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Vestibular and Optokinetic Nystagmus in Ketamine-Anesthetized Rabbits

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Abstract: The aim of this work has been to analyze the modification of vestibular and optokinetic nystagmus in animals after administration of therapeutic doses of ketamine. Three healthy rabbits (two reds and one white), weighing between 2.5 and 3 kg, were submitted to electronystagmography recording. The rabbits, head blocked, were placed on a Tönnies rotatory chair in the middle of a rotatory cylindrical chamber, the internal area of which was covered with 32 black vertical contrasts. All the rabbits underwent rotatory vestibular stimulation by stop test and optokinetic stimulation. After each test and a rest period for the animals, we administered 10 mg/kg of ketamine and performed the same ENG workup. In the first (red) rabbit, we collected eye-movement data at 3 minutes and 40 minutes after the intramuscular injection of a single dose of ketamine (10 mg/kg). In the second (white) rabbit, we performed ENG recording with the animal under anesthesia for the entire time of the test; in the third (red) rabbit, we analyzed the optokinetic response, from the administration of the drug until the end of its effects. Our data highlight the action of the drug on the structures that control the ocular movements and led to the conclusion of the presence of a second feedback integrator.

Key Words: electronystagmography; ketamine; optokinetic nystagmus; vestibular nystagmus

Ketamine, or ketamine hydrochloride, is frequently described as a "unique drug" because it has hypnotic (sleep-producing), analgesic (pain-relieving), and amnesic (short-term memory loss) effects; no other drug used in clinical practice combines these three important features [1]. The primary central nervous system (CNS) action of ketamine appears to be a noncompetitive block of NMDA (from the selective agonist N-methyl-D-aspartate). Glutamate is a major transmitter in central pain pathways. The excitatory effect of glutamate is mediated by two main types of ionotropic receptors: NMDA and the selective agonist α-amino-3-hydroxy-5-methyl-isoxasole-4-propionic acid. The NMDA receptors contribute to normal excitatory transmission and have special functions related to synaptic plasticity [2].

Ketamine binds to a specific site for phencyclidine (PCP), the NMDA receptor–gated channel, and inhibits the excitatory effect of glutamate selectively at these receptors; the analgesic effect and potency of ketamine correlate positively with its PCP site binding [3]. Previous studies had affirmed that the varied sensitivity to ketamine (of the different cerebral structures) depends on the specific density of receptors on the cellular membrane (high density on the hippocampal and cortical neurons; medium density on the cerebelli and thalamic neurons; low density on the substantia reticularis) [4].

The only exception is represented between the medium density on the thalamic cells and their low sensitivity to the drug; this depends on the different functional expression of the NMDA receptors and the possibility of the CNS structures to answer in a different way but so as to conform to specific demands [5].

The anesthetic effects of ketamine depend on its action on the cerebral cortex, hippocampus, and thalamus; ketamine spontaneous ocular movements are due to the action of the drug on the cerebellum and Purkinje cells [4]. Our interest in ketamine is derived from the experience of a few authors with such drugs as fentanyl [6] and with such substances as alcohol [7]; good vestibular knowledge of rabbits owing to previous research [8]; and personal experience demonstrating spontaneous ocular movements in pediatric patients after general anesthesia with ketamine.

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In our study, we examined the effect of ketamine on cortical and subcortical function by exploring evoked eye movements. On the basis of our previous studies, we analyzed the modification of vestibular and optokinetic nystagmus (OKN) in animals after administration of therapeutic doses of ketamine.

MATERIAL AND METHODS

We performed all experiments in compliance with the guidelines of the local authorities and of the European community for the care and use of laboratory animals; we examined three healthy rabbits (two reds and one white) weighing between 2.5 and 3 kg. To study the vestibular ocular reflex (VOR) and OKN, we subjected all animals to electronystagmography (ENG); no preparation was necessary for the ENG recording. We placed the animals on a Tönnies rotatory chair (Pro-800553/70 model, Freyburg, Germany) in the middle of a rotatory cylindrical chamber (2 meters in diameter and 1.9 meters in height). The animals' heads were affixed rigidly to a superstructure in the center of the chair in such a way that the semicircular horizontal canals were on the horizontal plane [9].

The rotatory cylinder was lighted from above by a 100-W bulb and was driven by a direct-current engine that turned it clockwise and counterclockwise up to 200 degrees/sec (maximum speed); preset acceleration ranged from 1 to 2 degrees/sec, and its internal area was covered with 32 black vertical contrasts [9]. We recorded ocular movements according to the usual method by means of a Tönnies electronystagmograph with eight channels, placing the electrode needles at the anterior and posterior eye angles [9]. We collected eye movement data after the intramuscular injection of ketamine (10 mg/kg), and movements were evoked by continuous rotation in the horizontal plane.

We evoked VOR in the dark by chair acceleration of 0.5 degrees/sec^2 to an angular velocity of 90 degrees/sec for 60 seconds, with rotatory vestibular stimulation by stop test from the constant angular velocity, in both the clockwise and counterclockwise directions.

We tested optokinetic reflex by a cylinder angular velocity of 30 degrees/sec for 60 seconds (in both clockwise and counterclockwise directions) in the light. When optokinetic stimulation had maintained a steady velocity for 60 seconds, we turned the lights off and allowed optokinetic after-nystagmus (OKAN) to decay to zero velocity.

On the basis of our previous studies, after each test and a rest period for the animals, we administered 10 mg/kg of ketamine and performed the same ENG workup. We collected data at 3 and 40 minutes after the first injection of ketamine. In the first (red) rabbit, we collected data from each eye movement at 3 and 40 minutes after the intramuscular injection of ketamine (10 mg/kg) as follows: right VOR, left VOR, right OKN, and left OKN. We chose the dose on the basis of previous dose-ranging studies. We performed optokinetic stimulation after a several-hours'-long rest period for the animal.

In the second (white) rabbit, we performed ENG recording with the animal under anesthesia for the entire testing period. We began 1 minute after the injection of ketamine (10 mg/kg); 15 minutes later, we administered a second dose of the drug (5 mg/kg); and 30 minutes after the beginning of the examination we administered a third dose of the drug (2.5 mg/kg). At 3 and at 40 minutes after the first injection of ketamine, we collected right VOR, left VOR, right OKN, and left OKN; we performed optokinetic stimulation after a several-hours'-long rest period for the animal.

In the third (red) rabbit, we collected eye movement data at 3 minutes and 40 minutes after the intramuscular injection of ketamine (10 mg/kg); we analyzed right OKN and left OKN from the administration of the drug until the end of its effects (40 minutes).

RESULTS

During the rotatory vestibular stimulation, all the rabbits presented spontaneous ocular movements with alteration of the quality of the shocks.

Examination 1: Red Rabbit

Three minutes after the injection of ketamine, the first (red) rabbit presented spontaneous ocular movements with alteration of the quality of the shocks; such modifications were present during constant-velocity rotation. After a stop test, ENG layout presented arrhythmic square waves with reduced amplitude and frequency. We confirmed such modifications during the second ENG recording, obtained 40 minutes after the beginning of the examination. The ENG layout was more regular but presented arrhythmic waves with reduced amplitude, frequency, and slow-phase velocity (SPV; Fig. 1).

Three minutes after the intramuscular injection of ketamine, ENG recording highlighted a reduced right OKN with arrhythmic waves; right optokinetic stimulation showed the presence of a left and reduced OKN. Forty minutes after the beginning of the examination, we repeated the right and left OKN recordings, which were present with reduced amplitude, frequency, and SPV (Fig. 2).

Examination 2: White Rabbit

In the second rabbit, 3 minutes after the injection of ketamine and after the stop test, ENG layout presented

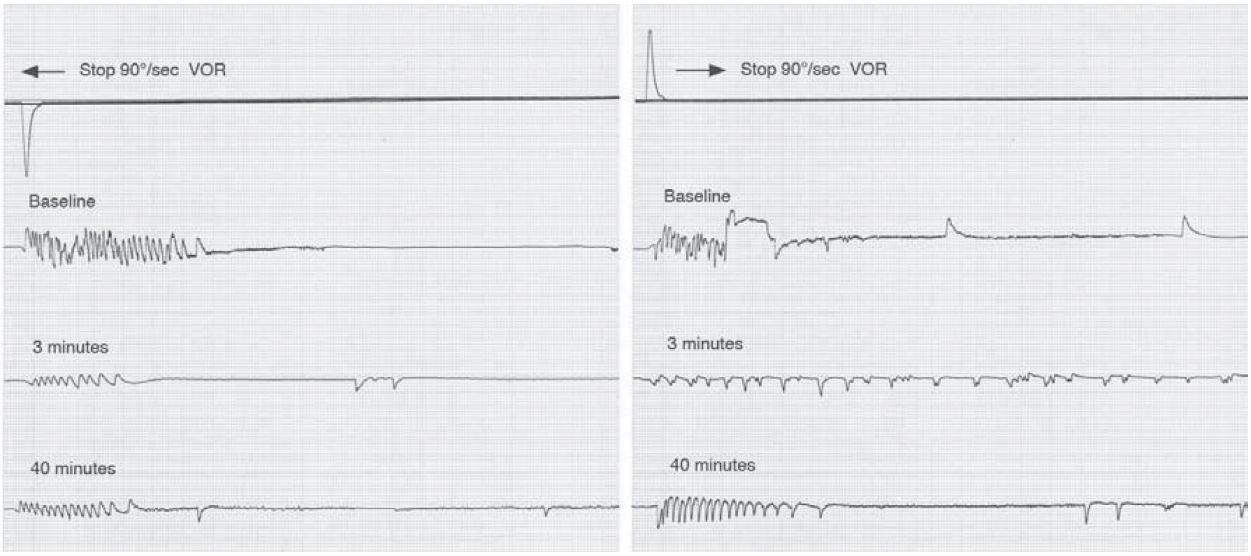

Figure 1. First (red) rabbit. Vestibuloocular reflex (*VOR*) recorded at 3 and 40 minutes after the intramuscular injection of ketamine (10 mg/kg).

arrhythmic square waves with reduced amplitude, frequency, and SPV. These data were confirmed by the second ENG layout, obtained 40 minutes after the beginning of the examination (Fig. 3).

During the optokinetic stimulation, at 3 minutes after the injection of ketamine, ENG recording showed an irregular right OKN with arrhythmic square waves followed by an irregular OKAN. The left OKN presented an irregular layout and waves with reduced amplitude, frequency, and SPV. These data were confirmed by the second ENG layout, obtained 40 minutes after the beginning of the examination (Fig. 4).

Examination 3: Red Rabbit

In the third rabbit, 3 minutes after the injection of ketamine, right OKN was present with reduced frequency, amplitude, and SPV; it presented arrhythmic waves with qualitative and quantitative alterations of the shocks. Left OKN was present with reduced frequency and

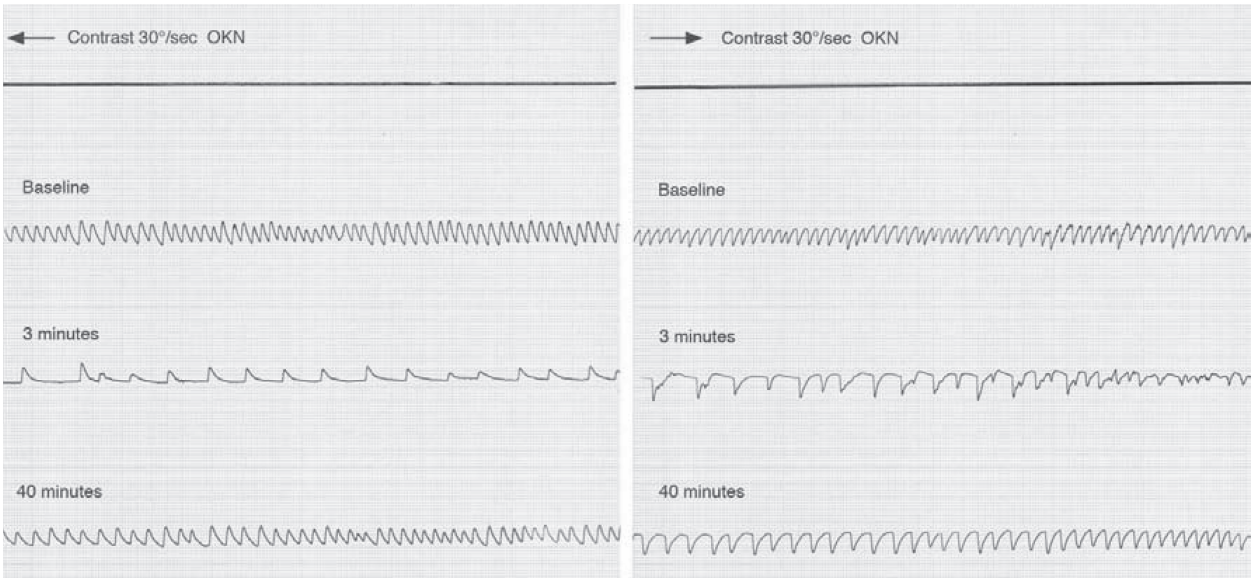

Figure 2. First (red) rabbit. Optokinetic nystagmus (*OKN*) recorded at 3 and 40 minutes after the intramuscular injection of ketamine (10 mg/kg).

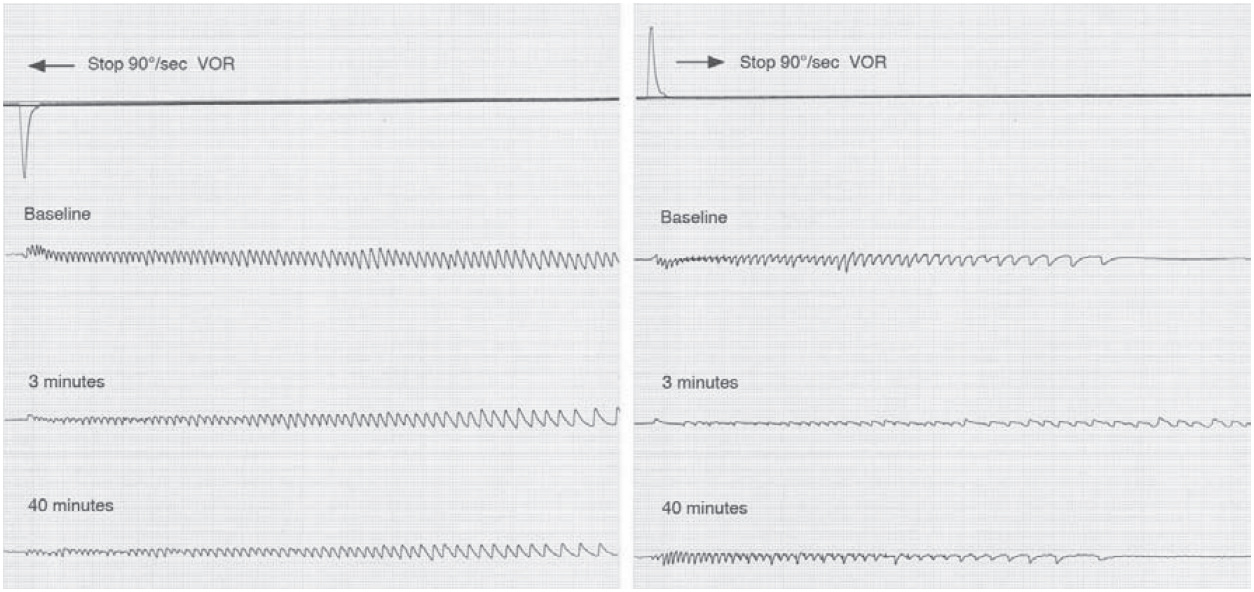

Figure 3. Second (white) rabbit. Vestibuloocular reflex (*VOR*) recorded at 3 and 40 minutes after the first intramuscular injection of ketamine.

amplitude, and it presented arrhythmic waves with qualitative and quantitative alterations of the shocks. The last two ENG recordings, obtained 40 minutes after the injection of ketamine, were more regular but presented a reduced amplitude, frequency, and SPV of right and left OKN.

DISCUSSION

Some studies have shown that ketamine causes schizophrenia-like positive, negative, and cognitive symptoms in normal healthy volunteers, including deficits in sensory processing and eye-tracking performance. An association between eye-tracking abnormalities and NMDA receptor antagonism is important because it suggests what neurophysiological mechanisms are related to eye-tracking abnormalities [10].

NMDA receptors are present on cells throughout the cortex, including the frontal-prefrontal cortex and the cerebellum, where they could play a functional role in eye-tracking abnormalities. Ketamine is a noncompetitive antagonist of the NMDA receptors in these regions;

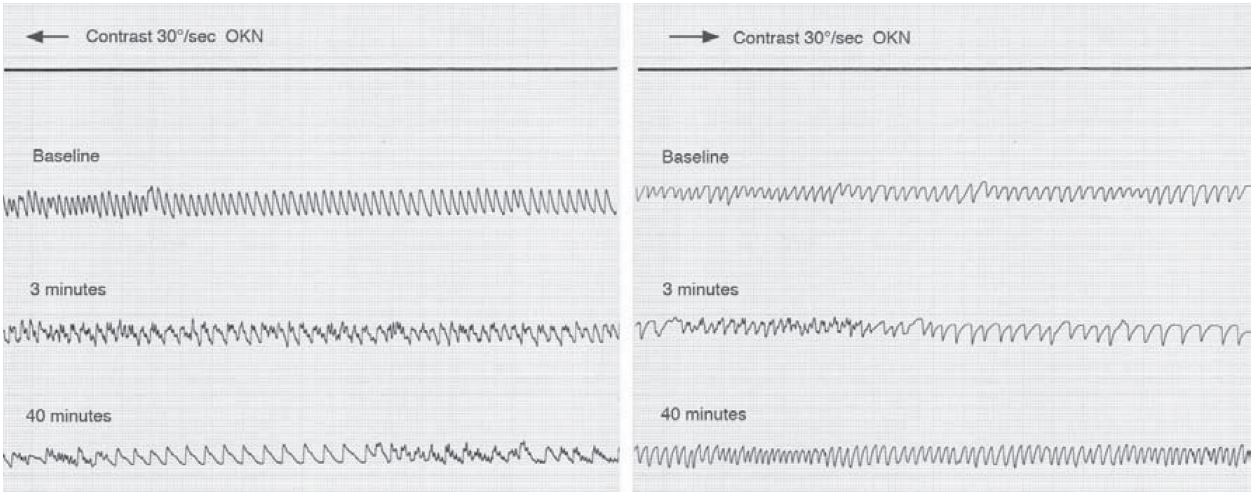

Figure 4. Second (white) rabbit. Optokinetic nystagmus (*OKN*) recorded at 3 and 40 minutes after the first intramuscular injection of ketamine.

thus, data from our study are consistent with a model of eye tracking mediated, in part, by NMDA receptors functioning within the frontal-thalamic-cerebellar circuit [11]. NMDA antagonism by ketamine is known to decrease neuronal activity potently in the cerebellum, an action that can explain the observed deficits in pursuit initiation and pursuit maintenance [12].

The eyes are moved by a combination of neural commands that code eye velocity and eye position: The eye position signal is supposed to be derived from velocity-coded command signals by mathematical integration via a single oculomotor neural integrator. For horizontal eye movements, the neural integrator is thought to reside in the rostral nucleus prepositus hypoglossi (NPH) and project directly to the abducens nuclei [13].

During the last 20 years, scientists have come to see that the NPH, which had been thought to be an accessory gustatory nucleus, is actually an oculomotor nucleus; several studies highlight the presence of NMDA receptors in the NPH. NMDA receptors are thought to be components of the "neural integrator" of the VOR, which generates a signal proportional to eye position. Our data suggest the action of ketamine on these structures [14].

Our data also highlight the action of the drug at the CNS level and the structures that control the ocular movements. In particular, the fact that the effect of ketamine on vestibular nystagmus is independent of the received stimulus suggests that ketamine, at least at higher dosages, induces a failure of the neural integrator (see Figs. 1, 4) [15].

The qualitative alterations of the shocks observed in vestibular nystagmus corroborate the hypothesis that the NPH is involved in the role of neural integrator for steady peripheral gaze and led to the conclusion of the presence of a second feedback integrator. A variety of alternatives have been suggested for the mechanism and site of the second integrator. Our results support the previous conclusion that the oculomotor neural integrator is not a single neural entity and that the mathematical integrative function for different oculomotor subsystems is most likely distributed among a number of nuclei [16].

OKN and OKAN are markedly altered but not abolished in experimental animals after cerebral lobectomy; this highlights a common subcortical neural integrator and validates the modification of the ENG record after ketamine administration [17]. In accord with these data, our OKN responses were horizontally bidirectional for monocular stimulation with qualitative and quantitative alteration of the shocks, indicating that ketamine did not eliminate cortical processing of the motion stimulus (see Figs. 2, 4).

These results, taken together, demonstrate that after ketamine administration, cortical circuits continue to processes visual patterns in a dose-dependent manner despite an animal's behavioral dissociation. Though evaluating perceptual experience is difficult under these conditions, oculomotor patterns revealed that the brain not only registers but acts on its sensory input, employing it to drive a sensorimotor loop and even responding to a sensory conflict by engaging in spontaneous perception-related state changes. Although many questions remain unanswered, we believe we have provided a starting point for new research that may find the answers to these questions.

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