2021 | BERLIN
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Kontakt: Dr. Luiza Bengtsson
Telefon: 030 94892931
E-Mail: LaborTrifftLehrer@mdc-berlin.de

9. FEBRUAR 2022 | BERLIN via Zoom
ORGANOIDE DES MENSCHLICHEN GEHIRNS ALIAS »MINI-GEHIRNE« ALS WERKZEUGE ZUR ERFORSCHUNG VON KRANKHEITEN DES NERVENSYSTEMS
Kontakt: Dr. Luiza Bengtsson
Telefon: 030 94892931
E-Mail: LaborTrifftLehrer@mdc-berlin.de

18. FEBRUAR 2022 | HEIDELBERG
GROSSHIRN AN NEBENNIERE – WAS SIE SCHON IMMER ÜBER STRESS WISSEN WOLLTEN
Kontakt: Prof. Dr. Andreas Draguhn/Susanne Bechtel
Telefon: 06221 544056
E-Mail: susanne.bechtel@physiologie.uni-heidelberg.de

24. FEBRUAR 2022 | TÜBINGEN
NEUROWISSENSCHAFTEN UND IMMUNOLOGIE
Kontakt: Prof. Dr. Uwe Ilg
Telefon: 07071 2980464 (Hertie-Institut)
07071 2982377 (Schülerlabor)
E-Mail: uwe.ilg@uni-tuebingen.de

16. MÄRZ 2022 ODER
17. MÄRZ 2022 | GÖTTINGEN
NEUROWISSENSCHAFTEN
Kontakt: Dr. Sylvia Ranneberg
Telefon: 0551 3851163
E-Mail: sranneberg@dpz.eu

16. MÄRZ 2022 | LEIPZIG
NEUE ENTWICKLUNGEN IN DER MIKROSKOPIE
Kontakt: Prof. Dr. Steffen Rossner / Dr. Max Holzer
Tel.: 0341 9725758 / 0341 9725759
E-Mail: rossn@medizin.uni-leipzig.de oder holm@medizin.uni-leipzig.de

Informationsmaterial für Lehrer finden Sie auf der Homepage der NWG.