Response to Comment on “Detection, Stimulation, and Inhibition of Neuronal Signals with High-Density Nanowire Transistor Arrays”

Brian P. Timko, Fernando Patolsky, Charles M. Lieber

Fromherz and Voelker make incorrect assumptions about our experiments that raise serious questions about the validity of their claims. We show that our calibrated signals are consistent with previously published data and a general model with biophysically relevant parameters. Additionally, the wide variation in previously published signal amplitudes suggests caution in applying and drawing conclusions from the models of Fromherz and Voelker.

Patolsky et al. (1) used arrays of silicon nanowire field-effect transistors to record neuronal signals along the individual axons and dendrites of cortical rat neurons. In their comment (2), Fromherz and Voelker claim that a physical rationale cannot be found for our recordings. However, there are three substantial deficiencies in their assumptions and analysis that raise doubts about the validity of their claims. First, Fromherz and Voelker make incorrect conclusions about the properties of our nanowire field-effect transistor (NWFET) devices and report signals that lead to greater than one order of magnitude error in the estimated voltage signals in our experiments. Second, they carry out analyses with models that either have no physical relevance to our experiments or use rigid parameters that cannot explain their own related published work. Third, the authors may not recognize the complexity of the interfaces between cells and conventional planar as well as newer nanoelectronic devices, which correspondingly leads to uncertainties in parameters used to describe such interfaces. Below, we address these three issues and show that (i) the average signal changes reported in our work yield calibrated voltages within the range of values reported for related studies using planar transistors by Fromherz and co-workers (3–5) and others (6), and that (ii) a general model applicable to our experiments and reported ranges of biophysical parameters yields signals consistent with our published results.

Central to the critique of Fromherz and Voelker (2) is their claim that our recorded signals correspond to a change of about −85 mV at the NW surface. This claim arises from their assumption that the NWFETs have a calibration factor of 1 nS/mV and that the typical recorded signal is 85 nS; unfortunately, neither of these assumed values is correct. First, the transconductance value used from Fromherz and Voelker to convert our measured signals to an estimated voltage is based on some of the earliest devices published by our group in 2001 (7) and estimated values for oxide capacitance (Cox) and hole-carrier mobility (μ). Given the substantial improvements in NWFET performance since this initial work (8–10), the Fromherz and Voelker assumption is not representative of devices produced after 2001. Below, we show that their assumption is conservatively an order of magnitude too small. Second, the results shown in figure 1 to 4 of (1) yield an average ± 1 standard deviation (SD) of 66 ± 21 nS for the signal change recorded by the NWFETs. Thus, the recorded signal value used from Fromherz and Voelker barely lies within +1 SD of those shown and is not statistically representative.

The device sensitivity can be assessed experimentally without assumptions. Data for two NWFETs representative of those used in (1) yield device sensitivities, ∆G/∆V_J, of 7 and 15 nS/mV (fig. S1). This measured range of sensitivity values is substantially above the estimate of Fromherz and Voelker. The low value they estimated can be attributed in large part to the small value of Cox used, which contrasts both calculated and experimental results (11, 12). A calculated value of Cox = 2.3 × 10^{-9} F/m, which is in good agreement with measured values (11, 12), yields a maximum sensitivity of 30 nS/mV for a device with the same geometry and carrier mobility as in (1) [see Supporting Online Material (SOM)]. Experimentally measured sensitivities of 20 to 30 nS/mV, which are substantially larger than the 10 nS/mV used in our analysis, have also been reported in recent studies (10).

Based on these results, we used a conservative calibration factor for the cell/substrate interface (∆G/∆V_J) of −10 nS/mV and the average NWFET/neuron conductance change of 66 nS from (1) to obtain a voltage ∆V_J = −6.6 mV, not −85 mV as claimed by Fromherz and Voelker (2). We note that this calibration is a reasonable approximation, but assuming the higher device sensitivity as reported recently for NWs prepared in a similar manner (10) would imply even smaller ∆V_J values of between −3.3 and −2.2 mV. Our calculated voltage, which is independent of the size of the device used for recording, is similar to values from at least three independent studies published by Fromherz and co-workers where ∆V_J values up to 8 mV were reported (3–5), and substantially smaller than the 30 mV value shown by Cohen et al. with mechanical pressure (6). For completeness, we tabulated the >10^5 range of voltage values reported by Fromherz and others for FET recording from neurons (table S1) and return to the important implications of this large range below. Therefore, the signals reported in (1) are consistent with values published in the literature, in contrast to the claims of Fromherz and Voelker.

Fromherz and Voelker (2) use models as a major basis for their claims, but their analysis has two important shortcomings. First, their models depend on the current flow model relevant to our experiments, Fromherz and Voelker make questionable choices for parameters to give a maximum value of −7 μV. The value they chose for Na⁺-channel conductance per unit area was for the soma and not the axon, the latter of which is known to have a much larger value (13, 14). Their selected value for r_J, which has not been measured in the specific case of axons, lies at the low end of a range that spans 10^4 (13, 16). In addition, another group not cited in (2) was unable to fit their opposite sign signals (17) and, thus, had to derive an entirely different model from Fromherz and Voelker to explain their data. Last, Fromherz and co-workers have measured both +4 and −4 mV signals simultaneously from two devices under the same cell. To qualitatively rationalize these signals, they invoke an inhomogeneous ion-channel distribution model (3). The models applied to our work, however, assume uniform average parameters, which cannot explain their results.

We find that the general current-flow model described by Fromherz and Voelker and the reported ranges of biophysical parameters yield signals consistent with our experiments. Specifically, the approximate potential change at the NWFET can be calculated from ∆V_J = (V_M − V_{Na}^-)/(1 − w_J^{-1} tanh w_J), where w_J = w_J/√(r_{J}g_{Na}^-/4). Using w_J = 1 μm, the estimated axon width crossing a NWFET, a reasonable
value for the Na\textsuperscript{+}-channel conductance of g\textsubscript{\text{Na}} = 200 mS/cm\textsuperscript{2} (13, 14), a mid-range sheet resistance of r\textsubscript{J} = 300 M\ohm/square (15, 16), and (V\textsubscript{M} − V\textsubscript{0}\text{\text{\textsubscript{Na}}}) = −50 mV yields ΔV\textsubscript{J} = −2.4 mV. This value is within a factor of 2 to 3 of our experimental value, −6.6 mV, and values reported by Fromherz and co-workers (3–5). Therefore, estimates using parameters within published biophysical ranges yield modeled signals consistent with those reported in (I).

It is important to emphasize that the interfaces between cells and devices have significant variability, which necessarily leads to considerable uncertainty in parameters used to describe these interfaces and correspondingly suggests caution in using and drawing conclusions from the models proposed by Fromherz and Voelker. A review of published data (table S1 and SOM text) shows that measurements from the same types of neurons and devices have yielded variations in signal amplitude of 400 and sometimes more. In certain cases, the values have been modeled, but not a priori predicted, by adjusting parameters, although other work published by Fromherz and co-workers and other groups could not be explained by their standard variation of parameters (5). This variability should not be surprising given that a living cell is a dynamic entity that will respond to the local environment of a device surface to yield, for example, inhomogeneous distributions of ion channels (3). In (5), Fromherz and Voelker invoke an argument of enhanced ion channels to qualitatively explain their larger signals, yet parameters such as those chosen to criticize our work cannot explain their own recordings.

In our own experiments, the NWFET device protrudes above the plane of the substrate surface (fig. S2), and the nonuniform NW-neuron interactions could lead to larger signals due to recruitment and enrichment of ion channels (18) or enhanced local pressures at the NW-neuron interface (6). Notably, recent studies of cultured rat neurons on carbon nanotubes show that these nanoscale wires generate tighter junctions than expected for typical planar substrates (19). Therefore, we can conclude that (i) in general, cell-device interfacial properties are highly variable, (ii) interfacial parameters could be enhanced by stronger interactions with nanostructured surfaces, such as NWFETs, and (iii) together these points challenge the rigid choice of parameters used by Fromherz and Voelker to justify their claims.

We have shown that the average signal changes reported in our work yield calibrated voltages within the range of values reported by several groups and thus conclude that our results are both physically reasonable and consistent with expectations from the existing literature. We have also shown that a current-flow model and reported ranges of biophysical parameters yield signals consistent with our experimental results. However, we emphasize that parameters used to describe the interfaces between cells and conventional planar or our nanoscale devices have substantial uncertainty, and thus we suggest caution in using and drawing conclusions from the models proposed by Fromherz and Voelker.

References and Notes
20. Harvard University has applied for a patent based on the work described in (I).

Supporting Online Material
www.sciencemag.org/cgi/content/full/323/5920/1429c/DC1
SOM Text
Figs. S1 and S2
Table S1
References
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