

Bones, Nerves and a Little Trk

Pain is one of the most common and unifying of human experiences. With the exception of certain diseases, everyone from newborn babies to centenarians felt it. And while some experience it more than others, it remains a vital biological process. Yet, for all of pain's universality, there is still a great deal unknown about what causes pain, and more specifically, what causes certain types of pain.

The basic function of nerve tissue is well understood—stimulation at synapses of nerve cells cause electrical impulses (with the help of a complex slew of different proteins and chemicals) to travel from a peripheral nerve through a chain of nerve cells, out to the spine and eventually up to the brain where the signal is decoded as pain. However, in some cases like bone pain, what triggers that first nerve cell to fire is not clear.

Bone pain can be caused by a variety of diseases. Patients with bone cancer, osteoarthritis, osteoporosis, fractures and other trauma, leukemia, osteomyelitis, and blood diseases like sickle cell anemia are all susceptible to having chronic pain¹. This pain, because of medicine's inability to fully control it, can lead to secondary issues of decreased mobility and other psychological and physical complications. Unfortunately, there are few treatments available for these people to seek out due to the imperfect understanding we currently have on what causes bone pain and therefore what can be done to attenuate it.

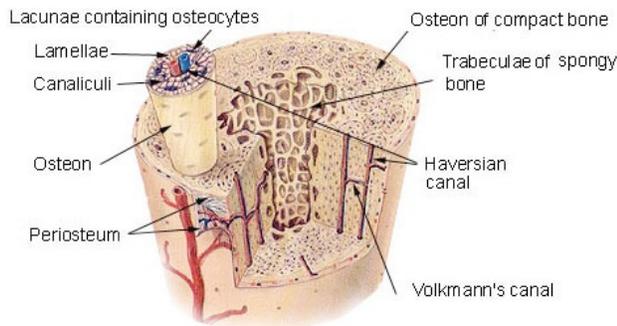
According to Castañeda-Corral et al., one reason for this imperfect understanding of bone pain is the medium in which the nerves themselves are imbedded. Instead of being embedded in soft tissue like in the muscle or the brain, the nerves in bone are surrounded by hard, mineralized tissue—calcified bone made primarily of crystals of hydroxyapatite².

¹ Linda J. Vorvick, "Bone Pain or Tenderness," *Medline Plus*, May 1, 2011, <http://www.nlm.nih.gov/medlineplus/ency/article/003180.htm>.

² G Castañeda-Corral et al., "The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A," *Neuroscience* 178 (March 31, 2011): 196-207.

Bone—specifically long bone where much of bone pain centers—is composed of three

Compact Bone & Spongy (Cancellous Bone)



main parts. There is an [interior](#) spongy or trabecular region, which contains less compact mineralized tissue and bone marrow and forms the core of the bone. Then there is the compact layer of cortical bone that forms the next most outer layer. This layer of bone contains the densest material and the most mineralized bone. However, throughout this dense layer are canals called haversian canals that contain blood vessels, and—the point of interest—nerves. The outer most layer of bone is an active, sensitive, thin layer of cells called the periosteum that has functions related to bone repair³. All three of these layers are

innervated in the bone and present potential casual areas of bone pain⁴.

It is also known what types of neural cells innervate the bone. For the most part, these neural cells are either thinly myelinated or peptide rich C-fibers that are not myelinated at all⁵ (myelin being a type of Schwann cell that wraps around neurons protecting their dendrites and helping conduct the electrical signaling)⁶. Most of these nerves fall under the classification of primary afferent nerves—the first line of action in transmitting signals from the peripheral nervous system⁷. Many of the peptide rich C-fibers which express calcitonin gene related peptide (CGRP) are un-myelinated while many of the myelinated fibers express neuro-filament 200 (NF200). In the periosteum, both these types of nerve cells crisscross in a mesh like network⁸. This high density of CGRP+ nerves on the outer most layer of bone is speculated to be involved in detecting pain-inducing substance and as such has been a point of study.

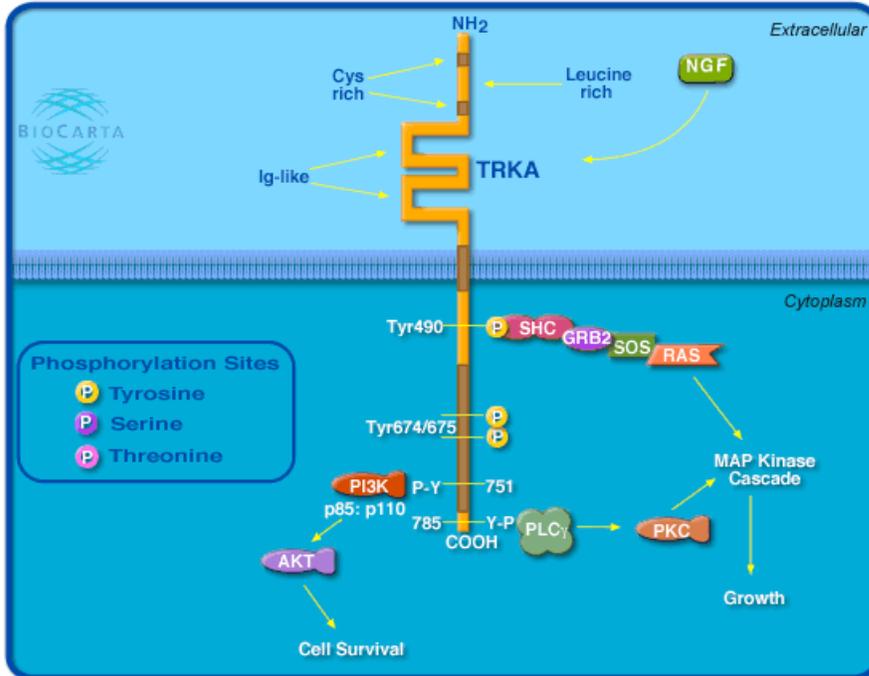
³ Elaine Nicpon Marieb and Katja Hoehn, *Human anatomy & physiology* (Upper Saddle River: Pearson, 2013), 173–190.

⁴ Castañeda-Corral et al., “The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A.”

⁵ Juan Miguel Jimenez-Andrade et al., “A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin: therapeutic opportunity for treating skeletal pain,” *Bone* 46, no. 2 (February 2010): 306-313.

⁶ Marieb and Hoehn, *Human anatomy & physiology*, 287–397.

⁷ Jimenez-Andrade et al., “A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin.”



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Genomics and Medicine

The densities of these different fibers vary depending on which section of bone they are in. CGRP+ and NF200+ fibers are highest in concentration in the periosteum, followed in density by the marrow, the mineralized cortical bone and finally cartilage in a ratio of 100:2:0.1:0⁹. In the interest of drug development, it is important to recognize that these nerve types have high levels of occurrence on the outside of the bone and low occurrence in the cartilage.

The long bone forms through a process of endochondrial ossification where cartilage is slowly converted into mineralized bone¹⁰. This it would make sense that enervation of the bone occurs post-cartilage or as a process of the ossification.

One reason it is so vital to identify the types of nerves that penetrate the bone is that in order to study and learn more about how they cause pain, we must first understand the specific workings of the proteins they express. For example, nerves taken from skin were long used as a model to try to understand musculoskeletal pain¹¹, but studies into the differences between these two tissues resulted in the understanding that they express a different range of neural cells. Skin tissue has been shown to express thickly myelinated fibers known as A-beta fibers, thinly myelinated fibers classified as A-delta fibers and, like in bone, peptide rich C-fibers. However, skin has additional peptide poor C-fibers in addition to the un-myelinated peptide rich ones. Bone on the other hand is limited to the thinly myelinated A-delta and C-fiber sensory neurons¹². Thus, it is the skeletal specific nerve fibers and the proteins they express which must be the target for possible bone pain treatments. Understanding the receptors on these cells and what binds to them would allow pharmaceutical scientists to craft a drug to block the particular receptor.

8 Castañeda-Corral et al., "The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A."

9 Ibid.

10 Marieb and Hoehn, *Human anatomy & physiology*, 183–185.

11 Jimenez-Andrade et al., "A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin."

12 Ibid.

In this vein, one receptor that has proved an interesting study and a possible target for future drug research is the Tyrosine Kinase A (TrkA) receptor which serves as the receptor for NGF (neural growth factor). NGF binds to TrkA and has been shown to induce neural cell growth. This particular pathway acts via the diagram shown (figure 2), taken from the pharmacological website Biocarta. While one pathway which involves the Harvey rat sarcoma oncogene (RAS or HRAS) protein or alternately Protein Kinase C, triggers the MAP kinase cascade and leads to cell growth, the other pathway involves the triggering of v-akt murine thymoma viral oncogene (AKT) and leads to a more stabilized cell condition¹³.

This pathway matters do to the particular way it bifurcates. Jimenez-Andrade and Mantyh showed that injected adjuvant into the knees of mice stimulated arthritic-like behaviors and, upon examination of the knee joints, found sprouting of nerves in the joint area. It is suggested that after inflammation or injury, something induces nerve growth¹⁴. In other words, injury stimulates the production of NGF and something additional that preferentially favors the pathway towards the release of RAS or PKC and the subsequent MAP kinase cascade resulting in growth. Understanding what causes both these things provides a possible treatment pathway.

The finding that there was a significant increase in the density of CGRP+ and NF200+ fibers on the periosteum of the bone in the neuroma-like structures formed upon injury to mice knees encourages the idea that these nerves are closely tied to pain generation. In addition to further supporting the theory that the nerves on the surface of the periosteum are important in driving pain, this is significant because both of these fibers (CGRP+ and NF200+) were found to be highly expressive of TrkA receptors. It was found that 80% of CGRP+ fibers expressed TrkA and 50% of NF200+ fibers where also TrkA positive in the periosteum. Furthermore, in the other mineralized bone and marrow sections it was seen that TrkA was expressed in over 80% of both CGRP+ and NF200+ fibers¹⁵. Stepping back, this means that that nearly all the un-myelinated and thinly myelinated fibers of the bone express this one receptor, which in turn makes them susceptible to regulation by potent NGF.

Before moving on to the pharmacological implications of such wide spread TrkA expression, there is one other protein that deserves some attention. An additional highly expressed protein that was found on nearly all CGRP+ and NF200+ fibers was Growth Associated Protein 43 (GAP43). About 93% of both CGRP+ and NF200+ fibers that cover the periosteum also express GAP43. Again, this differs from skin where only C-fibers seem to

13 Lilsa Eisenlohr, "Trka Receptor Signaling Pathway," *BioCarta*, n.d., http://www.biocarta.com/pathfiles/h_trkaPathway.asp#history.

14 Juan M Jimenez-Andrade and Patrick W Mantyh, "Sensory and sympathetic nerve fibers undergo sprouting and neuroma formation in the painful arthritic joint of geriatric mice," *Arthritis research & therapy* 14, no. 3 (2012): R101.

15 Castañeda-Corral et al., "The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A."

express GAP 43, whereas in the bone nearly all nerve fiber types express it¹⁶. This expression pattern of GAP 43 is important because it appears that high basal levels of GAP 43+ expressing nerve fibers may allow for greater sprouting of nerve fibers in response to trauma and injury¹⁷. The expression of GAP 43 has been seen to be up regulated in neural and glial cells undergoing differentiation, which ties to the fact that it appears to be involved in determining cell fate¹⁸.

GAP 43 and TrkA are important to understanding pain regulation because when CFA (an adjuvant) was injected into the knees of mice, a significant increase in nerve growth in the joint was exhibited. Not only was there a denser array of CGRP+, NF200+ and GAP43+ fibers, but the arrangement was also tangled and disorganized compared to the mesh like composition seen on normal periosteal bone surface¹⁹. It is hypothesized that this increased number of nerves—improperly organized and developed—is what causes the pain²⁰. Thus, treating this improper nerve growth of GAP43+ fibers along with the CGRP+ fibers (which as stated before are widely also expressive of TrkA) could arrest abnormal nerve sprouting and might control pain.

This has in fact already been recognized. Studies done on bone tumors have recognized the ability of NGF to affect nerve cell growth and sprouting. Trials utilizing mouse derived anti-NGF monoclonal antibodies in bone tumors have shown promising results in relieving pain. Sustained administration of the anti-NGF has seen noticeably reduced levels of CGRP+ and NF200+ nerve fibers²¹. Thus, it seems that TrkA, the receptor of NGF and widely expressed amongst much of the bone's nerve fibers, is a prime candidate for developing an effective nerve growth inhibitor and controlling pain. One of the interesting observations made during the study of TrkA receptor in the bone—specifically in bone cancers—is that while the malignant cells themselves do not express NGF there seems to be a significant

16 Ibid.

17 Ibid.

18 Yiping Shen, Shyamala Mani, and Karina F Meiri, "Failure to express GAP-43 leads to disruption of a multipotent precursor and inhibits astrocyte differentiation," *Molecular and Cellular Neuroscience* 26, no. 3 (July 2004): 390-405.

19 Jimenez-Andrade and Mantyh, "Sensory and sympathetic nerve fibers undergo sprouting and neuroma formation in the painful arthritic joint of geriatric mice."

20 Ibid.

21 Joseph R. Ghilardi et al., "Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint," *Arthritis & Rheumatism* 64, no. 7 (2012): 2223–2232.

amount of NGF driving the disorganized growth of CGRP+ fibers coming from immune system cells²². Here, again, a form of inflammation or injury causes a release of NGF factors that bind to the widely expressed TrkA receptor and creates excessive nerve cell growth which then causes bone pain.

As for GAP43+ fibers, it is unclear what triggers the growth of their “host” cells, although the observed stimulation by NGF of both TrkA+ and GAP43+ fibers (even though NGF does not bind to GAP 43) suggests that there is a relationship between the signaling pathway for TrkA and GAP43. Since GAP43 is thought to help in the differentiation of glial and neural cells, it seems likely that this could be a downstream effect of the NGF stimulation of TrkA—a possible kind of second trigger in the nerve growth process. Again, this suggests that the best course for effective therapy is looking at ways to mediate excessive nerve growth through blocking excessive NGF access to TrkA receptors.

Even though bone and joint pain affects millions of people—arthritis alone affects about 50 million people in the United States—there is not yet a good, targeted commercial therapy available to relieve the symptoms²³. It has been noted that in areas of concentrated bone pain there are an increased number of nerve fibers expressing CGRP, NF200, GAP43 and TrkA. In these same diseased joints, lightly myelinated and non-myelinated nerve fibers that usually grow in an organized mesh arrangement increase in density and disorganization. It was found that most nerve fibers in the bone express the TrkA receptor, which has a high affinity binding for NGF. Although NGF was not a factor produced by malignant cells in the bone, cells involved in the inflammatory response did express it. This makes the blocking of the TrkA-NGF complex a likely—indeed already promising—method of controlling future bone pain and forming a useful therapy to attenuate the large number of bone pain cases existing in the world.

22 Castañeda-Corral et al., “The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A.”

23 Ghilardi et al., “Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint.”

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