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Effects of the APV Injection into the Abducens and the Prepositus Hypoglossi Nuclei on the Generation of Eye Position Signal

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The prepositus hypoglossi nucleus (NPH) plays an important role in the gaze-holding system. It is a major part of the oculomotor neural integrator (NI) involved in the horizontal eye movements (Baker *et al.*, 1975; Baker and Berthoz, 1975). Deterioration of the NPH (induced either electrolytically or by injection of kainic acid) causes a serious failure in horizontal gaze holding (Cheron *et al.*, 1986; Cheron and Godaux, 1987; Cannon and Robinson, 1987). It has also been proposed that the eye position signal present in the abducens neurons is elaborated by a cascade circuit situated in the NPH (Delgado-García *et al.*, 1989; Escudero *et al.*, 1992). The neural components of this circuit comprise different proportions of velocity and position signals. Interestingly, the output of this integrating chain loop destined to the abducens nucleus is provided by the NPH "position" cells presenting during eye fixations a firing rate very close to that of abducens neurons. In this context, it was proposed in the past that the eye position signal could be partly elaborated by non-linear integration in abducens motoneurons (MN) (Barmack, 1974). Moreover, it was recently proposed that the incoming signals (sensory or premotor) may be computed by the motoneuron depending on activation of intrinsic membrane properties (Durand, 1993).

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micropipettes were then glued together: the function of the first was to record the antidromic field potential and neuronal activities of the abducens nucleus (1–2.5 Mohms of impedance); that of the second, connected to a picospritzer, was to inject 100 nl of a solution of APV, 0.2M).

The two glass micropipettes were glued in such a way that when the tip of recording microelectrode reached the centre of the abducens nucleus, the tip of the injection micropipette would be located in the target part of the NPH or the abducens. When APV was injected in the centre of the abducens nucleus, the tips of the two micropipettes were adjusted to be very close (less than 100 µm) allowing the recording of the neuronal activity in the injected zone. In one experiment this double micropipette assembly was glued with another recording micropipette in order to record simultaneously the neuronal activities in the right and left NPH when APV was injected in one NPH.

Spontaneous saccades, made under light, were examined in order to detect an eventual failure of the integration process of the saccadic command. In the case of a defect of the neural integrator, the saccade would be followed by an exponential post-saccadic drift toward a null position. The time constant of the exponential drift was computed as $T = (E_o - E_{null})/E$, where E_o is the eye position achieved at the end of a saccade, E_{null} the null position toward which the eye was moving and E the initial velocity of the drift.

At the conclusion of an experiment, a thin wire (50 µm in diameter), insulated except at its bevelled tip, was glued to a glass microelectrode. With this double electrode, small electrolytic lesions were made at (and 1 mm below) the point where the antidromic field potential was maximal. The cat was then killed with an overdose of pentobarbitone and perfused through the aorta with a 10% formalin solution. The brain stem was embedded in paraffin. Serial sections, 20 µm thick, were made. Every tenth section was stained with Cresyl Fast Violet. The locations of the injection sites were determined on the histological reconstruction with respect to the small electrolytic lesions.

Microinjections of APV in the abducens nucleus

Combined recording-microinjection pipettes were used for focal applications of APV (100 nl) in the centre of the abducens nucleus during extracellular recording of motoneurons or interneurons (Fig. 1). During these microinjections the gaze-holding system remained normal, not failure of the integration process of the saccadic command was detected (Fig. 1B). Moreover, the spiking behaviour of the abducens neurons during the intersaccadic fixation periods was not modified by the APV injection. This fact was illustrated for an interneuron (Fig. 1A,B) of the abducens nucleus. The absence of modification was also demonstrated by comparative analysis of the relationship between the firing rate and the horizontal eye position achieved during intersaccadic fixation periods in control and after the APV injection. Figure 1 illustrates this relation for an interneuron (C) and for a motoneuron (D). The slopes of their regression lines (Kf) were not different during the controls and following APV injection. For the interneuron, Kf was 9.4 spikes/sec/deg in control and 10.0 spikes/sec/deg after the APV injection. For the motoneuron, Kf was 15.5 spikes/sec/deg in control and 14.9 spikes/sec/deg after the APV injection.

The fact that the abducens motoneuron is a final pathway for the eye movement command makes the testing of this idea technically difficult. Is it possible to block at the motoneuronal level the eye position signal without affecting the eye velocity command?

Recently, microinjections of ketamine in the NPH (Cheron *et al.*, 1992) have induced a specific failure of the gaze holding without modification in the velocity of the saccades. As ketamine is an antagonist of the NMDA receptors (Anis *et al.*, 1993; Martin and Lodge, 1985), this suggests that NMDA receptors play a role in the building of the position signal by the NPH. Nevertheless, if ketamine has been shown to be a specific antagonist of NMDA receptors, it has also been reported to affect a variety of other ionic channels (Benoit *et al.*, 1986; Gage and Robertson, 1985). Consequently, the first aim of the present study is to test the involvement of the NMDA receptors in the building of the position signal using NMDA-antagonists other than ketamine. At the motoneuronal level it was demonstrated in the anaesthetized cat (Durand *et al.*, 1987) and rat (Durand, 1991) that electrophoretic applications of NMDA evoke a slow depolarization and a transient period of action potential burst followed by stable repetitive firing. Moreover, NMDA receptors activation leads to oscillations in hyperpolarized abducens MN. Ionophoretic applications of aminophosphonovalerate (APV) reduce or block the NMDA-induced responses but do not produce any change in membrane potential or conductance of the MN (Durand, 1991). In this circumstance, injection of APV in abducens nucleus in alert preparation provides an ideal tool for testing the contribution of the NMDA receptors of the abducens MN in the generation of the eye position signal.

The final aim of the present study is to compare the gaze-holding deficit produced by APV injection in the abducens and the NPH nuclei.

Experimental procedures

Five adult cats weighing between 2.5 and 3.5 kg were prepared for chronic recording of eye movements and discharges of identified abducens motoneurons and NPH neurons. Under general anaesthesia and aseptic conditions, several devices were chronically implanted. Scleral search coils were implanted subconjunctivally on both eyes. A bipolar stimulating electrode was placed on each VIth nerve at its exit from the brainstem. Three bolts were cemented to the skull for immobilizing the animal's head during the experimental sessions. A rectangular hole was drilled in the occipital bone. The dura mater was removed and a dental cement chamber constructed around the hole. Between recording 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 each experimental session, sterile saline and antibiotics were locally employed in order to maintain integrity of the opening. Local anaesthetics were also poured onto the dura mater in order to prevent any pain. The field potential of the abducens nucleus, evoked by stimulation of the abducens nerve, was recorded by a glass microelectrode. The centre of the abducens nucleus was determined by the maximal antidromic negativity. It was used as a point of reference. Two glass

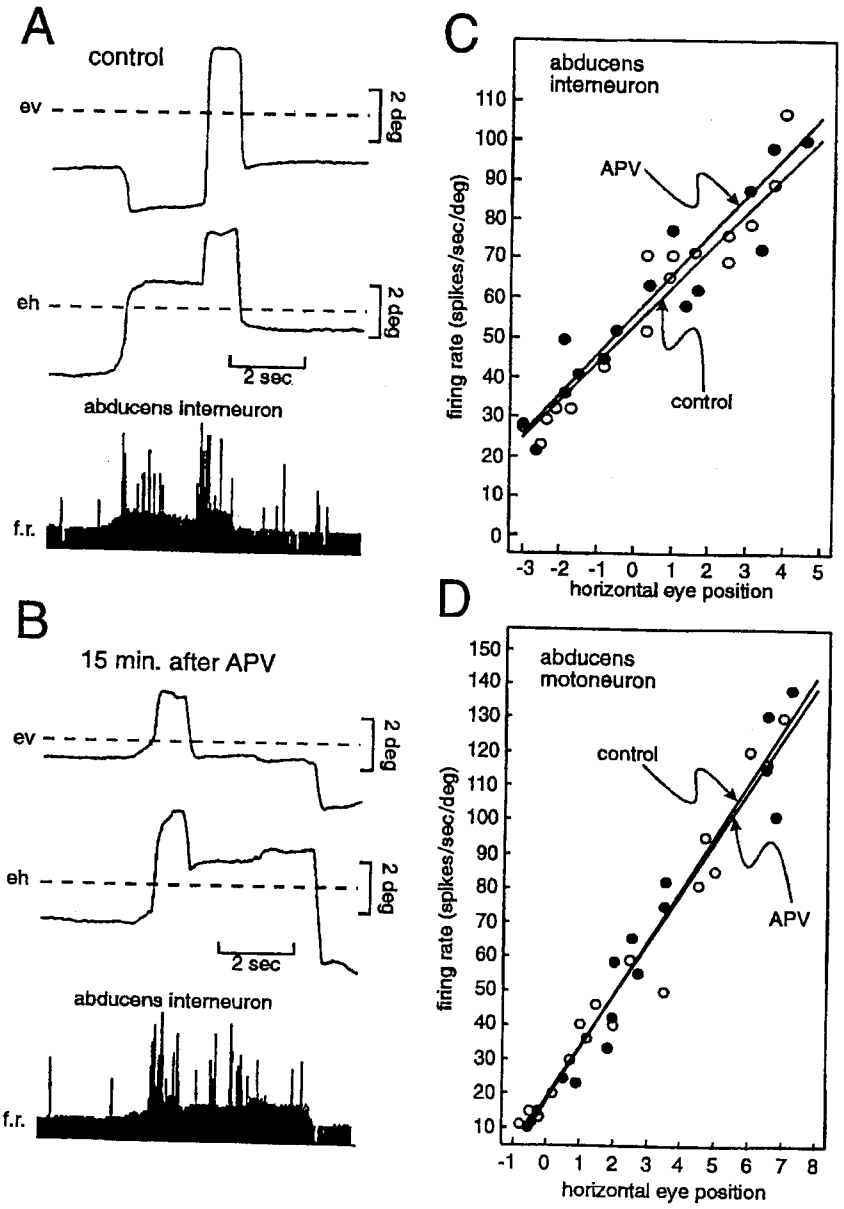

FIG. 1. Comparison of firing rate of abducens neurons before and after microinjection of APV in the centre of this nucleus. (A) and (B) Illustration of the firing rate of an abducens interneuron before and after the injection, respectively. Note the absence of modification in the gaze holding and in the related neuronal discharge. (C) and (D) Plots showing quantitative analysis of eye position sensitivity of the illustrated interneuron (C) and a motoneuron (D) before (dots) and after (circles) the APV injection.

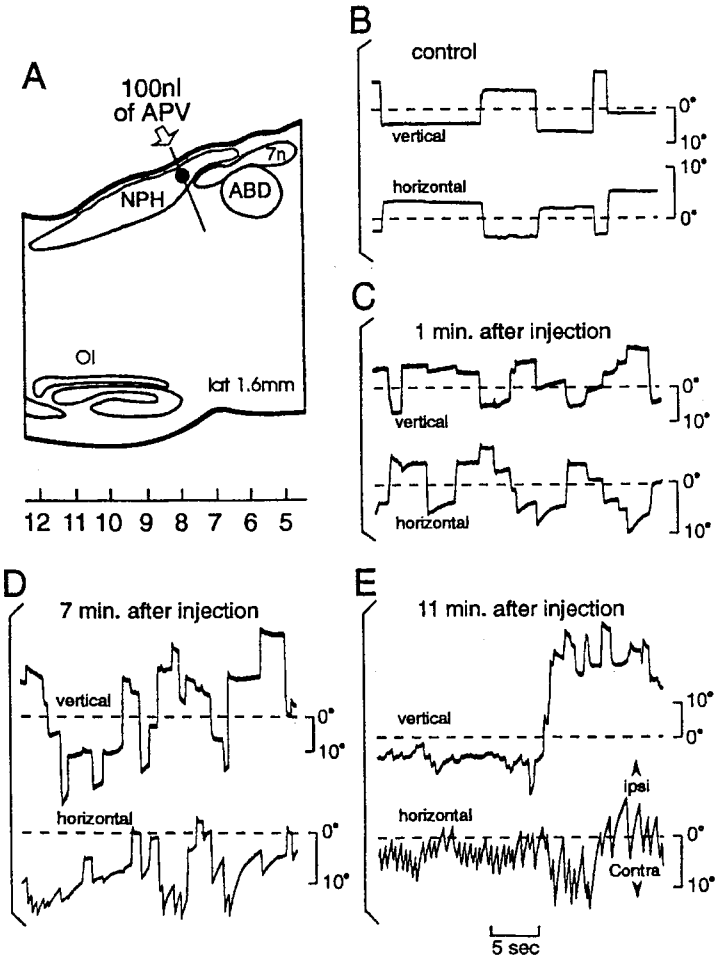

FIG. 2. Illustration of the gaze holding failures that could be observed after an injection of 100 nl of a solution of APV (0.2M) in a site located in the rostral NPH. (A) Map of the injection site on a parasaggital section at 1.6 mm from the midline. Abbreviations: NPH, nucleus prepositus hypoglossi; ABD, abducens nucleus; 7n, genu of facial seventh nerve; OI, inferior olive. (B) Vertical and horizontal components of spontaneous eye movements performed in the light before the injection (control). (C) – (E) Gaze-holding failures illustrated 1, 7 and 11 min after the APV injection.

Microinjections of APV in the NPH

Contrasting with the absence of APV effect in the abducens nucleus, a gaze-holding failure was observed every time the microinjection was performed in the NPH. A typical result is shown in Fig. 2. In this experiment, APV (100 nl) was injected in the rostral part of the right NPH. This microinjection caused a serious bilateral gaze holding-failure. Seven minutes after the injection, the time constant of the post-saccadic drift was as low as 0.4 sec (Fig. 2D). The gaze-holding defect was first

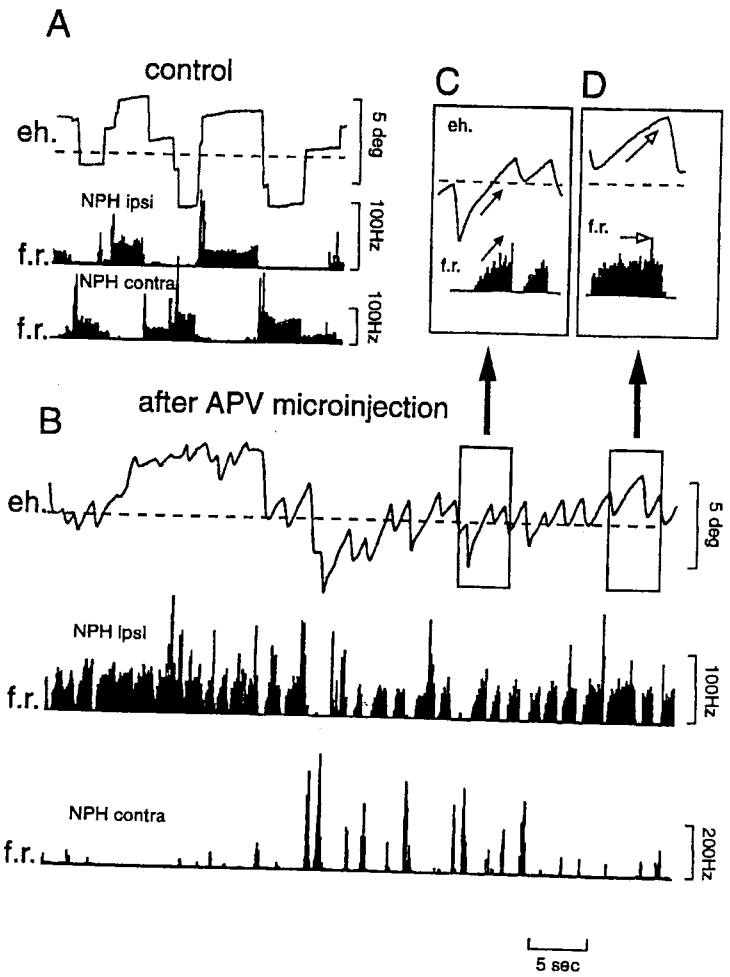

FIG. 3. Illustration of the experiment during which the neural activity of the right and the left NPH and the eye movements were recorded before (A) and after (B) APV microinjection into the right NPH (see text for more details.)

detected in less than 1 min (Fig. 2C). The slow phases were directed toward the side of the injection.

In one experiment, two NPH neurons, one in the ipsilateral NPH (the site of the APV microinjection) and another in the contralateral NPH, were simultaneously recorded just before and during the injection. This experiment is illustrated in Fig. 3. Before the injection the two NPH neurons, the ipsilateral and the contralateral ones, fired in a push–pull fashion (Fig. 3A).

The firing behaviour of these neurons corresponded to the "position-velocity" class of NPH neurons. The eye position sensitivities (*Kf*) were 8.2 and 11.5 spikes/sec/deg for the ipsilateral and the contralateral neuron, respectively (Fig. 3A).

During the gaze-holding failure induced by the unilateral injection of APV, the firing behaviour of the two neurons was drastically changed (Fig. 3B). Surprisingly,

the discharge frequency of the ipsilateral neuron was not decreased by the APV. On the contrary, this neuron discharged tonically during the slow phases of the APV-induced nystagmus directed to the side of the injection and was only inhibited during the contralaterally directed quick phases. During the same motor behaviour the tonic activity of the neuron situated in the contralateral NPH was lost. In this case only a short burst of activity was emitted during the contralaterally directed quick phase. The neuron of the ipsilateral NPH seemed to be disinhibited by the APV injection. During the slow phases of the nystagmus its firing rate presented a complex behaviour. In some cases, the firing rate of this neuron increased progressively during the slow phase reflecting some eye position sensitivity (Fig. 3C). This sensitivity was quantified during slow phases presenting a constant velocity. In these situations the variations of firing rate may be due to the change of eye position. However, the position sensitivity obtained in these circumstances reached values as high as 22 spikes/sec/deg during a range of position that never exceeded 1.5 deg. Moreover, during APV injection the firing rate of this neuron saturated around 55 spikes/sec/deg.

Discussion and conclusions

Although *N*-methyl-D-aspartate (NMDA) receptors have been found to be uniformly distributed on the membrane of the abducens motoneurons (Durand *et al.*, 1987), it was demonstrated here that the antagonist action of APV at this level was not able to produce any change in the generation of the position signal by neurons of the abducens nucleus during spontaneous eye movements. This result, associated with the fact that APV did not produce any change in membrane potential or conductance but reduced or blocked the NMDA responses of the motoneuron (Durand, 1991), seems to be incompatible with the idea of a possible involvement of the NMDA receptors in the final processing of the eye position command at the motoneuronal level. However, the absence of modification of *Kf* during the injection of APV in the abducens nucleus does not exclude a possible contribution of NMDA receptor activation in the control of the post-saccadic slide in the firing rate of the motoneuron. This post-saccadic slide is known to codify the transition between different positions of the eye (Pastor *et al.*, 1991). Its origin is uncertain but it seems likely that this slide signal is generated in premotor neurons (Delgado-García *et al.*, 1986, 1989) and probably adapted at the level of the motoneuronal membrane.

This result also corroborates the fact that direct electrical stimulation of the motoneuronal pool at a constant pulse rate does not produce a ramp in eye position as one would expect if mathematical integration of the stimulus had occured at this level (Skavenski and Robinson, 1973). In contrast, Cohen and Kotmatsuzaki (1972) provided, with a similar type of stimulation performed in the PPRF, the first experimental evidence for the existence of an oculomotor integrator in the brain stem.

The present microinjections of APV in the NPH confirm the key role of this nucleus in integration processing. Moreover, this finding indicates that the NMDA receptor-mediated neurotransmission in the NPH is necessary for the normal function of the neural integrator. However, the exact function of the NMDA receptors in this processing remains unknown. It is tempting to speculate about the different hypotheses which could explain the present result. The first hypothesis is that the NMDA receptors could be implicated in the closed self-excitatory chains of neurons.

This type of reverberating circuitry is known to be able to perform an integration function (Lorente de No, 1933, 1938) and may correspond to the cascade organisation of the left and the right NPH described by Delgado-García *et al.* (1989) and Escudero *et al.* (1992). Although this hypothesis could explain the ipsilateral gaze-holding failure produced by the APV (see Fig. 2C), the bilateral defect of the integrator necessitates a supplementary and a more complex site of action.

The present bilateral NPH recording during injection of APV in one side provided a surprising result (Fig. 3). The activity of this ipsilateral NPH neuron coding the position of the eye was not inhibited by the application of the NMDA antagonist, but on the contrary it discharged tonically and presented some saturation of its firing rate

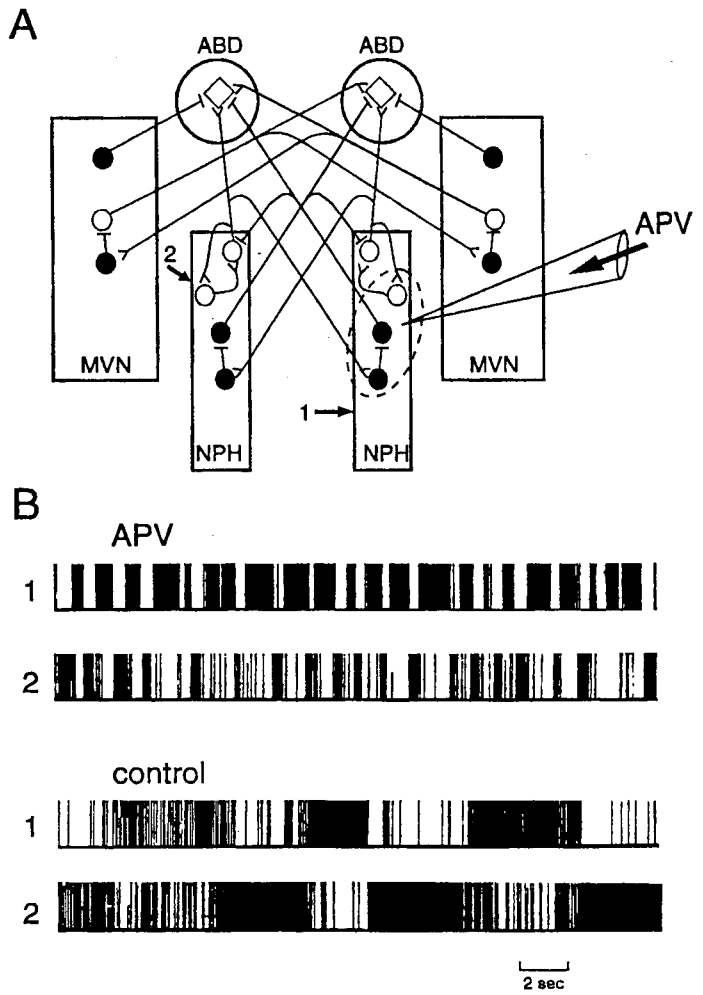

FIG. 4. (A) Diagram showing the possible pathways involved in the observed gaze-holding deficit and in the changes of the firing rate of neurons recorded in the APV-injected NPH (1) and in the non-injected NPH (2). (B) Simultaneous recording of the activity of two neurons in the injected (1) and non-injected (2) NPH before (lower traces) and after APV administration (upper traces).

during the curved slow phases of the APV-induced nystagmus. In contrast, the NPH neuron of the contralateral side seemed to be inhibited and was not able to contribute to the holding of the eye position in the contralateral direction. The oversimplified schema of Fig. 4, based on physiological and anatomical data, presents a second hypothetical explanation of this experimental fact. We propose the existence of a crossed push–pull connection between the two NPH based on the following assumptions: (1) the excitatory output of one side crossed the midline reaching the other side, (2) these reciprocal connections have an excitatory action on inhibitory neurons controlling the crossed inhibitory output of the NI, and (3) this latter inhibitory output exercises an inhibitory control on the ipsilateral excitatory output of the other side, the crossed excitatory connections being NMDA-dependent. Consequently, the application of APV in one side induces a disinhibition of the crossed inhibitory output of the NPH of this side. These hypothetical proposals could explain the bilateral deficit of gaze holding associated to a vestibular imbalance.

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