Functional Alterations of Cat Abducens Neurons After Peripheral Tetanus Neurotoxin Injection

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González-Forero, David, Rosa R. de la Cruz, José María Delgado-García, Francisco J. Álvarez, and Ángel M. Pastor. Functional alterations of cat abducens neurons after peripheral tetanus neurotoxin injection. J Neurophysiol 89: 1878–1890, 2003; 10.1152/jn.01006.2002. Tetanus neurotoxin (TeNT) cleaves synaptobrevin, a protein involved in synaptic vesicle docking and fusion, thereby preventing neurotransmitter release and causing a functional deafferentation. We injected TeNT into the lateral rectus muscle of adult cats at 0.5 or 5 ng/kg (low and high dose, respectively). In the periphery, TeNT slightly slowed motor axon conduction velocity, and at high doses, partially blocked neuromuscular transmission. TeNT peripheral actions displayed time courses different to the more profound and longer-lasting central actions. Central effects were first observed 2 days postinjection and reversed after 1 mo. The low dose induce depression of inhibitory inputs, whereas the high dose produce depression of both inhibitory and excitatory inputs. Simultaneous recordings of eye movement and neuronal firing revealed that low-dose injections specifically reduced inhibition of firing during off-directed saccadic movements, while high-dose injections of TeNT affected both inhibitory and excitatory driven firing patterns. Motoneurons and abducens interneurons were both affected in a similar way. These alterations resulted in modifications in all discharge characteristic analyzed such as background firing, threshold for recruitment, and firing sensitivities to both eye position and velocity during spontaneous movements or vestibuloocular reflexes. Removal of inhibition after low-dose injections also altered firing patterns, and although firing activity increased, it did not result in muscle tetanic contractions. Removal of inhibition and excitation by high-dose injections resulted in a decrease in firing modulation with eye movements. Our findings suggest that the distinct behavior of oculomotor and spinal motor output following TeNT intoxication could be explained by their different interneuronal and proprioceptive control.

INTRODUCTION

The firing properties and connectivity of central neurons are maintained by both retrograde and anterograde influences. Functional connectivity with the target is critical to support afferent innervation, electrophysiological properties, and the structural integrity of presynaptic neurons (for review, de la Cruz et al. 1996; Fitzsimons and Poo 1998; Purves et al. 1988; Titmus and Faber 1990). Target dependence is maximal during late embryonic and early postnatal development, being critical for neuronal survival (Cui and Harvey 1995). Adult neurons survive functional disconnection with their target, induced by either axotomy (Blinzinger and Kreutzberg 1968; Brännström and Kellerth 1998; Chen 1978; Eccles et al. 1958; Kuno and Llinás 1970a,b; Matthews and Nelson 1975; Mendell et al. 1974), neuromuscular blockade with botulinum neurotoxin (Pinter et al. 1991; Watson 1969), target depletion (de la Cruz et al. 1994), or axonal transport inhibition with colchicine (Cull 1975; Pilar and Landmesser 1972; Purves 1976). In addition, large alterations occur in the structure, firing, and electrical properties of parent neurons, suggesting that neuronal targets strongly influence the differentiation of presynaptic neurons and sustain their phenotypic and functional state (de la Cruz et al. 1996; Gustaffson and Pinter 1984; Huizar et al. 1975; Kuno et al. 1974).

Neuronal properties are also regulated by anterograde afferent synaptic and trophic activities, but functional modifications in signaling properties have not yet been quantified and described in experimental models that allow to specifically block afferent inputs without injury. The consequences of deafferentation in adult central neurons in vivo have been studied in motoneurons following muscle denervation (Blinzinger and Kreutzberg 1968; Brännström and Kellerth 1998; Mendell et al. 1974), but in this situation it is difficult to discern between the effects derived of target removal or afferent deprivation. Alternatively, others have used models of deafferentation induced by the lesion of specific afferent synaptic inputs (Cotman and Nieto-Sampedro 1984; Mendell 1984), which lead to compensatory responses in neuronal properties and synaptic inputs (Him and Dutia 2001; Weaver et al. 1997). However, these results need to be interpreted with caution in view of the limited regeneration capacity of the CNS after physical injuries (Goldberg and Barres 2000).

In this study, we used tetanus neurotoxin (TeNT) as a tool to induce prolonged and specific synaptic blockade. TeNT is a cistroidal neurotoxin that is transported retrogradely by motor axons to the motoneuron cell body from where it transsynaptically translocates to presynaptic boutons (Price et al. 1975; Schwab and Thoenen 1976) and blocks inhibitory neurotransmission (Brooks et al. 1957; Mellanby and Green 1981). Thus in the spinal motor system, TeNT commonly induces tetanic firing and muscle contraction. Alterations on excitatory syn-
apses onto motoneurons have also been reported with high doses (Bergey et al. 1987; Calabresi et al. 1989; González-Forero et al. 2002a; Kanda and Takano 1983). Usually TeNT bypasses the neuromuscular junction without affecting it, unless it is present at very high doses (Dreyer and Schmitt 1981). Here, we aimed at disconnecting abducens motoneurons from their inputs by injecting different doses of TeNT in the lateral rectus muscle. We have previously shown that TeNT induces changes in the expression of calcitonin gene-related peptide (CGRP) that were correlated with the levels of firing suggesting a form of activity-dependent regulation of neuronal phenotype (González-Forero et al. 2002b). Moreover, the firing regularity of abducens neurons varied in accordance with the alterations of the composition and synaptic strength (González-Forero et al. 2002a). In the present work we aimed to study the time course, amplitude and reversibility of changes in ocular movements, and firing patterns of antidromically identified abducens neurons in an alert behaving preparation. The results are compared with previous studies using target disconnection induced by axotomy (de la Cruz et al. 2000; Delgado-García et al. 1988), selective ablation of the target (de la Cruz et al. 1994b), or functional muscle denervation with botulinum neurotoxin (BoNT) (Moreno-López et al. 1997). Preliminary accounts of the present study were described in González-Forero et al. (2001a,b).

**METHODS**

Adult cats weighing 2.5–3.5 kg, obtained from authorized suppliers (Animal Supply Services, University of Córdoba, Spain), were used in this study. Animals (n = 4) were prepared for the chronic recording of eye movements and extracellular electrical activity in abducens neurons at different times postinjection. All experimental procedures followed the guidelines of the European Union Council Directive (86/609/EEC) and current Spanish legislation for the use and care of laboratory animals (BOE 67/8509-12 1988).

**Neurotoxin injection**

Cats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the left lateral rectus muscle isolated under a dissecting microscope and injected with toxin. A total of 0.5 or 5 ng/kg of TeNT (that will be referred to as low and high dose, respectively) dissolved in 5 μl of physiological saline was injected (Fig. 1A). Symptoms of systemic tetanus were absent in all the animals used. Two animals received low-dose injections and the other two received high-dose injections. TeNT was kindly provided by Dr. J. O. Dolly (Imperial College, London, UK).

**Chronic recordings**

Recording sessions started 2 wk postoperatively. Cats were restrained in a recording system with their heads immobilized. The recording system consists of a servo-controlled table that rotates around the vertical axis to induce the vestibulo-ocular reflex. Eye coils made with Teflon-insulated wires were sutured to the sclera, and eye movements were recorded using a magnetic coil frame by means of the magnetic field search-coil technique (Fuchs and Robinson 1966). Extracellular recordings were carried out with glass micropipettes having a resistance of 1–3 MΩ and filled with 2 M NaCl. The abducens nucleus was approached stereotaxically and located with the aid of the antidromic field potential produced by electrical stimulation of the ipsilateral VIth nerve (Fig. 1A; St.1). Abducens motoneurons and internuclear neurons were positively identified by their antidromic activation from the ipsilateral VIth nerve and the contralateral MLF, respectively, and by the collision test between the orthodromic and antidromic action potentials (Fig. 1B; St.1, St.2). Neuronal activity was amplified and filtered at a bandwidth of 10 Hz–10 kHz. Moreover, eye movements were recorded in the alert cat during electrical stimulation of the VIth nerve. Single pulses of 50 μs and at intensities
lower than 0.1 mA were used. Trains of stimuli lasted for 300 ms, and the stimulus frequencies ranged from 10 to 200 Hz, thus including activation frequencies covering the fusion frequency range for most lateral rectus muscle motor units (Shall and Goldberg 1992).

**Data storage and analysis**

Instantaneous firing frequency (i.e., the reciprocal of the interspike intervals) and eye and head positions were recorded and digitally stored for off-line analysis. For the purpose of illustration, upward deflections of eye position indicate eye movements to the left. Relationships between neuronal firing rate (FR, in spikes/s) and eye position (EP, in degrees) were obtained by linear regression analysis to calculate the slope, i.e., neuronal sensitivity to eye position ($k_r$ in spikes/°) and the intercept ($F_0$, in spikes/s), i.e., the neuronal firing rate at the primary position (0°). Therefore firing rate during fixations responded to the equation $FR = k_rEP + F_0$. Rate-velocity relationships during spontaneous saccades were also obtained by linear regression analysis after subtraction of the position component ($k_rEP$). Thus the equation used was $FR = k_rEP = r_EV + F_0$, where $r$ (in spikes/°/s) is the neuronal sensitivity to eye velocity (EV, in °/s). Sensitivities were also calculated separately for spontaneous eye movements occurring in the on- ($k_{on}$ and $r_{on}$) versus off-directions ($k_{off}$ and $r_{off}$). The on-direction for abducens neurons was that ipsilateral to the recording (left) site. Analysis of responses during vestibular stimulation was performed by multiple linear regression analysis, after selecting the slow phases of the vestibular nystagmus. The regression equation was $FR = F_0 + k_rEP + r_EV$, where the two regression coefficients represent the neuronal sensitivities to eye position ($k_r$, in spikes/°) and velocity ($r_v$, in spikes/°/s).

**RESULTS**

**Oculomotor movements following TeNT injections**

Following high- or low-dose TeNT injection, the amplitude and the velocity of eye movements and the oculomotor range covered by both eyes were dramatically reduced. Motor impairment was evident as early as 2 days after TeNT treatment and persisted for a period of ≥30 days postinjection. However, a progressive motor recovery was observed during this interval. In control animals, movement of both eyes was conjugate around the primary position (0°; Fig. 2A, top). Oculomotor range before injection was between +40.8 and −41.72° (+ indicating movement to the left, n = 4 animals) with the mean position situated in +0.89 ± 6.29° (SD) (Fig. 2B, ●). Ten days after a high-dose injection in the left lateral rectus muscle, eye

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**FIG. 2.** Eye field movements after TeNT application. A: x-y plots showing the oculomotor fields covered during 5 min of spontaneous eye movements before (control), 9 days after a high dose (5 ng/kg), or 7 days following a low-dose TeNT (0.5 ng/kg). Leftward (L) and upward (U) movements are shown positive, whereas rightward (R) and downward (D) movements are shown negative. Note that the left eye did not cross the horizontal primary position (0°) in the abducting direction after high-dose application. Following the low-dose injection, left eye movements were mainly confined to the left (abducting) hemifield. B: temporal course showing the oculomotor range restrictions and mean position deviation of the injected eye in the horizontal plane after high- (●) or low-dose (■) application. Motor symptoms were opposite for each dose and recovered partially after 30–40 days. C: velocity-to-position relationships obtained during horizontal saccades of the left eye before (●) and 15 days after treatment with high (●) and low dose (■). Slopes in control are 12.8 ± 11.1°/s ($r = 0.94$ and 0.95, respectively) for lateral (L) and medial (D) saccades. Slopes after the high dose were 5.7 and −7.3°/s ($r = 0.81$ and 0.85, respectively) and after the low dose were 9.7 and −8.15 ($r = 0.83$ and 0.80, respectively).
movements in the ipsilateral side were reduced in amplitude (between +1.01° and −36.13°) and limited to the right hemifield, showing a complete incapacity to cross the primary position and explore laterally in the abducting direction (Fig. 2A, middle). The central eye position shifted toward the right hemifield (−12.88 ± 6.1°) in the affected eye (left). Motor range of the right (noninjected) eye was also restricted (Fig. 2A, middle). However, this eye was able to cross the primary eye position. Frequently, right eye movements directed to the right were accompanied by depression (downward gaze). Similar movements have been observed after MLF transection and interpreted as secondary actions on vertical or torsional extraocular muscles (de la Cruz et al. 2000). These motor symptoms were readily observed 2–10 days after injection (Fig. 2B; ○; •). Eye movements recovered from toxin effects by 31–40 days postinjection. The treated eye recovered completely the mobility in the nasal hemifield, but abducting eye movements did not exceed 16° toward temporal positions and horizontal left gaze remained deviated in the nasal direction (−9.55 ± 5.18°; Fig. 2B; ○; •).

On the contrary, the low-dose TeNT injection caused motor deficits in the opposite direction. Thus movement of the injected eye was limited to lateral positions in the abducting direction, crossing only rarely the primary position toward the nasal hemifield (Fig. 2A, bottom). In the 2–10 day posttreatment interval, the horizontal motor range of the left eye was limited between +37.1° and −21.89°, and the mean ocular position was deviated to +8.93 ± 3.3° in the temporal hemifield (Fig. 2B; ▲; – – –). By 31–40 days, ocularmotor range (79% of control) as well as mean ocular position were partially recovered (Fig. 2B; ▲; – – –). The linear relationships between saccadic amplitude and velocity previously reported (Robinson 1981) also held after TeNT treatment (Fig. 2C), despite the slower velocity of saccades in the injected eye for any given amplitude compared with controls, although saccades of the same amplitude than control coursed at slower velocities.

Effects on neuromuscular junction transmission

To test possible peripheral actions of TeNT, eye movements were evoked by single or repeated electrical stimulation of the VIth nerve in high-dose treated animals and analyzed at several postinjection times. In controls, single pulse stimulation of the VIth nerve produced a movement deflection with average peak amplitude of 2.18 ± 0.67°. High-frequency train application at 150 Hz induced an eye displacement of 15.7 ± 3.21° (Fig. 3A). Following high-dose injection in the left lateral rectus muscle, the amplitude of the electrically induced eye movement was partially reduced. This effect was transitory and only evident between 4 and 8 days postinjection when the maximum reduction was obtained (approximately 50%; Fig. 3, B–D). Electrically evoked eye movements recovered at 12 (92%) and 16 days postinjection (97%). Similar results were obtained using train stimulation. An inflection in the upsweep phase of the movement was usually observed in control recording sessions during train applications. After the high-dose treatment, the inflection point was more evident (Fig. 3C, 8 d), perhaps because the unmasking of a retraction movement during depressed abduction movement. Single pulse or train evoked eye movements changed and recovered in parallel (Fig. 3D). These results suggest a partial and short-lasting blockade of neuromuscular transmission following high-dose injection of TeNT.

Alterations in axonal conduction velocity

Unitary identification of motoneurons and internuclear neurons was carried out by antidromic activation from the ipsilateral VIth nerve and contralateral MLF, respectively (Fig. 1B, top). Collision tests were used to confirm neuron identity (Fig. 1B, bottom). In control motoneurons the antidromic latency was 0.69 ± 0.13 ms (n = 111). After a low-dose TeNT injection, the mean antidromic latency increased significantly (P < 0.05, Student’s t-test) in motoneurons (0.78 ± 0.24 ms, n = 173), but average latency differences did not reach significance after high doses (0.73 ± 0.13 ms, n = 113). After grouping mean latency values depending on the postinjection time, we detected significantly longer activation latencies in the second (0.79 ± 0.154 ms) and third weeks (0.95 ± 0.364 ms) following the low-dose application (P < 0.05, 1-way ANOVA, Tukey test). Although average latency was not different in the high dose injected group at any postinjection time, the proportion of motoneurons with latencies longer than 0.8 ms was 45%, whereas this was only observed in 14.4% of control motoneurons. Histogram distributions of antidromic latencies measured in experimental motoneurons always showed the presence of a larger number of long-latency cells (>1 ms) relative to control (data not shown). The latency of internuclear neurons recorded following low- (0.79 ± 0.21 ms, n = 38) or high-dose application (0.74 ± 0.17 ms, n = 62) did not differ from controls (0.72 ± 0.20 ms, n = 86).

Qualitative changes in the firing of abducens neurons during spontaneous eye movements

Abducens neurons recorded before TeNT injection maintained a tonic and regular discharge during eye fixations, whose frequency was proportional to the horizontal eye position in the orbit (Fig. 4A). Their firing rate increased monotonically for ocular fixations at successive higher angular positions directed toward the ipsilateral side of recording (the on-direction) and decreased or eventually ceased as the eye moved toward more eccentric eye positions in the off-direction. Discharge activity was also related to eye movement velocity during saccades. High-frequency firing bursts occur during on-directed saccades (Fig. 4A, ●) and firing was reduced or paused preceding off-directed saccades (Fig. 4A, →). Motoneurons and internuclear neurons, both displayed similar firing characteristics, probably because they receive similar afferent innervation (Baker and Spencer 1981).

The firing pattern of abducens motoneurons was dramatically altered following a high-dose TeNT injection in the lateral rectus muscle. Changes were noticed as early as 2 days after TeNT application and lasted for about 1 mo. During the initial 15 days postinjection, treated motoneurons showed an overall reduction in firing rate and complete absence of firing modulation in relation to eye position and velocity (Fig. 4B1). Frequently, motoneurons recorded during this period exhibited reduced bursts for on-directed saccades (Fig. 5B1, ●), and pauses in firing were not present during off-directed saccades (Fig. 4B1, →). Initial signs of recovery in tonic and bursting behavior in the firing pattern of abducens motoneurons were
first observed by the end of the third week after TeNT injection (Fig. 4B2, but only for on-directed eye movements (Fig. 4B2, •). Eventually, motoneurons developed anomalous bursts of activity during off-directed saccades (Fig. 4B2, △). Both tonic and bursting components of the firing pattern in abducens motoneurons resumed to normal after 30–40 days.

The discharge characteristics of neurons recorded after low-dose injection was different to control and to the high-dose treatments. Low doses of TeNT typically altered the tonic-phasic discharge but did not cause its complete disappearance. Overall firing rate was increased (opposite to the high dose) and inhibitory signals during off-directed eye movements were lost (Fig. 4C). Firing activity did not diminish (Fig. 4C) and sometimes increased (Fig. 4C) preceding off-directed saccades and fixations. In contrast, the bursts and the tonic firing during on-directed eye movements were preserved (Fig. 4C, •).

Strikingly similar alterations were observed in the firing of internuclear neurons after high- and low-dose treatments. Following high-dose applications, ipsilateral abducens internuclear neurons displayed a discharge pattern with reduced firing modulation (Fig. 4D) for ocular fixations. Phasic activity was limited to reduced and transient bursts preceding both on- and off-directed saccades (Fig. 4D, • and △, respectively).

Quantitative changes in position sensitivity during spontaneous eye movements

For each abducens neuron, the relationship between firing rate and horizontal eye position was calculated. Linear regression analysis was used to obtain 1) the neuronal position sensitivity (\( k_s \) in spikes/s/°) as the slope of the regression line, 2) the firing rate at the primary position (\( F_0 \) in spikes/s) as the intercept of the regression line with the ordinate, and 3) the extrapolated recruitment threshold (in degrees) as \(-F_0/k_s^{-1}\) (Fig. 5A). These parameters were estimated from more than 50 different horizontal positions of the ipsilateral eye or contralateral eye for motoneurons and internuclear neurons.

Correlation coefficients obtained for control rate-position plots were always >0.8. The mean position sensitivity for control motoneurons and internuclear neurons was 6.72 ± 2.86
(n = 63) and 6.93 ± 2.59 spikes/s/° (n = 54), respectively (Table 1; P < 0.001, 1-way ANOVA, Tukey test). Firing rate at straight-ahead gaze (F₀) was 49.22 ± 20.45 spikes/s for motoneurons and 71.16 ± 27.59 spikes/s for internuclear neurons. Recruitment threshold ranged in control motoneurons between 6° and −23.48° with a mean value of −7.30 ± 5.69°. Internuclear neurons had a lower threshold (−10.23 ± 5.93°; range: 0.1° to −26°).

Early after the high-dose injection, abducens neurons exhibited a continuous and tonic firing almost unrelated to eye movements (Fig. 4, B1 and D). Thus the correlation coefficients obtained from regression analysis between eye position and firing rate were usually lower than controls. Only neurons with correlation coefficients higher than 0.65 were accepted for analysis. The sensitivities to eye position for both motoneurons and internuclear neurons were reduced by more than 80% after the high-dose injection of TeNT. Mean kᵣ values obtained in motoneurons and internuclear neurons recorded in the period 2–20 days posttreatment were 0.93 ± 0.53 and 1.26 ± 0.74 spikes/s/°, respectively (Table 1). A comparison of the rate-position lines obtained from a control motoneuron (●) and a motoneuron recorded 10 days after the high-dose application (○) is shown in Fig. 5A. Complete recovery toward control kᵣ values in the experimental motoneurons occurred within 1 mo (Fig. 5B). As a consequence of the continuous tonic firing and the reduced position sensitivity of abducens motoneurons after the high dose injection, the theoretical recruitment thresholds were lower than controls. Thus experimental motoneurons showed a more eccentric mean recruitment threshold (−38.45 ± 19.55°), which was significantly different (P < 0.001, 1-way ANOVA, Tukey test) from the control value (Table 1). As previously reported (González-Forero et al. 2002b), parallel changes in mean firing rate at primary eye position (F₀) were present in affected motoneurons (Fig. 5A). Mean F₀ for motoneurons recorded during the interval 2–20 days posttreatment was significantly reduced to 61% of the

FIG. 4. Effects of TeNT on the discharge pattern of abducens neurons during spontaneous eye movements. A: discharge of a control motoneuron. Traces in each panel are the horizontal position (in degrees) of the left (LH) and right eye (RH) and the histogram of the instantaneous firing rate (FR, in spikes/s). L and R indicate left and right movement directions, respectively. Control motoneurons displayed a tonic firing during fixations and burst (●) and pauses (→) during on- and off-directed saccades, respectively. B: same as A, but for high-dose treated motoneurons recorded at 7 (B1) and 16 days (B2) postinjection. Note in B1 the low-frequency tonic firing, the lack of modulation for eye fixations and off-directed saccades (→), and the reduced bursts for on-directed movements (●). As shown in B2, high-dose treated motoneurons presented a progressive recovery with partial reestablishment of the tonic-phasic behavior during on-directed movements (●), although they did not diminish firing rate during off-directed movements (→), and in some cases, pauses were even substituted by reduced burst activity (○). C: internuclear neuron recorded 6 days after the low-dose injection, showing discharge modulation during on-directed saccades (●) and fixations, but not for off-directed movements (→). D: internuclear neuron recorded 10 days after high-dose treatment showing partial loss of position signals, reduced bursts for abducting movements (●), and anomalous firing for adducting saccades (○).
control value (Table 1; \( P < 0.001 \), 1-way ANOVA, Tukey test). However, no differences were present in mean \( F_\theta \) for the group of affected internuclear neurons with respect to control (Table 1).

Low-dose treatments resulted in an overall increase in firing accompanied by reduced modulation during off-directed eye movements. Nevertheless, tonic and burst modulation for on-directed eye fixations and saccades persisted (Fig. 4C). Despite the unidirectional loss of modulation, mean position sensitivity did not differ from control at any postinjection period (Fig. 5D). However, an inflection point was frequently observed in the rate-position plots delimiting two different slope regions (Fig. 5C). After classifying the data according to the preceding saccade (on- or off-directed), we found that for each movement (on- or off-directed) the slopes were different. Thus while position sensitivities preceded by on-directed saccades (\( k_{\text{on}} \)) were similar to control at every postinjection time (Fig. 5D, \( \bullet \)), mean values of position sensitivity preceded by off-directed saccades (\( k_{\text{off}} \)) were significantly reduced in relation to control \( (P < 0.001; \text{Fig. 5D, } \circ) \). In particular, during the postinjection period between 2 and 20 days, mean \( k_{\text{off}} \) value for low-dose–treated motoneurons \( (2.52 \pm 1.05 \text{ spikes/s}^2) \) was significantly reduced with respect to \( k_{\text{on}} \) \( (5.54 \pm 1.78 \text{ spikes/s}^2) \) and \( k_{\text{on}} \) \( (5.90 \pm 1.94 \text{ spikes/s}^2) \) obtained in the same motoneuronal group, as well as in relation to control \( k_{\text{s}} \) (Fig. 5D; \( P < 0.001, 2\text{-way ANOVA, Tukey test}) \). The direction-dependent changes in the position sensitivity observed in low-dose–treated motoneurons were reversible and lasted for about 3 wk.
(Fig. 5D). Thereafter, \( k_{off} \) was not different to control \( k_{on} \). Although it has been reported that extracellular motoneurons present static hysteresis, that is, the tonic firing rate for the same eye position differs in about 15 spikes/s depending on the movement direction (Eckmiller 1974), it is not likely that the stationary behavior observed in low-dose treated motoneurons was related to hysteresis. The reason is that hysteresis would cause a lateral displacement of the rate-position plot, affecting only \( F_0 \) and recruitment threshold without a change in the slope \( (k) \). In conclusion, the directional effects observed in the discharge pattern of low-dose treated motoneurons probably result from a selective blockade of the afferent tonic inhibitory signals. Furthermore, the mean \( k_{on} \) and \( k_{off} \) values did not differ when calculated independently in control \((5.84 \pm 1.45\) vs. \(5.21 \pm 1.46\) spikes/s/\( \theta \)) or in high-dose–treated motoneurons \((1.02 \pm 0.97\) vs. \(0.52 \pm 1.05\) spikes/s/\( \theta \); \(P < 0.001\), 1-way ANOVA, Tukey test), which confirms the distinct alterations induced by the low dose of TeNT.

Following the low-dose injection, the mean \( F_0 \) increased, and recruitment thresholds were reduced in treated motoneurons (Table 1). However, these changes were parallel to position sensitivity alterations and normal parameters were reestablished in treated motoneurons after 20 days. During this period, mean \( F_0 \) increased by 30% and threshold decreased by 77% with respect to control \((P < 0.001; \text{Table 1; 1-way ANOVA, Tukey test})\). Similarly to the motoneurons, internuclear neurons showed reduced \( k_{off} \) compared with \( k_{on} \) \((P < 0.001; 3.61 \pm 2.35\) vs. \(6.88 \pm 2.60\) spikes/s/\( \theta \)) during the 20 days that followed low-dose injection.

### Table 1. Static and dynamic parameters of abducens neurons

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neuronal Type</th>
<th>( k_{on} ) (spikes/s/( \theta ))</th>
<th>( F_0 ) (spikes/s)</th>
<th>( \text{Th} ) (deg)</th>
<th>( r_s ) (spikes/s/deg/( \theta ))</th>
<th>( k_{on} ) (spikes/s/( \theta ))</th>
<th>( r_s ) (spikes/s/deg/( \theta ))</th>
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<tr>
<td>Control</td>
<td>Mn</td>
<td>(6.72 \pm 2.86)</td>
<td>(49.22 \pm 20.45)</td>
<td>(-7.30 \pm 5.69)</td>
<td>(0.96 \pm 0.20)</td>
<td>(7.09 \pm 2.92)</td>
<td>(1.27 \pm 0.49)</td>
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<td>Int</td>
<td>(6.93 \pm 2.59)</td>
<td>(71.16 \pm 27.59)</td>
<td>(-10.23 \pm 5.93)</td>
<td>(1.55 \pm 0.56)</td>
<td>(6.84 \pm 2.26)</td>
<td>(1.75 \pm 0.79)</td>
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<tr>
<td>5 ng/kg</td>
<td>Mn</td>
<td>(0.93 \pm 0.53)†</td>
<td>(30.67 \pm 10.28)†</td>
<td>(-38.45 \pm 19.55)†</td>
<td>(0.34 \pm 0.21)†</td>
<td>(1.64 \pm 0.99)†</td>
<td>(0.40 \pm 0.25)†</td>
</tr>
<tr>
<td></td>
<td>Int</td>
<td>(1.26 \pm 0.74)†</td>
<td>(55.27 \pm 25.84)</td>
<td>(-54.41 \pm 31.97)†</td>
<td>(0.31 \pm 0.20)†</td>
<td>(1.34 \pm 0.67)†</td>
<td>(0.45 \pm 0.36)†</td>
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<tr>
<td>0.5 ng/kg</td>
<td>Mn</td>
<td>(5.54 \pm 1.78)</td>
<td>(64.27 \pm 23.02)</td>
<td>(-13.12 \pm 6.31)</td>
<td>(0.82 \pm 0.36)</td>
<td>(7.30 \pm 2.93)</td>
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<td>Int</td>
<td>(7.48 \pm 4.07)</td>
<td>(69.03 \pm 19.72)</td>
<td>(-10.08 \pm 6.10)</td>
<td>(1.24 \pm 0.56)</td>
<td>(6.97 \pm 2.97)</td>
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Values are mean ± SD. Data were pooled from neurons recorded before (control) or during the 2- to 20-day post-injection time interval (after 5 or 0.5 ng/kg of TeNT). Daggers indicate significant differences with respect to control \((P < 0.001; \text{ANOVA})\). Mn, motoneurons; Int, internuclear neurons; \( k_{on} \), neuronal sensitivity to eye position during spontaneous eye movements; \( r_s \), neuronal sensitivity to eye velocity during spontaneous saccades; \( F_0 \), firing rate at the primary position (0 degrees); \( \text{Th} \), recruitment threshold for discharge; \( k_{on} \), and \( r_s \), neuronal sensitivities to eye position and velocity during the slow phases of the vestibulo-ocular nystagmus, respectively. The number of motoneurons and internuclear neurons in each group was 63 and 54 (control), 39 and 17 (5 ng/kg), and 56 and 12 (0.5 ng/kg).

Changes in firing during vestibular stimulation

Control abducens neurons modulated their activity in relation to slow and fast phases of the vestibulo-ocular reflex (Fig. 6A). Thus the firing rate profile showed a sinusoidal modulation during the slow phases of the nystagmus (Fig. 6A, ■), and
bursts (Fig. 6A, ●) or pauses (Fig. 6A, ▶) during the on- or off-directed fast phases of the reflex, respectively. Abducens neurons recorded after low- or high-dose TeNT injection showed altered discharge activity during vestibular stimulation, during both the slow and fast phases (Fig. 6, B–D). Following high-dose application, eye position and velocity sensitivity during vestibular stimulation were dramatically reduced ($P < 0.001$) by more than 70% with respect to control in both motoneurons and interneurons (Fig. 6, E and F, open bars; Table 1; $P < 0.001$, 1-way ANOVA, Tukey test). During on-directed fast phases, burst activity was partially preserved in high dose treated neurons (Fig. 6, C and D, ●). However, firing did not pause during off-directed fast phases (Fig. 6, C and D, ▶). The time course of recovery in mean eye position and velocity sensitivities for high dose treated motoneurons occurred in parallel, and after 3 wk, the mean $k_v$ and $r_v$ values did not differ significantly from control (Fig. 6, E and F, open bars).

Under the low-dose effects, an overall increase in firing rate was observed during vestibular modulation in abducens neurons. They exhibited very high-frequency bursts during on-directed fast phases (Fig. 6B, ●), and activity was not paused during off-directed fast phases (Fig. 6B, ▶). Despite these effects, $k_v$ showed no differences with control motoneurons at any postinjection interval (Fig. 6E, filled bars; Table 1). Mean values of $r_v$ for low-dose treated motoneurons only differed from control in the 2- to 10-day postinjection interval ($1.90 \pm 0.94$ spikes/s°/s; $P < 0.001$, 2-way ANOVA, Tukey test; Fig. 6F, filled bars). No differences were found in $k_v$ and $r_v$ values between control and low-dose treated internuclear neurons (Table 1).

**DISCUSSION**

The main finding of this study is that TeNT produced selective alterations of the firing patterns and sensitivities to eye position and velocity of abducens neurons. These alterations displayed distinct characteristics depending on the TeNT dose applied. During the initial 20 days that followed a high-dose injection, abducens neurons showed a highly regular and tonic low-frequency firing with reduced recruitment thresholds and sensitivities to eye position and velocity. After a low-dose TeNT injection, abducens neurons showed increased tonic firing with marked loss of position and velocity modulation during off-directed eye movements. In conclusion, low doses of TeNT markedly affected modulation of firing by inhibitory inputs into both motoneurons and internuclear interneurons. In contrast, a high dose of TeNT resulted in firing behaviors in both neuronal types that are consistent with the loss of both excitatory and inhibitory inputs (Gonzalez-Forero et al. 2002b).

**Effects of tetanus neurotoxin on neuromuscular transmission**

Some of the effects on ocular movements might be due to smaller peripheral effects of the toxin. The amplitude of eye displacement evoked by single or train stimulation after the
high-dose injection of TeNT was reduced by 50% relative to control, suggesting partial blockade of neuromuscular transmission. Previous studies suggested a differential neuromuscular sensitivity to TeNT related to muscle fiber types. Some authors suggested that neurotransmission onto slow muscles was blocked by lower doses of TeNT compared with fast muscles (Duchen and Tonge 1973), and the opposite conclusion was reported by others (Kretzschmar et al. 1980). Extracellular muscles contain fibers that resemble the histochemical and mechanical properties of fast skeletal fibers (Burke 1981; Shall and Goldberg 1992; Spencer and Porter 1988).

Decreased eye movements after VIth nerve stimulation could also be explained by adaptation of muscle properties to motoneuron activity patterns. Muscle contractile, metabolic, and histochemical properties are influenced by the motoneuronal firing pattern (Gordon et al. 1997; Kornell et al. 1987; Salmons and Sûrê 1976; Sketelj et al. 1998) and fast-to-slow transformation of muscle fibers has been shown during chronic low-frequency activation such as that induced by high doses of TeNT. However, amplitude changes in response to VIth nerve stimulation were seen as early as 4 days after TeNT, a time delay perhaps too short for the induction of activity-dependent adaptive modifications in muscle properties, which usually requires 2–3 wk (Gordon et al. 1997; Kornell et al. 1987). In TeNT-treated animals, high-frequency stimulation usually unmasked a sag in the upsweep phase of movement, which resulted probably from a combined eye retraction response added to the TeNT-depressed abducting movement. Motoneurons innervating cat retractor bulbí muscle are distributed in the abducens, accessory abducens, and oculomotor nucleus, and their axons course through the VIth and IIIrd nerves, respectively (Meredith et al. 1981). It is possible that VIth nerve stimulation activated lateral rectus and retractor bulbí motoneurons unmasking a subjacent retraction movement.

Why the extraocular muscles did not tetanize?

Analysis of recorded eye movements revealed the absence of spastic paralysis affecting injected lateral rectus muscles. The injected eye horizontal movements were restricted in amplitude and velocity and adopted eccentric orbital positions, but we never observed immobilization of the injected eye at extreme lateral positions. Low-frequency discharge activity displayed by high-dose treated motoneurons could explain the reduced lateral rectus muscle activation and, consequently, a lower tension toward the abducting direction.

Under the low dose, the horizontal movements of the injected eye were limited preferentially to the temporal hemifield. Mean horizontal eye position was displaced laterally, which was probably the consequence of motoneuronal hyperactivity. However, our recorded abducens neurons never presented a sustained firing above fusion frequencies for lateral rectus motor units whose mean value in the cat is approximately 170 spikes/s (Shall and Goldberg 1992). High-dose–treated motoneurons never discharged at frequencies higher than 50 spikes/s, and although low-dose treated motoneurons showed an overall increase in firing activity due to TeNT-disinhibition, they retained a partial capability to modulate their tonic discharge.

The present results suggest that discharge activity of abducens motoneurons in complete absence of tonic inhibition and modulated only by excitatory inputs is not necessarily tetanic, by difference to spinal motoneurons. It is possible that recurrent inhibition and proprioceptive control mechanisms present in the skeletomotor system and absent in the oculomotor system could contribute to potentiate the disinhibitory TeNT effects in spinal motoneurons. It has been reported that spasticity during local tetanus is more prominent in antagonistic extraocular muscles (Takano 1985), which are subject to greater recurrent inhibitory control by Renshaw cells (Burke and Rodomin 1977). TeNT blocks all the types of postsynaptic inhibition on spinal motoneurons, including Renshaw inhibition (Brooks et al. 1957). Another possible potentiation mechanism could occur through TeNT-induced γ-motoneuron hyperactivity (Benecke et al. 1977; Takano and Kano 1973) that would increase 1a afferent tonic excitatory synaptic drive onto homonymous α-motoneurons.

In conclusion, combined disinhibition and overexcititation could contribute to the symptomatic bases of tetanus in the spinal motor system. In the oculomotor system, there are not direct feedback recurrent inhibitory and excitatory loops (Keller and Robinson 1971; Ruskell 1999). Thus although disinhibition causes an increase in firing, it does not lead to either tetanic firing or muscle contracture.

Central effects of tetanus neurotoxin

TeNT injected in the lateral rectus muscle causes afferent synaptic blockade on abducens neurons showing a higher selectivity for inhibition (González-Forero et al. 2002b). We showed before that the low dose affects specifically to inhibitory postsynaptic potentials (IPSPs), whereas the high dose blocked both excitatory postsynaptic potentials (EPSPs) and IPSPs. Our present findings on the firing patterns and discharge characteristics of abducens neurons indicate that the effects can be explained by the selective blockade of the different groups of afferent neurons (prepositus, vestibular, and reticular) that terminate on the abducens nucleus (Escudero and Delgado-García 1988). Thus the low-dose selective blockade of inhibition would produce lack of pauses and modulation during spontaneous and vestibularly induced eye movements directed toward the off-direction. In addition, high-dose application would also reduce excitatory afferent signals leading to firing depression and revealing a dose-dependent action on excitatory neurotransmission. Although we cannot exclude the possibility that the firing of premotor neurons is also affected by the transsynaptic transport of the toxin, the effects observed in the firing of abducens neurons are perhaps best explained by the fact that presynaptic transmission is blocked (González-Forero et al. 2002b).

The prevalence of effects on inhibitory signals with lower doses indicate a higher susceptibility of inhibitory signals. Two possibilities have been proposed to explain the apparent affinity of TeNT for inhibitory synapses (Mellanby and Green 1981). One argument is based on the preferential localization of inhibitory synapses on the motoneuronal soma and proximal dendrites where TeNT would achieve higher concentrations. Alternatively, TeNT could be preferentially translocated into inhibitory synapses. In agreement with this proposal afferent terminations on abducens neurons from contralateral vestibular neurons (excitatory) are distributed on the dendrites and more distal to ipsilateral (inhibitory) synaptic inputs (Destombes and
Rouvière 1981). Likewise, as previously reported, EPSPs time courses were slower after high-dose TeNT (González-Forero et al. 2002a), suggesting a transition of synaptic inputs toward more distal dendritic compartments (Rall 1967). If retrogradely transported neurotoxin accumulates in a proximal-distal gradient, these synaptic changes can be explained by a preferential action on the more proximal synapses. However, a differential action based exclusively on afferent input localization seems improbable. For example, reticular excitatory and inhibitory terminations are distributed mainly on the somatic membrane of abducens neurons (Destombes and Rouvière 1981; Escudero and Delgado-García 1988; Grantyn et al. 1980), and despite their somatic distribution, the synaptic influence of excitatory burst neurons persisted after the low-dose administration, and although reduced, was also observed in the high-dose–treated neurons. Moreover, tonic inhibition supplied by contralateral prepositus hypoglossi neurons was sensitive to TeNT despite their location on distal dendrites (Escudero and Delgado-García 1988). Thus these observations suggest that the differential sensitivity of inhibitory and excitatory synapses to TeNT cannot be fully explained by their different spatial localization.

In our previous work, we reported no change in the resting membrane potential, action potential, or afterhyperpolarization amplitude and duration following TeNT treatment (González-Forero et al. 2002a). Similarly, TeNT did not alter the electrical properties of spinal motoneurons, striatal, or hippocampal neurons (Berger et al. 1987; Calabresi et al. 1989; Wiegand and Wellhöner 1979). Therefore the firing alterations observed here are best explained from the presynaptic action mechanism of TeNT and the blockade of selected synaptic inputs. However, we observed in the second and third week after the low-dose application, an increased latency of antidromic activation in treated motoneurons, which could indicate axonal alterations. Conduction velocity alterations reverted in the fourth week postinjection, in coincidence with the reestablishment of the normal firing pattern. Similar results have been reported in spinal motoneurons recorded at the initial states of local tetanus (Kanda and Takano 1983) and in several clinical cases of severe tetanus (Shahani et al. 1979). In relation to the present findings, Munson et al. (1997) demonstrated that chronic constant stimulation of the gastrocnemius muscle nerve induced a partial fast-to-slow transformation of deafferented motoneurons. Therefore the slower conduction velocity we observed in TeNT-treated motoneurons could be a consequence of their chronic firing patterns and not a direct action of TeNT. The expression, modulation or density of voltage-dependent sodium channels is tightly regulated by electrical activity and the concerted action of neurotransmitters (Desai et al. 1999; Sashihara et al. 1997). Therefore it is possible that the altered synaptic transmission and firing patterns contributed to change axonal properties.

Endocytic and transneuronal pathways

The onset of central actions 2 days after TeNT peripheral injection is in agreement with studies on the retrograde transport and accumulation of TeNT fragments injected peripherally (Horn and Büttner-Ennever 1990). Our results also indicate that TeNT-induced alterations derive from the central synaptic blockade and not from the functional disconnection of the muscle because 1) TeNT shows a higher affinity for central synapses, 2) peripheral effects had a shorter time course than central alterations observed in abducens neurons, 3) alterations in firing and synaptology also affected abducens internuclear neurons, and finally, 4) the firing of axotomized or target-deprived motoneurons and internuclear neurons (de la Cruz et al. 1994 2000; Delgado-García et al. 1988) differs in many aspects from TeNT treatment.

Firing alterations in abducens motoneurons after peripheral BoNT injection are similar to changes described here, but only occur after a longer delay (10–12 days). Also, following BoNT injection, there is an initial period that best resembles the axotomized state (Moreno-López et al. 1997). Both TeNT and BoNT can therefore block central synaptic transmission, preferentially inhibitory inputs, but TeNT shows a higher affinity and retrograde transport capability than BoNT. In addition, BoNT blocks neuromuscular neurotransmission and results in flaccid paralysis, while TeNT largely bypasses the neuromuscular junction.

The present findings do not allow us to determine how TeNT reaches the afferent terminals on motoneurons and internuclear neurons. Ultrastructural studies have showed that TeNT is transported intraxonally and retrogradely toward the motoneuronal soma (Price et al. 1975). Schwab and Thoenen (1976) proposed that transcellular TeNT migration occurs specifically at the synaptic contact sites. If we assume this is the mechanism of translocation, TeNT could induce functional partial deafferentation on the two neuronal types because their shared common inputs stemming from the same parent axons. Previous experimental manipulations disconnecting abducens motoneurons or internuclear neurons from their target (by axotomy or target ablation) have shown functional and structural deafferentation that exclusively affected the injured or disconnected neuronal group (de la Cruz et al. 1994, 2000; Delgado-García et al. 1988; Pastor et al. 2000). Terminal retraction could affect only axonal branches that do not receive trophic support from their target cells, without implicating axonal collaterals from the same arborization that terminate onto different targets (Bernstein and Lichtman 1999). Likewise, the injection of BoNT in the lateral rectus muscle, which has a mixed action blocking neuromuscular and central synapses, causes firing pattern alterations and synaptic disorganization exclusively in the motoneurons (Moreno-López et al., 1997; Pastor et al. 1997). In this latter case, synaptic stripping from the motoneuron surface also followed the functional blockade, but these were less profound than the blockade obtained with TeNT. Altogether, it seems that alterations in internuclear neurons after TeNT probably derive from the rapid and effective central accumulation of the toxin and its diffusion through shared terminal arborizations blocking synapses on both motoneurons and internuclear neurons. A similar mechanism could explain γ-motoneuron disinhibition and hyperactivity after intramuscular TeNT application (Takano and Kano 1973), since TeNT is not retrogradely transported by γ-motoneurons (Green et al. 1977). However, it cannot be excluded that TeNT translocation occurs also by paracellular pathways.

Functional recovery

Discharge alterations lasted for about 3 wk and a progressive functional recovery was observed during the following weeks. After 30–40 days, normal firing characteristics were reestab-
lished. A similar time course has been observed in other chronic studies (Brace et al. 1985; Collingridge and Davies 1980). The temporal course of TeNT action could be limited by metabolism and/or activation of compensatory mechanisms like formation of new synapses. The mean lifetime of TeNT in spinal cord cultures was estimated to be 5–6 days; however, elevated TeNT levels were detected four weeks (Habig et al. 1986). Neuromuscular paralysis and synaptic blockade induced by BoNT has comparatively a longer duration (≤2 mo) (Moreno-Lopez et al. 1997). Raciborska and Charlton (1999) suggested that the longevity of the BoNT-induced effects could result from the persistence of SNAP-25 fragments forming complexes with syntaxin molecules in the presynaptic membrane and preventing the insertion of de novo formed SNAP-25 molecules. Therefore functional reversibility after central blockade by TeNT could be delimited in time by several factors, such as mean TeNT lifetime, turnover rate of cleaved synaptobrevin molecules, and the replacement of nonfunctional synapses.

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