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Anesthesia/stereotaxic surgery exposure affects cognitive impairment and hippocampal synaptic plasticity dysfunction in male rats

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Abstract

Postoperative cognitive dysfunction (POCD) is a frequent complication after major surgery and anesthesia exposure, particularly in advanced aging. However, the effects of anesthesia/stereotaxic surgery (A/SS) on cognitive function in rats remain inadequately understood. This study aims to elucidate the effects of A/SS on cognitive function in male Sprague Dawley rats and explore potential alterations in hippocampal synaptic plasticity. Male Sprague Dawley rats, aged 6 months, were subjected to anesthesia (A) or A/SS, involving anesthesia with ketamine/xylazine (50/10 mg/kg, ip) and stereotaxic surgery to create a burr hole above the lateral ventricle. Anxiety-like behavior and locomotor activity were assessed using the open-field test one week post-surgery. Spatial memory and learning were evaluated through the Y-Maze test and novel object recognition test (NORT). Moreover, to determine hippocampal synaptic plasticity, we evaluated hippocampal long-term potentiation (LTP) at perforant pathway-dentate gyrus synapses. No statistically significant differences in anxiety and motor activity were observed between groups. Compared to the no-treatment group, the A/SS group rats exhibited a significantly ($p<0.05$) lower exploration of a novel arm and novel object in the Y-Maze and NORT, respectively. Furthermore, on postoperative day 7, LTP induced by high-frequency stimulation in the dentate gyrus region was attenuated ($p<0.05$) in the A/SS group compared to the no-treatment group. These findings indicated that A/SS induced cognitive decline and functional synaptic plasticity dysfunction. The results of the present study may be useful for future studies examining cognitive functions in experimental models involving such procedures.

Keywords: Stereotaxic surgery, anesthesia, postoperative cognitive dysfunction, long-term potentiation

1. Introduction

Postoperative cognitive dysfunction (POCD) stands out as a prominent postoperative complication frequently encountered in elderly patient populations (1). It is distinguished by manifestations such as memory deficits, cognitive dysfunction, psychomotor disturbances, delirium, or depressive symptoms (2). Findings derived from clinical investigations have demonstrated that POCD may correlate with heightened mortality rates, diminished quality of life, and prolonged hospital stays (3, 4). The hippocampus assumes a critical role in memory consolidation, stress response modulation, and various other cognitive processes. Numerous confounding factors may contribute to the onset of POCD. In addition to tissue disruption and postoperative pain inherent in any surgical intervention, the administration of general anesthesia and subsequent postoperative complications can independently lead to the manifestation of POCD.

Anesthesia entails a multifaceted procedure involving potent neurochemical volatile and/or intravenous agents aimed at inducing broad suppression of neuronal responsiveness and diminished functional interconnectivity (5). Ketamine functions as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, a critical glutamate receptor involved in synaptic plasticity, pivotal for learning and memory processes in the brain (6, 7). Its antagonistic action on the NMDA receptor is associated with detrimental effects on cognitive function. Prior research examining ketamine users has evidenced diminished frontal gray matter volume via magnetic resonance imaging and consistent neurocognitive deficits assessed through behavioral neurocognitive tests (8, 9).

Patients undergoing neurosurgical interventions pose a distinctive challenge concerning the onset of POCD (4). While cranial surgery shares similarities with other major surgical procedures in triggering immune responses and activating nociceptive pathways through local tissue injury, the complexity arises from the cognitive function's neural substrate. Distinguishing between direct neural tissue damage and indirect systemic factors such as inflammation or anesthesia in contributing to postoperative cognitive deficits proves challenging (10).

In the field of neuroscience, stereotaxic surgery is frequently used in techniques such as central injections, chronic electrophysiological recordings, and/or genetic manipulations. Moreover, ketamine/xylazine is commonly preferred as a reversible anesthetic in these chronic surgical interventions, as in the present study. Additionally, rather than

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performing craniotomy, smaller holes are often created to target specific experimental procedures. Nevertheless, the causative relationship between stereotactic surgery procedures and the onset of POCD remains uncertain. This study aims to investigate the impact of anesthesia and stereotactic surgery on learning and memory, utilizing electrophysiological and behavioral measures.

2. Material and Methods

2.1. Animals

Eighteen male Sprague Dawley rats (6-month-old), weighing between 300-350 g, were housed in conditions maintaining a 12-hour dark-light cycle (commencing at 6:00 AM), with ad libitum access to both food and water. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of Akdeniz University (with unique authorization number B.30.2.AKD.0.05.07.00/43).

2.2. Anesthesia/Stereotaxic Surgery Procedure

The rats were randomly placed into 3 groups: the control group, anesthesia (A), and anesthesia/ stereotaxic surgery group (A/SS). The control group did not receive any anesthesia or undergo surgical intervention. For A/SS, the rats were placed in a stereotaxic frame (Kopf Instruments, Model 962, Tjunga, CA, USA) with a heating pad under ketamine/xylazine anesthesia (50/10 mg/kg, intraperitoneal). Following the midline incision applied to the cranial region, the muscles and connective tissues were dissected, allowing access to the bony roof. The bregma was then exposed, and a burr hole (1.0 mm diameter) was drilled above the lateral ventricle site utilizing stereotaxic coordinates of 0.8 mm anteroposterior (AP) and 1.4 mm mediolateral (ML) relative to the bregma (Fig.1). Subsequently, the skin was closed with a 3.0 silk suture. Animals retrieved from the stereotaxic frame were administered meloxicam (1 mg/kg) as an analgesic and cefazolin sodium (30 mg/kg) as an antibiotic via intraperitoneal injection. This procedure is commonly used for intracerebroventricular cannulation surgery (11, 12). The “A” group of animals underwent the same surgery procedures, except their bony frameworks were not penetrated.

2.3. Behavioral Tests

We used the OFT to measure the anxiety-like behavior and locomotor activity, while spatial memory and learning were assessed using the Y-Maze and NORT.

The OFT was conducted using a square-shaped black mat with dimensions of 80x80 cm and a wall height of 40 cm (Fig. 1). The area was divided into 16 equal small squares, each measuring 400 cm². Among these squares, the central 4 were designated as the inner zone, constituting the testing area, while the surrounding squares comprised the outer zone. At the initiation of the experiment, individual rats were placed in the center of this area, and their movements were recorded over a 5-minute period. The animals were allowed to explore the open field apparatus for 1 minute to facilitate their adaptation on the sixth day. On the test day (day 7), the time spent and entry counts in both the inner and outer zones were monitored for each rat using the Ethovision XT video tracking system (Noldus Ethovision XT System, The Netherlands). Moreover, the total distance of movement and velocity in the box was calculated for motor activity (13).

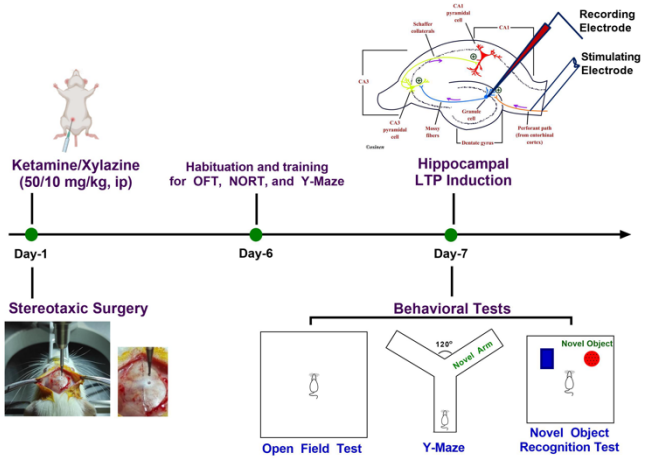

Fig.1. Representative flow chart of the experimental design.

The NORT was executed within an open field box featuring a wall height of 40 cm and a square base measuring 40 × 40 cm² with a black bottom (Fig. 1). During the training trial, rats were placed in the middle of the box, facing away from the objects, and were given three minutes to explore them. To eliminate olfactory cues, the objects were thoroughly cleaned with 70% ethanol between rats. After a 24-hour interval, the test phase occurred over 3 minutes. In the test phase, one object was replaced with another of different shapes and sizes, and the time spent investigating each object, as well as the overall time spent exploring both objects, was recorded for 3 minutes. The Noldus Ethovision XT System was utilized for measuring the time spent exploring, with the animal's nose serving as a reference point, ensuring that instances where animals approached the objects within 1 cm were recorded (14). The discrimination index (DI), a metric for assessing cognitive performance, was calculated using the following formula:

$$DI = \frac{(\text{Novel object exploration time} - \text{Familiar object exploration time})}{(\text{Novel object exploration time} + \text{Familiar object exploration time})}$$

The Y-Maze Test utilized an apparatus consisting of three identical arms, labeled as the start arm, the other arm, and the novel arm, arranged 120° apart around a central joining region (Fig. 1). In the training phase, rats were positioned at the start arm facing the wall and allowed to explore the maze for 10 minutes, with one arm (the novel arm) closed. Subsequently, the rats were returned to the home cage until the test phase 4 hours later, during which the previously blocked arm became accessible, and the rats were permitted to investigate all arms for 5 minutes. The behavior of each rat during the test was recorded by a digital camera and analyzed using the Noldus Ethovision XT System (15).

2.4. Hippocampal Long-Term Potentiation (LTP) Induction

Hippocampal LTP, a sustained enhancement in the efficacy of

synaptic connections between neurons, is a cellular phenomenon widely recognized to contribute to the mechanisms of learning and memory. Following completion of the behavioral assessment, rats were anesthetized with urethane (Merck-Millipore, 94300, Germany, 1.5 g/kg, intraperitoneal injection) and secured within a stereotaxic apparatus. Throughout the experiment, the rats' body temperature was maintained at a constant level of 37.0 ± 0.2 °C.

A bipolar tungsten electrode (platinum plated, 0.010 inch bipolar twisted, stainless steel, insulated except at its tips, Plastics One, Roanoke, VA, USA) was utilized to stimulate the medial perforant path in the left hemisphere (coordinates from bregma, in mm: AP, − 8.0; ML, 4.4; DV, 2–2.5 below the dura). This stimulation electrode was connected to the output of an isolator (ISO Flex, A.M.P.I Instrument, Jerusalem, Israel), which was further linked to a stimulator (MASTER 9, 7730, A.M.P.I Instrument).

A bipolar recording electrode was positioned 2.3 mm lateral to the midline and 3.8 mm posterior to bregma and inserted into the dentate gyrus (DG) until the maximum field excitatory postsynaptic potential (EPSP) was detected (depth range: 2.7–3.2 mm ventral). The optimal ventral positioning was determined through electrophysiological monitoring of the response evoked in the DG after perforant pathway (PP) stimulation (14).

An Input-Output (I/O) curve was generated by stimulating the medial perforant pathway (MPP) at various intensities using single pulse stimulation (0.1 ms biphasic square wave pulses at a frequency of 0.1 Hz). The stimulus intensity eliciting a population spike (PS) amplitude and field EPSP slope of approximately 50% of the maximal response were selected for subsequent experimentation (referred to as the test stimulus).

Following a 15-minute baseline recording, LTP was induced using a high-frequency stimulation (HFS) protocol consisting of 400 Hz stimulation (10 bursts of 20 stimuli, 0.2 ms stimulus duration for biphasic square wave pulses, with a 10-second inter-burst interval) at the test stimulus intensity. Post-HFS, LTP was monitored for 60 minutes to observe alterations in synaptic responses of the dentate gyrus (DG) neurons. Each animal underwent a single recording session to assess LTP (16, 17).

2.5. Statistical Analysis

Statistical analyses were conducted using Prism 9 software (GraphPad Software, Inc, La Jolla, CA). Data are expressed as mean ± standard error of the mean (SEM). Normality distribution was assessed using the Shapiro-Wilk test. Significance was evaluated using the non-parametric Kruskal-Wallis test with Dunn's test. A p value <0.05 was considered as statistically significant.

3. Results

3.1. Behavioural Measures

As shown in Fig.2, no statistically significant differences in anxiety and motor activity were observed between groups.

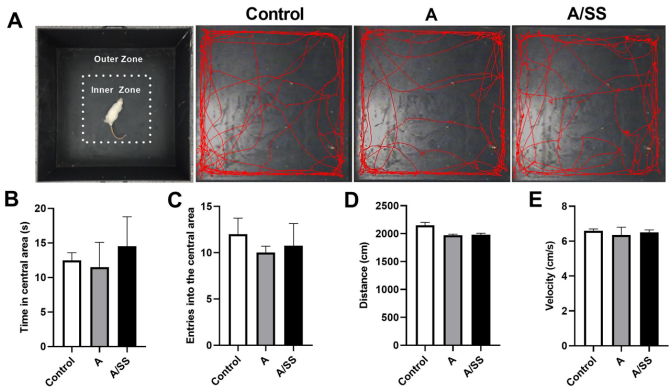

Fig.2. Effect of anesthesia/stereotaxic surgery on anxiety-like behavior and locomotor activity. Kruskal-Wallis test with Dunn's test was used to carry out to statistical comparisons, n= 6 rats per group

As shown in Fig. 3, the rats in the A/SS group displayed a significantly ($p<0.05$) reduced exploration of a novel object in the NORT, in contrast to the control group. Taken together, these findings suggest that exposure to A/SS affects the recognition memory of rats.

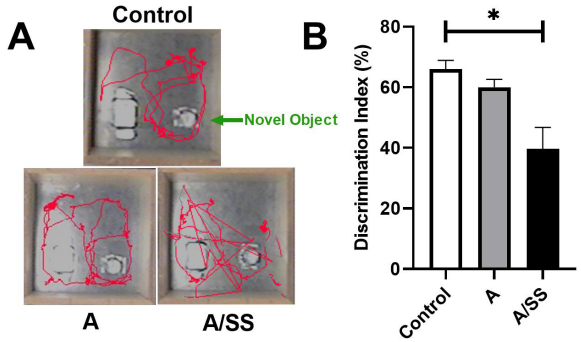

Fig.3. Effect of anesthesia/stereotaxic surgery on long-term recognition memory. Kruskal-Wallis test with Dunn's test was used to carry out statistical comparisons, n= 6 rats per group

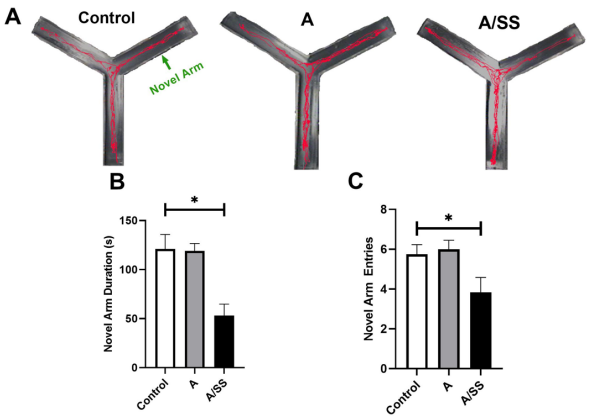

Fig.4. Effect of anesthesia/stereotaxic surgery on spatial memory and learning. Kruskal-Wallis test with Dunn's test was used to carry out statistical comparisons, n= 6 rats per group

As depicted in Fig. 4A, the path density for the A/SS rat in the novel arm was notably lower compared to the control rat during the test period. Statistical analysis demonstrated that the A/SS rats exhibited a significantly ($p<0.05$) reduced number of entries into the novel arm compared to the control rats (Fig. 4C). Additionally, they spent less time in the novel arm than the controls (Fig. 4B). Exposure to anesthesia alone did not result in a statistically significant difference for NORT and Y-Maze. These findings suggest that exposure to A/SS disrupts the short-term spatial memory and exploratory activity of rats.

3.2. Electrophysiological Recording

LTP induced in the PP and DG synapses was quantified as a percentage change relative to baseline recordings. Following HFS, alterations in PS amplitude and field EPSP slopes were assessed over a 60-minute period. The average increase in EPSP slope was notably diminished ($p<0.05$) in the A/SS group compared to the control group (Fig.5A-B). Furthermore, PS amplitude of field potentials was notably lower ($p<0.05$) in the A/SS group compared to the control group (Fig.5C-D). Exposure to anesthesia did not yield statistically significant alterations for EPSP. These data suggest that the observed attenuation of hippocampal synapses following A/SS implies the presence of an underlying electrophysiological mechanism potentially implicated in the pathogenesis of POCD.

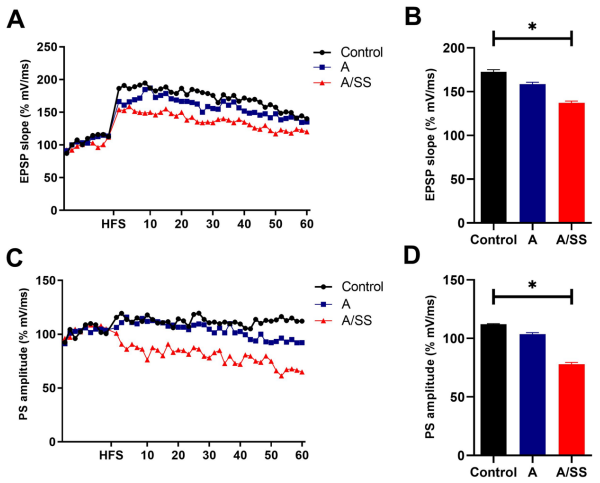

Fig.5 Effect of anesthesia/stereotaxic surgery on EPSP of DG field potential and DG field PS. (A) The EPSP slopes and (B) PS derived from DG synapses were recorded for all experimental groups. (B) The average EPSP slopes and PS amplitude values during the post-HFS periods were quantified and expressed as a percentage change from the baseline. Kruskal-Wallis test with Dunn's test was used to carry out to statistical comparisons, n= 6 rats per group

4. Discussion

This investigation provides insights into the influence of A/SS on cognitive function in male Sprague Dawley rats and its potential correlation with changes in hippocampal synaptic plasticity. The findings revealed that A/SS induced cognitive decline, as evidenced by decreased exploration in the Y-Maze and NORT. Additionally, a notable attenuation of LTP in the DG region suggested functional synaptic plasticity dysfunction in the A/SS group compared to the no-treatment group. Despite no discernible differences in anxiety and motor activity, the observed cognitive impairments emphasize the need for further investigation into the intricate relationship between surgical interventions, anesthesia exposure, and cognitive outcomes.

The disruption observed in spatial memory and learning subsequent to exposure to A/SS underscores the profound impact of such procedures on cognitive function. Spatial memory and learning are fundamental cognitive processes crucial for effective navigation and adaptation to the environment. The impairment of these cognitive faculties, evidenced by the marked decrease in exploration of a novel arm and object in the Y-Maze and NORT, respectively, suggests compromised acquisition and retention of spatial information. Such deficits may have far-reaching implications for diverse aspects of daily functioning, encompassing spatial orientation, decision-making, and problem-solving abilities. These findings underscore the imperative of considering the cognitive ramifications of surgical and anesthetic interventions, particularly in research environments where such procedures are prevalent. Further exploration into the underlying mechanisms responsible for A/SS-induced cognitive impairment holds promise for unraveling potential therapeutic interventions aimed at mitigating these effects and safeguarding cognitive function.

Exposure to A/SS, involving anesthesia with ketamine/xylazine and stereotaxic surgery, did not result in statistically significant variances in anxiety levels and motor activity among the experimental groups. This suggests that the observed cognitive effects were not confounded by alterations in these behavioral parameters.

Changes in synaptic connection as a result of neuronal activity form the basis of learning and memory (14). One of the methods for modeling the persistent strengthening of synapses is LTP recordings (14). In the present study, the effects of A/SS on cognitive performance and electrophysiological parameters in the PP and DG synapses were evaluated by LTP recordings for the first time. EPSP and PS provide information about the excitability of the cell and the neuron population that generates the action potential (18). The variation of the PS amplitude and EPSP slope was lower in A/SS groups during the 60-minute period after HFS. The results of the present study suggest a crucial role of A/SS in the weakening of strengthened synapses.

In conclusion, this study sheds light on the impact of A/SS on cognitive function in male Sprague Dawley rats and its potential association with alterations in hippocampal synaptic plasticity. These results contribute valuable insights for future studies exploring cognitive functions in experimental models involving similar procedures, potentially informing strategies for mitigating postoperative cognitive dysfunction.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions
Concept: O.S., Design: O.S., A.G.S., Data Collection or Processing: O.S., A.G.S., N.D., Analysis or Interpretation: O.S., A.G.S., Literature Search: O.S., A.G.S., N.D., Writing: O.S., A.G.S.

Ethical Statement
The study protocol was approved by the Animal Ethical Committee of Akdeniz University (with unique authorization number B.30.2.AKD.0.05.07.00/43).

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