Original Article

Expression patterns of nociceptin in rats following experimental tooth movement

Lina Liao[a]; Xiaochuan Hua[b]; Hu Long[a]; Niansong Ye[a]; Yang Zhou[c]; Sheng Wang[a]; Wenli Lai[d]

ABSTRACT

Objectives: To determine the expression levels of nociceptin following experimental tooth movement.

Materials and Methods: A total of 72 male Sprague-Dawley rats were divided into two groups: sham and experimental groups. For the experimental group, closed coil springs were used to mimic orthodontic force (80 g) between upper incisors and first molars, and the rats were killed at 0 hours, 4 hours, 12 hours, 1 day, 2 days, 5 days, 7 days, 10 days, and 14 days. All of these procedures were similar for the sham group, except for that no force was applied. The four rats killed at 0 hours without any intervention were used as the baseline control in each group. Trigeminal nucleus caudalis from both the ipsilateral and contralateral sides of force applications were obtained for immunostaining.

Results: Nociceptin was expressed in both the ipsilateral and contralateral sides of each group. Its expression levels started to increase on day 2, peaked on day 7, and returned to baseline on day 10 in the experimental group, while expression levels started to decrease on day 1 and returned to baseline on day 10 in the sham group. Moreover, the expression levels were similar between the ipsilateral and contralateral sides in each group.

Conclusion: The expression levels of nociceptin were elevated following experimental tooth movement. The anesthetic agent used in this study (chloral hydrate) may have an antagonism with nociceptin. Due to bilateral innervation of anterior teeth and bilateral projection of nerve fibers, the expression levels of nociceptin were similar between ipsilateral and contralateral sides. (*Angle Orthod.* 2013;83:1022–1026.)

KEY WORDS: Expression; Immunohistochemistry; Neuropeptide; Orthodontic tooth movement; Trigeminal nucleus caudalis

INTRODUCTION

Orthodontic pain, an inflammatory pain related to tooth movement,[1] is frequently encountered in orthodontic patients.[2] Its incidence has been reported to be 87%–100%.[2–4] Although various approaches have been used to relieve orthodontic pain,[5,6] none of them are truly effective. This may be attributed to yet-to-be-elucidated exact mechanisms underlying orthodontic pain. However, it has been established that many neuropeptides are implicated in orthodontic pain, but still many others are yet to be determined.[1,7–9]

Therefore, discovering the roles of novel neuropeptides can help elucidate the mechanisms underlying orthodontic pain, thereby helping develop an effective approach in relieving orthodontic pain.

Nociceptin, or orphanin FQ, a 17-amino-acid neuropeptide, is the endogenous ligand for opioid receptor-like receptor (ORL1).[10] Although it shares a strong structural similarity with other typical opioids, it has a

[a] PhD Graduate Student, State Key Laboratory of Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China.

[b] Masters Graduate Student, Huiying Dental Clinic, 55# Huashan Nan Road, Kunming, Yunnan, China.

[c] Masters Graduate Student, State Key Laboratory of Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China.

[d] Professor, State Key Laboratory of Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China.

The first two authors contributed equally to this work.

Corresponding author: Dr Wenli Lai, State Key Laboratory of Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Ren Min South Road, No.14, Section 3, Chengdu 610041, China
(e-mail: wenlilai@hotmail.com)

Accepted: April 2013. Submitted: February 2013.
Published Online: May 8, 2013

very distinct mechanism of action.[11] A large body of evidence indicates that nociceptin executes a nociceptive effect at the supraspinal level but an antinociceptive effect at the spinal level,[12–18] particularly in the trigeminal system.[15,19,20] The pain signals from the orofacial region are processed through three stations before cortical perception: they are initially received by trigeminal neurons located at trigeminal ganglia, replayed to the trigeminal nucleus caudalis located at the medulla oblongata, then transmitted to the ventral posterior nucleus of the thalamus, and last perceived by the sensory cortex.[21] Among them, the trigeminal nucleus caudalis, located in the caudal part of the medulla oblongata, is a very important sensory relay for orthodontic pain.[1]

However, to date, nociceptin has not been investigated in the trigeminal nucleus caudalis to see how it contributes to orthodontic pain. Therefore, we conducted this study to determine the expression levels of nociceptin in the trigeminal nucleus caudalis following experimental tooth movement.

MATERIALS AND METHODS

Animals

A total of 72 male Sprague-Dawley rats (age: 2 months; weight: 200–250 g) were used in this study. The rats were provided by the Animal Experimental Center, Sichuan University, Chengdu, China. This study was approved by the Ethical Committee of West China School of Stomatology, Sichuan University and State Key Laboratory of Oral Diseases. They were divided into two groups, ie, sham group (n = 36) and experimental group (n = 36). For the experimental group, closed, coiled springs were employed to mimic the orthodontic forces (80 g) between the upper incisors and first molars, and the rats were killed through cervical dislocation at 0 hours, 4 hours, 12 hours, 24 hours, 2 days, 5 days, 7 days, 10 days, and 14 days after force application (four rats for each time point); the process was similar for the sham group, except that no force was applied in the sham group. In particular, the four rats killed at 0 hours without any intervention were used as the baseline control for each group.

Tissue Sample Preparations and Immunohistochemistry

The trigeminal nucleus caudalis at both ipsilateral and contralateral sides of force applications was taken, fixed in paraformaldehyde (4%) for 30 minutes, and embedded in paraffin. Tissue sections were cut at 10 μm and deparaffinized for immunostaining. Following antigen retrieval (microwave oven-citrate buffer), tissue samples were rinsed in phosphate-buffered saline (PBS) three times, blocked with goat serum, and incubated at 37°C for 3 hours with a specific antibody against nociceptin (1:500; Chemicon, Temecula, CA, USA). Then, tissue samples were washed in PBS three times, incubated with biotin-labeled anti-rabbit (Vector, Burlingame, CA, USA) IgG (1:200) at 37°C for 40 minutes, rewashed in PBS three times, and incubated with alkaline phosphatase-labeled streptavidin (Vector, Burlingame, CA, USA) (1:200) at 37°C for 30 minutes. Alkaline phosphatase was employed for visualization. PBS was substituted for the primary antibody in the blank control.

Visualization and Quantification

Visualization of immunoreactive neurons was achieved by using a microscope (Model Bx51; Olympus, Tokyo, Japan). For each rat, the percentage of immunoreactive neurons among all neurons was calculated in each of six randomly consecutive fields, and the mean percentage was used for the quantification of expression levels.

Statistical Analyses

One-way analysis of variance was employed to analyze the difference in nociceptin expression among different time points in each sham and experimental group. A paired *t*-test was used to compare the difference in nociceptin expression between the ipsilateral and contralateral sides of the force application in each group. All the statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, Ill).

RESULTS

Expression of Nociceptin in the Trigeminal Nucleus Caudalis

Under the microscope, neurons positive for nociceptin were stained with red. The results showed that nociceptin was expressed in both the sham and experimental groups, including the baseline control. Moreover, both the ipsilateral and contralateral sides of force application expressed nociceptin (Figure 1).

Chronological Changes of Nociceptin Expression

As shown in Figure 2, in the experimental group, as compared to baseline control (0 hours, 14.93 ± 2.91), which received no intervention, the expression levels of nociceptin remained similar at 4 hours (17.92 ± 2.06, $P > .05$), 12 hours (15.38 ± 2.89, $P > .05$) and 1 day (18.90 ± 1.83, $P > .05$). As compared with baseline control (14.93 ± 2.91), the expression levels started to increase on day 2 (21.72 ± 3.88, $P = .018 < .05$) and day 5 (26.76 ± 1.75, $P = 1.33 \times 10^{-5} < .05$),

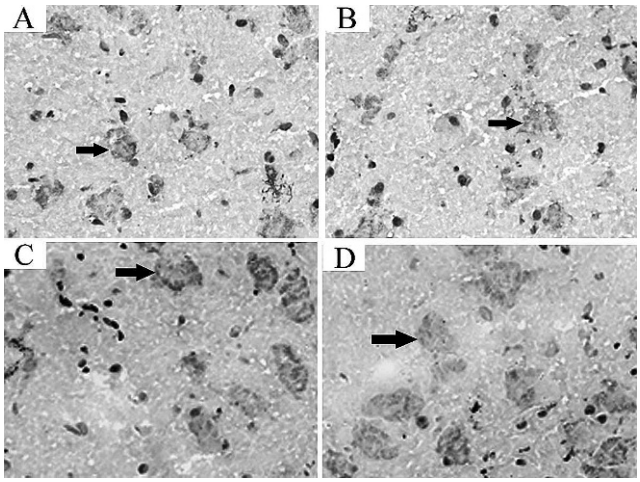

Figure 1. Immunostaining for nociceptin. Positive neurons were stained in red, as indicated by the arrows. (A) Neurons positive for nociceptin at the ipsilateral side in rats at baseline; (B) neurons positive for nociceptin at the ipsilateral side in rats (day 7) in the sham group; (C) neurons positive for nociceptin at the ipsilateral side in rats (day 7) in the experimental group; (D) neurons positive for nociceptin at the contralateral side in rats (day 7) in the experimental group.

peaked on day 7 (40.61 ± 2.12, $P = 2.02 \times 10^{-12} < .05$), and returned to baseline on day 10 (15.66 ± 1.08, $P > .05$) and day 14 (14.62 ± 3.06, $P > .05$).

In the sham group, in contrast to baseline control (14.70 ± 2.82), the expression levels of nociceptin did not differ significantly at 4 hours (15.08 ± 2.05, $P > .05$) and 12 hours (10.60 ± 0.97, $P > .05$). They started to decrease on day 1 (7.50 ± 0.66, $P = .001 < .05$), day 2 (7.36 ± 1.35, $P = .001 < .05$), day 5 (6.920 ± 0.507, $P = 4.9 \times 10^{-4} < .05$) and day 7 (9.480 ± 1.823, $P = .036 < .05$), and then returned to baseline on day 10 (11.10 ± 4.52, $P > .05$) and day 14 (14.58 ± 0.73, $P > .05$).

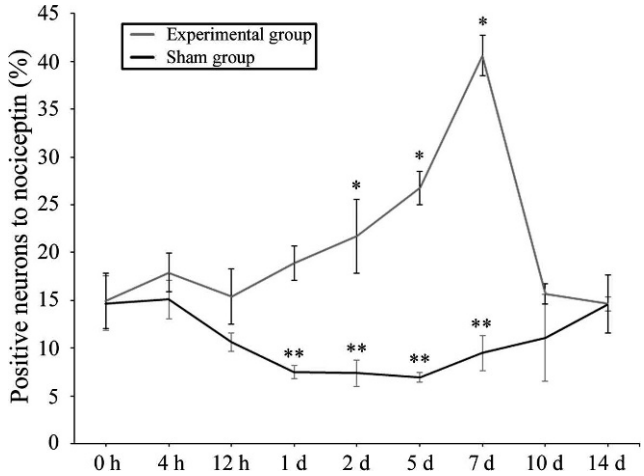

Figure 2. The percentage of neurons positive for nociceptin. The expression levels of nociceptin started to increase on day 2, peaked on day 7, and returned to baseline on day 10 in the experimental group (*indicates $P < .05$). In contrast, the expression levels of nociceptin began to decrease on day 1 and returned to baseline on day 10 in the sham group (**indicates $P < .05$).

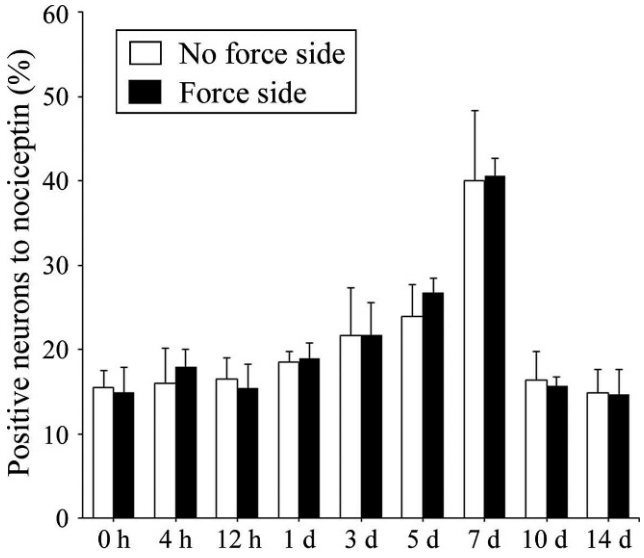

Figure 3. The comparison of expression levels of nociceptin between the ipsilateral side (force side) and contralateral side (no-force side) for the experimental group. The expression levels were similar among all the time points.

Comparison of Nociceptin Expression Levels Between the Force Side and the No-Force Side

As revealed in Figure 3, the paired *t*-test revealed that the expression levels of nociceptin were similar between the force side and the no-force side in the experimental group at baseline (0 hours, 15.46 ± 1.99 vs 14.93 ± 2.91), 4 hours (15.93 ± 4.19 vs 17.92 ± 2.06), 12 hours (16.51 ± 2.55 vs 15.38 ± 2.89), 1 day (18.53 ± 1.23 vs 18.90 ± 1.83), 2 days (21.61 ± 5.66 vs 21.72 ± 3.88), 5 days (23.98 ± 3.70 vs 26.76 ± 1.75), 7 days (40.08 ± 8.20 vs 40.61 ± 2.21), 10 days (16.33 ± 3.45 vs 15.66 ± 1.08), and 14 days (14.85 ± 2.72 vs 14.62 ± 3.06) (all $P > .05$).

Moreover, as shown in Figure 4, similar results were found in the sham group at baseline (0 hours, 14.27 ± 2.56 vs 14.70 ± 2.82), 4 hours (13.00 ± 3.28 vs 15.08 ± 2.05), 12 hours (9.88 ± 1.02 vs 10.60 ± 0.96), 1 day (7.09 ± 0.22 vs 7.50 ± 0.66), 2 dasy (6.53 ± 2.28 vs 7.36 ± 1.35), 5 days (6.61 ± 0.63 vs 6.92 ± 0.51), 7 days (8.29 ± 2.38 vs 9.48 ± 1.82), 10 days (10.12 ± 4.60 vs 11.10 ± 4.52), and 14 days (13.21 ± 1.88 vs 14.58 ± 0.73) (all $P > .05$).

DISCUSSION

We showed that nociceptin was expressed in the trigeminal nucleus caudalis in both groups and that both the ipsilateral and contralateral sides of force application expressed nociceptin. Expression levels of

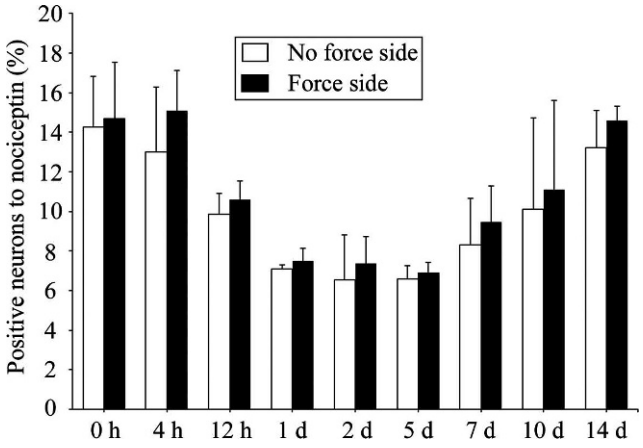

Figure 4. The comparison of expression levels of nociceptin between the ipsilateral side (force side) and contralateral side (no-force side) for the sham group. The expression levels were similar among all the time points.

nociceptin started to increase after force application on day 2, peaked on day 7, and then return to baseline on day 10 in the experimental group, while in the sham group expression decreased after force application on day 1 and then returned to baseline on day 10. Furthermore, the expression levels of nociceptin did not differ between ipsilateral and contralateral sides of force application for both groups.

Experimental tooth movement can mimic orthodontic pain observed in clinical settings. Recent studies indicate that plenty of neuropeptides (eg, CGRP, substance P, and NMDA) are expressed in trigeminal neurons and the nucleus during tooth movement.[1] These nociceptive neuropeptides play an important role in pain transmission and modulation. Being an antinociceptive neuropeptide, nociceptin has been revealed to be colocalized with those aforementioned nociceptive neuropeptides (eg, CGRP and SP) in trigeminal neurons and to exhibit an anti-nociceptive effect in the trigeminal system.[20] This anti-nociceptive effect of nociceptin is achieved through inhibition of nociceptive neuropeptides.[15,20,22] In our present study, we found that the expression levels of nociceptin started to increase on day 2, peaked on day 7, and returned to baseline on day 10. This finding was inconsistent with our previous studies, in which nociceptive neuropeptides started to increase on day 1 and returned to baseline on day 7.[7,8] We attribute this time lag of nociceptin as compared with nociceptive neuropeptides to a potential feedback that may be used to limit nociceptive signals. Specifically, in the early stage, nociceptive signals dominate with elevated levels of nociceptive neuropeptides. Later on, the increased levels of nociceptive neuropeptides induce the expression of nociceptin, which would in turn inhibit these nociceptive neuropeptides. However, the exact underlying mechanisms are largely unknown.

Ironically, we found that the expression levels of nociceptin started to decrease on day 1 and returned to baseline on day 10 in the sham group. In the present study, sham interventions included anesthesia, tooth separation, and ligation of ligature wire, without spring activation. Theoretically, the procedures other than anesthesia were noxious stimuli and, therefore, would induce the expression of nociceptin, since nociceptin exerts an antinociceptive effect in the trigeminal system.[20] Thus, we can only attribute the decrease in the expression of nociceptin in the sham group to anesthesia, and we suggest an antagonism may exist between the anesthetic agent (chloral hydrate) and nociceptin, since both exert antinociceptive or analgesic effect.

Our results showed that the expression levels of nociceptin were similar between the ipsilateral and contralateral sides of force application in both groups. This finding was in agreement with the study by Balam et al.[23] in which preproenkephain was shown to be expressed in both ipsilateral and contralateral trigeminal nucleus caudalis following experimental tooth movement. In our present study, forces were applied between central incisors and first molars and, thus, both central incisors and first molars received the experimental forces. Since anterior teeth receive nerves from both sides,[24] we attribute similar expression between ipsilateral and contralateral sides to bilateral innervation of central incisors. However, this cannot explain similar expressions in both sides for Balam et al.[23] In this study, the experimental forces were achieved through inserting elastic modules between the first and second molars, so no forces were applied on anterior teeth. It has been well documented that nerve fibers are projected to bilateral trigeminal nuclei after leaving the trigeminal ganglion.[25] Thus, in addition to bilateral innervation of central incisors, we suggest bilateral projections of nerve fibers from the trigeminal ganglion can account for similar expression levels of nociceptin in both sides.

CONCLUSIONS

- The expression of nociceptin was elevated following experimental tooth movement, suggesting a potential antinociceptive role of nociceptin in tooth movement.
- The anesthetic agent used in this study (chloral hydrate) may have an antagonism with nociceptin.
- Due to bilateral innervation of anterior teeth and bilateral projections of nerve fiber after leaving the trigeminal ganglion, the expression levels of nociceptin were similar between the ipsilateral and contralateral sides of force application.

ACKNOWLEDGMENT

This work was supported by NSFC (National Natural Science Foundation of China) grants 81070858 and 81100778.

REFERENCES

1. Krishnan V. Orthodontic pain: from causes to management—a review. *Eur J Orthod*. 2007;29:170–179.
2. Bergius M, Berggren U, Kiliaridis S. Experience of pain during an orthodontic procedure. *Eur J Oral Sci*. 2002;110: 92–98.
3. Erdinc AM, Dincer B. Perception of pain during orthodontic treatment with fixed appliances. *Eur J Orthod*. 2004;26: 79–85.
4. Khattab TZ, Farah H, Al-Sabbagh R, Hajeer MY, Haj-Hamed Y. Speech performance and oral impairments with lingual and labial orthodontic appliances in the first stage of fixed treatment [published online ahead of print October 18, 2012]. *Angle Orthod*. doi:10.2319/073112-619.1
5. Xiaoting L, Yin T, Yangxi C. Interventions for pain during fixed orthodontic appliance therapy. A systematic review. *Angle Orthod*. 2010;80:925–932.
6. He WL, Li CJ, Liu ZP, et al. Efficacy of low-level laser therapy in the management of orthodontic pain: a systematic review and meta-analysis [published online ahead of print September 2012]. *Lasers Med Sci*. doi:10.1007/s10103-012-1196-y
7. Yang Z, Wang Y, Luo W, et al. Trigeminal expression of N-methyl-D-aspartate receptor subunit 1 and behavior responses to experimental tooth movement in rats. *Angle Orthod*. 2009;79:951–957.
8. Yang Z, Cao Y, Wang Y, et al. Behavioural responses and expression of P2X3 receptor in trigeminal ganglion after experimental tooth movement in rats. *Arch Oral Biol*. 2009; 54:63–70.
9. Deguchi T, Yabuuchi T, Ando R, Ichikawa H, Sugimoto T, Takano-Yamamoto T. Increase of galanin in trigeminal ganglion during tooth movement. *J Dent Res*. 2006;85: 658–663.
10. Henderson G, McKnight AT. The orphan opioid receptor and its endogenous ligand—nociceptin/orphanin FQ. *Trends Pharmacol Sci*. 1997;18:293–300.
11. Lambert DG. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. *Nat Rev Drug Discov*. 2008;7:694–710.
12. Katsuyama S, Mizoguchi H, Komatsu T, et al. Antinociceptive effects of spinally administered nociceptin/orphanin FQ and its N-terminal fragments on capsaicin-induced nociception. *Peptides*. 2011;32:1530–1535.
13. Ko MC, Naughton NN. Antinociceptive effects of nociceptin/orphanin FQ administered intrathecally in monkeys. *J Pain*. 2009;10:509–516.
14. Mika J, Obara I, Przewlocka B. The role of nociceptin and dynorphin in chronic pain: implications of neuro-glial interaction. *Neuropeptides*. 2011;45:247–261.
15. Wang XM, Zhang KM, Long LO, Mokha SS. Orphanin FQ (nociceptin) modulates responses of trigeminal neurons evoked by excitatory amino acids and somatosensory stimuli, and blocks the substance P-induced facilitation of N-methyl-D-aspartate-evoked responses. *Neuroscience*. 1999;93:703–712.
16. Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev*. 2001;53:381–415.
17. Rizzi A, Nazzaro C, Marzola GG, et al. Endogenous nociceptin/orphanin FQ signalling produces opposite spinal antinociceptive and supraspinal pronociceptive effects in the mouse formalin test: pharmacological and genetic evidences. *Pain*. 2006;124:100–108.
18. Ko MC, Wei H, Woods JH, Kennedy RT. Effects of intrathecally administered nociceptin/orphanin FQ in monkeys: behavioral and mass spectrometric studies. *J Pharmacol Exp Ther*. 2006;318:1257–1264.
19. Bongsebandhu-Phubhakdi S, Phisonkulkasem T, Srikiatkhachorn A. Nociceptin/orphanin FQ modulates cortical activity and trigeminal nociception. *Headache*. 2011;51: 1245–1253.
20. Wang XM, Zhang KM, Mokha SS. Nociceptin (orphanin FQ), an endogenous ligand for the QRL1 (opioid-receptor-like1) receptor; modulates responses of trigeminal neurons evoked by excitatory amino acids and somatosensory stimuli. *J Neurophysiol*. 1996;76:3568–3572.
21. Crossman AR, Neary D. Cranial nerves amd cranial nerve nuclei. In: *Neuroanatomy: An Illustrated Colour Text*. Philadelphia, PA: Elsevier Health Sciences; 2000:103–116.
22. Capuano A, Curro D, Dello Russo C, et al. Nociceptin (1–13)NH2 inhibits stimulated calcitonin-gene-related-peptide release from primary cultures of rat trigeminal ganglia neurones. *Cephalalgia*. 2007;27:868–876.
23. Balam TA, Yamashiro T, Zheng L, Murshid Ahmed S, Fujiyoshi Y, Takano-Yamamoto T. Experimental tooth movement upregulates preproenkephalin mRNA in the rat trigeminal nucleus caudalis and oralis. *Brain Res*. 2005; 1036:196–201.
24. Nord SG. Bilateral projection of the canine tooth pulp to bulbar trigeminal neurons. *Brain Res*. 1976;113:517–525.
25. Hansen JT, Koeppen BM. Sensory pathways II. In: *Atlas of Neuroanatomy and Neurophysiology: Selections from the Netter Collection of Medical Illustrations*. Teterboro, NJ, USA: Icon Custom Communications; 2002:81.