Author's response to reviews

**Title:** Effect of Glenohumeral Forward Flexion on Upper Limb Myoelectric Activity during Simulated Mills Manipulation; Relations to Peripheral Nerve Biomechanics.

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**Author's response to reviews:** see over
Reviewer's report

Title: Effect of Glenohumeral Forward Flexion on Upper Limb Myoelectric Activity during Simulated Mills Manipulation; Relations to Peripheral Nerve Biomechanics.

Version: 1
Date: 26 May 2014

Reviewer: Rod Whiteley

Level of interest: An article whose findings are important to those with closely related research interests

Reviewer's report:

Major compulsory Revisions:

1. **Question:** The magnitudes of the EMG signal seen in this study are objectively very small in size, with the possible exception of the upper trapezius. Of course the researchers are not meaning to be deceptive in any way, however presenting such data as relative changes can be misleading: a change from 1 microvolt to 2 is a 100% increase, but most likely simply background noise. As the authors point out, normalizing to an MVC is not appropriate, however normalizing to a submaximal movement would have been – eg: simply holding the subject’s arm to 45° abduction, or flexion or similar. This would allow the reader to infer the magnitude of these protective responses in real terms – ie, how much force is actually occurring as the subject’s nervous system “exerts this protective” reaction?

**Answer:** The authors would like to thank the reviewer for the constructive comment. As the reviewer already pointed out, the magnitude of the EMG signal seen in this study are objectively very small in size. This may be because we are likely dealing with reflex-mediated muscular contractions in response to painful stimuli, rather than the classical muscular contraction found during active movements and known to produce high amplitude myoelectric signals. We too hypothesized that part of the recorded signal might include background noise. For this reasons we took all the appropriate steps to exclude this possibility, namely: In the data collection process we used a laptop working on battery, and the cables connecting the electrodes to the EMG device were twisted as to diminish the loops.

The power spectrum of each individual signal was checked for 50Hz peaks using the Discrete Fourier Transformation function included in our BTS system, Smart Analyzer, called DFT Powerspectrum, before accepting the signals for further analysis and discharging the volunteers.

Moreover, before submitting the manuscript for review, we sent the records to be checked by another laboratory. The Biosignal Analysis and Medical Imaging Laboratory at University of Eastern Finland, Kuopio (Finland) checked the signals by calculating the Fourier-based spectrum with the Welch’s averaged periodogram method (length of overlapping epochs was 1000 ms and overlap was 75 %). No peaks were observed in the spectra in the 50 Hz frequency bin, allowing the investigators to refuse the existence of power line interference in the recorded signals. This information has been inserted into the manuscript, lines 131-144.
Regarding the signal normalization: As stated also in 2012 Early Career Researcher School in New Methods of Investigation in Neuroscience, organized during the International’s Society of Electrophysiology and Kinesiology (ISEK) Biennial World Congress which took place in Brisbane, Australia, normalizing the EMG data to MVC might be not advisable as:
- MVC does highly depend on the subject’s training and his/her understanding of what MVC is
- Its high variability may actually also increase the variance of the presented data.
- In my personal view, even more perplexities may arise when choosing to normalize the EMG data recorded during the activation of a Nociceptive Flexor Reflex to EMG data collected from active movements.

However, we do share the reviewer’s opinion that in some cases normalization may be advisable. In the literature there are quite no investigations trying to quantify and analyze muscular reactions to neural mechanical loads in in-vivo and structurally intact asymptomatic human subjects. For this reason we consider the presented results as part of a developing process aimed at i) presenting information useful for investigators exploring this area and ii) improve the used methods. In our new experiments, in which we are currently analyzing quantitative parameters of such muscular activations, we have indeed normalized the data to those recorded during rest, and during strong muscular stretch. The overall trends are extremely similar to the ones presented here. We accept the reviewer’s comment and listed this point both as a limitation and suggestion for further improvement. We have also added a short discussion on the matter in lines 387-393.

Again, we wish to thank the reviewer for this comment.

2. Question: I appreciate the fact that the authors have essentially provided all the raw data in Figure 4, however it’s not appropriate to represent the data in figure 4 as a continuous line for each subject, rather each category (muscle group in this case) should have each data point represented by an individual marker. From this graph it is evident that the absolute values of EMG for all muscles (except upper trapezius) in all conditions in nearly all subjects are extremely low, likely less than 25 microvolts. If the authors wish to display the individual data points (which I encourage) then I would suggest a categorical scatter plot with perhaps the addition of a “Mean ± SD” point for each muscle (if the data is normally distributed) or a median ± 25th percentiles if it is not.

Answer: We thank the reviewer for this comment. Originally we decided to use this way of presenting data in EMG research bounding values gathered from the same acquisitions, thus connecting the raw data with a continuous line, following the positive feedbacks on our previous scientific publication on the same matter. At that time, it emerged that this figure was well accepted as it provided some direct visual information on the generally decreasing myoelectric trends.

However we do agree that this figure is not standard. For this reason we here propose an alternate figure. As the presented variables are gathered independently, a categorical scatter plot may not be best indicated. We here propose to use boxplots that allows the presentation of added information along with myoelectric trends, as median value ± 25th percentiles.

Moreover, the caption to figure 4 has been changed and inserted in lines 315-320. It now reads: Directional change in myoelectric values during the standard pre-manipulative stretch for Mills manipulation and the Varied position. Significant changes (P<.05) are marked with an asterisk. Note the significant decrease of myoelectric activity and variance of the signal in all the test muscles in the Varied position. No significant difference in myoelectric activity was found between right and left sides (P>.05).

Abbreviations: SMM - Standard Mills Manipulation.
We think this represents an improvement and added value for the attentive reader, and hope the reviewer will appreciate the change. However, if the reviewer thinks that Figure 4 should be omitted from the manuscript, we are willing to do so.

3. Question: Table 2: Presenting data as percentage change and observed power/p value can be misleading. Since your data are likely correlated, and perhaps not normally distributed, you may wish to consult something like: Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front. Psychol. 4:863. doi:10.3389/fpsyg.2013.00863.

Answer: The effect size has now been calculated dividing the mean value by the Cohen’s $d$, value, as suggested in Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front. Psychol. 4:863. doi:10.3389/fpsyg.2013.00863. The new values have now been inserted in table 2 and a short description added to statistical methods in lines 283-284.
Again, we thank the reviewer for this constructive suggestion.

4. Question: Similarly, please place confidence intervals on the bars in Figure 3

Answer: 95% Confidence Intervals (CI) were calculated using t-distribution and are now presented on the bars in Figure 3.

Minor Essential revisions:

1. Question: Line 10: “Since” is redundant

Answer: we feel that the grammar is correct, but the sentence is still very long and it would be better to break it up into pieces. The new sentence now reads:

Since this study focuses on normative responses which may in the future serve as reference material for the Mills manipulation in patients with lateral elbow pain, the maneuver relevant to this study is the radial neurodynamic test (RNT). Using buckle force transducers in cadavers, this test has been shown to apply a significant magnitude of tensile force to the radial nerve, as well as to the medial, posterior and lateral cords of the brachial plexus [7]. The same test has also been shown to produce symptoms its end range in asymptomatic subjects [8]. Lines 10-16.

2. Question: Line 43: “the effects” not “these effects”

Answer: Corrected – Line 45
3. **Question:** Lines 68-71: ii) whether non-specific neural and muscular effects of Mills manipulation could be controlled with forward flexion of the shoulder girdle joint; with “non-specific” meaning “effects that are not the direct target of the manipulation”. I find the term “Non-specific” confusing in this context.

**Answer:** Following the notion that the mills manipulation maneuver is extremely similar to the one Von Lanz and Wachsmuth (1959) specifically designed to apply mechanical tension to the radial nerve and its posterior interosseous branch, the present paper investigates two points: i) whether any discernible pattern in electromyographic activity in selected muscles would emerge during the pre-manipulative stretch for Mills manipulation (possibly for mechanical protection of nerves) and ii) whether such non-specific neural and muscular effects could be controlled with forward flexion of the shoulder. In this context, non-specific would mean ‘those tissues that were not the specific target of the maneuver’, such as nerves. With that, if such non-specific or collateral neural effects were found to occur, it may then be valuable to control them during clinical performance of the technique. As short discussion has been added in lines 72-75.

4. **Question:** Line 278 “Measurement” should read “Measurements”

**Answer:** Corrected – Line 291

Overall, we find that the manuscript improved significantly and would like to thank the reviewer for his contribution.
Reviewer’s report

Title: Effect of Glenohumeral Forward Flexion on Upper Limb Myoelectric Activity during Simulated Mills Manipulation; Relations to Peripheral Nerve Biomechanics.

Version: 1
Date: 4 June 2014
Reviewer: Leanne Bisset

Level of interest: An article of importance in its field

Reviewer’s report:

This paper investigated the mechanical effects on the radial nerve through changes in the pre-manipulation position for a Mill’s elbow manipulation. Overall, I commend the authors on a well-controlled study design and a nicely written paper.

I make the following comments and suggestions:

1. **Question:** Lines 148-149: how did you define the center of the muscle bellies?

   **Answer:** We defined the center of the muscle bellies following the European Recommendations for Surface Electromyography - SENIAM suggestions for electrode placement in Surface Electromyography (2000), with the electrode placement site being located at 50% of the line between the anatomic points listed in the SENIAM suggestions. Additionally, we visually compared the marked points with those provided in the maps from Konrad’s text book ABC of EMG (2005) and Freiwald et al. (2007). For Brachioradialis muscle we followed the directions in O’Sullivan and Gallwey (2002).

   References:

2. **Question:** When collecting EMG data from muscles that undergo some degree of movement, how can you be sure that the muscle fibres underlying the surface electrodes is the same between positions? For example, moving from shoulder abduction to shoulder flexion may mean that position of the electrodes overlying the upper trapezius, pectoralis major and biceps brachii muscle have moved -did you confirm that there was still muscle activity recorded from relevant muscles between positions, prior to formal testing?
The possibility that changes in shoulder position may be a potential confounder for measurement of EMG amplitude in muscles around the shoulder (i.e. those muscles that change length with change in shoulder position) needs to be explicitly mentioned as a limitation in the discussion.

**Answer:** We thank the reviewer for such comment. We do agree that the position of surface electrodes may change with respect to the muscle fibres from the underlying muscles and we see that this may be particularly true for Pectoralis major muscle. We confirm that before commencing the investigation we tested the signal collected from each muscle in order to check the quality of the signal and ascertain that myoelectric activity could be recorded in each test position.

As the presented analysis is based on within-subject comparison, the electrode positioning in respect to the orientation and position of muscle fibres from the underlying muscles is less likely to have influenced the presented data on subjective muscular reaction to neural loading. However, this is a common issue with surface electromyography investigations that may partly be avoided with the use of needle electrodes. We have inserted a small discussion on this matter in lines 394-398 and listed it both as a limitation and suggestion for further improvement.

We do appreciate the reviewer’s comment on this point as it stimulated constructive discussion between the authors of this study.

3. **Question:** Methods: was the study participant required to actively hold their shoulder in the test positions, or was this a passive position where the investigator supported the arm? Can you please make this clear in the methods?

**Answer:** In this investigation we recorded the changes in myoelectric activity during two different positions of the upper limb hypothesized to apply different degrees of strain to the peripheral nerves and brachial plexus. Those were passive positions where the investigator supported the subjects’ arm.

We thank the reviewer for this comment and do agree that this point needed to be clarified in the methods section. We have thus reformulated the paragraph in lines 230-234.

4. **Question:** Line 229: this shoulder read "....while maintaining the 90 degrees elevation in the frontal plan."

**Answer:** Corrected, line 239. We thank the reviewer for this correction.

Overall, we find that the manuscript improved significantly and would like to thank the reviewer for her contribution.