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On-going electroencephalographic rhythms related to cortical arousal in wild-type mice: the effect of aging

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ABSTRACT

Resting state electroencephalographic (EEG) rhythms reflect the fluctuation of cortical arousal and vigilance in a typical clinical setting, namely the EEG recording for few minutes with eyes closed (i.e., passive condition) and eyes open (i.e., active condition). Can this procedure be back-translated to C57 (wild type) mice for aging studies? On-going EEG rhythms were recorded from a frontoparietal bipolar channel in 85 (19 females) C57 mice. Male mice were subdivided into 3 groups: 25 young (4.5–6 months), 18 middle-aged (12–15 months), and 23 old (20–24 months) mice to test the effect of aging. EEG power density was compared between short periods (about 5 minutes) of awake quiet behavior (passive) and dynamic exploration of the cage (active). Compared with the passive condition, the active condition induced decreased EEG power at 1–2 Hz and increased EEG power at 6–10 Hz in the group of 85 mice. Concerning the aging effects, the passive condition showed higher EEG power at 1–2 Hz in the old group than that in the others. Furthermore, the active condition exhibited a maximum EEG power at 6–8 Hz in the former group and 8–10 Hz in the latter. In the present conditions, delta and theta EEG rhythms reflected changes in cortical arousal and vigilance in freely behaving C57 mice across aging. These changes resemble the so-called slowing of resting state EEG rhythms observed in humans across physiological and pathological aging. The present EEG procedures may be used to enhance preclinical phases of drug discovery in mice for understanding the neurophysiological effects of new compounds against brain aging.

1. Introduction

Alzheimer's disease (AD) is the most prevalent progressive neurodegenerative disorder across aging (Bastos Leite et al., 2004; Braak and Braak, 1995; Glodzik-Sobanska et al., 2005). Recent guidelines propose a diagnostic algorithm using physiopathological and topographical biomarkers of AD (Dubois et al., 2014). The physiopathological markers would be mandatory to confirm the diagnosis of dementia of AD type. These physiopathological biomarkers include amyloid beta (Aβ)-42 and tau in the cerebrospinal fluid or map them in the brain by ligand positron emission tomography (PET; Förstl and Kurz, 1999). Topographic markers are suggested to track the disease progression. These topographic markers included maps of brain hypometabolism obtained by

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fluorodeoxyglucose-PET and maps of brain atrophy or abnormal structural connectivity obtained by magnetic resonance imaging.

Unfortunately, these PET and magnetic resonance imaging methodologies are not easily translated into preclinical research using mouse models of AD for fundamental and applied research (i.e., drug discovery). For this reason, other kinds of biomarkers are being developed. Among them, a promising biomarker is derived from on-going electroencephalographic (EEG) rhythms (Babiloni et al., 2013; Schroeter et al., 2009). These rhythms are an emerging feature of the mammalian brain. They are mainly generated by the synaptic currents associated with the synchronization or desynchronization of the activity of many cortical pyramidal neurons, due to cortical and subcortical signals (Pfurtscheller and Lopes da Silva, 1999). Two main conditions are typically used to probe these neurophysiological synchronization and desynchronization mechanisms in a clinical setting. In a "passive" behavioral condition, the subject remains in relaxed wakefulness (resting state) with eyes closed for few minutes. This mode is contrasted with a more "active" behavioral condition in which the subject remains in relaxed wakefulness with eyes open for few minutes (monitoring the surrounding environment). In the resting state eyes-closed condition, EEG rhythms show the highest power (density) at about 8 and 12 Hz in posterior cortical areas, the so-called dominant alpha rhythms (Pfurtscheller and Lopes da Silva, 1999). The higher the alpha power, the lower the cortical arousal, the lower the vigilance. After eyes opening, alpha rhythms exhibit a power reduction (i.e., desynchronization) as a reflection of increased cortical arousal related to higher vigilance (Pfurtscheller and Lopes da Silva, 1999).

EEG power exhibited a different reactivity to eyes opening in normal elderly subjects (Nold) compared with AD subjects. It has been repeatedly reported a lower reduction (reactivity) of the posterior alpha power in AD and MCI patients than that in Nold subjects (Babiloni et al., 2010; Jeong, 2004; Stam et al., 1996; Stevens and Kircher, 1998; van der Hiele et al., 2007). This poor reactivity of alpha power predicted a deterioration of higher functions in subjects with cognitive decline (van der Hiele et al., 2008). These results were confirmed by the analysis of magnetoencephalographic rhythms in the same resting state conditions (Berendse et al., 2000; Kurimoto et al., 2008).

Can these EEG topographic markers be translated to preclinical AD research in rodents? A logical premise for the back-translation of EEG topographic markers from human to rodents is the existence of common neurophysiological mechanisms. Active brain state was associated with high cholinergic activity and hippocampal theta (6–9 Hz) rhythms both in humans and rodents (Buzsáki et al., 2003; Moruzzi and Magoun, 1949; Vanderwolf, 1969; Zhang et al., 2010). In both species, alertness was associated with enhanced power of low-voltage fast frequencies in EEG rhythms (i.e., beta rhythms spanning about 14–30 Hz), whereas nonrapid eye movement sleep and drowsiness were characterized by the enhanced power of high-voltage slow frequencies in EEG rhythms (i.e., delta and theta rhythms spanning about 1–7 Hz; Marshall and Born, 2002; Vyazovskiy et al., 2005). Anxiety has been shown to increase the power of low-voltage high frequencies in the resting-state EEG rhythms in both humans and rodents (Oathes et al., 2008; Sviderskaia et al., 2001). Finally, cholinergic and monoaminergic drugs caused similar effects on spontaneous ongoing EEG rhythms in humans and rodents (Coenen and Van Luijtelaar, 2003; Dimpfel, 2005; Dimpfel et al., 1992).

A limitation of the mentioned EEG studies in rodent models is that across prolonged EEG recordings, spontaneous ongoing EEG rhythms included several behavioral states of the animals. These studies are characterized by the continuous EEG recording for long periods (several days), including active mode (i.e., gross movements, exploratory movements, or locomotor activity), awake passive mode (immobility or small movements of trunk, head, and forelimbs), sleep, and instinctual activity (i.e., drinking, eating, mating, and so forth). Extended EEG recording of this experimental procedure (i.e., tens of hours) presents another disadvantage. It is quite different with respect to the EEG recording in the typical clinical setting in humans. In that setting, EEG recording lasts few minutes in humans in a relaxed wakefulness. This limitation was dealt with in the IMI PharmaCog project, a European academia-industry partnership (Innovative Medicine Initiative, http://www.imi.europa.eu/content/pharma-cog). As a solution to this problem, we identified 2 convenient and translational conditions of EEG recordings for mice. The "passive" condition was defined as a mode of relaxed wakefulness with no or minimal animal movements in the cage (no sleep), whereas, the "active" condition was defined as a mode characterized by spontaneous exploratory movements in the cage. In principle, this methodology is cost-effective and time-saving. If validated, it will imply relatively short EEG recordings for few hours to collect few minutes of artifact-free data for any animal behavioral condition of interest. In the present exploratory study, standard EEG recordings and 2 behavioral conditions were used to test the hypothesis that on-going EEG rhythms reflect changes in cortical arousal and vigilance in freely behaving mice, and are sensitive to aging stages.

Table 1
Features of the C57 mice (for the sake of simplicity, wild type [WT]) for the following electroencephalographic (EEG) recording centers: Janssen Research and Development (Belgium), H. Lundbeck A/S (Denmark), Mario Negri Institute (MNI, Italy), and University of Verona (UNIVR, Italy)

Center	N	Sex (F/M)	Age
Janssen	12	5F/7M	12 mo
Lundbeck	34	14F/20M	4.5, 15, and 24 mo
MNI	23	23M	6, 12, 14, and 24 mo
UNIVR	16	16M	5, 12, and 20 mo

Key: F, female; M, male.

2. Methods

2.1. Animals

Eighty-five (19 female; range of age: 4.5–24 months) wild-type (WT) (C57BL6) mice were used in the present study. WT mice were subdivided in 3 groups: young (N = 25; age: 4.5–6 months), middle-aged (N = 37; 19 female; age: 12–15 months), and old (N = 23; age: 20–24 months) mice. The data were collected from 1 Belgian center (Janssen Research and Development, Pharmaceutical Companies of J&J), 1 Danish center (H. Lundbeck A/S), and 2 Italian centers (Mario Negri Institute for Pharmacological Research of Milan, MNI; University of Verona, UNIVR). Table 1 reports the amount, age, and sex of the WT mice for each center. Procedures involving mice and their care were conducted in line with the institutions' guidelines that were in strict conformity with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; U.S. National Research Council, 1996, Guide for the Care and Use of Laboratory Animals). The respect of these guidelines was carefully controlled by the members of the IMI PharmaCog project, devoted to ethics of research.

2.2. Presurgery phase (3 weeks)

For 3 weeks before surgery, mice were acclimated to the respective institution for habituation of light switched on–off. Mice were housed at a constant temperature (18 °C–22 °C) and relative

humidity (55%–65%). They were maintained in a standard 12-hour light–dark cycle (light hemi-cycle typically spanning from 6 AM to 6 PM) with free access to food and water. Gentle handling for about 5–10 minutes a day was applied to reduce the general stress. Such stress was evaluated continuously along all the duration of the experiments by veterinary experts of each center. These experts evaluated standard behavioral and physiological indices such as animal muscle relaxation, abnormal respiration, grooming and hair coat (piloerection or greasy, possibly reflecting reduced grooming), motor postures (hunching or cowering in the corner of the cage, lying on one's side, lack of movement with loss of muscle tone), absence of alertness or quiescence (inattention to ongoing stimuli), changes in body weight, preservation of regular drinking and eating activities, presence of vomit, and intense or frequent vocalizations.

2.3. Surgery

The mice were anaesthetized (i.e., inhalation of isoflurane 5% or equithesin; 1% pentobarbital + 4% chloral hydrate, 3.5 mL/kg) and treated with the systemic analgesics and antibiotics (surgical care according to local guidelines). Stainless steel or nichrome-insulated monopolar depth electrodes were used for electrophysiological recordings. A reference electrode was placed in the cerebellum. A ground electrode was put in the left temporal bone. EEG electrodes were implanted in the frontal and parietal cortex. These electrodes were wired with a multipin socket. Alternatively, wireless transmitters were used. The animals were placed inside the cage and connected to a recording apparatus either through a swivel allowing animals to move freely or through a wireless device. Table 2 reports stereotaxic coordinates in a standard brain atlas for the electrode implantation (The Mouse Brain coordinates by Franklin and Paxinos, 1997).

2.4. Quiet postsurgery period (1 week)

After the surgery, the animals were single-housed for a continuous period of at least 2 weeks until the experimental day, at the same temperature and humidity conditions. The period of 2 weeks elapsing between the surgery and experimental day was selected by the veterinary and ethology experts of each preclinical recording center preliminarily to the beginning of the present study. The experts also evaluated whether animals showed unnatural behavior, abnormal anxiety or stress, and symptoms of illness (by the means of the standard behavioral and physiological indices adopted by each center) during the 2-week elapsing between the surgery and experimental day. Typical cage size of the single-house was 45 cm (length) × 24 cm (width) × 20 cm (height). Light intensity was 90–110 lx in the room, 60 lx in the cage during the light period, and less than 1 lx during the dark period. The mice were treated with the systemic analgesics and antibiotics, during a standard postsurgical period of 1 week. The week immediately after the surgery, animals underwent a period of recovery with no handling treatment nor EEG recordings.

2.5. Handling postsurgery period (1 week)

In the week after the quiet postsurgery period, the mice received no EEG recordings. Gentle handling for about 5–10 minutes a day was applied to reduce the general stress induced by the housing and experimenters. Such stress was controlled by the evaluation of the animal muscle relaxation and standard behavioral indices of stress in freely behaving mice. Furthermore, the animals were gently plugged and unplugged several times (for wired systems only) to familiarize with the procedure of EEG recording and reduce the global stress.

Table 2
Stereotaxic coordinates for the implantation of EEG electrodes in the mouse brain according to a standard atlas

Electrode	Stereotaxic coordinates
Reference	AP:−6, ML:+2
Ground	AP:−2, ML:+2.5
Frontal	AP:+2.8, ML:−0.5
Parietal	AP:−2, ML:+2

AP, anterior-posterior; ML, mid-line. The Mouse Brain coordinates by Franklin and Paxinos, 1997.

2.6. Experimental day

Experiments were performed during both the dark and light phases. During the EEG recording period, the mice received no handling treatment. EEG recordings started after the second hour of the beginning of the light (dark) period. Recording sampling frequency was at least 250 Hz with anti-aliasing bandpass analog filters (Janssen: 250 Hz, Lundbeck: 1000 Hz, MNI: 1600 Hz, and UNIVR: 500 Hz; 0.16–100 Hz passband filter). No notch filter was used.

Table 3 summarizes the time flow of the treatments and procedures adopted in the experimental sessions. Days are referred to the surgical event.

2.7. Determination of the behavioral mode of the mice

An important step of the data analysis procedure was the classification of the behavioral mode of the animal during the EEG recordings in terms of the mentioned passive condition and active condition. Specifically, 2 experimenters of the recording center used visual inspection (i.e., video of the animal), instrumental markers of the movement (actigraphy and so forth), and/or EMG activity to classify recording epochs lasting 10 seconds into behavioral classes. The discrepancy between the 2 raters was less than 5% of the total classified epochs in a control test with 2 raters (Angelisa Frasca and Susanna Lopez). The recording epochs with different behavioral classification from the 2 raters were not considered in the subsequent spectral and statistical analyses. Of note, the low value of the discrepancy between the 2 raters was because the procedure for the behavioral classification was accurately established before the beginning of the experimental phase within the PharmaCog Consortium. The behavioral classes were as follows:

1 Active condition: Each epoch of the active condition showed overt exploratory movements for most of the period. These movements were characterized by ample displacements of

Table 3
Time flow of the experimental procedures (including EEG recordings) before and after the surgery

Time flow of the treatments and procedure before and after the surgery		
Period	Days	Treatments and procedures
Presurgery	−21 to −1	✓Habituation to light switched on–off ✓Gentle handling for about 5–10 min a day
Surgery	0	✓Anesthetic procedure ✓Therapy with systemic analgesics and antibiotics ✓Electrode placement
Quiet postsurgery	+1 to +7	✓Therapy with systemic analgesics and antibiotics ✓No gentle handling ✓No EEG experiment
Postsurgery	+8 to +14	✓Facilitating the adaptation by plugging and unplugging several times the animal ✓Gentle handling for about 5–10 min a day ✓No EEG experiment
Experimental day	From +15	✓No gentle handling ✓EEG recording

the trunk, head, and/or forelimbs. They should not be confounded with movements associated with instinctual activities (vide infra).

2 Passive condition: Each epoch of the passive condition showed a substantial immobility of the animals for most of the period (no sleep). This condition could include small movements of the trunk, head, and/or forelimbs. Noteworthy, the experimenter did not consider the epochs in which animals stayed continuously immobile for 20 seconds or more as a passive condition. This was to avoid the risk of "passive condition" being misclassified during a period in which the animal was sleeping.

3 Sleep state: Each epoch of the sleep state showed immobility of the animals for the whole period (no sleep). Furthermore, the epoch should be part of longer periods of immobility lasting several minutes with signs of muscle relaxation. As previously mentioned, a particular attention was devoted to avoiding the misclassification of the passive condition and sleep state.

4 State of instinctual activities: Each epoch of the state of instinctual activities showed movements such as cleaning, drinking, eating, mating, and so forth for most of the period. As previously mentioned, a particular attention was devoted to avoiding the misclassification of this state and active condition.

5 Undefined: Each epoch classified as undefined showed a mix of the other behavioral classes or lack of clarity about the behavioral situation of the animal.

Noteworthy, the experiment did not use EEG data to classify the epochs to avoid circular logic. Based on the analysis of the behavioral states, the first 5 minutes of artifact-free EEG epochs classified as active condition were selected for the EEG data analysis. The same procedure of selection was followed by the epochs of the passive condition. The final data analysis was performed on the EEG epochs of the dark phase of the wake-sleep cycle in which the distinction between the passive condition and sleep state was more reliable than in the light phase was. The advantage of this option was not surprising as mice are nocturnal animals and showed few drowsiness or sleep periods in the dark phase.

2.8. EEG data analysis

The behavioral epochs of the active and passive state were segmented off-line in consecutive epochs lasting 2 seconds each. The 2-second EEG epochs with muscular, EEG, electrocardiographic, instrumental, or other artifacts were detected by 2 independent experimenters of the center for the centralized EEG data analysis and were discarded. As previously mentioned, EEG data were recorded by a monopolar montage with 2 exploring electrodes implanted in the frontal and parietal cortex and a reference electrode placed in the cerebellum. To reduce the head volume conductor effects, we re-referenced the EEG data to a frontoparietal bipolar channel. To this aim, we subtracted the EEG signal recorded at the parietal electrode from that recorded at the frontal electrode. The subsequent spectral analysis was performed on EEG epochs re-referenced to that frontoparietal bipolar montage.

2.9. Spectral analysis of the EEG data

The artifact-free EEG epochs of the active and passive state were used as an input for the analysis of EEG power (density). This analysis was performed by a standard (Matlab; MathWorks, Natick, MA, USA) FFT algorithm using Welch technique and Hanning windowing function with 1-Hz frequency resolution. A normalization of the results of FFT analysis was obtained by computing the ratio between EEG power at each frequency bin with the EEG power value averaged across all frequency bins (0–100 Hz). After this normalization, the EEG power lost the original physical dimension and was represented by an arbitrary unit scale. According to this scale, the value of "1" was equal to the power value averaged across all frequency bins. The following EEG frequency bands were selected for the statistical comparisons: 1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz. These narrow bands were selected to avoid any a priori assumption on the composition of EEG frequency bands in mice. In the same line, sharing of a frequency bin by 2 contiguous frequency bands is a widely accepted procedure to avoid any assumption about the physiological distinction of 2 contiguous ones.

2.10. Statistical analysis

Two sessions of statistical analysis were performed by Statistical 10.0 package to test the primary hypotheses of the present study. The first session tested whether the WT mice as a whole group showed differences in EEG power between active and passive conditions, thus reflecting changes in cortical arousal and vigilance. To test this hypothesis, an analysis of variance (ANOVA) used the normalized EEG power as a dependent variable. The ANOVA factors were condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The center recording the EEG activity was used as a covariate. The hypothesis would be confirmed by the following 2 statistical results: (1) a statistical main effect of the factor condition or a statistical interaction effect between the factors condition and band ($p < 0.05$); and (2) a post hoc test indicating statistically significant differences in the normalized EEG power between the active and the passive condition (Duncan test, $p < 0.05$, 2-tailed).

The second session examined whether the WT mouse groups showed differences in EEG power, thus reflecting changes due to the effect of aging. To test this hypothesis, we computed the difference in the normalized EEG power between the active and the passive condition (active minus passive). An ANOVA used this difference as a dependent variable. The ANOVA factors were group (young, middle-aged, old; independent variable) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The center recording the EEG activity was used as a covariate. The hypothesis would be confirmed by the following 2 statistical results: (1) a statistical main effect of the factor group or a statistical interaction effect between the factors group and band ($p < 0.05$); and (2) a post hoc test indicating statistically significant differences in the normalized EEG power (active minus passive) in the old group with respect to the others (Duncan test, $p < 0.05$, 2-tailed). In this statistical session, only male mice were considered ($N = 66$; 25 young, 18 middle-aged, and 23 old), to avoid the confounding effects of the sex.

The following other 2 sessions of statistical analysis tested (secondary) control hypotheses.

A third statistical session tested the effect of sex on the previously mentioned spectral EEG marker. In this statistical session, the variable age was paired including only 37 middle-aged mice (i.e., 19 females and 18 males). Indeed, this age group had a sufficient amount of mice to perform the statistical comparison of interest. To test this hypothesis, an ANOVA used the difference of the normalized EEG power (active minus passive) as a dependent variable. The ANOVA factors were group (female, male; independent variable) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The center recording the EEG activity was used as a covariate. The hypothesis would be confirmed by the following 2 statistical results: (1) a statistical main effect of the factor group or a statistical interaction effect between the factors group and band ($p < 0.05$); and (2) a post hoc test indicating

statistically significant differences in the normalized EEG power (active minus passive) in the female group with respect to the male group (Duncan test, $p < 0.05$, 2-tailed).

A fourth statistical session tested the sensitivity of the previously mentioned spectral EEG marker to the passive and active conditions in the 4 recording centers separately (i.e., Janssen, Lundbeck, MNI, and UNIVR). To test this hypothesis, 4 ANOVAs (1 for any recording center) used the normalized EEG power as a dependent variable. The ANOVA factors were condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). For any ANOVA, the hypothesis would be confirmed by the following 2 statistical results: (1) a statistical main effect of the factor condition or a statistical interaction effect between the factors condition and band ($p < 0.05$); and (2) a post hoc test indicating statistically significant differences in the normalized EEG power between the active and the passive condition (Duncan test, $p < 0.05$, 2-tailed).

3. Results

3.1. Normalized EEG power during active and passive conditions

Fig. 1 (top) shows the grand average (N = 85) of the normalized EEG power spectra for the active and passive conditions in all WT mice as a whole group. These spectra showed an EEG power peak at 2–4 Hz (i.e., delta range) that was higher in the passive condition compared with the active one. Furthermore, there was another EEG power peak at 6–8 Hz (i.e., theta range) that was higher in the active condition compared with the passive one. In the figure, the difference in the EEG power between the active and passive conditions (i.e., active minus passive) is also reported. It is observed a negative peak of the EEG power difference at 2–4 Hz, reflecting the maximum EEG power peak in the passive condition. Furthermore, there was a positive peak of the EEG power difference at 6–8 Hz, reflecting the maximum EEG power peak in the active condition.

Fig. 1 (bottom) reports the individual values of the normalized EEG power for all WT mice. The values refer to the 2 behavioral conditions (i.e., active, passive) and 8 frequency bands (i.e., 1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The distributions of these individual values showed no remarkable outliers. Fig. 1 (bottom) also illustrates the results of a statistically significant ANOVA interaction ($F[7, 581] = 13.39$, $p = 0.0001$) between the factors condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). All WT mice were considered as a whole group. Duncan-planned post hoc testing showed that the EEG power was significantly higher in the passive compared with the active condition at 1–2 Hz ($p = 0.000004$), 2–4 Hz ($p = 0.000005$), and 4–6 Hz ($p = 0.000005$). In contrast, this power was significantly higher in the active compared with the passive condition at 6–8 Hz ($p = 0.00001$) and 8–10 Hz ($p = 0.000004$). The present results showed statistically significant differences in the EEG power between the passive and active conditions in the WT mice as a whole group ($p < 0.05$).

3.2. Effect of age on the normalized EEG power

Fig. 2 (top) shows the grand average of the difference of the normalized EEG power spectra between the active and passive conditions (i.e., active minus passive) in the 3 mouse groups classified based on the age (i.e., the young, middle-aged, and old ones). Of note, the group of the old mice exhibited a dominant negative peak of the EEG power difference at 1–2 Hz.

Fig. 2 (bottom) shows the mean values (±standard error) of the difference in the normalized EEG power between the active and passive conditions (i.e., active minus passive) illustrating a statistically significant ANOVA interaction ($F[14, 434] = 2.034$, $p = 0.014$) between the factors group (young, middle-aged, and old) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). Duncan-planned post hoc testing unveiled the specific statistical differences among the groups of male mice. Compared to the young and middle-aged mice, the old mice were characterized by a dominant EEG power difference (i.e., active minus passive) at 1–2 Hz in the low-frequency delta band ($p < 0.05$). Also, the old mice showed a dominant EEG power difference at 6–8 Hz ($p < 0.05$), whereas the other groups displayed this effect at 8–10 Hz ($p > 0.05$).

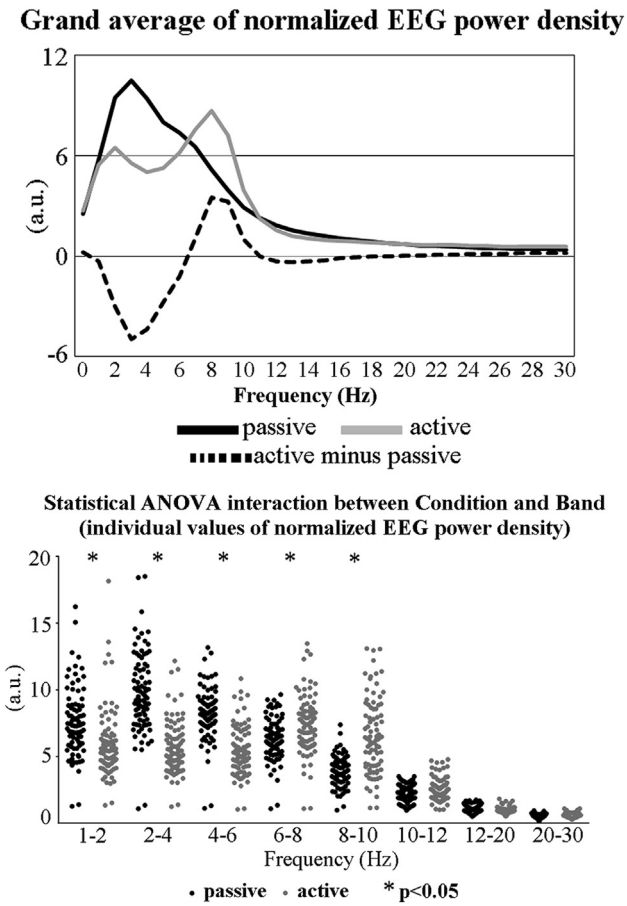

Fig. 1. (Top): Grand average of the normalized electroencephalographic (EEG) power density spectra relative to a bipolar cortical frontoparietal channel in 85 C57 adult mice (for the sake of simplicity wild type [WT]). The EEG power density spectra range between 0 and 30 Hz. The EEG recordings were performed in a passive (i.e., awake quiet wakefulness with immobility or small movements) or active (i.e., exploratory movements) condition. These recordings refer to the dark phase of the wake-sleep cycle (i.e., the phase of animal activity). In the figure, the difference in the EEG power density between the active and passive conditions (active minus passive) is also reported. (Bottom): Individual values of the normalized EEG power density for all WT mice for the 2 conditions (active, passive) and the 8 frequency bands (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). A statistically significant analysis of variance interaction ($F[7, 581] = 13.39$, $p = 0.0001$) between the factors condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz) was found. The asterisks indicate the EEG frequency bands at which the normalized EEG power density presented statistically significant differences between the passive and active conditions (Duncan post hoc testing, $p < 0.05$).

3.3. Effect of sex on the normalized EEG power

Fig. 3 (top) shows the grand average of the difference in the normalized EEG power spectra between the active and passive

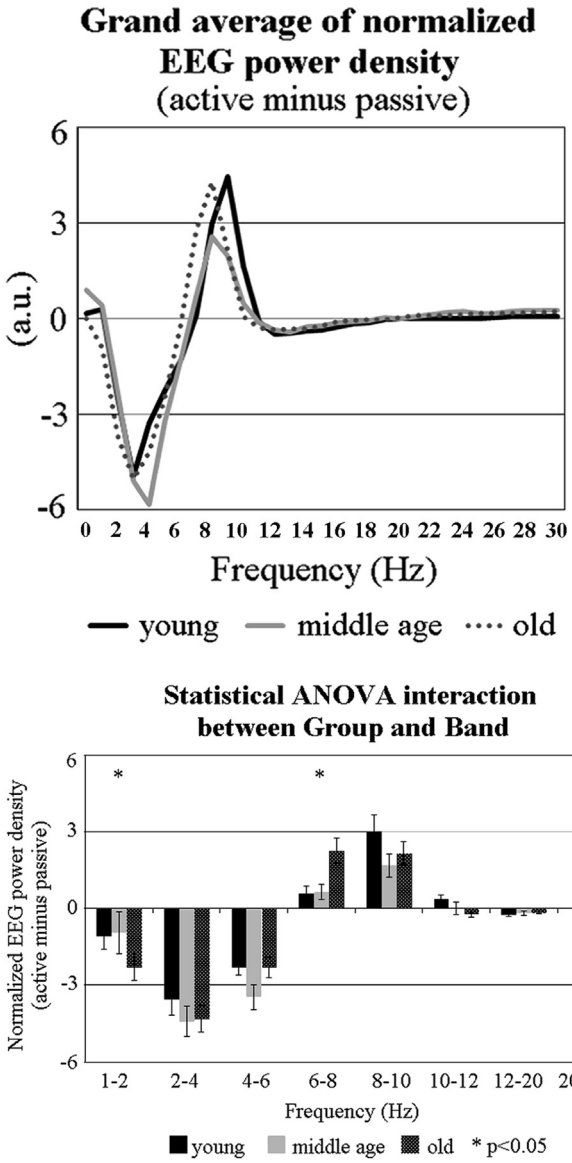

Fig. 2. (Top): Grand average of the difference of the normalized EEG power density spectra between the active and passive conditions (active minus passive) obtained averaging the spectral values in the young (N = 25), middle-aged (N = 18), and old (N = 23) male wild-type (WT) mice considered separately. The normalized EEG power density (active minus passive) refers to the frequency range between 0 and 30 Hz. (Bottom): Mean values (±standard error) of the difference of the normalized EEG power density between the active and passive conditions (active minus passive) illustrating a statistically significant analysis of variance interaction (F[14, 434] = 2.034, $p = 0.014$) between the factors group (young, middle-aged, and old) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The asterisks indicate the EEG frequency bands at which this difference of the normalized EEG power density presented statistically significant differences between the old mice compared with the young and middle-aged male mice (Duncan post hoc testing, $p < 0.05$).

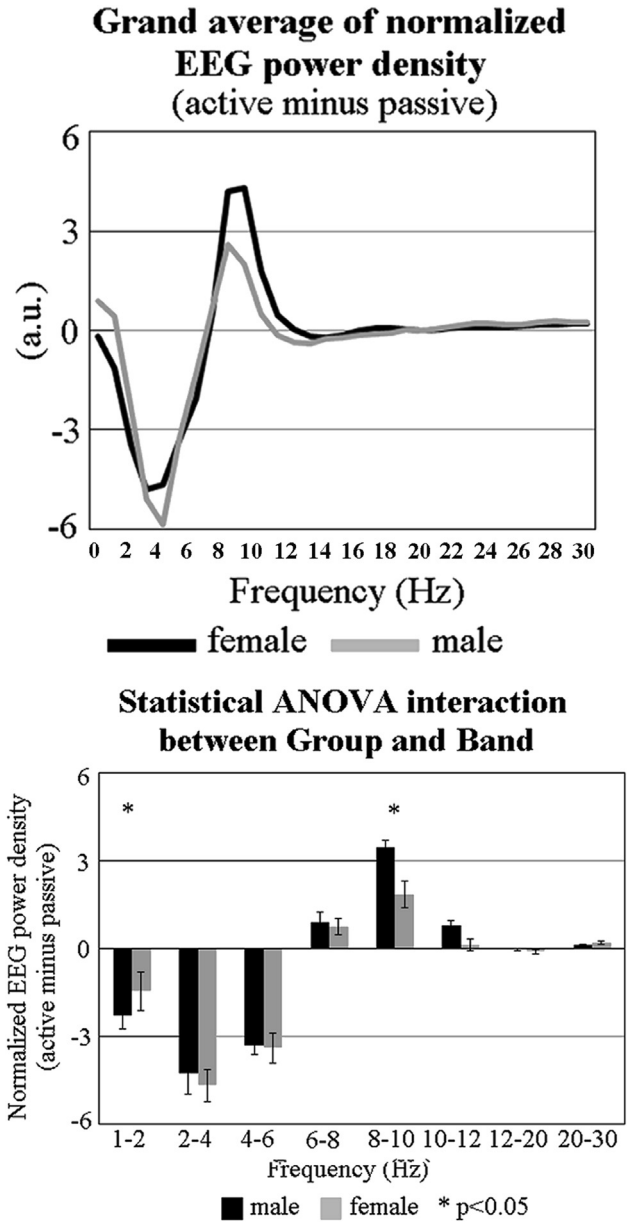

Fig. 3. (Top): Grand average of the difference of the normalized EEG power density spectra between the active and passive conditions (active minus passive) obtained averaging the spectral values of the female (N = 19) and male (N = 18) middle-aged wild-type (WT) mice. This difference of the normalized EEG power (active minus passive) refers to the frequency range between 0 and 30 Hz. (Bottom): Mean values (±standard error) of the difference of the normalized EEG power density between the active and passive conditions (active minus passive) illustrating a statistically significant analysis of variance interaction (F[7, 238] = 2.386, $p = 0.022$) between the factors group (male, female) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). The asterisks indicate the EEG frequency bands at which the difference of the normalized EEG power density (active minus passive) presented statistically significant differences between the female and male mice (Duncan post hoc testing, $p < 0.05$).

conditions (i.e., active minus passive) in a subgroup of female mice and in a subgroup of male mice. Compared with the male mice, the female mice exhibited the greatest negative values of the EEG power difference in the delta range (i.e., 1–2 Hz). Also, these female mice showed the greatest positive values of the EEG power difference in the theta and alpha range (i.e., 8–10 Hz).

Fig. 3 (bottom) illustrates the mean values (±standard error) of the difference in the normalized EEG power between the active and passive conditions (i.e., active minus passive) illustrating a statistically significant ANOVA interaction (F[7, 238] = 2.386, $p = 0.022$) between the factors group (male, female) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). Duncan-planned post hoc testing unveiled the specific statistical differences among these mouse groups. Compared with the male mice, the female mice showed a dominant EEG power difference (i.e., active minus passive) at 1–2 Hz in the low-frequency delta band ($p = 0.02$). Compared with the male mice, the female mice also showed a dominant EEG power difference at

8–10 Hz ($p = 0.002$). These results suggest a general greater EEG reactivity in the female mice compared with the male mice in both passive and active conditions.

3.4. Reliability of the spectral EEG markers among the recording centers

Fig. 4 (top) shows the grand average of the normalized EEG power spectra for the active and passive conditions in the WT mice of any recording center considered separately (i.e., Janssen, Lundbeck, MNI, and UNIVR). In all recording centers, these spectra showed a clear EEG power peak at 2–4 Hz (i.e., delta range) that was higher in the passive condition compared with the active one. Furthermore, there was another clear EEG power peak at 6–8 Hz (i.e., theta range) that was higher in the active condition compared with the passive one in Janssen, Lundbeck, and MNI recording centers. On the contrary, this peak at 6–8 Hz was slight in UNIVR recording center. In the figure, the difference in the EEG power between the active and passive conditions (i.e., active minus passive) is also reported for all recording centers. As in the grand average of all WT mice as a whole group, a negative peak of the EEG power difference at 2–4 Hz reflected the maximum EEG power peak in the passive condition in all recording centers. In all recording centers but one (i.e., UNIVR), there was also a clear positive peak of the EEG power difference at 6–8 Hz, reflecting the maximum EEG power peak in the active condition.

Fig. 4 (bottom) shows the mean values (±standard error) of the difference in the normalized EEG power between the active and passive conditions (i.e., active minus passive) illustrating the results of the 4 ANOVAs, 1 for any recording center considered separately (i.e., Janssen, Lundbeck, MNI, and UNIVR). In all ANOVAs, there was a statistically significant ANOVA interaction ($p < 0.00001$) between the factors condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). Duncan-planned post hoc testing confirmed in all the single ANOVAs the statistical differences among the passive and active conditions observed in the main analysis in all WT mice considered as a whole group. Compared with the passive condition, the active condition showed a statistically significant dominant negative EEG power difference (i.e., active minus passive) at 1–2 Hz and 2–4 Hz in all recording centers ($p < 0.005$). Also, the active condition showed a statistically significant dominant positive EEG power difference at 6–8 Hz in all recording centers but UNIVR ($p < 0.05$) and the 8–10 Hz in all recording centers ($p < 0.01$). More details on the results of this statistical session are reported in Table 4. The reduced differences of the EEG power density between the behavioral active and passive states in UNIVR mice, mainly due to a very slight increase of theta rhythms during the behavioral active state of the UNIVR mice, was probably due to the relative high amount of the old mice (20-month old) of UNIVR recording center (7 old mice for a total amount of 16). As the on-going cortical EEG rhythms differed across aging (see Fig. 2), the averaging between the young, middle-age, and old mice may have caused the reduced theta increase during the behavioral active state in the UNIVR mice.

4. Discussion

In humans, resting state EEG rhythms reflect the fluctuation of cortical arousal and vigilance in a typical clinical recording setting, namely the EEG recordings for few minutes of subjects in the state of eyes closed (i.e., passive condition) and eyes open (i.e., active condition). The higher the cortical EEG power at a given frequency, the higher is the synchronization of cortical pyramidal neurons at that frequency (Pfurtscheller and Lopes da Silva, 1999). Can this basic procedure be back-translated to C57BL6 (WT) mice for aging studies, this strain being the genetic basis for the mouse mutants modeling AD processes?

Here, we report that the WT mice showed substantial differences in the EEG power between passive and active conditions mimicking those of the mentioned clinical setting for humans. Compared with the passive condition, the active condition exhibited a decrease of the EEG power at 1–2 Hz in the so-called delta range. Also, there was an increase in the EEG power at 6–10 Hz in the so-called extended theta range. This difference was more pronounced in female than male mice.

4.1. On-going cortical EEG rhythms in WT mice differ between the passive and active conditions

The present results indicate that theta power of on-going EEG rhythms is sensitive to an increased cortical arousal and vigilance in WT mice during exploratory movements. These findings lead support to previous evidence showing that on-going theta rhythms (6–9 Hz) were correlated with the amount of motor activity in mice and rats (Buzsáki et al., 2003; Kelemen et al., 2005; Pickenhain and Klingberg, 1967; Vanderwolf, 1969). Furthermore, these rhythms increased in power in awake states associated with attentive or motor activities in rats (Maloney et al., 1997). In the same vein, amphetamine did induce both increased theta power and hyperlocomotion in rats (Páleníček et al., 2013).

The present results also suggest that delta power of on-going EEG rhythms reflects low cortical arousal and vigilance in WT mice in awake quiet wakefulness. These findings challenge the traditional view that on-going delta rhythms are negligible in awake (healthy) rodents, and primates, whereas they are dominant in nonrapid eye movement stages of the sleep (Crunelli et al., 2015; Lörincz et al., 2009a, 2009b; Steriade, 1993, 2000, 2003; Steriade and Amzica, 1998; Steriade et al., 1993). In this regard, the present results extend the following pieces of previous evidence challenging that traditional view. In rodents, a state of quiet wakefulness (i.e., similar to the current passive condition) induced dominant low-frequency (<5 Hz) and large voltage fluctuations in the membrane potential of cortical neurons and in cortical EEG and local field potentials (Crochet and Petersen, 2006; Petersen et al., 2003; Timofeev et al., 2000; Vyazovskiy et al., 2011; Zagha et al., 2013). These fluctuations interacted with incoming signals from visual, auditory, and somatosensory cortex in behaving animals (Bennett et al., 2013; Haider et al., 2013; Hromádka et al., 2013; Okun et al., 2010; Polack et al., 2013; Zhou et al., 2014). Furthermore, cortical delta rhythms exhibited increased power when rats were in quiet wakefulness with respect to states associated with attentive or motor activities (Maloney et al., 1997). Moreover, on-going delta rhythms and low-frequency/large voltage fluctuations were reported in membrane potentials, multiunit activity, and local field potentials in the cerebral cortex of awake nonhuman primates (Lakatos et al., 2005, 2008; Tan et al., 2014). In human primates, widespread on-going delta rhythms were recorded from the scalp in relation to physiological sleep (Simon and Emmons, 1956), consciousness disorders (Simon and Emmons, 1956), and pathological aging with cognitive impairment (Babiloni et al., 2007, 2009, 2014, 2016). In awake epilepsy patients, intracerebral EEG recordings showed ample on-going delta rhythms in circumscribed regions of the intact cerebral cortex during quiet wakefulness (Sachdev et al., 2015).

4.2. On-going cortical EEG rhythms in WT mice differ across aging

In the present study, the population of WT male mice was subdivided into 3 groups based on age: young (25 mice of

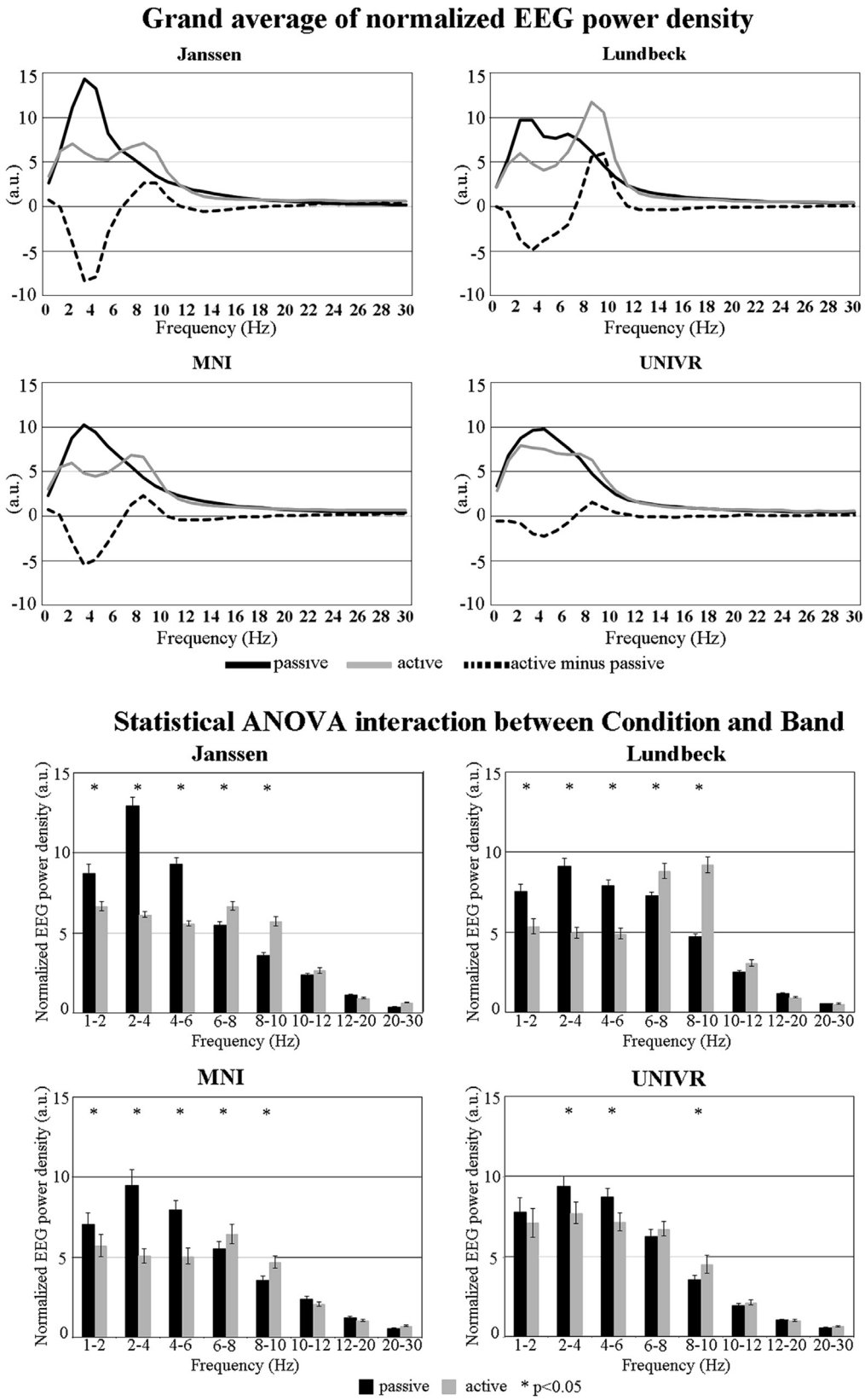

Fig. 4. (Top): Grand average of the normalized EEG power density spectra for the 4 recording centers considered separately. Specifically, these centers were the following: Janssen Research and Development (Belgium), H. Lundbeck A/S (Denmark), Mario Negri Institute (MNI, Italy), and University of Verona (UNIVR, Italy). The EEG power density spectra range between 0 and 30 Hz for the active and passive state. The difference between the active and the passive state (active minus passive) is also reported. (Bottom): Mean values (±standard error) of the normalized EEG power density illustrating a statistically significant analysis of variance interaction effect (Janssen: $F[7, 77] = 32.28$, $p = 0.00001$; Lundbeck: $F[7, 231] = 88.65$; MNI: $F[7, 154] = 21.64$, $p = 0.00001$; UNIVR: $F[7, 105] = 5.80$, $p = 0.00001$) between the factors condition (passive, active) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz). Asterisks indicate the EEG bands at which the normalized EEG power density presented statistically significant differences between the passive and active conditions (Duncan post hoc testing, $p < 0.05$).

Table 4
Results of the statistically significant interaction ($p < 0.05$) between the factors condition (i.e., passive, active; independent variable) and band (1–2 Hz, 2–4 Hz, 4–6 Hz, 6–8 Hz, 8–10 Hz, 10–12 Hz, 12–20 Hz, and 20–30 Hz) of 4 ANOVAs using EEG power (density) as a dependent variable

Center	ANOVA interaction between the factors condition and band Duncan post hoc results
Janssen	$F(7, 77) = 32.28$, $p = 0.00001$ 1–2 Hz ($p = 0.0003$), 2–4 Hz ($p = 0.00002$), 4–6 Hz ($p = 0.00002$), 6–8 Hz ($p = 0.04$), and 8–10 Hz ($p = 0.0002$)
Lundbeck	$F(7, 231) = 88.65$, $p = 0.00001$ 1–2 Hz ($p = 0.00001$), 2–4 Hz ($p = 0.00004$), 4–6 Hz ($p = 0.00004$), 6–8 Hz ($p = 0.00003$), and 8–10 Hz ($p = 0.00001$)
MNI	$F(7, 154) = 21.64$, $p = 0.00001$ 1–2 Hz ($p = 0.002$), 2–4 Hz ($p = 0.00004$), 4–6 Hz ($p = 0.00004$), 6–8 Hz ($p = 0.03$), and 8–10 Hz ($p = 0.005$)
UNIVR	$F(7, 105) = 5.80$, $p = 0.00001$ 2–4 Hz ($p = 0.00001$), 4–6 Hz ($p = 0.00003$), and 8–10 Hz ($p = 0.01$)

ANOVA-dependent variable was the normalized EEG power density. The ANOVAs refer to the following 4 EEG recording centers: Janssen Research and Development (Belgium), H. Lundbeck A/S (Denmark), Mario Negri Institute (MNI, Italy), and University of Verona (UNIVR, Italy). In the table, the results of a post hoc Duncan testing are also reported ($p < 0.05$).
Key: ANOVA, analysis of variance.

4.5–6 months), middle-aged (37 mice of 12–15 months), and old (23 mice of 20–24 months). EEG results showed some peculiar features in the former group. In the passive condition, EEG power in the delta band (i.e., 1–2 Hz) was higher in the old group than that in the young and middle-aged groups, whereas the EEG power peak in the active condition was slower as frequency in the Nold group (i.e., 6–8 Hz) than that in the other groups (i.e., 8–10 Hz). These results suggest a general "slowing" of the delta and theta rhythms in the old mice. In this line, they extend previous evidence showing a slowing of the frequency peak of on-going theta rhythms in awake C57 mice along physiological aging (Wimmer et al., 2013). In that previous study, there was no distinction of behavioral passive and active states during wakefulness, so it can be hypothesized that the overlapping of these states hides the aging effects on delta band.

4.3. Translational value of the present results

What is the translational value of the present results for the research on both physiological and pathological aging?

First, the present study unveiled the interspecies differences of on-going EEG rhythms in wakefulness between WT mice and humans. It is well known that in awake healthy humans, alpha rhythms (8–12 Hz) dominate in posterior areas of cerebral cortex in relaxed wakefulness, as a reflection of low cortical arousal and vigilance (Klimesch, 1999; Klimesch et al., 1997; Pfurtscheller and Klimesch, 1992). Power of these rhythms is dramatically reduced during perceptual, memory, and motor demands, as a reflection of increased cortical arousal and vigilance (Babiloni et al., 1999; Klimesch, 1999; Klimesch et al., 1997; Pfurtscheller and Klimesch, 1992; Sergeant et al., 1987; Van Winsum et al., 1984). The same dynamic of on-going alpha rhythms is reproduced in a convenient clinical setting as the conditions of resting state eyes closed (i.e., passive condition) and eyes open (i.e., open condition), lasting few minutes each (Babiloni et al., 2010). In the present study, the pattern of the EEG activity was quite different in WT mice. The mice showed neither a power peak in the alpha range (8–12 Hz) during the passive condition nor the reduction of this power peak during the active condition. Rather, changes in the cortical arousal and vigilance were reflected by on-going delta and theta rhythms recorded for few minutes. Despite these interspecies differences, we posit that the present passive and active conditions are a useful translational paradigm for the neurophysiological study of the fluctuation of cortical arousal and vigilance in mice across aging.

Second, this translational paradigm can be used in multicenter studies on physiological aging in mice. Indeed, the present experimental procedures for the classification of the animal behavior and the EEG recordings provided results quite repeatable across 4 qualified recording centers. These procedures were defined in the IMI PharmaCog project (www.pharmacog.org) by researchers coming from academia and the pharmaceutical industry. Therefore, these procedures have incorporated needs and views of both perspectives. Overall, the present study represents the first cross-validation of the mentioned behavioral and EEG procedures in a public-private research network.

Third, the mentioned translational paradigm can be used in multi-centric studies on a mouse model of AD. Indeed, the present spectral EEG markers of cortical arousal in WT mice can been considered a promising back-translation of abnormal spectral EEG markers observed in AD patients placed in resting state condition (Babiloni et al., 2010; Bennys et al., 2001; Bonanni et al., 2008; Claus et al., 1999; Huang et al., 2000; Lehmann et al., 2007; Ommundsen et al., 2011). Compared with groups of normal elderly subjects, groups of AD patients with dementia were characterized by the following EEG markers: (1) higher power of widespread delta (<3 Hz) and theta rhythms (4–7 Hz); (2) lower power of posterior alpha rhythms (8–12 Hz) with a slowing of the alpha peak frequency; (3) lower power of high-frequency beta (14–30 Hz) and gamma (around 40 Hz) rhythms (Adeli et al., 2005; Babiloni et al., 2007, 2009, 2013, 2014, 2016; Dierks et al., 2000; Huang et al., 2000; Jeong, 2004; Ponomareva et al., 2003; Prichep et al., 1994; Wolf et al., 2003); and (4) lower reduction of power of posterior alpha rhythms (Babiloni et al., 2010). In the framework of the PharmaCog project, we evaluated whether the present spectral EEG markers, reflecting changes in cortical arousal, were altered in single and double mutant transgenic mouse model of AD (i.e., PDAPP and TASTPM mice) compared with WT mice. Future studies will report the outcomes of these EEG comparisons as first positive impact of the present study (WT vs. PDAPP mice; WT vs. TASTPM mice).

5. Conclusions

Resting state EEG rhythms reflect the fluctuation of cortical arousal and vigilance in a typical clinical setting in humans, namely the recording of EEG rhythms for few minutes in the states of eyes closed (i.e., passive condition) and eyes open (i.e., active condition). Can this basic procedure be back-translated to WT (C57) mice for aging studies? On-going EEG data were recorded in young (4.5–6 months), middle-aged (12–15 months), and old (20–24 months) mice in passive state (no sleep) and exploratory movements. A few minutes of artifact-free EEG rhythms related to the 2 conditions were considered for the spectral EEG analysis. Compared with the passive condition, the active condition induced a decrease of EEG power at 1–6 Hz and its increase at 6–10 Hz in all mice as a group.

Concerning the aging effects, the passive condition showed higher EEG power at 1–2 Hz in the old group than that in the young and middle-aged groups. Furthermore, the active condition exhibited a maximum EEG power at 6–8 Hz in the former group and 8–10 Hz in the latter groups.

In the present conditions, delta and theta EEG rhythms reflected changes in cortical arousal and vigilance in freely behaving WT mice across aging. These changes resemble the so-called slowing of resting state EEG rhythms observed in humans across physiological and pathological aging.

In perspective, after some further cross-validation, shorter EEG recording sessions could be systematically planned and performed. Furthermore, the selection of the short EEG segments of interest and the determination of animal behavior could be done by semi-automatic procedures. As a result, there will be beneficial effects not only for the translational validity of the new preclinical EEG procedures but also for the experimental costs and the experimenter and animal wellness.

Disclosure statement

The authors have no actual or potential conflicts of interest to declare.

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References

Adeli, H., Ghosh-Dastidar, S., Dadmehr, N., 2005. Alzheimer's disease: models of computation and analysis of EEGs. Clin. EEG Neurosci. 36, 131–140.
Babiloni, C., Carducci, F., Cincotti, F., Rossini, P.M., Neuper, C., Pfurtscheller, G., Babiloni, F., 1999. Human movement-related potentials vs desynchronization of EEG alpha rhythm: a high-resolution EEG study. Neuroimage 10, 658–665.
Babiloni, C., Cassetta, E., Binetti, G., Tombini, M., Del Percio, C., Ferreri, F., Ferri, R., Frisoni, G., Lanuzza, B., Nobili, F., Parisi, L., Rodriguez, G., Frigerio, L., Gurzì, M., Prestia, A., Vernieri, F., Eusebi, F., Rossini, P.M., 2007. Resting EEG sources correlate with attentional span in mild cognitive impairment and Alzheimer's disease. Eur. J. Neurosci. 25, 3742–3757.
Babiloni, C., Del Percio, C., Lizio, R., Infarinato, F., Blin, O., Bartres-Faz, D., Dix, S.L., Bentivoglio, M., Soricelli, A., Bordet, R., Rossini, P.M., Richardson, J.C., 2014. A review of the effects of hypoxia, sleep deprivation and transcranial magnetic stimulation on EEG activity in humans: challenges for drug discovery for Alzheimer's disease. Curr. Alzheimer Res. 11, 501–518.
Babiloni, C., Frisoni, G.B., Pievani, M., Vecchio, F., Lizio, R., Buttiglione, M., Geroldi, C., Fracassi, C., Eusebi, F., Ferri, R., Rossini, P.M., 2009. Hippocampal volume and cortical sources of EEG alpha rhythms in mild cognitive impairment and Alzheimer disease. Neuroimage 44, 123–135.
Babiloni, C., Infarinato, F., Aujard, F., Bastlund, J.F., Bentivoglio, M., Bertini, G., Del Percio, C., Fabene, P.F., Forloni, G., Herrero Ezquerro, M.T., Noè, F.M., Pifferi, F., Ros-Bernal, F., Christensen, D.Z., Dix, S., Richardson, J.C., Lamberty, Y., Drinkenburg, W., Rossini, P.M., 2013. Effects of pharmacological agents, sleep deprivation, hypoxia and transcranial magnetic stimulation on electroencephalographic rhythms in rodents: towards translational challenge models for drug discovery in Alzheimer's disease. Clin. Neurophysiol. 124, 437–451.
Babiloni, C., Lizio, R., Marzano, N., Capotosto, P., Soricelli, A., Triggiani, A.I., Cordone, S., Gesualdo, L., Del Percio, C., 2016. Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. Int. J. Psychophysiol. 103, 88–102.
Babiloni, C., Lizio, R., Vecchio, F., Frisoni, G.B., Pievani, M., Geroldi, C., Claudia, F., Ferri, R., Lanuzza, B., Rossini, P.M., 2010. Reactivity of cortical alpha rhythms to eye opening in mild cognitive impairment and Alzheimer's disease: an EEG study. J. Alzheimers Dis. 22, 1047–1064.
Bastos Leite, A.J., Scheltens, P., Barkhof, F., 2004. Pathological aging of the brain: an overview. Top. Magn. Reson. Imaging 15, 369–389.
Bennett, C., Arroyo, S., Hestrin, S., 2013. Subthreshold mechanisms underlying state-dependent modulation of visual responses. Neuron 80, 350–357.
Bennys, K., Rondouin, G., Vergnes, C., Touchon, J., 2001. Diagnostic value of quantitative EEG in Alzheimer disease. Neurophysiol. Clin. 31, 153–160.
Berendse, H.W., Verbunt, J.P., Scheltens, P., van Dijk, B.W., Jonkman, E.J., 2000. Magnetoencephalographic analysis of cortical activity in Alzheimer's disease: a pilot study. Clin. Neurophysiol 111, 604–612.
Bonanni, L., Thomas, A., Tiraboschi, P., Perfetti, B., Varanese, S., Onofrj, M., 2008. EEG comparisons in early Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease with dementia patients with a 2-year follow-up. Brain 131 (Pt 3), 690–705.
Braak, H., Braak, E., 1995. Staging of Alzheimer's disease-related neurofibrillary changes. Neurobiol. Aging 16, 271–278 discussion 278–84.
Buzsáki, G., Buhl, D.L., Harris, K.D., Csicsvari, J., Czéh, B., Morozov, A., 2003. Hippocampal network patterns of activity in the mouse. Neuroscience 116, 201–211.
Claus, J.J., Strijers, R.L., Jonkman, E.J., Ongerboer de Visser, B.W., Jonker, C., Walstra, G.J., Scheltens, P., van Gool, W.A., 1999. The diagnostic value of electroencephalography in mild senile Alzheimer's disease. Clin. Neurophysiol. 110, 825–832.
Coenen, A.M., Van Luijtelaar, E.L., 2003. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav. Genet. 33, 635–655.
Crochet, S., Petersen, C.C., 2006. Correlating whisker behavior with membrane potential in barrel cortex of awake mice. Nat. Neurosci. 9, 608–610.
Crunelli, V., David, F., Lőrincz, M.L., Hughes, S.W., 2015. The thalamocortical network as a single slow wave-generating unit. Curr. Opin. Neurobiol. 31, 72–80.
Dierks, T., Jelic, V., Pascual-Marqui, R.D., Wahlund, L., Julin, P., Linden, D.E., Maurer, K., Winblad, B., Nordberg, A., 2000. Spatial pattern of cerebral glucose metabolism (PET) correlates with localization of intracerebral EEG-generators in Alzheimer's disease. Clin. Neurophysiol. 111, 1817–1824.
Dimpfel, W., 2005. Pharmacological modulation of cholinergic brain activity and its reflection in special EEG frequency ranges from various brain areas in the freely moving rat (Tele-Stereo-EEG). Eur. Neuropsychopharmacol. 15, 673–682.
Dimpfel, W., Spüler, M., Wessel, K., 1992. Different neuroleptics show common dose and time dependent effects in quantitative field potential analysis in freely moving rats. Psychopharmacology (Berl) 107, 195–202.
Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., DeKosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 13, 614–629.
Förstl, H., Kurz, A., 1999. Clinical features of Alzheimer's disease. Eur. Arch. Psychiatry Clin. Neurosci. 249, 288–290.
Franklin, K.B.J., Paxinos, G., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Glodzik-Sobanska, L., Rusinek, H., Mosconi, L., Li, Y., Zhan, J., de Santi, S., Convit, A., Rich, K., Brys, M., de Leon, M.J., 2005. The role of quantitative structural imaging in the early diagnosis of Alzheimer's disease. Neuroimaging Clin. N. Am. 15, 803–826.
Haider, B., Häusser, M., Carandini, M., 2013. Inhibition dominates sensory responses in the awake cortex. Nature 493, 97–100.
Hromádka, T., Zador, A.M., DeWeese, M.R., 2013. Up states are rare in awake auditory cortex. J. Neurophysiol. 109, 1989–1995.
Huang, C., Wahlund, L., Dierks, T., Julin, P., Winblad, B., Jelic, V., 2000. Discrimination of Alzheimer's disease and mild cognitive impairment by equivalent EEG sources: a cross-sectional and longitudinal study. Clin. Neurophysiol. 111, 1961–1967.
Jeong, J., 2004. EEG dynamics in patients with Alzheimer's disease. Clin. Neurophysiol. 115, 1490–1505.
Kelemen, E., Morón, I., Fenton, A.A., 2005. Is the hippocampal theta rhythm related to cognition in a non-locomotor place recognition task? Hippocampus 15, 472–479.
Klimesch, W., 1999. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Res. Brain Res. Rev. 29, 169–195.
Klimesch, W., Doppelmayr, M., Schimke, H., Ripper, B., 1997. Theta synchronization and alpha desynchronization in a memory task. Psychophysiology 34, 169–176.
Kurimoto, R., Ishii, R., Canuet, L., Ikezawa, K., Azechi, M., Iwase, M., Yoshida, T., Kazui, H., Yoshimine, T., Takeda, M., 2008. Event-related synchronization of alpha activity in early Alzheimer's disease and mild cognitive impairment: an MEG study combining beamformer and group comparison. Neurosci. Lett. 443, 86–89.
Lakatos, P., Karmos, G., Mehta, A.D., Ulbert, I., Schroeder, C.E., 2008. Entrainment of neuronal oscillations as a mechanism of attentional selection. Science 320, 110–113.
Lakatos, P., Shah, A.S., Knuth, K.H., Ulbert, I., Karmos, G., Schroeder, C.E., 2005. An oscillatory hierarchy controlling neuronal excitability and stimulus processing in the auditory cortex. J. Neurophysiol. 94, 1904–1911.
Lehmann, C., Koenig, T., Jelic, V., Prichep, L., John, R.E., Wahlund, L.O., Dodge, Y., Dierks, T., 2007. Application and comparison of classification algorithms for recognition of Alzheimer's disease in electrical brain activity (EEG). J. Neurosci. Methods 161, 342–350.
Lörincz, M.L., Geall, F., Bao, Y., Crunelli, V., Hughes, S.W., 2009a. ATP-dependent infra-slow (<0.1 Hz) oscillations in thalamic networks. PLoS One 4, e4447.
Lörincz, M.L., Kékesi, K.A., Juhász, G., Crunelli, V., Hughes, S.W., 2009b. Temporal framing of thalamic relay-mode firing by phasic inhibition during the alpha rhythm. Neuron 63, 683–696.
Maloney, K.J., Cape, E.G., Gotman, J., Jones, B.E., 1997. High-frequency gamma electroencephalogram activity in association with sleep-wake states and spontaneous behaviors in the rat. Neuroscience 76, 541–555.
Marshall, L., Born, J., 2002. Brain-immune interactions in sleep. Int. Rev. Neurobiol. 52, 93–131.

Moruzzi, G., Magoun, H.W., 1949. Brain stem reticular formation and activation of the EEG. Electroencephalogr. Clin. Neurophysiol. 1, 455–473.
Oathes, D.J., Ray, W.J., Yamasaki, A.S., Borkovec, T.D., Castonguay, L.G., Newman, M.G., Nitschke, J., 2008. Worry, generalized anxiety disorder, and emotion: evidence from the EEG gamma band. Biol. Psychol. 79, 165–170.
Okun, M., Naim, A., Lampl, I., 2010. The subthreshold relation between cortical local field potential and neuronal firing unveiled by intracellular recordings in awake rats. J. Neurosci. 30, 4440–4448.
Ommundsen, N., Engedal, K., Øksengård, A.R., 2011. Validity of the quantitative EEG statistical pattern recognition method in diagnosing Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 31, 195–201.
Páleníček, T., Fujáková, M., Brunovský, M., Horáček, J., Gorman, I., Balíková, M., Rambousek, L., Syslová, K., Kačer, P., Zach, P., Bubeníková-Valešová, V., Tylš, F., Kubešová, A., Puskarčíková, J., Höschl, C., 2013. Behavioral, neurochemical and pharmaco-EEG profiles of the psychedelic drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rats. Psychopharmacology (Berl) 225, 75–93.
Petersen, C.C., Hahn, T.T., Mehta, M., Grinvald, A., Sakmann, B., 2003. Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc. Natl. Acad. Sci. U. S. A. 100, 13638–13643.
Pfurtscheller, G., Klimesch, W., 1992. Functional topography during a visuoverbal judgment task studied with event-related desynchronization mapping. J. Clin. Neurophysiol. 9, 120–131.
Pfurtscheller, G., Lopes da Silva, F., 1999. Event-related EEG/MEG synchronization and desynchronization: basic principles. Clin. Neurophysiol. 110, 1842–1857.
Pickenhain, L., Klingberg, F., 1967. Hippocampal slow wave activity as a correlate of basic behavioral mechanisms in the rat. Prog. Brain Res. 27, 218–227.
Polack, P.O., Friedman, J., Golshani, P., 2013. Cellular mechanisms of brain state-dependent gain modulation in visual cortex. Nat. Neurosci. 16, 1331–1339.
Ponomareva, N.V., Selesneva, N.D., Jarikov, G.A., 2003. EEG alterations in subjects at high familial risk for Alzheimer's disease. Neuropsychobiology 48, 152–159.
Prichep, L.S., John, E.R., Ferris, S.H., Reisberg, B., Almas, M., Alper, K., Cancro, R., 1994. Quantitative EEG correlates of cognitive deterioration in the elderly. Neurobiol. Aging 15, 85–90.
Sachdev, R.N., Gaspard, N., Gerrard, J.L., Hirsch, L.J., Spencer, D.D., Zaveri, H.P., 2015. Delta rhythm in wakefulness: evidence from intracranial recordings in human beings. J. Neurophysiol. 114, 1248–1254.
Schroeter, M.L., Stein, T., Maslowski, N., Neumann, J., 2009. Neural correlates of Alzheimer's disease and mild cognitive impairment: a systematic and quantitative meta-analysis involving 1351 patients. Neuroimage 47, 1196–1206.
Sergeant, J., Geuze, R., van Winsum, W., 1987. Event-related desynchronization and P300. Psychophysiology 24, 272–277.
Simon, C.W., Emmons, W.H., 1956. EEG, consciousness, and sleep. Science 124, 1066–1069.
Stam, C.J., Jelles, B., Achtereekte, H.A., van Birgelen, J.H., Slaets, J.P., 1996. Diagnostic usefulness of linear and nonlinear quantitative EEG analysis in Alzheimer's disease. Clin. Electroencephalogr. 27, 69–77.
Steriade, M., 1993. Cholinergic blockage of network- and intrinsically generated slow oscillations promotes waking and REM sleep activity patterns in thalamic and cortical neurons. Prog. Brain Res. 98, 345–355.
Steriade, M., 2000. Corticothalamic resonance, states of vigilance and mentation. Neuroscience 101, 243–276.
Steriade, M., 2003. Cerebello-cerebral interactions during states of vigilance. Cerebellum 2, 82–83.
Steriade, M., Amzica, F., 1998. Coalescence of sleep rhythms and their chronology in corticothalamic networks. Sleep Res. Online 1, 1–10.
Steriade, M., Nuñez, A., Amzica, F., 1993. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J. Neurosci. 13, 3266–3283.
Stevens, A., Kircher, T., 1998. Cognitive decline unlike normal aging is associated with alterations of EEG temporo-spatial characteristics. Eur. Arch. Psychiatry Clin. Neurosci. 248, 259–266.
Sviderskaia, N.E., Prudnikov, V.N., Antonov, A.G., 2001. Characteristics of EEG signs of anxiety in human. Zh. Vyssh. Nerv. Deiat. Im. I P Pavlova 51, 158–165.
Tan, A.Y., Chen, Y., Scholl, B., Seidemann, E., Priebe, N.J., 2014. Sensory stimulation shifts visual cortex from synchronous to asynchronous states. Nature 509, 226–229.
Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T.J., Steriade, M., 2000. Origin of slow cortical oscillations in deafferented cortical slabs. Cereb. Cortex 10, 1185–1199.
van der Hiele, K., Bollen, E.L., Vein, A.A., Reijntjes, R.H., Westendorp, R.G., van Buchem, M.A., Middelkoop, H.A., van Dijk, J.G., 2008. EEG markers of future cognitive performance in the elderly. J. Clin. Neurophysiol. 25, 83–89.
van der Hiele, K., Vein, A.A., van der Welle, A., van der Grond, J., Westendorp, R.G., Bollen, E.L., van Buchem, M.A., van Dijk, J.G., Middelkoop, H.A., 2007. EEG and MRI correlates of mild cognitive impairment and Alzheimer's disease. Neurobiol. Aging 28, 1322–1329.
Vanderwolf, C.H., 1969. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr. Clin. Neurophysiol. 26, 407–418.
Van Winsum, W., Sergeant, J., Geuze, R., 1984. The functional significance of event-related desynchronization of alpha rhythm in attentional and activating tasks. Electroencephalogr. Clin. Neurophysiol. 58, 519–524.
Vyazovskiy, V.V., Kopp, C., Bösch, G., Tobler, I., 2005. The GABAA receptor agonist THIP alters the EEG in waking and sleep of mice. Neuropharmacology 48, 617–626.
Vyazovskiy, W., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., Tononi, G., 2011. Local sleep in awake rats. Nature 472, 443–447.
Wimmer, M.E., Rising, J., Galante, R.J., Wyner, A., Pack, A.I., Abel, T., 2013. Aging in mice reduces the ability to sustain sleep/wake states. PLoS One 8, e81880.
Wolf, H., Jelic, V., Gertz, H.J., Nordberg, A., Julin, P., Wahlund, L.O., 2003. A critical discussion of the role of neuroimaging in mild cognitive impairment. Acta Neurol. Scand. Suppl. 179, 52–76.
Zagha, E., Casale, A.E., Sachdev, R.N., McGinley, M.J., McCormick, D.A., 2013. Motor cortex feedback influences sensory processing by modulating network state. Neuron 79, 567–578.
Zhang, W., Savelieva, K.V., Suwanichkul, A., Small, D.L., Kirkpatrick, L.L., Xu, N., Lanthorn, T.H., Ye, G.L., 2010. Transmembrane and ubiquitin-like domain containing 1 (Tmub1) regulates locomotor activity and wakefulness in mice and interacts with CAMLG. PLoS One 5, e11261.
Zhou, M., Liang, F., Xiong, X.R., Li, L., Li, H., Xiao, Z., Tao, H.W., Zhang, L.I., 2014. Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex. Nat. Neurosci. 17, 841–850.