The brain during free movement – what can we learn from the animal model

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Abstract

Animals, just like humans, can freely move. They do so for various important reasons, such as finding food and escaping predators. Observing these behaviors can inform us about the underlying cognitive processes. In addition, while humans can convey complicated information easily through speaking, animals need to move their bodies to communicate. This has prompted many creative solutions by animal neuroscientists to enable studying the brain during movement. In this review, we first summarize how animal researchers record from the brain while an animal is moving, by describing the most common neural recording techniques in animals and how they were adapted to record during movement. We further discuss the challenge of controlling or monitoring sensory input during free movement.

However, not only is free movement a necessity to reflect the outcome of certain internal cognitive processes in animals, it is also a fascinating field of research since certain crucial behavioral patterns can only be observed and studied during free movement. Therefore, in a second part of the review, we focus on some key findings in animal research that specifically address the interaction between free movement and brain activity. First, focusing on walking as a fundamental form of free movement, we discuss how important such intentional movements are for understanding processes as diverse as spatial navigation, active sensing, and complex motor planning. Second, we propose the idea of regarding free movement as the expression of a behavioral state. This view can help to understand the general influence of movement on brain function.

Together, the technological advancements towards recording from the brain during movement, and the scientific questions asked about the brain engaged in movement, make animal research highly valuable to research into the human "moving brain".

Introduction

Unlike humans, animals will not voluntarily sit still during the execution of scientific experiments. However, since they are also not able to respond to behavioral probing through speaking and only in a few cases can answer by a button press or a directed eye movement, animals actually need to move their bodies in order to convey information about the outcome of cognitive processes. Therefore, when studying the role of the brain in cognitive functioning, animal researchers have to embrace the movements of their experimental subjects rather than suppress them. Scientists indeed have come up with many diverse techniques to make this possible.

In comparison, studying the human brain, there seems less need to provide participants with the freedom to move. Language gives us the possibility to convey complex information without actually needing to enact it. Even looking at locomotion as the epitome of free movement, there are many possibilities to test single aspects of this while restricting the actual freedom to move to a minimum. Freely moving through space is an intentional action based on spatial navigation, active sensing and motor planning. Using elaborate setups including virtual reality, treadmills, and focusing on planning very small movements these aspects can be studied in humans without allowing actual locomotion. However, this is only possible to a limited extent, especially concerning the complex interplay between these processes. Additionally, recent behavioral research has made very clear that the movement of the whole body can also affect a surprising number of cognitive functions, which on first sight seem to be independent of large body movements. These include memory, attention, and sensory

integration (Schmidt-Kassow et al., 2013; Schmidt-Kassow et al., 2014; Kirsch et al., 2017; Schaefer et al., 2010; McMorris and Graydon, 2000; Smith et al., 2010). Animal studies have shown similar effects in freely moving animals, whilst concurrently starting to uncover how brain activity is affected by free movements (Niell and Stryker, 2010; Polack et al., 2013; Saleem et al., 2013). Since neurophysiological measurements in humans during free walking are exceedingly rare, this review focuses on locomotion in animals and omits other research on free movements such as from the arm or hand. Human research might profit most readily from these animal studies during locomotion.

Despite the fact that portable recording systems were developed in animals out of sheer necessity to allow the animal to convey information through body movements, findings from ongoing animal research can be most useful for human research in this field. The benefit can be at least twofold: 1) the technical advancements enabling researchers to study the animal brain during large movements might inspire technologies to record neural activity in the moving human brain, and 2) the ongoing animal research might help to formulate hypotheses or shape promising experimental questions based on results from the animal model. It is our belief that the full potential of these benefits has not yet been met.

This review is divided into two main parts: first, we address the methodological developments and challenges in research on freely moving animals. Second, we discuss experimental questions in animal research that specifically address the interaction between brain activity and free movements with a focus on locomotion in mammals. We discuss how important the role of free locomotion is for understanding spatial navigation, active sensing, and complex motor planning. Additionally, we propose the idea of regarding movement as the expression of a behavioral state. In this view, free locomotion is the most active state of a wide range of behavioral states, including sleeping and being quietly awake. We suggest thinking about freely walking as a specific behavioral state since it calls for very specific processing of external stimuli and generating motor output. This view helps to understand the general influence of movement on brain function.

Methodological approaches

Recording brain activity during movement

Broadly speaking, the techniques for recording neural activity from the brain fall into two categories. There are those that directly measure electrical activity from neurons using e.g. microelectrodes, electroencephalography (EEG) or electrocorticography (ECoG), and those that measure neuronal activity indirectly by imaging the metabolic activity of neurons, such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and calcium imaging. These sets of methods have been refined to record from moving animals in many ways. For this review, we will describe the mobile versions of the above-mentioned techniques and refer to excellent reviews that specifically cover these techniques individually. It is also possible to measure neural dynamics intracellularly in moving animals (Lee et al., 2006). However fascinating, these methods have no comparable application in humans. We therefore will omit these techniques as well as those that actively manipulate neuronal activity like microstimulation or optogenetics, and focus on recording techniques only.

<u>Direct measurements - electrophysiology</u>

Neuronal activity can be assessed directly through the use of electrodes which measure the electrical communication of neurons.

Micro-electrodes

Recording electrical activity from neurons in the animal brain is mostly done through extracellular recordings. In a typical set-up, part of the skull is removed, and single electrodes or electrode arrays (optionally with multiple contact points – often referred to as laminar electrodes) are either being inserted for every experimental session or implanted permanently (Okun et al., 2016) (Fig 1A). In the latter case, the skull and skin are placed back. The inserted electrodes measure voltage differences with respect to a neutral reference, for example from the skull. These voltage differences reflect either action potentials (called spiking) from single neurons (single unit activity – SUA) or populations of neurons (multi-unit activity – MUA), or the local field potential (LFP), which is an aggregate measure of local changes in electrical fields caused mainly by dendritic activity of the neurons. It contains no spikes but rather slow changes in the electric potential (Fig 1B). Which type of neuronal activity is recorded depends mostly on the surface area of the electrode; single electrodes usually have a small surface area and are therefore well-suited to pick up SUA, whereas electrode arrays and laminar electrodes typically have larger surface areas and therefore mostly pick up MUA and LFP. The neuronal activity, i.e. LFP and spiking, is then transmitted to an amplifier and passed on for storage and further processing. In a conventional set-up, this transmission is realized via wires connecting the electrodes to the amplifier and then to the storage. These systems are called *tethered systems*. Typically, the animal's head is immobilized during a recording session via a head-post secured to the skull, which allows for task designs in front of a computer screen, as well as reduces mechanical stress onto the amplifiers and excessive muscle activity from the head (Fig 1C). In order to record from neurons in a brain that is not stationary a number of adaptations are necessary, in particular to the transmission of the neural signals. One approach is to use rather long wires that run from the animal's brain to the amplifier, which are mounted elaborately to ensure that the animal can move without getting entangled, step on or rip out the wires. These systems allow long-term recordings with multiple electrodes at a high sampling rate (Fig 1D, left).

However, being tethered by wires can cause some distress to the animals (Kramer et al., 2001), and is specifically limiting for animals which do not mostly move on the ground but fly or climb and jump (Roy and Wang, 2012). Additionally, whenever the animal moves, the tethering wires also move, which interacts with electrostatic fields in the recording set-up, resulting in transient noise on the neural signal termed mains hum. To counteract this noise, the animal's body must be grounded with a low-impedance wire; however, this ground wire will itself add mains hum and movement artifacts to the signal (Chang et al., 2011).

To overcome these problems, there is a second approach in which a battery-operated system attached to the animal's body transmits the signals wirelessly. These systems are often referred to as *telemetric recording systems* (Fig 1D, right). Since in this case, there is no wired connection to the animal the mobile part of the technical devices (recorder plus transmitter/storage) must be carried by the animal and must obtain their power from a battery alone. This limits the possible time of recording and low power consumption of transmission is therefore necessary. Additionally, all equipment (electrodes and transmitter) must be small enough to be comfortably carried around by the animal, either strapped to the body, fixed to the head or worn subcutaneously (Chang et al., 2011). These challenges are shared with intracortical recording approaches in humans.

As early as 1982, successful recordings were done with a telemetric system; a rabbit wore a radio transmitter on a collar and neuronal activity in the hypothalamus was recorded from multiple electrodes (Summerlee and Paisley, 1982). Since then, a number of telemetric recording systems have been developed for various species, including birds (Nieder and Klump, 1999), mice (Fan et al., 2011), rats (Szuts et al., 2011), primates (Roy and Wang, 2012; Chestek et al., 2009), and sheep (Perentos et al., 2017). Ongoing developments constantly improve the size and weight, electrode number, and transmission distance. We want to briefly mention some of the fascinating advancements that have been achieved to date: 64 electrodes have been recorded in a rat from up to 60 m away, even if the rat runs in a tunnel (Szuts et al., 2011). Fifteen electrodes have be recorded from a wireless setup allowing a marmoset to freely jump or climb (Roy and Wang, 2012). Even rats swimming in a water maze can be measured with a wireless system carried on the head (Pinnell et al., 2016). Mice, long posing a problem due to their small size, are by now also subject to wireless measurements (Fan et al., 2011). This development can be of great importance keeping the large amount of transgenic mouse models in mind (Weiergräber et al., 2005). New technological advancements permit researchers to greatly increase the number of recorded electrodes. For example, bidirectional wireless communication made it possible to perform pre-processing on data recorded with a multi-electrode implant over multiple cortical areas in rhesus monkeys. This reduced the amount of information transferred wirelessly, allowing for the recording of up to 512 electrodes (Schwarz et al., 2014).

All above-mentioned examples pertain to recordings with electrodes deep in the brain (depth electrodes), which can pick up voltage changes from one or more single cells. The single neuron level is largely ignored by human clinical neurology (Cash and Hochberg, 2015). However, insights from animal research will become more and more relevant as clinically indicated invasive recording techniques spread in human research. For example, certain movement disorders are treated with deep brain stimulation. During surgery, electrodes are placed within subcortical nuclei such as the basal ganglia. These micro-electrodes have a very small contact area and are therefore well-suited for single unit recordings. Once the correct site for implantation is found, a macro-electrode (with slightly larger contact area) is implanted for (deep brain) stimulation, which can then also be used for measuring population responses while the subject is awake after the surgery (Thompson et al., 2014). Neocortical single unit recordings are obtained from semi-chronic depth electrodes to localize epileptic foci for surgical removal in patients suffering from intractable epilepsies (see Engel et al., 2005 for review). To better characterize these foci, a linear multi-electrode with 20–24 contacts was developed which even allows laminar single unit recordings in humans (Ulbert et al., 2001; Ulbert et al., 2004). The above-mentioned examples show that SUA and MUA activity can be available for the human brain. Such recordings in animals might therefore provide a basis for future wireless recordings of these signals in the freely moving human.

ECoG

Another approach for localizing epileptic foci is via electrocorticography (ECoG) where voltage differences presumably caused by post-synaptic potentials synchronized over a large number of neurons, are picked up with electrode grids lying on the brain's surface beneath the skull and the dura mater (Ojemann et al., 2013). Such subdural ECoG is applied in animals and has also been developed into wireless systems. Rats can wear tiny single-channel ECoG systems in a backpack, which can run without a battery (Chang and Chiou, 2013). For larger primates, implantable and wireless multi-channel devices have been developed, able to provide long-term ECoG recording using a wireless rechargeable battery (Piangerelli et al., 2014). Implantable wireless ECoG grids with 64 electrodes

have been tested in monkeys (Mestais et al., 2015). While ECoG is applied in humans on a regular basis (if medically indicated), the application of wireless ECoG systems is still pending in humans. This could be an excellent case for combining knowledge gathered from animal and human data.

EEG

While the technique of ECoG seems almost directly comparable in humans and animals, EEG is less common in animal research and rather different in approach. One problem is that the human approach of placing an EEG cap on the head of the subject is rather prone to artifacts due to muscle activity and movement of the electrodes caused by the movement of the subject. Especially for moving animals, the human EEG approach is not very suitable. For a long time, rodent EEG techniques employed screw electrodes that are fixed to the skull of the animal. Due to the limited space on a rodent head, only a restricted number of such screw electrodes could be placed on their small skull. Recently, a wireless high-density polyimide-based microelectrode array allowing the recording of up to 26 active EEG and 2 active EMG channels on the skull of a rat has been developed (Stienen et al., 2016). From a translational perspective, especially the research on sheep using wireless EEG (Perentos et al., 2017) seems promising. Their approach using three different types of electrodes (epi- and subdural screw electrodes, disc electrodes and sharp microelectrodes) allows the recording of signals comparable to those recorded with human EEG, as well as more spatially specific neuronal activity, including spikes. Such approaches can be also particularly helpful to bridge animal invasive recordings to human non-invasive data since human wireless EEG systems have become available with increased quality and channel number.

<u>Indirect measurements – brain imaging</u>

Neuronal activity cannot only be assessed directly by measuring the electrical communication of neurons, but also indirectly by imaging their metabolic activity.

Calcium imaging

Two-photon calcium imaging measures changes in intracellular calcium concentration as a readout for neuronal activity. Within a neuron, the intracellular calcium concentration is constantly varying. When the neuron becomes active, calcium flows into the neuron due to the opening of voltage-gated calcium channels in the cell membrane. Dependent on the neuronal cell type and the cellular sub-compartment, more or less voltage is necessary to open the calcium channels (Catterall, 2000). The increase in calcium concentration during activation is often amplified by calcium release from intracellular calcium stores (Tsien and Tsien, 1990). On the other hand, calcium-binding proteins called buffers, such as parvalbumin, will negatively influence the calcium concentration. The amount of calcium in the neurons can be assessed via calcium-sensitive fluorescent indicators. These fluorescent molecules bind calcium ions and thereby change their fluorescence properties. There are two groups of calcium indicators: first, there are chemical dyes which are either injected into single cells (e.g. using a microelectrode or micropipette) or which label multiple neurons at the same time by various methods of loading (e.g. via air pressure pulses or electroporation) (Fig 2A). Second, there are genetically encoded calcium indicators (GECI), which are proteins expressed in all cells or specific cellular subtypes. The change in fluorescence properties of these calcium indicators due to calcium release can then be viewed using a fluorescence microscope (Fig 2B). A fluorescence microscope shines light within a certain wavelength range at the tissue via a laser, and then separates the much weaker emitted fluorescence from the excitation light via a dichromatic mirror. Only the emission light

should reach the detector/detecting photomultiplier tube (PMT) that transforms the light signal into an electrical signal. A common type of fluorescence microscope is called a two-photon microscope. A two-photon microscope uses two low-energy photons to excite the fluorescent calcium indicator so that it emits light. A scanner controls the exact location of excitation. This approach allows increased penetration depth and efficient light detection, while at the same time reducing damage to the tissue. The spiking activity can then be inferred from the recorded light signal (Fig 2C; see e.g. Chen et al., 2013; Grienberger and Konnerth, 2012 for review).

Recently developed mobile calcium imaging set-ups transfer a varying number of microscope components to the animal's head. For example, a complete miniaturized two-photon microscope has been developed that can be head-mounted (Fig 2D). It is connected to the laser and the control setup by a flexible tether which contains an optical fiber and electrical wires (Helmchen et al., 2001); (Piyawattanametha et al., 2009). Others minimized the size and weight of the mobile component by just placing the objective lens and the dichromatic mirror on the animals head while the rest of the components are external and immobile (Sawinski et al., 2009; Flusberg et al., 2005; Engelbrecht et al., 2008). To date, only systems suited to be carried by animals >70 g (e.g., rats) have been demonstrated to achieve calcium imaging with cellular resolution during freely moving behavior (Sawinski et al., 2009). An integrated fluorescence one-photon microscope with lower spatial resolution has been developed to be used in even smaller animals (Ghosh et al., 2011). For reviews, see Kerr and Nimmerjahn, 2012 and Hamel et al., 2015).

PET

Closer to methods applied in human neuroscience are other neuroimaging methods used in animal research, such as magnetic resonance imaging (MRI) and positron emission tomography (PET). While MRI can only be used in a stationary fashion, PET can be used in moving animals. A further advantage of PET is that it not only images metabolic activity, but also receptor occupancy and the activity of transporters and enzymes in the brain. The basic idea of PET is that a radioactive tracer coupled to a biologically active molecule (e.g. fludeoxyglucose) is injected into the subject. This molecule will be taken up by tissue, dependent on the metabolic activity. The uptake of the molecules will increase the concentration of the positron-emitting radionuclide (tracer). The PET scanner detects pairs of gamma rays emitted by the positron-emitting tracer. The assumption is that brain activity leads to locally increased metabolic activity in the active brain area, thereby leading to a measurable increase in radioactivity. The tracers can also be incorporated into molecules that bind to specific receptors. Historically, there has been an emphasis on dopamine transmission; now tracers for subtypes of serotonin, cannabinoid, and metabotropic glutamate receptors and others exist (for review see Virdee et al., 2012).

An early successful attempt to use PET on moving animals involved the use of small PET scanners along with fast optical tracking techniques. While the rodent is confined within a tube, information from the tracking device is used for motion correction (Weisenberger et al., 2005). This approach is now feasible in animals free to roam within a small space by keeping the rat's head within the PET field of view via a robot-controlled motion adaptive animal chamber (Zhou et al., 2013). While this approach will always limit the area the animal is allowed to stride across, another approach involves a miniaturized PET detector ring (RatCAP) which is surgically attached around a rat's head in a rigid manner (Schulz et al., 2011; Vaska et al., 2004). This pioneering approach eliminates the relative motion between the detector ring and the animal's head, offering motion-free functional brain images of a relatively

unrestrained animal. The development of a wearable PET scanner for humans is a very exciting prospect (Bauer et al., 2016).

(F)NIRS

Near infrared spectroscopy (NIRS) is an increasingly popular technology for studying brain function. Like MRI, it provides measures of metabolic activity, however, a NIRS set-up is portable. Metabolic activity is measured through concentration changes in both oxygenated- and deoxygenated hemoglobin. For red and near-infrared light, oxyhemoglobin (HbO_2) and deoxyhemoglobin (HbR) are the most significant absorbers in blood or tissue. Using red and near-infrared light from light-emitting diodes to penetrate through the brain to monitor their variation of relative optical transparency, the relative concentration changes of HbO_2 and HbR in relation to cerebral blood flow and oxygen metabolism can be calculated and transferred into current or voltage by photodiodes. This signal then has to be transmitted for storage and further analysis. In a wireless near-infrared spectroscopy system for rats, the signal acquisition module can drive the red and infrared light sources and acquire signals obtained from the photodiodes (Kuo et al., 2013). Such a setup could be used in freely moving small animals. A miniaturized wireless NIRS sensor has been successfully applied in freely moving sheep to investigate mood-modulated cerebral responses to a positive emotional stimulus (Muehlemann et al., 2011). Results from these animal studies could potentially be directly compared to (f)NIRS studies carried out in humans (Plichta et al., 2006).

Challenges for controlling the sensory input during free movement

Recording during free movement does not only pose challenges to keeping the recording equipment stable. When studying the sensory systems in moving animals the sensory input cannot be precisely controlled anymore. Concerning vision, there have been two ways of dealing with this problem. The first one is to record all visual input the animal receives while freely moving about, by mounting a small, lightweight camera on the head. This has been done in cats freely exploring natural environments (Betsch et al., 2004). Equally important to this endeavor is a reliable system for tracking the gaze of both eyes. An ocular-videography system has recently been developed that uses two lightweight head-mounted cameras for recording the movement of both eyes in rats (Wallace et al., 2013) . To our knowledge, neither filming the animal's visual input nor tracking its eye movements has been combined with concurrent neural recordings yet; this remains one of the big challenges for research in freely moving animals.

Another way to deal with this problem is to put the animal on a spherical treadmill, commonly made from a Styrofoam ball (Fig 3A). This allows for fixating the head of the animal, while the animal can move the rest of its body. Originally developed in insect research (Dahmen, 1980), this approach has been very successfully adapted for rodent research (Dombeck et al., 2007). In such a set-up, visual input can be controlled precisely by a virtual reality system (provided that gaze direction is known), consisting of either several monitors (Saleem et al., 2013), or a spherical dome (Harvey et al., 2009; Schmidt-Hieber and Häusser, 2013) around the animal on which the virtual environment is projected (Fig 3B). This allows for the rat or mouse to explore the virtual space and solve tasks (Hölscher et al., 2005; Thurley and Ayaz, 2016). Recently, an actual maze floating in the air has been developed through which head-fixed rodents can run (Nashaat et al., 2016). Virtual reality has also been used for testing behavior in fruit flies freely flying in a wind tunnel (Fry et al., 2008), and in monkeys, treadmills have recently been applied successfully to investigate primary motor cortex activity while the animal is walking (Foster et al., 2014). While until recently, such set-ups were not available for humans, the fast

development of virtual reality in combination with natural body movements for the gaming community, opens up new possibilities and can inspire exciting experiments.

Do restrained movements in virtual reality sufficiently mimic free movement? Experiments in which rodents explore virtual spaces suggest they do, since these uncover the same underlying neural circuitry as that involved in navigation through an actual space (Dombeck et al., 2007; Domnisoru et al., 2013; Schmidt-Hieber and Häusser, 2013). However, one study has directly compared virtual reality (which provides only visual cues about the environment) with real world environments and found that the real world activates this neural circuitry much stronger than the virtual reality. This suggests that animals usually also rely on sensory input other than vision for navigation (Ravassard et al., 2013). In addition, restraining an animal is typically not the ideal method for studying issues relating to spatial navigation: head-fixed walking rodents tend to rotate excessively (Dombeck et al., 2009), something which can be partly overcome by training the animals. Also, virtual reality is typically applied as a 'closed-loop system'; a system where the locomotion of the animal is used as feedback to update the sensory input coming from the virtual environment. The interaction between the animal and the environment must be parametric; that is, movements of the animal must map onto changes in some parameter space, which in turn correspond to updates of the virtual world. These parameters must be 'tuned', such that the animal's movements result in virtual reality updates of a certain expected magnitude. While certain feedback loops are maintained during virtual reality experiments, several others are broken; for example, head restraint can disrupt the elaborate control systems that animals possess to regulate the position of the head and eyes (Zeil et al., 2008). Lastly, certain behaviors, such as complex social interaction, may prove to be nearly impossible to study using virtual reality (but see Kohatsu et al., 2011), and therefore studying the neural circuitry underlying these behaviors will require recordings in freely roaming animals. That said, virtual reality can also significantly enhance real world set-ups, for instance with the possibility of providing conflicting sensory information to the brain (Chen et al., 2013; Kautzky and Thurley, 2016; Saleem et al., 2013).

In general, we can conclude that while studying moving animals, the sensory input cannot be precisely controlled. Although significant steps have been made towards this in the visual domain, other sensory input is not being controlled, let alone sufficiently monitored.

Experimental questions

Movement as an intentional action

Moving animals execute motor behavior; their muscles contract and relax in an organized fashion, as a result of which the animal moves. This can happen stereotypically, such as in reflex or in cases where a sensory input leads to a highly predefined motor output due to overtrained response behavior (e.g. button presses or saccades to a target). Free movement, however, is much more than this. Freely moving animals not only passively react to incoming information from the surround but also act on this information in an active, flexible manner. Therefore, free movement implies *intentional action*; flexibly based on incoming sensory input, the animal decides what movement to make, when to make it, and whether to move at all (Brass and Haggard, 2008). This means a freely moving animal has the freedom to choose. Just to what extent the possibility to freely move is an integral part of a general intention to execute actions is nicely illustrated by the necessity of free movement for behaviors seemingly unrelated to the movement, such as vocalization in songbirds (Long et al., 2010) and marmosets (Roy and Wang, 2012). When restrained in their free movement, animals will simply not display these behaviors albeit the motor act of vocalizing would be perfectly possible. The dependence of such behaviors on free movement underscores the importance of movement in the animal's general intention to interact with its environment.

A fundamental form of free movement is walking, which results in a change in spatial location. Intentionally moving through space is called *spatial navigation*, and it implies that the animal possesses a mental representation of the space it moves through. Immediately upon moving, the impact of this movement on the surroundings is evaluated by the senses, assessing if the new sensory input should lead to another intention to move. This can include taking in more or more specific sensory input. This process is referred to as *active sensing,* a behavior permanently observed in freely moving animals. The intentional nature of free movement extends to the final stages of movement production; even the output signal from the motor cortex is heavily dependent on the intention of the animal on how to execute the movement, in a process called *motor planning*.

In this section, we will look at some basic cognitive processes that can only be observed and studied during free movement. We will focus on spatial navigation, active sensing, and motor planning, but there are many more. We will highlight how crucial free movement is when studying these cognitive processes in animals, and briefly emphasize the potential of this research for answering important questions about the human brain.

Spatial navigation

Successfully moving through a space is something we do effortlessly all the time, yet in fact it is the final outcome of a complex chain of computations performed by our brains. Let's take a look at the major cognitive processes involved. Importantly, the brain has to keep track of any changes in spatial location. Information about the position of the animal in the spatial surrounding comes from the sensory input of the environment. When we move, this sensory input changes and will provide information about where we move to. However, the sensory input on the retina caused by moving objects in our immediate surroundings can be indistinguishable from the sensory input when we move about. The brain therefore needs a way to discriminate between *self-motion* and motion of the surround (Fig 4A). This is thought to be achieved by the use of an efference copy or collorary discharge; a prediction about the sensory consequences of self-motion which can be subtracted from the actual sensory input (Crapse and Sommer, 2008). The so identified self-produced changes in sensory input can then be used to calculate one's own movement through the environment.

The animal can integrate the information on self-motion and external cues from the environment to compute its own position and change in location, i.e. to know the path it has travelled. This process is called *path integration* (Fig 4B; Barlow, 1964; Etienne and Jeffery, 2004). The information about the spatial environment given by one's own position and the path travelled can now be used to construct a *spatial map* of the surroundings. If this process was successful, the environment has become familiar to the animal. Now, the spatial map can be used flexibly to reach certain locations without the necessity to retrace a previously taken path.

Animal research has been instrumental in unravelling the neural mechanisms behind these cognitive processes. A major step towards understanding these processes started with recordings from the hippocampus of freely moving rats in 1971, which revealed cells firing only when the rat was at a particular place in the testing environment (O'Keefe and Dostrovsky, 1971). These cells have been termed *place cells*, and they are the neural substrate of a spatial map of the environment (Fig 4C). Each neuron of the hippocampal cell population fires at a different location, such that the entire spatial surroundings of the rat are represented (Wilson et al., 1994). What gives rise to this spatial map in the hippocampus? The input into the hippocampus is given by the medial entorhinal cortex (MEC) (Fig 4C). MEC neurons that project to the hippocampus also exhibit sharply tuned spatial firing, much like place cells in the hippocampus. However, in the MEC each cell has multiple firing fields, meaning it fires at multiple places in the rat's spatial environment (Fyhn et al., 2004). These firing fields are not random, but neatly arranged in a grid-like pattern (Fig 4C) which led to the name *grid cells.* They provide the basic elements of a system for spatial navigation (Hafting et al., 2005), possibly by linearly combining

their multiple receptive fields to generate single receptive fields for place cells (O'Keefe and Burgess, 2005, McNaughton et al., 2006).

If place and grid cells are used by the animal as a spatial map, how do these cells construct this spatial map based on the changing position of the animal during movement, i.e. how is information about path integration stored? A key role seems to be reserved for a network of cells in presubiculum and anterior thalamus called *head-direction cells* (Fig 4C; Taube, 1998). These neurons encode information about the animal's directional heading, independently from visual cues; they even provide information about the movement direction in complete darkness (Chen et al., 1994). They are also directly connected to the MEC, and it is thought that in this way, they update the angle of the grid-like pattern stored in the grid cell population with respect to the head of the animal. Another important structure for path integration seems to be the parietal cortex. Rats and humans with parietal cortex lesions fail to acquire spatial tasks and remember positional relationships (Kolb et al., 1983, Takahashi et al., 2012).

It thus seems that a network of place, grid, and head-direction cells is key to maintaining and updating the spatial map of the animal. Sensory areas, mainly the vestibular and visual system, feed into this network to provide the necessary information from the direct environment. Studies in macaque have pointed to the medial superior temporal area (MST) and the ventral intraparietal area (VIP) as key areas for the calculation of self-motion (Britten, 2008). Both areas are part of the motion system and their neurons possess complex receptive fields: in MST, the receptive fields contain a vestibular component, whereas VIP neurons possess somatosensory receptive fields that correspond to the visual receptive fields. For example, a VIP neuron with a foveal receptive field might have a somatosensory receptive field on the nose or the mouth, whereas a neuron with a more peripheral visual receptive field might have a somatosensory receptive field on the shoulder or arm (Duhamel et al., 1997).

Intriguingly, recent studies have also implicated a role for primary visual cortex in navigation. Neurons in V1 in the mouse integrate information on optic flow as well as self-motion, indicating that V1 participates in a multimodal processing system that integrates visual motion and locomotion during navigation (Saleem et al., 2013). When there is a mismatch between the calculated motion signals in V1 and the visual feedback the mouse receives, certain neurons in V1 are activated (Keller et al., 2012). These mismatch neurons signal local deviations of actual visual flow from visual flow predicted based on self-motion. They could therefore underlie the detection of objects moving relative to the visual flow caused by self-motion (Zmarz and Keller, 2016).

Most of the work on spatial navigation has been done in rodents, although place cells have also been described in monkeys (Ludvig et al., 2004). Evidence for place and grid cells in the human brain has obviously been sparse. Recordings in epilepsy patients with implanted electrodes in the hippocampal formation have revealed both place cells (Ekstrom et al., 2003) and grid cells (Jacobs et al., 2013). Importantly, these cells were found while the patients were engaged in a virtual motion task and not actually moving about. Spatial navigation in humans has indeed been studied mainly in immobile volunteers engaged in virtual reality settings (Rodriguez, 2010; Doeller et al., 2010). If the presence of place and grid cells in animals actually moving about can be taken as a ‘gold standard’ for spatial navigation, then the evidence of such cells in humans immersed in virtual reality suggests that virtual motion systems are a good proxy of an actual brain during movement. However, as discussed above, virtual reality cannot mimic all relevant aspect of free movement, and to what extent sensory input other than visual input plays a role cannot be assessed to date. Additionally, having humans actually navigate in a real environment while measuring their brain activity would add to our current knowledge on spatial navigation, the understanding of complex ongoing cognitive computations that cannot be easily tested in the animal.

Active sensing

When moving about the environment, an animal is not only engaged in spatial navigation but also selectively samples and processes the external world. This is called active sensing, behavior greatly dependent on the animal's freedom to roam about. Active sensing strategies have been described for audition (Kondo et al., 2012) and vestibular sampling (Carriot et al., 2017) as well as for vision, olfaction, and tactile input (as will be discussed below).

Rodents deliberately touch the objects surrounding them with their whiskers to build up an internal representation of their environment. Many tasks rely on this active behavior. Gap-crossing is a skill for which free movement of the whiskers is essential: the animal perches at the end of a raised platform and uses its whiskers to localize a second platform before crossing the gap to retrieve a reward. The initial stages in the brain for active sensing are well-known: all whiskers are arranged in a certain topographical order on the snout, which translates into a fixed topographical order in a part of the brain called barrel cortex. Neurons in barrel cortex fall into three classes: 'whisking cells', which fire during whisking per se, 'touch cells', which fire upon contact with an object, and 'whisking/touch cells', which fire during both types of event (Szwed et al., 2003). These neurons work together to use either their firing rate or precise spike timing to encode an object's position relative to the rodent's head (Diamond et al., 2008). Apart from object localization, active sensing is also used for object identification. Research in this respect has focused mainly on texture discrimination, which happens by the same barrel cortex neurons during the late part of their response (Von Heimendahl et al., 2007). Also humans use an active strategy to touch objects for texture discrimination or to assess other physical features. Actually, if humans and rats are given an object localization task using vibrissae (rats) or plastic rods attached to the fingertips (humans), both species apply active sensing processes in a similar manner (Horev et al., 2011).

Another form of active sensing is observed in olfaction. Sniffing is closely related to respiration and therefore is an ongoing process. However, strong modulations are observed dependent on the movement state of the animal. While sniffing behavior is highly variable and dynamic, active rodents spend the majority of time sniffing at frequencies above 4 Hz while passive rats mostly stay below a 2 Hz sniffing frequency (Wachowiak, 2011). The frequency of sniffing is thought to be modulated in order to acquire the stimulus more quickly rather than to directly influence the low-level neural processes underlying odor perception (Wesson et al., 2009). A compelling example of context-specific sampling strategies is found in hunting dogs. While they sniff at up to 4 – 6 Hz when tracking the scent of prey on the ground, dogs will stop sniffing and run forward when tracking the same scent in the air, forcing a continuous stream of air into the nose for up to 40 sec (Steen et al., 1996). Also humans will change their sniffing pattern in response to sensory input (Johnson et al., 2003); how such a pattern is affected by ongoing movement like walking is not known.

Saccadic eye movements is a well-studied field in human research. These eye movements follow an active sensing strategy to maximize relevant information input per fixation in humans (Yang et al., 2016). Whether such strategies follow the same optimization process throughout different body states is not known. Studies from animal research advocate carefully investigating eye movement strategies during free movements; the movements of the eyes in head-restrained rats are conjugate and infrequent, whereas in freely moving animals, both eyes are highly mobile and eye movements are asymmetrical, keeping the animal's binocular visual field above it. This behavior seems indicative of a strategy to detect predators coming from above during movement (Wallace et al., 2013). The same study highlights another interesting point by showing that these eyes movements are specific to free movement; head-restrained rats, even when running on a spherical treadmill, do not exhibit these eye movement patterns.

Motor planning

Free movement requires the animal not only to decide *when* and *whether* to move, but also *how* the movement should be executed (Brass and Haggard, 2008). This latter process is known as motor

planning (Gnadt and Andersen, 1988; Sanes and Donoghue, 1993). Traditionally, motor planning has been studied in the context of reaching and grasping, in seated, immobile humans or primates (Castiello, 2005). Such a set-up has made it possible to test hypotheses about the motor system with a great deal of control and precision. However, it limits investigations to a small subset of the full capability of the motor system.

Recordings in motor cortex during unconstrained arm movement show great diversity in neural responses (Aflalo and Graziano, 2007). Existing models, developed with constrained behavioral task data, were not able to capture the neural variability in these free movement data. This result exposes a weakness in the prevailing course of study: the limits of conventional task design can lead to impoverished models of the neural underpinnings of motor planning (or any other cognitive process). Wireless recordings in the motor system of primates allow the study of not only free arm movement, but free movement of the entire body, including jumping and running. It also opens up the possibility of continuous recording for days instead of hours, thus capturing an almost unlimited range of free movements and behaviors. Due to these developments, a new class of experiments can emerge. Instead of primates repeating the same constrained movement thousands of trials, long and complex datasets of free behavior can be used for data mining to answer experimental questions (Gilja et al., 2010). This requires experimenters to move away from multiple trial averaging and embrace single 'trial' analysis, for which sophisticated analysis techniques have been developed in recent years (Brown et al., 2005; Yu et al., 2009).

One example of motor planning behavior of which our understanding can benefit greatly from such free movement recordings, is reaching and grasping in near and far space. Decades of research in movement constrained primates using a so-called memory-guided reach task have shown a network of areas, including the primary motor cortex, dorsal premotor cortex, and posterior parietal cortex, to be involved in planning a reach and grasp movement in near space (Mushiake et al., 1991; Murata et al., 1996). However, premotor cortex neurons activated by both the execution as well as the observation of movements (so-called mirror neurons) fire differentially depending on whether the movement happens in near (within arm's reach) or far (beyond arm's reach) space (Caggiano et al., 2009). Moreover, a subset of these neurons changes their activity according to the possibility that the monkey will interact with the object. Additionally, human patients with brain lesions in these areas can have neglect either in near or in far space (Halligan and Marshall, 1991; Vuilleumier et al., 1998). Recent work in humans on the hand-blink reflex (a blink elicited by stimulating the nerve at the wrist) showed that the motor system acts predictively on the transition from near to far space and vice versa: the hand-blink reflex was present only when the hand approached (and not receded from) the face (Bisio et al., 2017). These results suggest that a reaching movement towards an object in far space might be planned differently by these areas than a reaching movement towards an object in near space, a hypothesis which is currently being tested with wireless recordings from freely moving monkeys performing goal-directed reaching tasks (M Berger and A Gail, personal communication).

An important goal in recording and analyzing motor activity from freely moving primates is the construction and improvement of brain-machine interfaces (BMIs). The idea is simple: the translation of the cortical activity from the motor network into muscle movements is being used to control artificial limbs. Huge progress has been made in recent years in decoding complex motor activity patterns, and it is now possible to have monkeys navigate a wheelchair (Rajangam, 2016) and walk freely after a spinal cord injury (Capogrosso et al., 2016) using their brain activity. These studies are directly aimed at restoring mobility to severely paralyzed patients, and as such, human research could learn an incredible lot from these studies in primates.

Movement as an active state

Considering free movement within the framework of intentional action highlights the importance of free movement in a host of cognitive processes. However, movement, as compared to the absence of movement, can also be regarded as the expression of a bodily state accompanied by an underlying brain state. The brain perpetually transitions though a continuum of more or less active states, ranging from deep sleep through the various sleep stages, to the various awake states such as drowsiness, passively alert, and highly awake. Also included in this spectrum are certain non-natural brain states such as anesthesia and the vegetative state. The top of this scale, a state in which the animal or human is highly alert and active, often involves movement in the form of walking or running. The relationship is bidirectional; free movement can influence the animal's brain state and vice versa.

The view that the brains of animals moving about are in a behaviorally active state has a long history: studies in freely moving cats in the 1980s focused on subcortical nuclei such as the locus coeruleus, and their role in various arousal states (Rasmussen et al., 1986; Abercrombie and Jacobs, 1987). These studies found the activity of noradrenergic neurons in the locus coeruleus to be highly dependent on the behavioral state of the cats, with activity increasing as the cats transitioned from REM sleep to slow wave sleep to quiet waking to active waking (Rasmussen et al., 1986). These neurons were most active, however, with the application of a stressor such as visual threats, forced treadmill running (Rasmussen et al., 1986) or loud noise (Abercrombie and Jacobs, 1987). These studies suggest a general role for the locus coeruleus in the brain's response to stress.

The relationship between movement and brain state has been frequently investigated using spherical treadmills in combination with virtual reality (Fig 3B). Mice running on a spherical treadmill typically show increases in firing rate and local field potential power in the gamma frequency range, both in primary visual cortex (V1; Niell and Stryker, 2010) as well as in cerebellar granule cells and interneurons (Ozden et al., 2012). The pronounced firing rate increases in the visual cortex, without changes in spontaneous firing or stimulus selectivity, suggest a change in gain of these neurons. This is in line with the finding that locomotion reduces surround suppression in primary visual cortex, allowing V1 neurons to integrate over larger regions of visual space (Ayaz et al., 2013). V1 interneurons, as well as neurons in the lateral geniculate nucleus (LGN), do not increase their firing with locomotion (Niell and Stryker, 2010), although recent results do report increased firing rates already in the LGN (Erisken et al., 2014). These changes in visual cortex activity are transient, but locomotion can also have long-term effects on the cortex, as recent findings on sensory deprivation and cortical plasticity show. Recovery from sensory deprivation is slow and incomplete in adult visual cortex. Visual stimulation during locomotion dramatically enhances recovery in the mouse (Kaneko and Stryker, 2014) as well as induces a rapid and persistent increase in cortical responses (Kaneko et al., 2017). Both effects are specific to the particular visual stimuli viewed by the animal during locomotion.

How do these changes in neuronal activity with locomotion come about? Whole-cell recordings in V1 during locomotion showed decreases in membrane potential variability and an enhancement in the amplitude of visually evoked sub-threshold responses, which led to an improved signal-to-noise ratio and performance in a visual detection task during running (Bennett et al., 2013; Polack et al., 2013). These changes in membrane potential could be induced by nicotinic (Fu et al., 2014) and noradrenergic (Polack et al., 2013) inputs from the basal forebrain to vasoactive intestinal peptide (VIP)-positive neurons in mouse V1 during locomotion. Another key structure in the initiation of running is the mesencephalic locomotor region (MLR). Stimulation of the MLR in awake, head-fixed mice can induce both locomotion and increases in the gain of cortical responses (Lee et al., 2014). MLR stimulation below the threshold for overt movement similarly changed cortical processing, revealing that MLR's effects on cortex are dissociable from locomotion. This result agrees with recent findings on the effects of arousal and locomotion on activity in mouse V1. Arousal (as measured by pupil dilation) suppressed spontaneous firing, increased the signal-to-noise ratio of visual responses, and

reduced noise correlations. In contrast, increased firing in anticipation of and during movement was attributable to locomotion effects (Vinck et al., 2015). These findings suggest complementary roles of arousal and locomotion.

How important it is to consider this active state when interpreting measures of neuronal activity is shown by studies on cerebral blood volume and neural activity in the somatosensory and frontal cortex of head-fixed mice during locomotion (Fig 5A). In the frontal cortex, cerebral blood volume did not change during locomotion (Fig 5C), but firing rate and gamma-band power both increased (Fig 5B), indicating a decoupling of neural activity from the hemodynamic signal (Huo et al., 2015). These changes were resistant to pharmacological manipulations of heart rate, suggesting they arise from central processes (Huo et al., 2015). These results show that hemodynamic signals are not faithful indicators of the mean neural activity in the frontal cortex during locomotion. As mobile PET imaging becomes available for use in human subjects and NIRS is applied more frequently, researchers should be aware of this strong dependence of the hemodynamic signal on the behavioral state.

Conclusion

In conclusion, free movement can affect a surprising number of cognitive functions, which on first sight seem to be independent of large body movements. Besides the obvious necessity of motion for investigating motor activity, these other cognitive functions need to be considered also in human experimentation. We have shown that animal research that has been done in the fields of spatial navigation, motor planning and active sensing can directly help to understand these cognitive domains in humans. While the investigation of cognitive processes during free movement is intensified, more processes might be discovered that are significantly interlinked with free movement. Moreover, it is important to consider that the effect of movement reaches beyond specialized domains, impacting brain function in general, as we can think of moving as the top end of a continuum of arousal states. This urges us to take the behavioral state of our subject into account every time we interpret measures of neuronal activity. Also this is a highly important consideration for human research.

As the development of portable EEG, ECoG and PET devices for humans advances, the more comparable and therefore the more important the results from work on freely moving animals will become. The same holds for increased possibilities to measure single cell activity in humans.

We are optimistic that these technical advancements, in combination with the right scientific questions, will start to reveal a much more realistic and complete picture of brain function when considering free movement compared to what we have held until now. Human research in this field can profit greatly from ongoing animal research.

Figure and Figure Legends

Figure 1

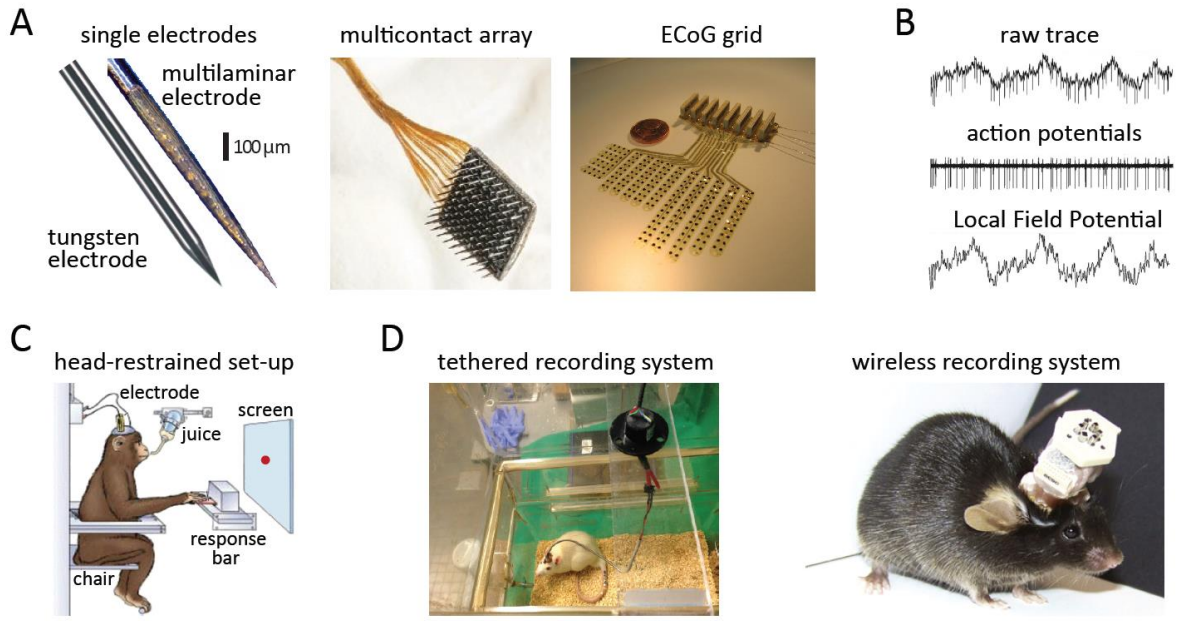

Figure 1. Electrophysiology. (A) Four examples of electrodes commonly used in animal electrophysiology. Tungsten and multilaminar electrodes, as well as multicontact arrays, are used for recording single unit or multi-unit activity; ECoG grids record local field potentials. Tungsten electrodes record activity from a local cell population; multilaminar electrodes can record activity across the cortical column; multicontact arrays can record activity from part of a cortical area; ECoG grids can record activity across multiple brain areas. *Adapted with permission from Plexon Recording, Blackrock Microsystems, and Birthe Rubehn. The multilaminar electrode depicted is known as a V-probe and is manufactured by NeuronElektrod and exclusively distributed by Plexon; the multicontact array depicted is known as a Utah Array.* (B) Raw activity trace recorded with an electrode (top), which can then be high-pass filtered to obtain the action potentials (middle) or low-pass filtered to obtain the local field potential (bottom). (C) Monkey in a head-restrained recording set-up. (D) Tethered (left) and wireless (right) recording systems in rodents. *Adapted with permission from (Fluri et al., 2015) (left) and (Battaglia et al., 2009) (right).*

Figure 2

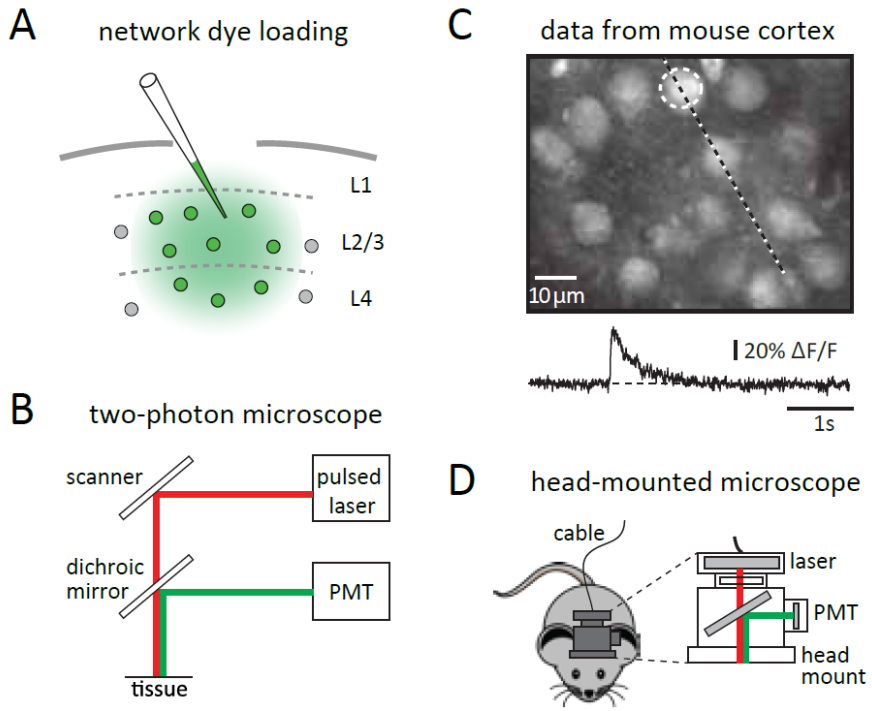

Figure 2. Calcium imaging. (A) First, the neural tissue is exposed to calcium-sensitive fluorescent indicators, in this example by 'acute' network loading: injection of a chemical dye. (B) Once the tissue has incorporated the calcium indicators, it can be imaged, for example by a two-photon microscope. A pulsed laser emits photons, which are steered towards the tissue by a scanner. Excitation light and much weaker emission light from the tissue are separated by a dichroic mirror. The emitted fluorescence is then detected by a photo-multiplier tube (PMT), which transforms it into an electrical signal. (C) Example image data from the mouse cortex obtained with two-photon microscopy. Looking at the activity in a single neuron (white circle) across time shows the calcium transient (bottom trace). The peak in the trace is caused by sensory stimulation. (D) In some head-mounted microscopes, all components are packed together into a compact microscope worn on the head. *Adapted with permission from (Grienberger and Konnerth, 2012) (A-B) (Stosiek et al., 2003) (C) and Mightex Systems (D; http://www.mightexsystems.com/applications/WP002112816.html).*

Figure 3

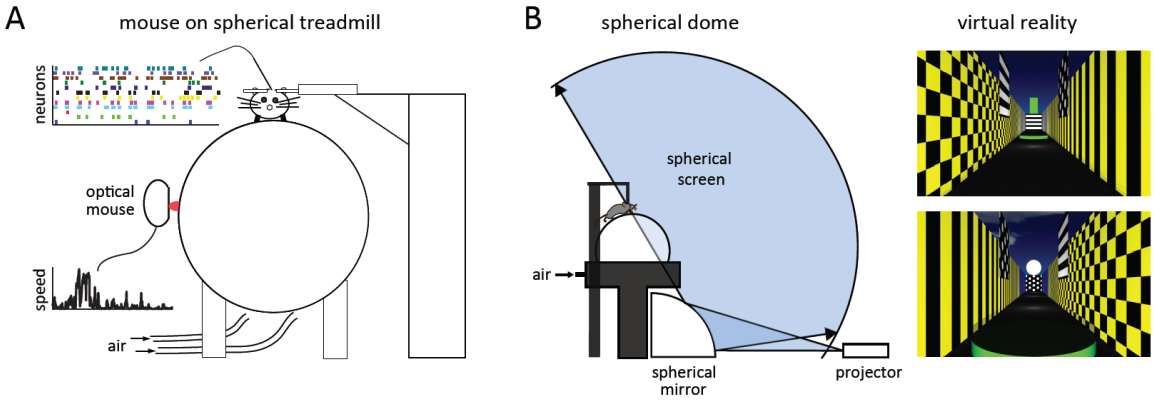

Figure 3. Head-fixed yet mobile mice. (A) Set-up with spherical treadmill. Air keeps a Styrofoam ball afloat, while an optical mouse tracks the movements of the ball. A mouse sits on top of Styrofoam ball and can move the ball with its legs, while being recorded from. *Adapted with permission from (Niell and Stryker, 2010)*. (B) Spherical treadmill surrounded by a spherical screen (left) on which a virtual environment can be projected (right). *Adapted with permission from (Schmidt-Hieber and Häusser, 2013).*

Figure 4

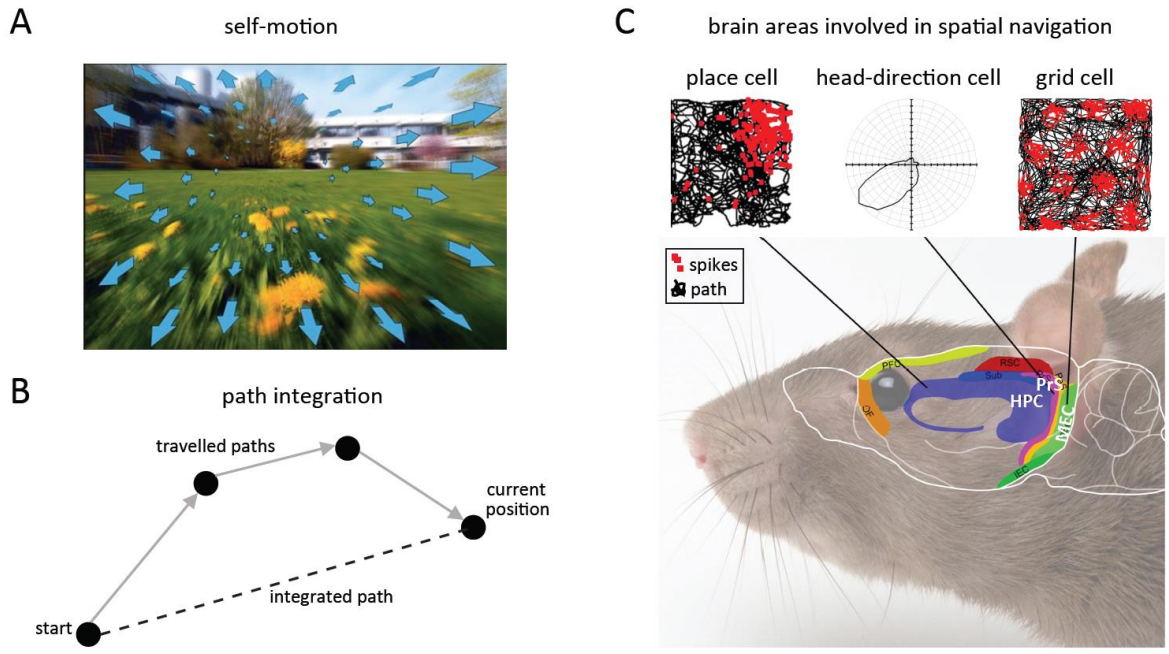

Figure 4. Spatial navigation. (A) Self-motion. The arrows indicate the optic flow generated by self-motion as one moves towards the trees in the background. (B) Path integration. Once the current position is reached, one can use the travelled paths to calculate the integrated path. This integrated path provides the direction and distance for a potential return journey. (C) Brain areas involved in spatial navigation in rodents. In the hippocampus (HPC), presubiculum (PrS) and medial entorhinal cortex (MEC), place cells, head-direction cells, and grid cells are found, respectively. On the top, 'receptive fields' of a place and a grid cell and a polar plot of a head-direction cell are shown. *Adapted with permission from (Marozzi and Jeffery, 2012) (top) and (Grieves and Jeffery, 2017) (bottom).*

Figure 5

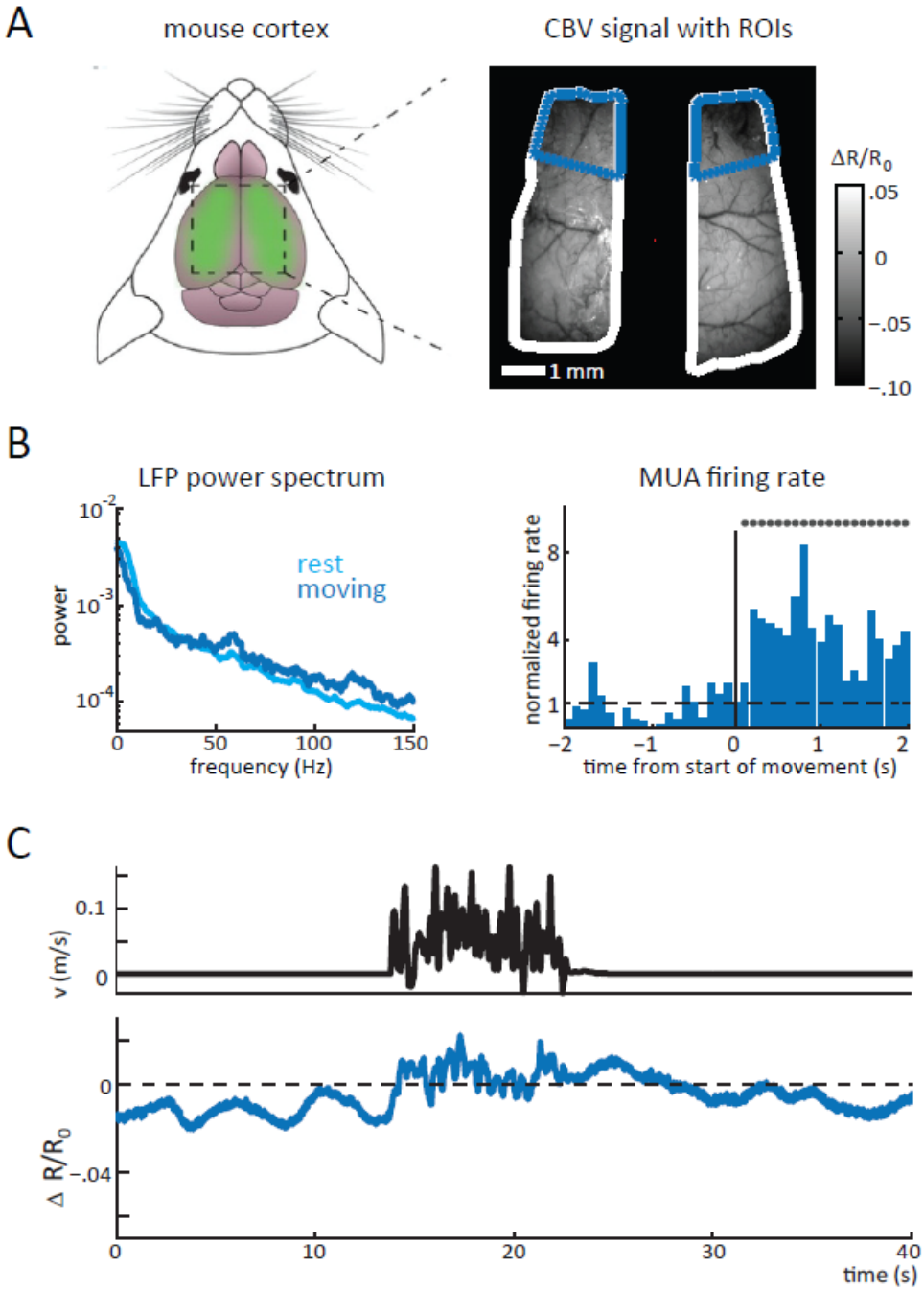

Figure 5. Measuring hemodynamic and neural activity during an active state (running) and an inactive state (sitting still). (A) Window for measuring cerebral blood volume (CBV) in a rat brain, with two regions of interest (ROIs) in the frontal cortex. (B) During running, neural activity increased compared to sitting still, as evidenced by the power in the local field potential (LFP, left) and multi-unit spiking activity (MUA, right). (C) However, the cerebral blood volume trace (bottom) showed no significant difference between running and sitting still (c.f. the velocity trace on top). *Adapted with permission from (Huo et al., 2014).*

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