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The memory stages of a spatial Y-maze task are not affected by a low dose of ketamine/midazolam

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ABSTRACT

Anesthetics, such as the ketamine/midazolam combination, are used in research with animals and in human clinical practice; thus, it is essential to clarify the potential effects of these anesthetics on memory. This study aimed to evaluate how a low dose of the ketamine/midazolam combination affects the acquisition, consolidation, or recall of a spatial memory task. Thirty-three adult male C57BL/6 mice were divided into four treatment groups: unanesthetized control animals and three groups of animals treated with 40 mg/kg of ketamine and 10 mg/kg of midazolam administered in a single intraperitoneal injection. The different treatment groups received the same anesthetic dose at different time points, to study the acquisition, consolidation, and recall of spatial memory in the Y-maze task. The percentage of correct choices was measured. Six mice were killed 4 days and 12 days after anesthesia for histopathological analyses. There were no differences between treatment and control groups regarding the acquisition of spatial memory, measured as the slope of the learning curve, or in the percentage of correct choices in the consolidation or recall periods of the task. Similarly, no differences were detected between groups regarding the number of cells per square millimeter in the visual and retrosplenial cortex, in the dentate gyrus, and in the CA1 and CA3 regions of the hippocampus. Hence, a low dose of the ketamine/midazolam combination did not impair memory processes or brain integrity in adult mice, suggesting that this combination is unlikely to cause cognitive complications.

1. Introduction

Anesthesia acts as a reversible intoxication that depresses the activity of the central nervous system by interfering with the biochemistry and the electrophysiology of the brain (Alkire et al., 2009). However, several studies have shown that anesthetics may also affect gene expression, protein synthesis, and cellular functions, among other effects that may persist after the drug is eliminated and interfere with cognitive processes (Perouansky and Hemmings, 2009).

The use of ketamine alone has been decreasing because of its dissociative and psychedelic effects (Copeland and Dillon, 2005). However, in combination with midazolam, ketamine is administered to laboratory animals and humans, especially at low doses, to reduce anxiety, promote amnesia, and provide analgesia (Chudnofsky et al., 2000; Slonim and Ognibene, 1998). Although this combination is considered a safe protocol, its potential effect on memory in adult individuals has been seldom studied. *N*-methyl-D-aspartic acid (NMDA) receptor antagonists, such as ketamine, disrupt the induction of long-term potentiation in the hippocampus, thus compromising learning and memory (Morris et al., 1986). Moreover, drugs that act on the γ-aminobutyric acid A (GABAA) receptor, such as midazolam, also affect memory processes (Izquierdo and Medina, 1997).

Memory has been described (Abel and Lattal, 2001) as being composed by three stages; acquisition, consolidation, and recall of information. Newly acquired information is labile; thus, a period of consolidation of minutes to days must occur to allow the integration of this information into memory. Later, the subject may need to recover the information previously encoded from memory, i.e., memory recall. Each stage may be disrupted by environmental factors, such as anesthesia. There are several indications that memory acquisition is affected by ketamine in humans (Morgan and Curran, 2006) and animals (Pitsikas and Boultadakis, 2009), and by midazolam in humans (Reder et al., 2006) and animals (Ishitobi et al., 2009), but little is known about the effect of the two drugs used in combination. The disentanglement of the effects of these drugs on memory consolidation and recall is more complicated and the results pertaining to these two stages are not

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consistent because it is difficult to determine the precise moment of the occurrence of each process (Boultadakis and Pitsikas, 2011; Chrobak et al., 2008).

In previous studies, we found that a lower anesthetic concentration of isoflurane impaired the learning of spatial tasks compared with higher concentrations of the drug and with no anesthesia at all (Valentim et al., 2008, 2010). Similarly, a recent study performed by our group showed that high doses of ketamine/midazolam (75 mg/kg/10 mg/kg) did not interfere with the acquisition of a spatial radial maze and motor task 24 h to 9 days postanesthesia (Valentim et al., in press). Hence, we designed this study to understand if a lower dose of this combination would have an effect on memory processes. We used a single low anesthetic dose of ketamine/midazolam to evaluate if the minimal concentration required to provoke dorsal recumbence caused memory deficits. This study intended to replicate sedation procedures in animals and provide potential translational research to humans.

In more detail, this study aimed to evaluate how the acquisition, consolidation, or recall of a spatial task may be influenced by a single administration of a low dose of ketamine/midazolam in combination, and how this drug combination affects the brain of adult mice.

2. Material and methods

All procedures were carried out under personal and project licenses from the Portuguese national competent authority for animal protection, Direcção Geral de Veterinária, the principles of laboratory animal care of which were followed in this study.

2.1. Animals

Male C57BL/6 mice (5–6 months of age) bred in the animal facility of the institute (F2–F3 offspring of animals bought from Charles River, Barcelona, Spain) were distributed among eight cages (Makrolon type II cage, Tecniplast, Dias de Sousa, Alcochete, Portugal) with 3–5 animals per cage. Thirty-three mice were used for behavioral tests; among those, 18 mice were killed for histopathological analyses. Each cage was provided with standard corncob litter (Probiológica, Lisbon, Portugal), a piece of tissue paper, and a cardboard tube. Water and rodent pellets (4RF25-GLP Mucedola, SRL, Settimo Milanese, Italy) were provided *ad libitum*. A food restriction schedule was established (Valentim et al., 2008) 1 week before the beginning of the appetitive Y-maze task. The animals were weighed throughout the experiment to ensure that they did not lose more than 15% of their initial weight. The animals were kept in a room with controlled temperature (21 ± 1 °C) and humidity (55%). Lights were kept on a 12/12 h cycle, with lights off at 20 h.

2.2. Anesthesia

Mice of the same age were randomly assigned into experiments 1, 2, and 3. Within experiments, animals were distributed to either the control group (unanesthetized animals, $n=8$), which received intraperitoneal (i.p.) saline injections, or to the treatment group (groups I, II, or III), which received 40 mg/kg of ketamine (Imalgene, Merial, Portugal; 100 mg/ml) and 10 mg/kg of midazolam (B. Braun Medical, Barcelona, Spain; 5 mg/mL) in a single i.p. injection.

Injection and animal restraint were always performed by the same person. After i.p. injection, the animal was placed in a type III cage until it lost the righting reflex (loss of the capability of returning to ventral recumbence). Then, the animal was placed in dorsal recumbence on a homeothermic blanket covered with a cap to avoid burns. This allowed maintaining the body temperature at 37 ± 2 °C by connecting the homeothermic blanket to a rectal thermal probe (50–7061-F, Harvard Apparatus Ltd., Kent, United Kingdom). The animals wore a face mask that delivered 100% oxygen with a flow of 0.6 l/min. A cuff and a transducer connected to a pressure meter (LE 5001, Panlab, Barcelona, Spain) were placed on the tail base to monitor the pulse rate and arterial pressure.

The dose administered did not lead to loss of the pedal withdrawal reflex, and a pilot study showed that this dose allows animals to be kept in dorsal recumbence for 30–40 min after anesthesia administration. Animals were considered as being awake when they recovered the righting reflex, when they stretched the front paws trying to recover the right posture, or when they reacted to the approach of the researcher's finger (stretching the nose to explore and poking). These considerations were made because of the muscular rigidity provoked by ketamine, which makes it difficult for animals to turn into an upright position. Control animals that received saline injections were placed for 2 min in the type III cage (average time until loss of the righting reflex in anesthetized animals). To avoid isolation stress in the unanesthetized control animals, these were returned to their home cage thereafter.

2.3. Appetitive Y-maze test

This protocol was used to test spatial reference memory (Deacon et al., 2008).

2.3.1. Apparatus

A Y-maze used was made of beige polypropylene and included a central polygonal area of 14 cm in diameter, to which three arms were attached (50 cm long, 9 cm wide, and surrounded by a wall that was 0.5 cm high). A food well was located 5 cm from the distal end of each arm, where the reward, commercialized pellets for laboratory animals (Dustless Precision Pellets, 20 mg, chocolate-flavored rodent purified diet, Bio-Serv, Frenchtown, New Jersey), was placed. The maze was located in a laboratory with prominent distal extramaze cues, and was elevated 80 cm above the ground on a central stand on which the entire maze could be rotated.

2.3.2. Habituation

The habituation was performed over 5 days before the start of the test. On the first day, animals were allowed to explore the apparatus for 10 min in cage groups. Then, each mouse was placed alone in the apparatus for 4 min. The second day included two trials; only during the second trial five reward pellets were scattered throughout the Y-maze. These trials ended when the mouse ate all the pellets or when 5 min had elapsed. On the third day, two trials with rewards in the two choice arms were performed and the animal started from different arms. If the rewards were eaten before 5 min had elapsed, the food wells would be rebaited. On the fourth day, the pellets were placed in the two choice arms for five trials. Each trial ended when 3 min had elapsed or when the mouse had eaten both rewards. This procedure was repeated in the fifth day, for 10 trials of 2 min each.

2.3.3. Test

On the day following habituation, mice received a saline or a ketamine/midazolam injection. The testing of mice in the Y-maze started 24 h later (Figs. 1–3).