Slowing of the Hippocampal $\theta$ Rhythm Correlates with Anesthetic-induced Amnesia

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ABSTRACT

Background: Temporary, antegrade amnesia is one of the core desirable endpoints of general anesthesia. Multiple lines of evidence support a role for the hippocampal $\theta$ rhythm, a synchronized rhythmic oscillation of field potentials at 4–12 Hz, in memory formation. Previous studies have revealed a disruption of the $\theta$ rhythm at surgical levels of anesthesia. We hypothesized that $\theta$-rhythm modulation would also occur at subhypnotic but amnestic concentrations. Therefore, we examined the effect of three inhaled agents on properties of the $\theta$ rhythm considered critical for the formation of hippocampus-dependent memories.

Methods: We studied the effects of halothane and nitrous oxide, two agents known to modulate different molecular targets (GABAergic $[\gamma$-aminobutyric acid] vs. non-GABAergic, respectively) and isoflurane (GABAergic and non-GABAergic targets) on fear-conditioned learning and $\theta$ oscillations in freely behaving rats.

Results: All three anesthetics slowed $\theta$ peak frequency in proportion to their inhibition of fear conditioning (by 1, 0.7, and 0.5 Hz for 0.32% isoflurane, 60% $N_2O$, and 0.24% halothane, respectively). Anesthetics inconsistently affected other characteristics of $\theta$ oscillations.

Conclusions: At subhypnotic amnestic concentrations, $\theta$-oscillation frequency was the parameter most consistently affected by these three anesthetics. These results are consistent with the hypothesis that modulation of the $\theta$ rhythm contributes to anesthetic-induced amnesia.

What We Already Know about This Topic
- Hippocampal $\theta$ rhythm, a characteristic oscillation at a frequency of 4–12 Hz, has been associated with memory formation
- Disruption of this rhythm impairs learning and memory

What This Article Tells Us That Is New
- Subhypnotic concentrations of three inhaled anesthetics with distinct molecular actions slowed hippocampal $\theta$ rhythm in proportion to their inhibition of fear conditioning
- Slowing of hippocampal $\theta$ rhythm is a possible mechanism for disruption of hippocampus-dependent memory by anesthetics

A MNESIA is one of the essential desirable elements of the anesthetic state along with unconsciousness (hypnosis) and immobility. The postanesthesia recall of contextually rich (episodic) memories in particular is highly undesirable and a potential cause of morbidity.

Behavioral anesthetic effects are attributed to interactions with specific proteins (as opposed to nonspecific effects on lipid membranes). Indeed, anesthetic interactions with numerous plausible molecular targets have been and continue to be documented extensively.1,2 Clinically used inhalational anesthetics are chemically diverse. Despite different receptor-level activity profiles and potencies, all inhaled anesthetics impair learning and memory at concentrations that are subhypnotic and, typically, are only a fraction of the standard concentration required for immobility in surgery.3–6 For example, both the alkane halothane, which markedly enhances $\gamma$-aminobutyric acid receptor type A-mediated inhibition, and the non–$\gamma$-aminobutyrategric (non-GABAergic) gas nitrous oxide suppress inhibitory-avoidance training at comparable lipid solubility–corrected concentrations.7 Likewise, the anesthetic isoflurane and the nonimmobilizer 1,2-dichlorohexafluorocyclobutane (F6 or 2N, an experimental drug)
supress fear conditioning (FC) at similar concentrations.\textsuperscript{5,8} It is noteworthy that both substances are more potent inhibitors of FC to context (hippocampus-dependent) than FC to tone (hippocampus-independent).\textsuperscript{5,8} The preferential sensitivity of hippocampus-dependent learning to suppression by anesthetic-like compounds is not related to specific molecular targets in any obvious pattern. Isoflurane enhances GABAergic activation of the cerebrum and inhibits glutamatergic synaptic transmission\textsuperscript{9,10} and blocks hippocampal long-term potentiation\textsuperscript{11} whereas F6 has no known effect on these processes.\textsuperscript{12,13}

These observations prompt the question of whether all anesthetics similarly affect a single amnesia-promoting molecular target or whether aggregate actions on different targets converge at some higher level of signal integration of particular importance for hippocampal learning and memory. The experiments presented here investigate the latter possibility.

The hippocampal \( \theta \) rhythm is a prominent network activity of the “online” hippocampus and can be separated into atropine-sensitive (type 2) and atropine-resistant (type 1) components. Type 1 \( \theta \) is suppressed by surgical levels of anesthesia.\textsuperscript{14} Indeed, based on this observation, it was proposed more than 30 yr ago that suppression of this noncholinergic activation of the cerebrum may mediate the behavioral effects of anesthesia.\textsuperscript{14} Since then, substantial evidence has accumulated demonstrating that the \( \theta \) rhythm serves an essential network-level role in hippocampal learning and memory.\textsuperscript{15,16} For example, \( \theta \) oscillations facilitate plasticity\textsuperscript{17} and support mnemonic processes requiring interregional signal integration.\textsuperscript{18–20} Conversely, suppression of the \( \theta \) rhythm impairs learning and memory.\textsuperscript{21–25}

We hypothesized that modulation of type 1 \( \theta \) oscillations might serve as a common network-level mechanism of anesthetic-induced impairment of hippocampus-dependent learning and memory. If this hypothesis were correct, some measure of \( \theta \) activity should vary with anesthetic concentrations in the amnestic (but subhypnotic) range. We tested this hypothesis by analyzing the effect of three inhaled anesthetics on type 1 \( \theta \) oscillations. We found that \( \theta \) frequency—but not other parameters of the \( \theta \) rhythm—changed systematically with anesthetic dose. We conclude that these results are consistent with the hypothesis that slowing \( \theta \) frequency correlates with suppression of hippocampus-dependent learning and memory.

### Materials and Methods

#### Behavioral Experiments

Experiments were approved by the University of California, San Francisco, Institutional Animal Care and Use Committee. Naive adult (aged 70–90 days) male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used in these studies. The rats were housed 3 to a cage (\( 42 \times 25 \times 20 \) cm) with food and water available \textit{ad libitum}. The rats were kept on a 12-h light-dark cycle, with transitions occurring at 6:00 AM/PM. All experimental procedures took place during the light cycle. All rats were handled daily (~20 s) for a week before each experiment.

An equilibration chamber, which was similar to the rats’ home cages, was used for anesthesia delivery before FC. It was covered with a plastic slab pierced with two small conduits that allowed inflow and sampling of gases. A 17-cm diameter hole in the slab was capped by a \( 20 \times 17 \)-cm plastic cylinder. This chimney-like structure allowed the escape of excess gases as well as rapid movement of rats into and out of the equilibration chamber with minimal disturbance to anesthetic concentrations. Gas inflow was delivered to the circuit in a 5 l/min oxygen flow through a vaporizer (Isotec 4; Datex, Los Angeles, CA) set to the desired concentration. We used a gas chromatograph (Gow-Mac Instrument Co., Bethlehem, PA) equipped with a flame ionization detector to measure concentrations of inhaled anesthetics. The 4.6-\( \times \)0.22-cm column was packed with SF–96. The column temperature was 100°C. The detector was maintained approximately 50°C warmer than the column. The carrier gas flow was nitrogen at a flow of 15–20 ml/min. The detector received 35–38 ml/min hydrogen and 240–320 ml/min air. Primary standards were prepared, and response linearity of the chromatograph was determined. We also used secondary (cylinder) standards referenced to primary standards. An infrared analyzer (Datex Instrumentarium Corp., Helsinki, Finland) was also used to continually monitor anesthetic concentrations in addition to the sampling described above.

The FC chambers (32 \( \times \) 25 \( \times \) 25 cm) were constructed of clear acrylic. These airtight chambers allowed the continuous delivery of inhaled anesthetics at a constant concentration by vaporizer. Two 2-cm diameter conduits on opposing sides of the chamber allowed inflow and the outflow of gases to all four chambers. The grid floor used to deliver shock was composed of 19 stainless steel bars, each 4 mm in diameter, spaced 16 mm center to center. These floors were connected to a shock delivery system (Med Associates, Inc., St. Albans, VT). The chambers were wiped down with a pine-scented cleaner (5% pine-scented disinfectant; Midland, Inc., Sweetwater, TN) before and after each session. In the room where training took place, the overhead fluorescent bulbs were left on and a ventilation fan provided background noise (65 dB). The appearance, odor, and texture of the chambers and room comprised the training context.

Four rats, counterbalanced for group assignment, were trained at a time. After a 3-min baseline exploratory period in the chambers, rats received three 30 s tones (2,000 Hz, 90 dB) coterminal with a shock (1 mA, 2 s) pairings, separated by 60 s and were removed from the chamber after an additional 30 s.

The next day, rats were tested for fear to the training context and fear to tone without anesthetics. For the context test, each rat was placed back in the chamber where it was trained for 8 min in the absence of tone and shock. For the tone test, groups of four rats were transported in separate plastic pots (\( 14 \times 15.5 \) cm) to a different context in a separate room. Test chambers, which were equipped with a
speaker, were triangular with an acrylic floor (28 × 25 cm) and two acrylic sidewalls (28 × 22 cm) at a 45° angle. Chambers were wiped down with acetic acid (1%; Thermo Fisher Scientific, Waltham, MA) before and after each training session. The room appeared dark to the rats, being lit by a single red 30-W red bulb. White noise (65 dB) was used for background noise. The order of the context and tone tests was counterbalanced, so that half of each treatment group was tested to context first, tone second, and vice versa. "Freezing," defined as the absence of all movement except that necessary for respiration, is an innate defensive fear response in rodents and a reliable measure of learned fear.24,25 Each rat’s freezing behavior was scored by an observer blinded to the treatment history every 8 s during the observation period (i.e., 60 observations per rat for each experiment). A percentage was calculated by dividing the number of freezing observations by the total number possible during the observation period. Experiments were conducted with separate groups of eight rats for each of the three agents as summarized in table 1. One rat in the 1% halothane group died during the equilibration portion of the experiment before training, leaving one group with seven rats. We considered the minimal alveolar concentration (MAC) for halothane, isoflurane, and nitrous oxide to be 1.1, 1.38,26 and 221%.27

### Statistical Analysis

For FC, we used nonlinear regression (SPSS, Chicago, IL) to calculate EC<sub>50</sub> values and the maximum value of the dose-response curve for context- and tone-conditional freezing.

The following equation was used in the regression: freezing = A × (isoflurane<sup>n1</sup>/isoflurane<sup>n1</sup>/EC<sub>50</sub>), where freezing indicates the percentage of time rats displayed freezing behavior during the observation period, A is maximal freezing value, and n1 is the Hill coefficient. Results of FC experiments are presented as mean ± SE.

### Electrophysiological Experiments

All experiments were conducted according to guidelines specified in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) and were approved by the University of Wisconsin Animal Care and Use Committee (Madison) and have been described previously.28

### Animal Husbandry

Young adult male Sprague-Dawley rats were housed in the animal care facility of the University of Wisconsin with free access to food and water. Thirteen rats underwent electrode implantation. After electrode implantation, the animals recovered for at least 7 days before being exposed to volatile agents. After each exposure, animals were observed until full recovery and then transferred back into their home cages. Each animal was exposed to an agent only once per day.

### Electrode Preparation

Linear microwire array recording electrodes with four sites spaced 200 μm apart were fabricated from 30 μm formvar-insulated nichrome wire.29 In brief, a piece of polytetrafluoroethylene tubing of 300 μm ID was cut along its long axis, and one half of the tubing was used as a form for the electrode array. Four holes were punched in the tubing at 200-μm intervals, and a single piece of 35 μm formvar-insulated NiChrome wire (CFW-188-0012-HFV; California Fine Wire Co., Grover Beach, CA) was passed through each hole. Each wire was bent at a 90° angle to lie parallel to the inside wall of the form. The hemicylinder of tubing with the microwire bundle was filled with epoxy resin (Epoxylite 6001-M; Epoxylite Corp., St. Louis, MO) and heat cured. After discarding the polytetrafluoroethylene tubing, the hardened resin hemicylinder formed the shank of the electrode, and the wires were then broken off at right angles to the long axis of the array to form recording sites. Finally, the resin matrix was sharpened at the tip to facilitate pen-
etration of the brain, with the closest electrode approximately 25 μm away from the tip. The bare wires of the electrode array were connected to a Neuralynx EIB-16 interface board with an Neuralynx HS-16 Omnetics nanoconnector (Neuralynx, Inc., Tucson, AZ) suitable for connecting to a small headstage.

**Electrode Implantation**

For implantation, animals were anesthetized with isoflurane and placed in a stereotactic apparatus. Miniature stainless steel machine screws were inserted through the right and left frontal, right parietal, and left occipital bones and advanced until contact was made with the dura. They served as anchors for the electrode assembly and as surface electrodes, with the screw in the occipital bone used as animal ground for reference. An additional hole was drilled 3.0 mm caudal to bregma and 2.0 mm lateral to the midline. The electrode array was advanced 2.6 mm below the surface of the cortex. After an animal had undergone all planned experiments, it was sacrificed to verify electrode location. Histologic examination showed that electrodes were successfully implanted into the CA1 region of the dorsal hippocampus in eight rats and the signal from the electrode closest to the hippocampal fissure was used for field-potential analysis.

**Experimental Procedures**

On the day of an experiment, the animal was placed into a custom-made 10-l chamber made of clear acrylic and equipped with ports for drug injection, gas sampling, and connection to the recording equipment. The footprint of the area that could be explored by the rat was 19 × 15 cm, which did not allow running. Adhesive tape sealed the lid of the chamber and all ports. Five minutes of acclimation were followed by a 15-min control period, after which a loading dose of the drug to be tested was injected. Distilled water was given animal. The number of 4,096-point segments under-

**Behavioral Scoring**

For electrophysiological experiments, the observer classified behavior as immobile, exploring, grooming, or undefined. Exploring was considered any behavior that involved movement of the animal’s head or body that was not grooming-related, and included walking, sniffing, or manipulating any of the objects present in the tray. Grooming included scratching as well as face and paw washing. Immobile did not include assumption of the sleep posture (curling up), which was infrequent and classified instead as undefined. In practice, only “exploring” and “immobile” behaviors, corresponding to type I and type II behaviors, respectively, were observed with sufficient duration and frequency for data analysis.

**Data Acquisition and Analysis**

Electroencephalographic signals were amplified using a unity-gain HS-16 headstage preamplifier to reduce movement-related artifacts with a Lynx-8 s-stage amplifier (both from Neuralynx, Inc.) bandpassed between 1 and 325 Hz and digitized at 1,000 Hz using a Digidata 1200 A/D converter (Molecular Devices, Sunnyvale, CA). Data acquisition and processing were controlled with the pClamp software suite (Molecular Devices) and stored for analysis on a Pentium-based personal computer.

Data analysis was performed primarily with custom-written routines in MATLAB (MathWorks, Natick, MA). Origin (OriginLab Corp., Northampton, MA) and Instat (GraphPad Software, Inc., La Jolla, CA) were used for graphical presentation and statistical analysis. In a preprocessing step, raw data were passed through a digital bandpass filter with an attenuation of 40 dB/octave (IIR Butterworth and MATLAB filtfilt forward and reverse filtering routine resulting in zero phase shift) designed for the extraction of signals in the θ-frequency band. The −3 dB (corner) frequencies were 4 and 12 Hz. Artifacts occurred primarily from mechanical causes and were excluded from analysis together with adjacent data points (0.5–1 s, as necessary). Subsequently, data were sorted by behavior. Raw and filtered data were subdivided into segments of 4,096 points each (~4 s) with 30% overlap (1,365 points). Shorter data segments were not analyzed. All parameters described below were computed for each segment and averaged with other segments obtained for the same drug condition and behavior for a given animal. The number of 4,096-point segments underlying each spectrum ranged from 1 (isoflurane at high concentrations) to more than 100 (drug-free control, 30% N2O) and is indicated for each spectrum shown.

For spectral analysis, we multiplied data segments with a Hamming window (4,096 points) and computed their fast
Fourier transforms, from which power spectral density was derived (i.e., generally corresponding to the Welch method). From the power spectral density, we extracted the power in distinct frequency bands (the integral of the power spectral density within the frequency ranges detailed above) as well as amplitude and frequency of the $\theta$ peak. Autocorrelograms were produced with a modified MATLAB routine (xcorr) and were normalized such that autocorrelations at zero lag were identical to 1.

**Statistical Analysis**

Unless indicated otherwise, all numerical results are expressed as changes relative to time- and behavior-matched control experiments. Only animals exposed to a specific drug were included in that drug’s control group. Linear regression lines were fitted according to the model: $Y = A + (B \times X)$, with the parameters $A$ (intercept) and $B$ (slope) estimated by the least squares method. The correlation between drug concentration and change in the measured parameter was considered significant if the null hypothesis of zero slope could be rejected at a $P$ value of less than 0.05. We assumed a normal distribution for all $\theta$-related variables. Excel (Microsoft Corp., Redmond, WA) and Origin were used for statistical analysis. Results are reported as mean $\pm$ SD. We compared drug effects on $\theta$ parameters using two-tailed paired $t$ tests.

**Results**

**Three Diverse Anesthetics Differentially Suppress FC**

We examined the amnestic potency of isoflurane, halothane, and nitrous oxide by testing animals at drug concentrations that, based on published data and our own preliminary results, we expected to bracket the EC$_{50}$ concentrations for FC to context and to tone. As shown in figure 1, all three anesthetics incrementally suppressed freezing to context and tone, reaching essentially complete suppression of learning and memory at the highest concentrations tested. For all three drugs FC to context was more sensitive to inhibition than FC to tone. In percentage of inspired gas, EC$_{50}$ concentrations derived from the concentration-response relationships for halothane, isoflurane, and nitrous oxide were 0.14 $\pm$ 0.09, 0.28 $\pm$ 0.03, 42 $\pm$ 3, respectively, for FC to context and 0.30 $\pm$ 0.05, 0.43 $\pm$ 0.05, 57 $\pm$ 6, respectively, for FC to tone.

**Separation of $\theta$ versus Non-$\theta$ States**

Electrophysiological data were obtained from eight animals in which we confirmed the location of the recording electrode by histology congruent with local field potential patterns. The behavioral state determines the pattern of hippocampal local field potentials in the awake, unrestrained animal. Type 1 $\theta$ rhythm is typically present during behaviors generally described as “exploratory.” Our behavioral classification of “exploring” versus “immobile” allowed a separation of high and low $\theta$ states as illustrated in figure 2. During im-

![Fig. 1. Inhaled agents suppress fear conditioning at subanesthetic concentrations.](http://anesthesiology.pubs.asahq.org/pdfsaccess.ashx?url=/data/journals/jasa/931101/)
mobility, local field potentials in the hippocampus were characterized by irregular, low frequency activity (fig. 2A). The irregular nature of the fluctuations is reflected in the rapid damping of the side-peaks in the autocorrelogram (fig. 2B). By contrast, during behavior classified as “exploring,” the hippocampal electroencephalogram was characterized by prominent, rhythmic activity in the \( \theta \) band (fig. 2C). The regularity of this oscillation is illustrated by the presence of multiple peaks in the autocorrelation (fig. 2D). Theta peak frequency under drug-free control conditions was \( 7.3 \pm 0.24 \) Hz for “exploring.” Peak frequency observed during “immobile” was slower, at \( 6.2 \pm 0.3 \) Hz (7.1–7.6 and 5.6–6.8 Hz, \( n = 8; P = 0.00018 \)), indicating effective separation of behavioral states with respect to the \( \theta \) rhythm with type 2 (dominant during “immobile”) slower than type 1. Only \( \theta \) exploring (type 1) was used for further analysis.

\( \theta \) Rhythm Persists under Amnesia-inducing Conditions

Exploratory activity occurred in the presence of memory-impairing concentrations of anesthetic agents accompanied by prominent rhythmic activity in the \( \theta \) band (fig. 3A). Power spectra obtained from these experiments reveal that peak amplitude and power (area under the curve) were not substantially altered, but that the frequency of the \( \theta \) peak was slowed by isoflurane, nitrous oxide, and halothane (fig. 3B). The illustrated example shows the effect of the drugs at concentrations that suppress hippocampus-dependent memory to a comparable degree (fig. 1).

\( \theta \) Frequency Is Reduced by Diverse Agents

All tested anesthetic agents caused a concentration-dependent slowing of \( \theta \) peak frequency as shown in the spectra of figure 4A–C. For all anesthetics, we observed significant slowing at the lowest concentration producing amnesia. Isoflurane had the most pronounced effect on peak frequency: \( 0.32 \pm 0.03 \% \) slowed the \( \theta \) peak frequency by 1 Hz (7.3 \( \pm 0.2–6.3 \) \( \pm 0.4 \) Hz, \( n = 7; P = 0.00029 \)); 60% \( \text{N}_2\text{O} \) slowed the \( \theta \) peak by 0.7 Hz (7.4 \( \pm 0.2–6.7 \) \( \pm 0.2 \) Hz, \( n = 8, P = 0.0013 \)); and halothane (0.24 \( \pm 0.01 \% \)) reduced the frequency by 0.5 Hz (7.4 \( \pm 0.2–6.9 \) \( \pm 0.1 \) Hz, \( n = 6, P = 0.0108 \)).

To facilitate the comparison between agents with different potencies, we normalized the effect on \( \theta \) frequency to the individual agents’ amnestic \( EC_{50} \) for inhibition of FC to context as determined in behavioral experiments. The results for all experiments are summarized as a concentration-response relationship in figure 4D. The correlation coefficients for all three least-squares linear fits were similar and significantly different from zero (\( R = 0.84, 0.80, \) and 0.80 for isoflurane, nitrous oxide, and halothane, \( P \) value of less than 0.001 for each). The slopes of the regression lines were steeper for isoflurane and nitrous oxide (–0.59 and –0.5) than for halothane (–0.19).

Anesthetic Effects on \( \theta \) Power and Rhythmicity

To test for a relationship with respect to anesthetic-induced amnesia, we analyzed three measures of \( \theta \) power (amplitude of the peak, the spectral power within \( \pm 1 \) Hz of the peak, and the power in the 4–12 Hz band) analogously to \( \theta \) frequency and grouped them by concentration. Summaries are shown in figure 5, A and B (power 4–12 Hz not shown). In contrast to the effect on \( \theta \) frequency, no common systematic change is apparent for the three drugs.

We also investigated whether anesthetics affected the rhythmicity of \( \theta \) oscillations. We expressed the rhythmicity as the height of the second peak in the autocorrelogram (fig. 2, B and D). As can be seen from figure 5C, none of the agents had a systematic effect on the regularity of \( \theta \) rhythm during exploratory activity.

Discussion

There were two principal findings from this study. First, that isoflurane, halothane, and nitrous oxide all inhibited hippocampus-dependent FC more effectively than hippocam-
was obtained from rabbits undergoing classic (delay) condi-
tions. All three traces show expression of the \( \theta \) rhythm and nitrous oxide (N\(_2\)O; 60%, bottom) during exploratory activity. 

Fig. 3. Hippocampal activity at subhypnotic amnestic concentrations. (A) Raw hippocampal electroencephalographic traces recorded from one individual under amnestic concentrations of halothane (0.25%, top), isoflurane (0.32%, middle), and nitrous oxide (N\(_2\)O; 60%, bottom) during exploratory activity. All three traces show expression of the \( \theta \) rhythm in the presence of amnestic concentrations of these agents. (B) Spectrograms obtained from concatenation of all epochs spent exploring during anesthetic exposure (same experiments shown in A: 20, 15, 4, and 14 segments for halothane, isoflurane, nitrous oxide, and control, respectively) reveal comparable slowing of the \( \theta \) peak frequency by all three agents at the concentrations applied (control, 7.6 Hz; isoflu-
rane, 6.6 Hz; N\(_2\)O, 6.6 Hz; halothane, 6.8 Hz).

First, we studied. Second, each agent slowed \( \theta \) frequency in proportion to its impairment of FC. These results are consistent with the hypothesis that a variety of agent-specific interactions with molecular targets converge to a uniform effect on an important network characteristic that is essential to proper hippocampal function during learning, in this case, \( \theta \) rhythm frequency. The degree of slowing observed at equivalently amnestic concentrations, however, varied—possibly reflecting the diversity of affected molecular targets.

**\( \theta \) Rhythm and Memory**

Evidence accumulated during past decades provides extensive support for the importance of the \( \theta \) rhythm in learning and memory. Some early evidence for a role of \( \theta \) oscillations was obtained from rabbits undergoing classic (delay) condi-
tioning. Although this learning task is not strictly hippocampus-dependent, rabbits acquired the learning task faster if \( \theta \) rhythm dominated their hippocampal electroencephalogram just before the presentation of paired stimuli.\(^{30,31}\) Conversely, administration of the conditioned-unconditioned stimulus pair during non-\( \theta \) states slowed learning whereas synchronizing stimuli with spontaneous \( \theta \) oscillations dramatically accelerated acquisition of conditioned reflex.\(^{32,33}\)

Because the importance of the hippocampus increases when a learning task requires temporal processing, even nonspatial tasks that are made contiguous (e.g., by imposing an empty “trace” interval between the conditioned and unconditioned stimulus) become hippocampus-dependent. Under the trace paradigm, neuronal responsiveness in the hip-
ncampus to a conditioned stimulus developed before behavioral expression of the conditioned reflex in the individual that acquired the reflex.\(^{34}\) Hence, it is not surprising that \( \theta \) power positively correlated with learning in trace eye-blink conditioning in the early stages of learning.\(^{35}\)

Although most published work does not discriminate be-
tween the influence of \( \theta \) power and frequency, recent data provide support for a role of \( \theta \) frequency per se for the rate of learning. Baseline \( \theta \) frequency recorded during quiet waking and paradoxical sleep (i.e., not during learning) positively correlated with the rate of learning in rats subjected to an operant-conditioning paradigm.\(^{36}\) Hence, both higher \( \theta \) frequency and increased \( \theta \) power at baseline appear to improve learning performance.

The results of these observational studies are complemented by investigations that actively modulated the \( \theta \) rhythm by phar-
macological, physical, or genetic means and observed the effects on learning and memory. Injection of tetracaine into the medial septum ablated hippocampal \( \theta \) but preserved “upstream” rhyth-
micity, leading to severe learning impairment that was, however, largely reversible by rhythmic exogenous stimulation of the fornix that generated \( \theta \)-like synchronized activity in the hippocampus.\(^{23}\) Pan and McNaughton\(^{21}\) slowed \( \theta \) oscillations ei-
ther by injection of chlordiazepoxide or systemic cooling, testing learning and memory using a Morris water maze. They found that hippocampus-dependent learning was sensitive to changes in \( \theta \) frequency. In fact, slowing \( \theta \) oscillations by 0.35–0.5 Hz\(^{37}\) produced a measurable memory impairment but slowed oscillations by 1 Hz produced a substantial impairment.\(^{21}\) Likewise, mice with a deletion of the \( \gamma \)-aminobutyric acid transporter type 1 displayed slowed type 1 \( \theta \) rhythm, reduced long-term poten-
tiation, and impaired hippocampal learning.\(^{38}\) By contrast, drug-induced acceleration of \( \theta \) frequency by 5–10% above baseline improved learning.\(^{39}\) These results thus indicate that learning is sensitive to changes in \( \theta \) frequency by amounts comparable with those produced by amnestic concentration of the agents we studied.

Changes in \( \theta \) frequency may not, however, be the only means by which drugs can influence memory via \( \theta \)-rhythm modulation. The cannabinoid CP55940 was shown to impair learning in hippocampus-dependent delayed spatial alternation tasks in proportion to the degree of selective suppression of \( \theta \).
power, a correlation independent of locomotion speed. Like-
wise, we showed previously that suppression of \( \theta \) power without a change in \( \theta \) frequency produced by the nonimmobilizer 1,2-dichlorohexafluorocyclobutane (F6, 2N) also correlated with impairment of contextual FC. In fact, the muscarinic receptor blocker scopolamine accelerates \( \theta \) oscillations but impairs learning and memory. Scopolamine, however, also suppresses \( \theta \) power. Therefore, scopolamine’s suppression of \( \theta \) power may override its effect on \( \theta \) frequency, resulting in a net proamnestic effect.

Anesthetic-induced Suppression of Learning and Memory

Considering the importance of amnesia as an anesthetic end-point during surgical procedures under general anesthesia, its mechanisms have not been studied extensively. From the variety of learning types that exist in mammals, those subserving what is commonly referred to as episodic and episodic-like memory in humans and animals, respectively, are tightly linked to the intact functioning of the medial temporal lobe in general and the hippocampal formation in particular. Contextual FC requires an intact hippocampus and is the primary target of our investigation (note that the terms “episodic,” “explicit,” “conscious,” and “declarative memory” are closely related and require intact hippocampal processing). However, to place our experiments into the framework of existing literature, we also tested FC to tone as a hippocampus-independent control paradigm.

Fig. 4. Anesthetics slow \( \theta \) rhythm. Spectrograms illustrating the concentration-dependent slowing of \( \theta \) rhythms by halothane (A), isoflurane (B), and nitrous oxide (N\(_2\)O) (C). Exploratory activity was absent in rats exposed to isoflurane concentrations above 0.5 minimal alveolar concentration, but animals continued to explore spontaneously under concentrations of halothane approaching 1.0 minimal alveolar concentration. (A) Spectrograms based on 47, 3, 6, and 14 segments for control and halothane 0.26, 0.49, and 1.06%, respectively. (B) Spectrogram for immobility under 0.69% isoflurane demonstrating the absence of a \( \theta \) peak during immobility. Based on 106, 14, 5, and 284 segments for control and isoflurane 0.3%, 0.69% exploring, and 0.69% immobile, respectively. (C) Spectrograms based on 14, 43, and 4 segments for control, and 30 and 60% N\(_2\)O, respectively. (D) Summary of all experiments. The effect of three anesthetics on \( \theta \) frequency is plotted as a function of anesthetic concentration. Lines represent linear regression fits and are bracketed by 95% confidence intervals. To facilitate comparison, anesthetic concentrations are expressed as multiples of \( EC_{50} \) amnesia for contextual fear conditioning (FC) (see Fig. 1) and x-values were scattered to reveal overlying data points for control and N\(_2\)O 30%.
Most data on isoflurane-induced memory suppression are derived from FC-based experiments. Similar to nitrous oxide, isoflurane-impaired learning and memory at subhypnotic concentrations and contextual (i.e., hippocampus-dependent) FC was more sensitive than cued (i.e., hippocampus-independent) FC. It is noteworthy that the EC50 of isoflurane for contextual FC that we determined in rats (0.28%) is close to its EC50 for suppression of episodic memory in human volunteers (0.24%).

Less is known about halothane. The existing data suggest only that very low halothane concentrations (behaviorally subhypnotic, but not further defined) do not affect acquisition (learning) but substantially impair retention (memory), although even “surgical” concentrations of halothane (up to 2%, but not further specified) fail to suppress completely trace conditioning to tone (a hippocampus-independent task). We found that halothane showed the greatest discrimination between the two forms of FC with a more than two-fold difference in EC50 (0.14 ± 0.09 and 0.3 ± 0.04%, respectively). An alternate way of expressing this finding is that halothane is the weakest inhibitor of hippocampus-independent learning (relative to its lipid solubility), as found in a recent study using inhibitory avoidance training as a learning paradigm.

When tested under identical conditions, the three anesthetics we studied suppressed FC to context at lower concentrations than FC to tone (EC50 Tone/EC50 Context were 1.35, 1.5, and 2.1 for nitrous oxide, isoflurane, and halothane, respectively). A similar differential amnestic profile was previously reported for the nonimmobilizer 1,2-dichlohexafluorocyclobutane (EC50 Tone/EC50 Context, 1.7). We interpret these results as indicating that hippocampal processing increases the susceptibility of learning and memory to interference. Based on our electrophysiological findings, we suggest that this result might be attributable to the dependence of the hippocampus on large-scale synchronization by the rhythm, a dependence not shared by other important memory-processing structures, including the amygdala. An alternative (but not mutually exclusive) explanation is that local expression of critical types of receptors or other circuit elements that produce or regulate synaptic plasticity are more sensitive to anesthetics in the hippocampus than in the amygdala. For this to be the case, however, one would have to postulate region-specific differences in the sensitivity of receptors to tested drugs. Such quantitative studies have not been performed.

How could slowing of the rhythm affect learning and memory? Hippocampal synaptic plasticity (and, by extension, hippocampus-dependent learning and memory) is known to depend on precise synchronization of cell spiking with the phase of ongoing network activity. This dependence may not be shared by other important memory-processing structures, including the amygdala. An alternative (not mutually exclusive) explanation is that local expression of critical types of receptors or other circuit elements that produce or regulate synaptic plasticity are more sensitive to anesthetics in the hippocampus than in the amygdala. For this to be the case, however, one would have to postulate region-specific differences in the sensitivity of receptors to tested drugs. Such quantitative studies have not been performed.

Fig. 5. Anesthetics did not systematically modulate theta power and rhythmicity. No common pattern of anesthetic effect on theta peak amplitude (A), theta power (B), or theta rhythmicity (C) was apparent.
Is There a Single Molecular Target that Mediates Effects on θ Rhythm?

We postulated that the actions of several inhaled agents on a diverse set of molecular targets converge on the θ-oscillation circuitry to produce a common network-level effect: a disruption of the endogenous temporal metric that interferes with the distinctive “what-where-when” associations of hippocampus-dependent episodic declarative memory. However, is it possible that modulation of a single molecular target is responsible for the observed effect? One such candidate could be a member of the K2P channel family. TREN-1 potassium channels are enhanced by halothane and nitrous oxide and TREN-1 knockouts are less sensitive to immobility and loss of righting reflex, although some researchers are skeptical of a causal relationship between mutation and anesthetic resistance. However, despite extensive studies, there is no reported phenotype of TREN-1 knockouts with respect to learning, memory, or electroencephalographic patterns. Nevertheless, considering the heavy expression of TREN-1 in the septum, an important contribution of these channels to anesthetic-induced changes in θ rhythm is a possibility. Alternatively, and perhaps more likely, a range of actions on different targets by each anesthetic may lead to slowing of the θ rhythm.

Conclusion

In summary, we have found a correlation between the concentration-dependent suppression of hippocampus-dependent learning and a reduced frequency of the hippocampal θ rhythm. This correlation, which was present for three different inhaled anesthetics that modulate different sets of molecular targets, supports the hypothesis that modulation of the hippocampal θ rhythm contributes to anesthetic-induced amnesia.

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