CLINICAL RESEARCH PROTOCOL

Mechanism Exploration of General Anesthesia-Induced Altered Consciousness via Cortical-Thalamic Network Modulation and Development of a Precision Anesthesia Depth Monitoring Device

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In accordance with Good Clinical Practice (GCP) requirements and the Measures for the Administration of Investigator-Initiated Clinical Research in Medical and Health Institutions, I will diligently fulfill my responsibilities and will personally conduct or directly supervise this clinical research. I have read and confirmed this protocol. I agree to perform the duties of the Principal Investigator in accordance with applicable Chinese laws and regulations, the Declaration of Helsinki, current Chinese GCP requirements, the Measures for the Administration of Investigator-Initiated Clinical Research in Medical and Health Institutions, and this protocol. These responsibilities include oversight of all investigators in their implementation of the protocol and the establishment of appropriate measures for clinical research quality management.

During study conduct, I will adhere strictly to the requirements of this protocol. Any protocol amendment will be implemented only after approval or filing, as applicable, by the Clinical Research Management Committee and the Ethics Committee, except when immediate action is required to protect the safety, rights, or interests of research participants.

Principal Investigator Signature: _____

Date: _____

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1. Background and Rationale

The mechanism by which general anesthetics produce reversible loss of consciousness remains a central unresolved question in anesthesiology and a major barrier to the implementation of precision anesthesia. In current clinical practice, the lack of mechanism-based tools for precise monitoring of anesthetic depth creates risks at both ends of the dosing spectrum. Inadequate anesthesia may lead to hemodynamic instability, intraoperative awareness, and patient movement, thereby interfering with surgery; excessive anesthesia may suppress respiratory and cardiovascular function and, in severe cases, threaten patient safety. At the same time, general anesthesia provides a controllable, reversible, and repeatable means of manipulating the level of consciousness, making it an especially useful experimental paradigm for investigating the biological basis of consciousness. A mechanistic understanding of general anesthesia is therefore important not only for anesthesiology itself, but also for the development of precision anesthesia monitoring and for fundamental consciousness research.

Our preliminary clinical electroencephalography (EEG) studies suggest that altered functional connectivity within large-scale brain networks may be an important mechanism underlying anesthesia-induced loss of consciousness. The core brain regions and targets through which anesthetics produce these changes, however, remain incompletely defined. At the animal level, we used functional magnetic resonance imaging (fMRI) to investigate the neural mechanisms of propofol-induced loss of consciousness. After loss of consciousness, marked changes in functional connectivity were observed among frontal-hub cortical regions - including the ventromedial prefrontal cortex (vmPFC), dorsomedial prefrontal cortex (dmPFC), and anterior cingulate cortex (ACC) - and several thalamic nuclei connected to the cortex, including the thalamic reticular nucleus (TRN), central medial thalamus (CMT), paraventricular thalamic nucleus (PVT), and mediodorsal thalamic nucleus (MD). These findings suggest that these regions and their associated circuits may be central to the mechanisms by which propofol alters consciousness. We further examined the roles of the prefrontal cortex (PFC), TRN, glutamatergic projections from vmPFC to TRN, and CMT in propofol-induced alterations of consciousness. Collectively, these results support the hypothesis that frontal-hub cortical regions and their connected thalamic nuclei constitute key nodes and circuits through which general anesthetics alter consciousness.

Because the mechanisms of anesthesia-induced loss of consciousness remain incompletely understood, clinically available depth-of-anesthesia monitors are not yet truly mechanism based. Most currently used systems are predominantly single-modality devices and perform linear analyses of signals derived from a single source, such as EEG or cerebral oxygenation. These approaches have substantial room for algorithmic improvement and often cannot provide real-time, precise assessment of the patient's level of consciousness. The brain is intrinsically a highly complex nonlinear dynamical system, and a single parameter is unlikely to capture its full dynamic state. Mechanistically informed extraction of multimodal features that specifically track consciousness may therefore enable more accurate monitoring and facilitate precision anesthesia.

This project adopts a translational, multiscale strategy. EEG will first be used in both clinical and animal studies to identify brain regions and neural circuits closely associated with transitions in consciousness. Candidate targets will then be validated and investigated mechanistically using advanced neuroscience approaches. Transcranial temporal interference stimulation (tTIS) will subsequently be used to modulate key brain regions and to examine their causal relationship with consciousness. Finally, leveraging an existing hardware platform, the project will integrate targeted stimulation with multimodal signals including EEG, cerebral oxygenation, and magnetic resonance imaging, and will apply deep-learning methods to develop a multimodal system for precision monitoring of anesthetic depth. The overall aim is to advance precision anesthesia while contributing to a mechanistic understanding of consciousness.

2. Current Evidence, Knowledge Gaps, and Scientific Premise

2.1 Why Understanding the Mechanisms of General Anesthesia Matters

2.1.1 Mechanistic understanding as a foundation for precision anesthesia

The mechanisms of general anesthesia are extraordinarily complex and remain an unresolved problem in neuroscience [1]. Theoretical explanations have evolved from nonspecific lipid theories to specific molecular-target hypotheses [2]. With advances in neurophysiology, neurobiology, neuroanatomy, and especially neural-circuit tracing, a circuit-level model of general anesthesia has gained broad acceptance: anesthetic agents alter states of consciousness by modulating specific neural nuclei, circuits, and networks [3]. Existing evidence also reveals substantial heterogeneity. The lateral hypothalamus (LH), ventral tegmental area (VTA), nucleus accumbens (NAc), basal forebrain (BF), and their associated circuits have all been implicated in anesthesia-related regulation of consciousness [4-8]. A single anesthetic can simultaneously activate or inhibit multiple circuits [9,10], while different anesthetic classes may act through distinct neural pathways [11]. Thus, despite substantial progress, the core neural regions and circuits that mediate anesthetic-induced changes in consciousness remain to be fully defined, particularly with regard to how mechanistically diverse anesthetics converge on similar states of depressed consciousness.

This unresolved mechanism is also a major bottleneck in the development of precise depth-of-anesthesia monitoring. Current clinical monitors are usually EEG-based and derive proprietary indices that reduce complex signals to a single numerical estimate of unconsciousness [12]. Although convenient, such indices are only indirectly related to consciousness and may not generalize across anesthetic classes [13]. Their clinical value may be further limited in children, in whom neural plasticity changes rapidly, and in older adults, in whom degenerative changes are common. Inadequate precision in monitoring anesthetic depth is associated with clinically important complications, including emergence delirium in children, postoperative delirium and perioperative neurocognitive disorders in older adults, and intraoperative awareness with possible long-term psychological consequences such as post-traumatic stress and sleep disturbance [14,15]. A mechanistic understanding of general anesthesia is therefore essential to developing monitoring systems that are robust across anesthetic agents and age groups, thereby supporting individualized anesthesia and reducing perioperative complications.

2.1.2 Mechanistic understanding as a route to the biology of consciousness

The biological basis of consciousness was listed by Science in 2005 among the major unanswered scientific questions and remains incompletely understood [16]. General anesthesia provides a uniquely tractable experimental model for consciousness compared with natural sleep or pathological disorders of consciousness such as coma and the vegetative state [17]. Natural sleep is a spontaneous circadian process in which transitions in consciousness occur gradually and are influenced by multiple internal and external factors, making precise control difficult. Pathological disorders of consciousness, by contrast, are commonly associated with complex structural brain injury and may be irreversible or difficult to switch rapidly between states. General anesthesia allows the level of consciousness to be titrated by drug dose, enabling transitions from wakefulness to sedation, surgical anesthesia, and burst suppression, while pharmacokinetic properties also permit prediction of recovery after drug discontinuation. Its controllability, reversibility, and repeatability therefore make general anesthesia one of the most powerful experimental paradigms for consciousness research. However, the anesthetic mechanisms themselves must be clarified if anesthesia is to be used rigorously as a model system for consciousness.

In summary, elucidating the mechanisms of general anesthetics may simultaneously promote mechanism-based monitoring of anesthetic depth, reduce anesthesia-related complications, and advance understanding of the neural basis of consciousness. Such knowledge may also provide a conceptual framework and potential targets for future treatment of patients with disorders of consciousness.

2.2 Brain Network Functional Connectivity as a Candidate Macroscopic Mechanism

2.2.1 Functional connectivity changes during anesthetic-induced loss of consciousness

Although the mechanisms underlying anesthetic-induced loss of consciousness have not been completely defined, accumulating evidence indicates that cortical brain networks are important targets of general anesthetics. Prior EEG studies have demonstrated marked changes in interregional cortical functional connectivity during altered consciousness induced by propofol, dexmedetomidine, ketamine, and sevoflurane [18-20]. In our previous work in surgical patients, 32-channel EEG was used to systematically characterize the effects of propofol, remimazolam, esketamine, and dexmedetomidine during anesthetic induction [21-23]. Functional connectivity was quantified using the weighted phase lag index (wPLI) and directed phase lag index (dPLI). All four agents produced significant decreases in anterior-to-posterior connectivity - particularly frontal-to-parietal wPLI and dPLI - after loss of consciousness, suggesting that disruption of frontoparietal functional connectivity may represent a shared macroscopic mechanism across pharmacologically distinct anesthetics.

These observations have also been supported in rodents [24]. Our group found that isoflurane-induced loss of consciousness was associated with reduced functional connectivity within the frontal cortex and between frontal and parietal regions. Taken together, human and rodent EEG findings suggest that disruption of cortical network organization is likely to play a key role in anesthesia-related changes in consciousness. Targeting cortical network dynamics may therefore provide a tractable route for identifying common mechanisms across anesthetic agents. Nevertheless, the core brain regions or circuits that initiate these connectivity changes and mediate loss of consciousness remain unclear.

2.2.2 Role and limitations of EEG in identifying neural correlates of anesthetic-induced changes in consciousness

EEG is a core clinical tool for real-time, noninvasive assessment of cortical electrical activity and is widely used to monitor anesthetic depth and level of consciousness [25]. By recording scalp electrical potentials generated by neuronal activity and transforming complex EEG signals into interpretable measures, EEG provides a practical window into brain state [26]. However, several limitations constrain its use. First, scalp EEG has relatively low spatial resolution and primarily reflects cortical activity, limiting direct localization of deep brain structures involved in changes in consciousness [27]. Second, EEG is vulnerable to electromyographic, ocular, and environmental electromagnetic artifacts that may obscure biologically relevant activity [28]. Third, EEG interpretation can depend heavily on expert judgment, potentially leading to interobserver variability and reduced sensitivity to low-amplitude or transient patterns. To address some of these limitations, our group has developed a Gaussian copula-based analytical framework that improves the characterization of distributed cortical signals and provides a more efficient approach to EEG-based assessment of consciousness [29-31].

2.3 Frontal-Hub Cortico-Cortical and Cortico-Thalamic Networks as Candidate Core Circuits

2.3.1 Frontal-centered cortical networks and anesthetic-induced changes in consciousness

The prefrontal cortex (PFC) is a major higher-order cortical region implicated in consciousness [32]. It integrates information conveyed through ascending brainstem-thalamocortical systems and sends descending signals to thalamic structures, thereby participating in recurrent information-processing loops that support conscious function [33]. Experimental studies in rodents have shown that activation of the PFC can modulate arousal and reverse anesthetic states [34]. In our previous work, general anesthesia disrupted frontal-temporal and frontal-occipital connectivity and impaired information transfer across cortical regions. We also observed a shift in network hubs from posterior parietal and occipital regions during wakefulness toward frontal regions after loss of consciousness [21]. This reorganization of hub structure may contribute to the reduction in cortico-cortical functional connectivity observed during anesthesia.

Comparable findings have been observed in rodents. We found reduced connectivity within the frontal cortex and between frontal and parietal regions after isoflurane-induced loss of consciousness [35]. Other groups have shown by mouse fMRI that connectivity among prefrontal, lateral cortical, and association networks progressively decreases as isoflurane concentration increases [24]. Human and rodent evidence therefore converges on frontal-centered cortical networks as important components of anesthesia-induced loss of consciousness. Modulation of these networks may help reveal mechanisms shared across anesthetic classes.

2.3.2 The cortico-thalamic network in anesthetic-induced changes in consciousness

In addition to frontal-centered cortico-cortical networks, substantial evidence implicates cortico-thalamic communication in anesthesia-related changes in consciousness. EEG and fMRI studies have shown disruption of cortico-thalamic functional connectivity after loss of consciousness induced by propofol [36], sevoflurane [37], and dexmedetomidine [38]. Mechanistic studies further suggest that anesthetics can interrupt action-potential transmission from superficial cortical layers to deeper cortical layers and the thalamus, producing desynchronization of cortical firing and cortico-thalamic decoupling [39]. This may represent a physiological substrate of anesthesia-induced unconsciousness. Multiple thalamic nuclei, including TRN, CMT, PVT, and MD, have also been shown by our group and others to modulate anesthetic state [40-42]. Because these nuclei are extensively connected with the cortex and participate in higher-order brain functions [43], cortico-thalamic information exchange is a strong candidate mechanism through which general anesthetics alter consciousness.

The thalamic reticular nucleus is the only entirely GABAergic nucleus of the thalamus and plays a critical role in both thalamocortical (TC) and corticothalamic (CT) information transfer [44]. TRN receives glutamatergic input from collateral branches of TC fibers and layer 6 corticothalamic neurons, as well as modulatory input to thalamic relay neurons, thereby supporting L6CT-TRN-TC feed-forward and TC-TRN-CT feedback inhibitory loops. Its position at the interface between cortex and thalamus provides an anatomical basis for widespread regulation of thalamic and cortical rhythms [45]. Our preliminary work showed that the anterior TRN actively participates in induction by propofol and isoflurane; propofol mildly depolarized PV- and SST-expressing TRN neurons, increased membrane input resistance and action-potential firing, and reduced action-potential threshold. We also identified a direct vmPFC-to-TRN projection. Optogenetic activation of this pathway prolonged emergence from propofol and isoflurane anesthesia and increased the frequency, amplitude, and duration of anesthetic-induced spindle activity. These observations suggest that the medial prefrontal cortex-TRN circuit may be one of the core circuits governing anesthetic-induced changes in consciousness.

The central thalamic region provides a key anatomical bridge between medial prefrontal cortex and the hippocampus, basal ganglia, amygdala, and other higher-order cortical areas. PVT, CMT, and MD have each been implicated in maintaining and promoting arousal [46,47], and rich reciprocal projections connect these nuclei with medial prefrontal cortex. During general anesthesia, anesthetics may suppress arousal systems through medial prefrontal-central thalamic circuits, reduce cortical connectivity, and thereby contribute to loss of consciousness. Our preliminary studies also support an important role for CMT in emergence from anesthesia. Whether medial prefrontal-central thalamic circuits constitute a shared core mechanism across anesthetics therefore warrants direct testing.

Defining the roles of cortico-cortical and cortico-thalamic networks in anesthesia-induced changes in consciousness could support targeted neuromodulation of key nodes to promote emergence and reduce complications. It may also advance understanding of the biological basis of consciousness and identify new targets for patients with disorders of consciousness. Integration of multimodal neurophysiological signals from these networks with advanced artificial-intelligence methods may, in turn, facilitate development of high-precision systems for dynamic monitoring of consciousness and anesthetic depth.

2.4 tTIS-Based Targeted Neuromodulation and Multimodal Monitoring

2.4.1 tTIS as a candidate noninvasive technology for deep-brain modulation of consciousness

Neuromodulation of core brain regions or circuits can deepen mechanistic understanding of consciousness and may also have translational value, for example by facilitating emergence from anesthesia. Existing noninvasive techniques such as transcranial direct-current stimulation (tDCS), transcranial alternating-current stimulation (tACS), and repetitive transcranial magnetic stimulation (rTMS) are widely used, but limited spatial resolution and insufficient ability to target deep structures constrain their effects. Noninvasive technologies capable of more selective deep-brain modulation are therefore of considerable interest.

Transcranial temporal interference stimulation (tTIS) is an emerging noninvasive neuromodulation technique. At least two pairs of scalp electrodes deliver two or more high-frequency alternating currents with slightly different carrier frequencies. Linear superposition within brain tissue produces a low-frequency envelope or beat pattern capable of modulating neural activity. Because tTIS may achieve improved targeting of deep brain regions, it has increasingly been investigated in animal and human studies [48-50]. A landmark study in 2017 demonstrated selective deep stimulation in mice: temporal interference fields modulated whisker, forepaw, and ear movements through motor-cortex targeting and activated c-Fos in the deep hippocampus without directly activating overlying cortex [51], supporting the potential of the technique for noninvasive modulation of deep neural structures.

Our team has established tTIS capability and has systematically evaluated its targeting performance in preliminary studies. In volunteers, we applied tTIS to the right amygdala and used computational modeling, functional MRI, and deep electrophysiological signals for multimodal and cross-scale validation. Concordant findings indicated that tTIS can effectively modulate neural activity in the targeted region. The present project will use this technology to intervene at key consciousness-related network nodes or circuits, with the aim of clarifying the neural mechanisms of anesthesia-induced changes in consciousness and providing a technical basis for translational, precision control of anesthetic depth or level of consciousness.

2.4.2 Development of a multimodal system for precision monitoring of anesthetic depth

Accurate monitoring of anesthetic depth and level of consciousness is a prerequisite for precision anesthesia. Current clinical systems include EEG-based monitors such as the Bispectral Index (BIS), Narcotrend, Cerebral State Index (CSI), and Index of Consciousness (IoC), as well as near-infrared spectroscopy (NIRS) systems based on cerebral oxygenation. Most are single-modality systems that perform linear analysis of parameters derived from EEG or cerebral oxygenation. Their algorithms remain imperfect, and many cannot provide real-time, high-precision assessment of consciousness. Because the brain is a complex nonlinear dynamical system, single parameters are unlikely to capture its full state. A mechanism-based multimodal approach may therefore provide more accurate assessment and better support precision anesthesia.

EEG remains central to most depth-of-anesthesia systems. To improve EEG-based monitoring algorithms, our group systematically compared 12 entropy measures for anesthetic-depth assessment and burst-suppression monitoring using isoflurane and sevoflurane datasets. Performance was evaluated using pharmacokinetic-pharmacodynamic modeling and prediction probability. Three permutation-entropy methods showed lower baseline variability and higher coefficients of determination and prediction probability than other approaches, with Revised Permutation Entropy (RPE) performing best. On this basis, we designed an anesthesia-depth monitoring system centered on rank-based entropy. The algorithm is computationally simpler than those used in several international commercial systems, is robust, and shows favorable pharmacodynamic properties. Preliminary clinical validation demonstrated good reliability and strong correlation with BIS. To move toward multimodal monitoring, we also developed an integrated EEG-NIRS system that uses three silver-chloride electrodes for two-channel EEG and two dual-wavelength light sources (760 and 850 nm) for six-channel NIRS acquisition.

Building on this platform, the project will integrate EEG-fMRI-NIRS multimodal data, extract brain-region features closely associated with state transitions in consciousness, and combine nonlinear theory, neural oscillatory measures, large datasets, and machine-learning algorithms to develop a precision anesthesia-depth monitoring system suitable for Chinese patients. The intended application is improved perioperative assessment of consciousness and implementation of precision anesthesia.

3. Study Objectives

1. To determine whether frontal-hub cortico-cortical and cortico-thalamic networks constitute core brain regions and circuits involved in anesthetic-induced changes in consciousness.
2. To investigate the roles and mechanisms of cortico-cortical and cortico-thalamic circuits in anesthetic-induced changes in consciousness.
3. To evaluate the role and mechanisms of tTIS neuromodulation during transitions in consciousness and to establish a clinical tTIS modulation database.
4. To construct a multimodal anesthesia-depth monitoring framework and develop a prototype device for precision monitoring of anesthetic depth.

4. Clinical Study Components

To test the proposed hypotheses and achieve the project objectives, the clinical research component comprises two complementary EEG studies.

4.1 Resting-State EEG Study: Time-Varying Brain Network Topology During Anesthesia-Induced Changes in Consciousness

Four anesthetic agents with different mechanisms of action - propofol, remimazolam, esketamine, and dexmedetomidine - will be studied. Multichannel EEG will be collected from patients or volunteers in different age groups across the trajectory from wakefulness to loss of consciousness and subsequent recovery. Dynamic brain-network analysis and time-varying feature extraction will be used to track the evolution of electrophysiological dynamics during transitions in consciousness and to identify core brain regions or circuits closely associated with these transitions.

4.2 Auditory Task EEG Study: Dynamic Changes in Information Integration During General Anesthesia

A continuous auditory task paradigm will be used to probe the neural representation of conscious content. Multilevel auditory stimuli will include pure tones (perceptual processing), speech deviations (semantic detection), and narrative sentences (information integration). Multichannel EEG will be recorded continuously across wakefulness, loss of consciousness, and recovery to track neural responses to external auditory information. Time-varying features of auditory brain responses, mismatch negativity, P300, late positive components, and task-related network reconfiguration will be examined to characterize the hierarchical loss and recovery of conscious content from low-level perception to higher-order semantic integration and to identify electrophysiological biomarkers associated with these changes.

5. Study Methods

5.1 Resting-State EEG Study

5.1.1 Study design and groups

Participants will be assigned according to anesthetic agent to one of four groups: propofol, remimazolam, esketamine, or dexmedetomidine. Each drug group is divided into three prespecified age subgroups: children aged 6-12 years (a key neurodevelopmental period), young adults aged 20-40 years (a period of mature and relatively stable nervous-

system function), and older adults aged 65-80 years (a period characterized by neural aging). Twenty participants are planned for each prespecified subgroup, with an overall target of 320 participant-visits across the clinical study components. In the pediatric subgroup, high-density EEG will be collected from surgical patients in the operating room. Young and older adult subgroups will consist of healthy volunteers monitored with 64-channel EEG. Data will be acquired under different anesthetic agents and across different states of consciousness, including wakefulness, loss of consciousness, and recovery.

5.1.2 Eligibility criteria

Inclusion criteria. American Society of Anesthesiologists (ASA) physical status I; ASA physical status II is permitted for older adults. Written informed consent must be obtained from all healthy volunteers and surgical patients; for children, consent must be provided by a legal guardian.

Exclusion criteria. History of neuropsychiatric disease, including epilepsy or dementia; known hearing or physical impairment that interferes with task performance; history of allergy to propofol, benzodiazepines, or other anesthetic agents used in the study; long-term use of sedative or analgesic medication for more than 1 month; disrupted sleep pattern within 14 days before the study, including shift work or insomnia; communication impairment or inability to cooperate with EEG acquisition.

5.1.3 Study procedures in young and older healthy volunteers

Preparation. Participants will undergo routine fasting from food and water for 8 hours before the study. After entering the observation room, noninvasive blood pressure, heart rate, and oxygen saturation will be monitored continuously. EEG acquisition will be performed in an environment designed to minimize electromagnetic interference.

Awake baseline. Participants will rest quietly with eyes closed for at least 10 minutes while awake EEG is recorded.

Assessment of consciousness. Throughout anesthetic induction and recovery after drug discontinuation, consciousness will be assessed every minute using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale. Loss of consciousness (LOC) is defined as two consecutive MOAA/S scores of 0 during induction. Recovery of consciousness (ROC) is defined as three consecutive MOAA/S scores of 5 after drug discontinuation.

Stepwise anesthetic induction. The target is to achieve LOC using the following dose-escalation protocols: propofol by target-controlled infusion (TCI), increasing the plasma target concentration by 0.5 microgram/mL every 5 minutes to a maximum of 5 microgram/mL; esketamine by intravenous infusion, increasing by 0.10 mg/kg/h every 5 minutes to a maximum of 0.50 mg/kg/h; dexmedetomidine by stepwise intravenous infusion in increments of 0.1 microgram/kg/h to a maximum of 0.7 microgram/kg/h; and remimazolam by intravenous infusion at 0.5, 1.0, 1.5, and 2.0 mg/kg/h, each level maintained for 5 minutes, until loss of consciousness, after which administration is stopped.

LOC maintenance. After LOC, the current drug concentration (the LOC dose) is maintained while EEG is recorded for at least 10 minutes, as specified in the protocol.

Recovery. After drug discontinuation, consciousness continues to be assessed every minute. Once ROC is achieved, EEG recording will continue for at least 30 minutes.

Post-procedure observation. Participants will be transferred to a recovery area and observed for at least 1 hour, with continued monitoring of vital signs and adverse reactions.

5.1.4 Pediatric study procedures

Children enrolled in this component will be surgical patients. High-density EEG will be recorded in the operating room under different anesthetic agents and across different states of consciousness. Criteria for determining consciousness state will refer to the adult procedure described above.

5.1.5 Outcome measures and analysis strategy

Primary outcomes. EEG features associated with level of consciousness, with emphasis on changes during anesthetic induction and recovery. Frequency-domain measures include delta-band power (0.5-4 Hz), alpha-band (8-12 Hz) power-related measures, and the theta/alpha ratio. Time-domain measures include the slow-wave index (SWI) or burst-suppression ratio (BSR), as appropriate for deep anesthesia. Spatial and synchronization-related outcomes include dynamic EEG measures.

Secondary outcomes. LOC and ROC times, slope of the anesthetic dose-response relationship, and incidence of adverse reactions.

Analysis strategy. Outcomes will be compared hierarchically across anesthetic groups and age subgroups while accounting for physiological variables such as heart rate and blood pressure.

5.1.6 Risk management

The operating room will be equipped with a defibrillator and emergency medications. In older adults, short-acting agents will be favored when applicable, for example esketamine rather than ketamine, to reduce delirium risk. In the pediatric subgroup, anesthetic depth will be monitored by dedicated personnel, with BIS maintained at 40-60.

5.1.7 High-density EEG data analysis

High-density EEG data will be analyzed across anesthetic agents, age groups, and anesthetic states (wakefulness, induction, maintenance, and recovery). Analyses will be performed in both sensor space and source space to characterize the spatiotemporal dynamics associated with the generation, maintenance, and loss of consciousness. Quantification will be organized around three complementary theoretical frameworks: complex-systems theory, complex-network theory, and Integrated Information Theory (IIT). The overall goal is to establish a macroscopic framework for tracking the evolution of consciousness and to provide methodological support for mechanistic inference.

Within the complex-systems framework, temporal complexity, spatiotemporal complexity, entropy, and critical dynamics will be evaluated in both sensor and source spaces. For spatiotemporal complexity, the project proposes a microstate-complexity approach in which the randomness and fluctuation of EEG microstate sequences are quantified to characterize changes in dynamic brain complexity across consciousness states. These analyses will test relationships between loss of consciousness and theoretical constructs such as complexity, entropy, and criticality.

For complex-network analysis, EEG channels will serve as nodes in sensor space, while source-reconstructed regions of interest (ROIs) will serve as nodes in source space. Graph-theoretical analyses will quantify conventional nodal and global metrics, including clustering coefficient, path length, small-world properties, and modularity. Higher-order networks will also be constructed to characterize large-scale information integration and propagation beyond conventional pairwise connectivity. These features will be used to explain mechanisms of loss and recovery of consciousness from both conventional and higher-order network perspectives.

To address the computational burden of IIT in high-dimensional EEG data, the project will use a Gaussian copula-based information decomposition approach to reduce computational complexity. Tensor-state synergistic entropy (TSSE) will be used to quantify synergistic and redundant information patterns across multiple brain regions, emphasizing their joint participation in complex cognitive activity. These analyses are intended to quantify information integration and differentiation during transitions in consciousness and to explore the emergence of conscious processing.

Together, these theoretical and computational approaches will be applied to high-temporal-resolution EEG to characterize how consciousness changes under different anesthetic agents and to construct a multi-theory mechanistic framework of consciousness.

5.2 Auditory Task EEG Study

5.2.1 Study design and eligibility

The anesthetic groups and eligibility criteria are the same as those described for the resting-state EEG study. The auditory task component described below is conducted in the young-adult subgroup.

5.2.2 Continuous auditory paradigm and study procedures

Preparation. The preparation procedures are the same as those described above.

Auditory stimulus sequence. Auditory stimuli will be presented continuously throughout the experiment in a pseudorandom sequence, including pure tones, white noise, single Chinese characters, words, sentences, another person's name, and the participant's own name. The stimulus-onset asynchrony (SOA) will be pseudorandomized between 3 and 8 seconds.

Stage 1 - Awake baseline with continuous auditory stimulation (10 minutes). Participants will rest with eyes closed while the continuous auditory sequence begins immediately. They will be instructed to listen naturally and not respond to the auditory stimuli. The study instructions also state that once per minute the investigator will ask the participant via intercom to clench a fist; during the final minute of this baseline stage, a command will be issued to confirm wakefulness.

Stage 2 - Anesthetic induction with consciousness monitoring and continuous auditory stimulation. Auditory stimulation will continue without interruption. The same stepwise anesthetic dosing regimens described above will be used until LOC. Once per minute, the investigator will issue a command such as "clench your right hand" or "clench your left hand." Each response will be classified as correct, incorrect, or absent. LOC is defined as two consecutive commands (two consecutive minutes) without a correct response; the LOC time is assigned to the first nonresponsive command. Once LOC is reached, dose escalation will stop and the current drug concentration will be maintained.

Stage 3 - LOC maintenance with continuous auditory stimulation. Auditory stimulation will continue without interruption. The drug concentration at LOC will be maintained. At least 10 minutes of LOC-state EEG-fMRI data will be collected. A behavioral command will be issued every minute to confirm that the LOC state is maintained.

Stage 4 - Recovery with consciousness monitoring and continuous auditory stimulation. Auditory stimulation will continue without interruption while anesthetic administration is stopped. A behavioral command will be issued every minute. ROC is defined as two consecutive commands with correct responses. The ROC time is assigned to the first correct response, provided that it is confirmed by the subsequent response. This stage ends when the ROC criterion is met.

Stage 5 - Post-ROC period with continuous auditory stimulation. Auditory stimulation and EEG recording will continue for at least 10 minutes after ROC. Behavioral commands will continue once per minute to confirm sustained recovery. After the 10-minute recording period is complete, the auditory task will be stopped and the experimental session will end.

5.2.3 Outcome measures

Primary outcomes. Task-specific analyses of auditory-evoked responses.

Secondary outcomes. LOC and ROC times, slope of the anesthetic dose-response relationship, and incidence of adverse reactions. Outcomes will be compared across anesthetic groups and age subgroups as prespecified in the protocol.

5.2.4 Auditory EEG data analysis

Event-related potentials (ERP). For each consciousness state (awake baseline, LOC maintenance, and post-ROC) and each stimulus category (pure tone, white noise, character, word, sentence, another person's name, and the participant's own name), ERPs will be averaged. Key components include N100 as a marker of early auditory processing, mismatch negativity (MMN) as a marker of pre-attentive detection of auditory change, N400 as a marker of semantic anomaly/semantic processing, and P300 as a marker sensitive to target stimuli and the participant's own name. A key question is whether components present during wakefulness, particularly N400 and P300, disappear after LOC and whether any disappearance is abrupt or gradual. Sliding-window analysis will be used to track changes in component amplitude.

Time-frequency analysis. Wavelet-based time-frequency analysis will be performed for each stimulus category. Event-related spectral perturbation and inter-trial phase coherence will be calculated, with particular attention to theta-band activity (4-8 Hz), associated with semantic processing, and alpha-band activity (8-12 Hz), associated with attention and inhibition.

Source-space ERP imaging. ERP components will be source-localized to cortical space to identify generators of N400, P300, and related responses. The analysis will test whether expected N400 sources, such as the left temporal cortex and inferior frontal gyrus, become inactive after LOC and will characterize changes in expected P300 sources, including temporoparietal junction and prefrontal regions.

Single-trial analysis and relationship to consciousness level. Single-trial linear regression will be used to estimate evoked-potential amplitude for each trial. Trial-level amplitude will be related to contemporaneous behavioral responsiveness coded from minute-by-minute commands (1 = responsive; 0 = unresponsive). A central question is whether P300 responses to the participant's own name decline gradually during the transition to LOC or disappear abruptly at a threshold, thereby addressing competing "all-or-none" and graded accounts of consciousness.

Parametric modulation by content gradient. Stimulus type will be encoded as a continuous semantic-load variable: pure tone = 0, white noise = 0.5, character = 2, word = 3, sentence = 4, another person's name = 3.5, and the participant's own name = 5. A parametric modulation model will be fitted in EEG source space, relating evoked-response amplitude in each ROI to semantic load. During wakefulness, ROIs showing a positive relationship with semantic load will be identified as candidate semantic-processing networks. During LOC, the analysis will test whether this semantic modulation disappears. The principal output will be a semantic modulation index, defined as the regression coefficient for semantic load, tracked across states of consciousness.

5.2.5 Risk management

Risk-management procedures are the same as those described for the resting-state study: the operating room will have a defibrillator and emergency medications available; short-acting anesthetics will be favored in older adults when applicable; and dedicated personnel will monitor anesthetic depth in pediatric participants, with BIS maintained at 40-60.

6. Feasibility

6.1 Strong scientific premise and preliminary data

Recent advances in neurophysiology, neurobiology, neuroanatomy, and neural-circuit tracing have established circuit-level models of general anesthesia as a major mechanistic framework. Nevertheless, the core regions and circuits that mediate anesthesia-induced changes in consciousness remain incompletely understood. In previous surgical-patient studies, our group used 32-channel EEG to characterize propofol, remimazolam, esketamine, and dexmedetomidine during induction. wPLI and dPLI analyses

showed significant reductions in anterior-to-posterior, particularly frontal-to-parietal, functional connectivity after loss of consciousness across all four agents. These findings support the hypothesis that disruption of frontoparietal functional connectivity may represent an important shared mechanism of anesthetic-induced unconsciousness.

At the animal level, our fMRI studies of propofol-induced loss of consciousness demonstrated marked changes in connectivity among frontal-hub cortical regions, including vmPFC, dmPFC, and ACC, and thalamic nuclei including TRN, CMT, PVT, and MD. We further investigated PFC, TRN, the glutamatergic vmPFC-to-TRN projection, and CMT in propofol-related changes in consciousness. These preliminary findings provide a strong theoretical and empirical basis for the proposed project.

6.2 Coherent experimental design

The project uses EEG monitoring in a cross-species, cross-age, multi-anesthetic framework to identify brain regions and circuits associated with level of consciousness. Candidate targets identified clinically will then be validated in animal models using advanced neuroscience techniques. Noninvasive tTIS will be used to modulate selected targets and assess their effects on consciousness, providing a bridge toward clinical translation. Finally, existing hardware platforms will be used to integrate EEG-fMRI-NIRS data, extract brain features associated with transitions in consciousness, and develop a precision multimodal depth-of-anesthesia monitoring device using large datasets and machine-learning methods.

6.3 Established experimental and clinical platforms

The central operating department at the applicant's institution conducts more than 80,000 anesthetic procedures annually and has established EEG acquisition and patient-monitoring infrastructure suitable for data collection in volunteers and patients under anesthesia. The research group has completed multiple EEG studies and has published more than 20 SCI-indexed papers as first or corresponding authors in journals including Anesthesiology, British Journal of Anaesthesia, Anesthesia & Analgesia, NeuroImage, and Journal of Clinical Anesthesia. The investigators therefore have substantial experience in EEG acquisition and analysis.

The home laboratory and collaborating laboratories - including the School of Basic Medical Sciences at Capital Medical University, Yanshan University, the Yulong Li laboratory at the School of Life Sciences, Peking University, and the Kexin Yuan laboratory at Tsinghua University - provide access to a range of advanced neuroscience technologies needed for the mechanistic components of the project. The team has also conducted prior clinical work using tTIS for targeted modulation of specific brain regions, and Professor Zhenhu Liang's collaborating group has extensive experience in instrument and device development.

6.4 Complementary multidisciplinary team

The Principal Investigator has focused on the mechanisms of general anesthesia, progressively expanding from depth-of-anesthesia monitoring to neural-circuit and macroscopic-network mechanisms of anesthetic-induced loss of consciousness and toward clinical translation of precision anesthesia. Team members have research experience in leading laboratories in China and abroad. During doctoral training, the Principal Investigator completed a joint training period in the Department of Anesthesiology at Washington University in St. Louis under Professors Michael Avidan and Ben Palanca, focusing on depth-of-anesthesia monitoring and postoperative delirium and receiving advanced training in EEG analysis, mechanisms of consciousness, and clinical study design.

Other team members provide complementary expertise across species and technologies: Dr. Yi Sun has worked in the Yulong Li laboratory at Peking University on neurotransmitter-probe development, application, and rodent mechanisms of general anesthesia; Dr. Jing Wang has conducted anesthesia-and-sleep research in the laboratory of Zhaofa Wu at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; and Sijie Li and Xuyang Wang have worked in the Kexin Yuan laboratory at

Tsinghua University on mechanisms of general anesthesia in nonhuman primates. Long-standing collaborations with the Baian Chen team at Capital Medical University and the Zhenhu Liang team at Yanshan University provide additional technical support for successful implementation and translation.

Overall, the project team has investigated mechanisms of anesthetic-induced loss of consciousness across rodents, nonhuman primates, and humans. The combination of a strong preliminary evidence base, multidisciplinary expertise, and broad collaborative infrastructure provides a solid foundation for successful completion of the project.

7. Study Features and Innovation

7.1 Distinctive features

1. Cross-scale integration: bidirectional validation between clinical and animal studies, in which clinical findings guide target selection for animal experiments and animal findings feed back to optimize the clinical strategy.
2. Life-course perspective: inclusion of children, young adults, older adults, and corresponding mouse models to examine age-related differences in anesthetic sensitivity across the developing, mature, and aging brain and to support age-adapted precision anesthesia.
3. Multimodal technology integration: high-density EEG, awake small-animal fMRI, optogenetic and viral-probe circuit manipulation, and noninvasive tTIS neuromodulation will be combined to investigate mechanisms from macroscopic brain networks to molecular and circuit-level processes.

7.2 Innovation

1. Technological innovation: application of tTIS to anesthesiology to overcome the limited depth penetration of conventional electrical stimulation and to investigate whether targeted tTIS can accelerate emergence from anesthesia.
2. Methodological innovation: establishment of a cross-scale validation platform that begins with human EEG-driven identification of candidate brain regions, proceeds to causal validation in animal models using optogenetic manipulation, and closes the loop by using these results to optimize tTIS targeting strategies.
3. Translational innovation: establishment of a multimodal anesthesia database using an EEG-fMRI-NIRS platform and development of a prototype precision anesthesia-depth monitoring device.

8. Data Collection and Management

8.1 Case Report Forms and Electronic Data Records

A hybrid data-collection model will be used, combining paper case report forms (CRFs) with electronic data files for data acquisition and management.

8.1.1 Data sources and collection plan

Clinical data. All clinical outcome measures, including events such as nausea and vomiting, baseline characteristics such as demographic information, medical history, and preoperative assessments, and study observations will be transcribed in a timely, accurate, and legible manner by trained clinical coordinators from source medical records (medical charts, nursing records, and anesthesia records) and direct assessments into study-specific paper CRFs.

Raw EEG data. Raw EEG signals acquired by the EEG system, including files in formats such as .edf and .set, will be named using anonymized participant identifiers (for example, Subject ID) and backed up to a designated secure server using encrypted storage media or secure network transfer.

Derived data. Brain-network measures derived from raw EEG, including nodal and global clustering coefficients, path length, spectral features, functional-connectivity matrices, and related variables, will be exported by analysts as structured electronic files such as .csv or .xlsx files.

8.1.2 Data transfer and monitoring

Completed paper CRFs will undergo on-site review, verification, and signature by the study monitor before being transferred to the clinical data-management unit. All electronic data files, including derived EEG metrics, will be transferred to the data manager using secure removable media or the hospital's internal encrypted network.

8.1.3 Data entry and quality control

Data from paper CRFs will be entered independently by two operators on separate workstations into the designated electronic data capture (EDC) system or encrypted research database. After entry, the two datasets will be compared and discrepancies will generate data queries for resolution.

8.2 Data Management

Data entry and coding. All data will be identified using a unique participant identifier to protect privacy. Data will be coded according to predefined rules; for example, adverse-event terminology will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

Confidentiality and security. Paper CRFs will be stored in locked cabinets accessible only to authorized personnel. Electronic data will be stored on password-protected computers and in encrypted databases. Database servers will be protected by firewalls and role-based access controls. Direct identifiers such as names and hospital identification numbers will be stored separately from research data and linked only through the unique participant identifier.

9. Statistical Analysis

This is an exploratory study and there is limited prior evidence on which to base a formal sample-size calculation. In similar studies, sample sizes have generally been determined empirically, with the largest groups typically containing no more than approximately 15 participants. On this basis, the study will include prespecified age strata of children aged 6-12 years, young adults aged 20-40 years, and older adults aged 65-80 years. At least 20 participants are planned per prespecified subgroup, with an overall target of 320 participant-visits across the clinical study components.

10. Ethical Considerations

10.1 Benefit-Risk Assessment

Potential benefits. Young and older adult participants may not receive a direct clinical benefit from participation, but the study may generate scientific knowledge that could contribute to safer and more effective treatment or monitoring strategies in the future. These participants will receive compensation of RMB 3,000 before tax. Pediatric participants may likewise receive no direct clinical benefit; their participation may provide new scientific information that could benefit future patients. Pediatric participants will receive compensation of RMB 500 before tax.

Risks related to study procedures. Potential risks include respiratory depression, airway obstruction, hypoxemia, or inadequate ventilation during anesthesia; discomfort, pain, or bruising at sites of venipuncture or blood sampling; and, rarely, syncope or infection at a puncture site. Metal electrodes used for 12-lead electrocardiography may cause discomfort or allergic reactions.

Other possible risks. Unexpected events related to study participation may include currently unknown drug-related risks, traffic accidents, or other unforeseen events.

EEG-related risks. Conductive EEG paste will be applied to the scalp. In rare cases, participants may develop a localized allergic reaction to the conductive gel, including papular skin eruptions.

10.2 Ethics Committee Approval

Ethics Committee approval must be obtained before study initiation and documented in a written communication to the investigator that includes the date of committee review and the date approval was granted. Any protocol amendment after approval will be submitted to the Ethics Committee in accordance with applicable procedures and regulatory requirements. During the clinical study, any safety-related issue - including amendments to the protocol or participant informed consent form and any serious adverse event (SAE) - must be reported to the Ethics Committee in a timely manner. Study completion or premature termination must also be reported.

10.3 Informed Consent

The informed consent form may be used only after review and approval by the Ethics Committee.

11. Quality Control and Quality Assurance

The investigators and funding party will each establish appropriate quality-assurance systems, fulfill their respective responsibilities, adhere strictly to the protocol, and apply relevant standard operating procedures (SOPs) to ensure effective quality control and quality assurance throughout the clinical research.

11.1 Quality Assurance During Study Conduct

11.1.1 Before study initiation

1. Protocol and SOP training: all investigators, coordinators, and operators will undergo centralized training before initiation to ensure full understanding of the protocol, clarity regarding individual responsibilities, and standardized performance of TEAS procedures, EEG acquisition, CRF completion, and related SOPs.
2. Equipment calibration and validation: all key equipment, including TEAS instruments and EEG systems, will be uniformly calibrated and performance-validated before study initiation to ensure reliable operation, accurate data acquisition, and consistency across study settings.
3. Authorization: only personnel who have completed required training and competency assessment will be authorized to perform assigned study procedures.

11.1.2 During the study

1. Routine monitoring: an independent clinical research associate (CRA) will conduct regular on-site monitoring. Activities will include, but are not limited to, source data verification (SDV) against CRFs, review of the informed-consent process, confirmation of protocol compliance, review of adverse-event documentation and reporting, and evaluation of maintenance and use records for study equipment.

2. Protocol compliance: all protocol deviations will be documented, reported, and evaluated for impact. The monitor will support investigators in implementing corrective and preventive actions when necessary.
3. Data quality control: study data will be recorded and transferred in a timely, accurate, and complete manner.

11.1.3 After study completion

1. Document and data archiving: all study-related documents, including signed informed consent forms, CRFs, monitoring reports, and raw data such as EEG recordings, will be organized and archived in accordance with applicable regulations and GCP principles to ensure long-term availability and security.
2. Close-out audit: after study completion, the funding party or a delegated independent quality-assurance function may audit the overall conduct of the study and related documentation to verify compliance and data quality.

11.2 Quality Assurance for Data Transfer, Analysis, and Reporting

11.2.1 Data entry and transfer

1. When an EDC system is used, built-in logic checks will evaluate data range, format, and internal consistency in real time.
2. When paper CRFs are double-entered independently, software will compare the two entries. Any discrepancy will generate a query for investigator verification and correction.
3. All electronic data transfers will use secure encryption to protect data integrity and confidentiality.

11.2.2 Data cleaning and logical review

1. The data manager will regularly run predefined programmatic logic checks to identify inconsistencies, outliers, and missing values and will generate data queries as needed.
2. All queries, responses, and data modifications will be recorded in a complete audit trail.

11.2.3 Data verification

1. A defined proportion of data will undergo source data verification by comparison of CRF entries with original medical records, nursing records, and raw electronic-instrument outputs. Error rates will be calculated to assess data quality.

11.2.4 Statistical analysis quality assurance

1. The statistical analysis plan (SAP) will be finalized and filed before database lock.
2. All statistical analysis code will be independently reviewed to confirm consistency with the SAP and correct execution.
3. Sensitivity analyses will be performed for key results to assess robustness of the primary conclusions.

11.2.5 Reporting quality assurance

1. Study reports will be written strictly on the basis of the prespecified statistical results to ensure accurate and complete presentation of findings.

2. The final study report must be reviewed and approved by the Principal Investigator.

12. Expected Outcomes

1. Integrate clinical and preclinical models to identify key brain regions involved in anesthetic-induced changes in consciousness and characterize associated network maps, neural circuits, and molecular targets; use tTIS to validate the activity of these target regions during loss and recovery of consciousness under general anesthesia.
2. Use clinical EEG and artificial-intelligence methods to identify age-spanning temporal and spatial features of anesthetic depth; develop a self-supervised deep-neural-network framework for anesthesia-depth monitoring and a prototype next-generation monitoring device.
3. Publish 4-6 high-impact SCI-indexed papers and file 4-6 national invention-patent applications, including software copyrights where applicable.
4. Train 6-8 young investigators and graduate students and support 8-10 attendances at academic meetings related to anesthesiology and neuroscience.

13. Annual Research Plan

Year 1

Clinical EEG will be used to characterize brain-network changes across age groups and anesthetic agents. Small-animal EEG and electromyography will be integrated to evaluate anesthetic effects under different drugs, together with awake small-animal MRI to characterize network differences across wakefulness, loss of consciousness, and recovery. Clinical EEG will be integrated with artificial-intelligence methods to screen whole-brain targets associated with age- and drug-dependent changes in consciousness and to extract key EEG features. Data will be organized, one review article will be prepared, and 2-3 team members will attend national neuroscience or anesthesiology meetings.

Year 2

At the animal level, advanced neurobiological techniques will be used to validate candidate brain regions or circuits, particularly frontal-hub cortico-cortical and cortico-thalamic circuits, and to investigate their neurobiological mechanisms. tTIS will be used to modulate selected targets and evaluate effects on consciousness in mice, and awake small-animal MRI will be combined with advanced neuroscience techniques to investigate mechanisms of tTIS-mediated modulation. At the clinical level, the study will examine the effects of tTIS targeting on recovery of consciousness across age groups and anesthetic agents and will collect multimodal EEG, cerebral oxygenation, and MRI data to establish a clinical tTIS modulation database. Data will be organized, 2-3 papers and 1-2 national invention patents will be prepared, and 2-3 team members will attend national neuroscience-related meetings.

Year 3

EEG-fMRI-NIRS multimodal data will be integrated to extract key brain-region features associated with transitions in consciousness. A new multimodal anesthesia-depth monitoring framework will be developed using nonlinear theory, neural oscillation measures, and neurovascular-coupling algorithms. A prototype precision anesthesia-depth monitoring device will then be developed. The project aims to publish 2-3 academic papers, file 2-3 national invention patents, and support 3-4 team members in attending interdisciplinary medical-engineering conferences.

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