Damage to sensory nerves may result in a painful neuroma. This results from aberrant proliferation of nerve endings in the stump,\(^1\) and may result in neuropathic pain because of persistent abnormal excitability of sensory nerve endings.\(^2\) A number of techniques have been described to reduce the incidence of neuromas in sharply transected nerves. These include burying in muscle,\(^3\)\(^4\) capping with vein,\(^5\) and even oblique transection of the nerve.\(^6\)

In the management of peripheral nerves, where neuromas are typically encountered and studied, these are sharply transected where indicated in such situations as amputation surgery or trauma. In the senior author’s (B.G.) practice, however, a significant number of patients present for migraine surgery, with one of the trigger sites requiring ablation of the zygomaticotemporal branch of the facial nerve.\(^7\) The small caliber of the nerve and the surgical approach allow avulsion or transection and possibly transection and folding of the nerve. Another situation where avulsion of nerves may occur is in surgical approaches to the upper craniofacial skeleton and midface, where the supraorbital, supratrochlear, or infraorbital nerves may be damaged close to the orbital rim where the available muscles (corrugator and

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depressor supercilii and procerus muscles) are removed for therapeutic or aesthetic reasons. An unanswered question, therefore, is the effect of nerve avulsion or folding on formation of neuromas.

In this study, we investigated the effect of four different techniques of nerve ablation on the incidence and degree of neuroma formation. These four methods included nerve avulsion, nerve transection, folding a nerve, and sharply transecting a nerve and burying in muscle, which served effectively as the negative control for neuroma formation.

**MATERIALS AND METHODS**

**Surgical Procedures**

Thirty adult Sprague-Dawley rats were used in this study, with both legs of each animal subjected to different interventions using a sural nerve model. The 60 nerves studied were assigned randomly to a different intervention, with experimental groups as follows: group 1, avulsion; group 2, transection; group 3, folding; and group 4, muscle burying. Control nerves (n = 15) were obtained from both legs of other animals of the same rat species and age, not subjected to any intervention, that were used for microsurgical practice by plastic surgery residents, for the purposes of all analyses. The study was approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Animals were anesthetized through induction with intraperitoneal injections of ketamine hydrochloride (100 mg/kg) and xylazine (5 mg/kg), and subsequently maintained under inhalational anesthesia for the duration of the operation. An incision was made on the dorsal surface of the leg from the mid thigh to the mid leg. The biceps femoris was divided and the sural nerve exposed (Fig. 1, left). The identity of the nerve was confirmed through proximal dissection to the trifurcation of the tibial, common peroneal, and sural nerves (Fig. 1, right).

For group 1 (avulsion), a 1-cm segment of nerve was removed through proximal and distal avulsion using a nontraumatic forceps, with care taken not to crush the nerve. The nerve was gripped 2 mm distal to the trifurcation point with a smooth forceps. A second forceps was then used to grip the nerve distal to the point held by the first forceps, and steady moderate traction in a cephalic direction was used to avulse the nerve proximally. In a similar fashion, the nerve was avulsed distally using steady moderate traction in a cephalic direction to create a 1-cm nerve gap. For group 2 (transection), a 1-cm segment of nerve was removed through sharp transection of proximal and distal nerves. For group 3 (folding), a 1-cm segment of nerve was removed through sharp transection. The proximal nerve stump was then folded onto itself for a length of 5 mm and secured with a single 6-0 Ethilon suture (Ethicon, Norderstedt, Germany). For group 4 (muscle burying), a 1-cm segment of nerve was removed through sharp transection. The proximal nerve stump was then buried in a pocket created in the gastrocnemius muscle and secured with a single 6-0 Ethilon suture. All surgical procedures were performed under an operating microscope by the first author (H.C.), with a uniform 1-cm nerve gap created for all experimental groups. Skin incisions were closed with interrupted 5-0 Vicryl (Ethicon) sutures. Animals were

![Fig. 1. A rat sural nerve model was used for this study. (Left) Exposure was achieved through a dorsal incision on the leg with splitting of the biceps femoris. (Right) The sural nerve was identified through proximal dissection to the trifurcation of the tibial, common peroneal, and sural nerves.](image-url)
housed in the animal facility at the Case School of Medicine and had access to unlimited food and water. Postoperative analgesia was achieved with twice-daily injections of buprenorphine (0.01 to 0.05 mg/100 g) for 3 days.

Animals were killed after 3 months, and the entire proximal nerve segment was explanted for analysis. Table 1 summarizes the experimental design.

**Experimental Methods**

Nerves were embedded in paraffin and cut into 5-μm sections for histologic evaluation ($n = 10$ from each group), or processed for real-time polymerase chain reaction for amplification of mRNA ($n = 5$ from each group). Histology stains used included hematoxylin and eosin and Masson trichrome. Immunostaining was performed against S-100 to identify phenotypically differentiated Schwann cells. Histomorphometric analysis ($n = 5$ from each group) was performed using a Leica MZ16F stereomicroscope (Leica Microsystems, Bannockburn, Ill.) and Leica LAS Image analysis software (Leica Microsystems), with the second author (E.M.) performing the analysis blinded to different experimental groups. Nerve cross-sectional area was analyzed on transverse histologic section, and the ratio of neural to connective tissue was calculated within the confines of the epineurium. Sections stained against Masson trichrome was used for analysis. The total area stained blue and red was used to determine nerve cross-sectional area. The ratio of areas stained red (against axons) compared to blue (against collagenous connective tissue) was used to determine the ratio of neural to connective tissue. Three separate transverse section slides of the widest part of each nerve specimen were analyzed separately and the mean designated as the value for each specimen. Histopathologic examination was performed with the aid of a board-certified neuropathologist (M.L.C.) in a single-blind fashion to ensure accuracy of analyses. Because the aim of the study was to compare formation of neuromas following different nerve ablation techniques, only basic measures of nerve survival were used in histomorphometric analysis for this experiment.

Real-time polymerase chain reaction was performed to assess for expression of ciliary neurotrophic factor and calcitonin gene-related peptide. Ciliary neurotrophic factor is a neurotrophic factor and supports and maintains normal function of axons, and is expected to be elevated with nerve regeneration.8,9 Calcitonin gene-related peptide is responsible for maintenance of neuropathic pain, and is an assay of mechanical allodynia.10,11 Thus, it is expected to be increased in neuromas. Quantification of mRNA was performed using Roche Sybr Green I Supermix (Roche, Indianapolis, Ind.) on a Roche Lightcycler480 real-time polymerase chain reaction instrument. Real-time polymerase chain reaction was conducted using 2.5 μl of diluted cDNA per 10-μl assay under the following conditions: denaturation for 10 minutes at 95°C, amplification for 45 cycles at 95°C for 10 seconds and 62°C for 1 minute, with fluorescence measured during the final 95 percent of each 60°C step. Melt curve analysis was performed from 55°C to 95°C at 0.5°C every 10 seconds with continuous fluorescence measurement. Expression of ciliary neurotrophic factor and calcitonin gene-related peptide mRNA was normalized to the mRNA levels of glyceraldehyde-3-phosphate dehydrogenase. The relative ratio of mRNA in treatment groups versus controls was determined using methods described by Pfaffl.12 Controls were obtained from sham-operated rats, with no intervention performed to the sural nerve.

**Table 1. Schematic Diagram of Experimental Design**

<table>
<thead>
<tr>
<th>Group 1 ($n = 15$)</th>
<th>Group 2 ($n = 15$)</th>
<th>Group 3 ($n = 15$)</th>
<th>Group 4 ($n = 15$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avulsion</td>
<td>Transection</td>
<td>Folding</td>
<td>Muscle burying</td>
</tr>
<tr>
<td>Histology ($n = 10$)</td>
<td>Histology ($n = 10$)</td>
<td>Histology ($n = 10$)</td>
<td>Histology ($n = 10$)</td>
</tr>
<tr>
<td>RT-PCR ($n = 5$)</td>
<td>RT-PCR ($n = 5$)</td>
<td>RT-PCR ($n = 5$)</td>
<td>RT-PCR ($n = 5$)</td>
</tr>
</tbody>
</table>

RT-PCR, real-time polymerase chain reaction.
Statistical Analysis

Multiple linear regression methods using JMP8 statistical software (SAS Institute, Inc., Cary, N.C.) were used to compare nerve cross-sectional area and neural-to-connective tissue ratio between different experimental groups and controls. Covariates used for nerve cross-sectional area were nerve size and presence of abnormal and invasive connective tissue. Covariates used for neural-to-connective tissue ratio were nerve size, status of degeneration, and shape. Because of the multivariate nature of the factor variables for each response, multiple linear regression methods were used to develop models for predicting each response. Only factor variables that were statistically significant ($p < 0.05$) were included in the models. Factors not included in the models all had values of $p > 0.10$. The goodness-of-fit for each model was based on a comparison of the square root of the mean square error from the regression analysis, with the pure error estimate from the repeated measurements of the same nerve using an $F$ test ($p < 0.05$) rather than relying on a test based on the $R^2$ value.

One-way analysis of variance with the Tukey-Kramer multiple comparisons test was performed using the GraphPad InStat version 3.05 for Windows 95/NT computer program (GraphPad Software, Inc., San Diego, Calif.) to compare relative expression of mRNA for ciliary neurotrophic factor and calcitonin gene-related peptide between experimental groups and controls.

RESULTS

All animals survived after surgery and did not exhibit movement patterns or distress postoperatively suggestive of neuropathic pain. There were no perioperative or late postoperative complications of surgery. No trophic changes or gait disturbance was observed following surgery.

Nerve cross-sectional area (Fig. 2, above) was highest in folded (mean, 8.8 ± 2.4 mm$^2$) followed by muscle buried (4.7 ± 0.7 mm$^2$) and transected (4.0 ± 0.4 mm$^2$) specimens. Nerve cross-sectional area in folded, muscle buried, and transected samples was statistically increased ($p < 0.05$) compared with control (3.1 ± 0.4 mm$^2$) and avulsed (2.2 ± 0.4 mm$^2$) specimens. Increased cross-sectional area of the proximal nerve stump is often indicative of a disordered bundle of overgrown terminal nerve fibers and suggestive of neuroma formation. This value was highest for transected (1.2 ± 0.1), muscle buried (1.2 ± 0.2), and folded nerves (1.2 ± 0.1), followed by control specimens (1.1 ± 0.1). Avulsed specimens (1.0 ± 0.1) had a statistically decreased neural-to-connective tissue compared with control specimens, correlating with decreased abnormal nerve tissue from neuroma formation.

Histologic analysis of specimens in groups 2 and 3 (transection and folding) showed evidence of disordered myelinated fibers with overgrowth of axons and loss of normal neural architecture. In contrast, specimens in groups 1 and 4 (avulsion and muscle burying) showed preservation of normal fascicular architecture with parallel arrangement of nerve fibers. Representative sections stained with Masson trichrome (Fig. 3) and S-100 are shown in Figure 4.

Relative mRNA expression of ciliary neurotrophic factor (Fig. 5, above) was lowest in muscle...
buried (4 percent of control) \((p < 0.05)\) and avulsed specimens (15 percent of control) \((p < 0.05)\) and higher in folded (52 percent control) and transected specimens (75 percent of control). The relative expression of ciliary neurotrophic factor for muscle buried and avulsed specimens was statistically lower compared with control specimens \((p < 0.05)\). No statistical difference was seen between folded and transected specimens and controls. These findings suggested that regeneration of the proximal nerve stump was strongly inhibited in muscle buried and avulsed groups.

Relative mRNA expression of calcitonin gene-related peptide for folded specimens was statistically higher compared with controls. This suggests that animals in this group experienced a significant degree of neuropathic pain. No statistical difference was seen between transected, avulsed, and muscle buried specimens and controls. A summary of results for different groups is presented in Table 2.

**DISCUSSION**

Prevention of neuroma formation is essential for achieving an optimal clinical outcome after nerve ablative surgery. Surgical procedures involving nerve ablation have evolved from transection of peripheral nerves during extirpative or trauma surgery of the extremities to, more recently, therapeutic interventions for chronic pain. The results of this study provide insights into the mechanisms underlying neuroma formation and suggest potential strategies for preventing or mitigating this adverse outcome.
pain\textsuperscript{13,14} and surgery for treatment of migraine headaches.\textsuperscript{7,15,16}

A favored technique for decreasing the incidence of neuroma formation is implantation of the proximal nerve stump into adjacent muscle.\textsuperscript{17,18} This was described in 1985\textsuperscript{17} and is used widely in clinical practice. Other described techniques\textsuperscript{19} include capping with a vein graft, transection with lasers, epineural sleeves, and end-to-side or end-to-end nerve anastomoses where possible. All of these varied techniques have the aim of blocking neurotropic signals from the distal nerve stump or preventing aberrant proximal nerve stump regeneration. Experimental studies have also linked a shorter nerve gap with increased incidence of neuroma formation.\textsuperscript{20}

In our clinical experience with ablation of smaller nerves in the head and neck for migraine surgery, techniques such as muscle burying or vein capping are technically difficult because of the surgical approach. Ablation of the zygomaticotemporal branch of the trigeminal nerve for treatment of migraine headaches\textsuperscript{21} in the practice of the senior author (B.G.) is achieved through an endoscopic brow-lift approach, with all dissection performed under endoscopic visualization. The small caliber of this sensory nerve allows nerve ablation to be most easily performed through either nerve avulsion or transection. An unanswered question in the literature involves which of these techniques, avulsion or transection, is more efficacious in prevention of neuromas.

The results of this study show that nerve folding, muscle burying, and transection lead to increased nerve cross-sectional area. Histology demonstrates that folding and transection result in increased neuroma formation. Transection and folding also lead to a very high mRNA expression of ciliary neurotrophic factor, correlated with nerve regeneration. Conversely, avulsed nerves had a very low cross-sectional area and low ciliary neurotrophic factor mRNA expression. Expression of ciliary neurotrophic factor in Schwann cells of injured nerves is linked to the

Fig. 4. Representative histologic sections of explanted nerves stained with S-100 (original magnification, \times100) (neural tissue stains brown). (Above, left) Group 1 (avulsion): normal nerve architecture is preserved. (Above, right) Group 2 (transection): evidence of neuroma formation. (Below, left) Group 3 (folding): evidence of neuroma formation. (Below, right) Group 4 (muscle burying): normal nerve architecture with maintenance of fascicular structure is shown.
nerve’s functional state, and studies have shown that ciliary neurotrophic factor is down-regulated when the nerve is demyelinated and undergoes Wallerian degeneration. Similarly, ciliary neurotrophic factor is up-regulated when nerve fibers regenerate. Ciliary neurotrophic factor is therefore a neurotrophic factor and supports and maintains normal function of axons, and is expected to be elevated with nerve regeneration. Thus, nerve avulsion appears to abrogate nerve regeneration effectively and reduce neuroma formation. A possible explanation for this could be that avulsion results in interruption of axons at multiple different levels, therefore inhibiting effective nerve regeneration and neuroma formation in a single level. In addition, nerve avulsion results in a more severe injury than transection, possibly preventing effective nerve regeneration and subsequent neuroma formation.

As the aim of migraine surgery and other interventions aimed at treating neuromas and other sources of chronic pain is to permanently eliminate the pain, another important issue is the incidence of neuropathic pain with different nerve ablation techniques. Calcitonin gene-related peptide has been implicated as one of the neuropeptides involved in peripheral nerve injury–induced neuropathic pain. Injecting calcitonin gene-related peptide into the spinal subarachnoid space was found to reduce pain threshold, whereas injection of calcitonin gene-related peptide antiserum could increase the pain threshold. Therefore, determining the effect of different techniques of nerve ablation on expression of ciliary neurotrophic factor and calcitonin gene-related peptide is also important for determining the degree of nerve regeneration and neuropathic pain.

Interestingly, calcitonin gene-related peptide mRNA expression was highest in the folded nerve group. This is consistent with findings from an experimental study where chronic constriction injury was found to result in increased autonomic fiber sprouting from skin, resulting in increased sensitivity of nociceptive fibers to sympathetic and parasympathetic stimulation. A similar constrictive mechanism would explain the increased level of neuropathic pain in rats with folded nerves. Conversely, the level of calcitonin gene-related peptide mRNA expression was lowest in the muscle buried group. It is possible that burying the proximal nerve stump in muscle abrogates the inflammatory response to pain, which has been

### Table 2. Summary of Results for Different Nerve Ablation Techniques

<table>
<thead>
<tr>
<th>Nerve</th>
<th>CSA (mm²)</th>
<th>N:CTR</th>
<th>CNTF mRNA (% of control)</th>
<th>CGRP mRNA (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avulsion</td>
<td>2.2 ± 0.4</td>
<td>1.0 ± 0.1*</td>
<td>15*</td>
<td>116</td>
</tr>
<tr>
<td>Transection</td>
<td>4.0 ± 0.4*</td>
<td>1.2 ± 0.1</td>
<td>75</td>
<td>137</td>
</tr>
<tr>
<td>Folding</td>
<td>8.8 ± 2.4*</td>
<td>1.2 ± 0.1</td>
<td>52</td>
<td>302*</td>
</tr>
<tr>
<td>Muscle burying</td>
<td>4.7 ± 0.7*</td>
<td>1.2 ± 0.2</td>
<td>4*</td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>3.1 ± 0.4*</td>
<td>1.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSA, cross-sectional area; N:CTR, nerve-to-connective tissue ratio; CNTF, ciliary neurotrophic factor; CGRP, calcitonin gene-related peptide.

*Statistically significant difference (p < 0.05) compared with control specimens.
implicated in the pathogenesis of neuropathic pain through up-regulation of interleukin-6 and increased calcitonin gene-related peptide release mediated in part by invading macrophages. Whether increased calcitonin gene-related peptide expression would necessarily correlate with a painful neuroma in humans has not been shown. However, what is clear is that calcitonin gene-related peptide expressing sensory neurons responds to painful stimuli.

This study is limited by a small sample size and short duration of follow-up. Future studies would aim at a longer duration of follow-up with more detailed neuropathologic histologic analyses, analyses of the distal nerve and cell body, and localization of RNA expression.

CONCLUSIONS
This study suggests that avulsion and muscle burying are both efficacious for ablation of peripheral nerves, and we would advocate the use of both techniques clinically. This study also suggests that nerve folding leads to neuropathic pain, assayed by calcitonin gene-related peptide expression. Avulsion offers an alternative to muscle burying when there is no muscle in the vicinity to bury the transected nerve.

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REFERENCES
New Submission Guideline: Level of Evidence

Beginning with submissions made July 1, 2011, and going forward, all manuscripts amenable to Level of Evidence grading need to indicate the clinical question addressed by the article and the Level of Evidence. The clinical question will be one of three categories: Diagnostic, Therapeutic, or Risk. Please use the ASPS Levels of Evidence and Grading Recommendations: Evidence Rating Scales to grade the level of evidence in your manuscript.

In general, the following types of articles are not gradable for level of evidence:

- Animal studies
- Cadaver studies
- Basic science studies
- Review articles
- Instructional course lectures
- CME courses
- Editorials
- Correspondence

As far as what is or is not ratable, the standard is to exclude basic science, bench work, animal, and cadaveric studies because the information gained from these studies is not something that can be applied directly to patient treatment decisions.

See the article “The Level of Evidence Pyramid: Indicating Levels of Evidence in Plastic and Reconstructive Surgery Articles,” in the July 2011 issue (Plast Reconstr Surg. 2011;128:311–314), for more information on determining the Level of Evidence of your manuscript.

NOTE: While we require authors to provide an initial Level of Evidence grade for their submissions, the final LOE grade for accepted papers will be determined and assigned by an independent panel of LOE experts, whose determination is final.