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Laboratory Investigation

LABORATORY INVESTIGATION

Electroencephalographic coherence and cortical acetylcholine during ketamine-induced unconsciousness

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Abstract

Background: There is limited understanding of cortical neurochemistry and cortical connectivity during ketamine anaesthesia. We conducted a systematic study to investigate the effects of ketamine on cortical acetylcholine (ACh) and electroencephalographic coherence.

Methods: Male Sprague–Dawley rats (n=11) were implanted with electrodes to record electroencephalogram (EEG) from frontal, parietal, and occipital cortices, and with a microdialysis guide cannula for simultaneous measurement of ACh concentrations in prefrontal cortex before, during, and after ketamine anaesthesia. Coherence and power spectral density computed from the EEG, and ACh concentrations, were compared between conscious and unconscious states. Loss of righting reflex was used as a surrogate for unconsciousness.

Results: Ketamine-induced unconsciousness was associated with a global reduction of power (P=0.02) in higher gamma bandwidths (>65 Hz), a global reduction of coherence ($P \leq 0.01$) across a broad frequency range (0.5–250 Hz), and a significant increase in ACh concentrations (P=0.01) in the prefrontal cortex. Compared with the unconscious state, recovery of righting reflex was marked by a further increase in ACh concentrations (P=0.0007), global increases in power in theta (4–10 Hz; P=0.03) and low gamma frequencies (25–55 Hz; P=0.0001), and increase in power ($P \leq 0.01$) and coherence ($P \leq 0.002$) in higher gamma frequencies (65–250 Hz). Acetylcholine concentrations, coherence, and spectral properties returned to baseline levels after a prolonged recovery period.

Conclusions: Ketamine-induced unconsciousness is characterized by suppression of high-frequency gamma activity and a breakdown of cortical coherence, despite increased cholinergic tone in the cortex.

Key words: acetylcholine; electroencephalography; ketamine; microdialysis; prefrontal cortex; unconsciousness

Ketamine is a unique anaesthetic drug that does not conform to most mechanistic frameworks of anaesthetic-induced unconsciousness.[1] Unlike many general anaesthetics, the γ-aminobutyric acid (GABA) receptor is not the primary molecular target of ketamine.[2–4] At a systems neuroscience level, ketamine does not appear to activate the sleep-promoting ventrolateral preoptic nucleus,[5] as typical GABAergic anaesthetics do.[5–7] Instead, ketamine activates the norepinephrine-producing locus coeruleus[5] and appears to depend, in part, on noradrenergic transmission.[8] At the neurophysiological level, ketamine enhances higher

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Editor's key points

- Ketamine, which acts by a unique non-GABAergic mechanism, might have distinct effects on long-range cerebrocortical interactions compared with other general anaesthetics.
- The effects of ketamine on electroencephalographic coherence, cortical acetylcholine concentrations, and behaviour were studied in rats.
- Ketamine-induced loss of consciousness was associated with global reductions in gamma power and cortical coherence similar to other general anaesthetics.

frequency electroencephalographic activity,[9 10] which can confound the algorithms in processed electroencephalographic devices intended for monitoring anaesthetic depth.[10 11]

Recent studies from our laboratory suggest that ketamine shares a network-level property with GABAergic drugs by disrupting information transfer,[9] phase relationships,[12] or both across the cortex. These findings are consistent with a study of ketamine in a cortical slice model that identified uncoupling of long-range corticocortical interactions.[13] However, studies of ketamine and cortical or thalamocortical connectivity in intact animal models are typically conducted with co-administration of xylazine, an α_2-adrenergic agonist.[14 15] Furthermore, there have been no carefully controlled studies of ketamine-induced unconsciousness in animal models that link disruptions of neurophysiological coupling with neurochemical events. Studies of ketamine and neurochemistry have focused on acetylcholine (ACh) but (i) without concomitant neurophysiological recordings,[16 17] (ii) using subanaesthetic doses of ketamine,[16 17] or (iii) without formal testing of loss of righting reflex (LORR) as a surrogate of anaesthetic-induced unconsciousness.[16] To address this gap in knowledge, the objective of the present study was to identify the relationship of electroencephalographic coherence, cortical ACh, and the state of ketamine-induced unconsciousness.

Methods

Experiments were conducted on adult (3- to 5-month-old) male Sprague–Dawley rats (n=11; Charles River Laboratories, Inc., Kingston, NY, USA) maintained on a 12 h light–12 h dark cycle (lights on at 06.00 h) with *ad libitum* food and water. The experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals and were in compliance with the *Guide for the Care and Use of Laboratory Animals* (8th Edition, The National Academies Press, Washington, DC, USA) and the ARRIVE guidelines.

Surgical procedures

Under surgical levels of isoflurane anaesthesia, rats were implanted with screw electrodes to record electroencephalogram (EEG) from frontal (Bregma: anterior 3.0 mm, lateral 2.5 mm), parietal (Bregma: posterior 4.0 mm, lateral 2.5 mm), and occipital areas (Bregma: posterior 8.0 mm, lateral 2.5 mm). A screw electrode was implanted over the nasal commissure to serve as a reference electrode. In addition, a craniotomy was performed over the prefrontal cortex (PFC) (Bregma: anterior 3.0 mm, lateral 0.5 mm, ventral 4.0 mm),[18] and a CMA/11 guide cannula (CMA Microdialysis, Harvard Apparatus, Holliston, MA, USA) was implanted 1.0 mm above the target area. Buprenorphine hydrochloride (Buprenex®; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA, USA) was used for pre- and postsurgical analgesia (0.01 and 0.03 mg kg^{-1}, s.c., respectively), and a presurgical single dose of the antibiotic cefazolin (20 mg kg^{-1}, s.c.; West-Ward Pharmaceutical Corp., Eatontown, NJ, USA) was administered. The EEG electrodes were mated with an electrode pedestal (Plastics One, Roanoke, VA, USA), and the entire assembly along with the guide cannula was affixed to the cranium using dental acrylic.

Electroencephalographic data acquisition

The electrode over the nasal commissure was used as a reference for monopolar EEG recordings from the frontal, parietal, and occipital cortices. The choice of monopolar EEG recordings was based on previously published animal studies[19–21] and on a study from our laboratory demonstrating that monopolar reference is best suited to detect genuine EEG phase synchronization.[22] Electroencephalographic signals were amplified (×5000) and filtered (0.1–300 Hz) on a Grass Model 15 LT system (15A54 Quad Amplifier, Warwick, RI, USA). Data were digitized at 1 kHz using an MP150 system and AcqKnowledge data acquisition software (version 4.1; Biopac Systems, Inc., Goleta, CA, USA).

Coherence and power spectral analysis

Data were first down-sampled to 500 Hz to reduce computation time, and an IIR notch filter was applied to remove 60 Hz line noise. We calculated coherence across cortical electrodes at individual frequencies from 0.5 to 250 Hz (in 0.5 Hz intervals) as magnitude squared coherence using the 'mscohere.m' function in the MATLAB Signal Processing Toolbox (MathWorks Inc., Natick, MA, USA).[21] To control for spurious coherence, the surrogate data method was used, wherein phases were randomized but the spectral content of the signals was maintained.[23] These estimates of spurious coherence were then statistically compared with the empirical data. Furthermore, frequencies at which obvious artifact was present on the individual spectrogram or coherogram were excluded from quantitative analysis and statistical comparison.

Absolute power spectral density (PSD) between 0.5 and 250 Hz was calculated based on the short-time Fourier transform using the 'spectrogram.m' function in the MATLAB Signal Processing Toolbox.[21] Relative power was calculated for each epoch by dividing the mean absolute power of each frequency band by the total power across the entire frequency range. Coherence and PSD were calculated for the following frequency bands: delta (δ: 0.5–4 Hz), theta (θ: 4–10 Hz), alpha (α: 10–15 Hz), beta (β: 15–25 Hz), low gamma (γ1: 25–55 Hz), medium gamma (γ2: 65–125 Hz), high gamma (γ3: 125–175 Hz), and ultrahigh gamma (γ4: 185–250 Hz). The data are reported as changes in global coherence, which was obtained by averaging the coherence values for individual channel pairs. Likewise, PSD values for individual channels were averaged and reported as changes in global PSD.

Quantification of acetylcholine release in prefrontal cortex

A CMA/11 microdialysis probe (1 mm cuprophane membrane, 0.24 mm diameter, 6 kDa) was perfused continuously with Ringer's solution (147 mM NaCl, 2.4 mM $CaCl_2$, 4.0 mM KCl, and 10 μM neostigmine; pH 5.8–6.2) at 2.0 μl min^{-1} using a CMA/400 syringe pump. Microdialysis samples were collected every 12.5