Clinical Research Protocol

Title of the Study: Effects of Preoperative Nasal Sedation with Dexmedetomidine and Eszopiclone on Postoperative Behavioral Changes in Children with Autism Spectrum Disorder

Source of the project:Horizontal Project

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1. Basis for the Proposal (This section primarily outlines the background of the study, including domestic and international research advancements, the novelty of the study, and the prominent clinical and scientific issues addressed. It proposes research hypotheses and includes a list of key references. If the study involves drug research, please specify whether the indicated indications are within the approved scope of use by the National Medical Products Administration (NMPA).)

1.1 Background of the Study, Domestic and International Research Progress, and Research Hypotheses

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with clinical phenotypes encompassing various subtypes, including typical autism, pervasive developmental disorder (PDD), and Asperger syndrome. The core symptoms of ASD primarily manifest as social interaction impairments, language communication deficits, and restricted, repetitive patterns of behavior and interests. ASD is also associated with numerous non-core symptoms, including anxiety, irritability, aggression, attention deficit and hyperactivity disorder (ADHD), depression, epilepsy, gastrointestinal and immune dysfunction, metabolic abnormalities, and sleep disorders [1].According to the latest epidemiological survey data from the World Health Organization (WHO), the global prevalence of autism spectrum disorder (ASD) in children exceeds 1%, with a sustained upward trend in recent years [2]. This phenomenon may be associated with multiple factors, including revisions in diagnostic criteria, increased public awareness, and changes in environmental factors [3].

Compared to normally developing peers, children with ASD may face more complex medical conditions and more frequent healthcare system visits [4]. Due to their inherent social communication impairments and sensory processing abnormalities, they struggle to adapt to unfamiliar environments and exhibit heightened sensitivity to external stimuli, posing unique challenges in seeking medical services and undergoing surgical procedures [5]. Perioperative adverse experiences can trigger significant physiological and psychological stress responses, with the incidence of Negative Postoperative Behavioural Changes (NPOBCs) being

markedly higher than that in healthy age-matched populations. This disparity holds substantial clinical implications for postoperative recovery and long-term quality of life [6].

NPOBCs (Non-Prominent Post-Anesthesia Complications) are one of the significant complications in pediatric anesthesia, exhibiting distinct biphasic characteristics in their onset: the acute phase (post-anesthesia recovery period) primarily manifests as postoperative delirium (ED), characterized by environmental disorientation and time-space cognitive disturbances; the long-term phase (several weeks to months postoperatively) presents with multidimensional behavioral abnormalities, including but not limited to sleep rhythm disorders, altered eating patterns, emotional regulation disturbances (irritability/anxiety states), iatrogenic phobias (e.g., white coat phobia/nightophobia), and reduced treatment compliance, forming a composite behavioral phenotype.Epidemiological data indicate that approximately 50% of pediatric patients undergoing general anesthesia experience NPOBCs [7], with a significant positive correlation observed between this occurrence and adverse perioperative outcomes such as prolonged hospital stays and increased medical costs.More importantly, if these behavioral changes persist without timely intervention, they may negatively impact children's emotional regulation and cognitive development, leading to reduced medical compliance and increased frequency of visits, thereby causing sustained harm to the physical and mental health of affected children [8].

Studies have shown that mutations in ASD-related genes (e.g., SHANK3, CNTNAP2) lead to abnormal synaptic pruning and excitatory/inhibitory imbalance, which may enhance the impact of perioperative stress on neural circuits [9,10]. Additionally, alterations in the neurotransmitter system of ASD children, particularly metabolic abnormalities in the dopaminergic and serotoninergic systems, have been widely reported. These signaling disorders may be associated with clinical manifestations such as postoperative anxiety and aggressive behavior [11,12]. Furthermore, ASD children exhibit abnormal pain perception. Clinical evidence indicates that developmental abnormalities in the dorsal root ganglion (DRG) alter the

function of pain conduction pathways, resulting in atypical postoperative pain expression and consequently affecting the timeliness of clinical interventions [13]. Finally, ASD patients demonstrate dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, characterized by increased cortisol secretion and delayed ACTH response. HPA axis dysfunction is also correlated with disease severity [14].As the severity of autism spectrum disorder (ASD) increases, baseline cortisol levels significantly decrease, ACTH levels rise, and the cortisol response to ACTH stimulation diminishes [15]. Dysfunction of the HPA axis mediates neurodevelopmental abnormalities, leading to impaired executive function and reduced emotional regulation, thereby forming a vicious cycle of "medical stress-behavioral abnormalities-health damage," ultimately significantly reducing the quality of life in affected children. These neurobiological abnormalities (including synaptic dysfunction, neurotransmitter disorders, and abnormal pain perception) interact pathologically with the inherent maladaptation to medical environments in ASD children, resulting in a higher incidence of postoperative non-postoperative brain contusions (NPOBCs) and more severe symptom manifestations in this population. Although postoperative NPOBCs in ASD children have garnered widespread attention from clinicians worldwide, current research remains limited, and systematic and effective prevention and management strategies are still lacking [16].

Perioperative management for children with autism spectrum disorder (ASD) aims to control preoperative anxiety, fear, and other emotions, reduce sympathetic nervous system excitability, and minimize stress responses. Special emphasis is placed on achieving comfortable care throughout all perioperative stages. Therefore, effective preoperative sedation is of significant importance in improving the perioperative experience for ASD children. Certain sedative agents, such as dexmedetomidine and esketamine, have demonstrated protective effects in the treatment of psychiatric disorders like depression, and are promising as potential sedative therapies to reduce the risk of postoperative non-painful bowel movements (NPOBCs) in ASD populations.

Dextromethorphan is a highly selective α2-adrenergic receptor agonist that has been widely used in pediatric anesthesia practice due to its excellent sedative, analgesic, and anxiolytic effects without affecting respiration. In the ASD population, Ahmend et al. [17] reported the use of dextromethorphan intravenously during pediatric magnetic resonance imaging (MRI), demonstrating that ASD children required the same dose of dextromethorphan as healthy children of the same age, with only a slightly longer recovery period and no other complications observed. Lubitsch et al. [18] similarly reported the successful use of dextromethorphan combined with midazolam for sedation during pediatric MRI, with its safety and efficacy partially validated. However, no existing studies have reported outcomes of non-postoperative behavioral complications (NPOBCs) in ASD children. Lee-Archer et al. [8] found that in the general pediatric population, dextromethorphan reduced the incidence of postoperative delirium and agitation, and continuous intraoperative infusion of dextromethorphan decreased the risk of NPOBCs within 28 days postoperatively. It remains unclear whether dextromethorphan provides the same or even superior protection against postoperative adverse behaviors in ASD children, which requires further investigation through well-designed, large-sample, randomized controlled clinical trials with higher evidence levels.

As a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, eszopiclone is characterized by rapid onset and significant analgesic effects. In recent years, its application in the treatment of mental disorders has become increasingly widespread, with preliminary studies demonstrating its efficacy in alleviating anxiety symptoms and improving mood states. A prospective observational study conducted by Yanling Liao et al. [19] revealed that among non-ASD children aged 2-5 years who underwent tonsillectomy, the combination therapy of eszopiclone and dexmedetomidine showed significant advantages over monotherapy: compared to eszopiclone alone, the combination therapy more effectively reduced the incidence of postoperative delirium; compared to dexmedetomidine alone, it significantly decreased the number of postoperative NPOBCs on day 7. Animal experimental

studies have shown that downregulation of methyl-CpG-binding protein 2 (MeCP2) gene expression in the hippocampus can induce behavioral abnormalities associated with autism-like traits in rats [20]. The MeCP2 gene is one of the few genes with a clear causal relationship in the pathogenesis of ASD, and eszopiclone intervention can effectively prevent the occurrence of these abnormal behaviors [21].In clinical case studies, Miriam Olivola et al. [22] reported a successful case of nasal ethylchloramine treatment for ASD combined with treatment-resistant depression, suggesting its potential safety and efficacy. However, only a limited number of case reports have indicated this potential, and large-scale randomized controlled trials are still lacking to verify the impact of ethylchloramine on postoperative NPOBCs in ASD children.

Despite the relatively low prevalence of ASD, this population exhibits a higher risk of postoperative NPOBCs due to their specific psychological-behavioral disorders, making the exploration of perioperative intervention strategies for ASD children particularly crucial. Dexmedetomidine and eszopiclone, as commonly used sedatives in pediatric anesthesia, have been well-established in terms of their sedative safety and efficacy. However, their impact on postoperative NPOBCs remains unclear. Existing studies are limited by insufficient sample sizes and single-arm designs, necessitating high-quality, multicenter randomized controlled clinical trials to provide more reliable evidence-based medical data.

In conclusion, this study aims to systematically evaluate the effects of dexmedetomidine and eszopiclone nasal spray on postoperative NPOBCs in children with ASD through an international multicenter, prospective, randomized, controlled trial. The study hypothesizes that dexmedetomidine and eszopiclone nasal spray can reduce the incidence of NPOBCs in ASD children postoperatively. The findings will provide critical reference for clinicians in decision-making within relevant fields, while also contributing to the optimization of perioperative management strategies for children with autism spectrum disorder (ASD), improving patients' perioperative experience, enhancing postoperative recovery quality, and thereby alleviating the burden on families and society. This study holds significant clinical and societal value.

1.2 Off-label Use

1.2.1 Domestic Levometomidine 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 are not well established. Therefore, this product is not recommended for use in this population.

1.2.2 Off-label Use in This Study

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

1.2.3 Expert Consensus on Off-Label Use

In Chapter 32 of the "China Anesthesia Guidelines and Expert Consensus" (2017 Edition) (the latest consensus), titled "Expert Consensus on Off-Label Use of Common Pediatric Anesthetic Drugs," there is clear evidence that dexmedetomidine is superior to midazolam as a preoperative medication for children. Dexmedetomidine can effectively reduce the tension and anxiety of separated children and parents, decrease delirium and post-anesthetic tremors, reduce postoperative agitation, improve perioperative analgesia, and reduce the use of postoperative opioids. Dexmedetomidine at 2.0 μg/kg has a better sedative effect than 1.0 μg/kg; intranasal administration is more effective than oral administration (Evidence Level 1a,2a, or 2b). In this study, the use of dexmedetomidine falls within the recommended scope of the "China Anesthesia Guidelines and Expert Consensus."

1.2.4 Current Research Status at Home and Abroad

Pediatric patients often experience anxiety and tension before surgery.

Preoperative sedatives can provide sedation to alleviate preoperative anxiety, thereby reducing pre-induction crying and improving participation and cooperation. In recent years, preoperative sedation for pediatric patients has been widely recommended and used. 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 those used in children during growth and development, exhibit inconsistent pharmacokinetics and pharmacodynamics, necessitating specialized administration. Additionally, the inherent conflict between pediatric populations and drug clinical trials has led to widespread delays in updating drug labeling for pediatric anesthesia. As a preoperative sedative for pediatric patients, dexmedetomidine has demonstrated a gradual increase in clinical use. Recent studies on the sedative effects of dexmedetomidine in preoperative settings have effectively validated its clinical efficacy and safety. Nasal administration of dexmedetomidine provides sedative and anxiolytic effects without causing respiratory depression, and the absence of nasal discomfort in pediatric patients ensures high patient acceptance.

In recent years, numerous clinical studies on the preoperative nasal administration of dexmedetomidine have been reported domestically and internationally. We have systematically reviewed five systematic evaluations published over the past decade to analyze literature on this topic [23-27]. Two of these systematic reviews and meta-analyses concluded that nasal dexmedetomidine is a safe and effective alternative to other drugs for pediatric preoperative sedation. In Kim et al. 's systematic review, 11 randomized controlled trials (RCTs) were selected, demonstrating superior sedative effects in children compared to oral benzodiazepines. Jun et al.' s systematic review included 13 RCTs, which showed that nasal dexmedetomidine provided effective preoperative sedation in children without serious adverse events. Jun et al. also reported that nasal dexmedetomidine achieved better sedation in children during separation from parents compared to other drugs and reduced the need for postoperative analgesics. Extensive research indicates that

preoperative nasal dexmedetomidine administration is becoming increasingly common and appears to be a superior choice among preoperative sedatives. Nasal administration eliminates the need for intravenous access, and the drug is absorbed through the nasal mucosa, avoiding hepatic first-pass metabolism. Theoretically, plasma concentrations are comparable to intravenous doses. As an α2-adrenergic agonist, dexmedetomidine is colorless, odorless, and does not cause respiratory depression. It exerts its sedative effect by reducing norepinephrine release in the raphe nucleus, producing a sedative state similar to natural non-REM sleep, thereby ensuring high safety and efficacy.

In summary, based on the actual application of dexmedetomidine in clinical practice, adhering to the principle of being most beneficial to patients, and considering the current research status both domestically and internationally, the clinical efficacy and safety of off-label use of dexmedetomidine are fully evaluated. The use of dexmedetomidine is reasonably applied without exceeding the recommended scope of use in the "China Anesthesiology Guidelines and Expert Consensus". A safety committee is established to monitor sedation-related adverse reactions in real time during the study period, and the study may be terminated if necessary.

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II. Research Content

2.1 Research Objectives and Significance

Primary objective: To compare the effects of right 美托咪啶 and eszopiclone nasal spray versus saline control on NPOBCs in children with ASD.

Secondary objective: To investigate the correlation between postoperative EEG characteristics and NPOBCs in children with ASD.

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

2.2.1 Research Content of the Project

This study planned to enroll 234 pediatric patients undergoing elective surgery and randomly divide them into three groups: dexmedetomidine nasal spray group (Group D): receiving dexmedetomidine 2.0 μg/kg nasal spray 30 minutes before anesthesia induction; ketamine nasal spray group (Group L): receiving ketamine 1.0 mg/kg nasal spray 30 minutes before anesthesia induction; saline control group (Group C): receiving an equal volume of saline nasal drops 30 minutes before anesthesia induction. The incidence of NPOBCs at 3 days, 7 days, and 28 days postoperatively in the three groups of ASD patients was observed, as well as the correlation between the EEG characteristics of the patients at the time of awakening and postoperative NPOBCs.

2.2.2 Key Issues to be Addressed

(1) This study aims to systematically evaluate the effects of dexmedetomidine and eszopiclone nasal spray on postoperative NPOBCs in children with ASD.

The research findings will provide critical reference for clinicians 'decision-making in relevant fields, while also contributing to the optimization of perioperative management strategies for children with autism spectrum disorder (ASD), improving patients' perioperative experience, enhancing postoperative recovery quality, thereby alleviating the burden on families and society, demonstrating significant clinical and societal value.

2.3 Study Design

2.3.1 Types of Research Designs and Research Centers

This study employed a dynamic stratified block randomization method with central randomization. Stratification was performed based on center (8 centers), age (0-6 years, 7-12 years), and surgical type (head and neck surgery, abdominal surgery, limb surgery). Within each stratification level, block randomization was conducted by pre-generating random block sequences of 6 or 9 units 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 pool to assign to that level until the block sequence was exhausted. Recruitment was completed once all block sequences in the pool had been allocated.

This study employed a double-blind design 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 the randomized allocation results from the central system and prepared the corresponding formulations. The investigational drug was administered via the same unmarked 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 was unaware of the administered drug. The intervention was completed and the patient was removed from the study. Subsequent investigators remained unaware of the group assignments. Postoperative follow-up was conducted by another independent investigator, and the data analysis was performed by a statistical expert not involved in the study.

This multicenter, prospective, randomized controlled trial enrolled 234 pediatric patients undergoing elective surgery from June 2025 to January 2027 at the following institutions: Children's Hospital of Nanjing Medical University, Zhongda Hospital of Southeast University, Children's Hospital of Xuzhou Medical University, Wuxi Children's Hospital of Jiangnan University, Changzhou Children's Hospital of Nantong University, Pizhou Hospital of Xuzhou Medical University, Dongtai Hospital of Nantong University (Dongtai People's Hospital), and Suqian Hospital of Nanjing Drum Tower Hospital Group.

2.3.2 Randomization and Blinding

This study employed a dynamic stratified block randomization method with central randomization. Stratification was performed based on center (8 centers), age (0-6 years, 7-12 years), and surgical type (head and neck surgery, abdominal surgery, limb surgery). Within each stratification level, block randomization was conducted by pre-generating random block sequences of 6 or 9 units and placing them into a

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2.3.3 Sample Size

Based on our preliminary study results, the incidence of adverse behavioral changes at 7 days postoperatively was 50% among autistic children undergoing day surgery. We hypothesize that dexmedetomidine or eszopiclone can reduce the incidence of these events by at least 50%, meaning the clinical minimum effective difference is 25%. Using PASS15.0 software to calculate the sample size with a 1:1:1 ratio, 70 cases per group would provide 80% power and a two-tailed significance level of 0.05. Considering a 10% dropout rate, the actual sample size required per group is 78 cases, with a minimum total sample size of 234 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 before surgery, investigators reviewed the surgical application form to determine eligibility based on the inclusion criteria. During the preoperative visit, basic patient information was collected, along with any risk factors that might cause perioperative respiratory adverse events. Informed consent was obtained from the patient's family members or guardians. All patients were required to fast and abstain from liquids before surgery: 6-8 hours for solid foods, 4 hours for breast milk, 4 hours for formula milk, and 2 hours for clear liquids. The patient's basic vital signs were recorded.

Thirty minutes prior to surgery, the child was escorted by parents into the anesthesia preparation room. A non-participating anesthesia nurse received the randomized allocation results from the central system and prepared the corresponding formulations. The study drug was administered via the same unmarked 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 was unaware of the administered drugs. The child was withdrawn from the study immediately after the intervention. Subsequently, continuous monitoring of the child's non-invasive blood pressure, heart rate, and pulse oximetry saturation was performed, with records taken every 5 minutes. Additionally, the child's sedation alertness (OAA/S) score was assessed every 5 minutes. Prior to entering the room, if the child exhibited severe anxiety or fear, the parents were first provided with verbal reassurance, along with behavioral interventions such as playing with cartoons or toys. If symptoms persisted, midazolam was administered for sedation, with relevant records kept.

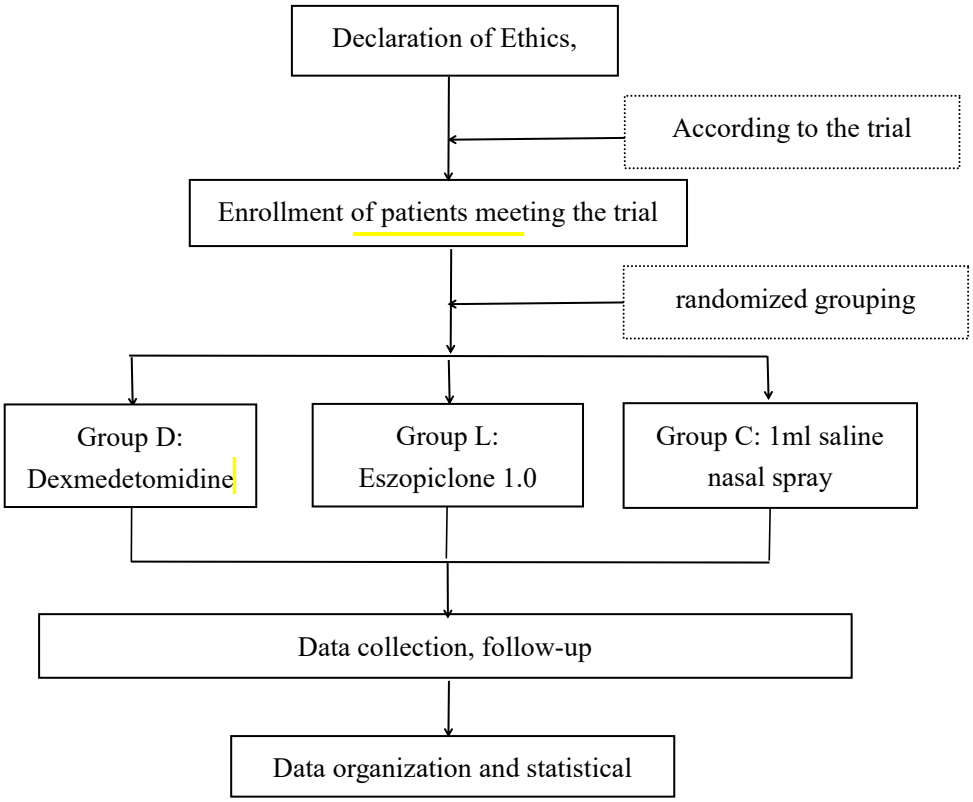
Upon admission, routine monitoring of heart rate, pulse oximetry, and non-invasive blood pressure was performed every 5 minutes. The method of anesthesia induction was selected by the lead anesthesiologist based on clinical practice, either via inhalation anesthesia or simple intravenous induction. For intravenous anesthesia induction, after establishing an open intravenous line, propofol 2-3 mg/kg and fentanyl 2 μg/kg were administered. For inhalation anesthesia

induction, 8% sevoflurane was inhaled, and after loss of consciousness, the intravenous line was established, followed by fentanyl 2 μg/kg or sufentanil 0.3 μg/kg. All patients received mivaclopramide 0.15 mg/kg or cisatracurium 0.15 mg/kg, after which a tracheal tube was inserted and connected to an anesthesia machine for mechanical ventilation. Mechanical ventilation was performed with either pressure-controlled or volume-controlled settings, with an intraoperative oxygen-to-air ratio of 1:1 and a flow rate of 2 L/min.

Anesthesia maintenance was achieved through continuous inhalation of 1% sevoflurane via anesthesia machine, with concurrent intravenous continuous infusion of propofol at a rate of 4-6 $mg \cdot kg^{-1} \cdot h^{-1}$, adjusted according to 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 $PetCO_2$ between 35-45 mmHg, with intraoperative AI monitoring to sustain anesthesia depth within the range of 40-60. Records included anesthesia and operative duration, fluid intake, and respiratory adverse events during the induction and maintenance phases.

Postoperative inhalation anesthesia is administered, and the patient is intubated and transferred to the Post-Anesthesia Care Unit (PACU) by another independent anesthesiologist. The endotracheal tube is removed after the child regains consciousness. The PACU stay time, tube removal time, and respiratory adverse events are recorded. Postoperative pain scores and PAED scores are documented after the child awakens. If additional sedative or analgesic medications are administered, the type and dosage are recorded. The patient is returned to the ward once the Steward score exceeds 4.

2.5.2 Technical Roadmap



2.6 Evaluation Indicators

① Primary outcome indicators

The incidence of adverse behavioral changes (PHBQ-AS scale) at 7 days postoperatively.

Secondary outcome indicators

The incidence of postoperative adverse behavioral changes at 3 days and 28 days after surgery;

③ Other outcome indicators

(1) 30-minute post-administration sedation score (MOAA/S scale);

(2) Post-sedation pain score (FLACC score);

(3) Postawake Delirium and Agitation Scale (PAED score);

(4) Electroencephalogram (EEG) characteristics of pediatric patients during surgery and postoperative recovery;

(5) incidence of other perioperative adverse events such as nausea, vomiting, and

allergic reactions;

(6) Awakening time and postoperative hospitalization duration;

(7) Parental satisfaction.

④General data collection

The child's age, gender, height, weight, ASA classification, surgical type, operative time, anesthesia duration, as well as the parents' educational level, the child's temperament type (measured using the Emotion, Activity, and Social Temperament Inventory), the parents' baseline anxiety level (measured using the State-Trait Anxiety Scale), and the child's Modified Yale Preoperative Anxiety Scale score.

2.7 Data Management and Statistical Analysis Plan, Confidentiality Plan

2.7.1 Data Management

Clinical research will establish corresponding data safety monitoring plans based on the level of risk. During the implementation phase of clinical research, all adverse events will be meticulously documented, properly managed, and tracked until they are adequately resolved or the condition stabilizes. Serious adverse events and unexpected incidents will be promptly reported to the ethics committee and the competent authority. The principal investigator will conduct regular cumulative reviews of all adverse events and convene investigator meetings when necessary to assess the risks and benefits of the study. This trial is a double-blind trial, with unblinding performed when necessary to ensure the safety and legitimate rights of participants. Independent data monitors will be assigned to oversee the study data, and an independent data safety monitoring committee will be established to monitor accumulated safety and efficacy data, determining whether to continue the study.

2.7.2 Statistical Analysis Plan

（1）Statistical analysis was performed using SPSS 26.0, with GraphPad Prism 8.0.2 for data visualization and MATLAB for EEG data processing.

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

（3）Continuous data with normal distribution within the group were expressed as mean ± standard deviation ($\bar{x} \pm s$), while non-normal data were presented as

median (M) and interquartile range (IQR). 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 continuous data with normal distribution at the same time point across three groups, one-way ANOVA was used. P-values for pairwise comparisons were adjusted using the Bonferroni correction. For continuous data with non-normal distribution across three groups, the Kruskal-Wallis H-sum test was applied, while Mann-Whitney U test was used for pairwise comparisons of non-normal data.

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

（6）The significance level was set at α=0.05, with P<0.05 indicating statistically significant differences.

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 registered in Excel by members of the Data Security Committee for storage. Electronic data is encrypted, and only authorized personnel are permitted to access and process the data. Access rights for researchers and other relevant personnel are strictly restricted, with data disclosure permitted only when necessary. The protocol is scheduled for regular review and updates to ensure compliance with the latest technologies, regulations, and requirements. Upon completion of all enrollment, an unblinded statistician conducts the analysis, reports the data results, and drafts the conclusions. This process does not disclose any personal patient information, and all patients have no access rights.

III. Case Selection

3.1 Eligibility Criteria

（1）American Society of Anesthesiology (ASA) classification Ⅰ~Ⅲ level ；

（2）2-12 years of age;

（3）diagnosed with autism by a psychiatrist;

（4）The patient was scheduled for elective surgery under general anesthesia.

3.2 Exclusion Criteria

（1）cardiac, thoracic and neurosurgical procedures;

（2）Congenital diseases or severe hepatic/renal dysfunction;

（3）Allergy to the investigational drug;

（4）Neuromuscular disorders, cerebral palsy, epilepsy;

（5）Other mental or neurological disorders;

（6）BMI ≥ 30 kg/m^2;

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

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

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

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

3.3 Exit and Termination Criteria

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

3.4 Duration of Study Participation

Participated in the whole process from April 2025 to March 2028.

3.5 Recruitment Process

No recruitment.

IV. Risk/Benefit Assessment of the Study

4.1 Research Benefits (Individual and Social Benefits)

(1) This study is expected to reduce the incidence of NPOBCs in children with ASD

after surgery through the administration of dexmedetomidine and eszopiclone nasal sprays.

(2) The findings of this study will provide clinicians with critical reference for decision-making in relevant fields, while also contributing to the optimization of perioperative management strategies for children with autism spectrum disorder (ASD), improving patients' perioperative experience, enhancing postoperative recovery quality, and thereby alleviating the burden on families and society, demonstrating significant clinical and societal value.

4.2 Research Risks

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

4.3 Risks and Protection for Special Populations

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

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

The Children's Hospital of Nanjing Medical University served as the primary responsible institution, with Director Zhang Li as the principal investigator and Sun Fei as the project liaison.

VI. Ethics in Clinical Research

Clinical research will comply with the World Medical Assembly's "Declaration of Helsinki" and other relevant 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, the investigator is responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their legal representative, and obtaining a 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 should be retained as part of the clinical research documentation for future reference. The privacy and data confidentiality of

participants will be protected throughout the study.

VII. Annual Plan

April 2025-May 2025: Ethics review and trial registration

June 2025-December 2026: Data collection and statistical analysis

January 2027-June 2027: Thesis writing, submission, and project completion

VIII. Evaluation 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 Publication: Authored and published 2 papers in SCI-indexed journals.

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 enhances the activity of prefrontal GABAergic neurons to regulate dysfunction in the amygdala-cortical circuit. The induced increase in δ-wave power may reduce 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 dissociation. Both agents target the 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 in children by 30%, while low-dose ketamine nasal spray has been demonstrated to decrease aggressive behavior in ASD children by 42%. Combined with the predictive model of theta/β wave power ratio established in our team's

preclinical trial, this provides theoretical and practical support for the correlation between intervention mechanisms and behavioral outcomes.

(2) Technical Feasibility: Nanjing Children's Hospital affiliated with Nanjing Medical University is a Grade III, Class A hospital with long-term experience in pediatric surgery, demonstrating exceptional technical expertise to provide the required surgical technical support for this study. The Department of Anesthesiology possesses extensive theoretical and clinical practical experience in pediatric anesthesia, with medical technical capabilities that are far ahead of the rest in China.

(3) This study was conducted at the Children's Hospital affiliated to Nanjing Medical University, Zhongda Hospital affiliated to Southeast University, Children's Hospital affiliated to Xuzhou Medical University, Wuxi Children's Hospital affiliated to Jiangnan University, Changzhou Children's Hospital affiliated to Nantong University, Pizhou Hospital affiliated to Xuzhou Medical University, Dongtai Hospital affiliated to Nantong University (Dongtai People's Hospital), and Suqian Hospital of Nanjing Drum Tower Hospital Group. These institutions boast superior geographical locations, advanced medical expertise, extensive regional patient distribution, a high annual volume of elective surgeries, and a rich pediatric case source, which can meet the required sample size for the study.

(4) The research team members have extensive prior research experience. Professor Zhang Li, the principal investigator of the project, serves as the Director of the Department of Anesthesiology at Nanjing Children's Hospital, a member of the Biomedical Committee of the Nanjing Association of Chinese Students in Europe and America, a high-level talent under Jiangsu Province's "Six Major Talent Peaks" program, and a visiting scholar at Cincinnati Children's Hospital and Dallas Children's Hospital in the United States. With years of clinical research experience, she possesses profound expertise in pediatric anesthesia.

(5) This study incorporated quality control feedback from experts in related fields such as pediatric surgery, anesthesiology, and the Department of Statistics. The research was conducted collaboratively by senior-title physicians, intermediate-title physicians, junior-title physicians, and master's/doctoral students, ensuring quality control at each level.

X. Investigator Information Form

1. Principal Investigator

surname and personal name	unit	Degree/Title	Ethical and GCP training	Phone/Email
Zhang Li	Children's Hospital of Nanjing Medical University	Doctor/Chief Physician	yes	13815406629/ drzhangli@njmu.edu.cn

2. Project team members

surname and personal name	unit	Degree/Title	Project task division	Ethical and GCP training
Sun Fei	Children's Hospital of Nanjing Medical University	Master/Attending Physician	Data acquisition and analysis	yes
Shen Fangming	Zhongda Hospital Affiliated to Southeast University	Doctor/Resident	Data acquisition and 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 certify the authenticity of the application content. I will fulfill the responsibilities of a principal investigator, strictly comply with national regulations pertaining to clinical research, ensure the timely completion of research tasks, diligently perform my duties, and submit relevant materials on

schedule. Should any inaccuracies or violations of regulations occur in the submission, I shall bear full responsibility.

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