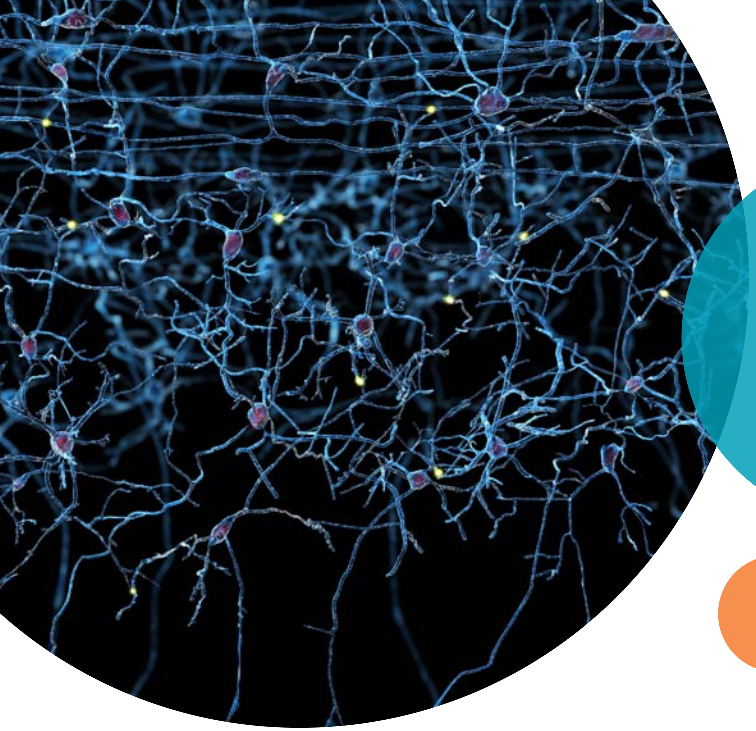

HumSilencer: A smart and simple feature for eliminating line-frequency noises across a wide range of electrophysiological applications

A major challenge within electrophysiology research is 50 or 60 Hz line frequency electrical noise, which can either distort or completely drown out the biological signal the researcher is attempting to acquire. Methods for tackling this problem already exist but are either slow and inefficient or run the risk of themselves distorting the data. The HumSilencer feature within the Axon™ Digidata® 1550A/B Series Low-Noise Data Acquisition System from Molecular Devices provides a quick, adaptive method to remove such noise, across a range of electrophysiological applications, without distorting the data.

eBook contents

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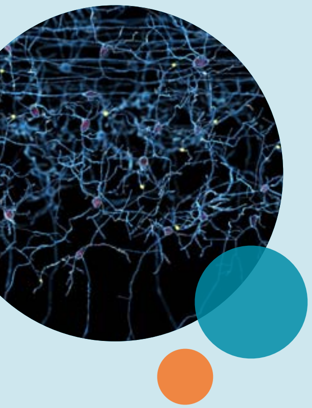


- Eliminating contamination from electrical noise is a major challenge in electrophysiology.

Introduction

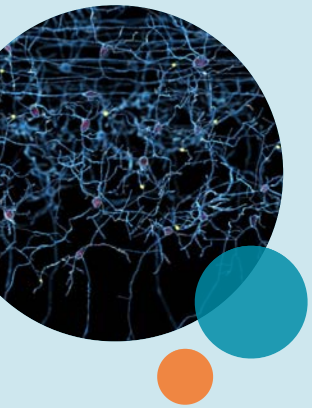
One of the major challenges in many electrophysiology laboratories is the appearance of 50 Hz or 60 Hz line-frequency noises and the associated high-frequency harmonics with acquired signals during the data acquisition process. Contamination with line-frequency noise can completely overwhelm biological signals of interest, making sensitive current or voltage measurements impossible. Even if the signal is not completely overwhelmed, this noise distorts the shape of the acquired signals. Therefore, minimizing and, if possible, entirely eliminating line-frequency noise is critical for many electrophysiologists.

Traditionally, one way of reducing noise is via a process of trial and error; to identify the sources of electrical noise and either eliminating or more usually shielding them. However, experiments need to be halted whilst the researcher embarks on this lengthy, time consuming and usually only partially effective troubleshooting process.

Notch filter or off-line filtering methods are sometimes used to eliminate the line-frequency noises. However, these filter-based methods are unable to eliminate electrical noise caused by high-frequency harmonics, and in some situations, can distort biological signals and reduce the overall data accuracy.

In order to rapidly and effectively eliminate line-frequency noise without distorting the underlying biological signal, we have developed the next generation of digitizer- the Axon™ Digidata® 1550A/B Series Low-Noise Data Acquisition System Plus HumSilencer™ Adaptive Noise Cancellation.

The HumSilencer feature provides a smart and simple way to eliminate 50 Hz or 60 Hz line-frequency noise and the associated high-frequency harmonics.



Electrical noise removed

The HumSilencer is an advanced, filter-free, adaptive technology that learns and removes local line-frequency noise patterns and associated high-frequency harmonics from incoming signals in less than one second. With a single mouse click on the “Subtract” checkbox in pCLAMP software, line-frequency noise is subtracted from the incoming signal during data acquisition, leading to a clearer biological signal with 50 ms (Fig 1).

- The HumSilencer eliminates electrical noise within one second.

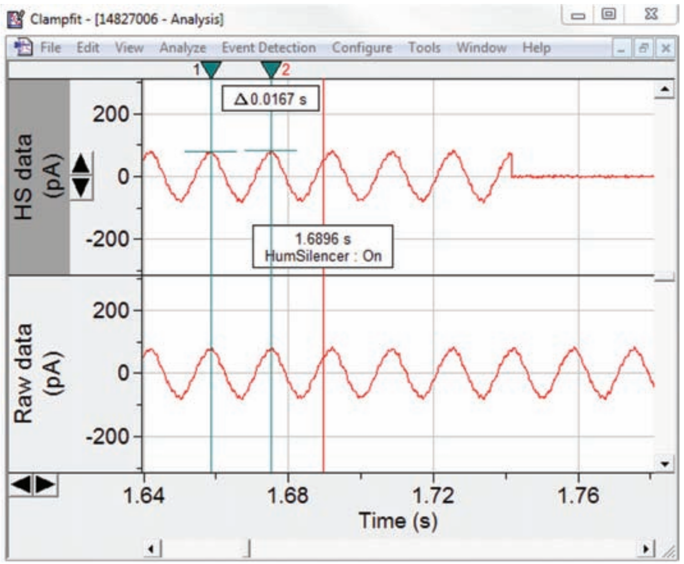
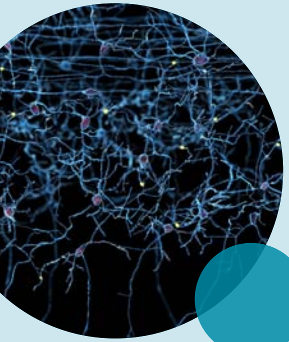


Figure 1. HumSilencer elimination of 60 Hz line-frequency noise. Recordings made from a model cell attached to an Axopatch 200B amplifier, with 60 Hz line-frequency noise introduced by a noise generator placed next to the model cell. Signals were digitized by a Digidata 1550A plus HumSilencer. Bottom trace: raw data; Top trace: same data with HumSilencer enabled at 1.6896 s (red line). Please note that the noise elimination takes place approximately 50 ms after the HumSilencer is turned on. The two vertical green lines indicate peak-to-peak time (0.0167 s).

The HumSilencer feature provides a smart and simple way to eliminate 50 Hz or 60 Hz line-frequency noise and the associated high-frequency harmonics.



Adapting to changes in noise patterns

The HumSilencer feature provides a fast adaptive rate (within 1 s) for changing noise patterns (Fig 2), digitizes a large range of input signals from -10 to +10 V, and eliminates noise amplitudes at the digitizer's analog input up to 20 V peak to peak.

- The HumSilencer quickly adapts to changing noise conditions.

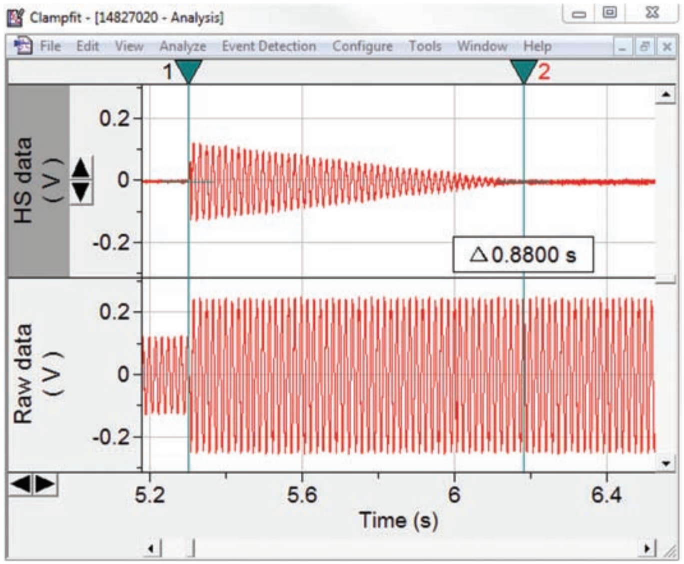
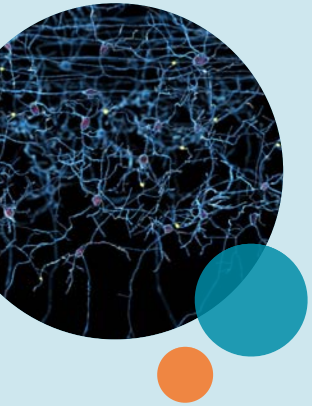


Figure 2. HumSilencer quickly adapts to changing noise conditions. Recordings made from a model cell attached to an Axopatch 200B amplifier, with 60 Hz line-frequency noise introduced by a noise generator placed next to the model cell. Signals were digitized by a Digidata 1550A plus HumSilencer. Bottom trace: raw data; top trace: same data with HumSilencer already enabled. At 5.3 s, the amplitude of the noise increases. In less than one second (time indicated by two vertical green lines, 0.8800 s), HumSilencer adapts to the changed noise conditions and eliminates the electrical noise, restoring the biological signal.

The HumSilencer feature provides a smart and simple way to eliminate 50 Hz or 60 Hz line-frequency noise and the associated high-frequency harmonics.



- The HumSilencer eliminates line-frequency noise across a wide range of electrophysiological applications.

The HumSilencer feature enables accurate measurement.

Single-channel recording

One of the most demanding applications in electrophysiology is single-channel recording (Fig 3). Typical currents from single-channel recording are in the order of single to tens of pico amperes (pA). In the presence of ~40 pA of line frequency noise, the small signal from the single-channel is undetectable (Fig 3, bottom panel). However, with HumSilencer enabled, the biological signal is revealed (Fig 3, top panel).

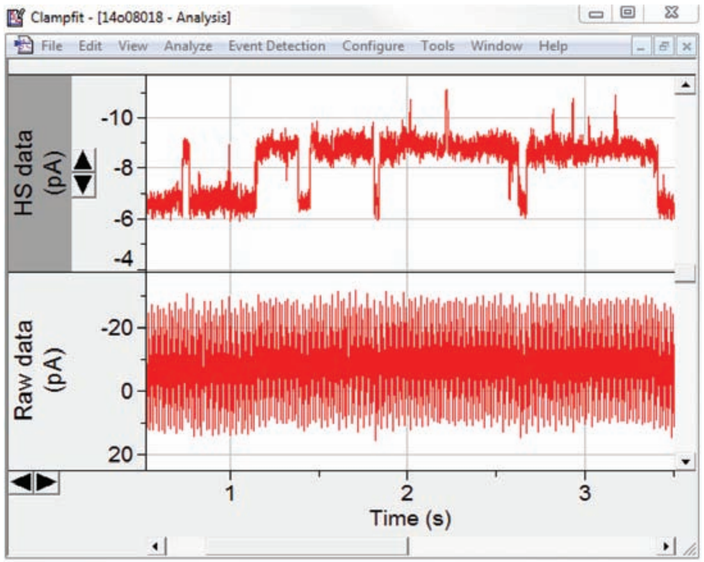
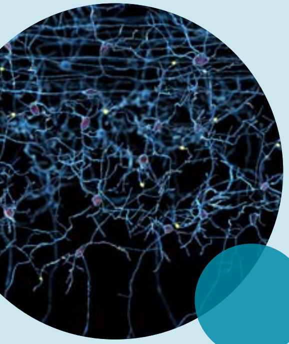


Figure 3. HumSilencer (HS) enables accurate signal measurement in single-channel recordings (data with HumSilencer enabled, raw data in bottom panel). Single-channel recording of the principal subunit of the olfactory cyclic nucleotide-gated channel (CNCA2). The excised membrane patch was isolated from HEK293 cells transfected with CNCA2, and the membrane clamped at +50 mV in the presence of 1 µM cGMP. Data courtesy of Tsung-Yu Chen, Ph.D. University of California, Davis.



- The HumSilencer eliminates line-frequency noise across a wide range of electrophysiological applications.

The HumSilencer feature enables accurate measurement.

Extracellular field-potential recording

A very different electrophysiological application is extracellular field-potential recording from brain slices (Fig 4). When an exogenous noise source is placed near the headstage during recording of extracellular population spikes (PSs), the shifting baseline prevents accurate measurement of the extracellular PS (Fig 4, bottom panel). When HumSilencer is enabled, the noise is removed, the baseline flattens, and the extracellular PS can now be accurately measured (Fig 4, top panel).

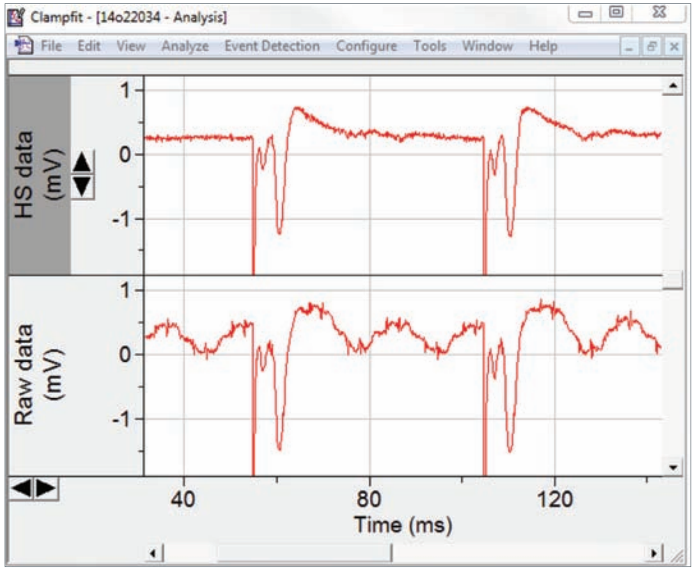
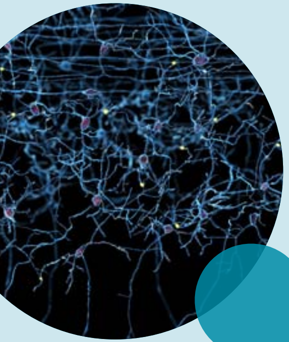


Figure 4. HumSilencer (HS) enables accurate measurement of extracellular field-potential recordings (Data with HumSilencer enabled in top panel, raw data in bottom panel). Representative extracellular population spikes (PSs) recorded from the CA1 pyramidal cell body layer of a mouse hippocampus brain slice. PSs are evoked by a pair of electrical stimuli with a 50 ms inter-stimulation interval on the Schaffer collateral pathway. 60 Hz line-frequency noise was introduced to the experiment as described above. Data courtesy of John Huguenard, Ph.D., Stanford University.



No signal distortion

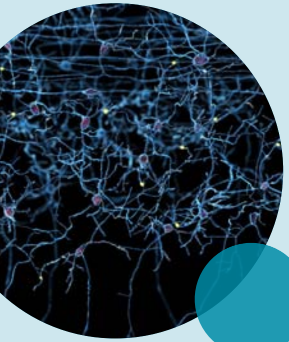
As previously mentioned, notch filter or off-line filtering methods for removing line frequency noises may distort the data. However, this is not the case with the HumSilencer feature. It is not a filter and does not have a filtering effect on acquired signals; nor does the HumSilencer system cause signal distortions such as frequency change, amplitude attenuation, phase shift, or DC voltage change.

To demonstrate that the HumSilencer does not distort acquired biological signals, we examined its effect on end-plate potentials and action potentials recorded from mouse muscle fibres (flexor digitorum brevis or interosseous). Two-electrode voltage-clamp recordings were made using the Axon Axoclamp™ 900A Microelectrode Amplifier. End-plate potentials and action potentials were evoked by injecting depolarizing current pulses to the muscle cell. The injection of a depolarizing current pulse (100 nA, 100ms) (Fig 5, bottom panel) evoked a train of action potentials (Fig 5, middle panel). With HumSilencer enabled, no distortion of the evoked action potential train, in terms of either frequency and amplitude of spikes, was observed (Fig 5, top panel).

We further measured the peak amplitude and time of peak amplitude of each spike of the action potential train. The peak amplitude was measured from the peak of the spike relative to the baseline, the resting membrane potential. The time of peak amplitude was measured from the time of occurrence of the spike relative to the beginning of the trace. There was no significant difference in peak amplitude or the time of peak amplitude of each spike of the train of action potentials obtained with and without HumSilencer enabled (Table 1). Together, this data shows that HumSilencer does not distort biological signals.

- The HumSilencer feature does not distort or filter acquired biological signals.

The HumSilencer does not distort biological signals.



No signal distortion, *continued*

- The HumSilencer feature does not distort or filter acquired biological signals.

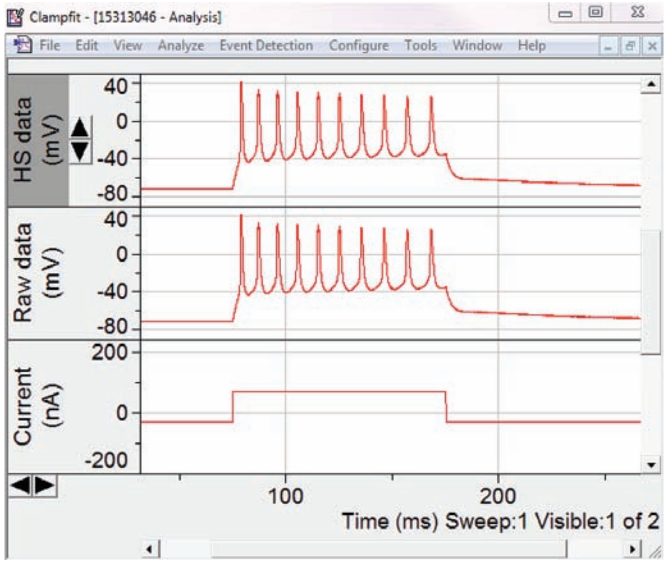
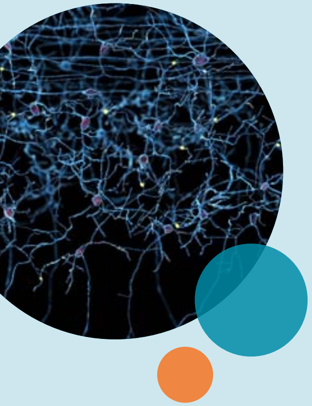


Figure 5. HumSilencer (HS) does not distort current-induced action potentials. A series of action potentials were evoked by injecting a depolarizing current pulse into the recorded muscle cell (HumSilencer enabled data in top panel, raw data in middle panel). The injected current pulse is 100 nA magnitude and 100 ms duration (bottom panel). The resting membrane potential of the muscle cell was -71 mV. Data courtesy of Andrew Voss, Ph.D. Wright State University.

The HumSilencer does not distort biological signals.



No signal distortion, *continued*

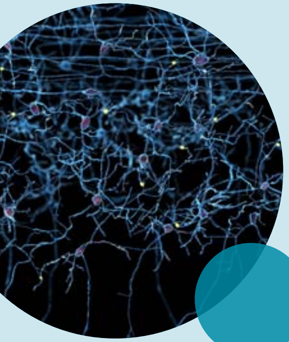
- The HumSilencer feature does not distort or filter acquired biological signals.

	1st Spike		2nd Spike		3rd Spike		4th Spike		5th Spike	
	Raw data	HS data	Raw data	HS data	Raw data	HS data	Raw data	HS data	Raw data	HS data
Peak Amp (mV)	112.8	112.9	105.3	105.3	103.9	104.0	102.9	103.0	102.2	102.2
Time of Peak (ms)	78.8	78.8	86.9	86.9	95.8	95.8	105.2	105.2	114.9	114.9

	6th Spike		7th Spike		8th Spike		9th Spike		10th Spike	
	Raw data	HS data	Raw data	HS data	Raw data	HS data	Raw data	HS data	Raw data	HS data
Peak Amp (mV)	100.9	101.0	100.0	100.1	99.0	99.1	98.2	98.3	97.1	97.2
Time of Peak (ms)	124.9	124.9	135.2	135.1	145.8	145.8	156.6	156.6	167.9	167.9

Table 1. Comparison of the peak amplitude and time of peak amplitude of each spike from the raw data trace vs spikes from the HumSilencer (HS) enabled trace shown in Fig 5.

The HumSilencer does not distort biological signals.



Signals are safe

- The HumSilencer feature does not distort or filter acquired biological signals.

The HumSilencer does not eliminate signals, even if they occur at the same frequency as much electrical noise.

To further investigate whether 60 Hz signals can be eliminated by the HumSilencer algorithm, 60 Hz end-plate potentials were evoked by injecting a 60 Hz series of depolarizing current pulses into the recorded muscle cell. The middle panel of Fig 6 shows that 60 Hz end-plate potentials were evoked by injecting a train of depolarizing current pulses (300 nA, 0.5ms) of 60 Hz frequency (Fig 6, bottom panel). With the HumSilencer enabled, the frequency and amplitude of the evoked 60 Hz end-plate potentials did not change (Fig 6, top panel). We measured the peak amplitude, time of peak amplitude, half-width, rise tau, decay tau, maximal rise slope, maximal decay slope, 10%–90% rise time, 10%–90% rise slope, 90%–10% decay time and 90%–10% decay slope of each event. There was no significant difference in any of the measured parameters for the evoked end-plate potentials obtained with and without HumSilencer enabled (data not shown).

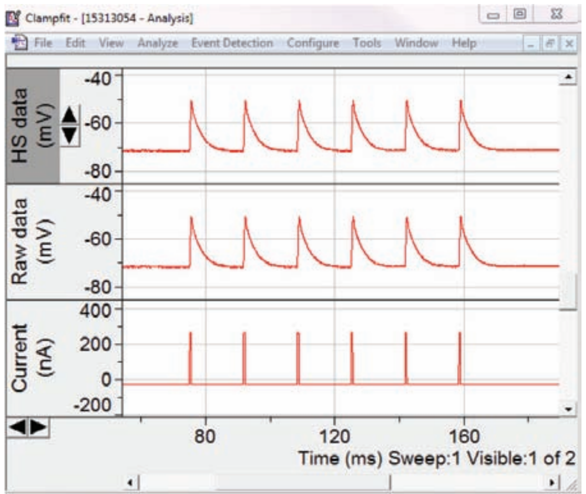
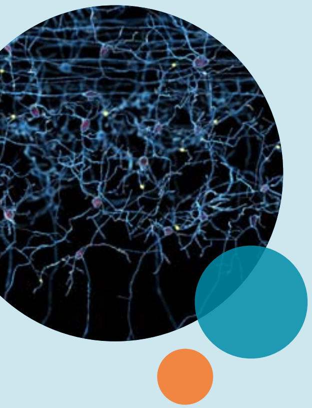


Figure 6. HumSilencer does not eliminate 60 Hz end-plate potentials. A train of 60 Hz end-plate potentials was induced by injecting a series of 60 Hz depolarizing current pulses into the recorded cell (HumSilencer enabled data in top panel, raw data in middle panel). The injected current pulse is 300 nA magnitude and 0.5 ms duration (bottom panel). The resting membrane potential of the muscle cell was -71 mV. Data courtesy of Andrew Voss, Ph.D. Wright State University.



Discriminating between signal and noise

- The HumSilencer feature does not distort or filter acquired biological signals.

To test whether the HumSilencer system is able to eliminate 60 Hz line-frequency noise without eliminating 60 Hz signals, exogenous 60 Hz interference was introduced to the recording system by placing a noise generator near the headstage. The introduced 60 Hz interference was picked up by the headstage and acquired along with the 60 Hz end-plate potentials (Fig 7, middle panel) evoked by injecting a 60 Hz series of current pulses (50 nA, 0.5 ms) (Fig 7, bottom panel). When the HumSilencer was enabled, the 60 Hz interference, but NOT the 60 Hz biological signals, was eliminated (Fig 7, top panel). We measured the time of peak amplitude of each spike of evoked end-plate potentials and found no significant difference in the between those obtained with and without HumSilencer enabled, (Table 2).

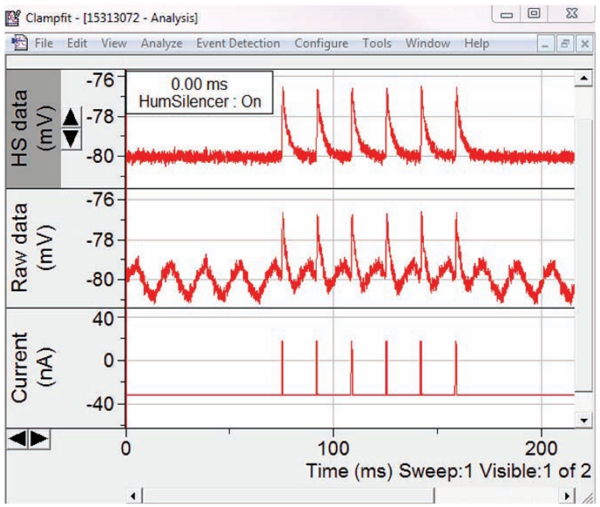
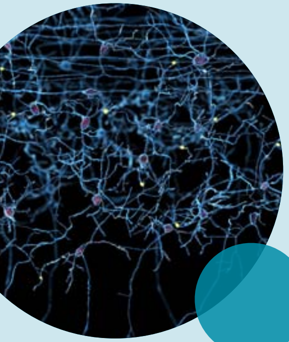


Figure 7. HumSilencer (HS) eliminates 60 Hz line-frequency noises but not 60 Hz end-plate potentials. A series of 60 Hz end-plate potentials were evoked by injecting a series of 60 Hz depolarizing current pulses (50 nA, 0.5 ms) into the recorded cell (bottom panel). 60 Hz end-plate potentials were purposely contaminated with exogenous 60 Hz line-frequency noises by placing an external noise generator near to the headstage (middle panel). Introduced 60 Hz interference, but not 60 Hz signals, was eliminated by HumSilencer system (top panel). The resting membrane potential of the muscle cell was -80 mV. HumSilencer was enabled in the top panel during the entire recording. Data courtesy of Andrew Voss, Ph.D. Wright State University.

The HumSilencer eliminates line frequency noise but not signals at the same frequency.



Discriminating between signal and noise, *continued*

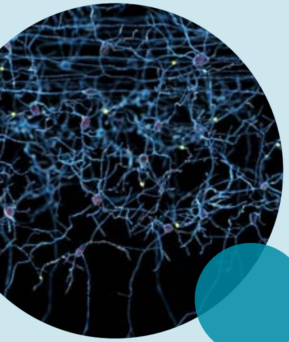
- The HumSilencer feature does not distort or filter acquired biological signals.

	1st Spike		2nd Spike		3rd Spike	
	Raw data	HS data	Raw data	HS data	Raw data	HS data
Time of Peak (ms)	75.5	75.5	92.1	92.1	108.7	108.7

	4th Spike		5th Spike		6th Spike	
	Raw data	HS data	Raw data	HS data	Raw data	HS data
Time of Peak (ms)	125.4	125.4	142.1	142.1	158.7	158.7

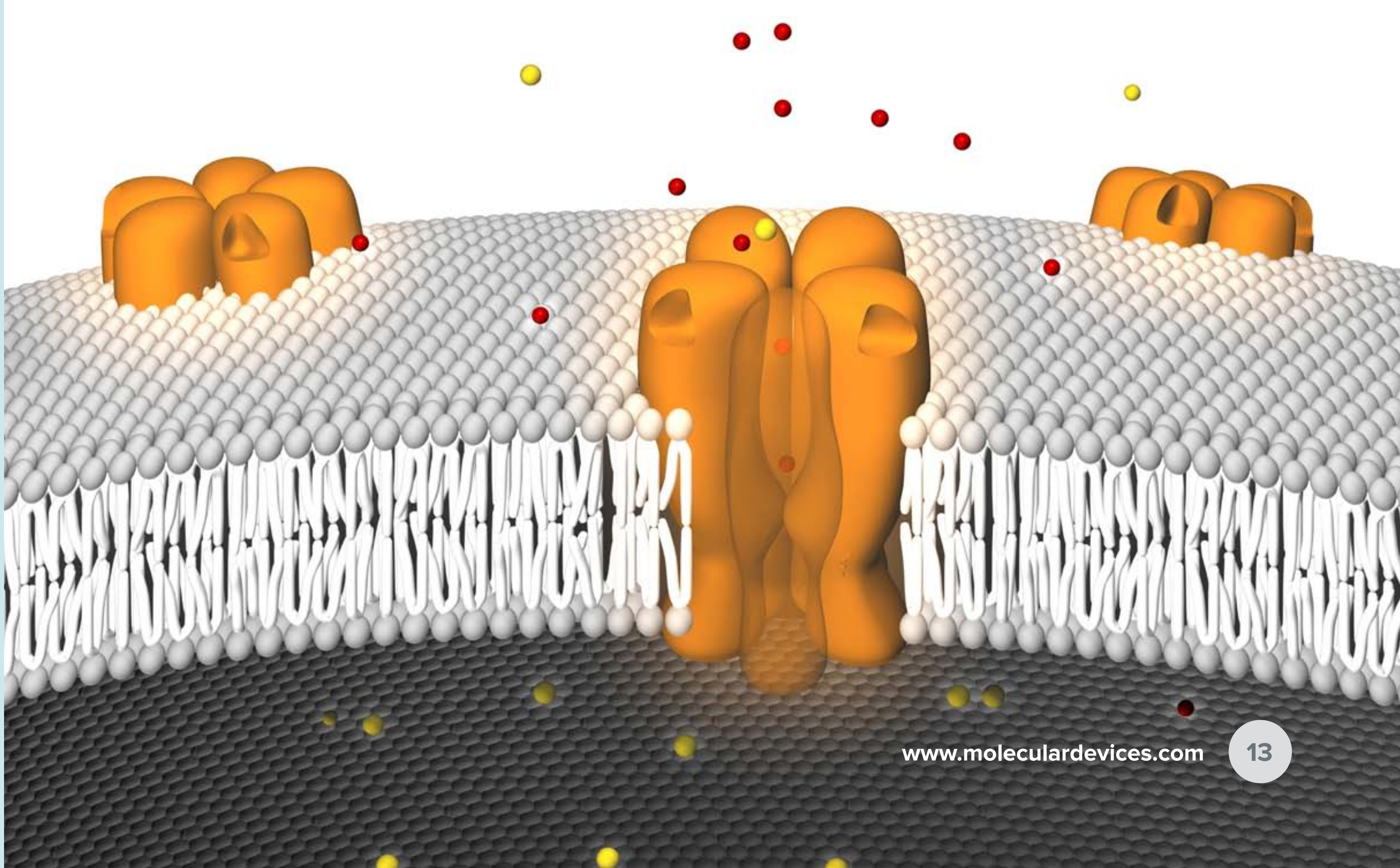
Table 2. Comparison of time of peak amplitude of each spike from the raw data trace vs. spikes from the HumSilencer (HS) enabled trace shown in Fig 7.

HumSilencer enables high-quality data acquisition.



Conclusion

We have demonstrated that the HumSilencer feature provides an extremely fast method of removing line frequency noise across a range of electrophysiological applications, revealing the underlying biological signal and improving the quality of data acquired. The HumSilencer's capabilities have been validated in two, very distinct, electrophysiological applications, and we have shown also that the HumSilencer can recognize and adapt to changes in the electrical noise pattern. Perhaps most importantly, we have shown that the HumSilencer feature removes line-frequency interference without distorting biological signals during data acquisition, in contrast to other methods of removing such interference.



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