Spontaneous eye movements were recorded before and after a microinjection (0.1–0.2 µl) of either APV (an NMDA receptor antagonist) or NBQX (a non-NMDA receptor antagonist) into the nucleus prepositus hypoglossi (NPH) of the alert cat. A unilateral injection of APV caused bilateral failure of the horizontal gaze-holding system; in the light, each saccade was followed by a post-saccadic drift. A unilateral injection of NBQX caused no sign of gaze-holding failure; in the light, spontaneous eye movements were unaffected; in complete darkness, a nystagmus with linear slow phases directed towards the injected side was observed. We conclude that NMDA receptors of the NPH neurones are involved in the gaze-holding system.

**Key words:** Nucleus prepositus hypoglossi; APV; NBQX; Oculomotor neural integrator; Gaze-holding; Alert cat

**Introduction**

In order to adequately control eye movements, the oculomotor neurones need to carry both a velocity signal and a position signal.1,2 However, all the signals that are at the origin of eye movements are velocity signals.3,4 From this apparent paradox, it has been proposed that a brain stem network converts velocity signals into position signals, through a mathematical integration of the input signals.5,6

Since the proposal of its existence, the oculomotor neural integrator has been the topic of extensive investigation. Pathological eye movements, similar to those that would result from the loss of the horizontal neural integrator were recorded after manoeuvres inducing incapacitation in the area of the nucleus prepositus hypoglossi (NPH) and the medial vestibular nucleus (MVN); i.e. electrolytic lesion of either the NPH or the MVN of the cat;7,8 ibotenic injections between the NPH and the MVN in the monkey;9,10 kainate injection into the NPH11 and muscimol injection into either the NPH or the central part of the MVN of the cat.12 Neurones carrying a strong eye-position related signal have been recorded in the NPH and the adjacent MVN both in the cat11,12 and in the monkey.13 Models have been proposed to explain how a neuronal network could perform mathematical integration of the signals it receives.1,13,14,15

The present study is devoted to the identification of neurotransmitters used by the oculomotor neural integrator. To address this question, we avoided using agonist agents, such as kainate and muscimol, that incapacitate neurones of the integrator network in a non-specific way by acting on receptors present on neurones of the integrator but not involved in the network of the integrator.

**Materials and Methods**

The experiments were performed on eight cats. Under general anaesthesia (xyloïdine-dihydrothiazin, Rompun, Bayer, 3 mg kg−1 and pentobarbitone, Nembutal, Abbott, 20 mg kg−1) and in aseptic conditions, three devices were chronically implanted. A scleral search coil was implanted subconjunctivally on both eyes. A bipolar stimulating electrode was placed on each abducens nerve at its exit from the brain stem. The position of each electrode was adjusted to produce a lateral eye movement following a 0.1 ms single pulse of 0.1 mA. This movement was checked under binocular control. Three bolts were cemented to the skull in order to immobilize the animal’s head during experimental sessions. At the end of the surgical procedure, a square hole (4 mm wide) was drilled through the occipital bone in order to provide an access to the brain stem via the cerebellum. The dura mater was removed and a dental cement chamber mounted around the hole. Between the experimental sessions the surface of the cerebellum was protected with a silastic sheet and the chamber sealed with bone wax. Terminal wires from eye coils and stimulating electrodes were attached to a socket cemented to the holding system. Eight days after surgery, each animal was trained to accept restraining conditions without stress. During the experimental sessions, sterile saline, antibiotics and local anaesthetics were used in order to prevent infection and suffering.

At the beginning of each injection experiment, the location of the centre of the abducens nucleus was mapped out in the alert cat, by recording the antidromic field potential evoked by stimulating the abducens nerve. The X-Y coordinates of the maximal antidromic negativity were noted. This electrode (rec-
FIG. 1. A Sketch summarizing the experimental setup. Parasagittal section through the nucleus prepositus hypoglossi (NPH) and the abducens nucleus (ABD). FG = genu of the facial nerve. HYP = hypoglossal nucleus. Two micropipettes are glued together in such a way that when one of them (recording micropipette) was positioned in the centre of the ABD, the tip of the second (injection micropipette) was positioned in the NPH. The optimal position of the recording pipette within the ABD was checked by recording the antidromic field potential evoked by stimulation of the abducens nerve. APV or NBOX was injected using an air pressure system. B. Diagrams showing the locations of the injection sites for NBOX. The top and bottom diagrams are at 1.2 mm and 1.6 mm from the midline, respectively. Left and right injection sites are pooled. C. Diagrams showing the locations of the injection sites for APV.

FIG. 2. Spontaneous eye movements before (A) and after (B) a microinjection of NBOX into the left NPH of an alert cat. Notice the appearance of a spontaneous nystagmus in complete darkness. The slow phases are directed towards the injected side. The vertical dotted line indicates the transition from light to darkness.

saline followed by 10% formaldehyde. Serial sections of 20 μm thickness were cut from the medulla andpons and stained with cresyl violet in order to verify the precise location of the injection sites.

**Results**

NBQX (1 μg μl⁻¹, diluted in 5.5% glucose, and adjusted to pH 8.1 with 1 M NaOH) was unilaterally injected 9 times into the NPH of 3 alert cats. The injection sites were localized at 1.2–1.6 mm lateral to the midline and at 0.4 to 4.5 mm behind the centre of the abducens-nucleus level (Fig. 1B). The pathological changes in the spontaneous horizontal eye movements were similar in the different experiments. A typical observation is illustrated in Figure 2. In complete darkness, about 5 min after the injection, a horizontal nystagmus developed with linear slow phases directed towards the injected side (right part of Fig. 2B). By contrast, in the light, the ocular movements were not affected and, in particular, gaze-holding remained normal (left part of Fig. 2B). The effects observed following the injection and depicted in Figure 2 lasted about 2 h and the maximal velocity achieved by the slow phases
reached $8.0^\circ$ s$^{-1}$ ($n = 20$ slow phases). For all 9 injections, the mean maximal velocity of the slow phases was $4.9 \pm 4.3^\circ$ s$^{-1}$. The nystagmus recorded in complete darkness started 1.5–5 min after the injection. The duration of this nystagmus ranged from 1 h 15 min to 2 h.

The pathological changes observed following an injection of APV into the NPH appeared quite different. Fourteen unilateral injections of APV (2.5 $\mu$g $\mu$l$^{-1}$, adjusted to pH 7.4 with 1 M NaOH, Sigma Chemicals) were performed on 5 cats. The 14 injection sites were located 1.2–1.6 mm lateral to the midline and at 0–4.3 mm behind the centre of the abducens nucleus (Fig. 1C). A typical experiment is illustrated in Figure 3. Three minutes after the APV injection, a bilateral gaze-holding failure occurred. In the light, saccades were followed by a centripetal post-saccadic drift (left part of Fig. 3B). At the worst of the deficit, the mean time constant of the exponential drift in the light was as short as $0.7 \pm 0.2$ s ($n = 30$ horizontal saccades). Recovery from this syndrome occurred after 90 min. When the animal was in complete darkness, a nystagmus with curved slow phases directed to the injection side was observed (right part of Fig. 3B).

For all 14 injections, the mean time constant of the exponential post-saccadic drift in the light was $0.8 \pm 0.2$ s. The observed gaze-holding failure started 2–5 min after the injection and ended 30 min–1 h 30 min after the injection.

In the light, after an APV injection, the null-position, towards which the gaze drifted back at the end of each saccade, did not coincide with the control zero-position, determined by computing the mean of the horizontal and vertical positions of the gaze recorded during a long period of time before the injection (about 6000 oculomotor positions). In 12 experiments, the null-position was shifted towards the injection side (mean deviation from the zero-position $\pm$ S.D. $= 4.3 \pm 2.3^\circ$) and in 2 experiments, towards the side opposite to the injection side (mean deviation $\pm$ S.D. $= 4.3 \pm 0.9^\circ$). In complete darkness, the curved slow phases of the injection-induced nystagmus were directed towards the injection side in 12 experiments and towards the side opposite to the injection site in 2 experiments.

**Discussion**

The major findings of the present study can be summarized as follows: (1) a unilateral injection of APV into the NPH caused a horizontal bilateral gaze-holding failure in the light and a nystagmus with curved slow phases in complete darkness; (2) a unilateral injection of NBQX, into the NPH produced a horizontal nystagmus with linear slow phases directed towards the injection site, without any gaze-holding failure.

The range of effect of the antagonists delivered by 0.1–0.2 $\mu$l microinjections is difficult to define with accuracy but probably corresponded to a diameter of 1–2 mm. Diffusion over a distance of 1–2 mm has been found in the substantia nigra of the rat, after injection of 0.1 $\mu$l [14C]GABA.$^{10}$ Similar values have been estimated for the pharmacological action of lidocaine administered by 0.1 $\mu$l injections into the medial longitudinal fasciculus.$^{21}$ We thus can reasonably surmise that our injection protocol resulted in diffusion of the antagonists throughout a substantial part of the target (NPH) without unwanted exposure of the neighbouring nuclei such as the MVN.

It could be hypothesized that APV produces a disturbance of the NPH neurones not by interacting with their NMDA receptors but by modifying the properties of their membrane in a non-specific way. However, microinjections of APV into the NPH do not cause any change in the resting rate of the neurones (unpublished observations). In other words, at the dose that we used and in our experimental conditions, APV affected the behaviour of the NPH neurones not by acting directly on their membrane but by interfering with the post-synaptic transmission of afferent signals. In a previous study, we have shown that microinjections of ketamine into the NPH also produced a gaze-holding failure.$^{22}$ Ketamine is a non-competitive antagonist of the NMDA receptor whereas APV is a competitive antagonist of the same receptor. The fact that two drugs acting on two different sites of
the NMDA receptor induce the same behavioural effect is strong evidence in favour of the involvement of the NMDA receptor in the horizontal gaze-holding system. The specificity of the effect of APV was confirmed by the fact that APV, an antagonist of the NMDA receptor, and NBQX, a highly specific antagonist of non-NMDA receptors, caused very different pathological movements.

**Conclusion**

We conclude that NMDA receptors of the NPH neurones are involved in generation of the eye-position signal performed by the horizontal oculomotor neural integrator.

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**References**


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