A Cholinergic Synaptically Triggered Event Participates in the Generation of Persistent Activity Necessary for Eye Fixation

Juan de Dios Navarro-López,1,* Juan Carlos Alvarado,2* Javier Márquez-Ruiz,1 Miguel Escudero,3 José M. Delgado-García,1 and Javier Yajeya4

1División de Neurociencias, Universidad Pablo de Olavide, 41013-Sevilla, Spain; 2Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157; 3Departamento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, 41012-Sevilla, Spain; and 4Departamento de Fisiología y Farmacología, Facultad de Medicina, Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, 37007-Salamanca, Spain

An exciting topic regarding integrative properties of the nervous system is how transient motor commands or brief sensory stimuli are able to evoke persistent neuronal changes, mainly as a sustained, tonic action potential firing. A persisting firing seems to be necessary for postural maintenance after a previous movement. We have studied in vitro and in vivo the generation of the persistent neuronal activity responsible for eye fixation after spontaneous eye movements. Rat sagittal brainstem slices were used for the intracellular recording of prepositus hypoglossi (PH) neurons and their synaptic activation from nearby paramedian pontine reticular formation (PPRF) neurons. Single electrical pulses applied to the PPRF showed a monosynaptic glutamatergic projection on PH neurons, acting on AMPA-kainate receptors. Train stimulation of the PPRF area evoked a sustained depolarization of PH neurons exceeding (by hundreds of milliseconds) stimulus duration. Both duration and amplitude of this sustained depolarization were linearly related to train frequency. The train-evoked sustained depolarization was the result of interaction between glutamatergic excitatory burst neurons and cholinergic mesopontine reticular fibers projecting onto PH neurons, because it was prevented by slice superfusion with cholinergic antagonists and mimicked by cholinergic agonists. As expected, microinjections of cholinergic antagonists in the PH nucleus of alert behaving cats evoked a gaze-holding deficit consisting of a re-centering drift of the eye after each saccade. These findings suggest that a slow, cholinergic, synaptically triggered event participates in the generation of persistent activity characteristic of PH neurons carrying eye position signals.

Key words: acetylcholine; glutamate; neuronal integrator; prepositus hypoglossi; saccades; oculomotor system

Introduction

In the oculomotor system, the presence of a neuronal integrator able to transform the eye velocity signal into a subsequent stable position signal has long been proposed (Robinson, 1981). The prepositus hypoglossi (PH) nucleus, a neuronal structure involved in the maintenance of eye position after horizontal eye movements, has been suggested to play an important role in the integration of the horizontal gaze (Moschovakis, 1997; Delgado-García, 2000). In support, it has been found that neurons in this nucleus project monosynaptically on extraocular motoneurons located in the abducens and oculomotor nuclei, as well as on many other structures involved in eye movement control (McCrea and Baker, 1985b). Extracellular recordings of PH neuronal activity during spontaneous and vestibularly and visually evoked eye movements in monkeys and cats have shown the presence of cells encoding pure eye position (López-Barneo et al., 1982; Delgado-García et al., 1989) or related position velocity and velocity position signals (López-Barneo et al., 1982; Delgado-García et al., 1989; McFarland and Fuchs, 1992). Moreover, permanent electrolytic lesions and transient pharmacological inactivations have shown that PH neurons are necessary for proper eye stability during positions of fixation (Cheron et al., 1986; Cheron and Godaux, 1987; Arnold et al., 1999; Moreno-López et al., 1996, 2002).

The source of eye velocity signals arriving at PH neurons is excitatory burst neurons located in the paramedian pontine reticular formation (PPRF), rostral and ventral to the abducens motor nuclei (Igusa et al., 1980; Bättner-Ennever and Horn, 1997). Although several mechanisms have been proposed to explain neuronal integration in the PH nucleus (Keller and Kamath, 1975; Cannon et al., 1983; Goldman et al., 2003), the neuronal processes involved have not yet been elucidated. Interestingly, it has been shown recently that ipsilateral excitatory and contralateral inhibitory projections between eye position-related cells located in goldfish brainstem area I could mediate...
neuronal integration (Pastor et al., 1994; Aksay et al., 2001, 2003). The synaptic feedback among those neurons seems necessary for the temporal integration (Aksay et al., 2003), and the sustained firing rate observed in the integrator could be explained by changes in the amplitude or rate of the synaptic inputs to the area 1 position-related neurons, rather than by neuronal intrinsic membrane properties (Aksay et al., 2001). Considering that position-related neurons of the goldfish area 1 are equivalent to “principal” cells identified in the cat PH nucleus (McCrea and Baker, 1985a,b; Escudero et al., 1992), it could be suggested that a similar circuit for the temporal integration is present in the latter neuronal center (Aksay et al., 2003). Additional information is needed regarding synaptic mechanisms underlying the integration process. Here, it is proposed that the interaction between PPRF excitatory burst neurons and meso-pontine reticular cholinergic neurons (Higo et al., 1990; Sembra et al., 1990; Barmack et al., 1992) is an important mechanism occurring at the synaptig level, which is necessary for the generation of the persistent neuronal activity recorded in PH neurons during eye fixations. Preliminary results have been published in abstract form (Navarro et al., 2003).

Materials and Methods

Preparation of slices. Experiments were performed in accordance with the European Union directive (609/86/EU) and current Spanish legislation (RD 233/89) for the use of laboratory animals in acute experiments. Female or male rats (23–33 d old; weight, 50–80 gm), raised in the animal colony of the University of Salamanca, were deeply anesthetized with halothane gas and decapitated. The skull and the first vertebral arch were removed, and the brain was excised and immersed in ice-cold (4°C) oxygenated artificial CSF (ACSF), in which NaCl (117 mM) was replaced with sucrose (234 mM) to maintain osmolarity. The brainstem was sectioned between the inferior colliculus and the obex, and sagittal slices (400 μm thick) were cut in cold oxygenated Ringer’s solution using a vibratome (series 1000; Technical Products International, O Fallon, MO). Collected slices were placed in an incubation chamber and maintained there for —2 hr, at room temperature. For recordings, a single slice containing PH nucleus and rostral PPRF was transferred to an interface recording chamber (BSC-HT and BSC-BU; Harvard Apparatus, Holliston, MA) and perfused continuously with ACSF composed of (in mMs) 117 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>P<sub>2</sub>O<sub>7</sub> and 11 glucose. The ACSF was bubbled with carbogen gas (95% O<sub>2</sub>/5% CO<sub>2</sub>) and maintained at 30 ± 2°C. Additional details of this in vitro preparation were published previously (Yajeya et al., 2000).

In vitro recordings. Intracellular records from PH neurons were obtained with borosilicate glass microelectrodes (140–180 MΩ; WPI, Sarasota, FL) filled with a potassium acetate solution (3 M) and connected to the headstage of an intracellular recording amplifier (VF180; Biologic, Claix, France). Micropipette tips were always directed toward the rostral third of the PH nucleus. Neuronal recordings were characterized by absolute values.

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Figure 1. Diagrams of stimulation and recording sites. A, A diagram illustrating the circuits present in the sagittal brainstem slice used in this study. Excitatory burst neurons (EBN) are located in the PPRF and project monosynaptically on abducens motoneurons (ABD Mn) and on PH neurons. Stimuli applied to the PPRF also activate descending cholinergic axons from pontomesencephalic areas. See Results for details and references. B, A photomicrograph of a PH neuron stained after intracellular recording. C, A drawing showing the reconstruction of the neuron illustrated in B. This neuron type corresponds to a principal cell (McCrea and Baker, 1985a). The two small arrows indicate initial trajectory of the axon. Scale bars: B, C, 50 μm. NVM, Medial vestibular nucleus.

 offline analysis. Additional details of this chronic preparation were published previously (Escudero et al., 1992; Moreno-López et al., 1996, 2002).

Analysis of data collected from in vivo experiments. During spontaneous eye movements, the alterations induced by drug injections in the PH nucleus consisted of a nystagmus with curved or straight slow phases separated by quick resetting movements and/or drifts toward a central position after eccentric saccades. Nystagmic slow phases with a duration >0.5 sec were fitted separately, by the least squares method, to a linear or single exponential equation and were considered to be linear, or exponential, when >80% of the analyzed phases had a correlation coefficient >0.99 or >0.90, respectively. For linear adjustment, the slope of the line was measured before and after each drug injection (see Fig. 9A, inset). In light, the posttacadic centripetal drifts evoked by drug injections did not correlate significantly to a single exponential equation. Thus, to characterize the temporal evolution of drug effects on the maintenance of gaze, the posttacadic centripetal drift was calculated as the area (in degrees by seconds) between the actual eye position drift and the theoretical “maintained” eye position for the 0.6 sec after each saccade (see Fig. 9C, inset). Because these area values depended on the velocity of the preceding saccade and on the eye position at the end of this saccade, a linear multiple regression test was performed to determine the coefficients for each injection. These coefficients were used to correct the area values by subtracting position and velocity effects. The corrected area, named here “missed eye position value” in degrees by seconds, was then expressed as the percentage of the maximum effect for each injection.

Drugs. All chemicals used in this study were applied by superfusion in the ACSF and introduced into the recording chamber by means of a perfusion pump (2115 Multiperspex pump; LKB-Bromma, Bromma, Sweden). The chemicals used were atropine sulfate, CNQX, APV, McNA-343, and TTX from Sigma (St. Louis, MO); pirenzepine (Tocris; Biogen Cientifica, S.L., Madrid, Spain); and methyllylcaconitine and carbachol (Research Biochemicals, Natick, MA). Atropine sulfate, McNA-343, pirenzepine, and carbachol were selected for the in vivo study.

Statistics. Unless indicated otherwise, the electrophysiological data are always expressed as mean ± SEM. In all cases, n represents the number of averaged neurons. Unless indicated otherwise, action and synaptic potentials were averaged (five or more) before quantitative analysis. Statistical analysis of collected data was performed using a paired Student’s t test and, when necessary, by one-way ANOVA. Statistical significance was determined at a level of p ≤ 0.05.

Results

Membrane properties of PH neurons

Sagittal slices used in this study allowed an easy identification of the anatomical limits of the PH nucleus (Fig. 1). Intracellular recordings (n = 125) were made from neurons located in the rostral third of the nucleus, because this is the place where principal cells projecting to the abducens nucleus and other oculomotor structures are located (McCrea and Baker, 1985a; Escudero et al., 1992). Recorded neurons did not present action potentials spontaneously at resting potential values (−66.6 ± 2.5 mV). The direct activation of these neurons by depolarizing current injections (0.1–0.6 nA) generated action potentials or a train of spikes with a slow adaptation in all of the cases. The mean input resistance of the neurons was 10.55 ± 0.6 MΩ, the mean membrane time constant was 29.9 ± 3.3 msec, and the threshold potential was 51.1 ± 1.0 mV. The spike amplitude was 76.6 ± 1.4 mV, with a duration of 1.28 ± 0.06 msec, a rise time of 0.28 ± 0.01 msec, a decay time of 0.32 ± 0.02 msec, and a half width of 0.62 ± 0.03 msec (Fig. 2A).

After DC injection (Fig. 2A) or electrical stimulation of the PPRF (Fig. 2B), PH neurons included in this study presented a biphasic afterhyperpolarization (AHP), consisting of a fast afterhyperpolarization (fAHP), continuing the repolarizing phase of the action potential, with an amplitude of 9.2 ± 1.0 mV, followed by a medium AHP (mAHP) with an amplitude of 13.2 ± 0.7 mV and a duration of 78.29 ± 4.19 msec (Fig. 2A). The peak of the fAHP was always negative (8.2 ± 1.3 mV) with respect to resting potential values. The fAHP increased in amplitude with membrane hyperpolarization and decreased with membrane depolarization (r = 0.71; p < 0.05). The two phases were separated by an afterdepolarization (ADP), which was generated 2.14 ± 0.21 msec after the spike. The ADP amplitude was voltage dependent, increasing with membrane hyperpolarization and decreasing with membrane depolarization (r = 0.68; p < 0.01).

Fifteen of the 125 recorded neurons were successfully filled. Morphological reconstruction of biocytin-injected neurons showed that they were fusiform or piriform in shape (25.3 ± 1.6 μm mean diameter), with three to six primary dendritic trunks...
leaving the soma in opposite directions (Fig. 1B,C). Primary dendrites divided into two or three secondary dendrites at 25–30 μm from the soma and extended radially around it, frequently reaching the paragigantocellular formation and parts of the medial vestibular nuclei included in the slice. For five neurons, the axon could be followed crossing the PH and entering the adjacent medial longitudinal fascicle. The morphology of these neurons is consistent with that of the principal cells identified in the PH nucleus of cats by HRP injections and projecting to many brainstem-located oculomotor sites, including the abducens nucleus (McCrea and Baker, 1985a).

Other neurons recorded in the anatomical limits of the PH nucleus (n = 27) did not present a biphasic AHP and did present ventrally directed axonal trajectories (n = 6) not reaching the medial longitudinal fascicle. Such neurons were considered to be "medusa cells" (McCrea and Baker, 1985a), projecting probably to cerebellar structures, and were not included in this study.

Response of PH neurons to ipsilateral PPRF stimulation
To characterize the synaptic nature of oculomotor signals afferent to PH neurons from the PPRF, this region was electrically stimulated at subthreshold values (Fig. 2B). The synaptic responses evoked on PH neurons were EPSPs in all cases (n = 35). The evoked EPSPs had a mean latency of 2.60 ± 0.32 msec, a duration of 68.8 ± 20.6 msec, a rise time of 8.07 ± 3.26 msec, a decay time of 20.5 ± 9.8 msec, and a half width of 21.2 ± 9.2 msec. The EPSP amplitude showed a graded nature, depending on stimulus intensity (Fig. 2B) and on resting membrane potential (data not shown), without any evident change in latency (with train stimuli up to 200 Hz), suggesting its monosynaptic nature.

Moreover, as shown in Figure 2, C and D, train stimulation (50–200 Hz, 100 msec) of the PPRF evoked a sustained depolarization of PH neurons exceeding the end of the train by hundreds of milliseconds. This depolarization was able to evoke a train of action potentials that reached peak frequencies ≤150 spikes/sec. The amplitude and duration of this depolarization were linearly related (r ≥ 0.95; p ≤ 0.001) to stimulus frequency during the train, with slopes of 0.023 mV/Hz and 1.44 msec/Hz, respectively (Fig. 2E,F).

Effects of DC stimulation on the firing properties of PH neurons
An attempt was made to evoke the same sustained depolarization of PH neurons by DC injection to check whether it was dependent on the intrinsic active properties of the membrane of PH cells. The depolarization of PH neurons (n = 25) by means of an injection of DC (0.1–0.6 nA) evoked spontaneous discharges ranging from 5 to 40 spikes/sec (Fig. 3A,B). This firing activity stopped immediately after the end of current-evoked depolarization, with no sign of any over-running depolarization.

Superfusion with TTX (1 μM; n = 7) blocked the action potentials elicited in PH neurons by depolarizing pulses (Fig. 3C).

These results indicated a Na⁺ dependence of action potentials in PH neurons. Moreover, the I–V relationships for PH neurons (n = 7), when voltage-dependent Na⁺ channels were blocked with TTX, were linear (r ≤ 0.75; p < 0.001) (i.e., without any
Glutamatergic nature of PPRF synaptic terminals on PH neurons

As shown in Figure 4A, the EPSPs evoked by subthreshold electrical pulses applied to the ipsilateral PPRF were completely blocked (n = 27) by superfusion with CNQX (10 μM), a specific blocker of AMPA-kainate receptors. This indicated that the excitatory synaptic response in PH neurons evoked by the subthreshold stimulation of the ipsilateral PPRF was essentially mediated by glutamate-activating AMPA-kainate receptors. Indeed, superfusion with APV (a specific blocker of NMDA receptors; 50 μM; n = 10), without the addition of CNQX, had no effect on EPSPs evoked by the electrical stimulation of the PPRF region, indicating that NMDA receptors were not present in this synapse. Furthermore, superfusion with atropine sulfate (a nonspecific antagonist of cholinergic receptors; 1.5–4.5 μM) had no noticeable effect on EPSPs evoked in PH neurons by single, subthreshold pulses presented to the PPRF region.

Postsynaptic and presynaptic effects of cholinergic drugs on the firing properties of PH neurons

It is known that train stimuli (>30 Hz) are able to activate cholinergic axons (Moises et al., 1995; Faber and Sah, 2002), and it has been described that cholinergic axons from pontomesencephalic reticular formation nuclei reach the PH nucleus (Carpenter et al., 1987; Higo et al., 1990; Semba et al., 1990). In this regard, we assumed that those cholinergic neurons and/or axons were also activated by the electrical stimulation of the PPRF and that the sustained depolarization evoked in PH neurons could have a cholinergic origin. Indeed, the superfusion with atropine sulfate (1.5 μM; n = 7) was able to remove the sustained depolarization evoked by a train applied to the PPRF area (Fig. 4B).

Superfusion of the slice with the cholinergic agonist carbachol (25 μM) was able to evoke a slow-building and long-lasting depolarization (peak at ≈12.3 mV; n = 25) of intracellularly recorded PH neurons. This depolarization even reached threshold values (80% of the cases; n = 25) to evoke action potentials (Fig. 5). The slope of PH neuron depolarization evoked by carbachol superfusion presented values ranging from 0.05 to 0.2 mV/sec (n = 15). This depolarization took place without any noticeable change in the input resistance of the recorded neuron, as calculated indirectly with the application of hyperpolarizing pulses (Fig. 5A).

The firing frequency evoked by the carbachol-induced depolarization was lower than that generated by DC-evoked depolarization at values close to threshold (peak values of 18.7 ± 2.6 against 41.9 ± 5.6 spikes/sec, respectively), indicating that carbachol-evoked peak firing of PH neurons represented only some 45% of that evoked by DC depolarization of the same neurons (n = 8). Moreover, action potentials evoked during carbachol-induced depolarization were 30% smaller (t = 3.63; p < 0.01) than those generated by DC injection (ranges: 30–70 against 60–80 mV, respectively) (Figs. 3, 5).

noticeable rectification in the physiological voltage values) (Fig. 3D). Accordingly, there was no sign in the postsynaptic membrane properties that could explain the sustained depolarization evoked by 100 msec trains of electrical stimuli applied to the PPRF. In consequence, this depolarization has a synaptic origin and is not related to intrinsic membrane properties.
The mAHP duration of the action potential was significantly 
\((t = 5.04; p < 0.001)\) longer in PH neurons depolarized with 
carbachol \((120.11 \pm 11.40 \text{ msec})\) than in those depolarized by 
DC injection \((72.83 \pm 6.39 \text{ msec})\). This change represented an 
increment of 65% in the duration of the mAHP, which could 
partly explain the differences in peak firing frequencies evoked by 
carbachol and DC injection indicated above (data not shown). 
The depolarizing effects of carbachol were abolished by superfusion 
with atropine sulfate \((1.5–4.5 \mu M; n = 14)\), indicating that 
the response was mediated by muscarinic receptors (Fig. 5B). To 
further characterize the subtype of muscarinic receptor involved 
in the carbachol-evoked depolarization, pirenzepine (a selective 
blocker of muscarinic M1 receptors) (Yajeya et al., 2000) was 
added to the perfusion solution \((0.5 \mu M)\). In all cases \((n = 7)\), 
the carbachol-evoked depolarization was abolished, indicating 
the involvement of M1 receptors in carbachol actions on the 
membrane of PH neurons (Fig. 5C). Interestingly, the level of depo-
larization evoked by carbachol was linearly related (slope, 1.094 
spikes/mV; \(r = 0.92; p > 0.001\)) with neuronal firing rate (Fig. 
5D). This relationship seems to be necessary \textit{in vivo} for evoking a 
proportional firing rate for each position of the eye in the orbit. 
When the effects of carbachol were blocked by superfusion with 
pirenzepine (Fig. 5C), the linear relationship was maintained, 
but with a smaller slope \((0.823 \text{ spikes/mV}; r = 0.95; p > 0.001)\). The 
noticeable hysteresis described here (i.e., different firing rates for 
the same resting potential, depending on the depolarizing or re-
polarizing direction of membrane potential values) has also been 
described in both abducens motoneurons and PH neurons \textit{in vivo} 
and is of physiological significance because of the opposite 
phenomena (extraocular muscles presenting greater strength for 
the same length when relaxing than when contracting) (Delgado-
García et al., 1989).

To confirm the postsynaptic nature of the depolarizing effects 
of carbachol on PH neurons, all possible action potentials sponta-
nously present were removed by TTX superfusion \((1 \mu M; n = 
8)\) of the slice (Fig. 6). The effective blockage of all action poten-
tials was checked by repetitive stimulation (at 0.2 Hz) of the 
PPRF. Figure 6B shows the progressive disappearance of EPSPs 
evoked in a PH neuron after TTX superfusion. During TTX su-
perfusion, the recorded cell did not evoke any action potential, 
even after DC injections (Fig. 6B, asterisk). In this situation, the 
superfusion with carbachol \((25 \mu M; n = 8)\) evoked a depolariza-
tion not accompanied by the generation of any action potential. 
The possibility of some additional presynaptic effects of cho-
linergic drugs on PPRF axon terminals on PH neurons was also 
checked, using the paired-pulse paradigm (Mennerrick and Zor-
rusky, 1995; Huang et al., 1996; Yajeya et al., 2000). It has been 
reported (Huang et al., 1996; Thompson, 2000; Fernández-de 
Sevilla et al., 2002) that any change in the response evoked by 
the second stimulus \((S2)\) in relation to the first \((S1)\) will be indicative 
of a presynaptic action \((100 \times S2/S1)\). Explored PH neurons \((n = 
10)\) presented a reduction \((61.00 \pm 0.33\%\) in EPSP amplitude 
evoked by the second stimulus \((S2)\) presented to the PPRF in 
relation to the first \((S1)\), at an interval of 50–100 msec (Fig. 7A, 
row 1). As shown in Figure 7A (row 3), carbachol superfusion \((25 
\mu M)\) was able both to decrease the amplitude of the EPSP evoked 
by the electrical stimulation of the PPRF by the S1 stimulus and to 
increase the S2/S1 ratio with respect to control values \((88.90 \pm 
0.05 \text{ against } 61.00 \pm 0.03\%\, p < 0.01)\). This effect could be 
considered presynaptic, because the depolarizing effect of carbachol 
on the postsynaptic PH neuron was canceled out by current-
holding of the postsynaptic cell, at resting membrane potential 
values. Superfusion with cacomitine \((0.1 \mu M)\), a potent antagonist 
of nicotinic receptors (Marchi et al., 2002), did not modify the 
presynaptic depressant effects of carbachol (Fig. 7). In contrast, 
atorpine sulfate \((1.5 \mu l)\) completely abolished the effects of car-
bachol, suggesting the presence of muscarinic receptors in the 
postsynaptic terminals of PPRF neurons projecting monosynap-
tically on PH neurons. Thus, the ACh has a double effect: a sus-
tained depolarization of postsynaptic PH neurons after PPRF 
activation and a presynaptic action, on PPRF axon terminals, 
decreasing glutamate release. The latter effect could help in a 
sharp termination of PPRF eye velocity signals on PH neurons 
(Delgado-García et al., 1989).

**Effects of cholinergic drugs on spontaneous eye movements 
in alert cats**

To characterize the effects of selected drugs on eye movements 
in alert behaving animals, agonists (carbachol and McN-A-343) 
and antagonists (atropine and pirenzepine) of cholinergic recep-
tors were unilaterally injected in the rostral PH nucleus (Fig. 8). 
Unilateral injections of carbachol \((40–120 \text{ nl}; 125 \mu l)\) and McN-
A-343 \((40–120 \text{ nl}; 250 \mu l)\) induced a nystagmus with linear slow 
phases directed contralaterally to the injected side. In both cases, 
the nystagmus was very much evident in darkness, whereas in 
light the nystagmus was reduced or absent. In all cases \((p < 0.05)\), 
nystagmus induced by carbachol was slower in reaching maxi-
imum values than that induced by McN-A-343 (Fig. 9A, B; Table 
1). Unilateral injections of atropine \((40–120 \text{ nl}; 100 \text{ ms})\) induced 
centripetal postsaccadic drifts in the light, with a time constant of 
0.196 ± 0.018 sec \((n = 4)\; \text{measured at peak response of the 
injected drug})\. In darkness, the atropine injection always induced 
a nystagmus with slow phases curved and directed ipsilaterally to
Signals have been recorded in the PH nucleus of cats and monkeys. These neurons carry pure (or closely related) eye position signals. For the horizontal oculomotor system, neurons carrying pure (or closely related) eye position signals have been recorded in the PH nucleus of cats and monkeys.

To maintain the position of the eye after a saccade, extraocular muscles generate eye position signals. For the horizontal oculomotor system, neurons carrying pure (or closely related) eye position signals have been recorded in the PH nucleus of cats and monkeys.

Discussion
Role of PH neurons in the generation of eye position signals
To maintain the position of the eye after a saccade, extraocular motoneurons need a persistent activity called “eye position signal” (Cannon and Robinson, 1987; Delgado-García et al., 1989; Fukushima et al., 1992; Moschovakis et al., 1996; Moschovakis, 1997). Because PPRF excitatory burst neurons fire only slightly in advance and during part of the ongoing saccade, a repeated question has been how do neuronal circuits implicated in eye movements generate eye position signals. For the horizontal oculomotor system, neurons carrying pure (or closely related) eye position signals have been recorded in the PH nucleus of cats and monkeys.

Role of ACh in the generation of eye position signals
Considering that persistent activity is independent of passive and/or active membrane properties of integrator neurons (Aksay et al., 2001), alternative membrane properties are required to explain the integration process. Recently, it has been demonstrated in goldfish that feedback activity among eye position-related neurons (excitatory ipsilateral and inhibitory contralateral) is necessary for temporal integration (Aksay et al., 2003). Because there is no anatomical evidence of connections between ipsilateral neurons that could explain the positive feedback (Aksay et al., 2000), a mechanism located at synaptic levels has been suggested that could operate for the short-term potentiation of synaptic inputs.
neuronal integration process, are consistent with this hypothesis. 

The sustained depolarization evoked by carbachol presented a slow course without significant variations of input resistance, which may be explained by induction of calcium-activated nonspecific cationic currents (Klink and Alonso, 1997; Haj-Dahmane and Andrade, 1999; Yajeya et al., 1999). Duration and amplitude of this depolarization depend on membrane potential due to cholinergic effects on voltage-dependent K⁺ currents (i.e., an M current) (Seeger and Alzheimer, 2001) or to a K⁺ inward rectification current (Washburn and Moises, 1992). A decrease in the amplitude of the action potentials, similar to that observed in the entorhinal and prefrontal cortices, was also observed (Cantrell et al., 1996; Klink and Alonso, 1997) and was attributed to a decrease in Na⁺ conductance, resulting from the increased availability of intracellular Ca²⁺ (Bulatko and Greeff, 1995; Klink and Alonso, 1997). Finally, carbachol induced a decrease in the firing rate that could be attributable to an enlargement in the mAHP as a consequence of increased intracellular Ca²⁺. Similar effects of carbachol-induced changes in the firing pattern have been described in the entorhinal cortex (Klink and Alonso, 1997; Egorov et al., 2002) and subiculum (Kawasaki et al., 1999). This modulation is important, considering that velocity (phasic) signals coming from the PPRF converge on PH neurons to be transformed into a position (tonic) signal that will be sent to the abducens nucleus to fix the eye after a horizontal saccade. Thus, ACh might play an important role in the integration process, modulating the firing pattern of PH neurons and facilitating the transformation of eye velocity signals into eye position signals. Moreover, ACh seems to have a depressant presynaptic effect on axon terminals of PPRF excitatory burst neurons. Consequently, ACh plays a complex role in the generation of persistent neuronal activity, contributing to the postsynaptic depolarization of PH neurons and to the presynaptic impairment of transmitter release, disfacilitating any immediate effect of excitatory burst neurons on PH cells after the train of impulses (i.e., after a saccade).

Pharmacological studies in vivo

In light, the pharmacological blockade of muscarinic receptors by atropine, and specifically of the M1 receptor by pirenzepine, induced an inability to maintain the eye position reached after saccades. In contrast, the less specific carbachol and the M1-specific McN-A-343 agonists did not affect the generation of eye position signals because no posttaccadic drifts were observed. In darkness, ACh agonists induced a nystagmus, the slow phases of which were linear (r > 0.995) and directed contralaterally to the injected side, whereas antagonists induced a nystagmus with ipsilateral curved slow phases. These results were exclusive to horizontal eye movements, indicating that there was no diffusion of the applied drugs to the neighboring vestibular nuclei. The pharmacological effects of ACh agonists were in agreement with the effects induced by blockade of the GABA and glycine receptors, using the same animal preparation (Moreno-López et al., 2002), and are in accordance with the depolarization of the PH neurons induced by muscarinic activation in vitro. The linearity of the slow phases of the nystagmus in darkness and the correct maintenance of the eye position in light are both indicative of a correct gaze-holding system. In contrast, results obtained with ACh antagonists (i.e., curved slow phases in darkness and postsaccadic centripetal drifts in light) were indicative of a loss of eye position in the horizontal plane. These findings, similar to those obtained by the blockade of glutamatergic receptors in the PH nucleus of alert cats (Moreno-López et al., 2002), suggest that activation of M1 receptors by ACh is necessary to generate correct eye position signals after an eye saccade in the activating direction. The absence of nystagmus in light suggests that the visual input is sufficient to override any effect induced by a higher concentration of ACh agonists.
Integrative properties of PH neurons

According to the present data, the persistent activity observed in PH neurons (Escudero and Delgado-García, 1988; Delgado-García et al., 1989; Escudero et al., 1992; McFarland and Fuchs, 1992) is the result, at least in part, of synaptic effects evoked by excitatory neurons in the PPRF and cholinergic neurons located probably in the pontomesencephalic region. These findings, together with the recent proposals regarding the feedback mechanism for temporal integration (Aksay et al., 2003), indicate that eye position signals arriving at the abducens nucleus could be originated in the PH nucleus by the effect of cholinergic inputs, subsequent to the depolarizing effects of glutamatergic PPRF inputs, and maintained by the participation of ipsilateral and contralateral reverberant circuits in the integrator (Aksay et al., 2003). Moreover, PH cholinergic neurons (Tighilet and Lacour, 1998) could be activated by collateral axons from integrator neurons, or they could represent an integral part of the involved circuits. The interplay of glutamatergic and cholinergic synaptic actions on the PH neurons described here is not unique because it has been reported recently that PH neurons are also involved in eye stability during head movements via the mediating action of nitric oxide release by some PH neurons on GABAergic afferents from the ipsilateral medial vestibular nucleus (Moreno-López et al., 1996, 2002). Thus, the PH nucleus could include more than one control mechanism to transform transient velocity signals into the persistent activity underlying eye positions of fixation.

Table 1. Time course of the nystagmus evoked by ipsilateral drug injections into the PH nucleus

<table>
<thead>
<tr>
<th>Drug</th>
<th>( t_{50} ) (min)</th>
<th>( T_{50} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbachol</td>
<td>9.9 ± 0.3</td>
<td>18.6 ± 1.0</td>
</tr>
<tr>
<td>McN-A-343</td>
<td>2.6 ± 0.4</td>
<td>15.3 ± 4.0</td>
</tr>
<tr>
<td>Atropine</td>
<td>7.4 ± 0.2</td>
<td>22.5 ± 3.6</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>3.8 ± 0.4</td>
<td>22.8 ± 3.6</td>
</tr>
</tbody>
</table>

\( t_{50} \) = Time necessary to reach the maximum effect; \( T_{50} \) = time elapsed between the injection and the moment at which 50% of the maximum effect was obtained in the response decay curve. Data are shown as means ± SEM. Data were collected from four or more effective injections of each drug.

References


Fukushima K, Kaneko CRS, Fuchs AF (1992) The neuronal substrate of eye stability during head movements via the mediating action of nitric oxide release by some PH neurons on GABAergic afferents from the ipsilateral medial vestibular nucleus (Moreno-López et al., 1996, 2002). Thus, the PH nucleus could include more than one control mechanism to transform transient velocity signals into the persistent activity underlying eye positions of fixation.


