

## Application Note 214: Eyeblink Startle Scoring for Prepulse Inhibition Studies

This Application Note describes the setup required to apply auditory and visual stimulus via SuperLab™ to elicit a startle response and record and analyze the EMG response of the *orbicularis oculi* muscle on a BIOPAC MP System. AcqKnowledge software will be used to determine the following measures:

- EMG startle onset (msec)
- Delta EMG startle onset (msec)
- Maximum EMG amplitude (mVolts)
- Stimulus (msec)
- Maximum EMG signal (msec)
- Time from stimulus to max EMG (msec)
- Area under the curve of the integrated EMG (msec)

These measures can easily be exported to Excel for statistical analysis.

If presenting only auditory stimulus, see Application Note [198](#) for a simplified setup.

## Equipment Required

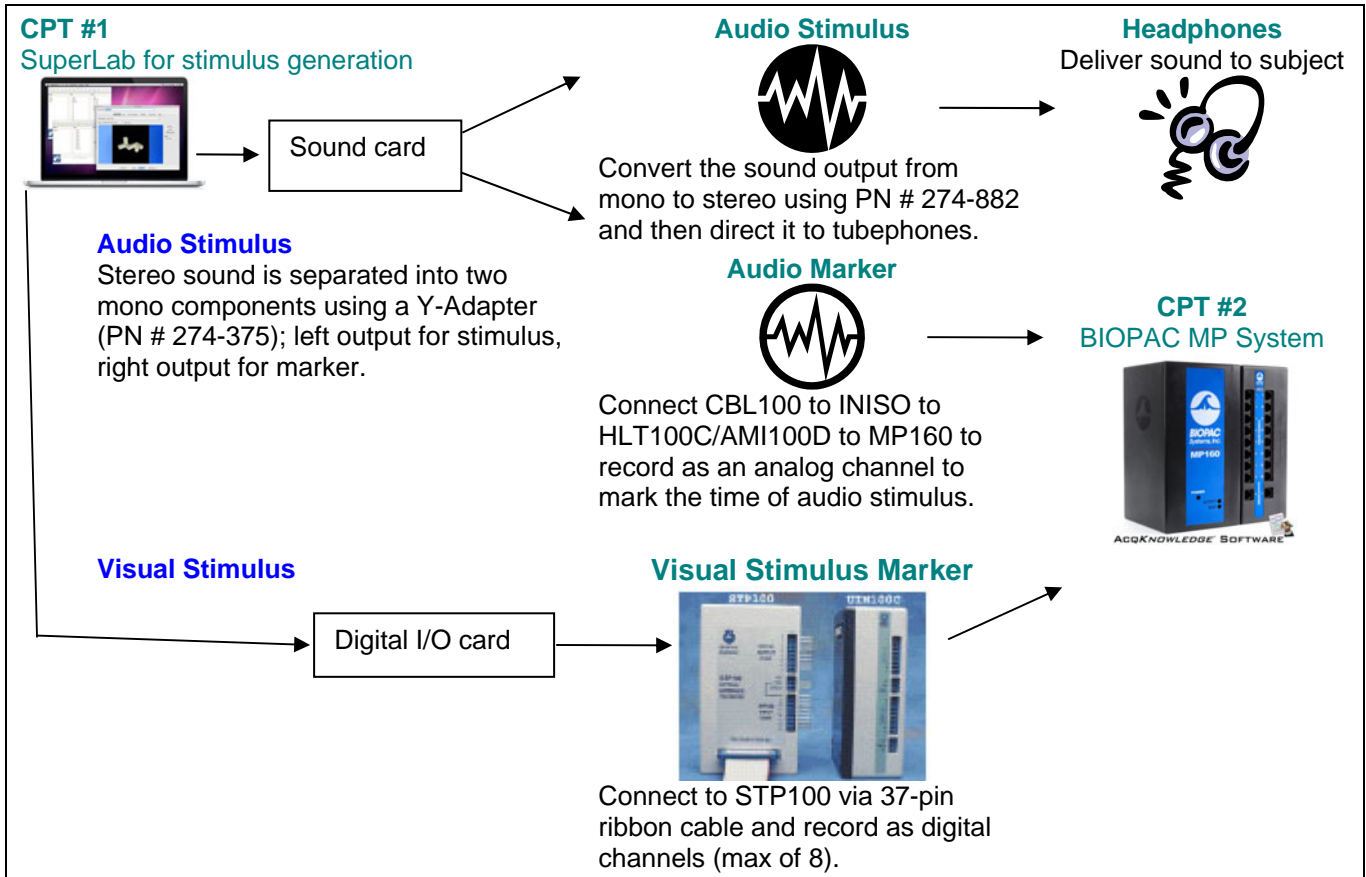
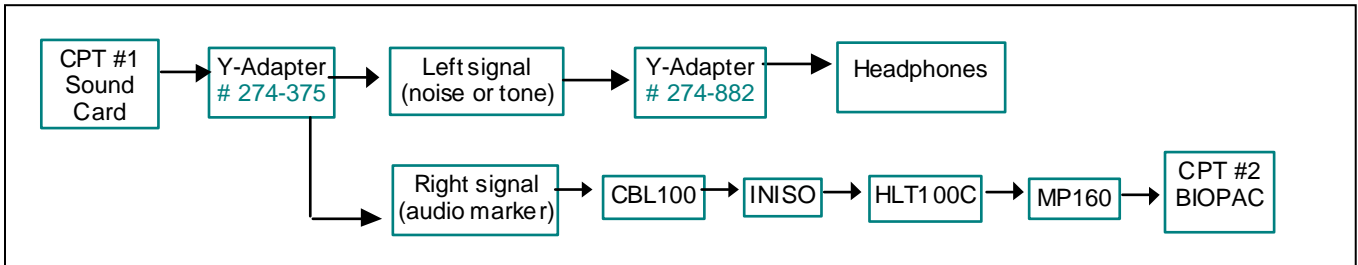
- [MP160 or MP150 System](#) (data acquisition unit and AcqKnowledge software)
- [EMG100C](#) amplifier
- [MEC110C](#) Modular Extension cable
- Electrodes & Leads
  - Reusable Electrodes*
    - [EL254](#) Small reusable Ag-AgCl electrode (unshielded) for GND\*
    - [EL254S](#) x2 Sm. reusable Ag-AgCl electrodes (shielded)
    - [ADD204](#) Adhesive collars
  - Disposable Electrodes*
    - [EL504](#) x 3 Cloth snap electrodes
    - [LEAD110](#) (unshielded) for GND\*
    - [LEAD110S-R](#) (shielded, red)
    - [LEAD110S-W](#) (shielded, white)
- [GEL100](#) Electrode gel
- [ELPAD](#) electrode site preparation pad
- [HLT100C](#) or AMI100D High-Level Transducer Interface
- [INISO](#) Input Signal Isolator
- [CBL100](#) 3.5 mm Phone Plug to 3.5 mm Phone Plug cable
- Headphones (TDH-39, 49 or 50 audiometric headphones or equivalent)
- SuperLab™ presentation package [STP100W](#) or SuperLab™ interface [STP100C](#)
- Sound generating software to create sound files (e.g. [Adobe® Audition™](#), previously Cool Edit)
- Audio adapters:
  - a) Y-Adapter: 3.5 mm to two 3.5 mm (e.g. Radio Shack Part # 274-375)  
This will split the stereo signal from the sound card into two mono signals.
  - b) Stereo-to-Mono Adapter: 1/8" Stereo plug to 1/8" mono phone jack (e.g. Radio Shack Part # 274-882)  
This will support the headphones and send the marker signal to the MP System.
- Sound level meter (e.g. Radio Shack Part # 33-2055)

\***GND Note** If recording EDA with this setup, the EDA will establish a ground and using a [CBL205](#) AC-Coupled electrode lead adapter is recommended for the additional ground.

## Auditory Stimulus Hardware Setup

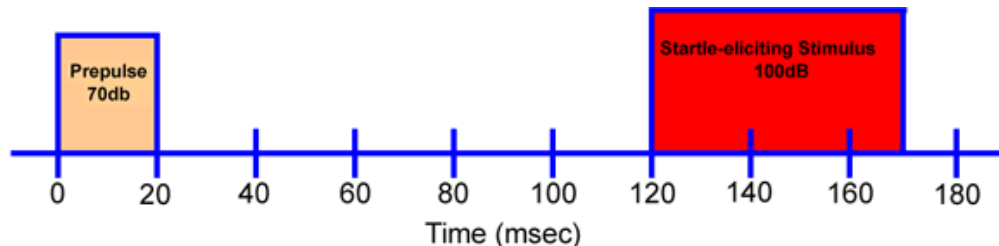
Accurate recording of the start of the auditory stimulus is critical for analysis. The stereo signal will be separated into left (L) and right (R) signals. A 2ms square signal will be used on the R stimulus signal to create a marker of startle. The mono L signal will be recombined into a stereo signal by using the Y-Adapter and output to headphones.

### Overview



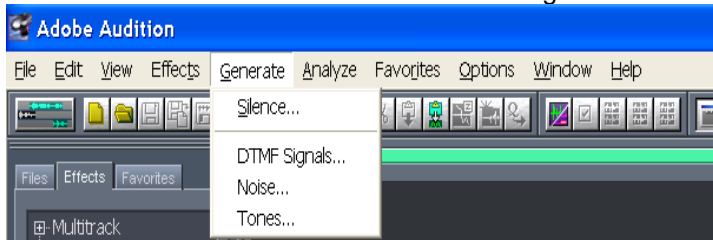
## Auditory Stimulus Signal Generation

Prepulse inhibition studies often use background noise at 70-75 db. Startle is elicited with a white noise pulse of 105 db for 50 ms. The prepulse signal can be 25 ms white noise of 90 db presented 30-240 ms before startle. Inter-trial intervals can vary from 8 to 30 seconds. Tones of various frequencies can be presented instead of white noise. A prepulse is illustrated below (at different frequencies than will be used in this note); see Application Note [198](#) for details.



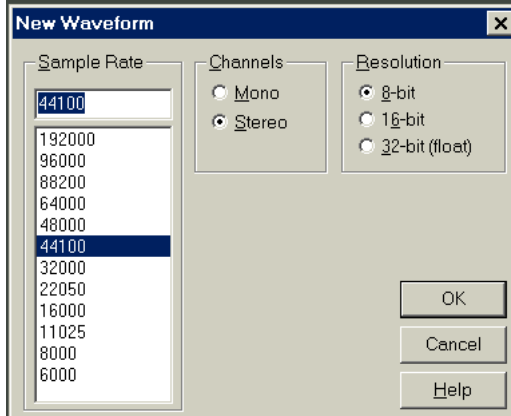
Prepare three distinct sound files of calibrated white noise (65, 90 and 105 dB) to construct the stimulus presentation; amplitude may vary depending on the protocol.

1. Launch [Adobe® Audition™](#).
2. Press F12 to toggle between Multitrack view and Edit Waveform view. The Edit Waveform view is used in this setup.
3. Click the Generate menu and select Noise to generate white noise:



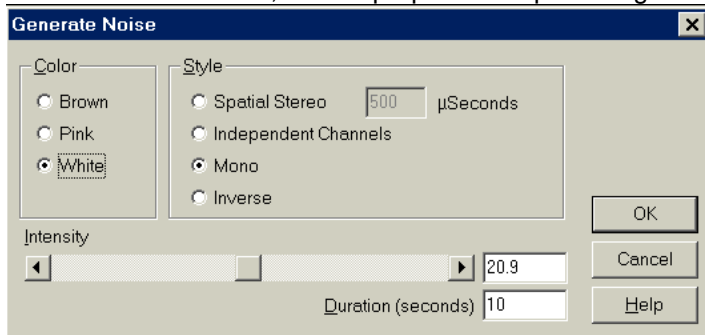
**NOTE:** In newer versions of [Adobe® Audition™](#), click Effects > Generate > Noise to access the white noise option.

4. Set the Sample Rate, Channels to Stereo, and Resolution to 8-bit.

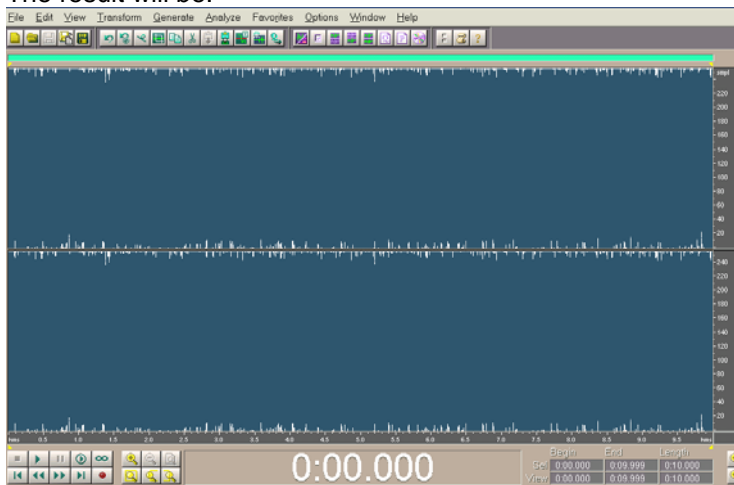


**NOTE:** 8-bit resolution may not be available on newer versions of [Adobe® Audition™](#).

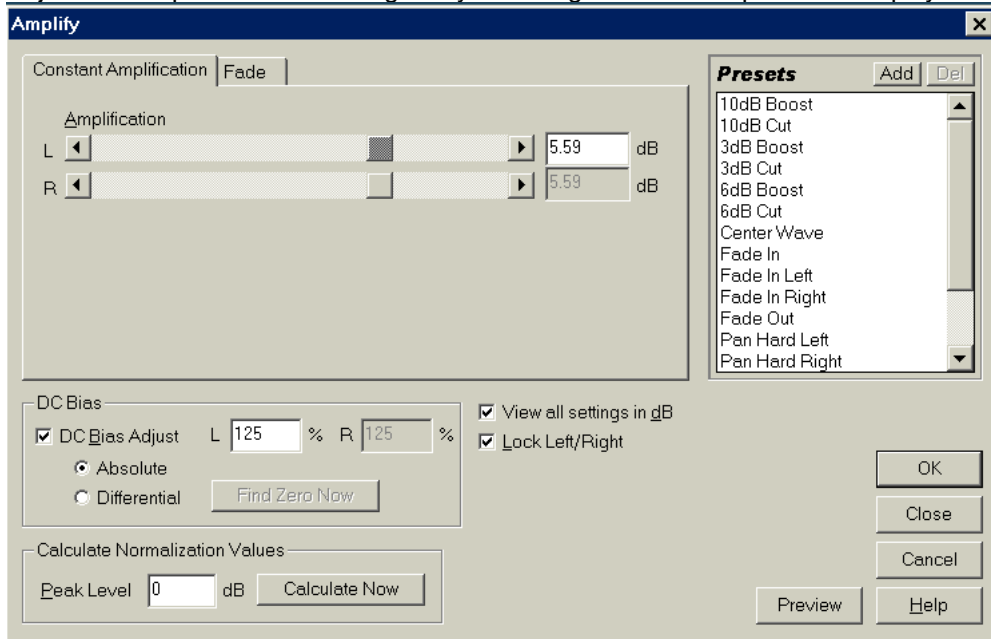
5. Select the Intensity of the sequence and the Duration of the sequence.
  - The Intensity is relative, as the output will be dependent on sound card, volume, and the tube phone used. For ease of calibration, set the prepulse and pulse segment Duration to 4 seconds.



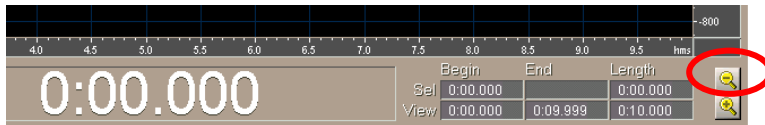
The result will be:



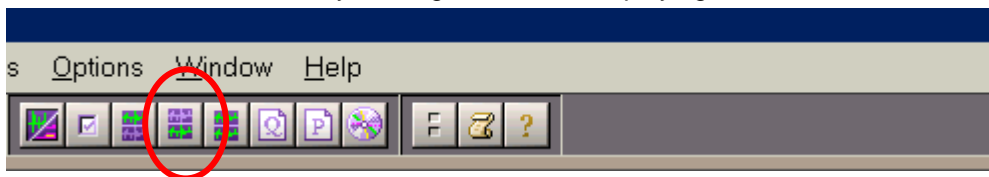
6. Place the sound level meter on the tubephones.
7. Click on the “Start” button (right pointing arrow, second row from bottom left) to play the sound.
8. Adjust the amplification as desired.
  - Adjust the volume of the tubephones.
  - Adjust the Amplification of the signal by selecting Effects > Amplitude > Amplify.



9. Change the vertical amplitude by clicking on the magnifying glass with a minus, located at the bottom right corner of the window.



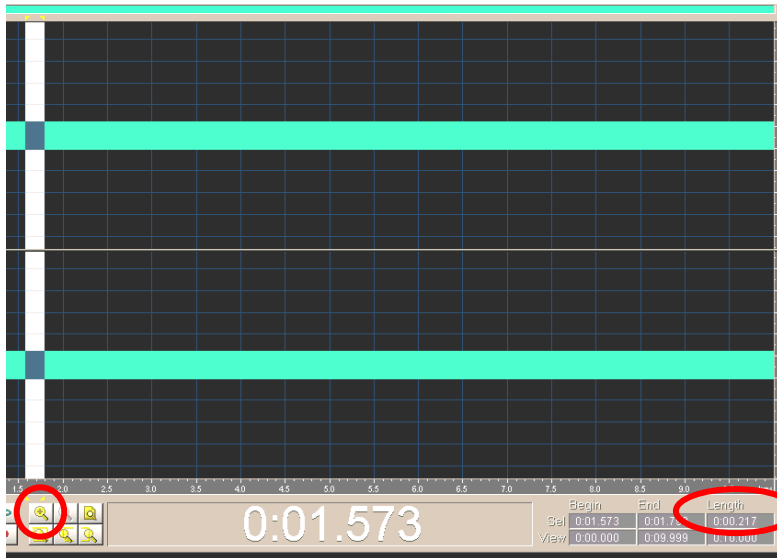
10. Starting with the baseline signal file (e.g. at 65 db), adjust the volume or the amplitude so that the sound meter reads the desired amplitude and the marker channel (R) is reduced to silence.
11. Choose Edit > Select Entire wave.
12. Select the bottom channel by clicking on the icon displaying the bottom channel.



13. Select Effect > Silence.
14. Save this transformed waveform as 65 dB.
15. Repeat steps 3-14 to create a 90 dB waveform.
16. Repeat steps 3-14 to create a 105 dB waveform.

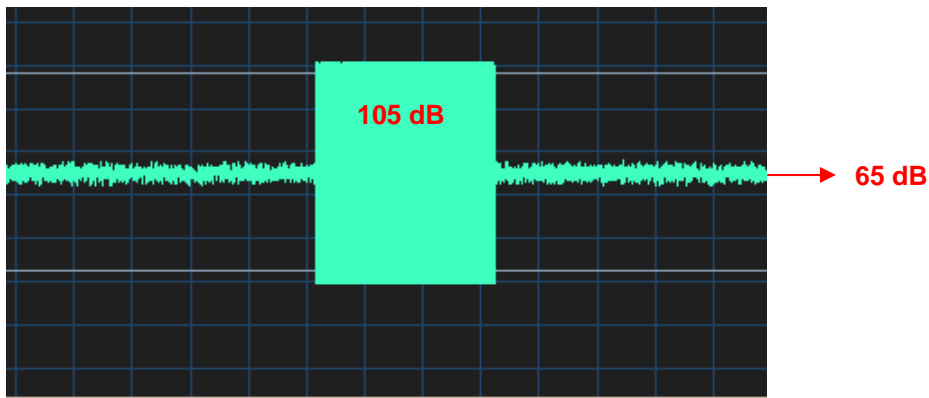
### Create a Startle Waveform

- Combine the 65 db file and the 105 dB file to create a Startle file.
  1. Open the 65 db file and the 105 db file.
  2. Select View > Display Time format and select Decimal (mm:ss.ddd).
  3. Use the zoom tool to highlight a 30 msec segment of the 105 db waveform (this segment will be pasted into the baseline waveform).

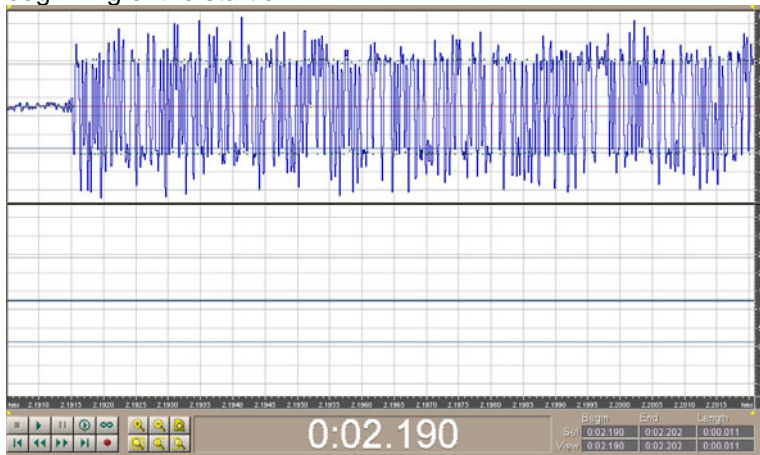


In this example the segment is 217 msec.

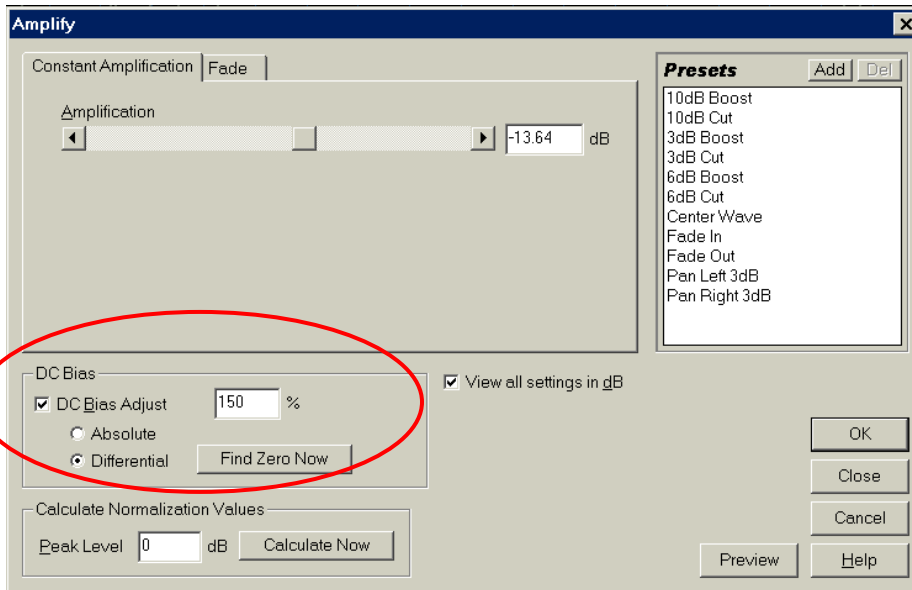
- Select Edit > Copy to remove the 30 msec segment from the zoomed section.
- Click in the background noise waveform (65 db) and Select Edit > Paste. The result should look like:



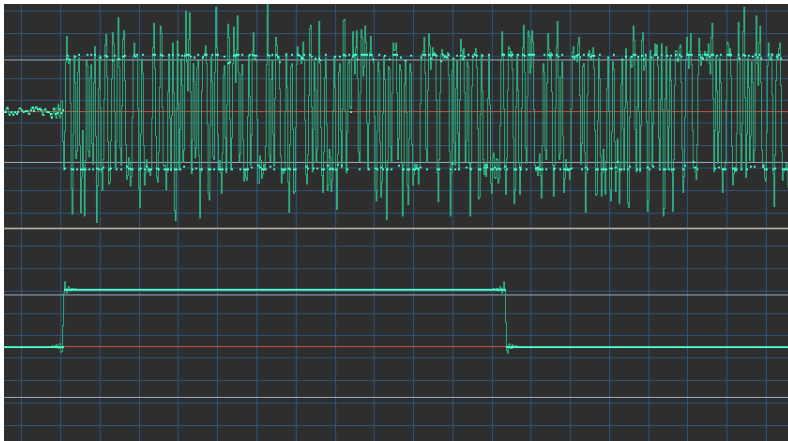
- Highlight the segment with the startle and zoom in to highlight a section of about 10 msec that includes the beginning of the startle.



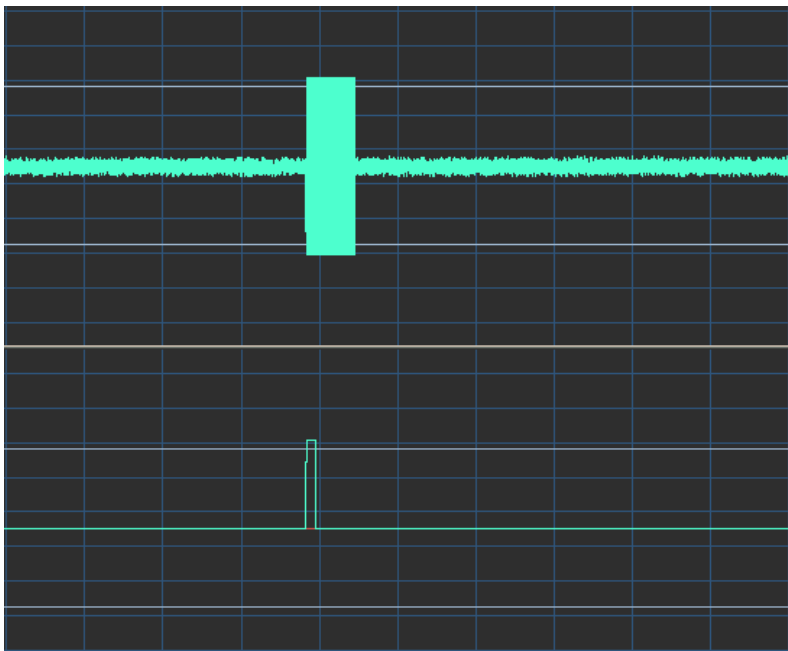
- Press CTRL-R to select the bottom waveform.
- Position the cursor exactly at the beginning of the startle, then click and drag for about 2 msec.
- Select Transform > Amplify and establish the following settings:



- The important setting here is **DC Bias adjust**. Change the % to distinguish different startles. This segment will be used to find the exact time of the startle and analyse the response. The result will be similar to:



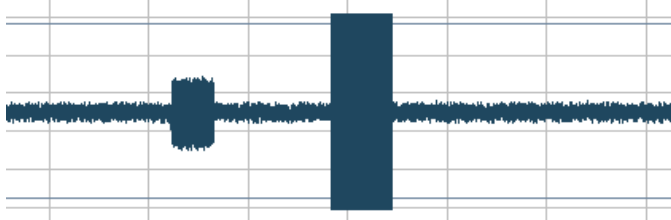
10. Zoom out so the display resembles the following:



11. Choose File > Save As and name as a Startle file.

## Create a Prepulse File

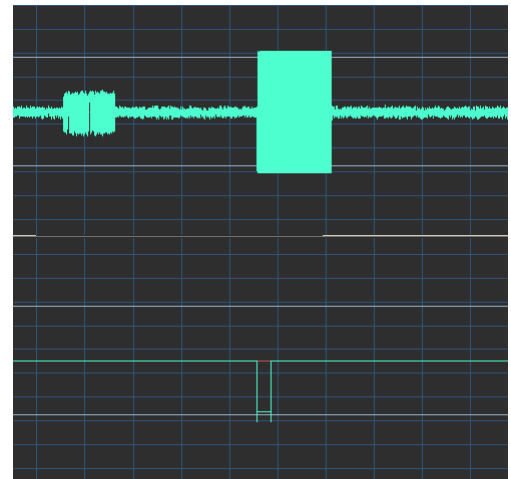
- Combine the 90 dB file and the Startle file to create a Prepulse file.
  1. Select 20 msec of the 90 db waveform.
  2. Choose Edit > Copy to transfer the 20 msec segment to the clipboard.
  3. Open the newly-created Startle waveform.
  4. Use the zoom tool to highlight a segment of about 100 msec before the startle (arbitrary).
  5. From the beginning of the startle, highlight a segment of 80 msec (prepulse of 20 msec and interval of 60 msec) and then paste the 90 db segment into the waveform. The waveform should resemble the following:



12. Choose File > Save As and name as a Prepulse file.

## Amplify this Sequence

1. Select the bottom channel and highlight the segment that was amplified for 5 msec.
2. Select Transform > Amplify and establish an Amplification of –125% to create a negative spike that will indicate that a prepulse has been presented.
  - If using different conditions, use different amplitude values.
  - The resultant waveforms should look similar to the graph shown at right:
3. Choose File > Save As and rename this waveform Marker.



## Create Additional Segments

The two main files can be used to present pulse-only startles and startles with a prepulse. Additional segments can also be created. These stimuli can be presented via two methods:

- a) Each 6-sec segment can be launched and presented just before an image is presented in SuperLab (see Application Note [201](#)).
- b) A longer segment of stimuli can be created using File > Open > Append.

## Set Up Recording Hardware

1. Set the EMG100C amplifier as follows: CH 1, Gain=5000, LP=500 Hz, 100 Hz HP=Off, HP=10 Hz.
2. Set the HLT100C/AMI100D on CH 16 (Sound Card→Y-Adapter→CBL100→INISO→HLT100C→CH16).

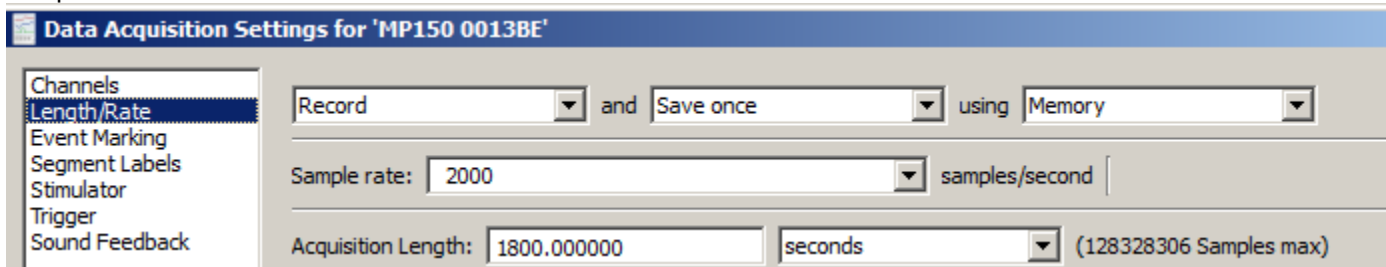
## Set Up Subject

1. The *orbicularis oculi* muscle surrounds the eye and causes the eye to close or blink. For best results, abrade the skin below the eye and apply gel before placing electrodes.
2. Apply electrodes and connect the leads.
  - Reusable: Use ADD200 adhesive disks to secure two EL254 electrodes on the *orbicularis oculi* muscle and one EL254S electrode on the forehead. Connect electrode cables to the MEC100.
  - Disposable: Secure two EL504 electrodes on the *orbicularis oculi* muscle and one EL504 electrode on the forehead. Connect LEAD110 (GND), LEAD110S-R, and LEAD110S-W.

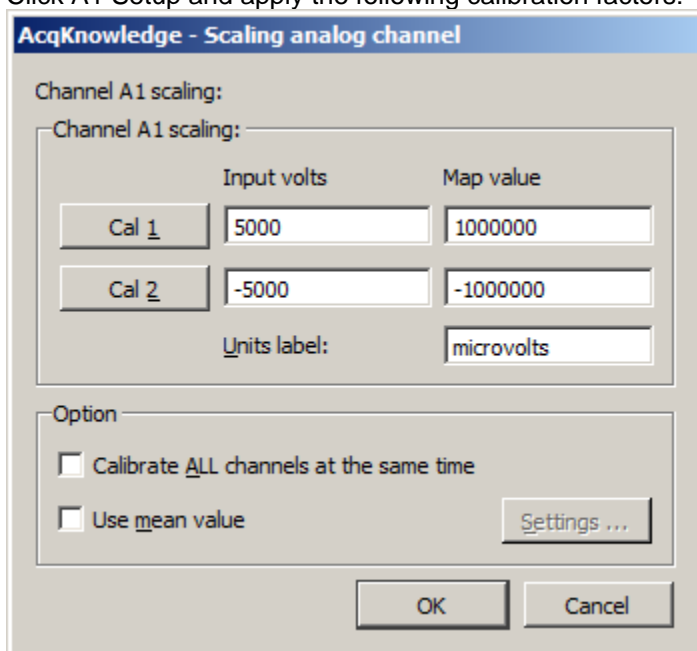
**GND Note** If recording EDA with this setup, the EDA will establish a ground. Use a CBL205 AC-Coupled electrode lead adapter for the additional ground.

### Set up AcqKnowledge Software

1. Select MP160/150 > Set Up Data Acquisition > Length/Rate and set to Record and Save once to Memory at 2000 samples/second.



2. Select MP160/150 > Set Up Data Acquisition > Channels (This setup requires two analog channels and four calculation channels.)
  - a) Set analog channel A1 for the EMG Signal
  - b) Click A1 Setup and apply the following calibration factors:



For more exact calibration, use the CBLCALC cable (see Application Note [102](#) for details).

3. Label Analog channel A16 as the Marker Signal (directed from the HLT100C or AMI100D).



4. Set calc. channel C0 to the IIR Filter Preset and set Source A1 EMG with a Band stop filter at 60 Hz.

**AcqKnowledge - Filter - IIR**

C0, Filter - IIR

Source: A1, EMG

Label: C0 - Filter - IIR

Preset: none

Output: Band Stop

Frequency

Fixed at 60 Hz

Sampling rate / 8

Line frequency

Q: 5.000000

New Preset OK Cancel

5. Set calc. channel C1 to the Filter Preset and set Source C0 Filter with a Band pass filter at 28 Hz (Low) to 500 Hz (High).

**AcqKnowledge - Filter - IIR**

C1, Filter - IIR

Source: C0, C0 - Filter - IIR

Label: C1 - Filter - IIR

Preset: none

Output: Band Pass Low + High

Low Frequency

Fixed at 28 Hz

Sampling rate / 8

Line frequency

High Frequency

Fixed at 500 Hz

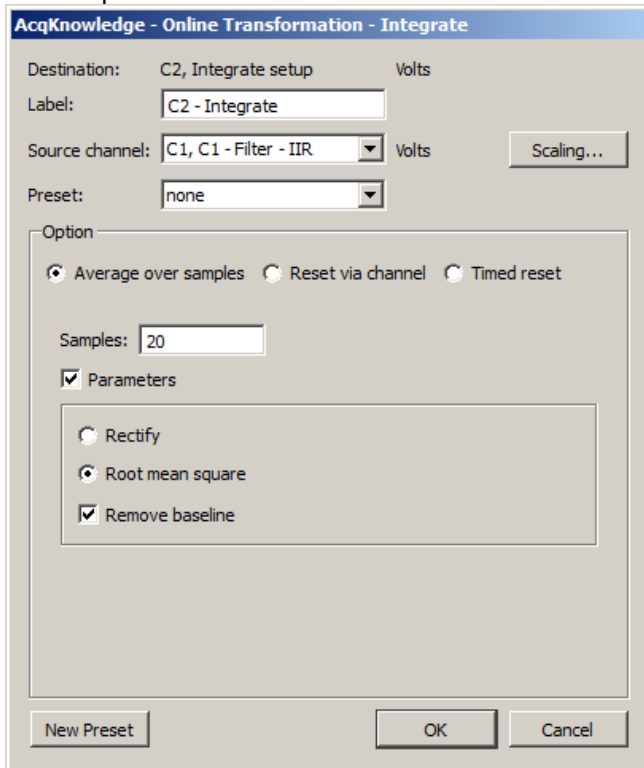
Sampling rate / 8

Line frequency

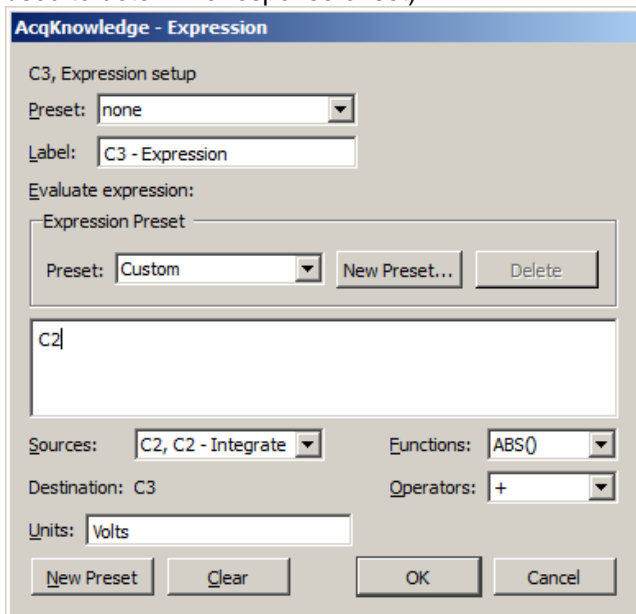
Q: 0.707000

New Preset OK Cancel

- Set calc. channel C2 to the Integrate Preset and set Source C1 to Average over 20 samples with Parameters, Root mean square and Remove Baseline checked.



- Set calc. channel C3 to the Expression Preset and set the Source to C2 (which will duplicate C2 Integrate so it can be used to determine response onset).



- To present images to the subject, open Digital channels D8 to D16 to correspond with the SuperLab setup.

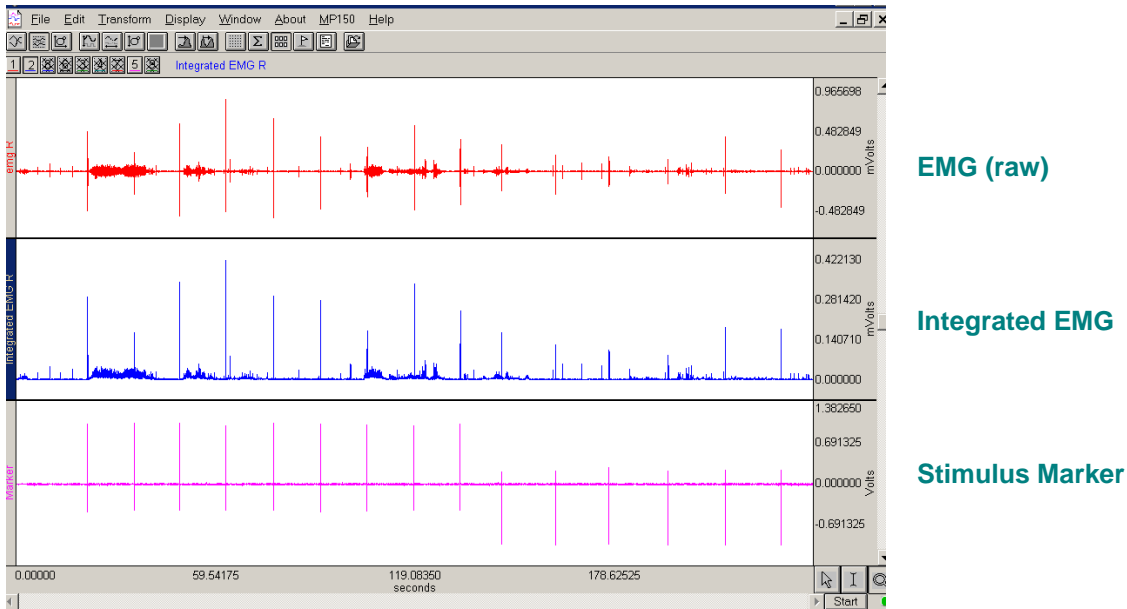
## Data Acquisition

- Use the *AcqKnowledge* “Start” and “Stop” buttons to begin and end the recording.
- Choose File > Save As and name the file.

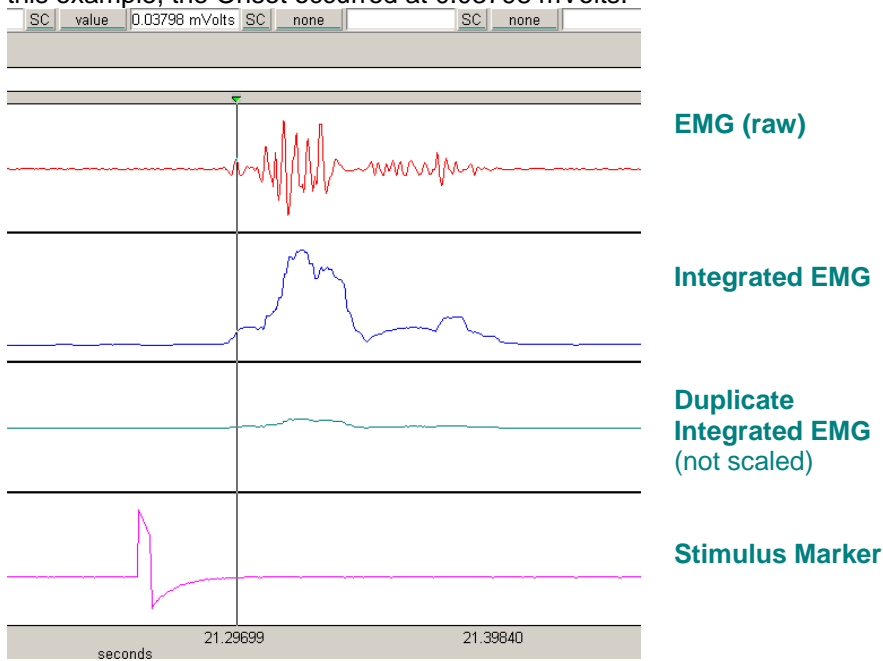
## Analysis

The recorded data should be similar to the following:

- In this example, each negative marker identifies a Prepulse.

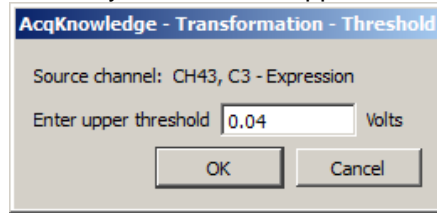
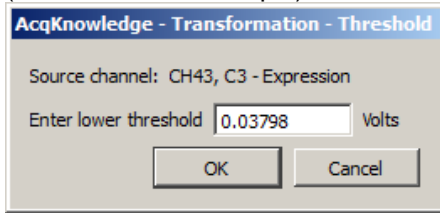


- As desired, hide channels (Alt-click on the channel box above the graph display).
  - Set a measurement for A3 Value.
  - Use the zoom tool (magnifying glass icon) to zoom in on the first marker of the experiment.
  - Place the cursor on the first positive deflection of the A1 EMG signal and note the A3 measurement value.
- The Value measurement from A3 Integrated EMG determines the Onset of the EMG activity following startle. In this example, the Onset occurred at 0.03798 mVolts.



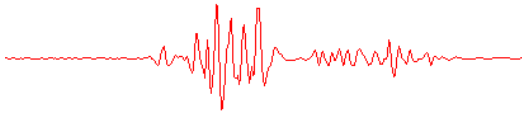
- Choose Edit > Select All.
- Click C3 (43) Duplicate Integrated EMG to select it as the active channel.

7. Choose Transform > Math Functions > Threshold and enter the measurement Value from step 4 for the Lower Value (0.03798 in this example) and then round up arbitrarily and enter an Upper Value (0.04 in this example).

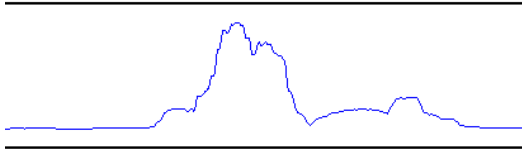


- These values will vary depending on individuals and electrode placement.

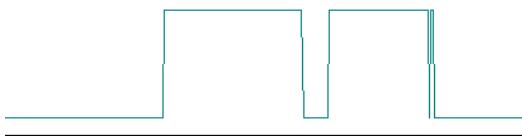
8. Choose Edit > Autoscale (with C3 still selected). C3 Dupe Integrated EMG should produce a signal oscillating between 0 and 1. The first rise in the signal after the startle indicates the Onset of EMG.



EMG (raw)



Integrated EMG

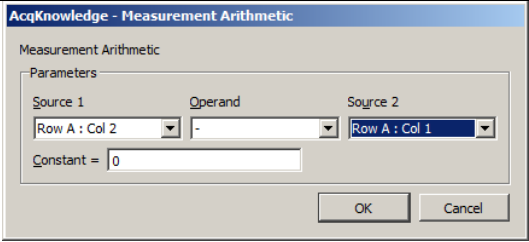


Response (from Threshold on Duplicated Integrated EMG)



Stimulus Marker

9. Set the following measurements:

Box Position	Channel	Measurement	Description
Row A Col 1	Marker	Max T	Determines time of startle
Row A Col 2	Duplicated Integration	Min T	Time of onset of EMG (absolute time)
Row A Col 3	N.A.	Calculate	 <p>Gives time of EMG onset from Startle</p>
Row A Col 4	Raw EMG	Max T	Time of maximum of EMG (absolute time)
Row A Col 5	N.A.	Calculate	Row A Col 4 Minus Row A Col 1 Gives time of maximum EMG onset from Startle
Row B Col 1	Raw EMG	Max	Maximum raw EMG after startle
Row B Col 2	Integrated EMG	Area	Measures Area under the integrated EMG
Row B Col 3	Marker	Max	Value of marker amplitude, for reference only

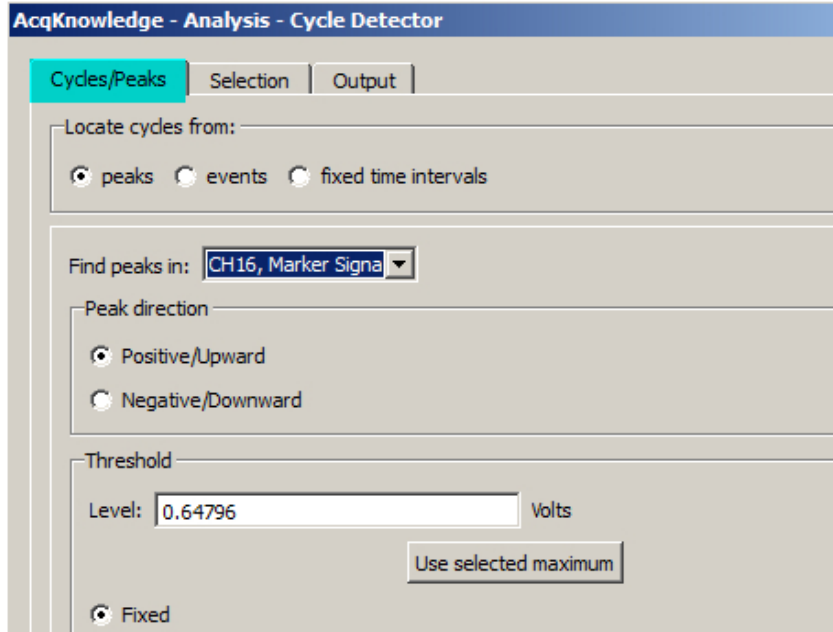
10. Click in A16 Marker and use the I-beam to highlight one peak.

- By highlighting the peak of the marker, the threshold is set automatically by the software.

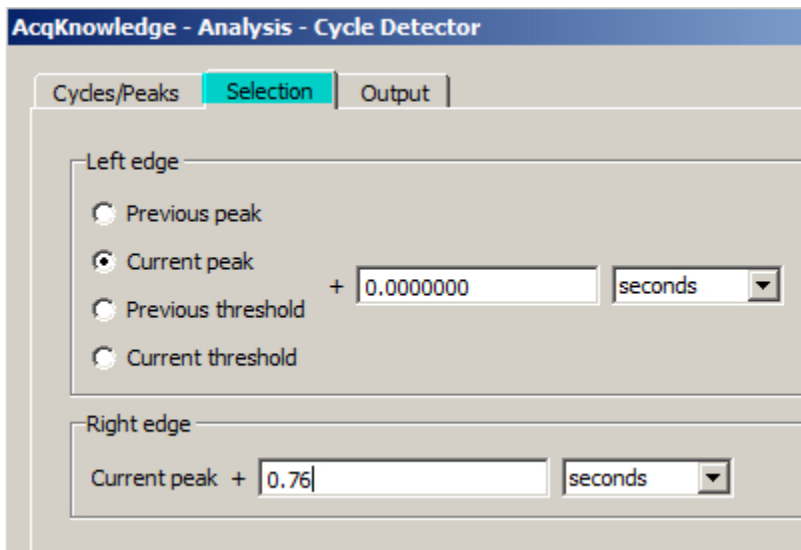
11. Select Analysis > Find Cycle.

- Startle responses recorded before 21 milliseconds are considered to be unrelated to the sound stimulus, while those occurring after 75 are considered as potentially random or consciously produced.

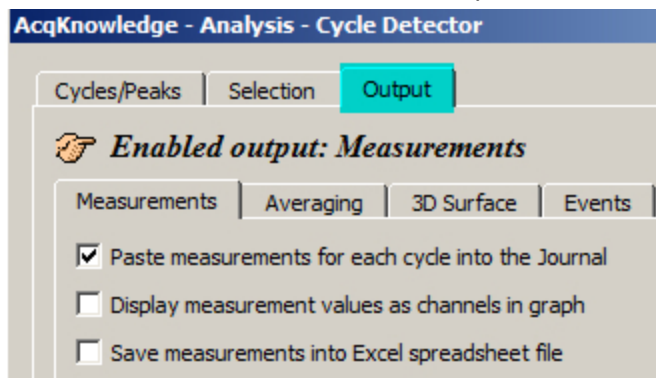
12. Set Find Cycle “Cycles/Peaks” to Positive peak. (Step 10 established the Threshold Level.).



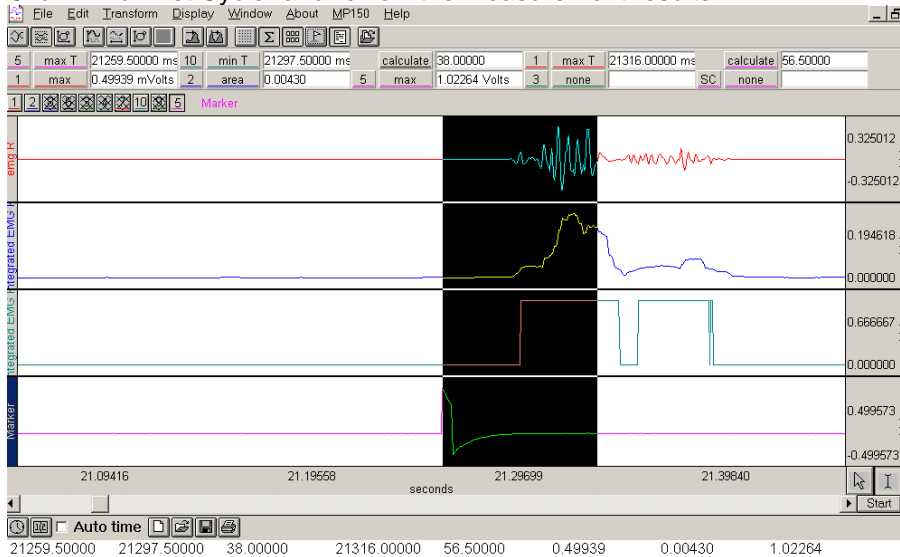
13. Under the “Selection” tab, set “Left edge” to Current peak + 0 sec, Set “Right edge” to Current peak + 0.076 sec.



14. Under the “Output” tab, select “Paste measurements for each cycle into the Journal.” The outputted measurements can also be saved to a spreadsheet.



15. Click "Find First Cycle" and review the measurement results.



- In this example: stimulus was at 21259 msec, EMG startle onset was at 21297 msec, delta EMG startle onset was at 38 msec, maximum EMG was at 21316 msec at 56.5 msec after stimulus, maximum EMG amplitude was 0.49939 mvolts and area under the curve of the integrated EMG was 0.00430.

16. Click the cursor to the right of the first startle.

17. For further peak analysis

- Choose Analysis > Find All Cycles" to automatically analyze the rest of the data, or
- Choose Analysis > Find Next Cycle" to look at each individual startle.

18. For statistical analysis, export all of the Find Peak measurement results from the journal to Excel.

21259.50000	21297.50000	38.00000	21316.00000	56.50000	0.49939	0.00430	1.02264
35176.00000	35210.50000	34.50000	35219.50000	43.50000	0.24103	0.00287	1.03455
48701.50000	48740.50000	39.00000	48766.50000	65.00000	0.59106	0.00422	1.03973
62479.00000	62509.00000	30.00000	62531.50000	52.50000	0.88702	0.00812	1.00189
76823.00000	76863.00000	40.00000	76890.50000	67.50000	0.65918	0.00396	1.03302
90892.00000	90928.50000	36.50000	90945.50000	53.50000	0.42883	0.00379	1.02081
104860.00000	104898.00000	38.00000	104919.50000	59.50000	0.30640	0.00199	1.01379
118924.50000	118960.00000	35.50000	118995.50000	71.00000	0.57074	0.00587	1.00067
132713.50000	132755.00000	41.50000	132782.00000	68.50000	0.40210	0.00340	1.02661
8 peaks found							

- Measurement results were automatically pasted to the journal as selected in Find Cycle setup (Step 14).