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Response

Response to "Sodium Current Inhibition by Nanosecond Pulsed Electric Field (nsPEF)—Fact or Artifact?" by Verkerk et al.

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It was nice to learn that our studies of nanosecond pulsed electric field (nsPEF) effects on membrane currents [Nesin et al., 2012; Nesin and Pakhomov, 2012] gained the attention of scientists outside the immediate field of bioelectromagnetics. The insight and constructive comments from scientists representing diverse areas are most welcome and help to identify the next research steps. Also, answering to critical comments gives the authors extra opportunity to convey more details about already published experimental data.

The comments by Verkerk et al. [2012] start with a basic introduction to the patch clamp method. They reiterate a well-known fact that the command voltage (V_c) is distributed between the series resistance of the pipette (R_s) and the cell membrane resistance (R_m), so that the clamped membrane potential (V_m) is actually less than V_c. This difference can be negligible for $R_s \ll R_m$, but may cause measurement errors when R_s is too high and/or R_m is too low. These considerations are thoroughly known by patch clamp practitioners and are emphasized in every relevant textbook (e.g., Molleman [2002]); hence, the reiteration appears somewhat redundant for a journal article.

Next, Verkerk et al. point to the effect of increasing the leak current (I_{leak}) by nsPEF. The increased I_{leak} reflects lower R_m and increased deviation of V_m from V_c. For a V_c of −80 mV (which we used as a holding potential), the development of $I_{leak} = 2{,}500$ pA translates into V_m depolarization from −80 to −70 mV, and holding the cell at a more depolarized V_m increases the inactivation of I_{Na}. Thus, Verkerk et al. hypothesize that the inhibition of I_{Na} by nsPEF was caused by an error in setting the holding membrane potential because of the huge I_{leak}.

This concern could be legitimate if Verkerk et al. used the right numbers. Regretfully, they did not. Instead, they arbitrarily chose a very large I_{leak} value of 2,500 pA, which has little relevance to the reported experiments. Why? There is no explanation in their paper. Apparently, using this heavily exaggerated value was the only way to support the ''artifact hypothesis.'' If we use the actual and typical experimental values of I_{leak} and estimate the artifacts using Figure 1 in Verker et al. paper, it becomes evident that the potential artifacts were too small even to be detected; or, in other cases, they were much smaller than the observed nsPEF effects.

Let us take a look at the actual I_{leak} values measured for V_c of −80 mV. In Figure 2B [Nesin et al., 2012], I_{leak} is only 50 pA (1.8 kV/cm nsPEF) or 200 pA (3 kV/cm). In Figure 2C, I_{leak} is also about 50 pA. Using Figure 1 in the article by Verkerk et al., the respective error in the holding voltage was just 1 mV (noise!) and the inhibition of I_{Na} was 1–2% (also just noise). In actuality, I_{Na} was inhibited by as much as 30–60%. Therefore, the ''artifact hypothesis'' by Verkerk et al. is irrelevant and fails to explain these experimental data.

Most of the other figures (Figs. 4–6 from Nesin et al. [2012], and Figs. 1, 3, and 5 from Nesin and Pakhomov [2012]) show data from cells that were ''patched'' prior to nsPEF exposure. Therefore, I_{leak}

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was measured much sooner after nsPEF (in 10–20 s), and its values typically were higher. In most of these experiments, and in most cells, a profound reduction in I_{Na} (two- to sixfold) was observed concurrently with I_{leak} values between 400 and 1,400 pA (note that I_{leak} values in Fig. 6 are shown for −90 mV and are 15–25% greater than at −80 mV). A typical I_{leak} value in cells that show a two- to sixfold inhibition of I_{Na} can be conservatively estimated to be about 1,000 pA for a −80 mV V_c.

For 1,000 pA I_{leak}, Figure 1 in the Verkerk et al. article predicts the reduction of V_m from −80 mV to −76 mV and a 10% decrease in I_{Na}. While these values are slightly above the ''noise'' level, they are far below the actual effect of I_{Na} inhibition by nsPEF. Therefore, the ''artifact hypothesis'' by Verkerk et al. again fails to explain the experimental data, although may account for a minor portion of the nsPEF effect.

Closer to the end of their comments, Verkerk et al. specifically discuss Figure 2C [Nesin et al., 2012], which shows a still inhibited I_{Na} minutes after I_{leak} had recovered. Somehow, Verkerk et al. again ignore the fact that the I_{leak} in these experiments was only 50 pA to start with. They further speculate that ''the voltage dependency of I_{Na} inactivation shifts toward more negative potentials in time after cells are patch clamped.'' However, there is no ''in time'' factor here. Apparently, Verkerk et al. ignored the notion (second paragraph on the same page) that ''the whole-cell configuration was formed 30–60 s prior to the scheduled data collection at 5, 10, or 15 min after exposure.'' In other words, the cells were left ''untouched'' until immediately before the measurements, so any discussions about the shift of ''voltage dependency of I_{Na} inactivation'' due to the prolonged holding of cells under patch clamp conditions are not applicable.

Notably, Verkerk et al. omitted any discussion of important Figure 7 [Nesin et al., 2012], which shows that I_{Na} may decrease with I_{leak} as low as 50 pA, or may increase despite I_{leak} as high as 1,500 pA. Poor correlation between I_{leak} and the inhibition of I_{Na} does not fit with the hypothesis of Verkerk et al.

It might also be useful for Verkerk et al. to take a look at Figure 9.5 in one of the referenced papers [Pakhomov and Pakhomova, 2010]. Using a potentiometric fluorescent dye concurrently with whole-cell patch clamp, we demonstrated that, within the studied limits, nsPEF exposure did not alter the accuracy of controlling V_m by V_c.

The data and arguments provided above are more than adequate to rule out the artifact hypothesis proposed by Verkerk et al. This hypothesis fails to explain the experimental data on all accounts, and that is why we did not discuss this type of artifacts in the original experimental papers. The nsPEF-induced inhibition of voltage-gated I_{Na}, I_{Ca}, and of certain but not other types of I_K (unpublished) is an intriguing and complex phenomenon that awaits detailed analysis. If Verkerk and coworkers are genuinely interested in this topic, they are most welcome to join the effort. We are open for ideas and proposals for collaboration.

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