Headers and Payloads inside of Nervous System as like as Digital Protocols

Oralia Nolasco, José Luis Leyva
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional
Mexico
onolasco@gdl.cinvestav.mx
luis.leyva@cinvestav.mx

ABSTRACT: *This paper presents the Nervous System as a digital communication protocol. The Nervous System is a network that allows two or more subnetworks to be able to communicate between them; it transmits information (afferent or efferent) as structured Messages. The structure of a Message is composed by a header followed of a payload. The header indicates to receptor the address where the information needs to be decoded (Broadmann area). The payload contains the information about the stimulus. To validate this proposal, the Engineers acquire raw bioelectrical signals in-vivo from sciatic nerve of the rats. The surgical protocol was supervised by CICUAL No. 0133-15.*

Keywords: Action Potentials, Header, Payload, T-Distributed EM, Message Structure, Protocol, Rat, Afferent Signals, Inter Spikes Distances, Nervous System, Communication Digital.

Received: 5 October 2016, Revised 12 November 2017, Accepted 17 November 2017

1. Introduction

The Nervous System (NS) is a communication network that allows the organism to interact appropriately with his environment [1][2][3][4][5]. The human body has sensory components are able to transduce stimuli from the external environment into Action Potentials (APs); the APs will be administered in Processing Centers (spinal cord, brain stem, thalamus and cerebral cortex) and then converted to sensations [6][7][8][9][10]. The processing centers command the APs to the motors units that will be convert into movements of the human body [11].

The study of the neurophysiological mechanisms of sensitivity began in 1925, when Edgar D. Adrian and Yngve Zotterman were the first to record an AP on a sensory nerve [12]. These authors discovered that the nerve carries information from the sensor receiver by modulating the frequency of electrical impulses [13][14][15]. Sensory information in the Central Nervous System (CNS) is processed in stages in the sequential relay nuclei of the spinal cord, brain stem, thalamus and cerebral cortex, known as:

Somatosensory Pathways [16]. NS Works hierarchically; The sensory receptors transmit information to the neurons of the first order, these neuron have peripheral axon that forms or innervates somatosensory receptors and a central process that synapses with 2nd afferent neurons in a spinal cord or brain stem nucleus; and then to synapses with higher order like thalamus-cortical area [17].

The NS is a complex communications network that interconnects around 100 billion neurons [18]. SN is comparable to any digital communications network because it activates and deactivates systems, guides and coordinates CNS processes for afferent and efferent ways [19].

In all communication systems the transmitters and receivers communicate with structured messages. The structure of message is a standard for them [20]. The structure of the message allows to the systems to decode and encode information avoiding communication errors [21]. The simplest digital structure of a message would be the header and the payload. In one-way communication, the header indicates the name of the receiver which must decode the information and the payload is the information to decode [22][23].

2. Hypothesis

This research aims to find the structure of the communication message between the Peripheral Nervous System (PNS). The PNS is the best place to acquire and analyze the signal; the reason is because here is the raw signal without processing. According to the technical literature, the first processing of the neural signals is in the spinal column. As the neural signal travels to the upper areas of the brain some information is added until it reaches its final decoding (possibly some of Broadmann's areas). For this reason, this research to acquires and works with raw signals of PNS without processing.

The advantage of avoiding the first coding and those of the upper zones is because throughout life each individual has created a series of neural connections at convenience that allow him to survive his own environment. Another proposal of this research is to assume that all individuals must handle the same type of raw signals. That's means that, like most of us, we have similar anatomies (eyes, nose, mouth, etc.), we shall generate the same message structure when we excite a skin sensor, but along various decoding (second and third order neurons) the individual adds "his signature" to his neural signal.

In the pain case, for example, the raw signal must be the same of all individuals, but some people feel more pain than others because each individual created different neural connections; thus, the decoding will be different for each individual, so each of them feels different perceptions of pain.

In the market there are prostheses that detect the presence of efferent neural signals in the fibers of the nerve and with them move the prostheses. When the prosthesis is moving, the eye feeds back to the brain and the brain learns, for an extend period of practicing the individual is able to move his prosthesis a convenience with perfect and exact movements, but in this case, the proposal is not to use the natural language of the NS. To have a "natural talking" with the CNS from the PNS the communication must be able to receive an immediate response without a previous learning period. The main goal of the present research is to decode the "natural talking" between the PNS and CNS. If this research is able to use a natural language between peripheral sensors and CNS, we may be able to find the structure of the message for different stimuli. With the correct structure, we could delete the pain messages in such a way that they do not reach their destination by changing one or more internal commands; with the correct message structures, the NS could be controlled.

3. Methods

The main goal of this research is to find the temporal structure of the message (header and payload) of low-threshold mechanoreceptors (LTMRs). To validate this proposal, this research used the rat NS like a model, see **Figure 1**. The general similarity of mammals NS allows rodents to be used as models, they share equivalences with humans like anatomy, biochemistry, physiology, neurological function and diseases [24][25].

The biological protocol of experimentation was submitted on PICUAL (web-platform of CINVESTAV), with the number **No. 0133-15** and approbation of CICUAL[2].

3.1 Signal Acquisition

This research focuses on afferent pathways of the PNS. The mechanical stimuli are in glabrous skin on hind legs of Wistar rats with weigh between 200-300g.

3.1.1 External Stimuli on the Skin

The LTMRs detect the fine touch on the skin. These sensors, when deformed mechanically, produce bursts of PAs that are transported through 5-12¼m diameter A-beta fibers with conduction velocities of 30-70 m / s [26].

The stimuli must be the same along the experimentation. Once the PAs burst is detected, the stimuli must be activated in the same area of the patient along the experimentation. The stimuli must be activated only LTMRs sensors, so, this proposal use VonFrey filaments calibrated at 4g-force pressures. With this pressure is ensuring that the nocireceptors are not activated. Likewise, in order to avoid stimulating thermoreceptors, it is proposed to keep the ambient temperature constant at 20 ° C and rat body temperature at 38 ° C with a thermal gel pad.

3.1.2 Intraneural Electrodes for Recording

The signal acquisition electrode is of the invasive type [27]. The electrode is placed on the sciatic nerve. The electrode is an array of 32 USEAs, "Utah Slanted Electrode Array"; they are multielectrodes array of microscopic needle types [28]. They acquire intraneural spikes ranging from 15μV amplitude.

The recording capabilities may be affected by biocompatibility with the patient, traumatic nerve damage at electrode insertion, a bad mechanical adjustment due to the rigid electrode structure, tissue softness, non-penetration of fascicle, and the forces produced by the immobilization of the transcutaneous connection cables have been subjects treated in many publications in the last years [29].

3.1.3 Electronic Circuit of Data Acquisition

The acquisition system is ®Plexon. The characteristics of neural signal recording were: 40kSps, gain of 20K, pre-amplification of 1K, the noise of the circuitry is measured with the cursors manually and once the patient is connected to the reference circuitry and he is within Faraday's cage. The noise of the circuitry was 40μV and a manual threshold of -7.263μV, 32 channels around 700 impedance values, they recording in real time with recording times are around 60s. The timing of the stimuli was 5 per recording in the seconds: 6, 9, 13, 17 and 20. The separation time of each stimulus is to ensure the recovery of the NS.

Offline Sorter software of ® Plexon offers the T-Distribution EM Scan, Valley Seeking and K-Means spike detection tools, all of them based on PCAs (Principal Components Analysis). Jutta Kretzberg et. al., evaluated the performance of two algorithms: T-distributed EM and valley seeking spikes (which ® Plexon offline spike sorter offers in its list of algorithms). They found that T-distributed EM is clearly superior to using artificial data and added noise [30]. Hartigan and Wong in 1978, compares the K-Means and T-Distributed EM spike clustering algorithms, the K-Means algorithm is a specific case of T-Distributed EM because it is based on Euclidian distance and *k* observations ; The problem arises when comparisons are made between global distances and several observations. In contrast, the T-Distributed EM is not based on the Euclidean distances it used a normalized quantities (values ranging from 0 to 1) and the maximum likelihood. In this proposal, we select T-Distributed EM for the detection of spikes in the recordings [31].

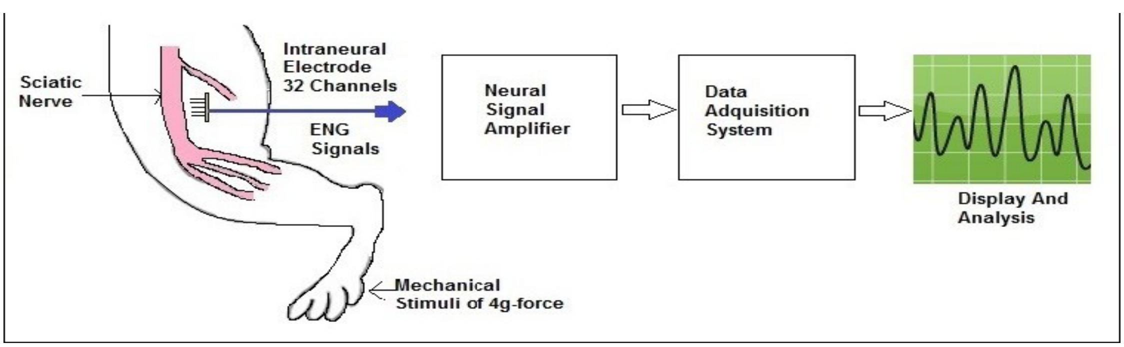

Figure 1. Schema of the neural signal acquired from the rat PNS

3.2 Signal Analysis

This research proposes to find the structure of the communication message between the PNS and the SNC through the intersection of subsets. The basis of the scanning is the matching elements within the symbol space. The space symbol is the recording file converted to the standard language, see Table 1. The space will be regrouped into finite subsets depending on the number of symbolizes created in the space recording time. The regroupings will be changing depending on the matching elements. The largest match subset within the subsets of space will be named the root element.

The greater number of coincidences within the subsets we could be sure that we have equal elements and we will regroup subsets with greater number of elements pretending to find the definitive structure that characterizes that recording.

3.3 ISDMessage-Symbols Software Tool

The ISDMessage-Symbols tool (Inter Spikes Distances to Symbols) is a proposal of this document that converts a PLX extension file to standard language, sees Table 1, forms subsets, and scans the recording and searches for the intersection of the elements into the subsets.

The intersected subsets could be regrouped depending on the number of intersecting internal elements:

- First Level: They are those subsets that intersect in 3 or 2 elements. That is, 3 or 2 of its elements are equal to the root element.
- Second Level: They are those subsets that intersect in 1 of its elements. That is, 1 of its elements is equal to the root element.
- Third Level: Are those remaining subsets. Generally, in this level these subsets intersect its elements with subsets of first and second level.

3.4 Symbols

The standard language consists of 30 symbols representing the spike-spike distance in milliseconds in neural recordings.

Symbol < ó = time(ms)			
1 <= 1.772	2 <= 3.375	3 <= 5.316	4 <= 7.088
5 <= 10.631	6 <= 14.175	7 <= 17.719	8 <= 24.856
9 <= 31.993	10 <= 35.129	11 <= 38.235	12 <= 45.875
13 <= 53.406	14 <= 56.542	15 <= 64.112	16 <= 85.524
17 <= 128.348	18 <= 153.329	19 <= 263.899	20 <= 338.899
21 <= 374.571	22 <= 438.037	23 <= 517.333	24 <= 731.454
25 <= 802.827	26 <= 838.514	27 <= 874.201	28 <= 909.887
29 <= 945.574	30 <= 1.05263s		

Table 1. Standard Language

3.5 Calibration Process

The calibration process is a proposal to choose the channels with which to work or characterize along the experimentation. For each in-vivo experiment its necessary recording 2 files (previously analyzed by the algorithm of T-Distributed EM):

- Baseline.plx

- Lidocaine.plx

The Baseline.plx and Lidocaine.plx files are recorded to select channels that have neural signals. The Baseline file has the recording of the biological noise, i.e. the reading of the system without stimulation. The lidocaine file is recording once placing the drug lidocaine over the nerve; it does immediately inhibit neural activity. Then, comparing the channels of both files is easily recognizing the neural channels. Finally, a correlation method is used between the neural channels to avoid redundant channels.

4. Results and Discussions

4.1 Patient#1 4g-force Subsets

As a result of the calibration process, from the patient#1 channel chosen are: 1, 3, 6, 12 and 32. In this case, these channels recorded 5 different fibers, depending of the type the fibers they could be activated conditional to the intensity of the stimuli, or they remain in their inactive state unless there is spontaneous activity.

The recording file f4.5620k01.plx has a cutaneous stimulation with the VonFrey filament number 4.56 equivalent to 4g-force. In this file the channel 6 increased in activity with respect to the Baseline.plx recording, that's means, that it has an A-beta fiber. The recording file f4.5620k01.plx has 1018 spikes in 45.9134 seconds.

The Table 2 is the result of the recording f4.5620k01.plx, with 283 different subsets and 300 total subsets. The Table 2 shows in red the first level subsets, the second level in blue and the third level in green. The subsets are clusters of 3 symbols. The root element of this space symbols (with the highest occurrence) is 395, i.e. the symbols 3, 9, 5. The symbols 1 and 2 are replaced by the letter "x" because they are very small distances. The rest of symbols that were not sub-set elements appear in black.

Channel 6
30 8 x 7 12 9 11 12 15 16 10 16 >>>
8 9 10 <<< 10 19 x 12 17 4 19 17 10 >>>
9 3 9 <<< 17 19 6 8 13 16 >>>
16 9 11 <<< 9 8 4 11 13 7 >>>
10 8 9 <<< 9 5 18 8 12 8 17 18 15 13 17 12 >>>
9 8 8 <<< 6 13 19 >>>
9 6 9 <<< 11 12 6 4 x 4 17 4 >>>
9 14 13 <<< 8 13 15 >>>
8 9 9 <<< 16 9 4 3 5 5 10 8 6 x 12 12 16 >>>
9 17 3 <<< 6 6 11 7 17 8 13 10 5 6 16 11 x 16 19 5 >>>
17 16 9 <<< x 9 9 9 x 8 5 18 15 17 19 >>>
9 15 3 <<< 8 x 13 5 16 >>>
5 9 9 <<< 10 7 11 18 9 18 17 11 8 11 8 x >>>
13 9 3 <<< 5 5 17 12 19 16 16 8 16 7 10 15 15 12 x >>>
17 4 9 <<< 11 6 15 >>>
17 7 9 <<< 16 5 3 16 9 9 15 18 15 >>>
9 12 8 <<< 8 7 3 11 9 17 5 15 17 6 5 3 17 7 6 >>>
3 16 19 <<< 5 13 9 15 8 14 5 12 18 9 5 6 13 13 8 x >>>
19 16 9 <<< 5 x 15 x 8 x 6 3 19 x 14 x 16 16 17 17 8 15 16 4 16 7 7 14 x 11 8 17 4 12 16 16 12 10 19 7 x 17 21 8 >>>
13 5 9 <<< 3 5 5 12 8 7 16 6 10 17 11 17 >>>
12 3 9 <<< 4 16 17 xx >>>
9 17 16 <<< 5 4 5 >>>
3 12 16 <<< 11 6 19 17 13 17 >>>
3 8 9 <<< 12 5 5 >>>
7 7 9 <<< 7 15 8 >>>
13 3 7 <<< 9 16 11 4 10 9 15 4 9 8 6 9 11 6 6 15 xxx 13 13 15 >>>
14 9 7 <<< 10 12 14 >>>
3 7 12 <<< 12 12 16 >>>
3 14 8 <<< 15 4 10 >>>
12 7 3 <<< 9 15 3 x 5 8 19 >>>

Table 2. Message-Symbols

4.2 Patient#1 4g-force Messages

The ISDMessage-Symbols scanning tool allows the identification of patterns. The matching pattern on all recordings is:

- *High priority subsets are followed by low priority subsets and in this sequence they reappear throughout the recording.*

The high-priority subsets are a proposal of this document that identifies the subsets of greater occurrence throughout the recording and that share symbols with the root element, as is the case of subsets of levels 1 and 2.

The subsets of low priority are the subsets that intersect each other with matching elements but with fewer occurrences within the recording and that do not share elements with the root element, as is the case of subsets of level 3.

Finally, the structure message is defines like:

• *The start Message is a subset of high priority and the end of Message is a subset of low priority.*

The Table 2 shows 300 subsets, like this ones: 3 4 9, 3 5 5, 3 6 6, 3 8 9, 3 9 5, 4 3 5, 4 3 8, 4 6 7, 5 3 7, 5 4 5, 5 8 6, 5 8 8, 5 9 5, 5 8 16, 5 8 19, 6 5 3, 6 5 9, 6 6 3, 6 6 6, 6 7 9, 6 8 8, 7 35, 7 7 9, 7 9 5, 7 9 9, 8 4 9, 8 6 9, 8 7 3, 8 8 9, 8 9 6, 8 9 9, 9 3 9, 9 4 9, 9 5 6, 9 6 8, 6 16 5, 6 19 6, 6 19 8, 6 6 11, 6 6 18, 6 7 17, 6 8 16, 6 9 16, 6 9 17, 7 11 8, 7 12 9, 7 15 6, 7 15 6, 7 15 8, and others.

The Table 2 shows only 28 of 79 total messages found on f4.5620k01.plx file; the starts message is defined with the “<<” character and the message end with “>>”.See Figure 2, register = 1.

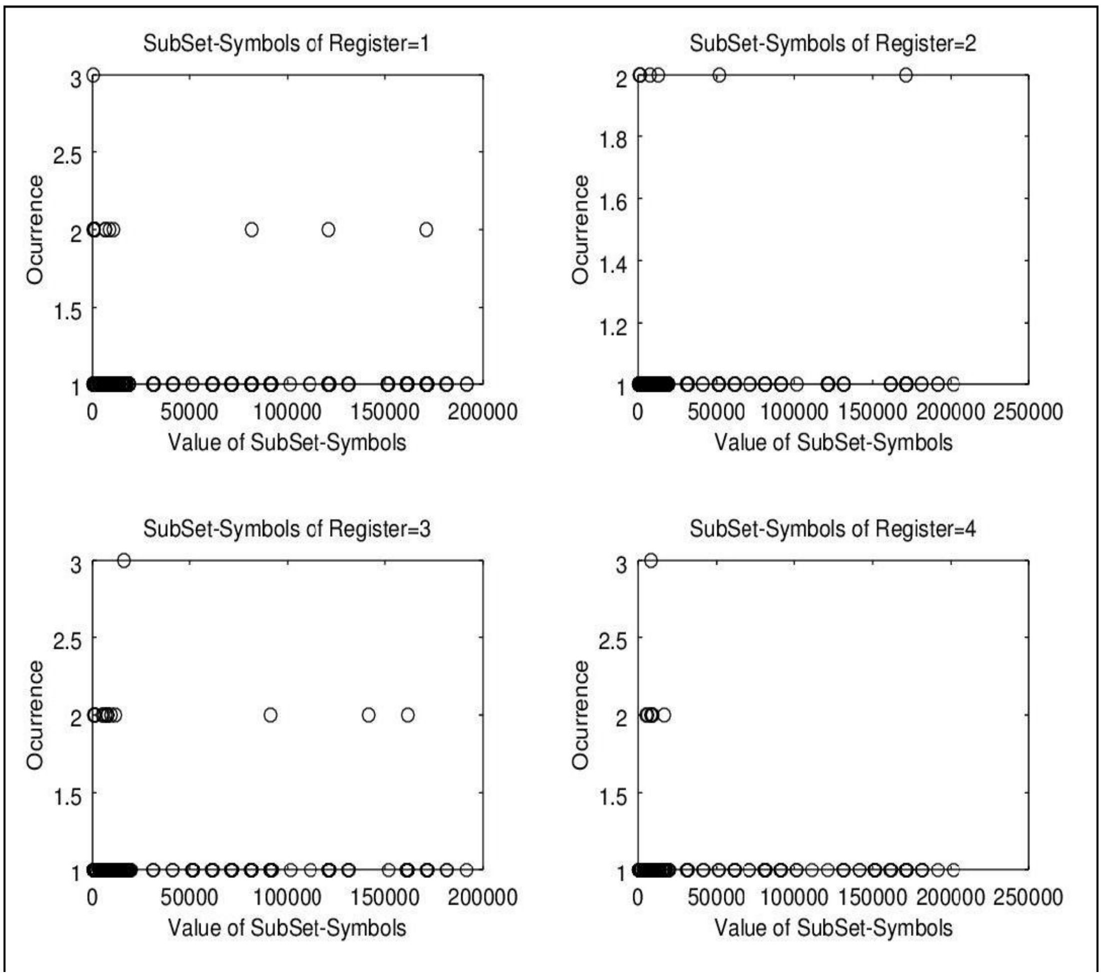

Figure 2. Subset comparative result of ISDMessage-Symbol tool scanning from Patient#1 with 4g-force

4.3 Patient#1 4g-force Headers

The Table 2 shows 49 different headers found on f4.5620k01.plx file, like: 3 8 9, 3 9 5, 4 3 5, 5 3 7, 5 9 9, 6 7 9,7 3 5, 7 7 9, 8 6 9, 8 8 9, 8 9 9, 9 3 9, 9 3 9, 9 4 9, 9 6 8, 9 6 9, 9 7 3, 9 8 8, 10 8 9,12 3 8, 12 3 9,12 7 3, 12 9 9, 13 3 7, 13 5 9, 13 9 3, 14 9 7, 16 6 3,17 4 9, 17 7 9,18 8 3, 21 7 9, 3 16 9, 3 7 12, 3 8 17, 4 15 9, 4 3 14, 6 9 16, 7 12 9, 7 9 19, 8 9 10, 9 12 8, 9 15 3, 9 17 3, 9 3 16 y 9 4 13. See Figure 3, register = 1.

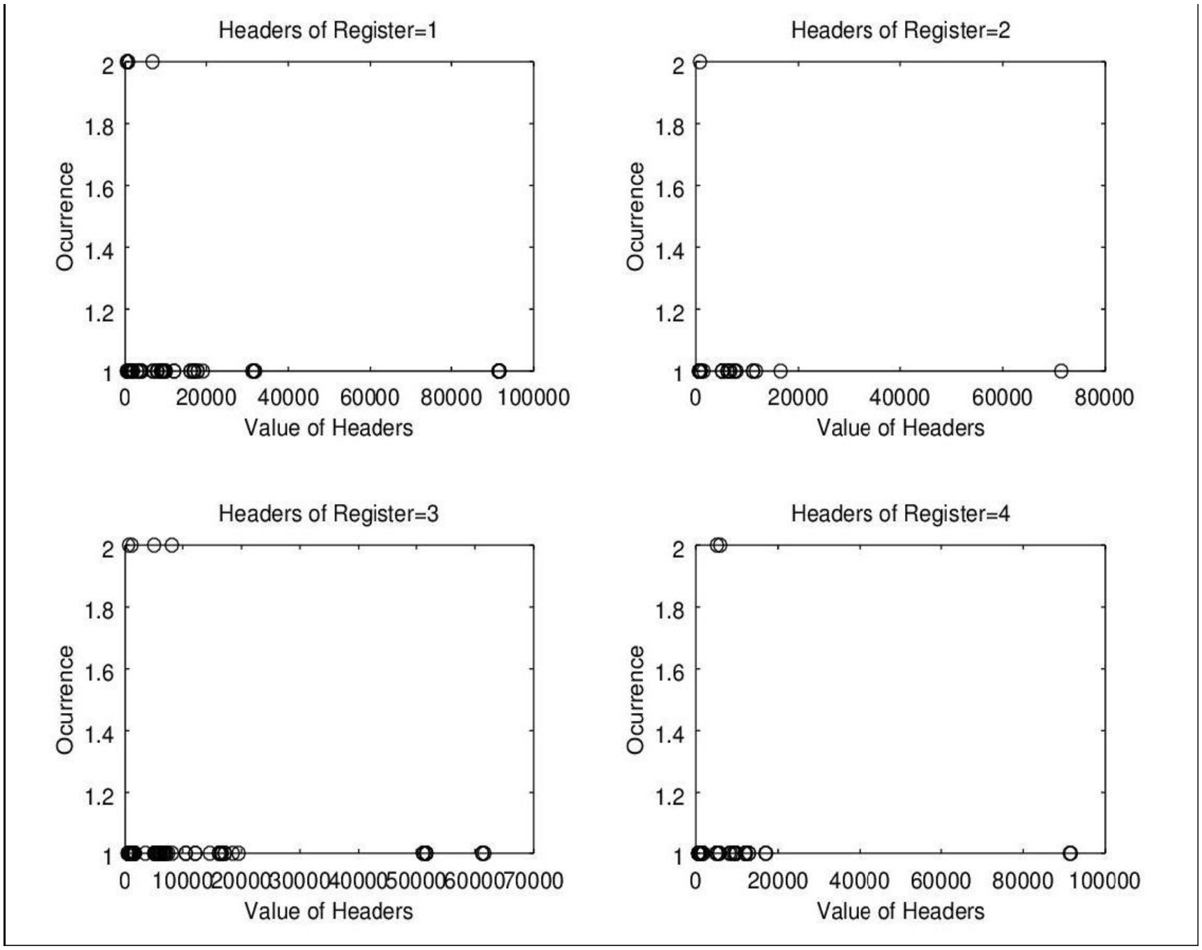

Figure 3. Header comparative result of ISDMessage-Symbol tool scanning from Patient#1 with 4g-force

4.4 Patient#1 4g-force Payloads

The Table 2 shows 20 longitudes payloads, these have since: 3 symbols, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 38, 40, 43, with an occurrence respectively of 18, 3, 10, 3, 5, 10, 4, 4, 7, 1, 2, 2, 2, 1, 1, 1, 2, 1, 1 y 1. See Figure 4, register = 1.

4.5 Others Recordings in Patient#1 4g-force

On channel 6, the f4.5620k02.plx register has 856 spikes in 36.7273s of recording, the ISDMessage-symbols software tool found 233 different subsets with 239 total subsets, see Figure 2, register = 2.

The root element is 7517, means, it is cluster of 7, 5, and 17 symbols. The Figure 3, register = 2, shows its headers values. The recording has 32 Messages with payloads longitudes of 3, 6, 8, 9, 10, 12, 13, 15, 17, 18, 24, 25, 35, 36, 37, 39, 42, 54, 59, 77, with a respectively occurrence of 4, 1, 1, 3, 1, 1, 2, 3, 1, 2, 2, 1, 2, 1, 1, 1, 1, 1, 1 y 2. See Figure 4, register = 2.

On the channel 6, the f4.5620k03.plx register has 1331 spikes in 56.385s of recording; the ISDMessage-symbols software tool found 353 different subsets with 369 total subsets.

The 659 root element, means, it is cluster of 6, 5 and 9 symbols, see Figure 2, register = 3. The Figure 3, register = 3, shows its headers values. This register has 94 messages, with longitudes in their payloads since 3 symbols, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 23, 25, 30, 38 with their respectively occurrence of 14 times, 1, 12, 3, 5, 8, 6, 5, 6, 7, 2, 5, 1, 3, 5, 4, 2, 1, 2, 1 y 1. See Figure 4, register = 3.

On the channel 6, the f4.5620k04.plx register has 727 spikes in 32.0499s of recording; the ISDMessage-symbols software tool found 199 different subsets with 207 total subsets.

The root element is 5916, means, it is cluster of 5, 9 and 16 symbols, see Figure 2, register = 4. The Figure 3, register = 4, shows its headers values. This register has 42 Messages, with longitudes in their payloads since 3 symbols, 5, 6, 8, 9, 10, 11, 12, 13, 17, 19, 20, 21, 23, 24, 25, 27, 30, 31 y 37, with their respectively occurrence of 4 times, 2, 3, 3, 5, 4, 1, 2, 2, 1, 3, 1, 1, 1, 2, 1, 1, 2, 1, 1 y 1. See Figure 4, register = 4.

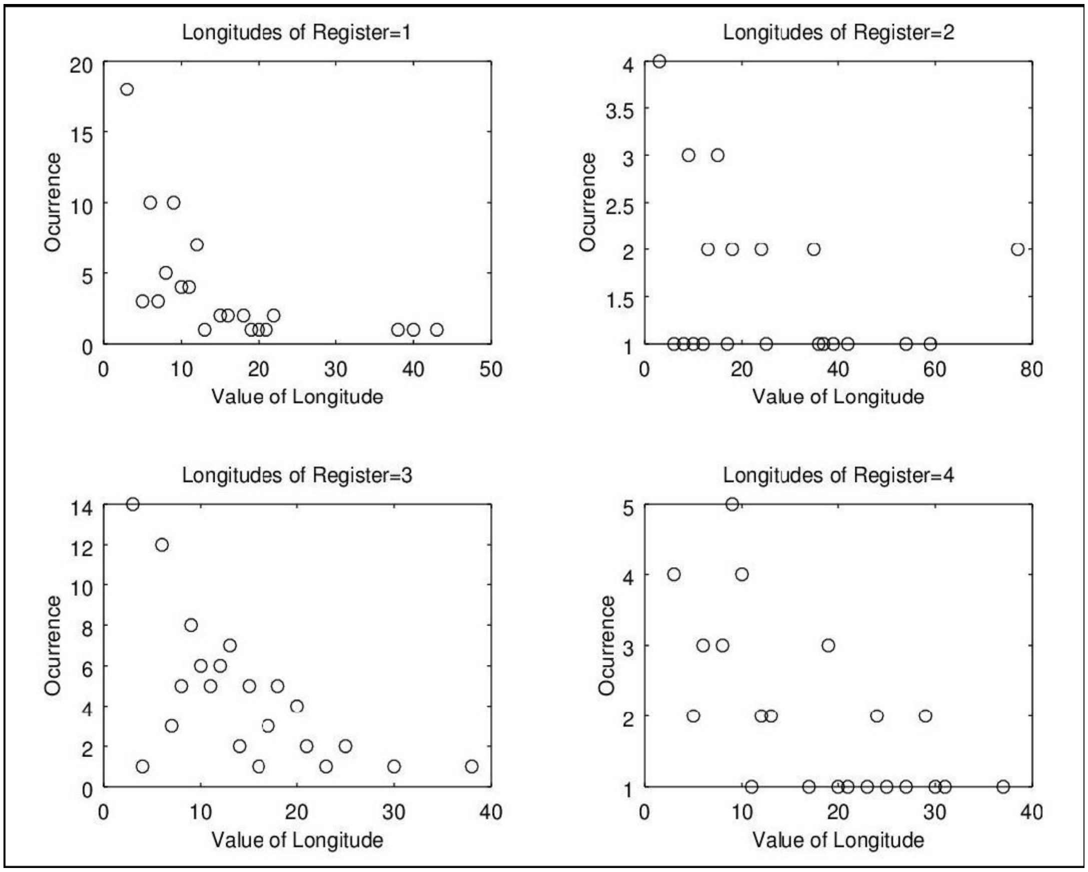

Figure 4. Longitude comparative result of ISDMessage-Symbol tool scanning from 4 different recordings of Patient#1 with 4g-force

5. Conclusions and Future Work

The SNP is the best place to acquire the signal without processing, lacking of the “signature” of each person.

The Figure 2, Figure 3 and Figure 4 are the results from recordings in the same patient under the same experimental situations it is easy to notice the similarity between the registers.

The repetitiveness of the occurrences on the subsets tell us about an intelligent structures defined within the bursts of PAs by cutaneous stimulating the skin of the patient. The repeated sequence of high priority, low priority and high priority subsets allows us to define the start and end of the message.

Initially, this research does not pretend to make a comparative among patients because it considers the individual as a unique being that by itself anatomy could handle raw signals different from than other patients.

Due to the uniqueness of the system, patients are managed independently to such an extent that prior to the initiation of the analysis the calibration process is performed and possibly in a future, the message structures shall be calibrated to each patient before being used.

The matching structures found into patient#1 (as shown in the Results section) is a breakthrough for this research. The coincidences in the structures tell us that the NS handles the stimulus by intelligent communication with temporized structure. And the NS must be handling a standard language known by the internal systems. Decoding the cutaneous communication code is practically the domain of the afferent system of PNS.

The possible reason why this research did not obtain a defined structure but only coincidences on subsets and not a complete matching may be the consideration of the "simple structure" proposed in the messages.

This document proposed a simple structure, header and payload, of the communication protocol and may not be adequate; and one should think in a complex structure with error detection algorithms, parity checking mechanisms; and protocols compared to telecommunication systems. Indeed, the coincidences in the subsets and their intersections speak about an intelligent and structured communication.

The nerve anatomy itself indicates that the myelin sheath prevents fibers from interfering with their signals with others. Just like wires have an insulation sleeve to prevent their signals from being limited or interfered with. Thousands of fibers travel through the nerve, and phenomena such as salutatory conduction communication cannot be a crude communication that increases or decreases its frequency with the intensity of the stimulus. PAs follow a structuring, are interpreted and codified with a standard that can be compared to any digital communication. The goal of this research is to find out which is the communication standard by reverse engineering.

In future work this research pretend use a message with a more complex structure with a calibration process; do not pretend to make a comparative among patients because it considers the individual as a unique.

Acknowledgment

The researchers are very grateful with Dr. Mario Romero for his counseling and bioengineering laboratory facilities at the University of Texas at Dallas. This work has supported by the National Council for Science and Technology of México.

References

[1] Hall, G. A. (2011). Textbook of Medical Physiology. USA: Saunders Elservier, 12a ed.

[2] Levy, B. ,Koeppen, Fisiología, S. B. (2009). Madrid: Elservier Mosby 6a ed.

[3] McNeal, D. R. (1976). Analysis of a model for excitation of myelinated nerve. *IEEE Tr. BME*, Vol. BME-23, p. 4. July

[4] Sweeney, J.D. ., J. T. (1987). Modeling of mammalian myelinated nerve for functional neuromuscular electrostimulation, IEEE 9th Ann. Conf. Eng. Med. Bio. Soc. p. 1577-1578.). Boston: IEEE.

[5] Loewenstein., W. R. (1959). The generation of electric activity in the nerve ending, Conference on Physicochemical Mechanism of Nerve Activity and Second Conference on Muscular Contraction, p. 367–387.

[6] Rattay, F. (1993). Modeling axon membranes for functional electrical stimulation. *IEEE Tr. BME*, Vol. 40, p. 12. December

[7] Rattay, F. (1998). Analisis of the electrical excitation of CNS Neurons. *IEEE Tr. BME.*, V. 45, p. 6. June

[8] W. A. Wesselink, J. H. (1999). A model of the electrical behaviour of myelinated sensory nerve fibres based on human data. Med. Biol. Eng. Comput., Vol. 37, p. 228-235.

[9] Y., O. (1991). Topographical differences in the vascular permeability of the peripheral nervous system. Neuropathological Laboratory, Department of Pathology I, University of Göteborg, Sweden (p. vol. 10. 26-33.). Göteborg, Sweden: Springer-Verlag . 1968. Milner T E, D. C. Cutaneos afferent activity in the median nerve during grasping in the primate Brain. Res Elsevier, Vol. 548: 228-41.

[10] Milner T E, D. C. (1991). Cutaneos afferent activity in the median nerve during grasping in the primate Brain". Res Elsevier,

Vol. 548. 228-41.

[11] Misgeld, T. N. (2007). *In Vivo* imaging of singal axons in the mouse spinal cor. *Nat. Protoc.*, Vol. 2, 263-268.

[12] Hoffer J A, L. G. (1987). Cat hindlimb motoneurons during locomotion. I. Destination, axonal conduction velocity and recruitment threshold . *J. Neurophysiol.*, Vol. 57 510-29. 1 February

[13] Kanamori, A. C. (2010). *In Vivo* imaging of retinal ganglion cell axons within the nerve fiber layer. Invest. Ophthalmol. Vis. Sci., Vol. 51 2011-2018.

[14] Plonsey, K. W. (1990). Point source nerve bundle stimulation: Effects of fiber diameter and depth on simulated excitation”. IEEE Tr. BME, Vol. 37, p. 688-698. July

[15] HJ., G. (1964). Comparative electron microscopic observations on the connective tissues of a peripheral nerve and a spinal nerve root in the rat. *Journal of Anatomy* , Vol. 98 17-24.

[16] Leung., C. (2011). Long-term *in vivo* imaging and measuremtn of dendritic shrinkage of retinal ganglion cell. *Invest. Ophthalmol. Vis. Sci.*, Vol. 52, 1539-1547.

[17] Dickinson R, F. N. (1995). The effects of temperature on the interactions between volatile general anaesthetics and a neuronal nicotinic acetylcholine receptor. *Br J Anaesth,* Vol. 116. 2949-56.

[18] P., I. D. (1961). The effects of temperature on the responses of Pacinian corpuscles. *The Journal of Physiology,* Vol.155 280-301. 1 FEB

[19] Mortimer, P. H. (1983). The effect of stimulus parameters on the recruitment characteristics of direct nerve stimulation. *IEEE Tr. BME,* Vol. 30, p.7. July

[20] Comer., D. E. (2000). Internetworking with TCP/IP - Principles, Protocols and Architecture (4th ed.). USA. Prentice Hall.

[21] Holzmann., G. J. (1990). Design and Validation of Computer Protocols. USA. Prentice Hall. October 11th 1990 .

[22] Perlman, R. (2000). Interconnections: Bridges, Routers, Switches, and Internetworking Protocols: Bridges and Routers (APC). Boston, MA, USA.: Addison-Wesley Longman Publishing Co., Inc.

[23] Vinton, G., Cerf, R. E. (1974). A Protocol for Packet Intercommunication, *IEEE Transaction on Communications*, 22 (5) 637-648. May 1974.

[24] Petrusz, P. (2005). The Handbook of Experimental Animal The laboratory Rat. University of North Carolina, Chapel Hill, U.S.A. Edited by Georg Kringe. Second Edition.

[25] Javier Longás Valién, J. C. Manual del anaestesiologo URPA y REA. Majadahonda (Madrid): Ergon. C/ Arbolea, ed. 1 . 2007.

[26] Ma Reina, A. L. (2000). Morfologia de los nervios periféricos, de sus cubiertas y su vascularización. Congreso de Anestesiología y Reanimación (p. 5). Madrid: Valmojado. agosto 2000.

[27] Prasad, Abhishek; Sanchez, Justin C. (2012). Quantifying long-term microelectrode array functionality using chronic in vivo impedance testing. *Journal of neural engineering*, vol. 9, no 2, p. 026028.

[28] Torfs, Tom, et al. (2012). Two-dimensional multi-channel neural probes with electronic depth control, *IEEE Transactions of Biomedical Circuits and Systems*, vol. 5, no 5, p. 403-412.

[29] Fitzgerald, James J.(2012). A regenerative microchannel neural interface for recording from and stimulating peripheral axons in vivo. *Journal of neural engineering*, . 9 (1) 016010.

[30] Kretzberg, Jutta, Thomas Coors, and Julia Furche. (2009). Comparision of Valley Seeking and T-Distributed EM algorithm for Spike Sorting. *BMC Neuroscience*, 10(1) 1.

[31] Shoham, Shy. Robust clustering by deterministic agglomeration EM of mixture of multivariante T-Distributions. *Pattern Recognition*, 35 (5) 1127-1142.