Global dissociation of the amygdala from the rest of the brain during REM sleep

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Global dissociation of the amygdala from the rest of the brain during REM sleep

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Keywords
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Abstract

Rapid-eye-movement sleep (REMS) or paradoxical sleep is associated with intense neuronal activity, fluctuations in autonomic control, body paralysis and brain-wide hyperemia. The mechanisms and functions of these energy-demanding patterns remain elusive and a global picture of brain activation during REMS is currently missing. In the present work, we performed functional ultrasound (fUS) imaging at the whole-brain scale during hundreds of REMS episodes to provide the spatiotemporal dynamics of vascular activity in 259 brain regions spanning more than 2/3 of the total brain volume. We first demonstrate a dissociation between basal/midbrain and cortical structures, the first ones sustaining tonic activation during REMS while the others are activated in phasic bouts. Second, we isolated the vascular compartment in our recordings and identified arteries in the anterior part of the brain as strongly involved in the blood supply during REMS episodes. Finally, we report a peculiar activation pattern in the amygdala, which is strikingly disconnected from the rest of the brain during most but not all REMS episodes. This last finding shows that amygdala undergoes specific processing during REMS and may be linked to the regulation of emotions and the creation of dream content during this very state.

195 words
Introduction

Several studies have shown a strong link between REM sleep (REMS) and emotions. According to Gujar and colleagues, REMS is strongly linked to the recalibration of the human brain’s reactivity towards specific emotions with a decreased reactivity towards fearful memory but a significant enhancement toward happy memories (Gujar et al., 2011). Moreover, several studies tend to show that the emotional memories are better recalled than neutral memories (Phelps, 2004) especially during REM-rich sleep (Groch et al., 2015; Nishida et al., 2009; Wagner et al., 2001). It is also now well established that REMS disturbances are often observed in PTSD cases. Mellman and colleagues have observed shorter and more frequent sleep bouts in trauma-exposed patients compared to non-injured ones (Mellman et al., 2002). These findings were later confirmed, as well as other observations stating that REMS disturbances led to impaired fear extinction learning, which might explain the development and maintenance of PTSD symptoms (Bottary et al., 2020; Pace-Schott et al., 2015; Spoormaker, 2018). A “sleep to forget, sleep to remember” (SFSR) model arose a few years ago, which divides the memories in two parts: the factual memory (the actual event), and its emotional part (the feelings and their intensity from the event) (Walker and van der Helm, 2009). The former is consolidated during REMS, while the latter is de-potentiated, meaning that the factual memory remains embedded in the hippocampus, while the intensity of the feelings attached to this memory is downscaled. This is considered as an “overnight therapy”, necessary to cope with distressful events and which is disturbed in cases of emotion-based disorders, such as depression or posttraumatic stress disorder (PTSD), as these disorders are often linked with REMS disturbances as previously stated. However, a few studies go against that model (Wiesner et al., 2015). The biological mechanisms of emotional regulation of memories involve an amygdala-hippocampal-medial prefrontal cortical (mPFC) network, whose intercommunications are enhanced by theta and ponto-geniculo-occipital (PGO) oscillations (or equivalent P-wave in rats), as well as elevated acetylcholine and cortisol levels during REMS (Hutchison and Rathore, 2015; van der Helm et al., 2011).

In addition to the regulation and processing of emotions, several other functions have been attributed to REMS, such as, for example, memory consolidation (which depends upon the hippocampal theta oscillations created by activation of GABAergic neurons in the medial septum (Boyce et al., 2016)), or the brain maturation specifically during early life REMS (Marks et al., 1995) and more precisely to aid sensorimotor system development through muscle twitches (Blumberg et al., 2013). Another function attributed to REMS is that of forgetting.
memories (see (Langille, 2019) for a review) probably through the activation of melanin-concentrating-hormone (MCH)-producing neurons (Izawa et al., 2019).

From a physiological point of view, complex brain circuits have been shown to play a role in REMS. For example, in the brainstem (Sastre and Jouvet, 1979), two subsets of glutamatergic neurons (so called REM-on neurons) located in the latero-dorsal tegmental area (LDT) and in the sub-latero-dorsal tegmental nucleus (SLD) have been described: the first one projecting to the forebrain, responsible for the generation of hippocampal theta rhythm and desynchronized cortical activity, the second one projecting to the brainstem and responsible for muscle atonia hence the suppression of motor activity (Peever and Fuller, 2017). Furthermore, the melanin concentrating hormone (MCH), known to play a role in the promotion of REMS, is synthesized by neurons in the hypothalamus (Vetrivelan et al., 2016). However, the downstream activations of these pathways are completely unknown. Taken together, these examples show that REMS is a complex brain state which involves many brain regions scattered across the brain, rendering the global investigation of REMS challenging. Apart from the hippocampal structure (Grosmark et al., 2012; Montgomery et al., 2008; Popa et al., 2010), a clear whole-brain electrophysiological characterization of REMS is currently missing. Though some fMRI/PET studies have investigated functional connectivity associated with REMS (Chow et al., 2013; Wehrle et al., 2007), the limited temporal resolution and complexity of these techniques impedes the characterization of whole-brain networks during single REMS episodes. Hence, a clear picture of global brain activity during REMS is missing.

Interestingly, a recent study using functional ultrasound (fUS) coupled to LFP recordings in rats, uncovered an intense hyperemic activity largely exceeding both NREMS and wake levels (Bergel et al., 2018). These large-amplitude hyperemic patterns in the hippocampus, thalamus and cortex occurred in phasic bouts, followed a sequential thalamus-hippocampus-temporal cortex pattern and were robustly preceded by fast-gamma oscillations in the CA1 region. Such intense hyperemic activity were also imaged using functional ultrasound in human neonates during active sleep (Demene et al., 2017). Another recent study confirmed this hyperemic activity during REMS, using intrinsic optical imaging (ISOI) in sleeping head-fixe mice (Turner et al., 2020). Notably, these findings are in line with the entrainment of arteriole diameter by gamma activity in the cortex of head-fixed mice during wake (Mateo et al., 2017). In both studies, the characterization of neurovascular interactions was restricted to the cortex. This is striking, as sleep is generally thought to be a period when the body is resting and its energy restored (Cirelli and Tononi, 2008; Schmidt, 2014; Siegel, 2005), yet this hyperemic activity seems to be extremely energy consuming. We assume that if such energy-demanding activity
has been maintained across evolution, it must have some important role for the survival of the animal, which does not seem to have been found yet.

As previous studies could not give a global view of REMS’s brain activity, we took advantage of fUS versatility to scan more than 250 brain regions over multiple coronal and sagittal planes during more than 600 REM episodes. This study thus provides an exhaustive characterization of global brain hemodynamics during rodent REMS. We demonstrate a clear dissociation between basal/midbrain structures and superficial ones, respectively activated in a tonic and phasic manner. We also disentangle the vascular structures involved in the irrigation of the brain during REMS episodes providing a detailed outlook of blood supply. Finally, we show that brain activity reveals a striking dissociation between the amygdala complex and the rest of the brain regions.

**Results**

This study aimed at investigating the large-scale hemodynamics during REMS, in particular in the amygdalar network. Using a chronic experimental approach developed previously (Sieue et al., 2015), which included a cranial window and the implantation of a permanent fUS-compatible plastic prosthetic skull, that also enabled the attachment of the ultrasound probe holder (Figure 1A). In this setup, the different regions of the brain were monitored in a series of coronal and sagittal planes, each acquisition lasting 30 minutes for 4-6h per day over the course of several days (Figure 1B). This resulted in a dataset of 84 recordings in n=8 animals, totaling 617 REM episodes recorded in 259 brain regions (Supplementary Figure S2), together with hippocampal local field potentials (LFP) recordings, accelerometer, and neck electromyogram (EMG) (Figure 1C, Supplementary Table 1).

**Distribution of CBV changes across brain regions during diverse arousal states**

Measurement of the CBV in n=3 rats, in a total of 72 recordings, in which the animal is spontaneously going through different arousal states (quiet wake - QW, active wake - AW, non-REM sleep - NREMS, REM sleep - REMS) revealed a quiescent level of CBV fluctuations in QW and NREMS (Figure 2A). AW however is associated with increased cortical CBV levels especially in the primary sensory areas, while REMS is characterized by increased CBV in all brain regions with strongest effect in the hippocampal and limbic structures.

As we aimed at studying in detail the hemodynamic changes in various parts of the brain during REMS, we next focused on the changes in a large number of regions of interests located under
our various imaging planes, by computing the percentage of CBV change during REMS. Calculations were performed using 1-3 min of either the QW or the AW for the baseline (Figure 2B). This double analysis shows consistently a large range of CBV changes in association with REM between different parts of the brain in both analyses. While the hippocampal formation, the periaqueductal grey (PAG), the superior colliculus (SC) and some parts of the cortex, (such as the cingulate and retrosplenial cortices) present a strong percentage of CBV increase during REMS, areas of the hypothalamus and laterally located cortices (auditory, rhinal, piriform cortices) present modest CBV increases during REMS (Figure 2B). This combined analysis demonstrates that REMS hyperemia is not only a state of intense activation with respect to QW and NREMS, which are known to quiescent states, but also to AW in all brain regions, with strongest effects in the hippocampus and midbrain structures. Detailed mean values of the CBV distributions in all regions across the different vigilance states are details in Supplementary Tables 2 & 3.

Dissociation between the basal brain and the superficial brain areas during tonic and phasic activations

We previously described strong hemodynamic changes, composed of both phasic and tonic components (Bergel et al., 2018). By thresholding vascular activity during REMS, we were able to extract a binary variable that accounted for the phasic component of REMS (seed phasic-REM, equals 2 during phasic activity, 1 during REM, 0 otherwise), that we used as a 'seed' for correlation analyses and compared it with another variable accounting for the tonic component of REMS (seed REM, equals 1 during REMS, 0 otherwise) (Figure 3A). Individual voxels taken in the superficial or deep structures of the brain show different activation profiles, voxels in basal brain structures showing a very tonic activation (sustained during a single REMS episode) while superficial pixels were active intermittently by phasic bouts, which was captured by the different cross-correlations functions obtained with seed-phasic REM and seed-REM (Figure 3B). This phenomenon was clearly visible on all correlation maps (each pixel displays the maximum of the cross-correlation function shown in 3B) generated with either seed: cortical structures were more strongly associated with REMS-phasic than with REMS (black arrows) on all brain sections (Figure 3C). This effect was confirmed in regional analysis across individuals and interestingly the timing associated with either seed variable yielded different information. Interestingly, timings associated with seed-REM captured the broad inter-episode fluctuations while those associated with REM phasic, revealed a precise sequence of activation between brain regions and captured the intra-REM fluctuations (Figure 3D). Moreover, a few brain regions stand out with very high correlation scores such as the
substantia nigra (SNi) \( (R_{\text{max phasic}} = 0.7702 +/- 0.0204, R_{\text{max}} = 0.7115 +/- 0.0173, N=3 \text{ animals}) \), the dorsal periaqueductal grey (dPAG) \( (R_{\text{max phasic}} = 0.8111 +/- 0.0277, R_{\text{max}} = 0.7408 +/- 0.0228, N=3 \text{ animals}) \), the superior colliculus (SC) \( (R_{\text{max phasic}} = 0.7706 +/- 0.0313, R_{\text{max}} = 0.6967 +/- 0.0340, N=3 \text{ animals}) \), the pretectal nuclei (PN) \( (R_{\text{max phasic}} = 0.7834 +/- 0.0398, R_{\text{max}} = 0.6667 +/- 0.0384, N=3 \text{ animals}) \) and preoptic area (POA) \( (R_{\text{max phasic}} = 0.7164 +/- 0.0240, R_{\text{max}} = 0.6803 +/- 0.0223, N=3 \text{ animals}) \), as was seen in Figure 2B. In both analyses however, all cortical regions, except parietal \( (R_{\text{max phasic}} = 0.5493 +/- 0.0260, R_{\text{max}} = 0.4708 +/- 0.0265, N=3 \text{ animals}) \) and retrosplenial \( (R_{\text{max phasic}} = 0.6607 +/- 0.0315, R_{\text{max}} = 0.5841 +/- 0.0272, N=3 \text{ animals}) \) cortices and all amygdala subregions (basal, lateral, cortical and residual amygdala) displayed lower correlation coefficients than other brain structures (Supplementary Table 4).

Selective contribution of vascular dynamics to the blood supply during REM sleep

The second major aim of this study was to elucidate the contribution of the vascular compartment. We first segmented all salient vascular structures in our imaging planes including parallel branches of the main cerebral arteries (anterior cerebral artery - acer, anterior choroidal artery - ach, middle cerebral artery – mcer, and posterior cerebral artery - pcer) and segments along on the anterior branch (anterior cerebral artery - acer, azygos of anterior cerebral artery - azac, azygos pericallosal artery - azp) (Figure 4A-B). We then investigated the temporal dynamics in these structures by re-aligning their time course to the start of each REMS episode (defined by hippocampal theta activity crossing a threshold) and averaged their activation profile, both from the onset and of REMS (Figure 4C-D and E-F). Quantifications show an increased CBV (expressed in percentage of variation relative to the QW baseline) at the beginning of REMS, a sustained level throughout the REM episode and finally a sudden drop at the end of the REM episode. This increased CBV in arteries was more pronounced (2-fold increase) in the arteries that vascularize the rostral part of the brain (acer, azac and azp), compared to the arteries that vascularize the medial and posterior parts of the brain (ach, mcer, pcer), confirming a general phenomenon of increased blood supply during REMS, but also an emphasis of this enhanced blood flow in the rostral part of the brain. Further analysis shows a significant propagation delay along the anterior branch with acer peaking earlier than azac and azp (acer: \( t_1 = -3.14 +/- 3.26s \), azac: \( t_1 = 1.73 +/- 6.50s \), azp: \( t_1 = 1.81 +/- 3.03s \) (Figure 4G). Such REM-associated increased CBV was observed at a lower level in veins with a surprising antagonist activity between two side-by-side veins: the longitudinal hippocampal vein (lhiv) and the azygos internal cerebral vein (azicv) (Supplementary Figure S3).

Atypical amygdala activity during REM sleep
When assessing inter-regional correlations in the CBV signal, the most striking pattern of activity was found in the amygdala and consisted of a robust disconnection from the rest of the brain, which was clearly visible on ‘functional connectivity’ matrices averaged over all REMS episodes (Figure 5A) and in the temporal fluctuations of individual recordings (Figure 5B). This effect is consistent with observation from previous figures: the amygdala showing both a relatively low-level of hyperemia during REMS compared to other regions (Figure 2) and low-correlation scores (Figure 3). Strikingly, the amygdala’s activity during REMS, showed a remarkably unique activation profile compared to the rest of the brain and long periods of strong fluctuation when the remainder of brain activity was silent (Figure 5B, second part of the episode). This effect was confirmed and strengthened using a seed-based approach taking either the regional whole-brain activity as a reference (Figure 5C, Supplementary Figure S4) or the amygdala (Figure 5D), which revealed are very strong and robust dissociation between the amygdala and all other brain regions. Interestingly, amygdala sub-structures seem to exhibit also very specific dynamics as shown by the heterogeneous correlation maps found by taking 5 different sub-regions (amygdalohippocampal area, posterolateral part: AHiPL, amygdalohippocampal area, posteromedial part: AHiPm, amygdalopiriform transition area: APir, basolateral amygdaloid nucleus, posterior part: BLP, posterolateral cortical amygdaloid nucleus: PLCo). These results suggest a strong dissociation between the amygdala and all other brain regions during specific epochs of REMS, but also among the amygdala itself.

Discussion

This study provides a whole-brain characterization of the cerebral and vascular structures involved in the atypical and large-amplitude vascular surges occurring during REMS. This study goes significantly deeper in the understanding of REMS-associated hyperemia, as it imaged a very large number of brain regions (257 regions) over hundreds of REMS episodes. We implemented fUS imaging in 2D imaging planes with light ultrasonic probes as it is compatible with both unrestrained movement and naturally induced sleep studies. 2D fUS imaging enables us to ensure that the animal is not restrained, behaves almost perfectly normally, and sleeps spontaneously. It is primordial as stressed and head-restrained animals are less eager to sleep and deprivation protocols are often used to acquire sleep data, which affects both the structure and nature of sleep episodes. As each imaging session could only image on one single 2D plane, we had to repeat the experiment a large number of times in order to achieve an almost full 3D coverage of the brain’s regional activity during REMS.
Although this approach of multiple 2D planes has the disadvantage to lose the temporal information regarding the coactivity of brain regions from different planes, we solved this difficulty by imaging from both coronal and sagittal planes, thus relying on a respectable number of co-activated regions in each single session.

Although recent technological demonstrations of full 3D fUS imaging using piezo-electric matrix arrays (Brunner et al., 2020; Rabut et al., 2019) or Raw-Colum arrays (Sauvage et al., 2018, 2020) are very promising, they remain to date limited in use, as the heavy weight and limited sensitivity of these probes requires the animal to be head-fixed, thus rendering sleep studies unsuitable and further from normal behavior.

A previous study by our lab has unraveled an intense hyperemic activity during REMS, which largely exceeded both NREMS and wake levels (Bergel et al., 2018). This hyperemic state is decomposed in a tonic component (the elevation of the baseline) and in a phasic one which is robustly preceded by fast gamma oscillations in the CA1 region. This finding is striking as sleep is supposedly a state in which energy levels are reconstructed (Cirelli and Tononi, 2008; Schmidt, 2014; Siegel, 2005), yet this activity must be highly energy consuming. This hyperemic activity has been confirmed by more recent studies, using intrinsic optical imaging (ISOI) in sleeping head-fixe mice (Turner et al., 2020). However, most imaging modalities used so far for REMS studies are either focused on the cortical part or have such a limited temporal resolution that it impedes deeper fundamental understanding.

Such hyperemic activity might be physiologically important as it was kept throughout evolution, despite its energy consumption. Moreover, a clear picture of global brain activity during REMS is still currently missing.

In this study, we used functional ultrasound imaging to gather data on more than 250 brain regions in both coronal and sagittal planes, thus providing a very exhaustive characterization of global brain hemodynamics during rodent REMS. We demonstrate a clear dissociation between basal/midbrain structures and superficial ones, respectively activated in a tonic and phasic manner. We also disentangle the vascular structures involved in the irrigation of the brain during REMS episodes providing a detailed outlook of blood supply. Finally, one of the most noteworthy result of this work is the striking global dissociation of the amygdala activity from the rest of the brain during the REM episodes.

Massive hyperemia observed across the whole brain and neurovascular coupling
A previous study has already shown a hyperemic activity during REMS in humans in some brain regions in human using positron emission tomography (Maquet et al., 1996). However, this study only presented a higher vascular activity correlated with REMS in pontine
tegmentum, left thalamus, both amygdaloid complexes, anterior cingulate cortex and right parietal operculum, and some regions with a negative correlation with REMS mainly in cortical areas.

One of the key findings of the present work is that hyperemia is global and spans throughout all of the forebrain that we were able to image (2/3 of total brain volume). Additionally, it was more sustained in the deep/midbrain structures (in particular in the hippocampus) than in the cortex, which activated in phasic bouts. Thus, REMS can be described as a state of tonic hyperemia in the forebrain that only partially spreads to the cortex. Also, activity in the different cortices were strongly heterogeneous, with strongest activations in the retrosplenial, limbic, motor and visual cortices but close to the levels of wake in the other sensory cortices (somatosensory, piriform). This is surprising as rats preferentially use odor and texture rather than vision. Hence, it is possible that hyperemia is associated with the reactivation of visual networks (geniculate, colliculus, cortex) or in link with memory (retrosplenial, septum, and hippocampus). Vascular hyperactivity specific to REMS in rats divides into tonic and phasic regimes, the latter exhibiting transient brain-wide hyperemic patterns, which we called vascular surges (VS). Bergel et al showed that these VS outmatched wake levels occasionally reaching up to a 100% increase in the cortical and hippocampal regions compared to a quiet wake state. Precursors to VS in the theta (6–10 Hz) and high-gamma (70–110 Hz) bands of hippocampal LFP, and the intensity of each individual VS was best accounted for by the power of fast gamma, suggesting a strong association between local electrographic events and massive brain-wide vascular patterns. These VS exhibit a strong link with LFP gamma power in some brain structures (Bergel et al., 2018). Although a high correlation was already found in former fUS imaging studies between the fUS signal and EEG recordings (Mace et al., 2013; Nunez-Elizalde et al., 2021; Sieu et al., 2015) and neural calcium activity (Aydin et al., 2020) in accordance with the neurovascular coupling model (Iadecola, 2017), the massiveness of the hyperemia during REMS cannot be unambiguously linked to the sole neural activity. Such very high hyperemia may also partly be linked to the metabolic demand of other cell type cells such as glial cells or the drainage of the glymphatic system occurring during sleep (Plog and Nedergaard, 2018).

Global dissociation of the amygdala and its substructures with the rest of the brain

Though it is known that dreams occur during both NREMS and REMS (Stickgold et al., 1994), REMS ones are more vivid and intense, thus raising the assumed link between emotions and REMS. In “The Interpretation of Dreams” by Sigmund Freud, Freud focuses on the importance of dreams and their understanding, as he considered them to be the gate to one’s unconscious
and emotional state (Freud, Sigmund, 1900). More recent studies have confirmed this link between REMS and emotions such as the beneficial effect of sleep (and especially REMS) on negative emotions, and even a link between sleep disturbances and the lack of such beneficial effects (see for review (Vandekerckhove and Wang, 2017)). The “sleep to forget, sleep to remember” (SFSR) model (Walker and van der Helm, 2009) also points towards a role of REMS on the regulation of the emotional valence of memories, while the factual component is encoded in the hippocampus.

Recent research has further revealed the function of sleep in regulating emotion to be primordial (Baglioni et al., 2010). Considering the major link between REMS and the regulation of emotions, it is of particular interest to highlight here a strongly dissociated activity of the amygdala compared to the rest of the brain. Indeed, a higher vascular activity in amygdala was already found correlated with REMS using positron emission tomography in humans (Maquet et al., 1996). Interestingly, our results also indicate that this amygdala hyperemia presents a lower correlation with the rest of the brain regions, depicting a global dissociation of the amygdala from other brain regions.

The function of sleep in regulating emotion was found to be essential (Krause et al., 2017). REMS physiology was associated with an overnight dissipation of amygdala activity in response to previous emotional experiences, altering functional-connectivity and reducing next-day subjective emotionality (van der Helm et al., 2011). It is believed that during REMS and through several sleep sessions, high co-activation of hippocampus and amygdala will strengthen the consolidation of an emotional memory while downscaling its intensity (Walker and van der Helm, 2009). Then, once an aversive memory is triggered during wake, the hippocampus will send a cue to the mPFC to inhibit the amygdala, so that this 3-way switch supposedly downscales the emotional response to the memory. Finally, during REMS, bursts of PGO waves were also recorded across the pontine tegmentum to the geniculate nuclei of the thalamus and the occipital cortex in humans. These PGO waves have been linked with memory consolidation and they enhance synaptic plasticity in the amygdala and dorsal hippocampus (Datta et al., 2008). Rats also appear to produce PGO waves following direct electrical stimulation of the amygdala (Deboer et al., 1998). Such link is thought to indicate a relationship between dreaming and specific epochs of alertness (Gott et al., 2017). Further investigations into rats have revealed important links between the PGO waves and memory-related gene expression in both the amygdala and hippocampus (Datta et al., 2008), while another study has correlated PGO wave density with successful consolidation fear extinction following traumatic stress (Datta and O’Malley, 2013).
Moreover, it is known for many years that the amygdala is electrophysiologically active during REMS (White and Jacobs, 1975) and was also confirmed more recently during NREMS (Girardeau et al., 2017). Our hypothesis is that during REMS, while the amygdala is active, it is only strongly activated when the rest of the brain is not and especially the mPFC resulting in the lower correlation observed in the connectivity matrix. Rats might need to regularly go through phases of emotional regulation during REMS to cope with every day’s accumulation of strong emotional memories.

We hypothesize that we image here this downscaling at play using functional ultrasound imaging during REMS. Finally, such a portable and wide field-of-view neuroimaging modality, as functional Ultrasound imaging provides an extensive picture of brain function and the interaction between large scale brain networks during sleep in rodents.
Methods

Animal Surgery

All animals received humane care in compliance with the European Communities Council Directive of 2010 (2010/63/EU). The experimental protocol used in this study was extensively reviewed and approved by the French CEEA (Comité Ethique pour l'Expérimentation Animale) n°59 Paris Centre et Sud under the reference number 2018061320381023. Adult Sprague Dawley rats aged 12-13 weeks were first put through a week of habituation with the experimenter and then underwent surgical craniotomy and implant of an ultrasound-clear prosthesis. Anesthesia was induced with 2% isoflurane and maintained with ketamine/xylazine (80/10 mg/kg), while body temperature was maintained at 37.0°C with a heating pad (Phymep, Paris, France). A sagittal skin incision was performed across the posterior part of the head to expose the skull. We excised the parietal and frontal flaps by drilling and gently moving the bone away from the dura mater. The opening exposed the brain from Bregma +4.0 to Bregma -9.0 mm, with a maximal width of 14 mm. An electrode was implanted stereotaxically and anchored on the edge of the flap. A prosthetic skull was sealed in place with acrylic resin (GC Unifast TRAD), and the residual space was filled with saline. We chose a prosthesis approach that offers a larger field of view and prolonged imaging condition over 4-6 weeks compared to the thinned bone approach. The prosthetic skull is composed of polymethylpentene (Goodfellow, Huntington UK, goodfellow.com), a standard biopolymer used for implants. This material has tissue-like acoustic impedance that allows undistorted propagation of ultrasound waves at the acoustic gel-prosthesis and prosthesis-saline interfaces. The prosthesis was cut out of a film of 250 µm thickness and permanently sealed to the skull. Particular care was taken not to tear the dura to prevent cerebral damage. The surgical procedure, including electrode implantation, typically took 6 hours. Animals were subcutaneously injected with anti-inflammatory drug (Metacam, 0.2 mg/kg) and prophylactic antibiotics (Borgal, 16mg/kg), and postoperative care was performed for 7 days. Animals recovered quickly and were used for data acquisition after a conservative one-week resting period.

Electrode design and implantation

Electrodes are based on linear polytrodes grouped in bundles of insulated tungsten wires. The difference with a standard design is a 90°-angle elbow that is formed prior to insertion in the brain. This shape enabled anchoring of the electrode on the skull posterior to the flap. An electrode was implanted with stereotaxic positioning micromotion. The prosthesis was then applied to seal the skull. Four epidural screws placed above the cerebellum and above the olfactory bulbs were used as references and grounds. The intra-hippocampal handmade
electrode was composed of 25 to 50 µm diameter insulated tungsten wires soldered to miniature connectors (Omnetics Inc, Minneapolis, US). Eight conductive ends were spaced 0.5 to 1 mm apart and glued to form a 5.5 mm-long, 100-150-µm-diameter bundle. The bundles were lowered in the dorsal hippocampus (left or right) at stereotaxic coordinates AP = -4.0 mm, ML = +/- 2.5 mm and DV = -1.5 mm to -4.5 mm relative to the Bregma. In addition to tungsten wires, we used copper wires (0.28 mm diameter) to measure the muscular activity (electromyogram EMG) in the neck muscles. Before each surgery, the relative position and distance between each recording site on the electrode (8 recording sites per electrode) was identified by measuring the impedance change, while lowering the electrodes in saline solution (Na-Cl 0.9%). The actual design was based on handmade electrodes with minimal spacing of 500 microns between recording sites and a maximal number of 8 recording sites per electrode. This allows to observe the characteristic phase reversal between the superficial and deep layers of the dorsal hippocampus, but not to quantify the cross-frequency coupling as with linear probes. Additionally, the surgical procedure is complex and there is variability between targeted structure and actual electrode position due to brain tissue movement (swelling) both during and after the surgery. See Supplementary Figure 1 for further details.

Electrode implantation verification

Electrodes sites' locations were verified post mortem via histology to reconstruct the tract of electrode bundles in the tissue. Each rat was euthanized and perfused with paraformaldehyde 4% to preserve the brains. Each brain was then cut using a vibratome to make 100µm-thick slices. The slices were then contrasted using hematoxyline/eosine coloration and scanned using a nanoscan. We then compared the slices with plates from Paxinos and defined the trajectory of implantation using the marks left by the electrode in the brain. Knowing the distances between the recording points, we could then define their position.

Recording sessions

After a recovery week following the surgical procedure, the animals were fit to be used in data acquisition. After applying a generous amount of centrifuged ultrasonic gel, the ultrasonic probe was put in place using a magnetic probe holder (home-made 3D designed and printed) and the headstage for LFP recordings was plugged onto the connector. The animal was then placed inside a box under an infra-red camera (to monitor the behavior) and the data acquisition started. The ultrasonic probe was placed randomly across the day and its position was changed after 2-3 30 min-acquisitions. A typical recording session scanned 4 different brain plans and lasted approximatively 6 hours with different breaks. All the probe and
headstage positioning and moving was realized without having to put the animal under anesthesia. At the end of the recording session, the window is cleaned from excess ultrasonic gel and the animal is replaced in its home-cage.

**LFP acquisition**

LFP, electromyogram (EMG) and accelerometer (ACC) signals and video were monitored continuously from video-EEG device for offline processing. Intracranial electrode signals were fed through a Blackrock Cereplex System using the Cereplex Direct Software Suite (version 7.0.6.0) developed by Blackrock Microsystems (Salt Lake City, UT, USA), together with a synchronization signal from the ultrasound scanner. LFP signals were pre-amplified and digitized onto the animal’s head which prevent artifacts originating from cable movement. A regular amplifier was used, and no additional electronic circuit for artifact suppression was necessary. A large bandwidth amplifier was used, which can record local field potentials in all physiological bands (LFP, 0.1-2 kHz). The spatial resolution of LFPs ranges from 250 µm to a few mm radius.

**Ultrasound Acquisition**

Vascular images were obtained via the ultrafast compound Doppler imaging technique\(^84\). The probe was driven by a GPU-based ultrafast ultrasound scanner (Iconeus One, Iconeus France, Neuroscan ART Inserm Software), relying on 24-Gb RAM memory. We continuously acquired N=4500 Doppler ultrasound images at 2.5 Hz frame rate for 30 minutes straight. Each Doppler frame is obtained using the accumulation of 100 successive compound plane-wave frames, each compounded frame corresponding to a coherent summation of beamformed complex in phase/quadrature (IQ) images obtained from the insonification of the medium with a set of successive plane waves with specific tilting angles\(^85\). Given the tradeoff between frame rate, resolution and imaging speed, a plane-wave compounding using eight 2°-apart angles of insonification (from -7° to +7°) was chosen. As a result, the pulse repetition frequency of the plane wave transmissions was 4000 Hz. To discriminate blood signals from tissue clutter, the ultrafast compound Doppler frame stack was filtered, removing the 60 first components of the singular value decomposition, which optimally exploited the spatiotemporal dynamics of the full Doppler film for clutter rejection, largely outperforming conventional clutter rejection filters used in Doppler ultrasound\(^86\).

**LFP analysis**
All analysis was performed in MATLAB (version R2017b, Mathworks, USA). NPMK package (version 4.5.3.0) developed by Blackrock Microsystems was used to import the raw LFP data into MATLAB. EEG was collected and high-pass filtered above 250 Hz. This, together with direct amplification onto the animal’s head via the INTAN chip from the Blackrock system, allows for quality and artifact-free LFP recording in the motor cortex and hippocampus during free running. EEG was then band-pass filtered in typical frequency bands including delta (1-4 Hz), theta (4-10 Hz), beta (10-20 Hz), low-gamma (20-50 Hz), mid-gamma (50-100 Hz), upper mid-gamma (80-120 Hz), high-gamma (100-150 Hz), upper high-gamma (130-180 Hz) and ripple (150-250 Hz). This division has been thoroughly described and proven to be functionally relevant for hippocampal electrographic recordings.

Sleep scoring
Signals from the EMG, ACC and LFP recordings were used to perform the sleep scoring. It allowed us to separate four different vigilance states: active wake (AW), quiet cake (QW), REM sleep (REMS) and non-REM sleep (NREMS). Each of these states is characterized by a combination of indexes drawn from the recorded signals. We computed the power of the EMG signal (as used in Katsageorgiou et al. 2015), the ratio theta/delta of the LFP signal and then manually thresholded these two parameters and the accelerometer signal to obtain indexes made of zeros and ones. The four different vigilance states are defined thus: AW = EMG 1 + ACC 1 + ratio 0/1; QW = EMG 1 + ACC 0 + ratio 0/1; REM = EMG 0 + ACC 0 + ratio 1; NREMS = EMG 0 + ACC 0 + ratio 0. Video was used from time to time to confirm the sleep scoring.

CBV maps & spatial averaging
Previous studies from our group have shown that the fUS signal tightly relates to neuronal activity and microscopic single-vessel hemodynamics. In order to build the CBV maps from the raw back-scattered echoes, radiofrequency (RF) signals are delayed and summed to form IQ matrices through a process known as beamforming. These matrices are then decomposed via Singular Value Decomposition (SVD) to decouple slow movements due to pulsatility and tissue motion from fast movements due to echogenic particles crossing a voxel during a full cardiac cycle (200 milliseconds). Importantly, Power Doppler images are computed by taking the power of the full Doppler spectrum, including a range of speeds in large and smaller vessels, with a typical inferior bound of 2-5 mm/s in axial velocity. This gives a signal proportional to the number of echogenic particles that have crossed a single voxel during 200 milliseconds (with a sufficient axial velocity) which is a good estimate of local cerebral blood flow (CBF). We thus can build Doppler movies with a sampling frequency of 2.5 Hz, which can
even be increased if needed up to the pulse repetition frequency (here 500 Hz) through the use of a temporal sliding window. To derive CBV maps from the raw Doppler movies, we performed voxel-wise normalization from a baseline period: depending on the analysis done afterwards, we either used 2 minutes of quiet wake, 2 mins of active wake, or the 20 seconds preceding the onset of a REM episode. We extracted the distribution for each voxel during this baseline period and computed a mean value, leading to one value for each voxel of the image. To derive a signal similar to $\Delta F/F$ in fluorescence microscopy, we subtracted the mean and divided by the mean for each voxel in the Doppler movie. This allowed normalization and rescaling of ultrasound data, yielding to an expression in terms of percent of variation relative to baseline (CBV % change). Each voxel was normalized independently before performing spatial averaging.

**Atlas registration**

Coronal recordings were registered to two-dimensional sections from the Paxinos atlas (Paxinos and Watson, 2017) using anatomical landmarks, such as cortex edges, hippocampus outer shape and large vessels below brain surface as a reference. We performed manual scaling and rotation along each of the 3 dimensions to recover the most probable registration. Once performed, regions of interest were extracted using binary masks. This process allowed us to derive vascular activity in 259 brain regions.

**Cross-correlation analysis**

We used Pearson’s cross-correlation score to quantify the association between REM episodes and the CBV activations in brain regions, or between brain regions, or between LFP signals and CBV activations in brain regions. To do so, we performed the cross-correlation computation on a large temporal window, depending on the couple analyzed. Regarding the CBV activations, we either used pixels (Fig. 5 C-E) or averaged values over brain regions or structures.

**LFP-CBV correlation analysis**

To assess the association between LFP events and CBV variables, we searched for correlations between each possible combination of LFP band-pass filtered signals and regional CBV variables. As neurovascular processes are not instantaneous, we considered possible delays between electrographic and vascular signals and thus computed cross-correlations functions between the two signals for any LFP-CBV pair and any lag in a given time window.
1.0 s to 5.0 s). We performed this analysis over pixel and regional variables, but only regional variables allowed for statistical comparison across recordings.

Identification of vascular structures

Another technical difficulty we encountered was the recognition of the imaging planes / registrations of these planes in a 3D map. Our laboratory recently developed such approach in mice, by co-registering a vascular atlas on the atlas from the Allen Brain Institute (Nouhoum et al., 2021). Such Approach will be available in the near future for rats, using recently published vascular and MRI atlas in rats. But at the time of these experiments, this was not available. Instead we used the few vascular atlas currently available in rats and mice (Scremin, 2015; Xiong et al., 2017) and in particular imaging plane, we performed Ultrasound Localisation Microscopy. This technique enables, through the detection and tracking of individual micro-bubbles injected intravenously that constitute contrast agents (Errico et al., 2015), to visualize the vasculature with a higher sensitivity and contrast, but also to measure the direction and speed of blood flow at a microscopic scale in both rodents (Errico et al., 2015) and human (Demené et al., 2021).

Statistics and Reproducibility

All statistics are given as +/- standard error of the mean, unless stated otherwise. Statistics in Fig. 3 are computed on n=11 animals over 42 recording sessions. Bar diagrams shown in Fig. 3 are computed by averaging the mean values of 22 recordings on 11 animals for the coronal planes and 20 recordings in 7 animals for the diagonal planes. Statistics are computed using a two-tailed Mann-Whitney test. The significance of Pearson correlation coefficients shown in Fig. 3 and Fig. 7 are assessed by computing the t-value (using $t = \sqrt{\frac{1-r^2}{n-2}}$) and reporting it in Student’s table with $n-2$ degrees of freedom. Statistical testing for correlation distributions were computed after Fischer transformation. Multiple comparison for regional analyses were accounted for using Bonferroni correction. Due to the difficult experimental constraints (difficult surgical procedure, precise electrode implantation, habituation and training required for the locomotion task) no replication attempt was performed in this study, but the results were robust and observable across individuals and recordings.
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Author contributions

A.B. and M.T. designed the experiment. A.B. designed the electrodes and performed the surgeries. M.M. crafted the electrodes, performed the training and recording sessions. A.B. programmed the software for multimodal data visualization and analysis. A.B. and M.M analyzed the behavioral, electrographic data, and ultrasound data. M.M. and S.P. performed ULM experiments and identified vascular structures. All authors discussed multimodal analysis. All authors wrote the paper.

Data availability

All data and software supporting the findings of this study are available from the corresponding authors upon reasonable request. Custom codes used for the collection of fUS data are protected by Inserm and can only be shared upon request, with the written agreement of Inserm.

Code availability

The code used to generate the results that are reported in this study is available from the corresponding authors upon reasonable request. Custom codes used for the analysis of fUS/LFP/video data used in this study are protected by Inserm and can only be shared upon request, with the written agreement of Inserm.

Competing interests

The Authors declare the following competing interests: M.T. is co-founder and shareholder in the ICONEUS company.

Materials & Correspondence

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List of Figures

Main Figures

Figure 1: Experimental design: plane-by-plane monitoring of large-scale brain hemodynamics, local field potentials and behavior in the same animal.

Figure 2: Distribution of cerebral blood volume across brain regions for the four vigilance states studied.

Figure 3: Topological dissociation between tonic activation in the basal brain and phasic activation in the superficial brain areas.

Figure 4: Spatiotemporal dynamics of the vascular supply during REM sleep suggests a preferential irrigation of anterior cerebral areas.

Figure 5: Segregated activity of amygdala nuclei during REM sleep.

List of Supplementary Figures

Supplementary figure S1: Example of location of LFP electrodes implantation in rat #2, showing the location of electrodes (A) in the rat brain atlas.

Supplementary figure S2: Details of brain regions and sub-regions imaged.

Table 1: Name of regions/subregions - number of recordings and of episodes per recording plane.

Supplementary figure S3: Dynamics of three veins highlighted in a sagittal plane.

Supplementary figure S4: Representation of the number of recordings used to calculate the average cross-correlation in the connectivity matrix.

Supplementary Table 2: Mean +/- sem values for the CBV distribution across the 4 different vigilance states for all regions of interests (baseline QW, Fig. 2B left).

Supplementary Table 3: Mean +/- sem values for the CBV distribution across the 4 different vigilance states for all regions of interests (baseline AW, Fig. 2B right).

Supplementary Table 4: Mean +/- sem values for the maximal correlation score Rmax with respect to the REM and REM-PHASIC variables for all regions of interests (Fig. 3D).
Figure 1: Experimental design: plane-by-plane monitoring of large-scale brain hemodynamics, local field potentials and behavior in the same animal.

(A) (Left) Top view of the fUS-LFP chronic surgical procedure. A large cranial window exposed the brain from Bregma AP +4.0 mm to Bregma AP +9.0 mm, which spanned approximately 3/4 of the total brain volume. LFP electrodes were implanted in one hemisphere underneath a sealed transparent polymer prosthesis (Right). Schematics of the volume accessible to fUS imaging with LFP implantation site. (B) Details of the recording protocol. Animals were placed in a familiar recording chamber and the ultrasound probe was fixed to a wearable headstage (see Methods) without anesthesia. They underwent sequential coronal (or sagittal) brain scanning: 60 min per plane, 4-6 planes per day, interleaved with 20 min breaks, during which they slept spontaneously, before being placed back in their home cage. (C) Typical plane-by-plane coronal scanning over 4 different planes (left) and associated temporal traces (right) during a single recording day (green: accelerometer Y-axis, grey: EMG neck signal, black: LFP dorsal hippocampus, color: regional CBV traces). Each recording was sleep scored using EMG, accelerometer and LFP (see Methods) and divided into 4 phases: active wake (AW, blue), quiet wake (QW, green), non-REM sleep (NREMS, yellow) and REM sleep (REMS, red). Each plane was imaged for 1h and CBV traces are expressed in % change relative to a baseline set of frames (see Methods).
Figure 2: Distribution of cerebral blood volume across brain regions for the four vigilance states studied (QW: quiet wake, AW: active wake, NREMS: non-REM sleep, REMS: REM sleep).

(A) Aggregated distributions of averaged CBV amplitude for the 4 different vigilance states (in 55 major brain regions for all coronal and sagittal recordings (N=3 animals, 72 recordings)), grouped by anterior cortical (dark blue, 7 regions), posterior cortical (blue, 6 regions), hippocampal (light blue, 7 regions), midbrain (cyan, 7 regions), thalamus (turquoise, 9 regions), amygdaloid complex (green, 5 regions), hypothalamus (orange, 7 regions) and others (yellow, 7 regions). The signal is expressed in delta CBV/CBV and the circles represent the means.

QW and NREMS are characterized by a relative brain quiescence in all regions. AW is associated with increased cortical CBV levels, especially in the primary sensory areas. REMS is characterized by increased CBV in all brain regions with strongest effect in the hippocampal and limbic structures. The baseline was taken as the first consecutive 1-3 minutes of QW per recording (B) Mean CBV values during REM sleep for all brain regions sorted in descending amplitude of percentage. For each recording, we computed the mean value of the REMS distribution (grey dots) and then computed the mean across recordings (color bar) for all brain regions (Left: baseline taken as the first 1-3 min of QW (similar as A), Right: first 1-3 min of AW). Consistent with previous observations, hippocampus, retrosplenial cortex, superior colliculus and periaqueductal grey are robustly activated during REM sleep. This allows for the quantification of REMS hyperemia for all brain regions relative to QW and AW independently. Error bars denotes standard error of the mean.
Figure 3: Topological dissociation between tonic activation in the basal brain and phasic activation in the superficial brain areas

(A) (Top) Typical fUS image (bottom left), seed variables (upper right) and CBV traces (right) in the whole brain (black) and two different voxels (blue: anterior cerebral artery, red: cingulate cortex) during a typical fUS recording. REMS episodes are denoted by shaded boxes. Note the difference in CBV dynamics between the basal and superficial voxels, showing respectively tonic (voxel 1, blue) and phasic (voxel 2) profiles. These are displayed concurrently with REMS “seed” variable (equals 1 during REM sleep, 0 otherwise) and REM-PHASIC seed (equals 2 during phasic REM sleep, 1 during non-phasic REM sleep, 0 otherwise).

(B) Cross-correlation functions between the two seed variables and the two voxel CBV traces. The coordinates of the peak ($T_{\text{max}}$, $R_{\text{max}}$) were extracted and used as a measure of the coupling strength between the CBV variable and associated state (C) Maximal Correlation ($R_{\text{max}}$) maps obtained by...
performs similar correlation analysis between the two seed variables and all CBV voxels traces in 4 different recording planes in one representative example (at Bregma +2.0 mm, +0.6 mm, -2.0 mm and -4.5 mm). Note how superficial voxels display stronger correlation in the REMS-phasic map than REM maps, suggesting that they track phasic activations better than deep voxels (black arrows) (D) Mean cross-correlation functions computed for the whole set of acquisitions, sorted in decreasing correlation strength by region, for the REMS seed (left) and REMS-phasic seed (right). Hippocampal, midbrain and hypothalamus structures display the strongest coefficient for both analyses with the highest scores for the periaqueductal grey, the substantia nigra and the superior colliculus. Note the dispersion in time lags in the REMS analysis showing that regions are not activated at the same time, as well as the smaller $T_{\text{max}}$ values in the REMS-phasic analysis centered on zero-lag, similar as in (B). Acronyms of the brain areas: see legend figure 2.
Figure 4: Spatiotemporal dynamics of the vascular supply during REM sleep suggests a preferential irrigation in anterior cerebral areas

(A) Typical Doppler images (in 5 imaging planes) illustrating the nature and location of the 6 arteries, where the hemodynamic changes were measured, respective to REM episodes (colored arrows, color code: in legend (B). (B) Hierarchical representation of the 6 different vessels studied, separated in two groups: one within the same branch and the others at the same level and in different branches (according to Xiong et al. 2017). (C & E) Representation of the delta CBV in each type of blood vessel over time during all the REM episodes, either aligned to the beginning of REM (top) or the end of REM episodes (bottom). (D & F): averaged delta CBV over time calculated from C & E, aligned either to the beginning of REM (top) or the end of REM episodes (bottom). Beginning and end of REM episodes are indicated by red dots in C & E or vertical lines in D & F. (G) Averaged representation for the azygos anterior cerebral artery (azac) centered on the beginning of REM and the 3 standard deviations of the averaged signal (σ). The table below shows the averaged times (in seconds) for each vessel to reach the 3 different standard deviations. Scale bar from A = 1 mm. Scale bars in C & E =
60 sec. In **C & E**: each color bar is applied for the blood vessels, irrespective of their alignment to the beginning or end of the REM episode.
Figure 5: Disconnection of the amygdala and sub-structures from the rest of the brain during REMS

(A) Connectivity matrix showing in color the average cross-correlation between the 47 different brain regions and nuclei (legend on the right) across all recordings and in all animals (N=3 animals, 72 recordings) (see supplementary figure S7 to have the number of recordings per pixels). White pixels correspond to cross-correlation couples where the number of recordings was either non-existent (because the two brain regions were not present on the same plane) or inferior to 5. (B - E) Example of one specific recording. (B) CBV dynamics of the entire brain (yellow), the amygdala (black) and two of its nuclei, the AHiPL (light blue) and the APir (dark blue). The colored patches on the background represent the different vigilance states (REM: red, NREM: yellow, AW: blue and QW: green). The dynamics are represented as an averaged value of the percentage of CBV compared to the baseline for each region. Note how the CBV changes dynamics mid-REM: the first part has both a tonic and phasic component while the second part presents only a tonic component. The amygdala and its nuclei keep the same tonic activity from the first part to the second while the rest of the brain (yellow) has a slightly lower CBV in the second part. (C & D) Pixel correlation maps with respectively, the ‘Whole Brain’ (D) and the ‘Amygdala’ (D) as seeds (highlighted in black). Note how the amygdala is not correlated with the rest of the brain in C and correlated only with itself in D. (E) Correlation map details for 5 different nuclei of the amygdala as seeds. For each correlation map, the highest possible correlation per pixel is represented (R_{max}).
Supplementary Figure S1: Example of location of LFP electrodes implantation in rat #2, showing the location of electrodes (A) in the rat brain atlas (Paxinos and Watson, 2017). The brain slices were cut from fixed brains (paraformaldehyde 4%) using a vibratome and were colored using a mix of hematoxyline/eosine. We could see the track of the electrode. This track was then translated to the corresponding Paxinos plate and the recording points are placed knowing the distances between each of them. Sometimes the track was visible on multiple slices, we then used them all to define an average position of the electrode.
Supplementary Figure S2: Details of brain regions and sub-regions imaged
Table 1: Name of regions/subregions - number of recordings and of episodes per imaging plane

| Plan span     | Ratio | Recordings | REM episodes | Important structures                                      |
|---------------|-------|------------|--------------|----------------------------------------------------------|
| c1 0–3.0 mm – 3–6.0 mm | 2 6 | 12          | Motor cts, Cg cts, Cpu                      |
| c2 0–2.0 mm – 3–4.0 mm | 2 4 | 9           | Motor cts, Cg cts, S1 cts, Cpu            |
| c3 0–1.0 mm – 3–5.0 mm | 3 6 | 12          | Motor cts, Cg cts, S1 cts, CPu, Mf        |
| c4 0–0.5 mm – 3–4.5 mm | 3 9 | 14          | Motor cts, Cg cts, S1 cts, CPu, GP, Amygdala, Striatum       |
| c5 0–1.0 mm – 3–2.0 mm | 2 9 | 21          | Motor cts, S1 cts, S2 cts, Thalamic, anterior hypothalamus, amygdala |
| c6 0–1.0 mm – 3–1.0 mm | 3 5 | 10          | RS cts, S1 cts, dorsal hippocampus, thalamus, hypothalamus, amygdala |
| c7 0–0.5 mm – 3–2.0 mm | 3 6 | 8           | RS cts, association cts, Au cts, dorsal hippocampus, thalamus, posterior hypothalamus, amygdala |
| c8 0–1.0 mm – 3–3.0 mm | 3 7 | 8           | RS cts, visual cts, Au cts, dorsal & ventral hippocampus, thalamus, midbrain, ncl, amygdala |
| c9 0–0.5 mm – 3–1.0 mm | 3 1 | 1           | RS cts, visual cts, Au cts, posterior hippocampus, SN, superior colliculus, PAC |
| c10 0–0.5 mm – 3–0.5 mm | 3 4 | 5           | RS cts, visual cts, Au cts, entorhinal cts, SN, superior colliculus, PAC |

| Sagittal plans | Ratio | Recordings | REM episodes | Important structures                                      |
|----------------|-------|------------|--------------|----------------------------------------------------------|
| s1 ML 0.0 mm – ML 1.0 mm | 2 5 | 10          | Cg cts, RS cts, Mf, superior colliculus, thalamus, preoptic area, hypothalamus, PAC |
| s2 ML 1.0 mm – ML 3.0 mm | 2 1 | 2           | Motor cts, association cts, visual cts, CPu, superior colliculus, thalamus, preoptic area, hypothalamus, hippocampus |
| s3 ML 1.0 mm – ML 5.0 mm | 2 2 | 0           | Motor cts, S1 cts, visual cts, CPu, thalamus, amygdala, hippocampus |
| s4 ML 1.0 mm – ML 4.0 mm | 2 4 | 5           | Motor cts, S1 cts, visual cts, CPu, GP, thalamus, amygdala, hippocampus |
| s5 ML 1.0 mm – ML 5.0 mm | 2 2 | 3           | S1 cts, visual cts, CPu, GP, amygdala, hippocampus |
Supplementary Figure S3: Dynamics of three veins highlighted in a sagittal plane.

(A) Identification of the veins in sagittal plane using both ultrafast doppler images (A) and super-resoluted images (B). These super-resoluted images, obtained following intravenous injections of microbulles were performed as previously described (Errico et al., 2015). Due to the high contrast and excellent spatial resolution (10 µm), they allow separation of the azicv and the lhiv (B). (C) Representation of all the REM episodes used to obtain the averaged values represented in (D) for each vessel, aligned either to the beginning of REM (top) or the end of REM episodes (bottom). Beginning and end of REM episodes are shown with red dots (C) or vertical lines (E). sss = superior sagittal sinus, azicv = azygos internal cerebral vein, lhiv = longitudinal hippocampal vein. Scale bar from A, B = 1 mm. Scale bars from C = 60 sec.
Supplementary Figure S4: Representation of the number of recordings used to calculate the average cross-correlation in the connectivity matrix.

Same connectivity matrix as presented in figure 5, with a detail of the number of recordings used to average the cross-correlation values for each couple of regions. Couples with no value (not in the same plan) or with less than 5 recordings were left blank.
| ROI                     | QW        | AW            | NREM       | REM        |
|------------------------|-----------|---------------|------------|------------|
| Auditory Cortex        | 5.7868+/-.15697 | 37.1996+/-.80978 | 0.3462+/-.5688 | 6.6340+/-.10478 |
| Basal Amygdala         | 1.6018+/-.5225  | 5.6608+/-.9649  | -0.0921+/-.2548 | 5.5405+/-.14347 |
| Basal Forebrain        | 1.1409+/-.2439  | 5.1509+/-.5190  | -0.0031+/-.1756 | 9.7729+/-.6557  |
| Brain Stem             | 0.2398+/-.4361  | 4.1396+/-.9820  | -0.1558+/-.7680 | 3.4508+/-.17961 |
| CA1 Region             | 0.7914+/-.12321 | 4.8180+/-.19208 | -1.2394+/-.8469 | 17.0400+/-.37020 |
| CA2 Region             | 0.4704+/-.5671  | 7.4421+/-.21325 | 0.6352+/-.5044  | 20.6952+/-.20479 |
| CA3 Region             | 1.7176+/-.3305  | 9.4156+/-.13691 | 0.3059+/-.2511  | 13.9788+/-.8461  |
| Callosum               | 2.4098+/-.11099 | 6.3190+/-.12515 | 0.1897+/-.3001  | 7.2116+/-.9570   |
| Centromedial Amygdala  | 0.7579+/-.6448  | 3.1817+/-.7822  | 0.8357+/-.4216  | 5.0101+/-.8538   |
| Cerebellum             | 2.3258+/-.9498  | 9.1911+/-.17161 | 1.0437+/-.7638  | 29.5915+/-.23301 |
| Cingulate Cortex       | 1.3414+/-.3823  | 6.6220+/-.12916 | 0.4761+/-.4251  | 6.6521+/-.8825   |
| Claustrum              | 1.5893+/-.5465  | 6.6290+/-.21158 | -0.2733+/-.2590 | 5.4707+/-.8742   |
| Cortical Amygdala      | 1.3824+/-.3467  | 5.3088+/-.8584  | -0.2647+/-.2509 | 9.7338+/-.9999   |
| DHypothalamus          | 1.0467+/-.4449  | 10.3465+/-.23308 | 0.1634+/-.8037  | 25.5931+/-.4557 |
| DPAG                   | 2.8642+/-.7395  | 11.8714+/-.17105 | 0.0837+/-.3730  | 23.6849+/-.19598 |
| D Thalamus             | 0.2463+/-.5677  | 6.4536+/-.16082 | 0.3637+/-.5333  | 28.2643+/-.6493  |
| Dentate Gyrus          | 1.7212+/-.5997  | 14.7010+/-.52934 | 0.1149+/-.4548  | 4.1117+/-.7391   |
| Entorhinal Cortex      | 1.4384+/-.5052  | 7.6595+/-.15693 | 0.4673+/-.3940  | 16.5768+/-.15768 |
| Fimbria                | 2.2808+/-.9696  | 10.6566+/-.12657 | -0.9665+/-.5195 | 19.7453+/-.2057 |
| Geniculate Nuclei      | 4.3455+/-.24888 | 19.6497+/-.10879 | 2.0653+/-.14858 | 39.9802+/-.95892 |
| Habenula               | 3.5227+/-.11527 | 20.2270+/-.59029 | 0.0440+/-.2328  | 5.0657+/-.7272   |
| Insular Cortex         | 1.3450+/-.4658  | 5.3580+/-.7865  | -0.3141+/-.2514 | 9.7669+/-.8908   |
| LHypothalamus          | 1.2768+/-.3538  | 7.8195+/-.26478 | -0.0001+/-.2735 | 4.5306+/-.9189   |
| Lateral Amygdala       | 0.2587+/-.5548  | 3.7981+/-.13879 | -0.5617+/-.9660 | 14.8495+/-.28558 |
| Limbic Cortex          | 1.4854+/-.6220  | 4.5750+/-.6474  | 1.1747+/-.15904 | 13.0582+/-.37483 |
| Mammillary Nuclei      | 1.8724+/-.6281  | 9.4580+/-.12000 | 1.3404+/-.4894  | 19.8980+/-.1483 |
| Motor Cortex           | 1.4013+/-.5588  | 4.8858+/-.7101  | -0.1812+/-.2267 | 10.5090+/-.13898 |
| Olfactory Nuclei       | 2.3012+/-.5188  | 7.1065+/-.10468 | 0.2850+/-.2925  | 11.9329+/-.1376 |
| Optic Chiasm           | 0.9216+/-.8053  | 4.4171+/-.12109 | 0.1058+/-.7873  | 6.2502+/-.21613 |
| Orbital Cortex         | 2.1532+/-.12222 | 9.8266+/-.15644 | 0.4033+/-.9469  | 17.7554+/-.8871 |
| Parietal Cortex        | 1.2446+/-.3161  | 6.2671+/-.12192 | 0.1090+/-.2444  | 5.3225+/-.6352   |
| Piriform Cortex        | 1.3882+/-.5386  | 3.5230+/-.5865  | 0.4547+/-.4002  | 8.7409+/-.8169   |
| Preoptic Area          | 1.0069+/-.6264  | 9.4958+/-.9164  | -0.9453+/-.7746 | 35.1367+/-.47926 |
| Pretectal Nuclei       | 1.6602+/-.4286  | 7.0316+/-.12288 | 0.2439+/-.2945  | 7.1467+/-.7049   |
| Residual Amygdala      | 1.0653+/-.8296  | 9.9170+/-.26916 | 0.0695+/-.7329  | 24.4478+/-.21223 |
| Residual Hippocampus   | 2.5178+/-.6987  | 10.4479+/-.20728 | -0.2279+/-.3646 | 16.8963+/-.19013 |
| Reticular Formation    | 2.7384+/-.11062 | 12.3676+/-.28341 | 1.6313+/-.7604  | 33.4812+/-.32270 |
| Retrosplenial Cortex   | 3.3458+/-.10677 | 29.4772+/-.96002 | 0.8047+/-.7215  | 3.8095+/-.10858 |
**Supplementary Table 2**: Mean +/- sem values for the CBV distribution across the 4 different vigilance states for all regions of interests (baseline QW, Fig. 2B left)

| Region               | CBV Mean | SD Mean | Increase Mean | Decrease Mean |
|----------------------|----------|---------|---------------|---------------|
| Rhinal Cortex        | 2.1024+/-0.5717 | 7.0898+/-1.0808 | 0.7225+/-0.5131 | 17.3999+/-1.6552 |
| Septum               | 3.1320+/-0.6347 | 17.1087+/-2.5500 | 0.1172+/-0.3448 | 11.2594+/-0.7554 |
| Somatosensory Cortex | 1.7327+/-0.4588 | 6.3076+/-1.0695 | 0.4798+/-0.2930 | 10.5455+/-1.1774 |
| Striata Terminalis   | 1.1932+/-0.3119 | 7.2476+/-1.1304 | -0.2030+/-0.2278 | 10.1025+/-0.7549 |
| Striatum             | 0.9648+/-0.6653 | 5.2135+/-0.9300 | -0.5224+/-0.5170 | 19.1332+/-2.5265 |
| Subiculum            | 0.6800+/-0.5103 | 4.8942+/-1.0113 | -0.5178+/-0.3777 | 10.7932+/-1.4303 |
| Substantia Nigra     | 0.9124+/-0.4935 | 8.7212+/-1.4514 | 0.6416+/-0.7780 | 31.4742+/-3.9782 |
| Superior Colliculus  | 1.1992+/-0.3188 | 4.6933+/-1.3283 | 0.0217+/-0.2481 | 8.3919+/-1.1375 |
| V Hypothalamus       | 1.0880+/-0.2856 | 8.4738+/-1.3042 | 0.3011+/-0.6933 | 17.2004+/-2.5963 |
| VPAG                 | 0.7015+/-0.3096 | 3.6291+/-0.7904 | -0.6895+/-0.5578 | 7.5384+/-1.2446 |
| VTegmental Area      | 2.6910+/-0.5688 | 10.2689+/-1.2613 | -0.2687+/-0.3699 | 16.1420+/-1.4954 |
| V Thalamus           | 1.9069+/-0.4437 | 8.1645+/-1.3321 | 0.9268+/-0.4178 | 17.7069+/-1.3700 |
| V Ventricles         | 0.6939+/-0.6561 | 8.8784+/-1.6241 | -0.1505+/-0.5498 | 21.5063+/-3.5277 |
| Visual Cortex        | 1.3200+/-0.4974 | 6.4417+/-1.1877 | -0.5886+/-0.2467 | 9.1062+/-1.0623 |
| Zona Incerta         | -6.2953+/-1.3004 | 1.6815+/-1.0117 | -9.6230+/-1.3086 | 7.7206+/-2.2207 |
| ROI                      | QW        | AW         | NREM        | REM        |
|--------------------------|-----------|------------|-------------|------------|
| AuditoryCortex           | -4.4469/-| 36.4204+/-| -13.5775+/-| -3.1272+/-|
| BasalAmygdala            | -4.3687/-| 1.9671+/-1.1995 | -6.8811+/-1.4208 | 3.1733+/-2.3426 |
| BasalForebrain           | -5.2101/-| 1.2732+/-0.5008 | -7.1589+/-0.6891 | 10.2627+/-1.1159 |
| BrainStem                | -5.9035+/-2.1049 | 0.8518+/-1.6710 | -6.7798+/-2.2167 | -0.3632+/-2.7877 |
| CA1Region                | -8.3571+/-2.4301 | 4.0982+/-1.9841 | -10.8342+/-2.4096 | 22.3698+/-4.2384 |
| CA2Region                | -3.7913+/-1.3209 | 0.7474+/-0.6802 | -7.4695+/-4.0885 | 26.8339+/-8.9677 |
| CA3Region                | -4.4590+/-1.8839 | 6.3085+/-2.7509 | -3.8667+/-1.7120 | 33.2459+/-3.8860 |
| CCallosum                | -6.0730+/-0.9833 | 4.1715+/-1.2771 | -8.4782+/-0.9159 | 15.4366+/-1.6477 |
| CentromedialAmygdala     | -4.4854+/-1.7404 | 1.8363+/-0.9903 | -7.7842+/-1.3291 | 4.5566+/-1.3720 |
| Cerebellum               | -3.8767+/-1.8943 | 0.4783+/-1.9922 | -3.7824+/-1.7112 | 4.0188+/-3.0613 |
| CingulateCortex          | -8.0699+/-2.2905 | 1.2906+/-1.7398 | -10.2722+/-1.7260 | 36.8495+/-3.4213 |
| Claustrum                | -6.6410+/-1.4306 | 0.8171+/-0.9997 | -8.1050+/-1.3874 | 2.9157+/-1.9052 |
| CorticalAmygdala         | -5.3411+/-1.9136 | 0.3614+/-1.0636 | -8.1340+/-1.9059 | 2.2715+/-2.5849 |
| DHyposothalamus          | -4.0436+/-0.9107 | 2.6229+/-0.8321 | -7.0116+/-0.9317 | 11.0661+/-1.6757 |
| DPAG                     | -11.4064+/-3.1876 | 2.7254+/-2.6541 | -12.7568+/-3.2102 | 29.5807+/-6.7963 |
| DThalamus                | -7.6097+/-1.4099 | 5.9815+/-1.6785 | -11.8718+/-1.3512 | 26.9156+/-3.0169 |
| DentateGyrus             | -3.1828+/-1.5634 | 6.8506+/-2.5213 | -2.6317+/-1.6607 | 50.4976+/-5.4953 |
| EntorhinalCortex         | -4.7584+/-1.6999 | 16.3836+/-8.6240 | -7.5583+/-1.7144 | 0.0482+/-2.3269 |
| Fimbria                  | -5.1630+/-1.6481 | 4.3182+/-1.7441 | -6.6391+/-1.5967 | 21.8098+/-3.1058 |
| GeniculateNuclei         | -8.5223+/-2.0491 | 3.6104+/-1.6281 | -13.7805+/-1.9447 | 20.9414+/-3.5540 |
| Habenulla                | -7.4091+/-4.8056 | 7.7684+/-2.9904 | -9.7830+/-4.9578 | 46.1511+/-7.4086 |
| InsularCortex            | -8.9266+/-2.2562 | 5.0387+/-2.5460 | -12.6938+/-2.1833 | -3.8520+/-2.7283 |
| LHypothesalamus          | -4.4432+/-0.8930 | 2.4463+/-0.7417 | -7.2988+/-0.8795 | 10.9517+/-1.4923 |
| LateralAmygdala          | -3.1274+/-1.4203 | 7.4087+/-3.8743 | -5.2623+/-1.6279 | 2.8431+/-1.6206 |
| LimbicCortex             | -5.5722+/-2.7903 | 0.5959+/-1.1912 | -7.7998+/-1.7349 | 19.2702+/-3.5153 |
| MammillaryNuclei         | -4.1504+/-1.1016 | 2.0957+/-1.2563 | -5.3201+/-1.8605 | 16.0648+/-5.0344 |
| MotorCortex              | -8.6114+/-1.5095 | 2.8231+/-1.5015 | -9.1055+/-1.5173 | 21.5187+/-2.6385 |
| OlfactoryNuclei          | -4.4318+/-1.0829 | 1.4045+/-0.6833 | -7.0398+/-1.0097 | 11.3959+/-1.9764 |
| OpticIasism              | -4.5589+/-1.1018 | 1.8637+/-0.6424 | -7.6767+/-1.0504 | 12.1792+/-2.1800 |
| OrbitalCortex            | -6.5127+/-2.2777 | -0.9016+/-1.3627 | -7.9612+/-2.0936 | 2.8914+/-3.3924 |
| ParietalCortex           | -9.3350+/-2.6983 | 1.2780+/-2.4263 | -11.9632+/-2.7566 | 16.7723+/-3.9223 |
| PiriformCortex           | -4.2277+/-0.8089 | 4.0292+/-2.1686 | -6.2695+/-0.7192 | 3.3446+/-1.2279 |
| PreopticArea             | -2.8299+/-0.8748 | 1.0106+/-0.7333 | -4.5556+/-0.7849 | 10.8105+/-1.3544 |
| PretectualNuclei         | -12.3657+/-2.7405 | 0.4230+/-2.6654 | -15.9815+/-2.1953 | 43.8170+/-7.3739 |
| ResidualAmygdala         | -5.2636+/-1.1838 | 2.8942+/-1.3167 | -7.6095+/-1.1935 | 4.5527+/-1.4551 |
| ResidualHippocampus      | -4.2053+/-2.2661 | 8.6159+/-2.7861 | -5.7642+/-2.5464 | 39.6513+/-4.9273 |
| ReticularFormation       | -7.4393+/-1.2544 | 4.3440+/-1.7523 | -11.8482+/-1.2873 | 16.5588+/-2.4643 |
| RetrosplenialCortex      | -6.3213+/-2.0491 | 6.6585+/-2.2692 | -7.8748+/-1.9717 | 45.3387+/-4.7122 |
| RhinalCortex             | -4.4391+/-3.2393 | 34.5624+/-16.5804 | -8.8900+/-2.2855 | -3.5716+/-2.3106 |
| Septum                   | -5.7718+/-1.4357 | 1.7830+/-1.1901 | -7.9044+/-1.3097 | 20.4387+/-2.0709 |
| Region                  | CBV Mean     | CBV SD       | T-Value     | P-Value     |
|-------------------------|--------------|--------------|-------------|-------------|
| Somatosensory Cortex    | -9.3377+/-1.4380 | 6.7547+/-2.3425 | -13.4172+/-1.2589 | 5.0823+/-1.7831 |
| Striata Terminalis      | -4.7805+/-1.4704 | 2.1954+/-1.2965 | -6.9175+/-1.4064 | 10.6657+/-1.9913 |
| Striatum                | -6.0404+/-0.8176 | 3.2813+/-1.5587 | -8.4743+/-0.6739 | 9.7605+/-1.2950 |
| Subiculum               | -6.5524+/-2.1136 | 0.0725+/-1.6295 | -9.1539+/-1.8840 | 26.9434+/-5.8091 |
| Substantia Nigra        | -6.5731+/-1.6709 | -0.2551+/-1.4404 | -8.7302+/-1.5176 | 11.4311+/-2.6285 |
| Superior Colliculus     | -6.2836+/-4.4265 | 5.8065+/-3.7991 | -7.3507+/-3.5073 | 48.2908+/-8.0123 |
| V Hypothalamus          | -2.5510+/-1.0256 | 3.3291+/-1.2337 | -4.7385+/-1.0545 | 10.5241+/-1.4040 |
| VPAG                    | -9.3643+/-2.0434 | 2.1737+/-1.5123 | -11.0977+/-1.9543 | 16.9619+/-3.1649 |
| V Tegmental Area        | -4.4603+/-1.1771 | 0.5131+/-0.8721 | -7.0823+/-1.3983 | 8.4525+/-2.4401 |
| V Thalamus              | -6.8070+/-1.1062 | 4.9364+/-1.3806 | -11.6275+/-1.0767 | 15.8079+/-2.3802 |
| V Ventricles            | -4.4172+/-1.2776 | 4.3142+/-1.2730 | -5.8823+/-1.3364 | 23.0586+/-2.3371 |
| V Visual Cortex         | -10.4059+/-1.8929 | 0.8217+/-1.8686 | -11.9236+/-1.9902 | 24.5472+/-6.8367 |
| Zona Incerta            | -6.2953+/-1.3004 | 1.6815+/-1.0117 | -9.6230+/-1.3086 | 7.7206+/-2.2207 |

**Supplementary Table 3**: Mean +/- sem values for the CBV distribution across the 4 different vigilance states for all regions of interests (baseline AW, Fig. 2B right)
| ROI                        | Rmax (REM)  | Rmax (REM-PHASIC) |
|----------------------------|-------------|-------------------|
| Auditory Cortex            | 0.2374 +/- 0.0391 | 0.1994 +/- 0.0390 |
| Basal Amygdala             | 0.4502 +/- 0.0404 | 0.4867 +/- 0.0413 |
| Basal Forebrain            | 0.6632 +/- 0.0188 | 0.7062 +/- 0.0209 |
| Brain Stem                 | 0.2581 +/- 0.1040 | 0.2823 +/- 0.1112 |
| CA1 Region                 | 0.5551 +/- 0.0339 | 0.6084 +/- 0.0396 |
| CA2 Region                 | 0.6607 +/- 0.0505 | 0.7855 +/- 0.0632 |
| CA3 Region                 | 0.6132 +/- 0.0203 | 0.7004 +/- 0.0201 |
| Callosum                   | 0.5915 +/- 0.0176 | 0.6528 +/- 0.0214 |
| Centromedial Amygdala      | 0.5944 +/- 0.0317 | 0.6194 +/- 0.0340 |
| Cerebellum                 | 0.4924 +/- 0.1120 | 0.5144 +/- 0.1170 |
| Cingulate Cortex           | 0.5828 +/- 0.0195 | 0.6556 +/- 0.0231 |
| Claustrum                  | 0.4259 +/- 0.0386 | 0.4683 +/- 0.0415 |
| Cortical Amygdala          | 0.4645 +/- 0.0424 | 0.4827 +/- 0.0472 |
| D Hypothalamus             | 0.6345 +/- 0.0209 | 0.6932 +/- 0.0258 |
| DPAG                       | 0.7408 +/- 0.0228 | 0.8111 +/- 0.0277 |
| D Thalamus                 | 0.6534 +/- 0.0156 | 0.7301 +/- 0.0174 |
| Dentate Gyrus              | 0.6446 +/- 0.0198 | 0.7296 +/- 0.0222 |
| Entorhinal Cortex          | 0.3404 +/- 0.0525 | 0.3771 +/- 0.0605 |
| Fimbria                    | 0.6093 +/- 0.0261 | 0.6792 +/- 0.0287 |
| Geniculate Nuclei          | 0.6042 +/- 0.0333 | 0.6837 +/- 0.0384 |
| Habenula                   | 0.6553 +/- 0.0577 | 0.7202 +/- 0.0765 |
| Insular Cortex             | 0.3547 +/- 0.0374 | 0.3881 +/- 0.0401 |
| LHypothalamus              | 0.6650 +/- 0.0260 | 0.7179 +/- 0.0289 |
| Lateral Amygdala           | 0.4480 +/- 0.0556 | 0.4668 +/- 0.0660 |
| Limbic Cortex              | 0.5841 +/- 0.0469 | 0.6458 +/- 0.0510 |
| Mammillary Nuclei          | 0.5832 +/- 0.0386 | 0.6563 +/- 0.0407 |
| Motor Cortex               | 0.4952 +/- 0.0168 | 0.5703 +/- 0.0192 |
| Olfactory Nuclei           | 0.6638 +/- 0.0243 | 0.6947 +/- 0.0276 |
| Opt Chiasm                 | 0.6120 +/- 0.0246 | 0.6449 +/- 0.0280 |
| Orbital Cortex             | 0.4305 +/- 0.0945 | 0.4651 +/- 0.1023 |
| Parietal Cortex            | 0.4708 +/- 0.0265 | 0.5493 +/- 0.0260 |
| Piriform Cortex            | 0.4583 +/- 0.0305 | 0.4904 +/- 0.0337 |
| Preoptic Area              | 0.6803 +/- 0.0223 | 0.7164 +/- 0.0240 |
| Pre-tectal Nuclei          | 0.6667 +/- 0.0384 | 0.7834 +/- 0.0398 |
| Residual Amygdala          | 0.5511 +/- 0.0264 | 0.5662 +/- 0.0294 |
| Residual Hippocampus       | 0.6369 +/- 0.0155 | 0.7292 +/- 0.0176 |
| Reticular Formation        | 0.6211 +/- 0.0213 | 0.6901 +/- 0.0252 |
| Retrosplenial Cortex       | 0.5841 +/- 0.0272 | 0.6607 +/- 0.0315 |
| Rhinal Cortex              | 0.2228 +/- 0.0376 | 0.2007 +/- 0.0448 |
| Septum                     | 0.6656 +/- 0.0240 | 0.7272 +/- 0.0266 |
| Region                | Mean Rmax | SEM Rmax |
|-----------------------|-----------|----------|
| Somatosensory Cortex  | 0.4012    | 0.0233   |
|                      | 0.4475    | 0.0276   |
| Striata Terminalis   | 0.6153    | 0.0222   |
|                      | 0.6519    | 0.0257   |
| Striatum             | 0.5881    | 0.0204   |
|                      | 0.6374    | 0.0237   |
| Subiculum             | 0.6794    | 0.0303   |
|                      | 0.7504    | 0.0294   |
| Substantia Nigra     | 0.7115    | 0.0173   |
|                      | 0.7702    | 0.0204   |
| Superior Colliculus   | 0.6967    | 0.0340   |
|                      | 0.7706    | 0.0313   |
| V Hypothalamus       | 0.7078    | 0.0154   |
|                      | 0.7467    | 0.0182   |
| VPAG                  | 0.6231    | 0.0495   |
|                      | 0.6851    | 0.0569   |
| VTegmental Area       | 0.6071    | 0.0534   |
|                      | 0.6545    | 0.0629   |
| V Thalamus            | 0.5925    | 0.0269   |
|                      | 0.6530    | 0.0302   |
| V Ventricles          | 0.6250    | 0.0142   |
|                      | 0.6806    | 0.0174   |
| Visual Cortex         | 0.5301    | 0.0256   |
|                      | 0.5951    | 0.0268   |
| Zona Incerta          | 0.5806    | 0.0306   |
|                      | 0.6643    | 0.0367   |

**Supplementary Table 4**: Mean +/- sem values for the maximal correlation score R_max with respect to the REM and REM-PHASIC variables for all regions of interests (Fig. 3D)