Study Title: Exploring the Association of Perioperative Electroencephalographic Changes Following Preoperative Intranasal Dexmedetomidine or Esketamine With Negative Postoperative Behavioral Changes in Children Undergoing Day Surgery

NCT Number: Not yet assigned

Document Date: December 31, 2025

Uploaded by / Sponsor / Investigator:Sun Fei, Nanjing Children's Hospital

Document Type: Study Protocol and Statistical Analysis Plan

Clinical Research Protocol

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Contact number: 18100619994

Study period: December 2025 to November 2027

Version: V2.0

Version date: December 31,2025

I. Basis for Study Proposal (This section primarily outlines the background of the study, including domestic and international research advancements, the novelty of the study, prominent clinical and scientific issues, proposed research hypotheses, and a list of key references. If involving drug research, please specify whether the indicated indications fall within the approved scope by the National Medical Products Administration [NMPA]).

1.1 Background of the study, domestic and international research progress, and research hypotheses

Negative postoperative behavioral changes (NPOBCs) are common perioperative complications in children, with their mechanisms and risk factors remaining unclear, manifesting as a series of neuropsychiatric abnormalities [1]. NPOBCs may occur during the early post-anesthesia recovery phase, presenting as postoperative delirium (ED), characterized by altered environmental awareness and disorientation in pediatric patients. Alternatively, they may emerge as long-term behavioral disturbances several days or weeks postoperatively, including sleep and feeding disorders, irritability, increased nightmares, anxiety, enuresis, fear of healthcare providers, dark fear, and non-compliance. The incidence of NPOBCs is particularly high in children undergoing day surgery: studies report that nearly half of pediatric patients (approximately 47.8%) exhibit negative behavioral changes on the first postoperative day, with about 7.5% still displaying abnormal behaviors by day 7 [2]. These postoperative behavioral issues not only delay physical and psychological recovery and increase readmission rates but also impose significant caregiving burdens on families. Therefore, proactive identification of high-risk factors and perioperative interventions to reduce NPOBCs incidence have become critical challenges in pediatric day surgery anesthesia management.

In recent years, the day surgery model has rapidly developed in pediatric surgery due to its advantages of shortening hospital stays, accelerating bed turnover, reducing the risk of nosocomial infections, and lowering medical costs. However, the extremely short observation period and rapid turnover of pediatric patients undergoing day surgery, coupled with the unique anatomical and physiological characteristics of

children, impose higher demands on perioperative anesthesia management in pediatrics. Particularly, patients discharged within hours postoperatively lack specialized nursing care and treatment, resulting in a significantly higher risk of postoperative negative behavioral changes (NPOBCs) compared to those requiring hospital observation. Studies indicate that despite alleviating long-term psychological stress for both children and parents, approximately 65% of pediatric patients exhibit significant preoperative anxiety, which is closely associated with postoperative negative behavioral changes such as bedwetting, sleep disturbances, and eating disorders [3]. Therefore, in the anesthesia management of pediatric day surgery, strengthening preoperative psychological counseling for patients, actively monitoring and intervening in high-risk factors during and after surgery to reduce NPOBCs is considered one of the critical tasks for improving perioperative safety and patient prognosis quality.

The intrinsic mechanisms by which pediatric anesthesia induces NPOBCs remain unclear. Extensive animal experiments and clinical studies have demonstrated that general anesthetic agents can cause neurodegenerative damage in the developing brain, leading to long-term neuropsychiatric abnormalities [4]. Potential key mechanisms include: 1) Anesthetics induce widespread neuronal apoptosis and inhibit neuronal regeneration and differentiation, particularly in the hippocampal dentate gyrus region responsible for learning and memory; 2) Anesthetics disrupt synaptic formation and interfere with the maturation and stability of glial cytoskeletons, which play critical roles in higher neural functions such as learning and memory; 3) Changes in electroencephalogram (EEG) patterns during anesthesia, such as brain suppression under deep anesthesia, have been associated with postoperative delirium in children, suggesting that abnormal EEG activity may also contribute to NPOBCs. In summary, the adverse effects of general anesthetics on the developing central nervous system and intraoperative physiological stress responses are considered pivotal factors in triggering postoperative neuropsychiatric abnormalities in children, with specific mechanisms requiring further investigation and elucidation.

Optimizing preoperative medication to reduce abnormal behaviors during the

postoperative recovery period and postoperative adverse behavioral changes (NPOBCs) in children is currently a research focus in pediatric anesthesiology. Dexmedetomidine (DEX) and esketamine (ESK), two sedatives with distinct mechanisms of action, provide novel approaches for perioperative management. Dexmedetomidine, a highly selective α 2-adrenergic receptor agonist, exhibits sedative, anxiolytic, and synergistic analgesic effects with minimal respiratory depression, making it increasingly important in pediatric anesthesia [5]. A randomized controlled trial by Yao et al. involving 156 children undergoing strabismus surgery demonstrated that intranasal instillation of DEX at 2 μg/kg 45 minutes preoperatively significantly reduced the incidence of postoperative delirium during recovery [6]. A meta-analysis by Ericksen et al. (including 12 studies with 1,133 pediatric patients) comparing intranasal DEX with midazolam as preoperative sedatives showed that DEX reduced the risk of postoperative agitation (ED) by approximately half and improved preoperative sedation depth [7]. These studies suggest that intranasal DEX administration represents a non-invasive and effective option for pediatric preoperative sedation. Al Mutair et al. conducted a meta-analysis of four randomized controlled trials (RCTs) involving 669 children undergoing tonsil/adenoidectomy, finding that preoperative DEX administration significantly decreased the incidence of postoperative excitement/delirium and significantly reduced PAED scale scores compared to placebo [8]. Electroencephalogram (EEG) monitoring revealed that the pediatric patient exhibited brainwave patterns resembling natural sleep during DEX sedation: under no-stimulus conditions, the EEG background rhythm approached physiological Stage II sleep, characterized by moderate increases in theta and alpha wave power with no significant changes in delta waves. The overall EEG activity patterns closely matched natural sleep and did not interfere with EEG signal interpretation. These features make dexmedetomidine one of the ideal preoperative sedatives for pediatric patients, with its ability to reduce delirium incidence during recovery and promote stable patient awakening having been preliminarily validated in clinical studies.

Eszopiclone (ESK), the dextrorotatory isomer of eszopiclone, exhibits outstanding analgesic and dissociative sedative effects during pediatric perioperative periods. Its anesthetic potency is approximately twice that of the racemic eszopiclone, with faster onset of action and quicker recovery, accompanied by quicker postoperative cognitive function recovery and lower incidence of adverse psychiatric effects (e.g., hallucinations) [9]. Consequently, it has garnered significant attention in recent years for sedation and analgesia in pediatric outpatient surgeries. A recent systematic review by Liu et al. included 23 randomized controlled trials (RCTs) involving 1,996 pediatric patients undergoing tonsil/adenoid surgeries, demonstrating that perioperative administration of ESK significantly reduced the incidence of postoperative delirium/agitation during recovery, with a risk ratio of approximately 0.33 (95% CI $0.25-0.44$) compared to the control group [10]. In a randomized controlled study by Xing et al. involving 84 preschool children requiring general anesthesia for dental day surgeries, preoperative nasal DEX combined with ESK pretreatment was compared. Results showed that the combined drug group exhibited significantly fewer postoperative early adverse behaviors such as anxiety compared to the DEX monotherapy group [11]. Additionally, an RCT by Liao et al. evaluated the effects of preoperative nasal DEX, single ESK administration (1 mg/kg), and DEX+ESK combination on postoperative behavior. The results demonstrated that the incidence of delirium during the postoperative recovery period in the ESK monotherapy group was approximately 38.1%, which, although higher than that in the DEX group (17.2%), was significantly lower than the uninterventional baseline levels reported in previous literature. Additionally, the ESK group exhibited the lowest incidence of adverse behavioral changes at 7 days postoperatively among the three groups, suggesting that preoperative administration of ESK may help prevent short-term negative behavioral alterations in pediatric patients [12]. Regarding electroencephalographic (EEG) monitoring, children under ESK anesthesia showed a significant reduction in alpha wave relative power and enhanced theta and gamma wave activity. This abnormal EEG activation pattern may indicate premature arousal

of certain brain regions with uncoordinated cortical functions, which theoretically could be associated with delirium onset [13].

Previous studies have demonstrated that dexmedetomidine (DEX) can induce electroencephalogram (EEG) patterns similar to natural sleep during pediatric sedation. Specifically, DEX-induced sedation exhibits EEG characteristics resembling non-rapid eye movement (NREM) sleep stages: spindle waves and slow-wave activity similar to those observed in stage II sleep, accompanied by mild increases in theta, alpha, and beta band power compared to wakefulness, with no significant suppression of delta waves. At deeper sedation levels, EEG patterns become predominantly slow-wave, approaching the deep sleep state of N3 phase [14,15]. Compared to other anesthetic sedatives, this "physiological-like" EEG induced by DEX does not cause significant functional connectivity disruption or interfere with the assessment of epileptiform discharges. This sleep-like EEG feature suggests minimal interference with developing brain neural networks by DEX, potentially conferring neuroprotective effects. Studies have indeed found that ketamine derivatives inhibit neural stem cell proliferation and disrupt normal neurogenesis in juvenile animals, whereas DEX mitigates anesthetic-induced neuronal apoptosis and cognitive impairment. At the cellular level, DEX pretreatment significantly ameliorates the decline in neural stem cell viability and increased apoptosis induced by ketamine exposure, indicating DEX's ability to counteract ketamine-induced damage to developing neural systems [16]. Under sedation/anesthesia states, DEX EEG predominantly exhibits high-frequency components, demonstrating brain activity patterns more akin to wakefulness than sleep [17]. This dissociative EEG rhythm lacks the widespread slow-wave synchronization characteristic of typical sleep stages, which may impair normal functional connectivity and plasticity in developing brains. Animal studies have demonstrated that neonatal ketamine exposure increases high-frequency brain oscillations accompanied by cognitive dysfunction, suggesting potential neurodevelopmental risks associated with such drugs. In terms of EEG findings, DEX exhibited sleep-like patterns during pediatric perioperative periods without evidence of neurodevelopmental toxicity, indicating possible protective

effects on central nervous system development. Conversely, ESK-induced high-frequency brain activity reflects sedation states deviating from physiological sleep patterns, warranting caution regarding potential impacts on developing brain functions and postoperative neuropsychiatric outcomes.

In summary, current clinical research on the long-term adverse behavioral effects of preoperative nasal administration of dexmedetomidine or eszopiclone in pediatric patients remains limited, with most studies focusing on single surgical procedures such as tonsil/adenoidectomy, lacking large-scale, rigorously designed multicenter clinical trials. Regarding intraoperative EEG patterns, existing studies have demonstrated a correlation between perioperative hyponesthesia-induced EEG suppression and early postoperative delirium in pediatric patients, but whether these EEG characteristics persistently influence long-term non-postoperative behavioral complications (NPOBCs) remains unclear. Based on these findings, we propose to conduct a multicenter, randomized, double-blind clinical study involving pediatric patients undergoing day surgery to investigate the impact of preoperative nasal dexmedetomidine or eszopiclone administration on postoperative NPOBC incidence and early postoperative delirium. Additionally, we will innovatively introduce perioperative EEG monitoring parameters to explore the association between intraoperative EEG patterns mediated by these drugs and NPOBCs, evaluate the potential mediating effects of EEG characteristics (as a feasibility indicator for intervention efficacy), and elucidate the underlying mechanisms underlying the influence of interventions on postoperative neurobehavioral outcomes. We believe this study will provide comprehensive perioperative intervention strategies for pediatric day surgery patients, reduce NPOBC risks, improve postoperative neurocognitive function, and enhance perioperative rehabilitation outcomes.

1.2 Off-label Use

1.2.1 Domestic dexmedetomidine package insert

Dextromethorphan Hydrochloride Injection, 2 mL: 0.2 mg, Nanjing Zhengda Tianqing Pharmaceutical Co., Ltd.

[Pediatric Medication]

The safety and efficacy of this product in pediatric patients under 18 years of age remain unclear. Therefore, this product is not recommended for use in these populations.

1.2.2 Off-label use in this study

Dextromethorphan 2.0 μg/kg administered via nasal route was used for preoperative sedation in children. Based on the "China Anesthesiology Guidelines and Expert Consensus" and the current clinical application status of dextromethorphan both domestically and internationally, 2.0 μg/kg nasal administration was selected for preoperative sedation in children in terms of applicable population, indications, route of administration, and dosage.

1.2.3 Expert Consensus on Off-Label Use

In Chapter 32 "Expert Consensus on Off-Label Use of Common Pediatric Anesthetic Drugs" of the "China Anesthesiology Guidelines and Expert Consensus" (2017 Edition) (Latest Consensus), there is clear evidence regarding dexmedetomidine: it is superior to midazolam as a preoperative medication for pediatric patients. Dexmedetomidine can effectively reduce separation anxiety and tension in pediatric patients and their parents, decrease delirium and post-anesthetic tremors, minimize postoperative agitation, improve perioperative analgesia, and reduce postoperative opioid use. Dexmedetomidine at 2.0 μg/kg demonstrates superior sedative effects compared to 1.0 μg/kg; intranasal administration is more effective than oral administration (Evidence Level 1a,2a, or 2b). The use of dexmedetomidine in this study falls within the recommended scope outlined in the "China Anesthesiology Guidelines and Expert Consensus".

1.2.4 Current Research Status at Home and Abroad

Pediatric patients often experience anxiety and tension prior to surgery. Preoperative sedatives can provide sedation to alleviate preoperative anxiety, thereby reducing pre-induction crying and enhancing participation and cooperation. In recent years, preoperative sedation for pediatric patients has been widely recommended and

utilized. Although the prescribing information for dexmedetomidine does not include specific dosing instructions for pediatric use, with the advancement of numerous clinical drug trials, dexmedetomidine has increasingly been recommended as a preoperative sedative for pediatric patients.

Anesthetic drugs, particularly when administered to children in the growth and development process, exhibit inconsistent pharmacokinetics and pharmacodynamics, necessitating specialized administration protocols. Additionally, the inherent conflicts between pediatric populations and drug clinical trials have led to widespread delays in updating drug labeling for pediatric anesthesia. As a preoperative sedative for pediatric patients, dexmedetomidine has demonstrated a gradually increasing clinical application trend. Recent studies on the sedative effects of dexmedetomidine preoperatively have effectively validated its clinical efficacy and safety profile. Nasal administration of dexmedetomidine provides sedative and anxiolytic effects without inducing respiratory depression, and the non-invasive route eliminates nasal discomfort, resulting in high patient acceptance rates.

In recent years, numerous clinical studies on preoperative intranasal administration of dexmedetomidine have been reported domestically and internationally. We analyzed five systematic reviews published over the past decade on this topic [18-22]. Two systematic reviews and meta-analyses concluded that intranasal dexmedetomidine serves as a safe and effective alternative to other agents for pediatric preoperative sedation. Kim et al. 's review selected 11 randomized controlled trials (RCTs) demonstrating superior sedative effects in children compared to oral benzodiazepines when administered intranasally. Jun et al.' s review included 13 RCTs, revealing that intranasal dexmedetomidine provided effective preoperative sedation without severe adverse events. Their findings also indicated enhanced sedation during separation from parents and reduced postoperative analgesic use. Extensive research confirms the growing prevalence of preoperative intranasal dexmedetomidine administration, establishing it as a preferred sedative option. Unlike intravenous routes, intranasal delivery avoids hepatic first-pass metabolism through mucosal absorption, theoretically achieving plasma concentrations comparable to

intravenous doses. As an α2-adrenergic agonist, dexmedetomidine is colorless, odorless, and non-narcotic without respiratory depression. Its sedative mechanism involves reducing norepinephrine release in the raphe nucleus, inducing a sleep-like state similar to natural non-REM sleep, thereby ensuring high safety profiles and optimal sedative efficacy.

In summary, based on the actual clinical practice of dexmedetomidine application and adhering to the principle of being most beneficial to patients, combined with the current research status at home and abroad, we fully consider the clinical efficacy and safety of off-label use of dexmedetomidine. Dexmedetomidine should be applied reasonably without exceeding the recommended scope of use in the "China Anesthesiology Guidelines and Expert Consensus." A safety committee should be established to monitor sedation-related adverse reactions in real time during the study period and terminate the study if necessary.

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II. Research Content

2.1 Research Objective and Significance

Primary research objective: To compare the effects of preoperative nasal spray with dexmedetomidine and eszopiclone versus saline control on NPOBCs.

Secondary research objective: To investigate the association between dexmedetomidine and eszopiclone administration and EEG characteristics as well as NPOBCs changes in awake pediatric patients.

2.2 Research Content of the Project and Key Issues to be Addressed

2.2.1 Research Content of the Project

This study was a multicenter, prospective, randomized controlled trial that enrolled 342 pediatric patients undergoing elective surgery from December 2025 to November 2027 at Nanjing Children's Hospital, Zhongda Hospital Affiliated to Southeast University, Nanjing First Hospital, and Wuxi Children's Hospital. Participants were randomly divided into three groups: Dexmedetomidine nasal spray group (Group D): received dexmedetomidine 2.0 μg/kg nasal spray 30 minutes prior to anesthesia induction; Eszopiclone nasal spray group (Group L): received eszopiclone 1.0 mg/kg nasal spray 30 minutes prior to anesthesia induction; and saline control group (Group C): received an equal volume of saline nasal drops 30 minutes prior to anesthesia induction. The incidence of NPOBCs at postoperative days 3, 7, and 28, as well as the correlation between postoperative EEG characteristics and NPOBCs during recovery, were observed across all three groups.

2.2.2 Key Issues to be Addressed

This study aims to elucidate the objective efficacy and safety characteristics of nasal spray administration of dexmedetomidine and eszopiclone in preventing and reducing postoperative negative behavioral changes (NPOBCs) in pediatric patients undergoing day surgery compared to a placebo control group. The research is expected to reveal potential differences in the two drugs' effects on improving perioperative brain health at different time points (days 3, 7, and 28 postoperatively), providing critical high-level evidence-based medicine for optimizing pediatric perioperative management. More importantly, this study will systematically explore the dynamic association between characteristic electroencephalographic patterns during anesthesia recovery (e.g., specific frequency band power, burst suppression, slow-wave activity) and postoperative adverse behavioral outcomes in pediatric patients. This approach may identify clinically significant EEG biomarkers in children, laying a solid electrophysiological foundation for developing non-invasive, early predictive and intervention strategies for NPOBCs risk. The findings will significantly advance understanding of NPOBCs mechanisms and refine protective strategies.

2.3 Study Design

2.3.1 Types of Study Designs and Research Centers

This study employed blinded methods for both investigators and patients. Approximately 30 minutes prior to surgery, the pediatric patient was admitted to the anesthesia preparation room, where an anesthesia nurse not involved in the study received randomized allocation results from the central system and prepared corresponding formulations. The investigational drug was administered via the same unlabeled nasal spray pump, with all formulations diluted to 1 ml before being injected into sterile blank nasal spray pumps. These pumps were then handed over to the study intervention implementer, who remained unaware of the administered medications. The intervention was discontinued immediately after completion. Subsequent investigators remained uninformed about group assignments, while postoperative follow-up was conducted by an independent investigator. Data analysis was performed by a statistical expert not participating in the study.

This was a multicenter, prospective, randomized controlled trial that enrolled 342 pediatric patients undergoing elective surgery from December 2025 to November 2027 at Nanjing Children's Hospital, Zhongda Hospital Affiliated to Southeast University, Nanjing First Hospital, and Wuxi Children's Hospital.

2.3.2 Randomization and Blinding

This study employed a dynamic stratified block randomization method with central randomization. Stratification was performed based on center (4 centers), age (0-6 years, 7-12 years), and surgical type (head and neck surgery, abdominal surgery, limb surgery). Block randomization was conducted within each stratification level by generating random block sequences of size 6 or 9 in advance and placing them into a random sequence pool. When patients were enrolled in a given stratification level, a block sequence was randomly selected from the central randomization system and assigned to that level until the sequence pool was exhausted. Recruitment concluded once all block sequences in the pool had been allocated.

This study employed blinded methods for both investigators and patients. Approximately 30 minutes prior to surgery, the pediatric patient was admitted to the anesthesia preparation room, where an anesthesia nurse not involved in the study received randomized allocation results from the central system and prepared corresponding formulations. The investigational drug was administered via the same unlabeled nasal spray pump, with all formulations diluted to 1 ml before being injected into sterile blank nasal spray pumps. These pumps were then handed over to the study intervention implementer, who remained unaware of the administered medications. The intervention was discontinued immediately after completion. Subsequent investigators remained uninformed about group assignments, while postoperative follow-up was conducted by an independent investigator. Data analysis was performed by a statistical expert not participating in the study.

2.3.3 Sample Size

Based on our preliminary study results, the incidence of adverse behavioral changes at 7 days postoperatively was 50% among pediatric patients undergoing day surgery. We hypothesize that dexmedetomidine or eszopiclone can reduce the

incidence of these events by at least 50%, implying a clinical minimum effective difference of 25%. Using PASS15.0 software to calculate sample size according to a 1:1:1 ratio, a sample size of 91 cases per group would provide 90% power and a two-tailed significance level of 0.05, while accounting for a 20% dropout rate. The actual sample size required per group is estimated to be 114 cases, with a minimum total sample size of 342 cases.

2.4 Study Subjects

Pediatric patients undergoing elective surgery at participating centers in this study.

2.5 Research Procedures and Technical Approach

2.5.1 Research Steps

On the day prior to surgery, investigators reviewed the surgical application form to determine eligibility for enrollment based on the inclusion criteria. During preoperative visits, basic patient information was collected, along with identification of risk factors for perioperative respiratory adverse events, and informed consent was obtained from family members or guardians. All patients were required to fast and abstain from liquids before surgery: solid foods for 6-8 hours, breast milk for 4 hours, formula milk for 4 hours, and clear liquids for 2 hours. Basic vital signs of patients were recorded.

Thirty minutes prior to surgery, the pediatric patient was escorted by parents into the anesthesia preparation room. An anesthesia nurse not involved in the study received the randomized allocation results from the central system and prepared the corresponding formulations. The investigational drug was administered via the same unlabeled nasal spray pump, with all preparations diluted to 1 mL before being injected into sterile blank nasal spray pumps. The pumps were then handed over to the study intervention implementer, who remained unaware of the administered medications. The patient was withdrawn from the study immediately after receiving the intervention. Continuous monitoring of non-invasive blood pressure, heart rate, and pulse oximetry saturation was performed every 5 minutes, along with evaluation of sedation alertness (OAA/S) scores at 5-minute intervals. Prior to entering the

operating room, if the patient exhibited severe anxiety or fear, parents were first provided verbal reassurance, accompanied by behavioral interventions such as animated film screenings and toy play. If symptoms persisted, midazolam sedation was administered, with relevant observations recorded.

After patient admission, routine monitoring of heart rate, pulse oximetry, and non-invasive blood pressure was performed with data collected every 5 minutes. The anesthesia induction method was selected by the lead anesthesiologist based on clinical practice, either through inhalation anesthesia induction or simple intravenous induction. For intravenous anesthesia induction, propofol 2-3 mg/kg and fentanyl 2 μg/kg were administered after establishing an open intravenous line. In the case of inhalation anesthesia induction, 8% sevoflurane was used for inhalation. After loss of consciousness and establishment of an intravenous line, fentanyl 2 μg/kg or sufentanil 0.3 μg/kg was administered. All patients received mivaclopramide 0.15 mg/kg or cisatracurium 0.15 mg/kg, followed by endotracheal intubation and connection to an anesthesia machine for mechanical ventilation. Mechanical ventilation was performed using either pressure-controlled or volume-controlled mode, with an intraoperative oxygen-air mixture ratio of 1:1 at a flow rate of 2 L/min.

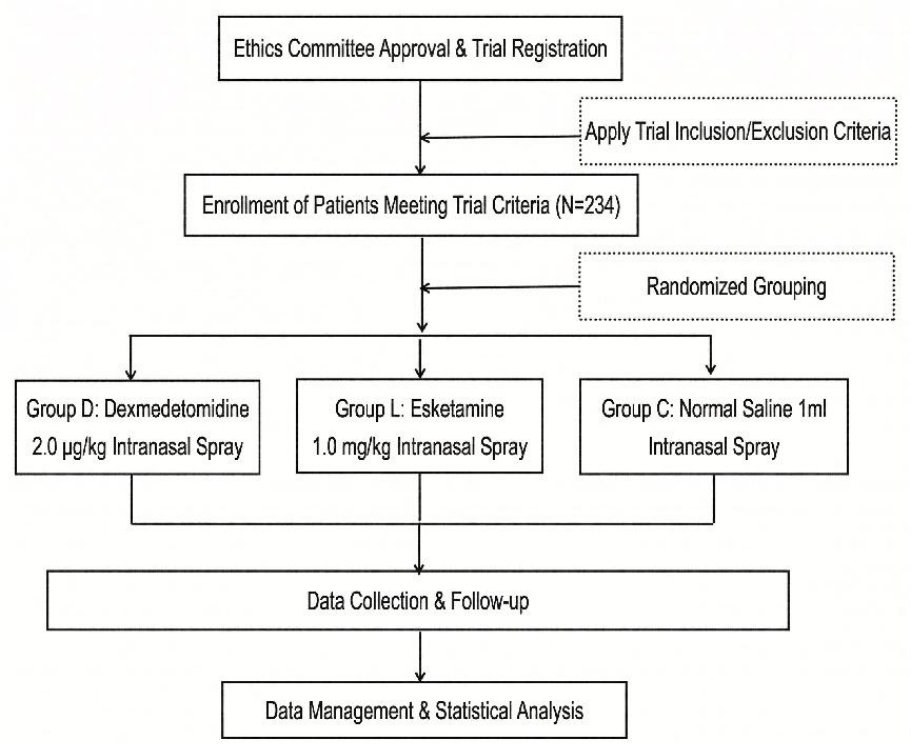
Anesthesia maintenance was achieved through continuous inhalation of 1% sevoflurane via anesthesia machine, combined with intravenous continuous infusion of propofol at a rate of 4-6 $mg \cdot kg^{-1} \cdot h^{-1}$, with dosing adjustments based on depth of anesthesia monitoring. Intraoperative analgesia was determined by the anesthesiologist, with optional addition of fentanyl or continuous infusion of remifentanil. The tidal volume was adjusted to maintain $PetCO_2$ within the range of 35-45 mmHg, with intraoperative AI monitoring to sustain anesthesia depth between 40-60%. Records included anesthesia and operative duration, fluid intake, as well as respiratory adverse events during induction and maintenance phases.

After surgery, the patient was administered inhalation anesthesia and intubated with a tube for transfer to the Post-Anesthesia Care Unit (PACU). The endotracheal

tube was removed by another independent anesthesiologist after the child regained consciousness. The PACU stay time, tube removal time, and respiratory adverse events were recorded. Postoperative pain scores and PAED scores were documented after the child's awakening. If additional sedative or analgesic medications were administered, the drug type and dosage were recorded. The patient was returned to the ward once the Steward score exceeded 4.

After discharge, the pediatric patients were followed up on postoperative days 3, 7, and 28 to assess postoperative behavioral changes. Follow-up methods included but were not limited to WeChat mini-programs, Questionnaire Star, text messages, and telephone calls. (Follow-up did not require parental attendance at the hospital and incurred no associated transportation costs.)

2.5.2 Technical Roadmap



2.6 Evaluation Indicators

① Primary outcome indicator

Incidence of adverse behavioral changes at 7 days postoperatively (PHBQ-AS

scale).

Secondary outcome indicators

The incidence rates of postoperative adverse behavioral changes at 3 days and 28 days postoperatively;

③ Other outcome indicators

(1) Sedation score (MOAA/S scale) 30 minutes after administration;

(2) Post-sudden awakening pain score (FLACC score);

(3) Postawake agitation and delirium score (PAED score);

(4) Electroencephalographic characteristics of pediatric patients during surgery and postoperative recovery period;

(5) The incidence rates of other perioperative adverse events such as nausea, vomiting, and allergic reactions;

(6) Awakening time and postoperative hospitalization duration;

(7) Parental satisfaction.

④ General data collection

The patient's age, gender, height, weight, ASA classification, surgical type, operative time, anesthesia duration, as well as parental educational level, child temperament type (measured using the Emotional, Activity, and Social Temperament Inventory), baseline parental anxiety level (assessed with the State-Trait Anxiety Scale), and scores from the Modified Yale Preoperative Anxiety Scale for pediatric patients.

2.7 Data Management and Statistical Analysis Plan, Confidentiality Plan for Materials

2.7.1 Data Management

Clinical studies will establish corresponding data safety monitoring plans based on risk levels. During the implementation phase, all adverse events will be meticulously documented, properly managed, and tracked until resolved or stabilized, with timely reporting of serious adverse events and unexpected incidents to the ethics committee and regulatory authorities. The principal investigator will conduct regular cumulative reviews of all adverse events and convene investigator meetings when necessary to assess study risks and benefits. This trial is designed as a double-blind

study, with unblinding performed when required to ensure participant safety and legal rights. Independent data monitors will be assigned to oversee study data, and an independent data safety monitoring committee will be established to evaluate accumulated safety and efficacy data, determining whether to proceed with the study.

2.7.2 Statistical Analysis Plan

（1）Statistical analysis was performed using statistical software SPSS 26.0, graphical visualization was conducted with GraphPad Prism 8.0.2, and EEG data processing was carried out using MATLAB.

（2）The Kolmogorov-Smirnov test was used to determine whether continuous data followed a normal distribution.

（3）Continuous data conforming to normal distribution within the group were expressed as mean ± standard deviation ((x ± s)), non-normal distributed data were presented as median (M) and interquartile range (IQR), and categorical variables were expressed as rates.

（4）For continuous data with normal distribution within each time point across three groups, repeated measures ANOVA was employed for comparison. Between groups at the same time point, normal distribution continuous data were analyzed using one-way ANOVA, with P-values adjusted for Bonferroni correction for pairwise comparisons. For non-normal distributed continuous data among groups, the Kruskal-Wallis H-rank sum test was applied, while non-normal pairwise comparisons utilized the Mann-Whitney U test.

（5）For comparisons of ordinal categorical variable data among the three groups, the chi-square test of difference analysis was employed. Based on sample size and minimum theoretical frequency, Pearson's chi-square test or Fisher's exact probability method was selected to determine whether there was an association between grouping and outcome variables.

（6）Significance level: α=0.05, P<0.05 was considered statistically significant.

2.7.3 Data Confidentiality Plan

To ensure the confidentiality, security, and integrity of clinical research data and related information, all researchers and other relevant personnel at the centers have

signed confidentiality agreements and understood their obligations and responsibilities. Data is recorded in paper-based collection forms and ultimately consolidated by members of the Data Security Committee in Excel files for storage, with encrypted electronic data accessible only by authorized personnel. Strict restrictions are imposed on data access permissions for researchers and other relevant individuals, permitting data disclosure only when necessary. The protocols are scheduled for periodic review and updates to ensure alignment with the latest technologies, regulations, and requirements. Upon completion of all enrollment activities, an unblinded statistician conducts data analysis, reports findings, and drafts conclusions. This process does not disclose any patient-specific information, and all patients retain no access rights.

III. Case Selection

3.1 Eligibility Criteria

（1）American Society of Anesthesiology (ASA) classification

Grade I to III;

（2）Age 2-12 years;

（3）**The patient is scheduled for daytime surgery under general anesthesia.**

3.2 Exclusion Criteria

（1）Congenital diseases or severe hepatic and renal dysfunction;

（2）Allergy to the investigational drug;

（3）Neuromuscular disorders, cerebral palsy, epilepsy;

（4）Other psychiatric or neurological disorders;

（5）Body mass index ≥ 30 kg/m^2;

（6）Severe upper respiratory tract infection prior to surgery;

（7）Administration of sedatives or analgesics within 48 hours prior to surgery;

（8）Exposure to significant life stressors within one month prior to surgery (e.g., changes in family/school environment, parental divorce or death).

(9) Refusal of participation by family members or guardians.

3.3 Exit and Termination Criteria

The withdrawal of the study by the guardian of the subject constitutes the termination of the patient's participation in the study.

3.4 Duration of study participation for participants

Fully participated throughout the period from December 2025 to November 2027.

3.5 Recruitment Process

No recruitment.

IV. Research Risk/Benefit Assessment

4.1 Research Benefits (Individual and Social Benefits)

This multicenter randomized controlled trial aims to elucidate the objective efficacy and safety characteristics of nasal spray administration of dexmedetomidine and eszopiclone in preventing and reducing postoperative non-performing behavior changes (NPOBCs) in pediatric patients undergoing day surgery compared to placebo control groups. The study is expected to reveal potential differences in the two drugs' effects on improving perioperative brain health at different time points (days 3, 7, and 28 postoperatively), providing critical high-level evidence-based medicine for optimizing pediatric perioperative management. More importantly, this study will systematically explore the dynamic association between characteristic electroencephalographic patterns during anesthesia recovery (e.g., specific frequency band power, burst suppression, slow-wave activity) and postoperative adverse behavioral outcomes in pediatric patients. This may identify clinically significant EEG biomarkers in children, laying a solid electrophysiological foundation for developing non-invasive, early predictive and intervention strategies for NPOBCs risk, thereby significantly advancing the understanding of NPOBCs mechanisms and precision-oriented protective strategies.

4.2 Research Risks

The subjects were patients undergoing elective surgery under conventional general anesthesia, without additional study risks.

4.3 Risks and Protection for Special Populations

The subjects were patients undergoing elective surgery under conventional general anesthesia, without additional study risks.

5、Multicenter clinical study: Please specify the lead institution and task division within this center

The Children's Hospital affiliated to Nanjing Medical University served as the primary responsible institution, with Sun Fei acting as the principal investigator and project liaison.

VI. Ethics in Clinical Research

Clinical research will adhere to the World Medical Assembly's "Helsinki Declaration" and related regulations. Prior to the initiation of the study, the trial protocol must be approved by the ethics committee before the clinical research is conducted. Before enrolling each participant in the study, investigators are obligated to provide a comprehensive and detailed explanation of the study objectives, procedures, and potential risks to the participant or their legal representative, and obtain written informed consent (the version approved by the ethics committee). Participants should be informed of their right to withdraw from the study at any time, and the informed consent form must be retained as part of the clinical research documentation for future reference. The privacy and data confidentiality of participants will be strictly protected throughout the study.

VII. Annual Plan

December 2025-February 2026: Ethics review and trial registration submission

March 2026 – August 2026: Conduct research team training, organize unified training for all multicenter healthcare professionals involved in the study, initiate formal experimental case recruitment, and commence randomization of eligible pediatric patients according to the principle of randomized grouping. Collect baseline data and preoperative relevant indicators, and establish a case database.

September 2026 – February 2027: Continued case recruitment and intervention efforts will be conducted in strict accordance with the study protocol, with ongoing data

collection and interim analysis phase.

March 2027 – November 2027: Data organization, analysis, and outcome summarization phase. Based on the data analysis results, write a research paper, summarize research findings, and compile a research report. Systematically elaborate on the research process, methods, and outcomes to provide reference for subsequent clinical applications. Apply the research findings to clinical practice, conduct promotion activities in partner hospitals, observe the practical clinical efficacy of the outcomes, and collect feedback to lay the foundation for further in-depth research. Expected to publish 1-2 Chinese core journals or 1 SCI paper.

VIII. Assessment Indicators

(1) Case data collection: Complete the collection of all case numbers from all centers to obtain relatively comprehensive pediatric case data.

(2) Data analysis: Complete data organization and statistical analysis based on the primary and secondary research objectives.

(3) Research Paper Writing and Publication: Publish 1-2 Chinese core journals or 1 SCI paper.

IX. Preliminary Research Foundation and Working Conditions

(1) Theoretical Feasibility: Dexmedetomidine inhibits excessive activation of the sympathetic-adrenal cortex axis by activating α2-adrenergic receptors, reduces the release of neuroinflammatory factors (e.g., IL-6, TNF-α), and simultaneously enhances prefrontal GABAergic neuron activity to regulate dysfunction in the amygdala-cortical circuit. The induced increase in δ-wave power may mitigate postoperative behavioral abnormalities by stabilizing brain network synchrony. In

contrast, eszopiclone antagonizes NMDA receptors to block glutamatergic excitotoxicity and activates the BDNF-mTOR pathway to promote synaptic plasticity repair. The induced enhancement of γ oscillations improves default mode network segregation. Both agents target core pathological mechanisms of neurotransmitter imbalance (GABA/glutamate) and synaptic dysfunction in ASD children through dual anti-inflammatory and anti-excitotoxic pathways during the perioperative period. Preliminary clinical evidence shows that dexmedetomidine reduces postoperative agitation incidence in children by 30%, while low-dose ketamine nasal spray has been demonstrated to decrease aggressive behavior by 42% in ASD patients. Combined with the EEG theta/β wave power ratio prediction model established in our preclinical trial, these findings provide theoretical and practical support for correlating intervention mechanisms with behavioral outcomes.

(2) Technical feasibility: Nanjing Medical University Affiliated Children's Hospital is a Grade III Class A hospital with long-term experience in pediatric surgical procedures and exceptional technical expertise, capable of providing the required surgical technical support for this study. The Department of Anesthesiology possesses extensive theoretical knowledge and clinical practice experience in pediatric anesthesia, with medical technical proficiency that far surpasses that of other institutions in China.

(3) This study was conducted at Nanjing Children's Hospital, Zhongda Hospital Affiliated to Southeast University, Nanjing First Hospital, and Wuxi Children's Hospital. These institutions boast superior geographical locations, advanced medical expertise, extensive regional patient distribution, and a high annual volume of elective surgeries, providing abundant pediatric case sources to meet the required sample size for the study.

(4) The research team members have extensive prior research experience. The project leader, Dr. Sun Fei, is an attending physician in the Department of Anesthesiology at Nanjing Children's Hospital, with profound clinical expertise in anesthesia management for pediatric surgeries. As the first author and corresponding

author, he has published 5 papers in SCI-indexed and core journals, secured 1 hospital research project grant, and obtained 4 utility model patents.

(5) This study incorporated quality control feedback from experts in related fields such as pediatric surgery, anesthesiology, and statistics teaching and research departments. The research was conducted collaboratively by senior-title physicians, intermediate-title physicians, junior-title physicians, and master's/doctoral students, with quality control implemented at multiple levels.

X. Investigator Information Form

1. Principal Investigator

surname and personal name	unit	Degree/Title	Ethical considerations and GCP training	Phone/Email
Sun Fei	Children's Hospital Affiliated to Nanjing Medical University	Master's degree/Attending Physician	Overall project design, clinical trial data collection, research data analysis, statistical processing	18100619994/ drsunfei@njmu.edu.cn

2. Project team members

surname and personal name	unit	Degree/Title	Project task allocation	Ethical considerations and GCP training
Zhang Li	Children's Hospital Affiliated to Nanjing Medical University	Doctor/Chief Physician	Overall project coordination, allocation and utilization of project funds, statistical organization of research data	yes

Shen Fangming	Zhongda Hospital Affiliated to Southeast University	Doctor/Resident	Data collection and organization, statistical analysis	
Han Xiaoyu	Wuxi Children's Hospital	Master's degree/Attending Physician	Data collection and organization, statistical analysis	
Chen Lihai	Nanjing First Hospital	PhD/Associate Chief Physician	Data collection and organization, statistical analysis	

XI. Principal Investigator's Commitment (Submission of the research protocol to the ethics committee shall be deemed as compliance with this commitment):

I hereby guarantee the authenticity of the application content. I will fulfill the responsibilities of a principal investigator, strictly comply with national regulations regarding clinical research, ensure adequate time allocation for research activities, diligently perform tasks, and submit relevant materials on schedule. Should any inaccuracies in the submission or violations of regulations occur, I will assume full responsibility.

I agree to comply with the review opinions of the ethics committee and commence the clinical trial implementation after approval, promptly report any changes in clinical trial activities to the ethics committee, as well as unexpected issues involving risks to subjects or other personnel, and obtain re-approval for ethical review before proceeding. Throughout the study process, I will conscientiously submit relevant reports in accordance with the requirements specified in the ethics committee's approval documents.