Methodologic recommendations and possible interpretations of video-EEG recordings in immature rodents used as experimental controls: A TASK1-WG2 report of the ILAE/AES Joint Translational Task Force

*Ozlem Akman, †Yogendra H. Raol, ‡†Stéphane Auvin, §§Miguel A. Cortez, ††Hana Kubova, ¶¶Marco de Curtis, §§§Akio Ikeda, ¶¶¶F. Edward Dudek, and ###Aristea S. Galanopoulou

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SUMMARY

The use of immature rodents to study physiologic aspects of cortical development requires high-quality recordings electroencephalography (EEG) with simultaneous video recording (vEEG) of behavior. Normative developmental vEEG data in control animals are fundamental for the study of abnormal background activity in animal models of seizures or other neurologic disorders. Electrical recordings from immature, freely behaving rodents can be particularly difficult because of the small size of immature rodents, their thin and soft skull, interference with the recording apparatus by the dam, and other technical challenges. In this report of the TASK1 Working Group 2 (WG2) of the International League Against Epilepsy/American Epilepsy Society (ILAE/AES) Joint Translational Task Force, we provide suggestions that aim to optimize future vEEG recordings from immature rodents, as well as their interpretation. We focus on recordings from immature rodents younger than 30 days old used as experimental controls, because the quality and correct interpretation of such recordings is important when interpreting the vEEG results of animals serving as models of neurologic disorders. We discuss the technical aspects of such recordings and compare tethered versus wireless approaches. We also summarize the appearance of common artifacts and various patterns of electrical activity seen in young rodents used as controls as a function of behavioral state, age, and (where known) sex and strain. The information herein will hopefully help improve the methodology of vEEG recordings from immature rodents and may lead to results and interpretations that are more consistent across studies from different laboratories.

KEY WORDS: Minimum standards, Rat, Mouse, vEEG, Ontogeny, Postnatal, Stereotaxic, Anesthesia, Cortical, Subcortical, Sleep, Awake, Spindles, Spectral analysis.
As discussed in the TASK1-working group 1 (TASK1-WG1) report of the International League Against Epilepsy/American Epilepsy Society (ILAE/AES) Joint Translational Task Force\(^1\) that describes video-electroencephalography (vEEG) studies in adult rodents used as experimental controls, a wide range of techniques for EEG recording are used in experimental studies. This also applies to immature rodents, but the lack of standardization for recording and reporting vEEG in immature rodents is probably even greater than for adult animals. The greater diversity of methods and recording systems in developing rodents is probably due to the various EEG electrophysiologic mechanisms in the developing mammalian brain. But there is currently no head-to-head comparison of the various methodologic and technology options for electrode implantation and EEG recordings of immature rodents, and these data allow crude only comparisons with human EEG studies.

**Key points**

- A standardized system for interpreting immature rodent EEG studies in experimental controls is needed; if widely accepted, interpretations across studies from different laboratories would be greatly improved.
- Careful consideration of the impact of peri-operative drugs and surgical protocols on the immature rodent brain is advised.
- Only a few studies have described the ontogeny of sleep-wake EEG patterns in immature control rodents, and these data allow crude only comparisons with human EEG studies.
- Improved technologies to allow detailed and rigorous analysis of the EEG patterns in immature control rodent EEG in a consistent manner across labs are needed to improve our understanding of the age-specific EEG patterns.

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\(^1\)These authors share first authorship.

\(^2\)These authors share senior authorship.

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from immature rodents, based on the experience of the authors and the cited literature. Because technical and surgery-related issues for EEG implantation as well as for signal acquisition have been covered in the recently published TASK1-WG1 and TASK1-WG5 reports, we refer only to those that specifically pertain to developing rodents.\textsuperscript{1,2} We do, however, mainly focus this article on vEEG recordings from immature rodents used as experimental controls because knowing the significant age-specific EEG changes observed during development is important for interpreting abnormal patterns. Discussion of vEEG patterns—epileptiform or other—that differ from those expected from control rodents adjusted for age or species is planned for a subsequent article.

**Methods**

Literature review included articles related to the topic that were available to the authors and articles cited therein, as well as those identified by the search string “(EEG OR electroencephalography OR electrocorticography) AND (mice OR mouse OR rat OR rodent) AND (development OR maturation OR immature OR developing OR postnatal) and (control OR normal)”. The search resulted in 219 articles that were screened for relevance. Additional literature search was done to retrieve literature on specific subtopics, by adding specific keywords, for example, anesthetics (“anesthetic OR isoflurane OR ketamine OR sevoflurane OR ether OR cryoanesthesia OR hypothermia”), sleep wake state (“sleep OR wake”). The content and recommendations of this article were discussed, reviewed, and agreed to by the co-authors through teleconferences or emails.

**Technical and Methodologic Issues in the Immature Rodents**

The TASK1-WG1 and TASK1-WG5 working groups of the ILAE/AIDS Joint Translational Task Force have reported most of the technical and methodologic issues (e.g., anesthetic agents, perioperative procedures and electrode types, recording issues) for EEG and vEEG recordings in adult control rodents.\textsuperscript{1,2} Here, we focus on specific issues related to immature rodents.

**Anesthetic agents**

Selection of anesthesia in electrode implantation surgeries in immature rodents needs to consider the age-specific differences in the anesthetic effects, pharmacokinetics, tolerability, and longer-term developmental and other biologic effects in the context of the study goals. Detailed discussion of the current evidence on such matters is beyond the focus of this manuscript and merits a systemic review. In the experience of the authors, isoflurane is a commonly used anesthetic in surgeries for electrode implantation in developing rodents. Due to its rapid and short-lasting effect, isoflurane can be easily titrated to effect and adjusted to tolerance, which can be advantageous when studying immature animals that could be more sensitive than adults to anesthesia. Prolonged or repetitive exposure to certain anesthetics, such as ketamine, may increase apoptosis in the brain of neonatal rodents \textsuperscript{≥}postnatal day 7 (P7).\textsuperscript{3} Concerns have also been raised that \textgammachainaminobutyric acid (GABA)-acting anesthetics may also enhance apoptosis in newborns; increased apoptosis has been observed, however, with prolonged \textsuperscript{≥}1 h exposure of P7 rats to isoflurane, whereas briefer (<1 h) sessions were not toxic.\textsuperscript{4,6} Isoflurane has also been shown to have age-specific effects on brain activity attributed both to developmental changes in neurotransmitter signaling systems (e.g., GABA\textsubscript{A} receptor signaling) and to increased connectivity with age.\textsuperscript{7} On the other hand, painful or stressful stimuli in the absence of anesthesia are reported to also trigger neuronal death, cause imbalance of excitatory and inhibitory transmitter systems, and enhance pain perception and behavioral and learning disabilities.\textsuperscript{8,9}

A variety of other anesthetic approaches have been used in pups. Although cryoanesthesia has been used in survival surgeries on rodents younger than 7 days old, in many institutions, it is not recommended for use in surgeries lasting longer than 15 min. Cryoanesthesia has produced more variability on physiologic measures (oxygenation, cardiorespiratory function) than what has been reported for inhaled anesthetics,\textsuperscript{10} whereas long-term adverse effects have been reported in hippocampal volumes, corpus callosum morphology, and performance in the water maze in adulthood with 30 min cryoanesthesia.\textsuperscript{11} Occasionally, parenteral anesthetics have been used in studies performing electrode implantation in immature rodents. However, some of them (e.g., pentobarbital, ketamine, fentanyl/droperidol) have also been reported to lead to increased mortality or be less effective in inducing anesthesia in 1–3 day old pups and can be associated with longer recovery times.\textsuperscript{12} Propofol has been reported to induce seizures in P4–6 rats,\textsuperscript{13} impair dendritic spines when GABA\textsubscript{A} receptor inhibition is still inefficient in newborns,\textsuperscript{14} as well as cause apoptosis, neuroinflammation, learning and memory deficits, sex-specific neurodevelopmental deficits, and altered endocrine responses to stress.\textsuperscript{15–18}

**Electrode types and placement**

In immature rodents, the choice of type, size, number, and placement of electrodes is affected by the small head size, softness of the skull, and the fact that the brain is growing during development. The existing atlases of the developing rodent brain may serve as helpful references for the placement of the electrodes, yet they do not cover all of the postnatal ages or strains of rodents, and thus some optimization of the coordinates used by the experimenters is required.\textsuperscript{19,20} Unlike in humans and similar to adult rodents, a standard protocol for EEG recording in immature rodents with recommendations for the number and location of recording electrodes has never been developed. The
decision on the type and location of electrodes is made based primarily on the features of the available recording EEG equipment, the goals of the study, and the age and species of the animals.

Stainless steel screw or wire electrodes are commonly used in developing animals. Implementation of methods of sterilization prior to implantation is important to minimize the resultant inflammation or possible infection from long-term use. The size of the epidurally placed electrodes needs to be carefully planned so as not to induce brain lesions. In pups with an incompletely ossified skull, application of Vetbond tissue adhesive (3M Animal Care Products, St Paul, MN, U.S.A.) prior to the creation of the burr holes and the electrode placement may help harden the skull and minimize the likelihood of producing deformities during electrode placement. Optimization of the technical procedures for electrode placement in each lab and the training of each new investigator is recommended so as to identify the optimal conditions that minimize the pressure during the opening of the burr holes and to avoid injury from excessive pressure or insertion of the electrodes beyond the dura. Examination of the brain histology is advised at the end of the recordings so as to examine for and account for such lesions when they occur.

No head-to-head comparisons in performance of various types of electrodes are available for neonatal rodents. Neonatal (i.e., a week old or younger) rat epidural EEG recordings have typically been done with 2–4 recording electrodes, which limits the possibility for extensive evaluation of the background organization. Some investigators use a screw to fix the electrode cap. When possible, symmetric bilateral placement of electrodes as well as anterior and posterior sets of electrodes, may allow investigators to obtain recording channels from symmetrical regions of the brain, and provide the opportunity to assess for unilateral or bilateral presence or anterior-posterior gradient of the observed rhythms and patterns, as done in humans. This, for example, will be useful to characterize the background activity in experimental control animals and to determine the lateralization and gross localization of observed patterns of activities, particularly when a new strain or model is studied.

Recording issues

Because the skull is continuously growing during development, one must be concerned that the head mount will restrict the growth of the skull or result in the loss of the head mount; therefore, vEEG recordings may be possible for only a few days or weeks under some conditions. The ongoing brain growth may cause changes in the location of electrodes if implanted within the parenchyma of the brain. Continuous, long-term vEEG recordings from immature rodents before weaning are also technically difficult because of the need to maintain the pup with the dam for feeding and maternal care. Monitoring of pups isolated from the dam and from the other pups of the litter offers the advantage that video analysis can be performed with an unobstructed view of the recorded pup; otherwise, the view of the pup may be blocked by the dam or other pups in the litter. EEG artifacts are also much more likely when the pup is recorded with the dam and litter. When pups are monitored separately from the dam and littersmates, however, the monitoring must be performed intermittently (i.e., periods with a duration of a few hours) to allow the pup to be returned to the dam for maternal care. Recordings from pups isolated from the dam must be done under temperature-controlled conditions, because the pups cannot thermoregulate adequately. Experimental designs, therefore, that control for these intermittent periods of maternal separation are important for future research.

Monitoring of pups in isolation is essentially required for tethered recordings. Although wireless recordings are possible in the presence of the dam and litter (Fig. 1), the quality of both the video and the EEG recordings is generally better when the pup is isolated. Recording sessions should include awake and sleep states for optimal characterization of brain activity.21,22 Additional studies are needed to better characterize sleep and wake states in immature rodents. The use of synchronous electromyography (EMG) recordings (e.g., using subcutaneous electrodes placed on nuchal muscles) offers the advantage of comparing brain activity with EMG activity, which may be useful in sleep scoring and/or determining EMG artifacts in the EEG. Synchronous video recordings often further help clarify age-specific behaviors and the presence or absence of EEG/EMG correlates or artifacts.23–28

Wired or tethered recordings

Tethered EEG recording systems have long been, and still are, the most commonly used approach for recording electrical signals from the brains of immature rodents. Wired EEG has allowed multiple-electrode recordings to localize the electrical events29 to various brain regions. Wired recording methods have the potential for use of microelectrodes to record local field potentials and extracellular action potentials. Most wireless systems suitable for use with immature rodents are not set up for recording within high-frequency bands (e.g., >100–160 Hz, see Table 1), whereas such recordings are relatively straightforward with wired approaches. Additional EMG or other biopotentials (e.g., electrocardiography [ECG]), along with EEG, may also be useful and are readily feasible with wired recordings. An ongoing concern with all EEG recordings, particularly from immature rodents, is the presence of movement artifacts due to the head mount, commutator, and wire connections, even when amplification is located on the head mount and when the connections appear to be well stabilized. Because of the thin and fragile skull of immature rodents, particularly during the first 2 weeks of life, it is important that investigators utilize head mounts and very thin wires that are stable but lightweight so that they can be well tolerated by the pup without limiting movement (e.g., see Figure 2 in Ref. 24).
Otherwise, large-amplitude and long-duration movement artifacts can be produced (see below). Tethered vEEG monitoring can be done in rats as young as P6, using systems that are specific for immature animals.23,24 Recordings in younger rats (P1–6) have been reported with other systems, although those studies described abundant EMG artifacts.21

**Wireless recordings**

Wireless recordings have the potentially important benefit that the animal is unrestrained by cables and can move more freely within the cage. A critical concern with wireless recordings, however, is whether the size/weight of the transmitter device and the associated battery is an impediment to unobstructed behavior. Thus, an important caveat is that wireless transmitters require power (i.e., batteries), which limit the duration of recording, the number of recorded channels, and the high-frequency components of the recordings; increasing any of these characteristics of the recording system requires additional power. The battery is generally the component that determines the size and weight of the device, and the combined transmitter and battery unit are either positioned internally (i.e., intraperitoneally [i.p.] or subcutaneously [s.c.]), on the head, or in a jacket or “backpack” worn by the pup; however, in young rodents,

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**Figure 1.**

Wired and wireless telemetry EEG recordings in immature rodents: prolonged continuous recordings with wireless methods and types of artifacts with wired and wireless approaches. A–C, Continuous monitoring for 48 h from a P7–P8 rat pup with dam using a wireless approach. Although video-EEG recordings with either wired or wireless recordings are optimal when performed when the pup is studied in isolation from the dam, wireless recordings can be obtained when the dam is present in the same cage; however, artifacts from the dam are still possible (see text and below). A rat pup was implanted with a wireless telemetry unit at P6 and housed with the dam and littermates in a cage positioned on a receiver base designed for an adult animal. A continuous recording of 48 h was made from a rat pup. Recording from Day 1 is shown in A, and from Day 2 in B; C shows background spontaneous electrical activity in a temporal expansion of part of the record in B (arrow below record in B). Downward arrows in A and B (above the traces) indicate “dropouts” of the EEG signal, which can occur when recordings are performed with wireless telemetry. In the top trace (A), the first 2 arrows mark “dropouts” that were too brief to see at this time scale (see below); however, the last 2 arrows show longer “dropouts” that can be seen as a flat part of the trace. This proof-of-concept experiment shows the possibility of conducting nearly continuous recordings—at least for 1–2 days at a time—in immature rats with the dam, but with interruptions of the recording. The rat pup was implanted at P6 (2–4% isoflurane) with a one-channel miniature wireless telemetry device (Epoch, Epitel, Inc., Salt Lake City, UT). The bandpass of the EEG signals with the wireless recordings was 0.1–120 Hz, 8 dB per octave. D–F, Examples of artifacts observed in wired and wireless telemetry recordings of EEG from awake, freely behaving immature rodents. Wired recordings, particularly from immature rodents, are susceptible to movement artifacts that arise from shifting of the connecting wires (D). Wireless telemetric recordings (E and F) can provide a signal with much smaller and fewer artifacts, but wireless recordings are also susceptible to movement artifacts, plus “dropout” artifacts that are shown in an expanded form in F. “Dropouts” in wireless recordings occur occasionally when the transmitter does not properly couple with the receiver (e.g., when the dam blocks transmission between the pup and the receiver). Reprinted with permission from Zayachkivsky et al.30

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the latter approach is problematic because the dam is likely to remove or damage it. However, the dam may also damage devices implanted on the head, and internal implantation i.p. or s.c. are particularly difficult in immature rodents.

Currently, wireless recording devices are available with either one of 2 different types of signal transmission: (1) radiowaves (i.e., radiotelemetry) or (2) capacitive coupling (Table 1). In radiotelemetry, a radiowave signal is generated in the transmitter and recorded with an appropriate receiver. Wireless recording via capacitive coupling involves a small transmitter with an amplifier that controls a pulse-width (i.e., frequency) modulation oscillator and a capacitive-coupled receiver with a frequency-to-voltage converter that recovers the original EEG signal. Although most of wireless systems have transmitters and batteries that are too large to use with immature rodents, systems designed for mice can be adapted for immature rats (see Table 1). Most wireless recording systems are limited to a single channel, although some have potential for 2 or more channels (Table 1). Radiotelemetry does not require line-of-sight conditions (see below), but it has the potential problem that cross-talk can occur if the animals are not separated by enough distance or if shielding is not used to isolate the animal cages electrically. Telemetry via capacitive coupling requires less power (i.e., batteries) than radiotelemetry, but the requirement of a direct line-of-sight between the transmitter and receiver antennas leads to more frequent disturbances in signal transmission (“signal dropouts”; Fig. 1) than with radiotelemetry, although objects between the transmitter and receiver can also cause signal dropouts with radiotelemetry. Both capacitive-coupled and radiotelemetry-based recording systems are only effective over short distances, although this issue may be more problematic with capacitive-coupled systems. Because a transmitter system based on capacitive coupling requires direct line-of-sight between the transmitter and receiver, the transmitter and battery must be positioned on the head of the recorded animal. Capacitive-coupled transmitter-battery systems are small enough to be positioned on the head of a postnatal day 6 (P6) rat pup with adequate power to allow recordings for weeks or even months. Anecdotal data suggest that similar recordings can be obtained from P12 mice. These systems have generally been designed to record within traditional EEG recording bands (see Table 1), which would imply potential bandwidth limitations for fast events, including high-frequency oscillations.

### Recommendations

- Selection of protocols for anesthesia or analgesia in immature rodents should consider, control for, and minimize potential age-specific neurotoxicity, while subjecting the animals to the minimum exposure time and dose necessary to minimize pain and ensure their well-being.
- Isoflurane anesthesia is generally well tolerated in immature rodents and is a common anesthesia method for electrode placement.
- Electrode placement systems that consider the small size and fragility of the skulls of developing rodents are preferred. When possible and permitted by the small size of the animal’s skull, bilateral and anteroposterior coverage with electrodes will allow for better evaluation of background activities.
- Reporting of the age- and species/strain-specific coordinates of the electrode locations should facilitate reproducibility of data and comparisons across studies and experimental groups.
- Use of synchronous video monitoring is important for the characterization of EEG patterns in relation to behavioral state and/or level of activity; the analysis of simultaneous behavioral (video) and EEG recordings may help differentiate electrical artifacts from valid electrical data.
- The description of the recording conditions should include the following: (1) the data acquisition system; (2) the type, number and location of electrodes; (3) the presence and properties of simultaneous video; (4) the conditions, duration, and frequency of EEG or vEEG recording sessions; (5) the total duration of EEG and vEEG recordings; (6) access to food and a description of housing; and

| Size | Location | Minimum animal weight | Channels (#) and battery (duration) | Transmission (RT or CC) | Biopotential bandwidth |
|------|----------|-----------------------|------------------------------------|------------------------|-----------------------|
| **DSI (New Brighton, MN)**<br>www.datasci.com | 1.1 cc<br>1.6 g | IP/SC | 17 g (SC)<br>20 g (IP) | 1 channel for 1 month<br>(second channel possible with half battery life) | Radiotelemetry | 3–100 Hz |
| **EMKA, Lomir Inc.**<br>(Malone, NY)<br>www.lomir.com | 1.8 cc<br>3.8 g | SC | 20 g (SC) | 1 Channel for 3 months | Radiotelemetry | 0.1–160 Hz |
| **Epitel, Inc (Salt Lake City, UT)**<br>www.epitelinc.com | 0.19 cc<br>(2 week)<br>0.5 g<br>(2 week) | Mounted on head | 14 g (on skull) | 4 channels for 2-weeks,<br>2 channels for 2 months, | Capacitive-coupled | 0.1–100 Hz |
| | 0.76 cc<br>(2 month)<br>2.3 g<br>(2 month) | | | 6 channels for 2 months | | 0.1–60 Hz |

### Table 1. Commercially available wireless systems

| Volume | Weight | Channels (#) and battery (duration) | Transmission (RT or CC) | Biopotential bandwidth |
|--------|--------|------------------------------------|------------------------|-----------------------|
| **4 channels for 2 weeks,**<br>2 channels for 2 months, | Capacitive-coupled | 0.1–100 Hz |
| **6 channels for 2 months,** | | | | |

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Tethered and wireless telemetry EEG systems can be used in immature rats, but the selection of the preferred system needs to consider the advantages and disadvantages of each system in relation to the experimental goals.

**Artifacts**

The occurrence of artifacts on EEG is a common problem observed in clinical and research study settings (see also TASK1-WG1 report\(^1\) on adult rodent EEG studies). The problem of movement artifacts and other spurious signals in immature rodents is similar to that in adult rodents, but much more challenging. Table 2 lists different types of physiologic and nonphysiologic artifacts, but the type of artifact most relevant to EEG recordings from immature rodents probably derives from movement. Aside from the presence/absence of the dam, the most critical difference between recording from isolated rodent pups and adults is the thinner and softer skull of the pups. These properties of the skull, combined with the much smaller size of the pups, translates to greater difficulty securing the headset to the skull, while still optimizing the ability of the pup to move around the cage without undue interference or restraint. Unstable connections of the wires to the headset/skull leads to substantial movement artifact in wired recordings. Figure 1 illustrates the large size and prolonged nature of typical movement artifacts that can arise when an animal moves with a tethered recording system. Similar to what can be seen while recording from adult animals, movement artifacts may be readily detected as an obvious artifact or may be difficult to discern from physiologic signals. Generally, movement artifacts are clearly linked to movement of the animal and are often so large that they cause repetitive saturation of the amplifier with a “steppy” waveform, unlike typical physiologic signals that are smaller and smoother. Movement artifacts are also possible with wireless recordings. The conceptual and practical difficulty, however, is that although one can frequently identify some obvious examples of movement artifacts, it is sometimes unclear how much of the recorded signal is artifact and how much is an actual physiologic event. Comparison of the quality of the vEEG patterns while the animal has various types of movements or when the experimenter is moving the EEG cables, or comparison of EEG with synchronous EMG recordings, may offer some insight into the presence or type of associated EEG artifacts. Intracortical or subcortical electrodes may reduce such artifacts, when such recordings are suitable for the experimental design.\(^31,32\) However, depth electrode recordings are more invasive than epidural electrodes and the possibility that additional electrode-induced brain pathology may occur needs to be considered and addressed. Loose implants may lead to movement or noise artifacts on EEG that are accentuated by the movement of the animal, whereas the lower signal-to-noise ratio may also result in the appearance of ECG and EMG signal on EEG.\(^21,31\) For example, Jouvet-Mounier et al.\(^21\) found that much of the EEG in P1–6 rats in the awake state was

| Artifacts                              | Possible solution                                                                                   |
|----------------------------------------|-----------------------------------------------------------------------------------------------------|
| 1. EMG, ECG, respiration artifacts     | Simultaneous recording of EMG and ECG may help identify the artifacts that correlate and/or are synchronous with the EMG and ECG signals. Careful observation correlating respiration with EEG signal may help determine if respiratory artifact is present. Intracortical or subcortical electrodes may reduce such artifacts, if suitable for the study design.\(^31,32\) |
| 2. Movement artifacts                  | Unity gain impedance matching head stage prior to amplification.\(^42,52\) Secure electrodes and cable connections to headset. Telemetry recording might limit such artifacts, if compatible with the study design. |
| 3. Cable movement related noise        | Preamp or unit gain head stage to amplify signal prior to leaving animal, shielding and grounding of the cable to reduce capacitance effects, use of a telemetry system to eliminate the need for a cable. Proper grounding, use of notch filter if needed after the acquisition, during signal analysis. |
| 4. Electrical artifacts 60 Hz in the US and 50 Hz in Europe | Proper grounding, use of notch filter if needed after the acquisition, during signal analysis. |
| 5. Environmental artifacts such as walking or movement of the experimenter near the recording unit can cause low frequency artifact | Proper grounding of the cables; use of a Faraday cage. |
| 6. Aliasing artifacts                  | TASK1-WG5\(^*\) report describes aliasing artifacts and anti-aliasing filters. Use of correct sampling rate (more than twice the frequency of the high-frequency filter) for analog-digital conversion. Use systems or monitoring conditions that do not interfere with the direct line-of-sight principle in capacitive-coupled wireless recordings, for example, tethered or radiotelemetry or monitor in isolation from dam and litter. |
| 7. Signal dropout artifact             | Use of correct sampling rate (more than twice the frequency of the high-frequency filter) for analog-digital conversion. Use systems or monitoring conditions that do not interfere with the direct line-of-sight principle in capacitive-coupled wireless recordings, for example, tethered or radiotelemetry or monitor in isolation from dam and litter. |

EEG, electroencephalography; ECG, electrocardiography; EMG, electromyography.
composed of EMG artifact under the recording conditions of their study. “Bridging artifacts” between 2 EEG electrodes located closely together and accidentally connected by conducive material (e.g., electrolyte gel or solution, silver epoxy) may generate similar signals in these 2 electrodes. Table 2 describes commonly observed artifacts in the EEG records of immature rodents, and the possible solution to eliminate or minimize the magnitude of the artifact, thus resulting in improved quality of the EEG recording.

**Ontogeny of EEG in Developing Rodents (Experimental Controls)**

Features of the EEG in immature rodents and patterns of background activity

**Definition, terminology, and sleep/awake differentiation in immature rodents**

Table 3 presents the terminologies and definitions used to describe the sleep-wake maturation and associated patterns of human and rodent EEG studies. The description of the behavioral state and sleep–wake cycles in humans has been standardized because of their usefulness in deciding whether a neonatal record is normal or abnormal. In humans, a behavioral sleep–wake state is said to be present when features of that state are present for 1 min or longer. In neonates, the sleep–wake cycle comprises 3 main states: awake, quiet sleep (QS), and active sleep (AS; Table 3). In the description of EEG features during early development, the continuous/discontinuous aspects of the EEG recording are important components. Discontinuity and the developmental appearance of continuous background is a key feature of EEG in newborn human babies and is typically used to describe whether there is continuity of EEG within a given sleep/awake state. In humans, discontinuity refers to the sudden interruption of background rhythms by long periods of suppression/quiescence on EEG (e.g., tracé discontinu in premature babies, seen in various states). In humans, continuity first appears in AS (28th–30th conceptional week [CW]), then in wakefulness (34th–35th CW), and finally in QS (38th–40th CW), although alternating periods of attenuated background may still be present in QS until the 46th CW (tracé alternant). In the healthy full-term neonate, the EEG background is continuous when during awake state or in AS.

As in humans, the evolution of the sleep/wake cycles during development is an important aspect of the evaluation and interpretation of the EEG activity in immature rodents. The study of sleep/wake states in immature animals has been done using various combinations of observations of the behavior of the animals, their eye movements, and their EMG and EEG recordings. The first studies mostly used criteria from behavior, EMG, and eye movements, but as the EEG correlates in each of the observed states became clearer, EEG features were incorporated to distinguish sleep states, at least in rodents older than P6. Table 4 presents the methodology and criteria used for staging the sleep/wake states as well as their defining features as described in immature rats. Table 5 presents the age-specific changes in the EEG background of immature rodents, as reported by various studies. Examples of the EEG background from developing control rats during different wake-sleep or behavioral states are presented in Figures 2–4, using EEG/EMG (Fig. 2) or EEG (Figs. 3 and 4) recordings and behavioral monitoring.

During the first 6 postnatal days, the frequency and intensity of the high-amplitude “wiggling” movements, the tonic extension of the neck muscles during wake periods, and the muscle twitches during the AS state highly limits the evaluation of the EEG or ECoG due to the masking effect of large EMG signals. The youngest age when QS has been described was P3 by Seelke and Blumberg, whereas it was first recognized on P7 by Jouvet-Mounier et al., probably due to the use of different criteria (see Table 3). The earliest age when separation of QS from paradoxical sleep (PS) or AS has been reported is P7. By comparison, in humans, clear differentiation of sleep and awake states can be done by EEG features around the 36th–37th CW, although the presence of continuity may help differentiate QS (discontinuous) from AS (continuous) as early as the 28th–30th CW.

QS is characterized by periods of muscular hypotonia, with no muscular twitches while the eyes are closed, even after the age of eye opening (P12–15 in rats). During the first week of life, QS periods can be brief (lasting for a couple of seconds) and become longer with age (exceeding 30 s by the end of the second postnatal week). The voltage is initially lower than in AS. Although the EEG activity tends to increase its amplitude and be more synchronized toward P10, the first signs of slow wave sleep (SWS) appear around P11. These SWS features include frontal delta (1–4 Hz) and sleep spindles (12–15 Hz). This differentiation of the EEG patterns across the sleep/wake cycles is followed by a period when the amplitude increases in all frequency bands during SWS EEG between the ages of P13 and P26, thus yielding the adult pattern of higher EEG amplitudes during SWS than those during awake and rapid eye movement (REM) sleep states. On P15, QS becomes similar to adult SWS, except that the voltage is still lower than in PS. Infrequent startles (synchronous unprovoked activation of different muscle groups) can be seen, which had been described previously as SWS with myoclonic twitches of “half-activated sleep.”

PS also is characterized by muscular hypotonia with eyes closed (even beyond the age of eye opening) but demonstrates frequent muscular twitches. Unlike startles, muscular twitches are independent muscular activation of limbs, tails, or nuchal muscles. Twitches with simultaneous
| Terminology                        | Humans                                                                 | Age of occurrence (CW) | Rodents                                                                 | Age of occurrence |
|-----------------------------------|------------------------------------------------------------------------|------------------------|-------------------------------------------------------------------------|-------------------|
| Wakefulness (W)                   | State with:                                                            |                        |                                                                         |                   |
|                                   | • eyes open (usually)                                                  |                        |                                                                         |                   |
|                                   | • either exploratory behavior or purposeful movements (active W) or     |                        |                                                                         |                   |
|                                   |   quiet immobility (quiet W)                                           |                        |                                                                         |                   |
|                                   | • EMG associated with tonic and phasic muscle activity due to           |                        |                                                                         |                   |
|                                   |   coordinated movements                                               |                        |                                                                         |                   |
|                                   | • EEG with age-appropriate awake patterns (see sections on alpha        |                        |                                                                         |                   |
|                                   |   posterior rhythm, central rhythm, continuity)                        |                        |                                                                         |                   |
|                                   | Any                                                                    |                        | Same as in humans, but eyes are closed prior to the eye opening age    | Any               |
|                                   |                                                                         |                        | (P12–15). See also sections on alpha posterior rhythm, central rhythm, |                   |
|                                   |                                                                         |                        | continuity and Table 4.                                               |                   |
| Active sleep (AS) or paradoxical  | Sleep state with:                                                     | 28–30 (first appearance)| Sleep state with:                                                      | P3 (first appearance) |
| sleep (P5)                        | • eyes closed and rapid eye movements,                                |                        | • eyes closed and rapid eye movements,                                 |                   |
|                                   |   muscular hypotonia with random,                                      |                        |   muscular hypotonia with random                                       |                   |
|                                   |   uncoordinated muscular sudden twitches and startles                  |                        |   muscular sudden twitches                                             |                   |
|                                   | • EEG similar to wakefulness                                           |                        |   EEG similar to wakefulness                                            |                   |
|                                   | • irregular respirations                                               |                        |                                                                         |                   |
| Quiet sleep (QS)                  | Sleep state with:                                                     | 28–30 (first differentiation from AS based on continuity)              | Sleep state with:                                                     | P3 (first appearance) |
|                                   | • eyes closed but no eye movements,                                    |                        | • eyes closed (even after eye opening day) but no eye movements,        |                   |
|                                   |   muscular hypotonia with no                                           |                        |   muscular hypotonia with no                                           |                   |
|                                   |   movements observed except for                                        |                        |   movements observed except for                                        |                   |
|                                   |   startles or tonic chin movements,                                    |                        |   startles or tonic chin movements,                                    |                   |
|                                   | • EEG discontinuous or with trace `al`                                |                        | • EEG discontinuous or with trace `al`                                  |                   |
|                                   | • regular respirations                                                 |                        |                                                                         |                   |
| Slow wave sleep (SWS)             | QS with frontocentral sleep spindles (12–14 Hz) and delta             | 44–49 (first appearance)                                            | QS with frontal sleep spindles (12–15 Hz) and delta                  | P1 (first appearance) |
| Distinction of QS from AS by EEG  | Sudden interruption of background rhythms by long periods of            | 28–30 (all states); Till 28–30 (all states); Till 34–35 (QS and     | Alterations of bursts of high amplitude activity with interburst      |                   |
| Discontinuity                      |   suppression/quinence in the EEG (e.g., trace `discontinuous in premu   |   W); Till 38–40 (QS)                                              |   intervals of very low activity. Clear distinction of discontinuous |                   |
|                                   |   ture babies, seen in various states). It is typically scored for     |                        | vs. trace `al` pattern has not been established. Discontinuity in       |                   |
|                                   |   each sleep-wake state separately.                                    |                        |   reference to specific sleep-wake states has not been studied, partly |                   |
|                                   |                                                                         |                        |   due to the rapid alterations of                                       |                   |
|                                   |                                                                         |                        |   states in immature rodents.                                          |                   |
|                                   |                                                                         |                        | Declines with age, possibly                                            |                   |
|                                   |                                                                         |                        |   EEG becomes continuous – P7–10, although some alternating patterns    |                   |
|                                   |                                                                         |                        | can still be seen.                                                     |                   |
| Terminology                  | Definition                                                                                       | Age of occurrence (CW) | Humans | Definition                                                                 | Rodents | Age of occurrence |
|-----------------------------|--------------------------------------------------------------------------------------------------|------------------------|--------|---------------------------------------------------------------------------|---------|-------------------|
| Continuity                  | The record appears continuous in each of the recognized states without period of quiescence. Trace alternant may, however, still be present. | 28–30 (AS); 34–35 (AS and W); 38–40 (AS, W, and QS; trace alternant is, however, still observed) |        | Although the definition is similar as in humans, the description of continuity for each state in rats has been less well defined due to the rapid cycling of sleep-wake states and the associated alteration of high vs. low voltage EEG. Unclear, possibly between P7–10 |
| Trace alternant             | Semiperiodic episodes (3–15 s) of generalized voltage attenuation (not of voltage quiescence). | 37–46                  |        | Not well defined. Episodic semiperiodic voltage attenuation have been described in P7–10 rats | P9–13   |                   |
| Slow activity transients (SATs) | High amplitude (up to 800 μV) slow transients that nest oscillations (0.1–30 Hz) and occur at the occipito-temporal regions at a rate of ~8/min. These are best seen with DC recordings. | 32–46 (period of occurrence) |        | High amplitude (396–800 μV), slow waves lasting 5.2–6.6 s with embedded delta activity. |         |                   |
| Delta brushes, beta-delta complexes, spindle bursts | Beta-delta complexes or delta brushes: Random 0.3–1.5 Hz waves (50–255 μV) with concomitantly bursts of low to moderate fast activity (8–12 Hz or 18–22 Hz, <75 μV). Recorded on routine scalp EEGs, as asynchronous/asymmetric patterns from central or occipital and temporal regions and are not helpful in deciding concomitant age. | 26 (first appearance); 26–33 (mostly in AS); 33–38 (mostly in QS) |        | Spindle bursts: alpha-beta activity nested in delta waves. Recorded so far with wide band extracellular recordings in association with contralateral limb movements (SI somatosensory cortex) or triggered by retinal waves (V1 visual cortex). | 1st–2nd week of life |                   |
| Theta bursts or temporal sawtooth | Bursts of 4–6 Hz activities at the midtemporal regions which rarely exceed ~2 s. They are bilateral, asynchronous, more frequent in AS than QS or W. | 24–26: onset; 30–32: maximal presence; 33: temporal alpha; 34: disappear |        | Unclear | Unclear |
| Sharp transients            | Sharp waveforms with a surface negative component lasting less than 200 ms which are seen in neonatal EEGs. They are present in frontal, central, temporal or occipital leads. These are indicative of immaturity and are not necessarily epileptic. | Frontal sharp transients: onset at 33–35; Present till 44 |        | Sharp or spike or spike and sharp wave forms have been described in neonatal rodent EEGs. Their significance has been unclear. No clear data on changes in amplitude, morphology or location with age. | Have been described in P7–13 rats. |                   |
| Posterior (alpha) rhythm    | Seen in wakefulness occipital regions (posterior dominant rhythm [PDR]). PDR is responsive (suppressed) to eye opening. | 3rd-4th postnatal months (first appearance but slower [3.5–4.5 Hz]); 12 months (5–6 Hz) 3 years (~8 Hz) 15 years (~10 Hz) |        | Rhythmic theta/alpha in wakefulness. Limited information on topography and reactivity in neonatal rodent recordings. | P12–14 (rhythmic theta in wakefulness) P27 (7–8 Hz rhythm in fronto-occipital regions [PS, W]) |                   |

|                |                                                                                                           |                        |        |                                                                         |          |                   |

Table 3. Continued.
rapid eye movement (REM; based on electrooculographic [EOG] recordings) have been described as early as P3 in rats. The background contains faster activities (17–22 Hz), higher amplitude than in QS, whereas it lacks the frontal delta seen in QS.

Wakefulness, on the other hand, is characterized by periods when pups have eyes open and are (1) either sitting motionless with eyes open (after the age of eye opening; i.e., “quiet wakefulness”), (2) or show coordinated, purposeful movements (grooming, head lifts, righting, exploratory movements, re-positioning, stretching; i.e., “active wakefulness”). Most studies describe increase in the frequencies of the background activities and their amplitudes during development. The EEG background in awake pups contained 12–17 Hz low-amplitude activities in P7–13 rats, which increased in frequency (17–20 Hz) and amplitude in P12–14 rats. Gramsbergen reported delta rhythms superimposed by low amplitude fast (5–15 Hz) activities in P9–10 rats and increases in amplitude and frequency in P10–13 rats. The EEG was predominantly low-amplitude delta with bursts of theta between P5–7 and progressively increased in frequency and amplitude between P10–12 (alpha [8–12 Hz] background with theta bursts) until P25–27. A 7–8 Hz fronto-occipital rhythm appears in PS and wakefulness after P26. Reports have suggested that the body temperature might modulate brain activity and sleep behavior.

In the rodent literature, there are some reports of discontinuity in immature rodents; however, its definition is not always described in a manner that allows comparisons with the use of this term in human EEG (e.g., whether it is state-dependent, or refers to emerging periods of suppressed vs. attenuated EEG or how long the periods of background attenuation/suppression last). “Discontinuous, low amplitude (20–30 µV at 4 days) cortical activity with superimposed EMG bursts accompanying muscle twitches” was described by Ref. 21 in P4 infant rats during sleep. Progressive increase in the continuity of the EEG with age was mentioned by Jensen et al., although no specific definition was given. In EEG recordings of 20 min duration from mice, Zanelli et al. reported alterations between periods of high amplitude sinusoidal activity and periods with lower amplitude activity in P5–10 mice, which was gradually replaced by continuous higher amplitude activity in older mice, but changes in sleep/wake states could account for some of these differences. Continuous background has been reported in P7–9 Sprague-Dawley rats, although periods of relative background attenuation could be seen. An increase in background continuity was described in P7 and older Sprague-Dawley rats by Zayachkivsky et al. Tucker and colleagues also described increases in continuity in the trace activity and decreased interburst intervals from P1 to P21 in amplitude-integrated EEG (aEEG) records from Wistar rats. However, no correlation was undertaken...
### Table 4. Criteria for sleep/wake staging in immature rats

| State                  | Methodology                                                                 | Criteria for scoring sleep/wake states in rodents | EEG                                                                 | Reference                                                                 |
|------------------------|-----------------------------------------------------------------------------|---------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------|
| **Methodology**         | Observation/Video                                                            |                                                   | At minimum, layout may include left frontal and left parietal screw electrodes (e.g., 00 – 96 × 1/16) with a reference (e.g., cerebellum). | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2005, 2008)21,27,28    |
| **Wakefulness (W)**     | EMG at nuchal muscles (bipolar) or masseters.                               | Visual observation                                | Winter observation EOG: Stainless steel hook electrodes (50 µm) placed between eyelid and orbit at nasal and temporal sides of each eye. | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2008); Gramsbergen (1970)21,27,53 |
|                        | Stainless steel EMG electrodes (hook or subcutaneous) placed on nuchal muscles. |                                                   | Optical changes in the background with gradual increase in amplitude and appearance of faster rhythms. Eventual appearance of theta/alpha rhythm. |                                                                            |
| **Quiet sleep (QS)**    | Quiet W: pup sits quietly, motionless, with no gross movements (>P14).       | Eyes open after the eye opening age (P12 –15).     | Age related changes in the background.                               | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2008); Gramsbergen (1970)21,27,53 |
|                        | Active W: coordinated movements that appear purposeful, but no locomotion.  |                                                   |                                                                      |                                                                            |
|                        | Movements may include grooming, mouth movements, head lifts, repositioning/ righting reflex movements, stretching, moving as if sniffing. |                                                   |                                                                      |                                                                            |
| **Slow wave sleep (SWS)**| Locomotion: typically after P9.                                                | Eyes open after the eye opening age (P12 –15).     | Age related changes in the background.                               | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2008); Gramsbergen (1970)21,27,53 |
| **SWS with myoclonic twitch or "half-activated sleep"** | Same as for QS.                                                              | Same as for QS.                                   | Delta slow waves with cortical spindles present (after P11).         | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2008); Gramsbergen (1970)21,27,28 |
| **Active sleep (AS) or paradoxical sleep (PS)** | Quiet behavior, muscular hypotonia, with occasional twitches.                | Same as for QS.                                   | Cortical spindles present.                                          | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2005, 2008)21,27,28 |
|                        | Muscular hypotonia with many muscular twitches.                              | Eyes closed, even after the eye opening age (P12 –15). | No cortical delta present.                                          | Jouvet-Mounier et al. (1970); Seelke and Blumberg (2005, 2008); Gramsbergen et al. (1970)21,27,28,53 |

**Startles:** discrete behavioral events characterized by simultaneous activation of multiple muscle groups, without relevance to environmental triggers.28,53 **Twitches:** independent, phasic, rapid, limb, vibrissae, and tail movements, most prominent between P5 and 15. **SWS with myoclonic twitch was not recognized in.**27
| Age  | Species  | Sex | Type of electrode/ montage | Location of electrodes | Background | Epileptiform activity | References |
|------|----------|-----|-----------------------------|------------------------|------------|----------------------|------------|
| P0   | Wistar   | NR  | Hook-shaped or spiral monopolar electrodes, subcutaneous, nuchal EMG | Frontal and occipital cortices | State NR: Mostly flat with low EMG activity; periods with irregular 6–12 Hz waves with high EMG activity | – | NR | Nagamura and Iwahara (1968) |
| P1–P3| Sprague Dawley | NR  | Tungsten wire | Frontal cortex, caudate, thalamus, dorsal hippocampus, lateral amygdala | State NR: Low-voltage irregular activity | – | NR | Snead and Stephens (1983) |
| P3–P4| Sprague Dawley | NR  | Tungsten wire | Frontal cortex, caudate, thalamus, dorsal hippocampus, lateral amygdala | State NR: Gradual improvement in synchronization and appearance of faster rhythms | Synchrony, organization, and voltage of the EEG activity increased with age | – | None | Jensen et al. (1991) |
| P5–7 | Long Evans rats | NR  | Epidural electrode plugs | Two electrodes, between bregma and lambda | State NR: Low voltage delta (1–3 Hz) with bursts of higher voltage theta (3–6 Hz). | – | None | Jensen et al. (1991) |
| P5–P8| Wistar Albino | NR  | Hook-shaped or spiral monopolar electrodes, subcutaneous, nuchal EMG | Frontal and occipital cortices | State NR: 6–12 Hz waves appeared: 6–8 Hz after body movement, 8–12 Hz at resting state and synchronous to respiratory rate, High voltage slow waves 4–3 Hz appeared at PN7 and increased by P15 | By P5, irregular EEG pattern became dominant. From P5 on, rhythmic 6–12 Hz waves were frequent | – | NR | Nagamura and Iwahara (1968) |
| P7   | Sprague Dawley | Male | Silver electrodes in superficial cortex, references placed at lambda | Parietal cortex | State NR: Continuous low (30 μV) amplitude activity with intermitting periods of voltage attenuation and no movement, alternating with periods of high amplitude activities with occasional twitches | Unclear if sharp waveforms are physiologic or pathologic, due to invasive EEG (unclear significance) | Spike and sharp wave like activity | Sampath et al. (2014) |
| P7   | Rats      | NR  | NR | NR | 12–17 Hz, 20–80 μV background | – | NR | Jouvet-Mounier et al. (1970) |
| Age   | Species               | Sex   | Type of electrode/montage | Location of electrodes | Background | Interpretation                                                                 | Epileptiform activity | References |
|-------|-----------------------|-------|---------------------------|------------------------|------------|--------------------------------------------------------------------------------|-----------------------|------------|
| P7–13 | Sprague-Dawley rats   | Male  | Stainless steel screw electrodes | Bilateral frontal and parietal | Continuous | Continuous | Spikes of unclear significance (immaturity or due to prior surgery) | Rare isolated spikes of unclear significance | Scantlebury et al. (2010) |
| P7–11 | Sprague-Dawley rats   | NR    | Stainless steel wire electrodes | Unilateral | Increasing power of α, θ between P7–8, stabilization between P8–11. Increasing power of β, γ between P7–10, stabilization between P10–11. | Rare isolated spikes of unclear significance | Some spike and sharp wave-like activity | Zayachkivsky et al. (2013) |
| P9    | Rats                  | NR    | NR                        | NR                     | QS: 12–15 Hz, 50 µV | No state specific EEG patterns | – | NR | Jouvet-Mounier et al. (1970) |
| P9–P10| White and black hooded Lister strain | NR    | Bipolar recordings with 4 Teflon insulated silver electrodes, 0.3 mm diameter. Actograph | Sensory-motor and visual cortices | At all states, 1–3 Hz irregular waves (50–100 µV). Intermittent superimposition of fast low amplitude (5–15 µV) activity on the slow waves. | No state specific EEG patterns | – | NR | Gramsbergen (1976) |
| P10–P13| White and black hooded Lister strain | NR    | Bipolar recordings with 4 Teflon insulated silver electrodes, 0.3 mm diameter. Actograph | Sensory-motor and visual cortices | 1–3 Hz, 75–125 µV waves with superimposed low amplitude 25 Hz waves | State 1 (no movement, regular respiration): High amplitude waves. Startles coincided with flat EEG. State 2 (twitches, irregular respiration): same as awake. | – | NR | Gramsbergen (1976) |
| P10–12| Long Evans rats        | NR    | Epidural electrode plugs   | Two electrodes, between bregma and lambda | State NR | Higher amplitude alpha (8–12 Hz) with bursts of theta (3–6 Hz). | – | – | Jensen et al. (1991) |
| P10   | Wistar Albino         | NR    | Hook-shaped or spiral monopolar electrodes, subcutaneous, nuchal EMG | Frontal and occipital cortices | State NR. | Slow (≤3 Hz) waves (no detail) | – | – | Nagamura and lwahara (1968) |
| P9    | Sprague-Dawley rats   | NR    | Screws attach to silver wires, nuchal EMG | Left frontal and parietal cortices, cerebellum as ground | No differences in power spectra across ages (P9 to P13) | No state-dependent difference among the QS, AS and wake states | Power spectrum of the EEG did not show any distinguishable peaks | NR | Seelke and Blumberg (2008) |
| P11   | Sprague-Dawley rats   | NR    | Screws attach to silver wires, nuchal EMG | Left frontal and parietal cortices, cerebellum as ground | No differences in power spectra across ages (P9 to P13) | QS: Appearance of a clear delta peak in the power spectrum of the EEG | First signs of adult slow-wave sleep appear around P11 | NR | Seelke and Blumberg (2008) |

Continued
| Age    | Species      | Sex | Type of electrode/montage               | Location of electrodes                                      | Background                                                                                                                                      | Interpretation                                                                 | Epileptiform activity | References |
|--------|--------------|-----|----------------------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------|------------|
| P13    | Sprague-Dawley | NR  | Screws attach to silver wires, nuchal EMG | Left frontal and parietal cortices, cerebellum as ground | No differences in power spectra across ages (P9 to P13)                                                                                 | Q5: The power of delta activity is higher than that in P11                         | –                     | Seelke and Blumberg (2008) |
| P14-15 | Sprague-Dawley | Screws attach to silver wires, nuchal EMG | Frontal and parietal cortices, cerebellum as ground | –                                                                                           | AS: Theta peak in the powerspectrum                                               | AS: High amplitude slow-wave activity, 2–4 Hz delta peak in power spectrogram | –                     | Seelke et al. (2005) |
| P11–14 | Rats         | NR  | NR                                      | NR                                                                                         | SWS: P11: Cortical spindles 12–15 Hz, 100–150 μV appear P12: 3–5 Hz with cortical spindles                                                        | –                                                                               | NR                   | Jouvet-Mounier et al. (1970) |
| P12–14 | Rats         | NR  | NR                                      | NR                                                                                         | Wakefulness: Fast background (17–20 Hz, 50–100 μV)                                                                                              | –                                                                               | –                    | Jouvet-Mounier et al. (1970) |
| P14–16 | Long Evans   | Male | Teflon coated wire with soft head plug   | Bipolar EEG recordings from somatosensory cortex | Awake: Relatively low EEG amplitude                                                                                                              | REM: Lower EEG amplitude than slow-wave sleep SWS: Higher EEG amplitude than wake or REM sleep | –                     | Vogel et al. (2000) |
| P15    | Rats         | NR  | NR                                      | NR                                                                                         | SWS: frequencies and patterns similar to adults, but with lower amplitude PS: 17–20 Hz, 50–100 μV, and theta frequencies (6–7 Hz); patterns similar to adults, but with higher amplitude | –                                                                               | NR                   | Jouvet-Mounier et al. (1970) |
| P15–17 | Long Evans   | NR  | Epidural electrode plugs               | Two electrodes, between bregma and lambda | State NR Increased frequency and amplitude compared to younger ages EEG patterns become similar to adults                                   | –                                                                               | None                  | Jensen et al. (1991) |
| P17    | Rats         | NR  | NR                                      | NR                                                                                         | EEG patterns across states become similar to adults                                                                                             | Amplitude of SWS increases between P17–P26                                       | NR                   | Jouvet-Mounier et al. (1970) |
| Age  | Species | Sex  | Type of electrode/montage | Location of electrodes | Background                                                                 | Sleep | Interpretation                                                                 | Epileptiform activity | References                                      |
|------|---------|------|---------------------------|------------------------|-----------------------------------------------------------------------------|-------|--------------------------------------------------------------------------------|-----------------------|------------------------------------------------|
| P10–P20 | Long Evans | NR   | Stainless steel screws, Nuchal EMG | Frontal and parietal cortices | NR                                                                          | NR    | SWS: By P14: spindles and delta waves are seen. REM: At P14–16 desynchronized EEG and intermittent theta activity | NR                   | Frank and Heller (1997) [37]                 |
| P12   | Wistar   | Male | Nichrome wires            | Frontal cortex         | NR                                                                          | NR    | High amplitude slow waves (SWS)                                                 | NR                   | Mirman and Corner (1982) [38]                |
| P12–P14 | Sprague Dawley | NR   | Tungsten wire             | Frontal cortex, caudate, thalamus, dorsal hippocampus, lateral amygdala | Similar to adult awake EEG (no detail)                                      | NR    | EEG is equivalent to adult awake EEG (spectral and visual analysis)             | NR                   | Snaed and Stephens (1983) [39]             |
| P4–P18 | White and black hooded Lister strain | NR   | Bipolar recordings with 4 Teflon insulated silver electrodes, 0.3 mm diameter, Actograph | Sensory-motor and visual cortex | NR                                                                           | NR    | State 1 (no movement, eyes closed): 14–18 Hz spindles superimposed on the 1–3 Hz waves. State 2 (eyes closed, twitches): after PN1 6.5–7 Hz over visual cortex | Increase in amplitudes of the higher frequency waves with age. By P14 spindles (frequencies from 14 to 18 Hz) occurs State 1 | Gramsbergen (1976) [40]          |
| >P20–25 | Wistar Albino | NR   | Hook-shaped or spiral monopolar electrodes, subcutaneous, nuchal EMG | Frontal and occipital cortices | NR                                                                           | NR    | Slow waves with spindles. Active sleep can be identified. EEG maturation complete by PN30 | --                  | Nagamura and Iwahara (1968) [41]          |
| P25–27 | Long Evans rats | NR   | Epidural electrode plugs | Two electrodes, between bregma and lambda | State NR                                                                    | --    | EEG frequency similar to adults (6–12 Hz) but with lower amplitude | None                 | Jensen et al. (1991) [42]                 |
| Age    | Species | Sex | Type of electrode/montage | Location of electrodes | Background | Epileptiform activity | Interpretation | References |
|--------|---------|-----|---------------------------|------------------------|------------|-----------------------|----------------|------------|
| P3–P6  | C57BL/6 mice | NR  | Stainless steel wire electrodes | Hippocampus            | State NR  | –                     | Low amplitude mixed frequency activities with briefer periods of higher amplitude mixed frequency activity. During higher amplitude activity, trains or bursts of spike wave activity were present. | Zanelli et al. (2014) |
| P7–P8  | C57BL/6 mice | NR  | Stainless steel wire electrodes | Cortex + Hippocampus   | State NR  | –                     | Longer periods of higher amplitude activity with a sinusoidal pattern. Spike wave discharges during periods of higher amplitude activity. | Zanelli et al. (2014) |
| P10    | C57BL/6 mice | NR  | Stainless steel wire electrodes | Cortex + Hippocampus   | State NR  | –                     | Continuous moderate amplitude activity with minimal variability and some interspersed sharper activities. Neonatal mouse EEG reaches a continuous background pattern by P10. | Zanelli et al. (2014) |
| P11–P12 | C57BL/6 mice | NR  | Stainless steel wire electrodes | Cortex + Hippocampus   | State NR  | –                     | Continuous with moderate amplitude activities in a sinusoidal pattern. Periods of high amplitude activity lengthened with advancing postnatal age and a continuous background activity pattern. | Zanelli et al. (2014) |

AS, active sleep; NR, not reported; PS, paradoxical sleep; QS, quiet sleep; REM, rapid eye movement; SWS, slow wave sleep.
between the EEG changes and the sleep-wake cycle in their study. Increase in continuity and amplitude of the EEG background between P4–14 rats was also reported by Ranasinghe et al., although sleep-wave staging was not done and criteria for scoring were not defined. A better definition of the developmental changes in the sleep/wake cycle and EEG correlates is needed.

**EEG spectral analysis during development and various EEG patterns in immature rodents**

Power spectrum analysis first shows a clear delta peak during QS at P11, which is followed by the appearance of a theta peak (4–6 Hz) and frontal fast activity during PS or active sleep (AS) at P13. When single channels of EEG electrical activity were analyzed for changes in integrated power and power spectral density, increases in power were observed with increases in age for each of the traditional EEG bands in rats (Fig. 5). After quantitative analysis of the data, the enhancement in power was particularly robust in the beta and gamma bands (Fig. 5).

**Figure 2.** Examples of EEG/EMG from a male Sprague-Dawley rat recorded on P7, P10, and P12. Panel A, Wake sleep scoring was done using behavioral (video monitoring), nuchal EMG recordings, and 2 EEG channels recording from the frontal and parietal regions (Right [R.] Frontal-Left [L.] Parietal; R. Parietal – L. Parietal) through stainless steel screw electrodes. EMG was recorded using 2 subcutaneous stainless steel wires placed over the nuchal muscles (2EEG/1EMG system for EEG/sleep recordings; Pinnacle Technology Inc, Lawrence, Kansas). Wakefulness was characterized by coordinated movements (indicated by green line under EMG), increase in the range of EEG frequencies and their amplitude with age. Quiet sleep (QS) was characterized by lack of EMG activity, low voltage EEG on P7 and appearance of delta (mostly frontal; 1 Hz to <4 Hz rhythms) at P10 and P12 (shown by red line). Increased fast activity is also seen in older ages; included fast alpha/beta rhythms embedded in the delta waves. Active sleep (AS) was indicated by low nuchal EMG activity and presence of EMG bursts during the muscular twitches and jerks (shown by blue *). The range of frequencies was similar in wakefulness and AS. Please note the difference in the type and voltage of activities recorded from the anterior and posterior EEG channels, which helps describe the anteroposterior organization of EEG activities. The insert presents an enlarged version of the delta activity seen in panel A (P10, quiet sleep, R. frontal – L. parietal channel). Panel B, Diagram of the location of the electrodes (stainless steel screw electrodes, red dots; stainless steel EMG wire electrodes, blue lines) on the pup’s skull. Acquisition filters were 0.5 Hz (low filter) and 1,000 Hz (high filter). The bar scale indicates the sensitivity and timescale. The figure was provided by Aristea Galanopoulou.
decline with age. In the rodent literature, different studies have reported (1) “no spontaneous spikes or rhythmic spike/wave discharges” in P5 or older Long-Evans rats,40 (2) “rare isolated spikes of unclear significance” in P7–13 control male Sprague-Dawley rats,23 (3) “waves of spike-like activity” in control P7 and older Sprague-Dawley rats, or (4) “some spike and sharp wave-like activity” in control P7 Sprague-Dawley rats.30,32 To our knowledge, systematic studies of the characteristics of these waveforms (i.e., state-dependence, rate of occurrence, ontogeny) have not been performed; their significance in previous studies is unclear, since they could be attributed to either immaturity or prior surgical interventions.

Other EEG patterns of immaturity

Unlike the human EEG, where various EEG patterns have been recognized, the EEG background in immature rodents has not been studied in detail; there is a lack of epidural recordings across ages and different sleep stages where hemispheric synchrony and EEG patterns (e.g., beta delta waves, delta brushes) have been analyzed. Using extracellular wide-band recordings of multiple-unit activity in somatosensory cortex (S1) of neonatal rats, “spindle bursts” were recorded in association with contralateral limb movements to sensory stimulation.46 These spindle bursts (alpha-beta activity nested in delta waves) were spatially confined and were proposed to correspond to the “delta-brushes” seen during quiet/slow wave sleep.

**Figure 3.** Examples of EEG from a male Sprague-Dawley rat recorded on P11 and P18. Intermittent vEEG recordings (A) using stainless steel screw electrodes placed at bilateral frontal and occipital regions and referenced with a screw electrode placed over the cerebellum (see diagram of electrode layout in panel B) were done. (A) Examples of awake and asleep (quiet/slow wave sleep and active sleep) EEG studies on P11 and P18, using a referential montage, with a cerebellar reference. The awake background shows a mixture of activities but also the emergence of a 6–7 Hz activity (indicated by the green line), better seen at the frontal leads, which becomes more prominent on P18. Quiet sleep or slow wave sleep shows prominent high amplitude delta activity maximal frontally (red line) with superimposed frontal maximal spindles (blue *). Active sleep shows a fast background and occasional twitches/jerks detected as EMG artifact. Please note the difference in the range of activities recorded at the frontal versus the occipital regions, suggesting that the location of electrodes may alter the recorded signal. Scoring of sleep/wake states was done using the EEG and video recordings. B, Diagram of electrode layout. C, Magnification of EEG segments showing frontal delta and spindles (slow wave sleep) or 6–7 Hz rhythmic activity (wakefulness). EEG was done using the Stellate EEG system (Montreal, CA) with a Lamont Pro-36 amplifier, sampling rate of 2 kHz. EEG is shown here using low and high frequency filters of 1 and 70 Hz, respectively. Time and sensitivity scales are shown for each age group. Please note the lower sensitivity in the P18 EEG recordings due to the higher amplitude signal at this older age. The figure was provided by Aristea Galanopoulou.
in human newborns. Similarly, spindle bursts were recorded at the V1 visual cortex and were triggered by spontaneous or evoked retinal waves transmitted to the lateral geniculate nucleus, prior to the age of eye opening. Sensory stimulus (whisker simulation) triggered spindle bursts were also reported from the barrel cortex of P1–6 Wistar rats. These authors reported that spindle bursts consisted of an N-methyl-D-aspartate (NMDA) and \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor generated delta wave while the fast oscillations were AMPA receptor mediated and had corticothalamic origin. These spindle bursts were present in the first week of life and declined during the second week. In infants, similar patterns have been described as slow activity transients (SATs). SATs have been detected with both stainless steel skull screw electrodes as well as DC-coupled Ag-AgCl electrode recordings from the cortical surface, but the latter were more accurate. SATs have also been reported in humans between 32 and 46 CW, and their developmental disappearance has been proposed to demark the period when GABA\(_A\)-receptor signaling becomes hyperpolarizing in the neocortex.

**Maturation of EEG to adult patterns**

A progressive increase in the amplitude and frequency range of the background EEG with age has been reported in

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**Figure 4.** Evolution of EEG activity during early development using a wired recording system. The 4 representative epochs show EEG activity from cortex for each of 4 days from postnatal day 7 (P7) to P10 recorded from Sprague-Dawley rats. The amplitude of the electrical activity increased with increasing age, from P7 to P10. Three behavioral states of the rat pup were observed during 3 corresponding EEG patterns for each epoch (P7 to P10). For each age (P7–P10, respectively), the EEG during the 3 different behaviors is shown below at an expanded time scale (A–C): A shows the EEG activity during the awake state, which involved crawling, stretching, and yawning; B illustrates the EEG when no movement was observed, except for limb twitches and whole body jerks; and C shows the EEG when no movement was detected. The 3 EEG patterns in this figure, corresponding to the observed behaviors, are similar to those reported earlier by Jouvet-Mounier et al. (1969), who recorded EEG from frontal cortex in P7 to P26 rats. These authors described the EEG corresponding to the awake state of the rat (A), to the early state of paradoxical sleep (B), and to quiet sleep (C). The EEG was recorded using a tethered system (Stellate Harmonie system, Natus Medical, San Carlos, CA, U.S.A.) with a silver wire (0.008 inch outer diameter) placed 2.5 mm behind the bregma and 3 mm lateral from midline sutures that was referenced to an electrode positioned near lambda in the same hemisphere. The electrode was placed just inside the cortex to obtain a good-quality signal. The EEG data were collected with a sampling rate of 1,000 Hz, the default gain of 4,000 \( \times \), and filters set at 0.5 Hz (low) and 70 Hz (high). The representative epochs of EEG shown in the figure were digitally filtered at 3.0 Hz (low) and 35 Hz (high) after the acquisition. The offline filters were applied to improve the visibility of amplitude differences between behavioral stages and across the ages. The figure is provided by Yogendra Raol.

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several studies. Newborn rats, as early as P131 and during the first week of life,21,40 have low-voltage EEG background. Developmental changes in the EEG background were observed in both cortical and subcortical sites such as the hippocampus.31 However, regional differences have been described: subcortical sites like the hippocampus may exhibit more activity than cortical sites in very young pups,31 whereas the visual cortex may have higher amplitude activity than the sensorimotor cortex in the first 2 postnatal weeks.39

The awake EEG was reported as similar to adults in P12–14 rats.31 However, maturation was described in older ages in other studies. The EEG background became almost similar to adults after P17, except that frontooccipital theta (7–8 Hz) appeared in PS and wakefulness after P26.21 In a different study utilizing 2 electrodes placed on the right and left parietal cortical areas, the authors also observed that the EEG around P25–27 became similar to adult rats with regard to the frequency range, albeit the amplitude was still lower than in adults.40 However, in Long-Evans rats, some qualitative differences in the patterns of the described EEG activities were observed at the various ages.40 The EEG was predominantly low-amplitude delta with bursts of theta between P5 and P7 and progressively increased in frequencies and amplitude between P10 and P11 (alpha [8–12 Hz] background with theta bursts) until P25–27.40 Although qualitatively the sleep-wake EEG of P30 rodents resembles the adult EEG, some quantitative differences in sleep-wake transitions have been observed between P34 and P71 rats.51 Adult rats have more microarousals and transitions between wake and light sleep, whereas P34 rats have fewer transitions from light SWS (delta with 6–15 Hz sleep spindles) to deep SWS (>70% delta slowing with reduced EMG and motor activity). Consequently, P71 rats spent more time in light SWS (26%) than P34 rats (17%) and less time in PS (7.6% vs. 11%, respectively).51

Recommendations

- Utilization of a widely accepted system to record and analyze EEG in rodents would help to characterize the background and determine the presence of EEG patterns in immature rodents at different ages.

Figure 5.
Age-dependent changes in background frequency bands in an awake freely behaving control rat pup. Each day between P7 and P11, background EEG was recorded from a set of 10 rat pups (A). For each of the EEG bands, power spectral density (PSD) in the EEG was estimated and the mean values were plotted with 95% confidence intervals (B). A substantive increase in power was observed between P7 and P8 across all frequency bands. Power in the beta and gamma bands progressively increased with age and showed a plateau at P10 and P11. Two recordings were conducted at P7 to verify stability and to evaluate the same-day variability of the signal. Measurements of integrated power were compared with analysis of variance (ANOVA) (C). An asterisk shows statistical significance. Recordings were performed as described in Figure 1. The bandpass for the wireless recordings was 0.1–120 Hz. (For methods, please see methods in Ref. 30). Reprinted with permission from Zayachkivsky et al.30 Epilepsia Open © ILAE

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Video-EEG in Immature Rodents
• Study of the known features of sleep-wake state differentiation and maturation may be helpful in evaluating the background EEG.
• More detailed studies of the ontogeny, maturation, and state differentiation of the immature rodent EEG using bilateral and anteroposterior coverage in experimental control rodents are needed.

CONCLUSIONS

Few modern studies have described attempts to outline the maturational changes of the EEG in immature rodents. The current evidence suggests that maturation of the EEG background occurs gradually through the first month of life. However, the technical difficulties in implanting EEG electrodes in very young rodent pups, and the utilization of various methods to score sleep-wake states while recording the EEG has hindered the creation of a uniform system for interpreting EEG and has prevented the creation of a detailed description of specific EEG patterns during development in immature rodents. The best existing description of the EEG in immature rodents refers generally to the frequency ranges and the types, amplitudes, and power spectra of the EEG in various sleep and awake states (plus the description of certain patterns of immaturity, such as SATs). More systematic studies with improved EEG technologies are needed to permit more extensive spatial coverage of the rodent brain at different developmental ages, which would allow generation of a system to better evaluate the organization, topography, and state-dependence of the EEG characteristics of immature rodents across different ages and species/strains. A broadly accepted system for the recording and interpretation of the EEG in immature rodents will be particularly useful for preclinical studies aiming to characterize the epilepsy and neurologic phenotype of rodent models of early life epilepsies, neurodevelopmental disorders, or other early life neurologic disorders, as is commonly done in clinical practice.

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CONFLICT OF INTEREST

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