Dynamics of abducens nucleus neurons in the awake mouse

John S. Stahl1,2 and Zachary C. Thumser1
1Neurology Division, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio; and 2Department of Neurology, Case Western Reserve University, Cleveland, Ohio

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Stahl JS, Thumser ZC. Dynamics of abducens nucleus neurons in the awake mouse. J Neurophysiol 108: 2509–2523, 2012. First published August 15, 2012; doi:10.1152/jn.00249.2012.—The mechanics of the eyeball and orbital tissues (the “ocular motor plant”) are a fundamental determinant of ocular motor signal processing. The mouse is used increasingly in ocular motor physiology, but little is known about its plant mechanics. One way to characterize the mechanics is to determine relationships between extraocular motoneuron firing and eye movement. We recorded abducens nucleus neurons in mice executing compensatory eye movements during 0.1- to 1.6-Hz oscillation in the light. We analyzed firing rates to extract eye position and eye velocity sensitivities, from which we determined time constants of a viscoelastic model of the plant. The majority of abducens neurons were already active with the eye in its central rest position, with only 6% recruited at more abducted positions. Firing rates exhibited largely linear relationships to eye movement, although there was a nonlinearity consisting of increasing modulation in proportion to eye movement as eye amplitudes became small (due to reduced stimulus amplitude or reduced alertness). Eye position and velocity sensitivities changed with stimulus frequency as expected for an ocular motor plant dominated by cascaded viscoelasticities. Transfer function poles lay at approximately 0.1 and 0.9 s. Compared with previously studied animal species, the mouse plant is stiffer than the rabbit but laxer than cat and rhesus. Differences between mouse and rabbit can be explained by scaling for eye size (allometry). Differences between the mouse and cat or rhesus can be explained by differing ocular motor repertoires of animals with and without a fovea or area centralis.

To execute an accurate movement, the brain must shape its outgoing commands so as to account for the mechanical properties of the controlled limb(s). As such, one approach to studying brain function begins with determining the mechanical properties of limbs, thereby specifying the brain’s computational goals. The neurophysiologist’s task is then to determine how the brain accomplishes the specified computations. Nowhere has this paradigm been so influential as in the ocular motor system, where for several decades the application of engineering concepts of control systems analysis has been a major factor in shaping the neurophysiological investigations of that system (Robinson 1964, 1981b). The approach is facilitated in the ocular motor system because the mechanical properties of the eyeball, extraocular muscles, and nonmuscular orbital tissues (an assemblage that could be considered a “limb of one joint”) are in many respects simpler than the mechanical properties of multijointed arms and legs. The mechanical properties of the eye (or in control systems terminology, the “ocular motor plant”) can be studied directly, for instance, by determining the motion of the eye after it is released from an eccentric position (Sklavos et al. 2005) or in response to electrical stimulation of the motoneurons (Anderson et al. 2009), or by determining length-tension or velocity-tension relationships of eye muscles (Barmack 1976; Goldberg et al. 1998; Quaia et al. 2009; Robinson 1964). A complementary approach has been to determine the firing rate properties of extraocular motoneurons with respect to eye position during eye movements having various temporal characteristics, including maintained fixations, slow sinusoidal oscillations, and saccades. This alternative approach has generated a considerable body of literature regarding the signaling relationships between extraocular motor nucleus firing and eye movement in multiple mammalian species, including the rhesus, cat, and rabbit (Davis-Lopez de Carrizosa et al. 2011; Delgado-Garcia et al. 1986a; Fuchs et al. 1988; Goldstein and Robinson 1986; Pastor and Gonzalez-Forero 2003; Robinson 1970; Robinson and Keller 1972; Stahl and Simpson 1995; Sylvestre and Cullen 1999).

Over the past decade the laboratory mouse has become increasingly popular for use in ocular motor studies, due largely to the ease with which genetic tools can be used in this species to explore ocular motor function through manipulations of the biochemistry, physiology, biophysics, or anatomy of the system’s neural and muscular components (Stahl 2008). Although several studies have already examined contractile properties of mouse extraocular muscles (McMullen et al. 2004; Yin et al. 2005; Zhou et al. 2009), other aspects of the ocular motor plant mechanics have yet to be explored, in particular the transformation between motoneuron firing and eye movement. A description of this relationship is an important precursor to exploring signal processing within the murine vestibular, optokinetic, and ocular motor circuits as discussed above. Additionally, descriptions of mouse ocular motor mechanics may ultimately lead to an understanding of how the mechanics are defined. The mouse eye is far smaller than those of the other mammals whose ocular motor mechanics have been studied before (i.e., human, rhesus, cat, and rabbit), and juxtaposition of the mouse mechanics with those of larger species should provide insight into how scaling affects mechanics.

We performed extracellular recordings of neurons within the mouse abducens nucleus and simultaneous eye movement recordings during yaw sinusoidal rotations in the light, and we used these data to determine the time constants for a classic model of the ocular motor plant in which the mechanics are dominated by cascaded viscoelastic elements. We have demonstrated that the mouse plant is “faster” than the larger plant of the rabbit but still slower than those of the cat and rhesus, both foveate species whose more sophisticated ocular motor...
METHODS

Animals and animal preparation. Experimental use of mice was approved by the Institutional Animal Care and Use Committee at Case Western Reserve University and conformed to the National Institutes of Health guidelines for the use and care of vertebrate animals. C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). Data were drawn from experiments in 9 mice, aged 2–12 mo at the time of the experiments.

Animals were prepared for recording by surgical implantation of an acrylic head-fixation pedestal as previously described (Stahl et al. 2000; 2006b). To improve the consistency of head orientation and thus facilitate locating the abducens nucleus, the pedestal construction was performed in a stereotactic frame in such a manner that when fixed in the recording apparatus, the pedestal held the animal’s lambda-bregma axis at a pitch-down angle of 18°, with 0° roll. A craniotomy was made through the interparietal bone and surrounded by a chamber built up from dental acrylic and fused to the pedestal. A permanent mark was molded in the interior of the chamber to act as a reference for subsequent neuronal recordings; all electrode tracks could thus be placed on a grid map referenced to this fiduciary mark. The dura was removed. Between recording sessions the craniotomy was closed by an application of melted bone wax.

Recording apparatus. During recordings mice were mounted on a servo-controlled turntable with the head pedestal fixed to a rigid armature and the body loosely confined in a plastic tube. The turntable was enclosed in the cylinder of an optokinetic drum, which remained stationary for these experiments and whose interior was painted with a high-contrast pattern. Eye movement recording was accomplished using pupil-tracking video oculography under infrared illumination as previously described (Stahl et al. 2000). Video sampling rate was 120 Hz. Video oculography was employed rather than magnetic search coil oculography (commonly considered the “gold standard” of oculography in larger animals) because it obviates the need to instrument the orbit. The presence of the coil has been demonstrated to alter significantly the eye movements in mice, potentially indicating an effect on orbital mechanics (Stahl et al. 2000). Thus a noninvasive recording technique was vital for experiments aimed at quantifying orbital mechanics. The horizontal and vertical positions of the pupil, horizontal and vertical positions of a reference corneal reflection, and pupil diameter were output from the oculography system as analog signals. These signals, along with turntable position, were passed through four-pole Bessel low-pass filters (corner frequency 200 Hz) and then stored to a computer via a digital acquisition system (System 3; Tucker-Davis Technologies, Gainesville, FL) at a rate of 500 samples/s.

Extracellular single-unit recordings were accomplished using 80-μm quartz-insulated platinum-tungsten fiber electrodes (Thomas Recording, Giessen, Germany). The electrode was held in a hydraulic manipulator (FHC, Bowdoin, ME), which in turn was angled and positioned using a miniature x-y manipulator mounted to the animal support. Neuronal signals were conventionally amplified and stored along with the eye and turntable position data.

Recording procedure and stimuli. Before each recording session, animals were treated with an ophthalmic solution of 0.5% physostigmine salicylate to limit pupil dilation. The mouse abducens nucleus is a 200- to 250-μm sphere, containing fewer than 200 neurons (Shaw and Baker 1986). This small nucleus was located in the initial recording session(s) by using a combination of stereotactic coordinates and trial and error. In practice, the abducens nucleus was easily recognized by several attributes: 1) a spatially restricted, dense collection of burst-tonic neurons excited during abduction of the ipsilateral eye; 2) depth more than ~300 μm below the floor of the fourth ventricle; 3) adjacency to the genu of the facial nerve, which itself was easily identified by the ability to evoke twitches of facial muscles by single shocks through the recording electrode; and 4) abducting eye movements evoked by modest electrical stimuli (e.g., 100 Hz, 8 pulses/train, 1.5 V) delivered through the recording electrode. Once located on our grid map, the abducens nucleus was easily returned to in successive recording sessions. Note that the anatomy of the mouse is such that there is little potential to include in the sample any neurons that are not part of the abducens nucleus. Neurons of the nucleus prepositus hypoglossi (which overlies the abducens nucleus in the mouse) were rarely isolated using our electrodes and in any case would be recognized as such by their shallow depth within the brain stem. On all other sides, the abducens nucleus is bordered by regions that lack either units excited by abducting eye movements or neuronal somas of any kind. Figure 1 shows some of the typical features of abducens nucleus neurons, including the extracellular waveform (Fig. 1A), robust sinusoidal modulation with peak and trough firing rates somewhat leading, respectively, the maximal abducted and adducted eye positions (Fig. 1B), bursting and pausing activity associated with saccades (Fig. 1C), and the generation of a predominantly abducting eye movement when electrical stimuli are delivered through the recording electrode where the neuron was recorded (Fig. 1D).

Once a neuron was isolated, samples of eye movement and neuronal activity were recorded during sinusoidal rotation in the light at 0.2 Hz, ±4.9° amplitude (the “basic” stimulus), followed by 0.1 Hz, ±5.0°; 0.4 Hz, ±4.8°; 0.8 Hz, ±3.6°; and 1.6 Hz, ±2.2° (which together with the basic stimulus made up our “Bode plot series”). Total harmonic distortion of the head rotational position was minimal, averaging 0.01, 0.01, 0.02, 0.04, and 0.04% at 0.1, 0.2, 0.4, 0.8, and 1.6 Hz, respectively. Sample length at the five stimulus frequencies was 50, 40, 25, 25, and 25 s. If the neuron remained isolated, a further “linearity series” was obtained at 0.2 Hz, ±2.0°, ±4.9°, and ±10° amplitudes. For cells that remained isolated, we obtained additional samples of the responses to the various frequencies and amplitudes of the earlier series. Note that on rare occasions we encountered abducens neurons that were wholly or largely silent during slow eye movements (i.e., they might fire only during saccades, or only a few spikes per stimulus cycle, or only during brief periods if the eye oscillation was occurring about an unusually abducted average position). Such cells could not be studied using our techniques and were excluded from sampling. (Although in theory such cells could be studied by first rotating the animal to drive eye position into the “on” range of the cell and then conducting further oscillations about this eccentric position, in practice mice will not maintain such offset eye positions once they are oscillated.)

Data analysis. The neuronal recording channel was processed to discriminate spikes using a commercial cluster analysis program (Offline Spike Sorter; Plexon, Dallas, TX). The resultant trains of spike times were convolved with a unitary Gaussian kernel (SD = 20 ms, width = ±100 ms) to generate firing rate frequency as a function of time, which was then resampled at an effective 500 samples/s to match the sample rate of the eye and turntable position signals. The raw pupil, corneal reflection, and pupil diameter signals were processed to determine horizontal eye position in degrees, using a trigonometric method previously detailed (Stahl 2002, 2004; Stahl et al. 2000), programmed in MATLAB (The MathWorks, Natick, MA). This position signal was then numerically smoothed with MATLAB’s “filtfilt” function (a zero-phase, forward and reverse digital filter, cutoff frequency 40 Hz) and differentiated using the five-point stencil algorithm to generate eye velocity.

The relationship between firing rate and eye movement was quantified using a strategy first applied to analyze abducens nucleus dynamics in the rabbit (Stahl and Simpson 1995). In this approach, firing rate is regressed on eye position and eye velocity to yield the “dynamic” (or “apparent”) eye position sensitivity (k) and dynamic eye velocity sensitivity (r). If the transformation from abducens
neuron firing rate to eye position (henceforth noted as VI→E) could be modeled by a one-pole (1P) Laplace transfer function (i.e., if the orbital mechanics could be modeled by a single elastic element in parallel with a single viscous element), then r and k would be constants and directly proportional, respectively, to the orbit’s coefficients of viscosity and elasticity. In fact, a 1P transfer function has been demonstrated in multiple species to be an insufficient model of the orbital plant, even for modeling slow sinusoidal eye movements (Fuchs et al. 1988; Stahl and Simpson 1995); for a more complex but still linear plant, r and k depend on stimulus frequency. Fortunately, provided that both the eye movement and firing rate are at least grossly sinusoidal, an equation relating the dynamic r or k to stimulus frequency can be developed for any given transfer function, and curve fitting of this equation to the dynamic r or k values can be employed to extract the parameters of any posited linear VI→E transfer function. The analytical approach amounts to the classical procedure of determining time constants from a phase vs. frequency plot, with the advantage that the method can be used on data in which the cycles of firing rate or eye movement are incomplete (for instance, due to saccades, losses of eye movement track, or silencing of the neuron).

Before using regression to obtain r and k, we preprocessed the data to exclude from analysis any portions of the record in which the eye track or neuronal isolation was lost. We also excluded any periods during which the neuron was silenced, since the linear regression equation does not accommodate such a nonlinear property as cutoff. In practice, cutoff was defined as any firing rate below 20 spikes/s. Firing rates below this level rarely fit the regression equation well, in large part because of the use of Gaussian kernels to generate neuronal firing rates generates an artifactual nonzero firing rate at the borders of each silent period, related to the tails of the Gaussian kernels. Another factor causing the poor fit in these peri-cutoff regions, however, may be the observation in rhesus that aducens neurons tended to shift abruptly from silence to 10–20 spikes/s, i.e., firing rates below 10–20 spikes/s (but greater than 0 spikes/s) were rarely observed (Fuchs et al. 1988), in violation of what would be expected from a purely linear transfer function. The regression process and the interpretation of the r and k values also assumes that the behavior is at steady state, describing a sinusoidal profile at a specific frequency. Thus saccades and their immediate aftermath, which violate these assumptions, were also deleted. (One consideration in the original selection of the stimulus amplitudes was to minimize the generation of saccades.)

Because animals were subject to variations in alertness, which would affect the VI→E relationship due in part to the amplitude nonlinearity property (see below), we also excluded any periods in the record in which eye gain fell below an arbitrary threshold of 0.25. We then averaged the remaining cycles that were free of deletions. In some instances, the various exclusions left us without any uninterrupted cycles to average. In this case, the subsequent treatment was guided by the nature of the interruptions. If the interruptions were saccades, then the record was analyzed without averaging, because any step changes in eye position incorporated in the averaged cycle would violate both the assumption of steady state and the requirement that the eye position be varying sinusoidally. If the interruptions consisted of neuronal silencing or brief losses of the eye track or neuronal isolation, then we averaged the record but subsequently excluded from analysis any points in the averaged cycle that incorporated points in the original cycles that would ordinarily have required exclusion. Note that averaging is very desirable because it reduces the noise in the independent variables (E, E’) and so increases the values of the regression coefficients (Levi 1973). Since eye velocity is inherently noisier than eye position, this effect would have the potential to depress r more than k, depressing the ratio r/k and altering the measured time constants. It also decreases the noise in the dependent variable (firing rate) and as such increases the coefficient of determination and makes more readily obvious, to graphical inspection, any systematic deviations of the regression fit from the empirical data. Averaging at each of the stimulus frequencies 0.1, 0.2, 0.4, 0.8, and 1.6 Hz was possible in, respectively, 87, 94, 91, 98, and 96% of the time. The lower rate of averaging at the lowest stimulus frequency reflects the fact that saccades were more common at the larger stimulus amplitudes employed for low stimulus frequencies, and also because it was more likely that interruptions of any type would occur as cycle period increased.

The firing rate (F) as a function of time was regressed against eye position (E) and velocity (E’) according to the firing rate equation

$$F(t) = rE(t) + kE(t) + c.$$  \hspace{1cm} (1)

In cases where analysis was performed on the nonaveraged data, the regression equation was augmented to

$$F(t) = rE(t) + kE(t) + c + m_1t + m_2t^2.$$  \hspace{1cm} (2)

The $m_1t$ and $m_2t^2$ are “dummy” terms intended to absorb variance related to slow drifts of eye position or firing rate, neither of which
relate to the stimulus frequency and thus are unlikely to be fit by the $E$, $E'$ terms alone (Stahl and Simpson 1995). The dummy terms were not used for averaged data because their quadratic form would have some multicolinearity with the form of a single-cycle sinusoid, which would cause the dummy terms to interact with the $E$ and $E'$ variables and artificially reduce the values of $r$ and $k$. Values of $r$ and $k$ (as well as the derived values $r/k$, phase, and magnitude sensitivity described below) were averaged across samples to yield a single value for the neuron for each stimulus and then averaged across cells to yield single values for the collection of cells.

From the $r$ and $k$ values for each sample, we calculated the derived quantity $r/k$. We also calculated firing rate phase with respect to eye position ($P$) and magnitude sensitivity ($z$) according to the following equations (Stahl and Simpson 1995):

$$P = \arctan(\omega r/k)$$

$$z = \sqrt{k^2 + (\omega r)^2},$$

where $\omega$ is stimulus frequency in rad/s. Magnitude sensitivity (in spikes s$^{-1}$ deg$^{-1}$) provides a measure of the depth of modulation of the neuron normalized to the amplitude of the eye movement, unrelated to the phase of firing with respect to the eye. It thus summarizes the depth of modulation as a single number, which is often more convenient than considering the $r$ and $k$ values individually. The curve of average $r/k$ vs. frequency was fit by a nonlinear least-squares process (using the MATLAB “cftool” function with the “Trust Region” optimization algorithm and resultant $r^2$ values adjusted for degrees of freedom) with the equation predicting $r/k$ variation with frequency for a two-pole, one-zero (2P-1Z) plant:

$$r/k = \frac{T_1 + T_2 - T_Z + \omega^2 T_1 T_Z}{1 + \omega^2 (T_1 + T_Z - T_1 T_Z - T_2)},$$

where $T_1$ and $T_2$ are the time constants of the poles, and $T_Z$ is the time constant of the zero. Analogous equations exist for $r$, $k$, magnitude sensitivity, and phase. However, determining the time constants from $r/k$ is preferable because 1) the ratio is less affected by the amplitude nonlinearity than are $r$, $k$, and magnitude sensitivity (see RESULTS); 2) there is less neuron-to-neuron variability of $r/k$ than any of our other indexes of activity (compare $A$ with the other panels in Fig. 6); 3) whereas $r$ and $k$ have been reported to differ between motoneurons (MN) and abducens internuclear neurons (INT), no significant differences have been demonstrated for their ratio; 4) whereas variation of phase with frequency is only partially related to the use of a 1P model to quantify a higher order plant, all of the variation of $r/k$ with frequency relates to this “mismatch”; and 5) possibly as a consequence of reason 4, fits to the $r/k$ vs. frequency curve converge more robustly and generate time constants with narrower confidence intervals than do fits to phase vs. frequency. The parameters were seeded with the following initial values: $T_1 = 0.001$ s, $T_2 = 10$ s, and $T_Z = 2$ s.

Gain ($G$) and phase ($P$) of the eye movement were calculated from the same data used for neuronal sensitivity analysis by first regressing eye position against head position ($H$) and head velocity ($H'$) according to the model equation:

$$E(t) = aH(t) + bH'(t) + c.$$  

$G$ and $P$ were then calculated from $a$, $b$, and stimulus frequency $\omega$ according to

$$G = \sqrt{(a\omega)^2 + b^2}$$

$$P = \arctan (a\omega/b).$$

This approach had the advantage over Fourier analysis of eye movement gain (used, for example, in Stahl et al. 2000) in that it could be used on incomplete cycles of stimulus and response, and thus could be applied to the identical data selected for determining $r$ and $k$.

Distributions of parameters were generated using the kernel method of probability density estimation (Silverman 1986) with Gaussian kernels (SD $= 2^\circ$, width $= 20^\circ$); parameters were selected empirically to produce the best possible resolution without generating excessive ripple due to the overvisibility of the individual kernels.

All averaged values are means $\pm$ SD except as noted. The coefficient of determination from regression is represented as $r^2$ and should be distinguished from the velocity sensitivity ($r$). Correlation analyses were performed using the MATLAB “corr” function, which computes a Pearson’s linear correlation coefficient ($r$, which we subsequently squared) with a two-tailed $P$ value representing the probability of obtaining the $r$ value (or better) if a relationship between the variables were actually lacking.

RESULTS

We obtained data during 0.2 Hz, $\pm 4.9^\circ$ oscillation (our basic stimulus) from 127 abducens nucleus neurons. An additional six units supplied data at other frequencies, but the data at 0.2 Hz were not usable due to problems with isolation, eye movement tracking, or animal behavior. For 53 units we obtained data over the complete frequency range of 0.1–1.6 Hz, and for 16 units we obtained the data required to assess linearity at 0.2 Hz. Eye movements made in response to the sinusoidal rotation were themselves strongly sinusoidal, as expected for the vision-augmented vestibuloocular reflex and supporting the use of our analysis method (see METHODS). Total harmonic distortion of the eye position signals measured 1.19 $\pm$ 1.36, 0.90 $\pm$ 1.31, 0.66 $\pm$ 1.31, 0.36 $\pm$ 0.50, and 0.35 $\pm$ 0.10% at 0.1, 0.2, 0.4, 0.8, and 1.6 Hz, respectively. The firing rate data were fit well by the linear regression model. Predictably, the coefficient of determination ($r^2$) was considerably higher when the regression was carried out on averaged data. For instance, at 0.4 Hz, where we were able to average the data in 91% of samples, the $r^2$ value averaged 0.93 $\pm$ 0.08 in $n = 119$ samples, compared with 0.79 $\pm$ 0.14 for the regression analysis of the nonaveraged version of those same samples. Because of the advantages of averaging, we used the $r$ and $k$ values from the averaged analysis wherever possible. The $r^2$ values at each stimulus frequency (with each cell contributing a single value averaged from one or more samples at the given frequency) averaged 0.79 $\pm$ 0.13 ($n = 67$), 0.87 $\pm$ 0.14 ($n = 127$), 0.90 $\pm$ 0.10 ($n = 68$), 0.94 $\pm$ 0.07 ($n = 93$), and 0.94 $\pm$ 0.12 ($n = 59$) at 0.1, 0.2, 0.4, 0.8, and 1.6 Hz, respectively. Inspection of the regression residuals did not reveal any systematic patterning of the residuals that was consistent across multiple neurons.

At 0.2 Hz, the average values of the measured indexes for the 127 neurons were $r = 2.77 \pm 1.88$ spikes/deg, $k = 7.95 \pm 3.56$ spikes s$^{-1}$ deg$^{-1}$, $c = 101.04 \pm 63.13$ spikes/s, $r/k = 0.35 \pm 0.14$ s, phase $= 22.76 \pm 7.83$ deg, and magnitude sensitivity $= 8.77 \pm 4.11$ spikes s$^{-1}$ deg$^{-1}$. Distributions of each index were examined (plots not shown); $r$, $k$, $r/k$, magnitude sensitivity, and firing rate phase with respect to eye position exhibited some positive skewing, whereas $c$ was more nearly symmetrical.

Recruitment properties. Multiple studies of extraocular motor nuclei have demonstrated that one can assign to each MN a recruitment eye angle $E_T$ at which the neuron first begins to fire as the eye deviates progressively in the pulling direction of the MN’s muscle (Delgado-Garcia et al. 1986a; Fuchs and Luschei 1970; Robinson 1970; Robinson and Keller 1972; Schiller 1970). Generally, $E_T$ values are determined during
steadily maintained eye positions, since during motion the eye velocity component of the firing rate would influence whether or not the neuron is firing at any given eye position. The current experiments were restricted to assessing firing rate modulation during continuous sinusoidal rotation. However, the ratio \( c/k \) obtained from the linear regression on the response to 0.2-Hz oscillation provides a substitute for \( E_T \) because the procedure for setting up the video oculography produces a facsimile of an absolute eye position, where \( E = 0 \) is defined as the animal’s horizontal rest position (Stahl et al. 2000). With eye positions all referenced to the rest position, one can pool the “c” data across sessions and/or animals. If one rearranges our firing rate Eq. 1 and compares it with an equivalent firing rate equation,

\[
F(t) = k[E(t) - E_T] + rE'(t),
\]

then \( E_T = -c/k \).

Figure 2 plots the distribution of \( c/k \), where \( c \) and \( k \) were determined at 0.2 Hz. The distribution has a bell-shaped form, which is also the case in other species for which the \( E_T \) of abducens nucleus neurons has been reported, including rhesus (Robinson and Keller 1972) and cat (Delgado-Garcia et al. 1986a). The mouse distribution extends past the rest position (\( c/k = 0 \)) to negative values, and examination of the area under the curve indicates that at the rest position, 6% (8/127 units) of the population has yet to be recruited. This is considerably lower than the 22% of the MN that are recruited on the abduction side of straight-ahead position in the monkey (extracted from Fig. 5 of Fuchs et al. 1988). On the other hand, the 50% recruitment position (the point that divides the distribution in Fig. 2 in half) is 13.8°, which is closer to the 50% recruitment position of 10° in the rhesus (again, extracted from Fig. 5 of Fuchs et al. 1988). In the cat, 14% of abducens MN are recruited on the abduction side of straight-ahead (Pastor and Gonzalez-Forero 2003). It should be noted that the distribution in our Fig. 2 underestimates the true extent of the distribution to the left of \( E = 0 \), since cells recruited far into the “on” direction of the muscle would be largely silent during slow eye movements about the \( E = 0 \) position and thus would have been omitted from our sample, as described in METHODS. In contrast, in experiments in trained monkeys, the animal can be made to hold far abducted eye positions, making it practical to study cells recruited far to the abducted side of \( E = 0 \) and, by their inclusion, shifting the \( E_T \) distribution in the direction of abduction.

In rhesus and cat abducens MN, \( k \) and in some studies \( r \) have been found to correlate with \( E_T \) (Delgado-Garcia et al. 1986a; Fuchs et al. 1988; Pastor and Gonzalez-Forero 2003; Sylvestre and Cullen 1999). Figure 3 plots our \( k \), \( r \), and \( r/k \) vs. \( c/k \) (our surrogate for \( E_T \)). Both \( k \) vs. \( E_T \) and \( r \) vs. \( E_T \) exhibit a weak but statistically significant correlation (\( k: r^2 = 0.077, P = 0.0016; r: r^2 = 0.038, P = 0.028 \)). Although weak, the correlations are of the same sense as found in other species, and it should be noted that inspection of the comparable plots for \( k \) in the cat (Fig. 14 in Delgado-Garcia et al. 1986a, Fig. 2 in Pastor and Gonzalez-Forero 2003) and in one study of rhesus (Fig. 11 in Sylvestre and Cullen 1999) suggests that even in cat and rhesus, the relationship can be very noisy. Moreover, our correlations with \( c/k \) are expected to be weaker than correlations with conventionally determined \( E_T \) because our setting \( E = 0 \) to the somewhat ill-defined “rest” position of the mouse

\[
f_{\text{PDF}}(c/k) = \frac{1}{\sqrt{-2\pi c/k}} e^{-\frac{E_T^2}{2(c/k)}}
\]

Fig. 2. Distribution of ratio \( c/k \), which approximates the recruitment eye position \( E_T \). Positive values of \( c/k \) denote direction of eye adduction. Dashed line at 13.8° indicates the eye position at which one-half of the neurons in the sample were recruited.

Fig. 3. Relationship of dynamic eye velocity sensitivity (\( r \), dynamic eye position sensitivity (\( k \), and \( c/k \) (all determined at 0.2 Hz) to recruitment angle, as determined by the ratio \( c/k \). Linear regression fits are superimposed on each plot.

\[1\] The relevant aspect of the setup procedure is where we adjust the angle of the camera/reference emitter assembly to align the reference corneal reflection with the average rest position of the pupil. Because mice have fairly reproducible rest positions, this procedure assures that the definition of \( E = 0 \) is at least roughly stable from one experimental session to another. Note that a more accurate procedure is possible in which horizontal eye position is referenced instead to the animal’s rostrocaudal axis, yielding eye position with respect to that axis (Oommen and Stahl 2008), but we had not yet developed this procedure at the time these data were collected.
eye is less reliable than defining $E = 0$ as a monkey fixates a target at the geometrical straight-ahead position.

**Assessment of linearity.** The fact that the firing rate modulation was modeled well as a function of the sinusoidal eye position and eye velocity indicates that the firing rate modulation was also sinusoidal, which in turn indicates that the $VI \rightarrow E$ transfer function is at least grossly linear. We tested how well $VI \rightarrow E$ obeyed the scaling (homogeneity) property of linear systems by determining the neuronal response at a single stimulus frequency and three different stimulus amplitudes. Figure 4 plots average magnitude sensitivity and neuronal phase vs. stimulus amplitude for the 16 neurons for which the data are available. Magnitude sensitivity declined monotonically with stimulus amplitude in 14 of the neurons, whereas the phase relationship was more irregular and generally less affected by stimulus amplitude. The effects of stimulus amplitude on all of the measured parameters were computed as the difference between the values of a given parameter for $10^\circ$ and $2^\circ$ stimulus amplitudes, expressed as a percentage value of the parameter averaged across the three stimulus amplitudes (Stahl and Simpson 1995). The resultant percentages were $P = 9\%$, $z = 67\%$, $r = 61\%$, $k = 67\%$, and $r/k = 12\%$. A similar pattern of increasing magnitude sensitivity with decreasing stimulus amplitude (as well as the lesser effects of stimulus amplitude on phase and $r/k$) was demonstrated for the rabbit (Stahl and Simpson 1995) and termed the “amplitude nonlinearity.”

This phenomenon of magnitude sensitivity increasing as response declines is treated further in Fig. 5, in which we plot magnitude sensitivity and $r/k$ vs. eye movement amplitude for all samples of the response to $0.2 \text{ Hz, } \pm4.9^\circ$ amplitude. The origins of the gain variation exhibited in this plot may include variations in state of alertness or animal-to-animal variation in average gain, although the animal-to-animal variations may themselves be driven, in part, by individual variation in the propensity to maintain alertness. Note also that the plot does not include any data obtained during periods of frank nonalertness, defined in this study as periods where eye gain fell below 0.25. To compare these data with the linearity assessment shown in Fig. 4, we calculated from those linearity data the average eye amplitude and associated average magnitude sensitivity (or $r/k$) at each of the three stimulus amplitudes and superimposed these three-point curves on the scatter plots. The scatter plot for magnitude sensitivity (Fig. 4, top) demonstrates that as eye gain declines, firing rate modulation fails to decline at the same rate. Comparison of these $0.2$-Hz data with the curve from the linearity series suggests that some part of the effect of performance gain on magnitude sensitivity derives from a change in the relationship between magnitude sensitivity and eye amplitude (Fig. 5). Declining eye gain is associated with higher magnitude sensitivity and is at least partly explained by the amplitude nonlinearity function defined in Fig. 4. In contrast, $r/k$ was independent of eye amplitude.
from the amplitude nonlinearity function. However, the apparent greater steepness of the data cloud suggests that a subtle decline in alertness could have an additional effect. Figure 4, bottom, shows that in contrast to the situation with magnitude sensitivity, \( \frac{r}{k} \) (from which the time constants are extracted, see below) is unaffected by performance gain.

Variation of parameters with stimulus frequency and estimation of time constants. Data were obtained at all 5 stimulus frequencies in 53 neurons. Plots of \( k \), \( r \), \( \frac{r}{k} \), phase, and magnitude sensitivity vs. stimulus frequency for each of those neurons (fine lines), along with the average of the curves (superimposed heavy lines), are shown in Fig. 6. We could also generate averaged curves using all 133 neurons, i.e., by including in the averages at each stimulus frequency the data from cells for which we failed to obtain a complete Bode plot. Since the averaged curves from the 53-neuron and 133-neuron data sets superimposed almost perfectly (plot not shown), we used the larger data set in time constant analyses (see below) to take advantage of its tighter confidence intervals.

Table 1 summarizes the dynamic measures obtained at all stimulus frequencies.

Figure 6 demonstrates that \( k \) increased, \( r \) decreased, and \( \frac{r}{k} \) decreased with increasing stimulus frequency. These are the relationships expected if the mouse orbital mechanics are dominated by a cascade of two or more viscoelastic elements, as has been demonstrated to be the case in other species (Fuchs et al. 1988; Stahl and Simpson 1995). The curve for \( k \) is quite flat across most of the frequency domain, which means that the dynamic \( k \) at 0.1 Hz is for most purposes equivalent to the value of \( k \) that would be obtained for steadily held (as opposed to
to sinusoidally varying) eye positions. Near equivalence of the flat portion of the dynamic $k$ curve and the true static $k (k_0)$ has been demonstrated in the rabbit (Stahl and Simpson 1995).

As described previously (Stahl and Simpson 1995) and briefly reviewed in METHODS, one can develop an equation relating $r/k$ to stimulus frequency for any linear plant transfer function and fit the equation to the empirical $r/k$ values to determine the time constants of the proposed transfer function. Figure 7 shows the results of this process for a 2P-1Z proposed plant, which yields time constants of $T_1 = 0.114 \pm 0.007$, $T_2 = 0.88 \pm 0.05$, and $T_Z = 0.50 \pm 0.04$ s ($r^2 = 0.996$). The fitted curve stays well within the $\pm 2$ SE confidence interval of the average $r/k$ values at all frequencies. To reduce the potential for $r/k$ outliers and skewing to over-influence the calculation of time constants (the $r/k$ distribution is positively skewed, see above), we repeated the fitting on the median values of $r/k$ at each frequency, yielding time constants of $T_1 = 0.08 \pm 0.01$, $T_2 = 0.76 \pm 0.07$, and $T_Z = 0.42 \pm 0.06$ s ($r^2 = 0.989$). The plot of the fit (not shown) is nearly indistinguishable from that shown in Fig. 7.

Abducens nucleus data sufficient to estimate time constants for a 2P-1Z plant (i.e., firing rate phase or dynamic $r$ and $k$ values obtained over a range of stimulus frequencies) have been published for the rabbit (Stahl and Simpson 1995), cat (Goldberg 1980), and rhesus (Fuchs et al. 1988). Figure 8 plots firing rate phase vs. stimulus frequency for these three species as well as the mouse. Superimposed on the data are the model phase vs. frequency curves for each species, where the time constants for the phase curve were generated from 2P-1Z fits to mean $r/k$ vs. frequency data. (Data are presented as phase and in semilogarithmic form because it is more intuitive than dynamic $r/k$, but time constants were determined from $r/k$ for all the advantages discussed above.) Where the source studies gave mean phases rather than $r/k$, the phases were transformed to $r/k$ by the equation $r/k = [\tan (P)]/\omega$. Rabbit data consist of identified but subsequently pooled MN and INT. Rhesus and cat data are for identified MN only. The time constants themselves are shown in Table 2. Table 2 also includes “rhesus, displacement,” an estimate of $T_1$ and $T_2$ from the return of the eye to its initial position following a perturbation generated by electrically stimulating the abducens nucleus (Anderson et al. 2009), as well as an additional estimate for the cat generated from the phase vs. frequency data for a single identified

\[
\begin{align*}
T_1 & = 0.11 \pm 0.007 \\
T_2 & = 0.88 \pm 0.05 \\
T_Z & = 0.50 \pm 0.04 \\
r^2 & = 0.996
\end{align*}
\]

Fig. 7. Determination of 2-pole, 1-zero (2P-1Z) time constants by fitting $r/k$ vs. frequency curve. Error bars indicate the $\pm 2$SE confidence interval of the mean $r/k$ data. Fit line is according to Eq. 5, where $T_1$ and $T_2$ are the time constants of the poles and $T_Z$ is the time constant of the zero. The $r^2$ value is the correlation coefficient.

Fig. 8. Firing rate phase vs. stimulus frequency curves for rabbit, mouse, cat, and rhesus, demonstrating the comparative dynamics in these 4 species. Fine lines are based on the 2P-1Z time constants determined by fitting the associated $r/k$ curves. For sources of rabbit, cat, and rhesus data, see text.

Abducens MN (Delgado-Garcia et al. 1986a) pooled with a single abducens INT (Delgado-Garcia et al. 1986b). It can be seen from Table 2 that the time constants are the longest in the rabbit, which lacks a retinal fovea, and the shortest in the rhesus, which possesses a fovea. The mouse occupies an intermediate position between the foveates and its fellow avoveate, the rabbit.

Modulation during nonalert states. On some occasions mice would enter a state in which the gain of the compensatory eye movements fell to very low levels. Such states presumably reflect depressed alertness, because they were uniformly associated with small pupil sizes (indicating reduced sympathetic drive) and could be ended by auditory stimuli that are presumably “alerting” (e.g., a sharp clap of the hands). These periods may be equivalent to the state of “quiet wakefulness” described in a study of eyelid conditioning, during which conditioned blink responses disappear (Boele et al. 2010). Figure 9 illustrates two examples of the phenomenon, obtained during recordings from 9 motoneurons (MN) and 7 internuclear neurons (INT) (Goldberg 1980). Cat 2 data are based on pooling 1 MN and 1 INT in a published study (Delgado-Garcia et al. 1986a, 1986b). Rhesus, displacement data include the comparable pole time constants for the rhesus determined from movements following electrical stimulation of the abducens nerve (Anderson et al. 2009).

Table 2. Comparison of time constants for two Voigt-element plants in four species

<table>
<thead>
<tr>
<th>Species</th>
<th>$T_1$ (s)</th>
<th>$T_2$ (s)</th>
<th>$T_Z$ (s)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.114</td>
<td>0.876</td>
<td>0.498</td>
<td>0.996</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.275</td>
<td>3.390</td>
<td>1.640</td>
<td>0.973</td>
</tr>
<tr>
<td>Cat</td>
<td>0.112</td>
<td>2.959</td>
<td>2.285</td>
<td>0.977</td>
</tr>
<tr>
<td>Cat 2</td>
<td>0.141</td>
<td>0.689</td>
<td>0.600</td>
<td>0.845</td>
</tr>
<tr>
<td>Rhesus</td>
<td>0.063</td>
<td>0.482</td>
<td>0.271</td>
<td>0.980</td>
</tr>
<tr>
<td>Rhesus, displacement</td>
<td>0.026</td>
<td>0.102</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Values are time constants for 2-pole, 1-zero (2P-1Z) plants, where $T_1$ and $T_2$ are time constants of the poles and $T_Z$ is the time constant of the zero, determined by fitting $r/k$ vs. frequency curves for mouse, rabbit (Stahl and Simpson 1995), cat, and rhesus (Fuchs et al. 1988). Cat data are based on mean data from 9 motoneurons (MN) and 7 internuclear neurons (INT) (Goldberg 1980). Cat 2 data are based on pooling 1 MN and 1 INT in a published study (Delgado-Garcia et al. 1986a, 1986b). Rhesus, displacement data include the comparable pole time constants for the rhesus determined from movements following electrical stimulation of the abducens nerve (Anderson et al. 2009).
cordings from different neurons. In both cases the transition from alert to nonalert state (or vice versa) was captured in the course of the record. Figure 9A was captured during 0.4 Hz, \( \pm 5^\circ \) oscillation. As the recording progressed, the eye oscillation and pupil size gradually declined, reaching the threshold of 0.25 gain (below which we did not analyze the data, as described in METHODS) by the sixth cycle (arrow). We superimposed on the actual firing rate the firing rate predicted from the \( r \) and \( k \) values determined from the region of the record where gain exceeded 0.25 (gray trace). Comparison of the actual and predicted firing rates indicates that actual modulation declined as the record progressed but still remained considerably greater than what would be predicted by the sensitivity parameters obtained during the more responsive condition. Additionally, the mean firing rate declined by about 20 spikes/s compared with the predicted mean. A reduction in mean firing rate could account for some of the attenuation of eye movement, since it would presumably shift neurons operating at low mean firing rates into complete silence, effectively reducing the number of muscle units available to generate active force. In Fig. 9B (obtained during 0.1 Hz, \( \pm 5^\circ \) oscillation), the animal was initially in the nonalert state. At approximately halfway through the record, the animal was alerted with a sharp clap. Pupil size immediately increased, and the amplitude of the eye movement response also greatly increased. Again, modulation of actual firing rate during the nonalert period greatly exceeded the predicted modulation and, in fact, remained unaffected by the change in behavioral responsiveness.

We systematically reviewed the database to identify records like those of Fig. 9 that contained similar transitions in alertness, as well as a sufficient duration of both alert and nonalert state data to allow us to determine \( r \) and \( k \) from the alert region and then compare the firing rates predicted by \( r \) and \( k \) with the modulation during the nonalert period. In the 37 records we identified, we found 4 different phenomena: 1) no effect, as illustrated in Fig. 9B; 2) firing rate effect, as illustrated in Fig. 9A (an effect that on occasion was sufficient to drive the neuron into silence); 3) modulation effect, as also shown in Fig. 9A; and 4) an effect we termed the “limpness effect.” In the lattermost case, as the animal became nonalert, the eye drifted temporally (i.e., into the on-direction of the neuron), but the firing rate did not increase accordingly. This behavior could lead to slackness of the muscle fiber and might reduce its ability to transmit force to the globe. As in the example of Fig. 9A, neurons could manifest some combination of the modulation, firing rate, or limpness effects. Moreover, in the very few examples we had in which we collected more than one record with varying alertness from a single cell, the cell could respond differently in different records. For instance, one cell provided us with one record that showed reduction in modulation, one record with a reduction of firing rate, three records with reduction of firing rate and modulation, and one record with no discernible response. Whichever effect was observed, all four effects share the common feature that firing rate modulation during the nonalert state was greater than predicted from the \( r \) and \( k \) values determined during alertness. The implications of this observation are treated further in DISCUSSION. As described

Fig. 9. Representative examples of firing rate and eye movement data captured during changes in alertness. A: example in which firing modulation and mean rate declined during the nonalert period. Note how eye movement and pupil size declined through the course of the record, which we interpreted as a reduction of alertness. Arrow indicates the point at which eye movement gain crossed the threshold below which we routinely excluded data from analysis. Firing rate modulation (bottom plot, black line) declined, but less so than did the firing rate modulation predicted from analysis of the alert period (shown as superimposed gray line). B: example in which change in state did not correlate with a change in firing rate. As the record opens, the animal is in a nonalert state. Arrow indicates the point at which the experimenter alerted the animal; pupil diameter and eye movement amplitude abruptly increased. Firing rate modulation and mean rate appeared unaffected. During the nonalert period, it greatly exceeded the modulation predicted from analysis of the alert data (gray line).
in METHODS, the periods of nonalertness were excluded from analysis and consequently do not affect the reported values of the firing rate parameters (e.g., $r$, $k$, phase, etc.) and the derived time constants.

**DISCUSSION**

Mouse abducens neurons exhibited firing rate properties during compensatory eye movements (eye movement in response to yaw oscillation in the light) that were in many respects identical to those of mammalian species studied previously. Their firing rate dynamics could be explained to a large extent by the assumption that the VI→E transformation is 2P-1Z, which is equivalent to saying that the lumped active and passive properties of the ocular motor plant—or at least those that are relevant to movements whose frequency content is encompassed by the 0.1- to 1.6-Hz range—are dominated by two cascaded “Voigt” elements, each of which consists of an elastic element (a spring) placed in parallel with a viscous element (a dashpot). As in the rabbit (the only other afoveate mammal in which VI→E has been determined, to date), there was an additional nonlinear property such that when eye movements became very small, the depth of modulation of the firing rate did not fall in proportion to the reduction in the amplitude of the eye movement (Stahl and Simpson 1995). One difference from the rabbit, however, was that most abducens neurons in the mouse were recruited into the firing population when the eye was still well on the adducted side of its central position. In contrast, both extraocular muscle tension and neuronal firing rate recordings in the rabbit suggest that most abducens neurons in that species are recruited near, or on the abducted side of, the central position (Barmack 1976; Stahl and Simpson 1995). The similarities of the mouse VI→E to those of previously studied species support the idea that studies in mice of selected aspects of ocular motor control should be applicable to other mammalian species. On the other hand, there are differences, most notably, the large quantitative difference between the dynamics of the mouse and larger species discussed below, that need to be recognized because they may well arise from qualitative differences in the ocular motor repertoire and the operation of the neural mechanisms that support the repertoire.

**Adequacy of 2P-1Z model.** In a classic early study of ocular motor mechanics, Robinson (1964) concluded that the plant is dominated by its viscoelastic properties, so much so that the mass of the eyeball is rendered relatively inconsequential. For some years Robinson’s fourth-order (3P-1Z) mechanical model was simplified to a first-order (1P) model for the purposes of exploring the relationships of MN firing to eye position (e.g., Delgado-Garcia et al. 1986a; Robinson 1970; Skavenski and Robinson 1973). Subsequently, it was recognized that the 1P simplified model was insufficient to adequately model the plant, even during its response to slow, sinusoidal variations in firing rate (Fuchs et al. 1988). Later, it was demonstrated that the $k$ and $r$ measures of the 1P model, whatever their limitations, could still be used to determine the time constants of higher order plants and that a 2P-1Z model derived from dynamic $k$ and $r$ measurements was adequate to summarize the VI→E of the rabbit (Stahl and Simpson 1995).

Although models of plant mechanics conventionally show an assemblage of single springs and dashpots, a contemplation of the actual anatomy would suggest that the viscosities and elasticities are distributed throughout the volume of the tissues, and as such, a cascade of multiple viscoelastic elements would likely be required to model the mechanics (Sklavos et al. 2005). Fortunately, whereas a large cascade would be necessary to generate a model isomorphic with the anatomy, in practice a cascade of a few elements whose time constants have been selected strategically is sufficient to capture most properties of the orbit; in the rhesus, a four-element cascade with time constants spaced roughly 1/decade spanning 10 ms to 10 s (Sklavos et al. 2005), or a seven-element cascade with time constants spaced 1/0.75 decades spanning 1.3 ms to 40 s (Quaia et al. 2009), is sufficient to model natural eye movements. Adding more elements or spacing the time constants more tightly either fails to improve the ability of the model to fit empiric data or degrades the robustness of the curve-fitting process (Anderson et al. 2009; Quaia et al. 2009; Sklavos et al. 2005).

The four- and seven-element models were determined in the rhesus by measuring the time course with which the eye returns to its central position following a perturbation. In practice, determining the shortest and longest time constants of the four-element model would be problematic using the technique of the current study, because it would be impractical to oscillate the animal at the frequencies at which the fastest and slowest mechanical elements become apparent. Very high frequencies risk losing neuronal isolation. For very low stimulus frequencies, large stimulus amplitudes are necessary to generate eye velocities sufficiently high to surpass the eye velocity noise and drive the regression analysis (recall from METHODS that a poor signal-to-noise ratio in an independent variable reduces the regression coefficient of that variable). However, the large stimulus amplitudes would trigger saccades, which would prevent the system reaching steady state and thereby obscure the influence of mechanical elements with very long time constants.

For the current analysis, we used a two-element model because it is the smallest number of Voigt elements that would generate the properties peculiar to cascaded viscoelasticities, which include the relationships of $r$ and $k$ to stimulus frequency, and the gradual relaxation of tension following a step change in eye position. The latter property, a manifestation of the zero term in the 2P-1Z transform, is termed the firing rate “slide.” Although we did not assess the presence of a slide in the current experiments, it has been demonstrated in every species in which it has been investigated, including rhesus (Goldstein and Robinson 1984; Sylvestre and Cullen 1999), cat (Davis-Lopez de Carrizosa et al. 2011), and rabbit (Stahl and Simpson 1995). Thus it can be assumed that it would be present if sought in the mouse; a quantitative model of the mouse plant must include at least one zero term. A 2P-1Z model explained well the variation of $r/k$ with stimulus frequency and left little unexplained variance to drive fitting to a more complex model (e.g., the 3P-2Z transform of a 3-Voigt element model). Thus a 2P-1Z model is an adequate model of the mouse plant for the purposes of understanding its mechanics over the 0.1- to 1.6-Hz frequency range. It allows for a comparison to other species’ mechanical parameters estimated from sinusoidal behavior over comparable frequency ranges. However, it is expected that a perturbation-release experiment such as those performed in the monkey (Anderson et al. 2009; Sklavos et al. 2005) would reveal the presence of additional

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zero-pole terms. Furthermore, a set of time constants derived from such a perturbation experiment might flank, rather than duplicate, the time constants obtained in this study.

It should be noted that recent studies of viscoelastic properties of passively stretched eye muscles have advanced significant departures from the cascaded Voigt element topology, including a nonlinearity in which stiffness increases with muscle length and a failure of forces to add linearly during sequences of closely spaced elongations (Quaia et al. 2009, 2011). However, since these data were obtained in anesthetized animals with muscles detached from the globe, it remains difficult to relate them to the setting of our experiments in which the ocular motor plant is intact, the animal is awake, and eye muscles are changing length actively under varying levels of innervation. In this setting, the cascaded Voigt element topology may still be the best available description of the ocular motor plant.

**Source of amplitude nonlinearity.** The VI→E relationship of the mouse proved to be grossly linear, as judged by the fact that both the eye movement and firing rate varied sinusoidally and by the generally excellent \( r^2 \) values from the multiple regression fits used to generate \( r \) and \( k \) values. However, a departure was demonstrated: as eye oscillations became very small in terms of both position and velocity, firing rate modulation failed to decline proportionately, causing both \( r \) and \( k \) to increase. The source of the amplitude nonlinearity in mice is unclear. In the context of the rabbit, it was suggested that the nonlinearity originated in part from the hysteresis property that was demonstrated during steadily maintained eye positions (Stahl and Simpson 1995). The suggestion was supported by the behavior of phase lead with respect to eye position, which increased with decreasing stimulus amplitude. The low degree of muscle tension near central eye positions found in the rabbit (Barmack 1976) could also fit with these ideas, since it might create a “dead zone” about the central eye position where firing rate could vary without causing much motion of the eye. Unfortunately, these explanations do not fit as well with the situation in the mouse, since phase lead did not increase with lowering eye amplitude, and Fig. 2 indicates that at the central position, as many as 90% of abducens neurons are already recruited (and presumably, so are those of the medial rectus), making a tension dead zone less likely. Thus it remains unclear whether the superficially similar amplitude nonlinearities of rabbit and mouse are the same phenomenon.

Another cause that has been proposed for decreasing sensitivity values with faster eye movements is a change in the mechanical properties of the antagonist muscle when its MN are silenced during very rapid eye movements (Sylvestre and Cullen 1999). However, this explanation is unlikely to apply to the current data because the silencing of the neurons was comparatively rare during these experiments, a result reflecting the recruitment distribution of the neurons and the fact that we investigated only slow eye movements, usually located about a central eye position. Another cause proposed for declining sensitivity values with faster eye movements is a nonlinear relationship between firing rate and tension (Anderson et al. 2009). Although this effect was also discussed primarily in the context of saccades and is thus of uncertain relevance to the slow eye movements we explored in the mouse, the actual eye movements in that rhesus study were somewhat slower than saccades, and inspection of Fig. 10 in Anderson et al. (2009) indicates that \( k \) and \( r \) were still increasing at the lowest peak velocities they explored and thus could continue to increase into the range of eye velocities we explored in the mice. One approach to exploring the amplitude nonlinearity in the mouse would be to determine whether there are nonlinearities in the transformation of firing rate to muscular force that are appropriate to explain the amplitude nonlinearity of VI→E, a strategy followed in the rhesus (Anderson et al. 2009).

**Impact of variations in alertness.** As demonstrated in Fig. 9, mice can enter a state in which the gain of the compensatory eye movements falls to very low levels but the firing rate modulation exhibits a lesser (or no) reduction. Some of the tendency for firing rate modulation to be relatively maintained while eye movement is attenuated is expected from the amplitude nonlinearity function shown in Fig. 4, which predicts that modulation will endure even as eye amplitude falls to very low levels. Examples such as that shown in Fig. 9B, however, indicate that during nonalert periods, the coupling between the firing rate of an individual neuron and eye position may be loosened or even severed. The ability of any one neuron to become apparently decoupled from eye position should not be surprising; because eye position is controlled by the aggregate activity of the entire population of motoneurons (not only the abducens motoneurons but also those of the medial rectus and, to a lesser extent, those of other extraocular muscles), a neuron whose activity diverges from the mean behavior of the population would appear to be uncoupled from the eye movement. An extreme example of the idea that neurons may become mathematically decoupled from eye position while remaining physically coupled to the eye muscles has been demonstrated in the rhesus, in which the firing rates of 15% of the abducens motoneurons relate better to the position of the contralateral eye than to the [ipsilateral] eye whose lateral rectus muscle they innervate (Zhou and King 1998). This result demonstrates that a neuron can be decoupled from the eye it is connected to without hypothesizing an actual disconnection due, for instance, to dysfunction at the neuromuscular junction. The finding that there was a variation in neuronal response to changes in alertness from neuron to neuron and from one alertness transition to another in a single neuron provides support for the idea that activity across the population of mouse abducens neurons is nonhomogeneous during nonalert periods.

Although the instances we observed of reductions in modulation during nonalert states could obviously contribute to reduced eye movement, the finding of a reduction in mean firing rate in some neurons could also have important effects on eye movement amplitude. The reduction in firing rate could reduce average muscle tension and thus alter the mechanics of the ocular motor plant. Along these lines, Anderson et al. (2009) argued that muscle stiffness must vary with animal state, based on their observations of effects of electrical stimulation in the abducens nucleus in alert vs. barbiturate-anesthetized monkeys. Mechanical properties of the ocular motor plant such as the series elasticity have also been demonstrated to vary with innervation level (Collins 1971). The reductions in firing rate we observed also imply that some motoneurons cease firing for some or all of each stimulus cycle (a phenomenon that we actually observed on occasion). Since the amplitude of eye movement reflects the sum of the modulation amplitudes of all neurons in the population, and since the modulation amplitude of any one neuron would be compressed...
(the difference between maximum and minimum firing rates would decrease) if it went into cutoff (because minimum firing rate cannot fall below 0 spikes/s), increases in the percentage of neurons going into cutoff would be expected to reduce average modulation depth and thus eye movement amplitude. Furthermore, the failure of some motor units to generate tension could engender a slackness of the muscle that interferes with the ability of other, still active motor units to move the eye. Since we strove to minimize the amount of data recorded in the frank nonalert state, the current data set does not permit a quantitative analysis of the VI→E transfer function in the nonalert state. Further systematic studies are required to determine the impact of behavioral state on both the distribution of neuronal activity within the abducens nucleus and the relationship between firing rate and whole muscle tension in the mouse. The current data are sufficient, however, to underscore the importance of recognizing periods of quiet wakefulness when measuring firing rate behavior of extraocular motor nuclei.

Of note, neurons that maintain their modulation in response to head rotation when compensatory eye movements are attenuated [for instance, by suppressing the vestibuloocular reflex (VOR) by having the animal track an object rotating with the head] are often claimed to be encoding a "sensory" vestibular signal (for instance, see King et al. 1976; Tomlinson and Robinson 1984). Since neither abducens MN nor INT have been shown to carry such sensory signals, records such as that shown in Fig. 9B raise the possibility that the sample contains data from units that are neither MN nor INT. There are multiple reasons why this possibility is unlikely. Vestibular nucleus neurons carrying a sensory vestibular signal in both rhesus (King et al. 1976; Tomlinson and Robinson 1984) and mouse (Beraneck and Cullen 2007) modulate approximately in phase with head velocity. In contrast, as shown in the phase vs. frequency plot of Fig. 6, no neuron in our sample modulated in phase with head velocity at any stimulus frequency. Second, all neurons in our sample for which we recorded at least one record in which firing rate failed to reflect an attenuation of behavior (as in Fig. 9B) exhibited bursting activity during temporally directed saccades. In contrast, no mouse vestibular nucleus neuron classed as vestibular-only exhibited saccadic bursts, and of the mouse vestibular nucleus neurons carrying both vestibular and eye movements signals, only about 20% generated bursts during saccades (Beraneck and Cullen 2007). Third, neurons of our sample overwhelmingly possessed negative-positive waveforms, consistent with a juxtasmatic extracellular recording and making it unlikely that we were recording fibers passing from hitherto undescribed, burst-tonic vestibular nucleus neurons carrying vestibular sensory signals. Given the multiple landmarks that were available to us to ensure we were concentrating our sampling within the abducens nucleus, it is also improbable that these juxtasmatic recordings were obtained from any of the vestibular nuclei. Fourth, and finally, we assessed whether neurons exhibiting the different responses to a change in alertness (i.e., no effect, reduced modulation, reduced firing rate, and limpdness effect) differed in terms of their r/k vs. frequency function. We focused on r/k because this is the parameter we used for our calculations of time constants, a central point of this study. For this analysis, we were limited to neurons for which we had a complete r/k vs. frequency plot as well as a record (1 of the 37 described above) containing a transition in alertness. We constructed one plot of r/k vs. frequency for each response type and included in the plot the r/k vs. frequency curve of any neuron that exhibited that specific alertness-associated phenomenon in at least one record. The different plots (shown in Fig. 10) are indistinguishable from each other. Therefore, even in the unlikely event that cells capable of exhibiting the behavior shown in Fig. 9B were neither MN nor INT, their r/k vs. frequency relationships were indistinguishable from that of the overall sample, and therefore they did not bias our measurements of the VI→E time constants.

**Interspecies comparison of mechanics.** The fact that the rabbit has longer time constants than the rhesus despite similar eye sizes has been argued (Stahl and Simpson 1995) to stem from the rabbit’s lower orbital stiffness (Barmack 1976). The lower stiffness was in turn attributed to the recruitment of rabbit MN beginning near the primary position. The situation appeared different in the mouse, since predicted thresholds extended well into the adducted field, reminiscent of the rhesus. Thus some of the shortening of the time constants of the mouse compared with the rabbit (and equivalently, the rightward shift of the mouse phase plot in Fig. 8) may reflect this difference in recruitment.

An additional explanation of the shortened time constants would be the smaller size of the mouse eye compared with the rabbit (~3-mm diameter vs. 18 mm (Hughes 1972; Remtulla and Hallett 1985)). In general, the rapidity of homologous movements of different species (e.g., heart rate, wing beating rate) scales as a power function of animal size (McMahon 1984), i.e., the rate of the movement (y) relates to animal mass (M) according to the allometric equation \( y = aM^{-b} \), where \( a \) and \( b \) are constants. The slope of these power functions in a

![Fig. 10. Separation of r/k vs. frequency data (extracted from Fig. 6) according to the response of neurons to transitions in alertness. Four different types of neuronal response to an alertness transition were observed, as described in text. The plots include only the neurons for which we captured at least 1 record with a transition in alertness. Since any neuron could exhibit a combination of effects in any 1 record or could exhibit different effects in different records, each neuron can appear in more than 1 plot. Mean response of the entire sample is reproduced from Fig. 6 (heavy line). Median response of the entire sample (dashed line) is also included. There is no obvious difference between the plots, rendering it unlikely that neurons with differing responses to changes in alertness represent special subpopulations.](http://jn.physiology.org/)
been argued to influence a number of mechanical properties about by differences in innervation. Innervation level has changes in the operating point of the mechanics brought only fast but also extremely accurate may have required the VOR in mice and gerbils (Kaufman 2002; Stahl et al. 2006b). Coordination in afoveates may be rather loose, as evidenced by direction of gaze of the two eyes. In contrast, interocular mals need to minimize the angular difference between the further augmented because these frontal-eyed, binocular animals need to make the distinction have demonstrated several differences between the MN and INT, not always consistent across species. INT were found to be recruited at more adducted positions than MN in cats (Delgado-Garcia et al. 1986b) and rhesus (Fuchs et al. 1988), whereas the INT k value was reported to be greater in cats but lesser in rhesus. In the rabbit, no significant differences were found between MN and INT for k and r; E_T was not reported in that study, because that study assessed eye positions relative to an arbitrary position that varied from session to session (Stahl and Simpson 1995). INTs have been estimated to make up 15% of the population of abducens neurons in the mouse, which is considerably less than the 20% in rabbit, 33% in cat, and 40% in rhesus (Shaw and Alley 1981; Shaw and Baker 1986; Steiger and Buttner-Ennever 1978). If the proportions of MN and INT in our sample reflect the balance within the abducens nucleus as a whole, then the inclusion of INT should be a minor problem. However, the anatomical distribution of INT and MN within the mouse abducens nucleus is also nonhomogenous, with INT more populous in ventral and rostral regions; if yields were greater in these regions (for instance, if neuron isolation were easier), then INT could make up a larger percentage of the sample than their 15% share of the nucleus.

Fortunately, there are two reasons why the impact of unrecognized INT on estimation of VI−E time constants may not be great, whatever the percentage within the sample. First, the time constants were taken from r/k, which is similar for MN and INT in rhesus, cat, and rabbit (Delgado-Garcia et al. 1986b; Fuchs et al. 1988; Stahl and Simpson 1995). Second, as noted above, INT were recruited in far adducted positions in both cats and rhesus. The mouse r/k vs. c/k curve (Fig. 3) is very flat, so if INT in this species possess the same relatively adducted (positive) values of E_T, then their r/k values do not differ greatly from those of the MN recruited at less adducted positions.

A second limitation of this study is that we examined firing rate dynamics only during the slow phases of the visual-vestibular nystagmus. It proved difficult to quantify the response during fast phases of nystagmus (which can be loosely termed “saccades”) because neuronal isolation during the saccadic burst usually became uncertain due to a combination of decreased spike height and recruitment of additional nearby units. Thus the current results do not provide insight into plant properties beyond our highest tested frequency, 1.6 Hz. Along the same lines, our finding of the amplitude nonlinearity means that the results should be extrapolated with great caution to eye

**Fig. 11.** Allometric relationships between plant dynamics and eye size of the 4 mammalian species for which the necessary data are available. For each of the species, the frequency at which the phase lead passes through 30° is plotted vs. eye size on a log-log plot. Dashed line connecting the data for the 2 afoveate species (mouse and rabbit) has a slope of −0.19.
movements whose amplitude exceeds the range we tested in this study.

Concluding statements. The current results reinforce the idea that the characteristics of the ocular motor plant are dominated by cascaded viscoelasticities, even in species with very different eye sizes. Thus an important task of the mammalian brain is to generate control signals that compensate for a viscoelastic load. This task has at times been diagrammed as if carried out by parallel pathways that contribute distinct components of the final signal, e.g., position, velocity, and slide components (Optican and Miles 1985; Skavensen and Robinson 1973). A literal interpretation of this concept, however, becomes less plausible as one considers higher order plants; a 3P-2Z plant, for instance, would require not one but two different discrete slide signals. Ultimately, it may prove more useful to conceive of the premotor circuitry as a general-purpose inverse transfer function, with no specific part of the network being identifiable as the source of a specific aspect of the final premotor signal.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.S.S. conception and design of research; J.S.S. performed experiments; J.S.S. and Z.C.T. analyzed data; J.S.S. and Z.C.T. interpreted results of experiments; J.S.S. and Z.C.T. prepared figures; J.S.S. drafted manuscript; J.S.S. edited and revised manuscript; J.S.S. approved final version of manuscript.

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