A Comparison of Sleeplike Slow Oscillations in the Hippocampus Under Ketamine and Urethane Anesthesia

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Sharma AV, Wolansky T, Dickson CT. A comparison of sleeplike slow oscillations in the hippocampus under ketamine and urethane anesthesia. J Neurophysiol 104: 932–939, 2010. First published June 10, 2010; doi:10.1152/jn.01065.2009. During sleep and anesthesia, a slow (≤1 Hz) synchronized rhythmic fluctuation of the network activity in the neocortex (nCTX) is prominent. This rhythm, called the slow oscillation (SO), corresponds to sequences of neuronal activity and inactivity (UP and DOWN states) in local and extended networks. Recently, this network pattern has also been described in the hippocampus (HPC) and, interestingly, it shows a dynamic correlation with ongoing SO in the nCTX. However, because of its transient nature during both sleep and urethane anesthesia, studying the SO in the HPC is difficult. Ketamine anesthesia is known to induce a uniform and continuous SO state in the nCTX, but its effects on HPC activity and HPC–nCTX interplay are unknown. Using multisite local field potential recordings, we investigated the effects of ketamine anesthesia on HPC activity and its relation to concurrent nCTX activity. We directly compared the spectral content and spatial distribution of laminar potentials and current source density (CSD) under ketamine to urethane anesthesia. Ketamine evoked stable bouts of SO in the HPC that had a peak frequency of 1.77 ± 0.08 Hz, slightly higher than that of urethane: 1.17 ± 0.07 Hz. Laminar voltage and CSD profiles of SO were highly correlated across ketamine and urethane anesthesia, although a higher degree of HPC–nCTX coherence at SO frequencies appeared under ketamine as opposed to urethane. In addition, activity in the gamma bandwidth (30 – 40 Hz) made up 4.7% of the power spectrum under ketamine, but accounted for only 2.0% of the power spectrum in urethane. Although some differences exist, the SO under ketamine anesthesia appears highly similar to that under urethane.

INTRODUCTION

Information processing in the hippocampus (HPC) is relevant to its mnemonic function (Alvarez and Squire 1994; Eichenbaum 2004; McGaugh 2000; Scoville and Milner 1957; Stefanacci et al. 2000). In this regard, dynamic and state-dependent changes in spontaneous synchronized pattern of hippocampal networks are especially salient (Alvarez et al. 1995; Buzsáki 1989; Mormann et al. 2008; Sejnowski and Destexhe 2000). During sleep there are two mutually exclusive large-amplitude rhythmic patterns of field activity in the HPC: theta (3–10 Hz) (Bland 1986; Buzsáki 2002; Buzsáki et al. 1983; Vanderwolf et al. 1978) and the slow oscillation (SO) (≤1 Hz) (Clement et al. 2008; Isomura et al. 2006; Wolansky et al. 2006). The latter pattern is representative of the deactivated state that characterizes non-rapid eye movement (REM) sleep stages in the forebrain more generally (especially in the neocortex [nCTX]) and the dynamic coordination of hippocampal and neocortical ensembles via the SO has been suggested to play a role in the consolidation of declarative memories (Axmacher et al. 2008; Bodizs et al. 2002; Isomura et al. 2006; Ji and Wilson 2007; Marshall et al. 2006; Mölle et al. 2004; Wolansky et al. 2006).

One of the problems with studying the SO in natural sleep (and even urethane anesthesia, which allows for the expression of sleeplike state alternations; Clement et al. 2008) is that it is highly transient; individual epochs, especially in the hippocampus, tend to be short-lived (Wolansky et al. 2006). In part, this has been addressed by studying individual instances of time-locked SO waves (Hahn et al. 2006, 2007; Isomura et al. 2006; Massimini et al. 2004), since they can also occur sporadically as K complexes in stage 2 of sleep (Amzica and Steriade 2002; Cash et al. 2009). However, this type of analysis where sampling occurs across different brain states precludes the idea that the SO can show organizational dynamics that are sleep-stage and brain-state dependent. Indeed, some of the controversy concerning the presence or absence of SO-related activity in the hippocampus is likely due to these state-dependent dynamics (Dickson 2010; Wolansky et al. 2006).

Because the neocortical SO was originally characterized using ketamine anesthesia and later extended to both the case of urethane anesthesia and the natural slow-wave sleep state (Steriade et al. 1993), many studies continue to use this preparation since it provides an excellent degree of brain state control in terms of both network dynamics and the maintenance of unconsciousness. Indeed, existing work suggests that at a number of levels, the SO apparent during both ketamine and urethane anesthesia and natural slow sleep shares many characteristics (Amzica and Steriade 1997; Steriade et al. 1993). Not surprisingly, however, some more recent work (Chauvette and Timofeev 2009) has highlighted differences at fine levels of network activity structure within cortical structures. The effect of ketamine anesthesia on hippocampal networks has not been systematically characterized, although there is preliminary evidence to suggest that it may also elicit slow waves in the HPC (Kayama and Iwama 1972; Kloosterman et al. 2004). An obvious advantage of using ketamine to study forebrain-wide SO is that it elicits long-lasting and nearly stationary bouts of SO (i.e., it is stable and only slightly variant with time) at least in the nCTX (e.g., Steriade et al. 1993) and thus might provide a good model for the study of hippocampal SO and cortical–hippocampal interactions during this activity stage.

By recording multisite local field potential (LFP) activity in the HPC and nCTX during both ketamine and urethane anesthesia, we were able to compare the spontaneous SO activity evoked in both cases at these sites and to elaborate on the interactions of both hippocampal and neocortical regions dur-
ing the SO. We found that the SO activity evoked by both types of anesthetics is comparable and thus both are likely to be good models of SO activity during natural sleep.

METHODS

Data were obtained from 19 male Sprague-Dawley rats. The average weight of the rats was 290 ± 12 g. All experimentation conformed to the guidelines established by the Canadian Council on Animal Care and the methods were approved by the Biological Sciences Animal Policy and Welfare Committee of the University of Alberta.

Solutions

A ketamine/xylazine cocktail was made, consisting of a solution of ketamine hydrochloride (100 mg/ml Ketalar; Bimeda-MTC, Cambridge, ON, Canada) mixed in a 10:1 ratio (by weight) with xylazine hydrochloride. The final solution after dilution was 50 mg/ml (ketamine) and 5 mg/ml (xylazine). Ethyl carbamate (urethane) was mixed in a nearly saturated solution at a concentration of 0.8 g/ml.

Surgery, electrode implantation, and recording

Animals were initially taken from their housing cages and placed in an enclosed Plexiglas induction chamber that was filled with gaseous isoflurane mixed with medical oxygen at a minimum alveolar concentration (MAC) of 4. After the loss of postural reflexes, the animals were transferred to a nose cone delivering isoflurane at MAC 2.0–2.5 and anesthesia was maintained at this level. A surgical level of anesthesia was assessed by lack of responsiveness to application of a pinch to the hindpaw. The animals were implanted with a jugular catheter containing either ketamine or urethane and, once inserted, isoflurane administration was discontinued. An anesthetic dose was administered intravenously (iv) (ketamine: 49.1 ± 2.9 mg/kg ketamine, 4.9 ± 0.3 mg/kg xylazine; urethane: 1.66 ± 0.03 mg/kg) by administering a small bolus of solution, 0.01 ml at a time. Once anesthetized, animals were placed in a stereotaxic apparatus.

Using stereotaxic coordinates measured from bregma, holes were drilled in the skull to allow access to the HPC (anteroposterior [AP], −3.3; mediolateral [ML], ±2.1) and frontal regions of the nCTX (AP, +3.2; ML, +2.1). For single electrode recordings, a Teflon-coated stainless steel wire with a bare diameter of 125 μm (A-M Systems, Carlsborg, WA) was implanted at the level of the HPC fissure, or just above it (dorsoventral [DV], −2.7 to −3.3). In addition, an identical wire was placed in the superficial frontal nCTX (DV, −0.25 to −0.85). To obtain a full laminar activity profile, a 16-channel linear multiprobe (MP) with 100 μm spacing between contacts (Neuronexus Technologies, Ann Arbor, MI) was implanted contralaterally to the single HPC electrode (AP, −3.3; ML, −2.1; DV, 0.0 to −4.0).

Single-channel recordings were amplified at a gain of 1,000 and filtered between 0.1 and 500 Hz using a 4-channel AC amplifier (Model 1700; A-M Systems). The MP recordings were amplified at a gain of 1,000 and filtered at a bandwidth between 0.7 and 500 Hz using a 16-channel head stage and amplifier system (Plexon, Dallas, TX). The differential (reference) channel for all recordings was the stereotaxic apparatus. All signals were digitized with a Digidata 1322A A-D board connected to a PC running AxoScope (Molecular Devices, Union City, CA). Signals were all sampled at ≥1,000 Hz.

Stimulation

In some experiments we confirmed the anatomical position of probe channels by stimulating commissural fibers from the contralateral CA3 subfield. In these cases we used a bipolar twisted wire (Teflon-coated stainless steel, 110 μm bare diameter; A-M Systems) electrode targeting the CA3 region (AP, −3.5; ML, −3.5; DV, −3.0 to −4.0).

Stimulation was elicited using an isolated constant-current pulse generator (Model 2100; A-M Systems) with a 0.2 ms biphasic current pulse at an intensity range of 130–200 μA every 8 s. Evoked potentials (EPs) were collected and averaged using ≥16 different stimulation epochs. These potential profiles were compared with previous work (Brankack et al. 1993; Wolansky et al. 2006) and across anesthetics to match the position of each probe channel.

Experimental procedures

Baseline spontaneous recordings were taken for a minimum of 5 min in all cases. These recordings were later used to assess basic spectral characteristics of spontaneous activity. In experiments involving the multiprobe, the position of the probe was adjusted in sequential advancements (500 μm steps) through the nCTX and HPC to a final (tip) depth of 4.0 mm. At each position, recording samples were taken as before. The first placement was made with the tip of the probe at a depth of 1.5 mm from the cortical surface. In the case of ketamine anesthesia, supplemental doses were administered throughout the experiment to ensure a similar level of anesthesia throughout. For urethane, we ensured that we obtained 5 min samples of the deactivated state showing prominent slow oscillations.

In some cases, following a complete profile sequence using ketamine, we carefully and slowly substituted iv urethane anesthesia (final dosage: 1.58 ± 0.03 g/kg) for ketamine. This was completed over a 30- to 40-min period by making repeated infusions of small (0.01 ml) iv bolus injections of urethane while monitoring heart and breathing rates. Once fully anesthetized under urethane (as evidenced by spontaneous state alternations in the LFP signals and a lack of withdrawal responses to hindpaw pinches), recordings were taken as previously described except that the probe was moved dorsally from its last position in 0.5 mm increments until it reached a depth of 1.5 mm. This was done to allow a direct comparison of the spontaneous activity profiles during ketamine to those during urethane in the same animals, permitting a clearer and more accurate comparison of the activity under the two anesthetics.

Following experiments using monopolar recording or stimulating electrodes, small lesions were made at the tips of the electrodes to visualize the tips. The lesions were achieved by passing a 1 mA DC current for 5 s using the isolated constant-current pulse generator. For experiments using the multiprobe, the probe was moved in two planes at its deepest position to make a more visible track.

Histology

Animals were transcardially perfused with physiological saline followed by 4% paraformaldehyde in saline. Brains were extracted and placed in a solution of 30% sucrose and 4% paraformaldehyde overnight. To obtain brain slices, the tissue was first frozen with compressed carbon dioxide and then sliced at a thickness of 48 μm using a rotary microtome (1320 Microtome; Leica, Vienna). Slices were mounted on gel-coated slides and were allowed to dry for ≥24 h before staining with thionin and coverslipping. Sections were viewed under a Leica DM LB2 microscope (Leica) to verify recording sites and digital photomicrographs were taken using a Canon Powershot S45 camera (Canon, Tokyo), which were later imported into Corel Photo Paint (Corel, Ottawa, ON).

Data analysis

Autopower, cross-phase, coherence, and autocorrelation functions were computed using code written in Matlab (The MathWorks, Natick, MA), then plotted using Origin (OriginLab, Northampton, MA) for field signals and certain signal pair combinations. Segments of data no less than 60 s in length were used to compute spectra and autocorrelations for slow oscillatory activity as well as large-amplitude irregular activity (LIA); data segments of 30 s were used to
compute spectra and autocorrelations for higher frequency activity (theta or gamma). Spectra were estimated by using Welch’s periogram method, using a series of 6 s long, sequential Hamming windows with 2 s overlap. Spectral values at the peak frequency were compared across anesthetics. We computed spectral profiles from multiprobe recordings using these same methods. Power values at the peak spectral frequency (the SO fundamental) were extracted and plotted by relative depth. As well, cross-phase and coherence for each channel were computed with respect to both the neocortical and contralateral HPC signal and the values for the SO fundamental were also plotted by depth. This was done for each sequential position of the probe.

Current source density (CSD) analysis was conducted on spontaneous as well as averaged EP profiles from the probe. This analysis eliminates complications due to volume conducted potentials by estimating transmembrane current flow based on computations that followed the logic of previous work (Freeman and Nicholson 1975; Ketchum and Haberly 1993; Rodriguez and Haberly 1989). In brief, the CSD is computed by estimating the second spatial derivative of voltage traces by using a three-point difference on the voltage values across spatially adjacent traces. Thus the differentiation grid size of the estimate is 300 μm because this is the distance spanning three channels of the multiprobe. Mathematically

\[
\text{CSD} = \left( f(p_{i-1}) - 2f(p_i) + f(p_{i+1}) \right) / d^2
\]

where \( f(p_i) \) is the signal obtained from probe channel \( i \) \((i = 2, 3, \ldots, 13 \text{ for } 14\text{-channel recordings}) \) and \( d \) is the distance between adjacent channels (0.1 mm). For traces from each end of the probe \((i = 1, 14)\), the differentiation grid was based only on the immediately adjacent channel \((i = 2, 13)\). We confirmed that the latter procedure yielded similar, if not identical, CSD results as the three-point differentiation method by successively eliminating probe end channels, then recomputing and comparing results. CSD traces were also analyzed using the same laminar spectral methods as described earlier.

Due to concerns about possible spatial mismatches of channel positions across the anesthetic conditions, we also simultaneously compared the distribution of CA3 stimulation EPs (within the HPC) as well as the spectral profiles of faster (gamma: 30–40 Hz) activity across both ketamine and urethane. Since the arrangement of these types of laminar profiles constitutes characteristic physiological markers of specific anatomical layers (in the case of EPs, the synaptic termination zones of known inputs and, in the case of gamma profiles, the localization of dense cellular layers), mapping these profiles across conditions allowed us to make any necessary adjustments corresponding to depth across the two conditions. Confirmation of the spatial localization of individual probe channels was also confirmed by mapping the spectral profile of theta rhythm under urethane, which shows a reversal at the beginning of stratum (s.) radiatum, just under s. pyramidale. If necessary, spatial adjustments were made across anesthetic data sets to ensure that the relative positions of probe channels matched up.

Gamma frequency profiles were conducted by extracting power values in the bandwidth of 30–40 Hz from spectra computed across all channels of the probe. This bandwidth was used since this was the range within the gamma band (30–80 Hz), with the highest peak power and greatest difference between anesthetics at our level of temporal resolution. By plotting these values as a function of depth, we were able to localize characteristic topographical regions of local maxima separated by minima. These regions corresponded to reversal points of gamma activity as measured by cross-phase measurements (compared with a fixed channel on the probe) and were thus likely to be densely packed cell layers such as the dentate granule cell layer (s. granulosum) and the CA1 pyramidal cell layer (s. pyramidale). These profiles were compared and, if necessary, adjusted for alignment across both ketamine and urethane conditions.

**RESULTS**

**Histological findings**

We confirmed the location of all recording and stimulation electrode locations. All CA1 recording positions were at, or close to, the level of the hippocampal fissure, in either the most apical dendritic zones of CA1 (s. lacunosum molecular [SLM]) or in the s. molecular layer of the dentate gyrus. Stimulation sites in the contralateral hippocampus were found in CA3 at the level of s. pyramidale. Multiprobe tracks were all in a plane that passed through the CA1 pyramidal cell layer, through the hippocampal fissure, and into the dentate gyrus. Summary placements and tracks for ketamine–urethane experiments as well as four of the seven urethane-only experiments are shown in Fig. 1. Three urethane-only experiments did not have histology available; however, based on electrophysiological criteria obtained during these experiments [LFP profiles in addition to the observation of multitunit (cell layer) activity when lowering electrodes] we are certain that they were at or near the hippocampal fissure in the CA1/dentate gyrus plane.

**Raw LFP characteristics of ketamine activity**

As others have previously shown (Amzica and Steriade 1997; Mölle et al. 2006), the ongoing and spontaneous neocortical LFPs in all animals anesthetized with ketamine showed a prominent and relatively stationary slow rhythm, with an average peak frequency of 1.67 ± 0.07 Hz. Concomitant with this cortical SO, a highly similar slow rhythmic activity also dominated the LFP recorded at the level of the hippocampal fissure (Fig. 2A). Across all experiments, the peak frequency of the HPC SO under ketamine was 1.62 ± 0.07 Hz. The peak frequencies of the SO in the HPC and nCTX in ketamine-anesthetized animals were not significantly different \( t(11) = 1.13, P = 0.28 \). This suggested that the SO peak represented a common mode of activity across forebrain structures. This idea was supported by high coherence values for the SO peak across hippocampal and neocortical sites \((0.92 \pm 0.02)\).

Qualitatively, this SO activity was similar to that observed in animals anesthetized with urethane only (Fig. 2B), although the latter tended to show a slightly lower average peak frequency \((1.02 \pm 0.02 \text{ Hz})\). Across anesthetic groups this peak frequency was significantly different \( t(17) = 6.01, P < 0.05 \). A
Further difference compared with ketamine was that the coherence of the SO activity across neocortical and hippocampal sites was lower (0.77 ± 0.08) and this difference was significant \( t(17) = 2.22, P < 0.05 \).

Similar differences were observed across anesthetics for experiments in which animals were anesthetized first with ketamine and then subsequently with urethane. The peak frequency of the SO was significantly higher under ketamine (1.77 ± 0.08 Hz) than that under urethane (1.17 ± 0.07 Hz) \( t(4) = 6.10, P < 0.05 \) (Fig. 3A). In addition, the average peak power of the SO was larger during ketamine (125.4 ± 24.99 mV^2) compared with that during urethane (78.38 ± 18.90 mV^2), although this difference was not significant \( t(4) = 2.09, P = 0.10 \) (Fig. 3B). We also observed a larger coherence value across neocortical and hippocampal sites for ketamine (0.96 ± 0.01) as opposed to urethane (0.87 ± 0.06, \( t(4) = -2.98, P < 0.05 \) (Fig. 3C). However, coherence values for the SO across homotopic points in the hippocampus were equally high across ketamine (0.97 ± 0.01) and urethane (0.92 ± 0.04) anesthesia and these values were not significantly different \( t(4) = 2.09, P = 0.10 \). The difference in neocortical–hippocampal coherence across anesthetics may be related to the fact that ketamine produces only stereotyped SO activity with no state alternations, whereas the SO under urethane is short-lived and gives way to both LIA as well as theta activity in the HPC. In the current study, the proportion of time spent in SO under urethane compared with other states was roughly half (i.e., 52 ± 5%). In ketamine, the proportion of time spent in SO was 100%.

Another prominent feature of the electrographic activity during ketamine was fast activity (gamma). This activity had a frequency range of 30–40 Hz across all experiments. Interestingly, the power in the gamma bandwidth was found to make up 4.7% of the total power across the spectrum under ketamine, whereas during urethane this proportion was significantly lower (2.0%) \( [t(4) = 2.96, P < 0.05] \).

**Laminar profile of the SO**

To better ascertain any potential differences of the SO evoked by ketamine versus urethane, we examined the laminar distribution of LFPs using the 16-channel linear multiprobe across its sequential positions through both nCTX and HPC. As has been previously shown, the raw signals corresponding to the SO under ketamine reversed phase completely in the nCTX in deeper layers (Steriade et al. 1993) (Fig. 4B). Indeed, this was also the case under urethane as we and others have previously shown (Steriade et al. 1993; Wolansky et al. 2006). As well, within the HPC, a local increase in the amplitude of the SO was observed in both the ketamine and urethane cases. This amplitude increased as the probe approached the interface between CA1 and the dentate gyrus (Fig. 4C). This suggests that the SO field potential profiles from the HPC were at least
partially the result of active current flow occurring close to the hippocampal fissure, despite the fact that no reversal point was observed within the HPC itself.

Using the same overlapping profiles in the same animals at incremental depths through the brain, we constructed complete laminar spectral profiles for the SO across both anesthetic conditions (Fig. 5). As shown, abrupt reversal points (as observed by a clear power minimum, accompanied by a phase reversal and a drop in coherence) were observed in the deep layers of the nCTX and a prominent maximum was observed concurrent with a phase shift in the middle of the HPC. Noticeably, the qualitative shapes of the profiles were highly similar across conditions. Indeed, any differences across experiments were within the spatial resolution of the probe (±0.1 mm). A more quantitative measure of the similarities between ketamine and urethane was obtained by computing the correlation between the amalgamated power profiles (averaged across overlapping depths) between the two conditions. The average correlation value for this comparison was \( r = 0.80 \pm 0.06, P < 0.05 \). This confirms that the shape of spectral power profiles was highly similar across both anesthetics.

**Current source density analysis**

To eliminate any possible influences of volume conduction in the local field potential recordings within the HPC, we computed the CSD. In certain cases, and as shown in Fig. 6A, we confirmed the alignment of probe channels across both anesthetic conditions by comparing the EP profiles elicited by stimulation of contralateral CA3. As can be observed (and as was the case in most experiments) the alignment of both the peak maximal negativity in the average potentials, in addition to the position of the major sinks evoked by stimulation, were well matched. When alignment was out by ≥100 μm, the spatial positions of traces were adjusted accordingly before comparison of spontaneous traces. As shown in the remaining panels in Fig. 6 and, indeed, across all experiments, the distribution of SO potentials and the computed CSD were highly similar across ketamine and urethane. The region showing the most prominent alternation of sinks and sources in both cases was consistently at the level of SLM. This is similar to results reported previously for both urethane and for natural sleep (Isomura et al. 2006; Wolansky et al. 2006) and suggests that current flow may be due to rhythmic SO inputs arriving at this dendritic layer.

By computing the spectral power of the spontaneous CSD at the peak SO frequency and plotting these values by depth we were able to show that these were also similar across anesthetics. As shown in Fig. 6E the qualitative shape of these power profiles was highly comparable in individual experiments and was quantitatively assessed by computing their linear correlation across depth. On average, the correlation across experiments was both high (\( r = 0.77 \pm 0.05 \)) and significant (\( P < 0.05 \)) in every case (\( n = 5 \)). A subtle difference between the two conditions was that the amplitude of the sink/source alternations produced under ketamine appeared larger than those produced during urethane. By taking the spectral power of these traces at the level of SLM, these differences were found to be significant: ketamine, \( 1.68 \pm 0.07 \text{ mV}^2/\text{mm}^4 \); urethane, \( 1.12 \pm 0.13 \text{ mV}^2/\text{mm}^4 \) \( [t(4) = 9.96, P < 0.05] \). Differences also existed in the coherence values calculated between the nCTX LFP and the CSD trace at the level of SLM. Under ketamine, the coherence between these two signals was 0.74 ± 0.05. However, under urethane the coherence value dropped to 0.30 ± 0.09. This difference was significant \( [t(4) = −3.41, P < 0.05] \).

**DISCUSSION**

Ketamine anesthesia has long been known to promote the SO in the nCTX (Amzica and Steriade 1997; Steriade et al. 1993). In the present study we have additionally shown that in this condition, the SO is a prominent feature of the electrographic activity of the HPC as well and that this local activity has a coherent relationship with ongoing neocortical SO that is similar, albeit stronger, to the case in both slow-wave sleep and under urethane anesthesia (Isomura et al. 2006; Wolansky et al. 2006). The laminar and CSD profiles of the SO within the HPC across ketamine and urethane anesthesia were also highly similar, suggesting that the neural mechanisms underlying both are comparable and thus both are likely good models of SO activity during natural sleep as well.

**SO mechanisms**

Throughout the forebrain, the SO cycle is manifested by repeating periods of depolarization (activity) and hyperpolar-
Our own work (Wolansky et al. 2006) in addition to that of others (Isomura et al. 2006) has previously demonstrated the subtle differences certainly exist between the anesthetized and naturally sleeping conditions (Chauvette and Timofeev 2009). Although CA pyramids may or may not show prominent UP and DOWN membrane potential fluctuations from the entorhinal cortex (Isomura et al. 2006; Wolansky et al. 2006). Indeed, the majority of single units in all hippocampal subfields are highly modulated by the slow rhythm (Wolansky et al. 2006). Although CA pyramids may or may not show prominent UP and DOWN membrane potential fluctuations (cf., Tsukamoto-Yasui et al. 2007), this has definitely been found in dentate gyrus granule cells and stratum lacunosum moleculare interneurons (Hahn et al. 2006, 2007; Isomura et al. 2006). This suggests that the hippocampal SO may be intimately dependent on cortical input, although it does not necessarily explain its dynamic coordination with neocortical ensembles.

Previous work has already established the similarities of neocortical SO across ketamine and urethane anesthesia to that found in natural sleep (Steriade et al. 1993, 2001), although subtle differences certainly exist between the anesthetized and naturally sleeping conditions (Chauvette and Timofeev 2009). Our own work (Wolansky et al. 2006) in addition to that of others (Isomura et al. 2006) has previously demonstrated the similarity of the hippocampal SO across urethane anesthesia and natural sleep. The present study has demonstrated that the profile and, indeed, the extracellular current flow that generates the hippocampal SO LFP are highly similar across ketamine and urethane. In both cases, it appears that strong and presumably excitatory afferent synaptic input arriving at the SLM is responsible for the rhythmic SO LFP within the hippocampus. It would be of substantial interest to determine whether this is mediated through neocortical inputs via the entorhinal cortex, which project to this very region (Lavenex and Amaral 2000; Witter et al. 1989, 2000). A suggestion for future research is to study the dynamics of single- and multunit activity in the HPC under ketamine in addition to the intracellular dynamics of hippocampal cells. We presume that given the stability and enhanced synchrony between hippocampal and neocortical sites that SO-related cellular activity will be much more prominent than that during either urethane or natural sleep (Isomura et al. 2006; Wolansky et al. 2006).

As well, intracellular studies would be of benefit to assess slow fluctuations in the infraslow range (<0.5 Hz), a range that was not explicitly examined here. Although it is possible that there may be differences between ketamine and urethane in the infraslow range, this is unlikely because prior work examining intracellular dynamics during in vivo recordings under both ketamine and urethane in the nCTX did not find any systematic differences across the two anesthetics (Steriade et al. 1993). In addition, previous work in the HPC using DC-coupled amplifiers to study infraslow activity found significant contributions only in this frequency range across state changes themselves (Brankack et al. 1993). Since our work was focused on a single state in the HPC (the SO), it is unlikely that infraslow activity...
contributed significantly to the phenomenon under study. However, all avenues will doubtless further our understanding of the relevance of the SO to hippocampal processing.

Given the similarities shown in the present study across ketamine and urethane anesthesia, our results suggest that both yield an appropriate model for the natural SO state in the forebrain. This is despite the differences in the known pharmacological actions of these drugs. Urethane is known to promote resting K+ conductances (Śceniak and Maciver 2006), as well as depressing both glutamatergic and GABAergic neurotransmission (Hara and Harris 2002), whereas the major action of ketamine is to antagonize N-methyl-D-aspartate (NMDA) receptor mediated transmission (Harrison and Simmonds 1985). It should be noted, however, that the in vivo anesthetic mechanisms of both these drugs (and for that matter, any anesthetic agent) are less well understood. However, the implication of these similarities is that promoting a condition of unconsciousness via both natural and anesthetic means induces a spontaneous and internally generated SO state throughout the forebrain.

Advantages of ketamine anesthesia

One of the problems with studying the SO in natural sleep and during urethane anesthesia is the very limited timeframe of each epoch. The current work provides a potential solution to this problem because ketamine appears to elicit a long-lasting and consistent SO state in the forebrain. This facilitates the study of the generation and interaction of the SO across the nCTX and HPC. An additional benefit of ketamine over urethane is its relatively high rate of metabolism and low toxicity. This does not preclude its use for chronic behavioral studies, as is the case with urethane. Preliminary work on the influence on ketamine itself (but not the SO) on information processing in the neocortico–hippocampal axis has already begun, specifically addressing memory interference (Chrobak et al. 2008). Certainly the present work could be used as a framework for assessing the role of the SO induced by ketamine in terms of information processing and consolidation. This would obviously have to be independent of its known pharmacological action to inhibit NMDA-receptor mediated transmission (Harrison and Simmonds 1985) and the demonstrated role of these receptors in long-term potentiation (LTP) (Harris et al. 1984). However, the role of synchrony between structures as well as synaptic plasticity that is dependent on synchronous activity (and not on NMDA receptor stimulation), even at slow frequencies (Habib and Dringenberg 2009), could reasonably be investigated.

Conclusions

Our findings support the use of ketamine anesthesia as a useful model for the SO in the forebrain, including both the hippocampal- and neocortices. Moreover, they suggest that the SO is a common mode of operation of the forebrain during both natural and anesthesia-induced unconsciousness. Further work will undoubtedly ameliorate our understanding of the neural mechanisms and pathways underlying this activity during natural sleep, eventually leading to a better understanding of its function in terms of brain operation during subsequent wakefulness.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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