In Vitro Properties of Neurons in the Rat Pretectal Nucleus of the Optic Tract

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Submitted 11 January 2007; accepted in final form 26 February 2007

Prochnow N, Lee P, Hall WC, Schmidt M. In vitro properties of neurons in the rat pretectal nucleus of the optic tract. J Neurophysiol 97: 3574–3584, 2007. First published March 7, 2007; doi:10.1152/jn.00039.2007. The nucleus of the optic tract (NOT) has been implicated in the initiation of the optokinetic reflex (OKR) and in the modulation of visual activity during saccades. The present experiments demonstrate that these two functions are served by separate cell populations that can be distinguished by differences in both their cellular physiology and their efferent projections. We compared the response properties of NOT cells in rats using targeted-directed whole cell patch-clamp recording in vitro. To identify the cells at the time of the recording experiments, they were prelabeled by retrograde axonal transport of WGA-apo-HRP-gold (15 nm), which was injected into their primary projection targets, either the ipsilateral superior colliculus (ISC), or the contralateral NOT (cNOT), or the ipsilateral inferior olive (iIO). Retrograde labeling after injections in single animals of either WGA-apo-HRP-gold with different particle sizes (10 and 20 nm) or two different fluorescent dyes distinguished two NOT cell populations. One projects to both the ISC and cNOT. These cells are spontaneously active in vitro and respond to intracellular depolarizations with temporally regular tonic firing. The other population projects to the iIO and consists of cells that show no spontaneous activity, respond phasically to intracellular depolarization, and show irregular firing patterns. We propose that the spontaneously active pathway to iSC and cNOT is involved in modulating the level of visual activity during saccades and that the phasically active pathway to iIO provides a short-latency relay from the retina to premotor mechanisms involved in reducing retinal slip.

INTRODUCTION

The nucleus of the optic tract (NOT) consists of a small and inconspicuous population of neurons intercalated among optic tract fibers passing through the pretectum. Considering its small size and apparent lack of distinct nuclear subdivisions, NOT has not been associated with a surprising variety of functions that appear remarkably conserved across mammalian species. The most extensively studied function of NOT is its role in the control of compensatory eye movements during the horizontal optokinetic reflex (OKR) (marsupials: Ibbotson et al. 1994; Volchan et al. 1989; rat: Cazin et al. 1980; Mustari and Fuchs 1990; rabbit: Collewijn 1975a; cat: Hoffmann and Fischer 1981; monkey: Ilg and Hoffmann 1996; Mustari and Fuchs 1988), whereas other neurons are responsive only to much faster stimulus velocities (marsupials: Ibbotson et al. 1994; Price and Ibbotson 2002; Schweigart and Hoffmann 1992; Sudkamp and Schmidt 1995; monkey: Inoue et al. 2000). In addition, in monkey neurons have been described whose activity is completely blocked by a saccadic eye movement (Mustari et al. 1997).

Thus the known response properties in vivo suggest that, even though NOT cells as a group prefer moving visual stimuli, some selectively respond to high-velocity movements and others to slow movements. However, with currently available in vivo methods, it has been difficult to determine with certainty whether NOT makes a similar contribution to each of its diverse targets or, instead, it consists of several distinct cell populations, each with its own function and pattern of connec-
tions. In the present experiments, we addressed these questions in rats by performing in vitro whole cell patch-clamp recordings on NOT cells that were prelabeled by retrograde axonal transport after injections of neuronal tracers in the destinations of their efferent projections. This approach allowed direct comparisons of the intracellular properties of pretectal cell populations with their identified projection targets. The results demonstrate that NOT includes several cell populations that can be distinguished both by their intrinsic membrane properties and by their efferent connections.

**METHODS**

**Retrograde labeling**

Experiments were performed on 27 Long–Evans hooded rats between 16 and 32 days postnatal age. Experimental procedures were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and approved by the Duke University Institutional Animal Care and Use Committee. For stereotaxic surgery, the animals were deeply anesthetized by an intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine hydrochloride (1 mg/kg) and the level of anesthesia was maintained by additional injections of ketamine. Using stereotaxic coordinates, the terminal zones of NOT neurons were injected with 0.5 μl of the retrograde axonal tracer wheat germ agglutinin (WGA)-apo-horseradish peroxidase (HRP)-gold (15-nm particle size; Sanbio, Beutelsbach, Germany, and E-Y Laboratories, San Mateo, CA). As a cell label, the (WGA)-apo-HRP-gold has an advantage over fluorescent labels of being resistant to fading, nontoxic to the neurons, and visible under bright-field illumination (Lee et al. 2001). In some experiments, two injections of different-size gold particles (10 and 20 nm), each in a different site, were made in single animals to determine whether one cell type projects to both sites. After surgery, animals were allowed to recover and survive for a period of 5–7 days, which is sufficient for retrograde axonal transport of the tracer (Lee et al. 2001). Although the particle clusters that appear in labeled cells are of much larger size than the gold particles, control tracer injections with a single particle size revealed that smaller particles lead to considerably smaller clusters than do the larger particles (Fig. 1). This allows a clear distinction between labels from different particle sizes in double-label experiments.

The results obtained with WGA-apo-HRP-gold were confirmed using double injections of fluorescent tracers. We used red textmarker dye (“Stabilo Red”; Stabilo, Heroldsberg, Germany) for cNOT and iIO injections and green textmarker dye (“Stabilo Green”) for injections into the iSC.

**Slice preparation for patch-clamp recording**

After injection of an overdose of ketamine and xylazine hydrochloride, animals were transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF) containing the following components (in mM): NaCl, 124; KCl, 5; NaH₂PO₄, 1.25; NaHCO₃, 26; MgSO₄, 2; CaCl₂, 2; and glucose, 10. The ACSF was continuously gassed with carbogen (95% O₂-5% CO₂). After removal of the brain from the skull, 350-μm-thick coronal slices were cut and kept at 35°C in ACSF for ≥1 h to allow the tissue to recover from the slicing procedure. For whole cell patch-clamp recording they were transferred to a submersed-type recording chamber and superfused at 3 ml/min with room-temperature ACSF.

**Whole cell patch-clamp recording**

Whole cell patch-clamp recordings from WGA-apo-HRP-gold–labeled neurons in the NOT were performed on a fixed-stage micro-

![FIG. 1. Distribution of gold particle cluster sizes after retrograde transport of wheat germ agglutinin (WGA)-apo-horseradish peroxidase (HRP), labeled with 10-nm-sized gold particles (gray bars, arrowheads in A) and 20-nm-sized gold particles (black bars, arrows in B). Although clusters are much larger than the original gold particles retrograde label from either particle size can be easily distinguished. Scale bar in A: 20 μm.](image-url)
in 30% sucrose buffer overnight at 4°C for cryoprotection. The brains
with injections of WGA-apo-HRP-gold were cut with a freezing
microtome into 40-μm-thick coronal slices through both the prete-
crum in its entire anterior–posterior extent and the tracer injection sites.
Sections were collected in 0.1 M phosphate-buffered saline, put on
gelatin-coated slides, and stained with 0.03% cresyl violet. After
injections of fluorescent dyes, the same procedure was performed,
except the sucrose rinses were omitted and 40-μm-thick coronal slices
were cut using a vibratome. Then, the brain slices were coverslipped
in 0.01 M phosphate-buffered saline containing 1% glycerol to avoid
drying. Injection sites and labeled somata were identified and photo-
graphed with an oil-immersion objective lens (×60) on a light
microscope.

Soma sizes of labeled neurons as well as sizes of gold particles
were analyzed using a computer-aided reconstruction system (Neu-
rolucida, MicroBrightField, Williston, VT). All chemicals used were
obtained from Sigma–Aldrich (Deisenhofen, Germany).

RESULTS

Two populations of NOT cells were distinguished in these
experiments. One population projects to the iIO, whereas the
other projects to both the iSC and the cNOT. Whole cell
patch-clamp recordings from cells that were identified by their
efferent projections confirmed that these are two distinct cell
populations; the cells that project to iSC and cNOT are toni-
cally active in vitro, whereas the cells that project to iIO
generate phasic responses.

Tonically active cells

All of the clamped cells that projected to the iSC were
tonically active (n = 15). For example, Fig. 2 illustrates the
results from one cell that was prelabeled by an injection of
WGA-apo-HRP-gold into the iSC (0.5 μl, 15-nm gold parti-
cles). The arrows in Fig. 2A indicate gold particles that were
transported to the cell soma from the injection site. The
homogeneous background in the soma and primary dendrite
(asterisk) was produced by the biocytin that diffused into the

cell from the patch pipette during the experiment, confirming
that the recordings were obtained from the prelabeled cell.
Figure 2B shows, in a current-clamp recording, that action
potentials evoked by current injections were sustained for the
entire duration of the depolarizations (500 ms) and varied in
frequency with the injected current or membrane potential.
Figure 2C illustrates that action potential frequency increased
with stepwise increases in the injected current. Figure 2D plots
the relationship between the response frequency and the mem-

FIG. 2. Results from a representative nucleus of the optic
tract (NOT) neuron retrogradely labeled from ipsilateral supe-
rior colliculus (iSC). A: clustered gold particles are present in
the soma and primary dendrite of the neuron (arrows). Homo-
geneous background label (asterisk) produced by biocytin dif-
fusion from the patch pipette during recording confirms that the
recording was from a prelabeled cell. Inset: location of the
neuron within the slice (m, medial; l, lateral; d, dorsal; v,
ventral). B: depolarizing current injection under current-clamp
conditions (duration 500 ms) evokes action potentials in a
regular firing pattern. Neuron’s firing rate varies with the
membrane potential. C: depolarizing current injections (ad-
justed to achieve +5 mV steps of the membrane potential)
increase the firing rate of the neuron, which was spontaneou-
ly active at resting potential (~62 mV). Interspike intervals (ISIs)
remain regular at all membrane potentials. D: firing rate plotted
as a function of membrane potential. Maximum firing rate (27
spikes/s) is reached at −30 mV.
FIG. 3. Representative NOT neuron labeled after an injection of WGA-apo-HRP-gold into the contralateral NOT (cNOT). A: arrows point to the clustered gold particles in the soma and primary dendrite that prelabeled the cell. Homogeneous background of biocytin confirms that the recording was from the prelabeled cell. B: depolarizing current injections during current clamp (5 and 10 pA, duration 500 ms) increase the firing rate as a function of the amplitude of the injected current. C: a sustained nonadapting tonic firing of action potentials (maximum firing rate: 18 spikes/s) is generated during current-clamp recordings at membrane potentials of $-60$ and $-40$ mV.

FIG. 4. Double labeling with 2 retrograde tracers. A: WGA-apo-HRP-gold with 20-nm-sized gold particles were injected into the superficial layer of the iSC and WGA-apo-HRP-gold with 10-nm-sized gold particles into the cNOT. B: distribution of retrogradely labeled neurons in a representative midbrain section containing caudal NOT. Double-labeled cells are shown in blue; cells labeled only from iSC or cNOT are shown in red and green, respectively. C: in 40-μm-thick coronal sections counterstained with cresyl violet, NOT neurons retrogradely labeled with WGA-apo-HRP-gold contain clusters with both particle sizes (inset). Clustered 20-nm-sized particles appear as prominent dots, in contrast to the smaller clusters of 10- nm-sized particles. Coappearance of 10- and 20-nm-sized particles within individual NOT neurons (arrows) indicates that the neuron sends axon collaterals to both the iSC and the cNOT.
brane potential; the action potentials increased in frequency with the amount of depolarizing current from fewer than five spikes/s when the cell was near its resting potential (−61.3 mV) to a peak of about 27 spikes/s when the membrane potential approached −25 mV. The mean input resistance of the tonically active cells was 323.7 MΩ (SD = 119.7); the mean time constant τ, which was conventionally calculated from the onset of long-lasting hyperpolarizing voltage steps, was 0.93 ms (SD = 1.09); and the mean resting potential was −55.1 mV (SD = 3.4). The membrane potential threshold for the generation of action potentials, defined as the potential at which the first spike appeared when the cell was continuously depolarized from −90 mV by increasing the amplitude of the current injections, was −59.6 mV (SD = 3.8).

Figure 3 illustrates the results from an experiment on a cell prelabeled after an injection of WGA-apo-HRP-gold in cNOT (Fig. 3A). Similar to the NOT–iSC cell illustrated in Fig. 1, the cell in Fig. 2 generated sustained action potentials that varied in frequency with the amplitude of the injected current in a current-clamp recording (Fig. 3B). Figure 3C shows trains of action potentials (two and 18 spikes/s, respectively) that were generated when the cell was clamped at a membrane potential of −60 mV (left) or −40 mV (right). Similar results were obtained for all cells that projected to cNOT (n = 8). The mean input resistance of NOT–cNOT cells (R_m) was 429.5 MΩ (SD = 106.0); the mean time constant τ was 1.81 ms (SD = 2.96); the mean resting potential was −55.5 mV (SD = 3.6); and the mean spike threshold was −56.6 mV (SD = 2.4). These parameters were not significantly different from those of the cells that projected to the iSC (P = 0.84 for membrane resistances, P = 0.35 for time constants, and P = 0.45 for resting potentials; P = 0.15 for spike thresholds, t-test).

To determine whether the NOT cells with in vitro tonic firing patterns constitute a single population that projects to both iSC and cNOT, single animals (n = 3) were injected with 20-nm-sized gold particles in iSC and 10-nm-sized particles in cNOT (Fig. 4A). Although the size of gold particles themselves are below the resolution limit of the light microscope, the two tracers can be distinguished by the size of the aggregates that occur in the labeled cells. Aggregates in iSC, where 20-nm-sized gold particles had been injected, had diameters >2.5 μm (mean size 3.0 μm, SD = 0.3), whereas aggregates of the 10-nm particles in iIO were <2.5 μm in diameter (mean 1.8 μm, SD = 0.2), which was significantly smaller (P < 0.0001).

The distribution of retrogradely labeled cells within the NOT in one representative midbrain section is depicted in Fig. 4B. Of the cells labeled from iSC (n = 267), 76% were double labeled; of the cells labeled from cNOT (n = 226) 90% were double labeled. Thus the majority of labeled cells contained both tracers, indicating that they projected to both targets. Figure 3C illustrates a cluster of five cells in rostral NOT, each of which contained both sizes of gold particles, confirming that single NOT cells project to both targets. The same conclusion was reached in the experiments illustrated in Fig. 5. In this case, the retrograde fluorescent tracer Stabilo Red was injected into the cNOT and Stabilo Green was injected into the iSC and clusters of cells with both labels (arrows) were present in NOT.

We also compared soma sizes of single-labeled and double-labeled cells. The average soma area of NOT neurons labeled only from iSC was 191.0 μm² (SD = 44.1); that of cells labeled only from cNOT was 212.3 μm² (SD = 46.1). Double-labeled cells had a mean soma area of 221.7 μm² (SD = 48.3). These differences were not statistically significant.

**Phasic cells**

Neurons prelabeled with WGA-apo-HRP-gold after injections in iIO (0.1 μl, 15-nm-sized particles) generated phasic or phasic–tonic, fast-adapting, or bursting firing patterns in response to intracellular current injection and never showed spontaneous activity at resting potential. An example is shown in Fig. 6. Figure 6A shows a prelabeled cell containing the gold particles (arrows) and the homogeneously distributed biocytin (asterisk). Figure 6B is a current-clamp recording showing that the cell generated a phasic–tonic firing pattern in response to depolarizing current injections leading to irregular maintained firing when higher depolarizing currents were injected. In general, NOT neurons prelabeled from the iIO showed less-uniform responses to intracellular depolarizations than neurons prelabeled from either iSC or cNOT. Figure 6C shows the irregular firing pattern of another prelabeled cell when injected currents induced small depolarizing steps of the membrane potential. In Fig. 6D, the response frequency is plotted against the membrane potential; the average firing rate increased with the amount of depolarizing current from fewer than five spikes/s at −40 mV to a peak of roughly 30 spikes/s at −25 mV. More positive membrane potentials resulted in a reduced firing rate and no spikes occurred above −15 mV.
The mean input resistance of the cells that projected to iIO neurons \((n = 17)\) was 200.7 M\(\Omega\) (SD = 59.4), the mean time constant \(\tau\) was 3.07 ms (SD = 2.28), the mean resting potential was -57.9 mV (SD = 4.9), and the spike threshold was -39.9 mV (SD = 7.9). Compared to the tonic cells that projected to iSC and cNOT, the phasic iIO-projecting cells were characterized by significantly lower input resistances \((P = 0.00012; t\text{-test})\), larger time constants \((P = 0.0122)\), more negative resting potentials \((P = 0.015)\), and, in particular, significantly more positive spike thresholds \((P < 0.0001)\).

A striking difference between iSC/cNOT- and iIO-projecting NOT neurons in vitro concerned the regularity of their ongoing firing. To analyze the temporal firing pattern, inter-spike intervals (ISIs) were calculated from the ongoing firing of spontaneously active cells and from activity of nonspontaneously active cells during maintained intracellular depolarization over a 10-s period. ISIs of NOT neurons that project to iSC and cNOT, the phasic iIO-projecting cells were characterized by significantly lower input resistances \((P = 0.00012; t\text{-test})\), larger time constants \((P = 0.0122)\), more negative resting potentials \((P = 0.015)\), and, in particular, significantly more positive spike thresholds \((P < 0.0001)\).

Comparisons of the soma sizes of labeled cells revealed that the average soma area of NOT neurons labeled from iSC was 227.4 \(\mu m^2\) (SD = 49.9), whereas that of cells labeled from iIO was 154.3 \(\mu m^2\) (SD = 40.2). Thus iO-projecting NOT cells had significantly smaller soma areas than those of iSC-projecting cells \((P < 0.001)\). In a comparison of soma shapes (Figs. 8B and 9) it appeared that iSC-projecting cells had spherical cell bodies whereas somata of iO-projecting cells were more elongated, particularly along the mediolateral axis.

**Fig. 6.** NOT neuron prelabeled with WGA-apo-HRP-gold (particle size 15 nm) after an injection into the ipsilateral inferior olive (iIO). A: gold particles are clustered in the soma and the primary dendrite (arrows). To confirm that the recording was from the prelabeled cell it was filled with biocytin from the patch pipette during whole cell patch-clamp recording (asterisk). B: in this cell, depolarization (5 mV above resting potential, *middle trace*) generated a phasic–tonic response with irregular action potentials. Increasing the membrane potential (30 mV above resting potential, *top trace*) leads to an increase of the number of the evoked action potentials; the firing pattern, however, remains irregular. C: action potentials generated by current injections that induce small depolarization steps at various holding potentials in another cell. Firing pattern is always irregular. D: firing rate as a function of membrane potential. Firing rate increases rapidly above spike threshold until maximum firing level. Further depolarization leads to reduced firing.
DISCUSSION

We examined the in vitro activity patterns of neurons in the NOT of the rat pretectal nuclear complex that were distinguished by differences in the destinations of their axons. Specifically, we examined NOT neurons that project to one or more of three structures: the iIO, the iSC, and the cNOT. These NOT cells could be distinguished by their responses to intracellular depolarization: one group responded with a transient fast-adapting pattern of activity or burst firing, whereas a second group responded tonically with only minor adaptation. The latter neurons were also spontaneously active when clamped at their resting potential, which was a unique feature of this population. These two response patterns were clearly associated with the connections of the cells; the neurons that projected to iIO responded transiently, whereas those that projected to iSC and cNOT were tonically active. This distinction between NOT cells that project to iSC and cNOT and those that project to iIO was confirmed by anatomical experiments. That is, injections of different retrograde axonal tracers into cNOT and iSC in single animals produced NOT cells with both labels, indicating that at least some NOT cells project to both structures. In contrast, after similar injections into either iIO and iSC or iIO and cNOT, individual NOT cells contained only one of the two labels, indicating that the cells that project to iIO are distinct from those that project to iSC and cNOT. These results demonstrate that NOT constitutes at least two cell populations and that these populations differ in both their firing properties and their patterns of connections.

NOT cell populations

Data in the literature on NOT anatomy and physiology obtained from a variety of mammalian species demonstrate that cell properties are very similar across different mammals (Gamlin 2005; Ibbotson and Dreher 2005; Simpson et al. 1988). Therefore it seems reasonable to generalize results between mammalian species.

Previous in vitro experiments in the rat demonstrated differences in the responses of NOT cells to intracellular depolarization (Prochnow and Schmidt 2004). One population of cells was characterized by tonic firing to intracellular depolarizations, an almost linear relationship between membrane potential and firing rate, and the generation of spontaneous activity. The activity pattern is characterized by a high degree of...
regularity leading to periodic peaks in activity autocorrelation. The association of these properties was confirmed in the present study. Although we did not specifically test for an autonomous generation of spontaneous activity, all cells that generated spontaneous activity at their resting membrane potential also responded with tonic firing to intracellular depolarizations. Furthermore, the spike frequency of the cells in this population was a linear function of the membrane potential and the spontaneous firing showed strong periodicity. Because these properties always appeared together in individual neurons, we conclude that they define a distinct population of NOT cells. The experiments with two retrograde tracers demonstrated that at least some of the cells in this population project to both iSC and cNOT. In contrast, NOT cells that project to iIO must be regarded as a separate NOT cell population, as can be derived from fundamental differences in their temporal activity pattern, which lacks any periodicity. This conclusion is consistent with earlier results from cat and rat showing that NOT cells that project to iIO send collaterals to the iNPH but not to the cNOT (Schmidt et al. 1995). Furthermore, NOT cells that project to iSC were previously characterized as being GABAergic (rat: Baldauf et al. 2003; Born and Schmidt 2004; rabbit: Nunes Cardozo et al. 1994). In contrast, NOT cells that project to iIO are not GABAergic (cat: Horn and Hoffmann 1987) and show immunoreactivity for glutamate (rat: Lewald et al. 1994).

A comparison of electrophysiological properties reveals that NOT cells that project to iSC and cNOT have higher input resistances, more positive resting potentials, and more negative spike thresholds than do cells that project to iIO. This constellation of properties is similar to the properties of spontaneously active NOT cells in an earlier study (Prochnow and Schmidt 2004), which also showed higher input resistance, more positive resting potentials, and lower spike thresholds than did NOT cells with phasic firing characteristics. Because the differences in firing were still present when the cells were pharmacologically isolated from synaptic input, by a substitution of calcium in the extracellular solution with cobalt (Prochnow and Schmidt 2004), they must result from differences in intrinsic physiological properties of the two cell populations.

Although both response properties and efferent connections distinguish between these two populations, other common criteria for differentiating between cell groups, such as differences in spatial distribution and morphology, do not appear to apply to NOT. That is, the cells labeled by transport from iIO, iSC, and cNOT were intermingled at similar topographical locations within the NOT. Furthermore, the morphology of the cells revealed by the biocytin label after recording did not reliably distinguish the two populations on a single-cell analysis. This result agrees with our earlier observation that spontaneously active NOT cells with tonic response patterns show dendritic morphologies similar to that of cells with phasic response characteristics (Prochnow and Schmidt 2004).

Although morphological comparisons of the biocytin-filled cells revealed no obvious differences, our results from the tracing experiments indicate that iIO-projecting cells on average have smaller somata than those of cells that project to iSC and cNOT. Even though this might seem contradictory to...
results from the biocytin-filled cells, the soma sizes of the recorded cells may be biased toward large cells because they are easier to detect and to patch-clamp than are small cells. Thus because there is overlap of the soma sizes between NOT cells that project to cNOT and iSC and cells that project to iIO, we probably have primarily recorded from large iIO-projecting cells. On average, NOT cells labeled from iSC and cNOT had soma sizes that are comparable to those of spontaneously active cells recorded from in a previous study (Prochnow and Schmidt 2004).

**NOT cells involved in the optokinetic reflex**

A priori, one might predict that the spontaneously active cells with tonic firing patterns are involved in the generation of slow compensatory eye movements during the horizontal optokinetic reflex. Indeed, in vivo the NOT cells that serve this function generate tonic responses to large moving visual stimuli as do functionally related cells in the adjacent dorsal terminal nucleus (DTN) of the accessory optic system (Cazin et al. 1980; for review, see Gamlin 2005; Ibbotson and Dreher 2005; Simpson et al. 1988). Because neurons in the left NOT–DTN generate eye movements only to the left and neurons in the right NOT–DTN generate movements only to the right (Cazin et al. 1980; Collewijn 1975b; Hoffmann and Schoppmann 1981; Mustari and Fuchs 1990; for review, see Gamlin 2005; Ibbotson and Dreher 2005), spontaneous activity may stabilize the eyes by maintaining an activity balance between the right and left NOT–DTN in the absence of appropriate visual stimuli. However, the NOT–DTN neurons involved in horizontal OKR generation project to the iIO (Gamlin 2005; Ibbotson and Dreher 2005; Simpson et al. 1988) and in our in vitro experiments none of the cells that projected to iIO was spontaneously active. Instead, all NOT neurons that projected to iIO showed phasic responses to intracellular depolarization and we assume similar responses are characteristic of DTN cells.

How might this in vitro response property be related to the known function of OKR–NOT neurons? In general, phasic responses allow rapid transmission of afferent spikes with high temporal precision. For the NOT cells that project to iIO, the main driving input arises from retinal ganglion cells (rat: Kato et al. 1992; rabbit: Pu and Amthor 1990; ferret: Klauer et al. 1990; cat: Hoffmann and Schoppmann 1981; Koontz et al. 1985; monkey: Perry and Cowey 1984). In cat, retinal afferents were classified as directionally selective ganglion cells that respond tonically to visual stimuli that move slowly across the visual field (Hoffmann and Stone 1985). Because the directionally selective ganglion cells are tuned to stimulus velocity (rabbit: Oyster 1968; Oyster et al. 1972) changing the firing rate at the retinal ganglion cell-to-NOT cell relay might confound the information necessary to achieve the required eye velocity. That is, a temporally precise transfer of changes in spike frequency might be better achieved by NOT cells that have phasic response properties than by spontaneously active cells that add self-generated spikes to the firing patterns generated by the retinal input.

**NOT cells that project to cNOT and iSC**

To our surprise, NOT cells that project to the cNOT and the iSC were tonicaily active in vitro. We expected these neurons would respond phasically because the only NOT neurons that are characterized by tonic responses in vivo are the OKR-related directionally selective NOT cells that project to the iIO and iNPH (Gamlin 2005; Ibbotson and Dreher 2005). In anesthetized animals, many NOT neurons respond to saccadic eye movements or shifts of the retinal image at saccadic velocities with short, high-frequency bursts (for review, see Gamlin 2005; Ibbotson and Dreher 2005). Previous studies demonstrated that neurons with these phasic responses project to either the ipsilateral dorsal lateral geniculate nucleus or to the ipsilateral extrageniculate visual thalamic nuclei (Schmidt 1996; Sudkamp and Schmidt 1995). The response properties of the NOT neurons that project to iSC and/or cNOT have not been characterized in vivo. Based on results in marsupials, it was previously argued that the NOT cells that project to cNOT contribute to the binocularity of the directionally selective NOT cells that project to IO (Ibbotson et al. 2002; Pereira et al. 1995). Because the responses of these cells to monocular stimulation of the ipsilateral eye are tonic, the expectation would be that the commissural NOT cells are also tonicaily active. In higher mammals, the binocularity of directionally selective NOT cells seems to depend on cortical input (ferret: Sengpiel et al. 1990; cat: Distler and Hoffmann 1993; monkey: Hoffmann et al. 1992), suggesting that the commissural NOT cells have a function different from conveying input from the ipsilateral eye. Whether cortical input is also responsible for NOT cell binocularity in lower mammals (rat: Schmidt et al. 1993; guinea pig: Lui et al. 1994) remains to be confirmed.
Without more information concerning the in vivo response properties of the NOT cells that project to cNOT and iSC, we can only speculate about their functions. The evidence that NOT is not retinotopically organized (Hoffmann and Schoppmann 1981) suggests that its influence is modulatory. Tonic activity of NOT neurons in vivo has been reported not only for OKR-related cells, but also for a population of NOT neurons in monkey that are effectively suppressed by saccadic eye movements in a nondirectionally selective fashion (Mustari et al. 1997). These neurons, termed pretectal omnidirectional pause neurons, could represent the spontaneously active cells reported here. We also know that SC-projecting NOT neurons, as many others, are GABAergic and that they project almost exclusively to non-GABAergic projection neurons in the superficial, visuosenory layers of the superior colliculus (Baldauf et al. 2003; Born and Schmidt 2004; Nunes Cardozo et al. 1994). If iSC-projecting NOT cells are the pretectal pause neurons, they could provide a tonic inhibitory input to collicular cells that might help to suppress the execution of unwanted saccades. Alternatively, iSC-projecting neurons could have properties in vivo different from those of the pause neurons and might be activated by fast image movements, as many NOT cells are. Then, these GABAergic NOT cells could provide inhibition to collicular cells that increases during saccades and reduces the likelihood that the saccade-induced shifts in the visual field trigger subsequent unwanted eye movements. In contrast, the GABAergic projection from NOT to dorsal lateral geniculate nucleus terminates exclusively on GABAergic interneurons and disinhibits relay cells in the dorsal lateral geniculate nucleus during saccades (Cucchiaro et al. 1991, 1993; Fischer et al. 1998; Wang et al. 2002). Thus NOT may contribute both to preventing unwanted saccades by attenuating visuomotor activity in the superior colliculus during saccades (Richmond and Wurtz 1980) and to maintaining the relay of visuosenory signals from the thalamus to the cortex during and immediately after a saccade (Büttner and Fuchs 1973; Judge et al. 1980).

In conclusion, the organization of NOT has been a puzzle. This small and, by usual morphological criteria, homogeneous nucleus has been associated with several apparently diverse functions ranging from modulation of the activity of visual thalamic relay cells during saccades to inhibition of the visual layers of the SC and, through its pathway to iO and iNPH, to mediation of the OKR. The question addressed by the present experiments is whether NOT is one nucleus that makes a single contribution to all of these diverse structures and functions or whether, in contrast, it constitutes several distinct nuclei that overlap spatially but differ in terms of their connections, physiology, and functions. The results demonstrate that NOT consists of several cell types that are anatomically and physiologically distinct. Tonic spontaneous activity is propagated by NOT neurons in a branced pathway to the iSC and the cNOT, whereas the NOT–DTN neuronal population that projects to the iO is characterized by phasic response properties in vitro.

REMARKS

This work was supported by an International Graduate School for Neuroscience (Ruhr-Universität Bochum) grant to N. Prochnow, Deutsche Forschungsgemeinschaft Grant SFB 509 (“Neurovision,” TP A8) to M. Schmidt, and National Eye Institute Grant EY-08233 to W. C. Hall.

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