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Transient Enhancement of Low-Threshold Calcium Current in Thalamic Relay Neurons After Corticectomy

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SUMMARY AND CONCLUSIONS

1. The alterations of voltage-sensitive calcium currents produced in thalamic cells by injury were investigated under voltage clamp using patch-clamp recordings in the whole-cell configuration.

2. One day after unilateral cortical ablation in immature rats (postnatal day 7), low-threshold transient calcium (T) currents in acutely isolated thalamic relay neurons (RNs) were increased by 68% compared with contralateral controls ($P < 0.001$). Three days after the operation, T currents in injured neurons were at 44% of control levels ($P < 0.001$). On the other hand, high-threshold (L) calcium currents in RNs did not change over the same interval.

3. To investigate the mechanism for the increase of T current, both kinetics and voltage dependency of activation and inactivation were examined. At a test voltage of −40 mV, the activation time constant decreased from 4.1 to 3.2 ms ($P < 0.05$); however, this small change was insufficient to explain the large increase in T current. Time constants for both fast and slow inactivation did not change significantly, nor did voltage dependence of activation or inactivation of thalamic T currents.

4. Methylphenylsuccinimide (MPS, 1 mM), a compound known to block T currents, was used to examine possible alterations in the pharmacological properties of T channels after injury. MPS was more effective in reducing T currents in normal versus injured RNs (24 and 20% reductions, respectively; $P < 0.05$), suggesting that pharmacological properties of T channels in the injured RNs may be different from those of the normal RNs.

5. These findings suggest that cortical injury producing thalamic degeneration alters the expression of T currents in RNs, mainly through changes in the number of available channels or channel conductance. We speculate that this early increase in T current may participate actively in the process of thalamic degeneration after cortical lesions, because the alterations of T current by injury are quite selective.

INTRODUCTION

Injury to the cerebral cortex brings about a degenerative reaction in thalamic relay neurons (RNs), which project to and receive input from the damaged cortical areas and are therefore axotomized and deafferented. One of the most interesting aspects of this thalamic retrograde degeneration (TRD) is the extreme sensitivity of involved neurons. For example, Chow and Dewson (1966) estimated that 80% of thalamic neurons in rabbit lateral geniculate body degenerated within 3 days after a unilateral ablation of visual cortex. Despite the wealth of morphological and biochemical information about this phenomenon, the cellular mechanisms have not yet been clearly identified.

Recently, it has been suggested that an "excitotoxic" process is the common mechanism for TRD induced in adult mice by either cortical ablation or kainate injection (Ross and Ebner 1990). In brief, the hypothesis is that excessive glutamate (Glu) released from axons of injured corticothalamic projection cells accumulates in the milieu of the affected RNs and induces overexcitation, which in turn leads to degeneration. It is, however, questionable whether such an excitotoxic process is the main mechanism for TRD. If this were the case, it would be expected that Glu would also be released from thalamocortical and corticothalamic collaterals in the thalamic nucleus reticularis (nRt) and produce excitotoxic degeneration of nRt neurons, because these cells are known to be highly vulnerable to the effect of kainate, an excitotoxin (Peterson and Moore 1980). However, nRT neurons do not degenerate during TRD, suggesting that the excitotoxic process itself may not be sufficient to lead to death of RNs. Because RNs but not nRt neurons are axotomized and deprived of target cells after a cortical lesion, axotomy itself may be an important factor that determines the fate of RNs. Direct trauma and subsequent loss of cytoplasm is probably not sufficient to explain axotomy-induced cell death, because not all of the axotomized neurons degenerate. A neurotrophic hypothesis has also been suggested to explain TRD (Matthews 1973) and supported by several in vitro and implantation studies (see Cunningham et al. 1988). According to this proposal, a neurotrophic factor, probably transported retrogradely to RNs from corticothalamic cells, is necessary for the maintenance of normal thalamic function and RN survival. However, colchicine, which blocks retrograde transport of horseradish peroxidase in thalamocortical axons, does not cause TRD (Ross and Ebner 1990). To the extent that such blockade might affect other axonal transport processes, this finding argues against the neurotrophic hypothesis.

It is interesting to note that these two explanations for TRD may require alterations of intracellular calcium ion concentration ($[Ca^{2+}]_i$). Ross and Ebner (1990) suggested that a Glu-induced excitotoxic process sustains high levels of $[Ca^{2+}]_i$ through activation of transient calcium channels (T channels) or channels coupled to *N*-methyl-D-aspartate receptors, resulting in a cascade of calcium toxicity followed by death. Calcium ions may also play an important role in the cell death that follows neurotrophic deprivation, as evidenced by protective effects of calcium channel blockers (Johnson et al. 1992; Koike et al. 1989; Rich and Hollowell 1990). These considerations lead to the hypothesis that axotomy of RNs produces cell injury through alterations in membrane calcium conductances. It is known that axotomy can lead to reorganization of voltage-gated conductances for sodium and potassium ions in mamma-

lian motoneurons (Laiwand et al. 1988; Sernagor et al. 1986). Evidence from invertebrate studies suggests that calcium action potentials are unaltered after axotomy (Goodman and Heitler 1979; Kuwada 1981); however, the effects of such injury on the several types of calcium currents now known to exist in neurons were not examined. Further, there are no data available relevant to possible alterations in calcium or any other ionic conductances in injured neurons of the mammalian brain. In the experiments reported here, we studied whole-cell calcium currents of injured RNs that projected to the area of a cortical ablation. Results show a selective short term upregulation of a low-threshold Ca current (T current) in these cells.

METHODS

Surgical procedures

Sprague-Dawley rats, ranging in age from postnatal day 7 (P7) to 10 (P10), were anesthetized with 0.4 ml of methoxyflurane solution and immobilized with adhesive tape. The skull was exposed and opened with a dental drill and fine scissors to produce a large window overlying the left hemisphere. The dura was opened, and a large cortical suction-ablation lesion of SmI (sensorimotor area I) (Zilles and Wree 1985) was made with a 20-gauge blunt needle. Care was taken to make the location and extent of lesions approximately equivalent in different animals. All surgical protocols were approved by Stanford Institutional Animal Care and Use Committee, and the investigators adhered to the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

Four lesioned animals were deeply anesthetized with pentobarbital sodium (50 mg/kg) and perfused with 50 ml of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) 1, 3, 5, and 7 days later. Standard histological paraffin sections were then prepared and stained with cresyl violet to assess lesion sites and cell changes in thalamus.

Immature animals were used because it is known that TRD is rapid and severe at young ages (Matthews et al. 1977), making it more likely that we would observe significant electrophysiological changes. Also, several anatomic (Durham and Woolsey 1984; Erzurumlu and Jhaveri 1990; Rice et al. 1985) and physiological studies (Armstrong-James 1975) show that the morphological and functional connections between the RNs of the ventrobasal complex (VB) and the target cells of layer IV are formed by P7, making it likely that the ablation would axotomize a large proportion of RNs. An added advantage of using the P7–P10 age group was the relatively high yield of isolated RNs that could be obtained (see below).

Dissociation

The method for acutely isolating thalamic cells has been described elsewhere (Coulter et al. 1989a). Animals were anesthetized with pentobarbital sodium (50 mg/kg) and decapitated, and a block of brain containing the thalamus was removed and placed in chilled oxygenated piperazine-*N,N'*-bis(2-ethanesulphonic acid) (PIPES)-buffered saline, taking care to mark the side ipsilateral to the cortical lesion. Coronal 500-μm-thick slices containing the VB of the thalamus were obtained using a vibratome (Lancer, St. Louis, MO). The thalamus was dissected free under a stereomicroscope. The thalamic slices were then incubated for 1 h in an enzyme solution containing collagenase (1 mg/ml) and protease (0.5 mg/ml) in PIPES-buffered saline. Slices were removed from the enzyme solution and rinsed twice with enzyme-free saline. We noted a whitish opaque area in thalamus including VB ipsilateral to the cortical lesion. Pieces of VB were cut from this area and triturated with fire-polished Pasteur pipettes. Dissociated cells were plated onto culture dishes (Lux, E & K Scientific, Saratoga, CA) and stored in an oxygenated chamber until use. Cells from the VB contralateral to the injured cortex were used as controls. The PIPES solution contained (in mM) 120 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 25 D-glucose, and 20 PIPES (pH 7.0).

Because rodent VB has practically no interneurons (Coulter et al. 1989a; Ross and Ebner 1990; Sherman and Koch 1990), we assume that most neurons isolated from rat VB are RNs. The rare, small monopolar or bipolar cells isolated from VB, which are known to be γ-aminobutyric acid (GABA)-positive (Huguenard and Prince 1992) were avoided. We selected only cells with triangular somata, which are known to be GABA-negative (Huguenard and Prince 1992). These RNs isolated from the VB ipsilateral to the cortical ablation were not obviously different in shape than those contralateral to the cortical lesion.

Electrophysiological recording

Whole-cell voltage-clamp recordings of calcium currents were obtained with an L/M EPC-7 amplifier (List Medical, Darmstadt, FRG) at room temperature (22–24°C). Patch electrodes (3–5 MΩ) were pulled on a Narishige electrode puller PP-83 (Narishige, Tokyo, Japan) from borosilicate glass capillaries (KG-33, Garner Glass, Claremont, CA; 1.1 mm ID, 1.5 mm OD). The external solution contained (in mM) 155 tetraethyl ammonium chloride, 3 $CaCl_2$, and 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid, pH 7.4. The pipette solution contained (in mM) 110 tris(hydroxymethyl)aminomethane (Tris) phosphate (dibasic), 28 Tris base, 11 ethylene glycol-bis-(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid, 2 $MgCl_2$, 0.5 $CaCl_2$, and 4 Na_2-ATP, pH 7.35. To reduce the run-down of the sustained calcium (L) current, an ATP regenerating system (Forscher and Oxford 1985) consisting of 20 mM creatine phosphate and 50 U/ml creatine phosphokinase (bovine heart, Sigma Type III) was added to the patch pipette solution. Drugs such as α-methyl-α-phenyl succinimide (MPS) were applied using a rapid exchange superfusion system (Johnson and Ascher 1987; Yellen 1982). The device consisted of several Teflon tubes arranged in parallel in one plane. Changes of solution were accomplished by aligning various barrels with the cell.

Leakage and capacitive currents were subtracted on-line from active responses using a P/4 protocol (Bezanilla and Armstrong 1977). Four hyperpolarizing commands, ¼ of the magnitude of the test pulse (P), were applied before each test pulse. The currents evoked by the hyperpolarizing pulses were added to that evoked by the depolarizing command (P), effectively subtracting the leakage and capacitive currents. All data were stored and analyzed using an INDEC Basic-23 system. Nonlinear simplex routines were used to obtain best-fitted Boltzmann and m^2h curves (Huguenard and Prince 1992). A 9-mV liquid junction potential was subtracted from all command potentials. Cell capacitance was determined by integrating the capacitive current transient elicited by a 5-mV hyperpolarization and dividing the resultant capacitative charge by the voltage change (5 mV). Significant differences between groups of data were determined using analysis of variance (ANOVA) and Student's *t* test as appropriate.

RESULTS

Nissl-stained histological sections demonstrated large cortical ablations that involved most of somatosensory cortex and surrounding zones, sparing subcortical white matter and deeper structures. Many normal-appearing neurons were seen in VB 1 day after surgery, but extensive loss of RNs was present after 3 days (see also Barron et al. 1973; Matthews et al. 1977).

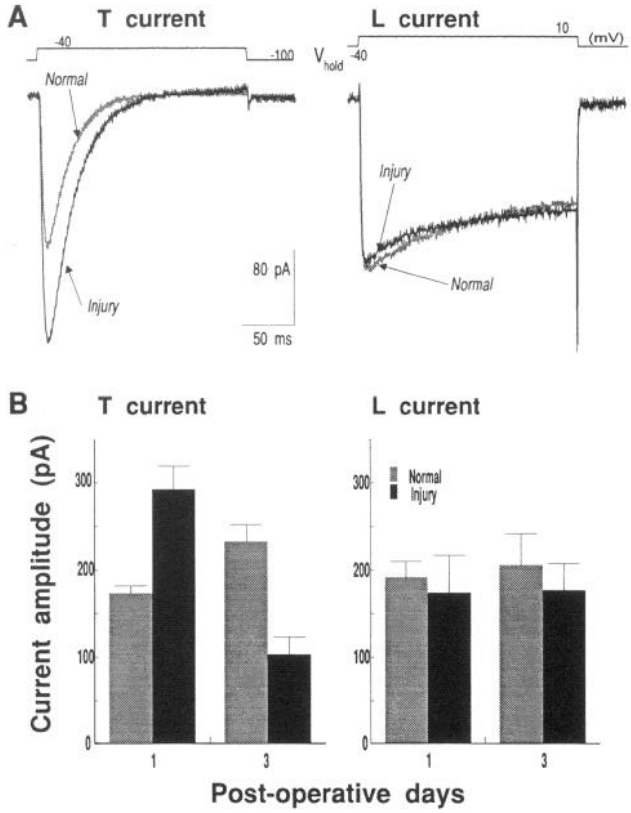

FIG. 1. Effect of injury on calcium currents in relay neurons (RNs). *A*: calcium currents of representative control and injured ventrobasal complex (VB) neurons 1 day after cortical ablation. A transient low-threshold (T) current and a sustained high-threshold calcium current (L current) are elicited by the voltage protocols shown in the *top* traces. The T current reached peak amplitude ~11 ms after the onset of the 200-ms voltage step and decayed completely during the depolarization (time constant for decay: 24.5 ms). The L current decayed slowly during the 200-ms step. Peak T current in the injured neuron was 69% larger than that in the control cell, whereas L currents were similar. T and L current traces are averages of 10 consecutive sweeps. Membrane capacitance (Cm), 17 μF; access resistance, 9.5 MΩ; series resistance (Rs) compensation, 80%. *B*: changes in calcium currents in thalamic VB cells induced by cortical ablation. Currents were elicited as in *A* from rat pups on the 1st [postnatal day 8 (P8)] and 3rd [postnatal day 10 (P10)] postoperative day. Dark bars: Injury. VB neurons ipsilateral to cortical lesion. Shaded bars: Normal. VB neurons contralateral to the injured site, used as controls. Data were collected and averaged from neurons isolated from 6 thalamic slices of 3 different animals, with no more than 3 recordings from each slice. Each sample was the average of 3–5 traces. Sample number, $n = 10$ for 1 day after operation; $n = 6$ for 3 days after operation. T and L currents in each group were from same cells. Error bars, SE. Note that T currents in control neurons increased significantly ($P < 0.01$) as the animals aged from P8 to P10 (*left* panel).

Effects of injury on Ca^{2+} currents in thalamic relay neurons

To isolate T and L currents in RNs, a voltage protocol was used (Fig. 1*A;* see Huguenard and Prince 1992). Voltage steps to −40 mV from a holding potential of −100 mV activate only T current (Fig. 1*A, left*), because the activation threshold for L current is above −40 mV (Coulter et al. 1989a; Fox et al. 1987), whereas a voltage step to +10 mV from a holding potential of −40 mV will activate only L current (Fig. 1*A, right*) because T current is inactivated at this holding potential (Fig. 5*C;* see Coulter et al. 1989a).

In initial experiments, calcium currents were recorded in neurons isolated from postnatal day 8 (P8) rats that had been lesioned at P7. Much larger T currents were obtained from VB neurons ipsilateral to the cortical ablation ("injured" neurons) (−293 ± 84 pA, mean ± SD; $n = 10$) than from the normal cells (−174 ± 26 pA; $n = 10$), as shown in representative traces of Fig. 1*A* (*left*) and group data in Fig. 1*B* (*left*). By contrast, 3 days after operation, much smaller T currents were present in the injured (−103 ± 49 pA; $n = 6$) than in the normal neurons (−233 ± 47 pA; $n = 6$) (Fig. 1*B*). Cortical ablation thus initially increased the amplitude of T currents by 68% (293 vs. 174 pA in injured vs. normal neurons; $P < 0.001$) and decreased T currents by 56% 3 days after operation (103 vs. 233 pA in injured vs. normal neurons; $P < 0.001$). No such dramatic changes occurred in L currents (Fig. 1, *A* and *B, right*). The yield of viable VB neurons and adequate recordings was much lower 3 days after surgery.

The histogram in Fig. 2 shows the distribution of T current amplitudes elicited in both normal neurons and neurons 1 day after cortical ablation (*Injury*). The center of Gaussian curve is shifted to the *right* in injured neurons, showing that T currents in these cells tended to have a higher amplitude. The range of the T currents in the injured group is also much broader than that in normal cells, suggesting that the injured neurons are not as homogeneous with respect to T current as normal cells. Three of 10 injured cells had T currents that were within the normal range. This suggests that some neurons isolated from the VB ipsilateral to the injured site may not be significantly altered with respect to T current generation, either because they did not project to the ablated area or because they were resistant to the effects of the lesion.

Mechanism of injury-induced T current enhancement

The possibility that the increases in T current amplitude were due to increases in RN membrane area was eliminated by using measurements of whole-cell capacitance as an index of cell size and making the assumption that specific membrane capacitance (1 μF/cm^2) was not altered by injury. Total capacitance of normal (14.7 ± 2.1 pF; $n = 8$) and injured neurons (14.7 ± 2.8 pF; $n = 8$) was not significantly different 1 day after injury, although the capacitance

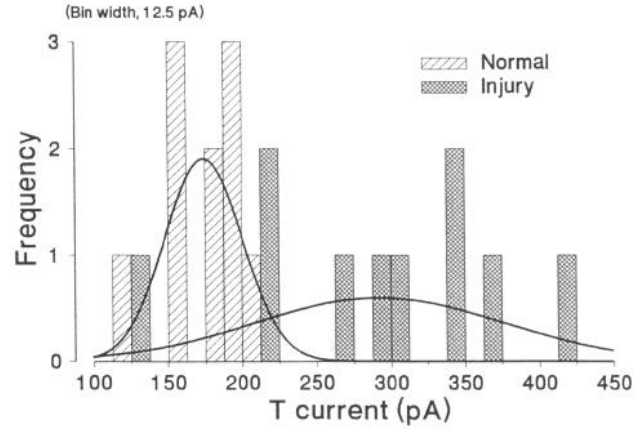

FIG. 2. Histogram of T current amplitudes from the *left* panel of Fig. 1*B*, 1 day postoperation. Values are peak inward currents, and normal distribution curves are superimposed.

of injured neurons (13.4 ± 3.4 pF; $n = 8$) was smaller than that of normal neurons (17.7 ± 2.8 pF; $n = 7$) 3 days after operation ($P < 0.05$).

Alternative explanations for the enhanced T current in injured neurons include changes in either kinetics or voltage dependence of activation or inactivation, an increase in the number of T channels available, or an increase in single-channel conductance. Some of these possibilities were examined.

To determine whether an increased rate of activation or slowed inactivation was responsible for the enhanced T currents, we measured the time constants for activation and inactivation by fitting individual traces to the following m^2h kinetic equation (Hodgkin and Huxley 1952; Huguenard and Prince 1992)

$$I_t = \left[1 - \exp\left(-\frac{t}{\tau_m}\right)\right]^2 \times \exp\left(-\frac{t}{\tau_h}\right) \times I_\infty \qquad (1)$$

where t is time, τ_h is the inactivation time constant, τ_m is the activation time constant, and I_∞ is an amplitude scaling factor. The best fit for the decay component was obtained with a double exponential relation. The decay component at a test potential of −40 mV (Fig. 3*A*) consisted of an initial fast component with a time constant of 17 ms, followed by a small, slow component that had a time constant of 193 ms. Although there were differences in both activation and inactivation rates between injured and normal cells (Table 1), the increase of τ_h did not attain statistical significance, whereas activation was significantly ($P < 0.02$) faster. To confirm the possible alterations in activation, we examined the voltage dependence of τ_m. If the activation process became more rapid after injury, we would expect the τ_m-voltage curve to shift to the left. ANOVA, however, failed to demonstrate a significant change in this relation, although slight increases in τ_m in injured neurons appeared at potentials more depolarized than −40 mV (Fig. 3*B*). In any case, the small changes in activation kinetics alone (Table 1) could not account for the large changes in current amplitude (Fig. 4*B*). We also calculated that the average decrease in τ_m, combined with the increase in τ_h, would result in a 16% increase in peak current (Fig. 4*C*). Obviously, these changes in kinetics cannot explain the >50% increase in current produced by injury, although they might contribute in a minor way.

A third candidate mechanism by which injury could enhance the T current might be a shift in the voltage dependence either of steady-state inactivation to more depolarized levels or of activation to more hyperpolarized levels, which would increase the number of available T channels that could be activated by a given depolarizing command. Figure 5*A* shows T currents in an RN in response to a voltage step protocol designed to elicit steady-state inactivation of this current. The peak current was progressively reduced as the prepulse potential became less negative, because of an increase in the level of steady-state inactivation. The *left* side of Fig. 5*C* shows the inactivation curves for the normal (▲, $n = 13$) and the injured groups (●, $n = 10$). In each cell, peak current values from the test pulses were normalized to the maximal current amplitude and the ratios then fit to the following Boltzmann relation with a power factor $N = 1$

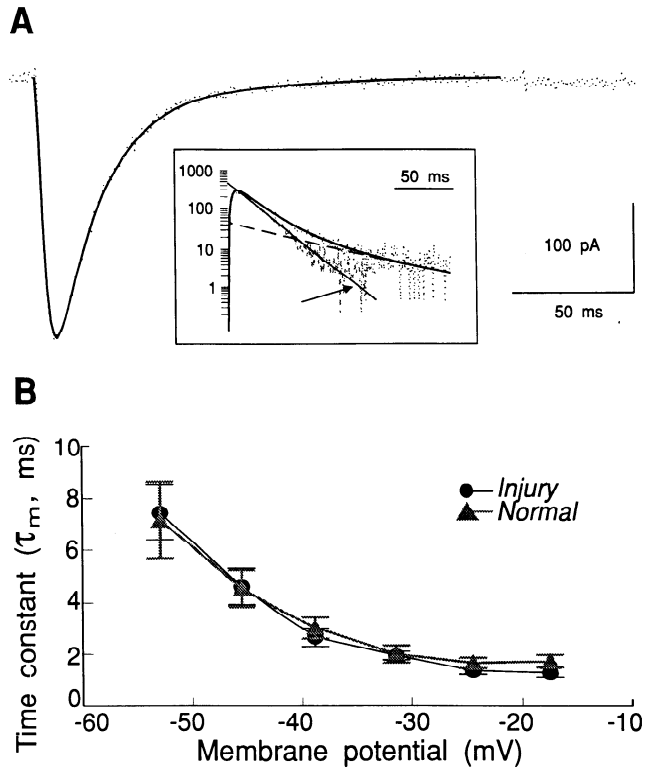

FIG. 3. Effect of cortical injury on kinetics of activation and inactivation of T current in RNs. *A*: time course of T current in a VB neuron injured by cortical ablation. The superimposed smooth curve represents the best fit to m^2h kinetics. *Inset*: semilog plot of the current. The decay followed a double exponential relation that had an rapid component followed by slower component. One of the linear fits (→; ——) represents the rapid component peeled from the slower one (- - -). Fitting parameters for activation time constant (τ_m): 3.7 ms; inactivation time constant (τ_h): 16.55 ms for fast inactivation and 193 ms for slow inactivation. *B*: relationship between τ_m and membrane potential. τ_m was obtained from T currents evoked by step commands to various membrane potentials between −80 and −24 mV from a holding potential (V_{hold}) of −100 mV. No significant differences in the τ_m-potential relation were found between the normal (▲, shaded line) and injured neurons (●, ——). Error bars: SE.

$$\frac{I}{I_{max}} = \left(\frac{1}{1 + \exp\left(\frac{(V_h - V_m)}{k}\right)}\right)^N \qquad (2)$$

where V_h is the membrane potential for 50% inactivation, V_m is the membrane potential, k is the slope factor describing the steepness of the relation, and N is the power factor that may represent the number of (in)activation gates per channel. The V_h for the normal neurons was −82.3 ± 4.3 mV ($n = 13$), compared with −82.8 ± 4.0 mV ($n = 10$) in the injured cells. The slope factor was also not affected and was 4.8 ± 0.5 mV/*e*-fold change for the normal ($n = 13$) and 4.5 ± 0.5 mV/*e*-fold change for the injured cells ($n = 10$). To determine whether the voltage dependence of activation was changed by injury, we made measurements of tail currents at hyperpolarized potentials and assumed that activation processes in both control and injured groups were similarly affected by inactivation, because no differences in kinetics and voltage dependence for inactivation were found between two groups (Table 1 and Fig. 5*C*). The current traces of Fig. 5*B* are responses to depolarizations

TABLE 1. *Comparison of the time constants for T current kinetics in normal and injured RNs*

Time Constants, ms	Control	Injury
τ_m	4.08 ± 0.20	3.24 ± 0.25*
τ_{h1}	18.93 ± 0.57	20.68 ± 0.79
τ_{h2}	222.33 ± 14.73	318.56 ± 83.45

Values are means ± SE, and were obtained from best-fit curves to T currents, elicited by a voltage step to −40 mV from a holding potential of −100 mV, in RNs isolated from 4 different brain slices of 2 different animals. n = 8 for each group. T current, low-threshold transient calcium current; RNs, relay neurons; τ_m, activation time constant; τ_{h1}, fast inactivation time constant; τ_{h2}, slow inactivation time constant. * $P < 0.05$ in 2-tail t test.

from −100 mV to various potentials (*top* traces). In each cell, tail currents at the arrow (1.5 ms after the test pulse) were normalized to the maximal value, and then the ratios were fit to the second-order Boltzmann relation (power factor N = 2; Huguenard and Prince 1992). The V_h was −59.3 ± 3.8 mV for control neurons (n = 11) and −60.0 ± 3.9 mV for injured cells (n = 11). k was −5.7 ± 1.3 mV/e-fold for the normal (n = 11) and −4.5 ± 0.7 mV/e-fold for the injured cells (n = 11). There were no significant differences in either V_h or k between two groups (Fig. 5*C*).

Effect of MPS on T current

The above results suggest that the increase in T current produced by cortical injury is not mainly due to the alterations in the voltage-dependent kinetics. Therefore, it appears that changes in whole-cell T currents are due to the increases in either the number of available T channels or the unit conductance. New T channels in the soma could either arise from nonsomatic membrane after axotomy, as has been proposed for Na^+ current (Gilly and Brismar 1989), or from de novo synthesis. We examined the possibility that the T current in injured cells, which was indistinguishable from that in normal cells with regard to gating properties, might have different pharmacological sensitivity. T currents in RNs are known to be reduced by the succinimide class of petit mal anticonvulsants (Coulter et al. 1989b, 1990), such as ethosuximide (ES) and MPS. Because MPS is greater in efficacy than ES (Coulter et al. 1990), we examined the effects of MPS on T currents in injured neurons. Typical results are shown in Fig. 6*A*. Application of 1 mM MPS reduced T current (elicited by the depolarization to −40 mV from −100 mV) in a normal RN by 29.9% (Fig. 6*A*), an effect comparable with the previous results (Coulter et al. 1990). On the other hand, the same concentration of MPS reduced the T current only 18.4% in the injured RN of Fig. 6*B*. Figure 6*C* shows the percentage of current reduction by MPS. In the normal group, one mM MPS reduced the amplitude of T current by 23.6 ± 3.1% (n = 5 animals). In the injured cells, the same concentration of MPS reduced the current by 20.2 ± 1.8% (n = 5 animals). Cortical ablation thus decreased the effectiveness of T current block by MPS by 17% (20 vs. 24% in injured vs. normal neurons; $P < 0.05$). It is, therefore, possible that the T currents increased by injury have a different sensitivity to MPS than T currents in normal RNs. These results provide indirect support for the conclusion that the increase in T current after injury is due to either alterations in existing channels or increase of a new type of channel with similar gating but different pharmacological properties.

DISCUSSION

The major finding in these experiments is that T currents in RNs are initially "upregulated" after axotomy and deafferentation. Similar changes have not been reported after injury to other mammalian neurons, although most studies have involved axotomized cells in spinal cord (Gustafsson 1979; Kuno et al. 1974; Pinter and Vanden-Noven 1989) and brain stem (Delgado-Garcia et al. 1988; Takada et al. 1980) rather than those within the brain, and specific classes of calcium currents have not been examined.

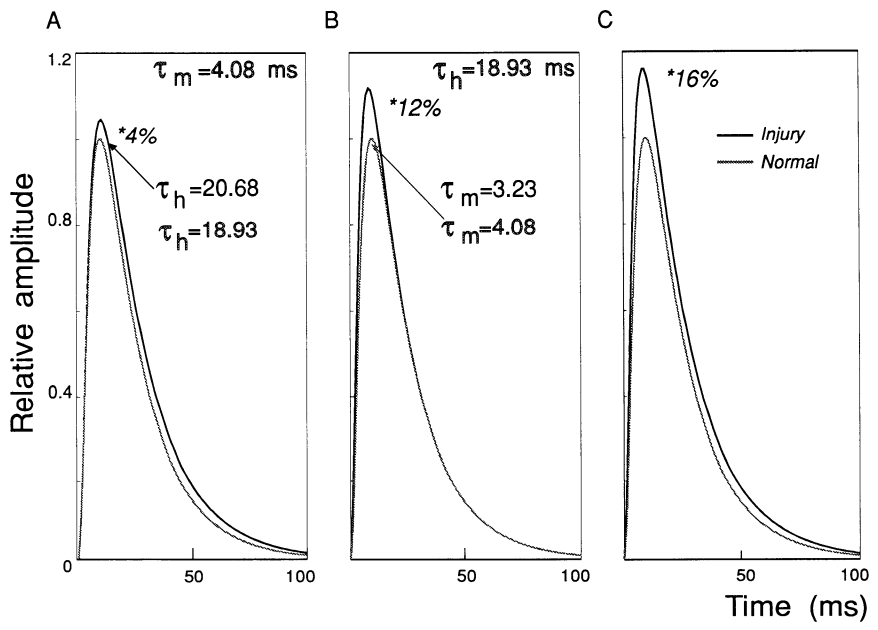

FIG. 4. Expected increases in T current based on the alterations of gating kinetics induced by injury. Relative increases of T currents in injured thalamic neurons (——) with respect to T currents in control cells (shaded lines) are 4, 12, and 16%, respectively, when different kinetic parameters for inactivation (τ_h in *A*), activation (τ_m in *B*), and both inactivation and activation (τ_h and τ_m in *C*) are considered. Calculated from m^2h kinetics with various time constant values in Table 1: 4.08 and 3.23 ms for τ_m and 18.93 and 20.68 ms for τ_h, respectively, for normal and injured cells.

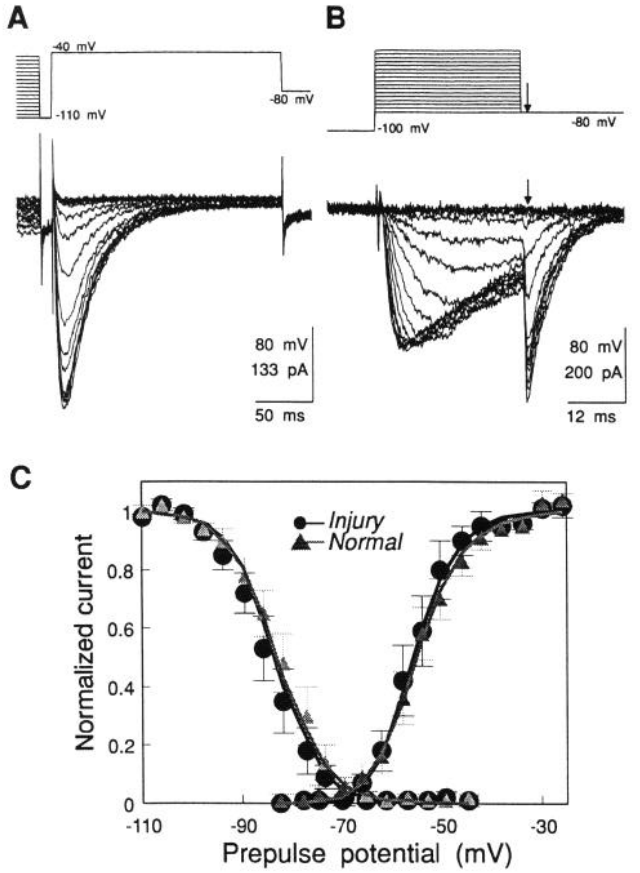

FIG. 5. Effect of cortical injury on the voltage dependence of steady-state inactivation and activation of thalamic T current. *A*: currents in an injured RN in response to voltage protocol (above) in which 1-s prepotentials in the range between −110 and −44 mV were followed by a voltage step to −40 mV. No leak subtraction was used for this protocol. *B*: T currents activated by 30-ms depolarizations from a V_{hold} of −100 mV to various potentials ranging from −80 to −24 mV in 4-mV steps. Return voltage steps to −80 mV generated tail currents. Recordings in *A* and *B* obtained from the same cell. *C*: inactivation-activation curve. The *left* side of this panel shows the inactivation curves for the normal (▲; mean ± SE, $n = 9$) and the injured cells (●; $n = 6$). Peak amplitudes of T current from the test pulses were normalized to the maximal current recorded from each cell. The normalized values were fit with a 1st-order Boltzmann relation (shaded line, control; ——, injured), from which the slope factor (k) and the voltage for 50% inactivation (V_h) were determined. On the *right* side of this panel are the activation curves for the normal (▲, $n = 7$) and the injured groups (●, $n = 7$). The amplitudes of tail currents 1.5 ms after the termination of the test pulse (→ in *B*) were normalized to the maximal value from each cell. Boltzmann parameters, k and V_h, were obtained by fitting the normalized values to a 2nd-order Boltzmann relation.

Significance of T-current increase in thalamic retrograde degeneration

Robust T currents, which play an important role in generating and maintaining thalamocortical oscillatory interactions (Steriade and Llinás 1988), may contribute indirectly to the extreme sensitivity of RNs to cortical injury. Given the potential importance of disorders of intracellular calcium homeostasis in the development of neural death (Choi 1987; Johnson et al. 1992; Murphy and Miller 1989; Siesjö 1988), it seems to be reasonable to speculate that enhanced T currents play a role in TRD. Indeed, the transient increase of T current in RNs after injury is selective in that neither sodium, potassium, nor L currents show a similar enhancement (unpublished data).

One of the problems in electrophysiological studies of neuronal responses to axotomy is the wide range of effects from cell to cell, even within homogeneous populations. The standard deviations of T currents in normal and injured RNs of P8 rats were 26 and 47 pA, respectively (Fig. 1*B*), indicating that T-current amplitudes in injured cells were much more variable than those in control neurons. The histogram of Fig. 2 shows that 10–30% of injured cells have T currents with amplitudes within the range of the distribution of the normal samples. This variability could be due to *1*) incomplete removal of the cortical area to which RNs in the VB project so that some RNs might have escaped axotomy and/or deafferentation at the time of the cortical surgery, *2*) inclusion of neurons from outside VB in the sample, or *3*) a heterogeneous response to injury among RNs. The first two possibilities appear less likely, because observation of thalamic slices before isolation showed that the entire VB area had a whitish opaque appearance on the injured side, and tissue for isolation came from this zone. It is interesting to note that 2 days after cortical ablation in P7 rats, ~30% of VB neurons survive (Matthews et al. 1977),

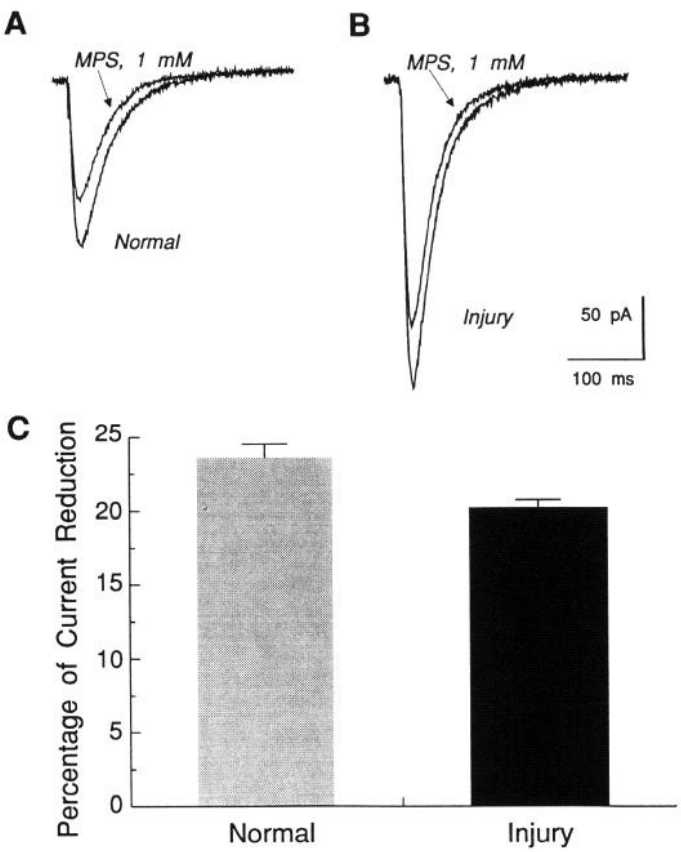

FIG. 6. Differences in effects of 1 mM α-methyl-α-phenyl succinimide (MPS) on T currents in normal and injured neurons. T currents were elicited by a voltage step to −40 mV from a V_{hold} of −100 mV. One mM MPS reduced T current by 30% in the normal cell (*A*) and by 18% in the injured neuron (*B*). *C*: MPS (1 mM) reduced T currents by 20% (average of 11 cells from 9 slices; $n = 5$ animals) in the injured RNs, which is significantly ($P < 0.05$) less than the 24% reduction (average of 11 cells from 9 slices; $n = 5$ animals) of T currents in the normal cells. Error bars: SE.

indicating that a significant population of thalamic cells (termed "residual neurons") remains unaffected by cortical ablation. It is possible that the injured RNs that did not show increases in T current were such residual neurons.

Possible mechanisms for the increase of T current

No significant changes in inactivation kinetics (Table 1) or voltage dependency of activation and inactivation (Fig. 5) were observed after injury. The small but statistically significant change in τ_m at −40 mV (Table 1) would not be sufficient to explain the large increase in current amplitude (Fig. 4). Therefore the enhancement of the T currents in injured RNs would have to be due to either an increase in the number of T channels or their unit conductance. The decreased activity of MPS on the enhanced T currents in injured cells (Fig. 6) suggests that the pharmacological properties of T current channels may have been altered and indirectly supports the possibility that a new type of T channel may be expressed after injury. This might occur through de novo synthesis, modification of existing T channels, or a combination of these processes.

One hypothesis for the transient increase of T currents in TRD is the presence of a trophic factor that is normally retrogradely or anterogradely transported from corticothalamic cells to RNs and acts to suppress the overexpression of T channels. The blockade of transport by axotomy would then result in the enhanced expression of T channels, a large influx of calcium through the overexpressed T channels into the injured cell during excitation, and a consequent intracellular cascade resulting in cell death. A role for trophic factors in TRD is suggested by several studies (Cunningham et al. 1988; Matthews 1973; Yamada et al. 1991), although neither the underlying cellular mechanisms nor the possible relationship of a trophic factor to voltage-sensitive calcium conductances in TRD has been elucidated.

Another possible mechanism for the increase of T currents would be a redistribution of T channels after injury, triggered in some way by changes in dendritic membranes (see Ruit et al. 1990; Yawo 1987). If the T channels were distributed in normal dendrites, retraction of distal dendrites, which is known to occur after axotomy (Braennstroem et al. 1992; unpublished observations), might be associated with an increased T channel density on proximal dendrites and soma. As a result, T currents would appear larger in the injured RNs than in normal cells. A similar redistribution of channels has been proposed to explain changes in dendritic and somatic sodium spike components after axotomy in cat motoneurons (Sernagor et al. 1986). Obviously, this explanation requires that T-type channels have a relatively selective dendritic distribution or more mobility in the membrane compared with L-type channels, because the L currents were not enhanced by injury.

The possibility that T current alterations play a role in TRD is highly speculative at this point. However, the selective enhancement of this conductance after cortical injury, its temporal pattern, and the fact that other voltage-sensitive currents show no similar upregulation (unpublished data) are findings that tend to support this suggestion.

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