Recording Startle Eye-Blink in Humans: Habituation, Pre-pulse Inhibition and Conditioning.

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This application note describes the use of the PowerLab unit with a Bioamp front-end to record startle eye-blink in humans. The software package "Scope" provides a simple interface for doing anything from simple demonstrations of basic learning principles (as in this application note) to the sophisticated recording and analyses of eye-blink EMG activity needed for research applications.

Introduction

The human startle eye-blink (SEB) is rapidly becoming one of the most widely used measures of the behavioural neuroscientist for several reasons:

- It is a simple, reliable biological measurement technique
- It can also be measured in animals and infants
- It is an extremely powerful index of biological information processing mechanisms as it can be modified by:
- The physical nature of the stimulus eliciting the eye-blink
- The temporal characteristics of these stimuli
- The instructions given to the subject
- The psychological characteristics of the subjects themselves

The typical SEB technique involves a series of presentations of a startle-eliciting stimulus (e.g. a loud tone). In addition, stimulus pairs of a non-startling stimulus (e.g. a soft tone) followed by startle eliciting stimulus are presented. The initial soft stimulus is referred to as the "lead" stimulus, and the interval between this and the loud tone it is paired with is referred to as the "lead interval".

The dependent measure of main interest in the SEB technique is the difference in amplitude of eye-blinks in the lead stimulus condition compared to the baseline condition when the startling stimulus is presented alone. The most robust effect observed is a decrease in eye-blink amplitude in the lead stimulus condition with a lead interval of 30-500 ms. This effect is known as pre-pulse inhibition (PPI).

Researchers have shown consistent evidence that SEB or SEB modification can show systematic differences:

- When attention is directed at, or away from, the startle or lead stimulus (Hazlett *et al.*, 1993).
- When subjects are exposed to different affective stimuli (e.g. pleasant vs unpleasant pictures; Vrana *et al.*,1988).

- In schizophrenic (Braff *et al*., 1992), psychosis prone (Chapman *et al*., 1994) and post traumatic stress disordered individuals (Morgan *et al*., 1995) compared to psychologically healthy control subjects

Due to such findings, researchers currently believe that SEB modification techniques provide unique and valuable information about human attentional, affective and disordered processing. (For an excellent review, see Filion *et al*.,1998).

The aim of the following exercise is to give a brief introduction to eye-blink startle measurement and modification techniques.

Equipment

- Any PowerLab unit with Scope software
- ML132 Bio Amp and MLA1340 Bio Amp cable
- MLA1090 Electrode cream
- 3 MLAWBT-9 EEG/EMG Recording electrodes
- sound amplifier with BNC leads & headphones
- 70% alcohol preparation & cotton wool
- surgical tape
- tissues

Setting up the equipment

Ensure all equipment is turned off. Ensure the PowerLab is connected to your computer as outlined in the users manual. If necessary, connect the Bioamp to the PowerLab unit as outlined in the users manual. Plug the BNC lead from sound amplifier directly into the positive and negative outputs (or stimulator outputs) from the PowerLab unit. Plug the headphones into the amplifier. The PowerLab will generate the sound stimuli you need through the amplifier to the headphones.

Method

The electrical measurements taken when measuring eye-blink electromyogram (EMG) are known as *differential*. This is because the electrical signal measured is the difference between electrode sites. The electrode sites are classically located on a vertical plane through the center of the eye. One electrode is above the eyebrow and the other on the cheekbone. An earth is also connected to offset 50 Hz electrical interference. This electrode is often placed on the earlobe with an ear-clip connector (MLA IME electrode) or behind the ear. (See Figure A).

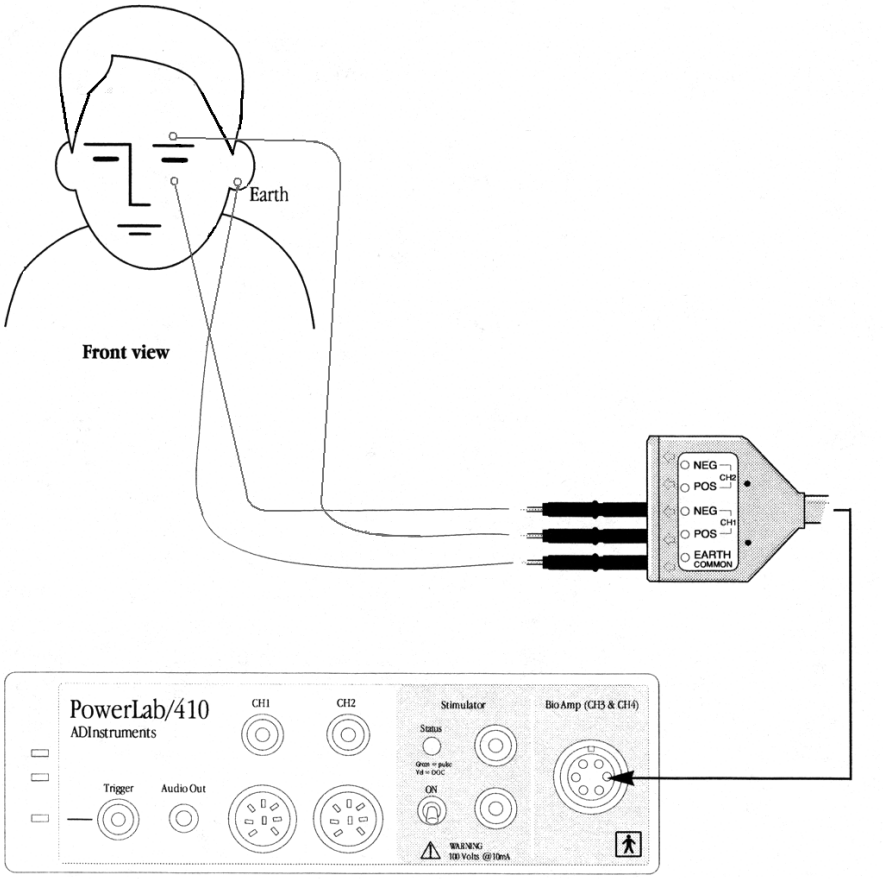

Figure A. Electrode placement for measuring eye-blink electromyogram.

Electrode Placement:

1. Measure and mark the positions above and below the eye.
2. Place EEG paste in the electrode cups.
3. Clean each site with 70% alcohol solution, then fix the electrodes with medical tape.
4. Connect the subject to the patient lead. Ensure the earth electrode is plugged in the green socket marked “earth” on the patient lead.
5. Ask the subject to relax and avoid moving as much as possible.

Safety

The Bio Amp is specially designed to have no direct electrical connection from the patient to ground. Thus, protecting your subject from any electrical faults. To maintain this patient-isolation, NEVER connect your subject to anything other than the patient lead, which must only be connected to the Bio Amp. The sound amplifier with headphones should not exceed a maximum output of 100 dB, and should not be delivered other than outlined in the following procedures. For guidelines regarding the safe delivery of auditory stimuli, consult appropriate international safety standards.

Bioamp Settings

Turn on the sound amplifier, PowerLab unit and computer, in that order.
Open Scope.

You now need to set up Scope for eye-blink EMG recording using the Bio Amp. The recommended settings for the Bio Amp are shown in Figure 1.

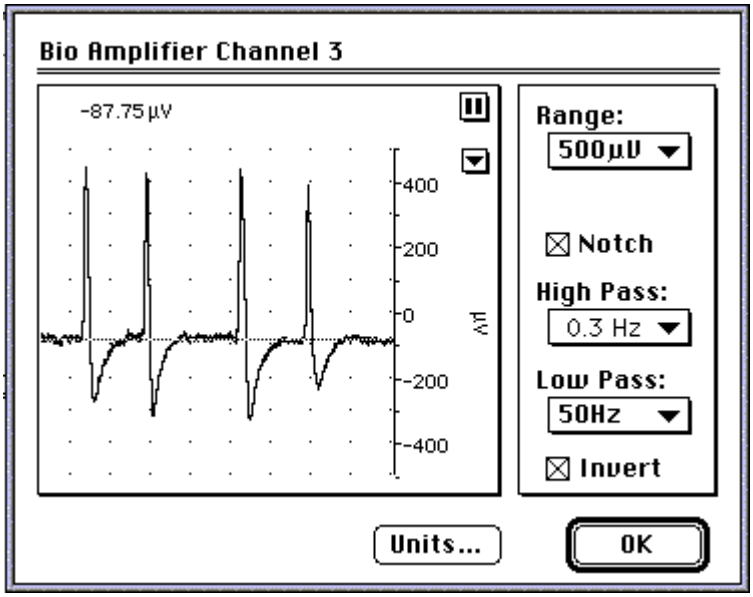

Figure 1. The Bio Amplifier dialog box.

The Bio Amp should have a recording range of 500 µV. Time Base should set to at least 512 samples over 1 sec or 400 Hz. Click the Bioamp button. You are now looking at the ongoing eye-blink EMG of your subject. A recording range of 500 µV with High Pass at 0.3 Hz and Low Pass at 50 Hz is best. However, if your signal is noisy (most likely due to a poor connection of electrodes to the subject), you can select the notch filter. If it is still noisy, you can set the Low Pass filter as low as 20 Hz and still achieve a useful recording. Ask your subject to blink a few times. You should see a clear definite EMG activation.

Stimulator Settings

You now need to set up Scope to deliver startling sound stimuli to your subject by clicking the mouse of your computer. You will also need to view this on the Scope display with the resulting blink EMG. The settings of the Stimulator are shown in Figure 2.

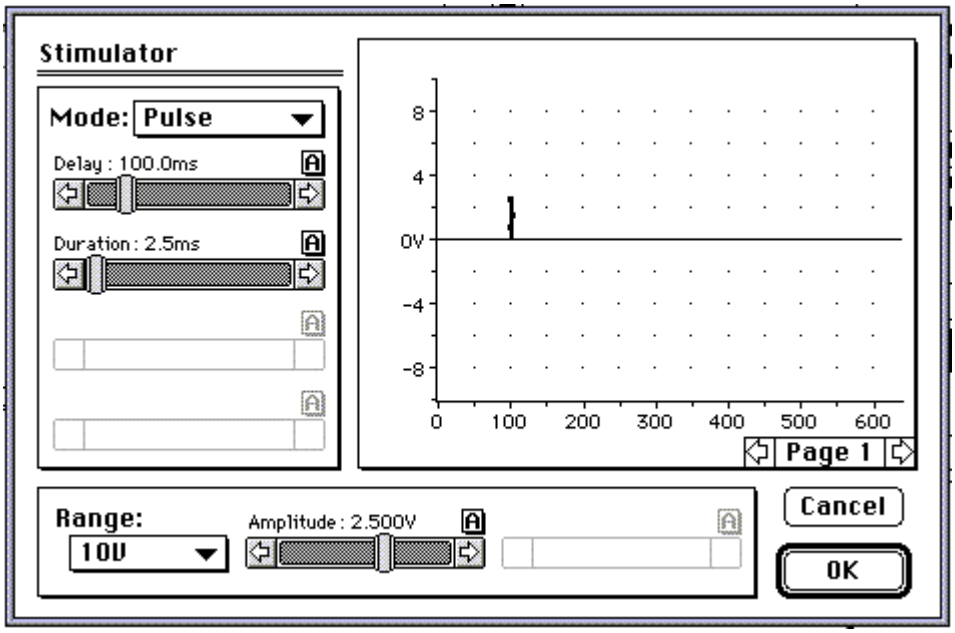

Figure 2. The Stimulator dialog box.

Under the Setup menu, open the Stimulator. Change the Mode to Pulse. Set the Delay to 100 ms. Set the Duration to 2.5 ms. You now you need to set the Amplitude of the stimulus. The Amplitude settings will depend on the requirements of the sound amplifier you use. Essentially, you want to deliver a loud pulse to your subject of no more than 100 dB. Write down the amplitude of the stimulus.
You are now ready to record startle eye-blinks.

Exercise 1 (PowerLab 410):
Eye-blink Startle and Habituation to a Loud Random Auditory Stimulus

1. Explain to the subject that loud tones will be presented randomly via the headphones, and to remain as still and relaxed as possible.
2. Ask the subject to put the headphones on, remain still and to focus at a spot on a nearby wall.
3. Observe the stimulus parameters in the Stim box, ensure these are the correct settings.
4. You will be also be randomly presenting a lead stimulus before about half of the tone presentations. You will create a lead stimulus by calling to your subject as you click your mouse button to deliver the tone. Call a single word out each time like "Now". Remember, only do this for about half of the presentations in a random fashion, and *write down what presentations you did this for!!!*
5. Click the "Start" button in Scope and observe the associated blink response.
6. Continue presenting the stimulus about 30 times, at random intervals (about 1-5 secs) remembering to randomly call to your subject in about half the presentations
7. Save the file as "Master".
8. Along the bottom of the screen in Scope, each stimulus presentation can be observed by clicking the appropriate page. View each presentation and delete all presentations that had no lead stimulus. Save this file as "Lead".

9. Open the file “Master” again. Delete all presentations that had a lead stimulus. Save this file as “Control”.
10. Open the file “Control”. Compare the first presentation to the last. The last presentation should be markedly reduced in amplitude compared to first presentation. This is because the subject has adapted to the tone. The cursor will give time and amplitude of its position in the “Cursor” box in the top right-hand corner of the Scope program.
11. Compare the average peak eye-blink amplitude in the “Control” condition to the “Lead” condition. Also compare the average latency to peak eye-blink amplitude. Calculating average parameters is very easily done under Scope by simply pressing the X page in the bottom corner of the screen. Using this feature and moving the cursor appropriately, you can establish the average peak amplitude of the eye-blink and the average time to this peak in the X page. See Figure 3.

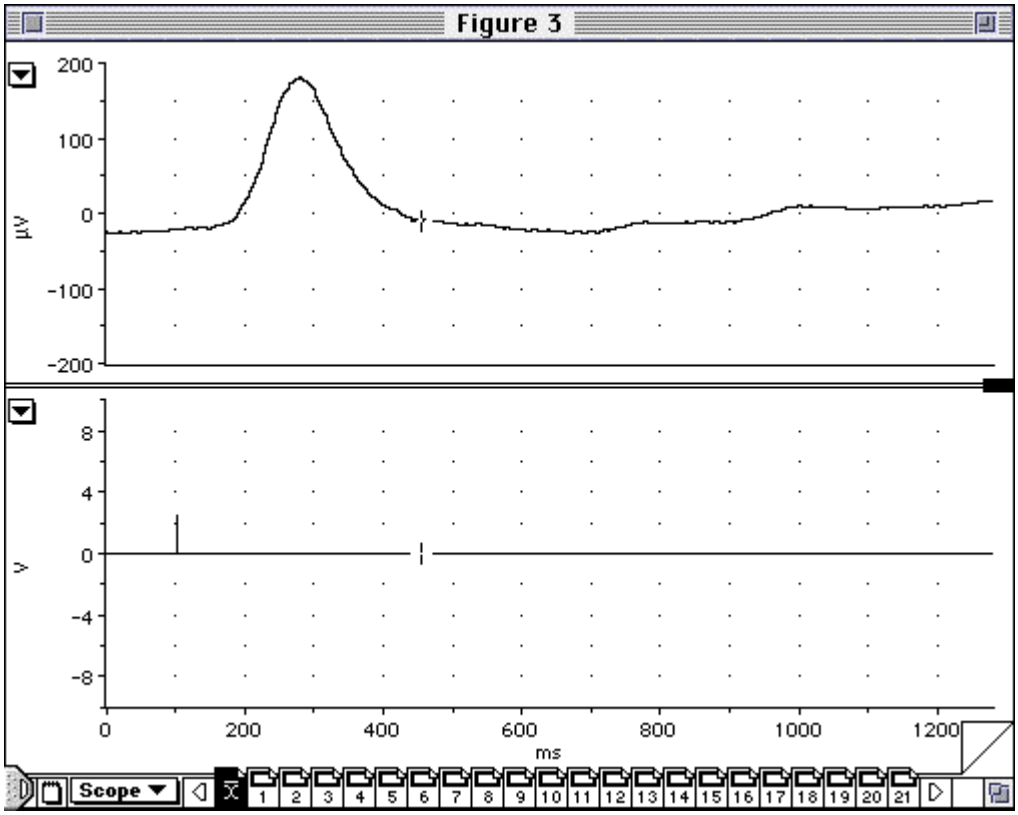

Figure 3. Eye-blink data.

Further Investigation

1. Try manipulating attention to the startling stimulus by observing subjects’ responses while they are completing a complex task. For example, the experimenter could hold up a card with a number and ask each subject to count backward from that number in lots of 13. A few tones could be presented during this task. As a control condition, a few tones could be presented while the subject counts forwards, in ones, from a number presented by the experimenter. These two tasks could be repeated several times until about 20-30 tone presentations from each condition are collected for comparisons. When task demands are high, what would you expect from the eye-blink startle response?
2. Try investigating startle eye-blink amplitude and latencies under different subject-affect conditions. For example, one could contrast SEB when viewing randomly presented pictures of attractive people and pictures of car accidents. What would

you expect in terms of SEB amplitude and/or latency?

Exercise 2 (PowerLab X00 series):
Eye-blink Startle, Pre-pulse Inhibition and Habituation to a Loud Random Auditory Stimulus

1. Essentially, you want to deliver a soft pulse to your subject of no more than 50 dB (A) followed by a loud pulse of no more than 100 dB. Under the "Setup" menu, open the "Stimulator". Change the Mode to Double. Set the Delay to 150 ms. Set Duration A and Duration B to 2.5 ms. Set the Interval to 500 ms. You now you need to set the Amplitude of A and B. The Amplitude settings will depend on the requirements of the sound amplifier you use. Write down the amplitude of A.
2. Explain to the subject that tones will be presented randomly via the headphones, and to remain as still and relaxed as possible.
3. Ask the subject to put the headphones on, remain still and to focus at a spot on a nearby wall.
4. Observe the stimulus parameters in the Stim box, ensure these are the correct settings.
5. Click "Start" in Scope and observe the associated blink response.
6. Quickly open the Stimulator and set the amplitude of Stimulus A (lead stimulus) to zero. Exit the Stimulator and click start to deliver the startling stimulus on its own.
7. Present this stimulus a few times with a random interval of about 30-60 secs.
8. Quickly open the "Stimulator" and reset the amplitude of Stimulus A (lead stimulus) back to its original value. Exit the "Stimulator" and click start to deliver the lead and startling stimulus together
9. Present this stimulus once or twice with a random interval of about 30-60 secs.
10. Continue this pattern of presentation (points 9 – 13) for about 30 trials, conducting each trial at random intervals (about 30-60 secs). This is to ensure the subject can not recognize the lead trials by a delay between presentations.
11. Save the file as "Master".
12. Along the bottom of the screen in Scope, each stimulus presentation can be observed by clicking the appropriate page. View each presentation and delete all presentations that had no lead stimulus. Save this file as "Lead".
13. Open the file "Master" again. View each presentation and delete all presentations that had a lead stimulus. Save this file as "Control"
14. Open the file "Control". Compare the first presentation to the last. The last presentation should be markedly reduced in amplitude compared to first presentation. This is because the subject has adapted to the tone. The cursor will give time and amplitude of its position in the "Cursor" box in the top right-hand corner of the Scope program.
15. Compare the average peak eye-blink in the "Control" condition to the "Lead" condition. Also compare the average latency to peak eye-blink. Calculating average parameters is very easily done under Scope by simply pressing the X page in the bottom corner of the screen. Using this feature and moving the cursor appropriately, you can establish the average peak amplitude of the eye-blink and the average time to this peak in the X page. See Figure 4.

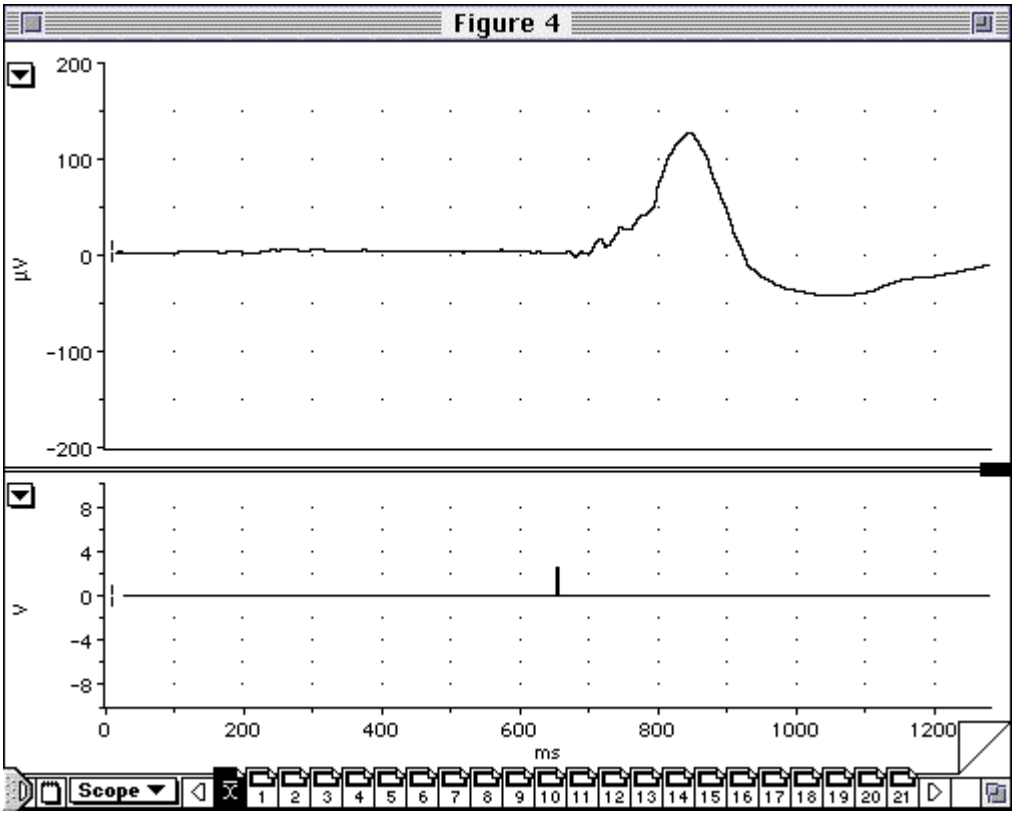

Figure 4. Eye-blink data.

Exercise 3 (PowerLab X00 series):
Classical Conditioning the Eye-blink Startle Response

When presenting a soft tone pre-pulse immediately before a loud tone (the unconditioned stimulus: UCS) an eye-blink startle response (unconditioned response: UCR) will be elicited by the loud tone. However, after repeated presentations, the pre-pulse should become associated to UCS and elicit an eye-blink (conditioned response: CR) when presented alone.

1. Prepare a new subject as described in steps 1-5.
2. Under the Setup menu, open the Stimulator. Set the Mode to Double. Set the Delay to 150 ms. Set Duration A and Duration B to 2.5 ms. Set the Interval to 100 ms. Set Amplitude of A and B the same as the previous soft and loud stimuli. Explain to the subject that two randomly presented tones will be presented to them, one soft one, followed shortly by a louder one. Write down the amplitude of B.
3. Ask the subject to put the headphones on, remain still and to focus at a spot on a nearby wall.
4. Press “Start” to present both the soft and loud tone together.
5. Present this stimulus a few times with a random interval of about 30-60 secs.
6. Quickly open the “Stimulator” and set the amplitude of Stimulus B (startling stimulus) to zero. Exit the “Stimulator” and click start to deliver the soft stimulus on its own.
7. Quickly open the Stimulator and reset the amplitude of Stimulus A (lead stimulus) back to its original value. Exit the Stimulator and click start to deliver the lead and startling stimulus together

8. Present this stimulus about three or four times with a random interval of about 30-60 secs.
9. Continue this pattern of presentation (points 9 – 13) for about 30 trials, conducting each trial at random intervals (about 30-60 secs). This is to ensure the subject can not recognize the conditioning trials by a delay between presentations.
10. Save the file as “Conditioning”.
11. Along the bottom of the screen in Scope, each stimulus presentation can be observed by clicking the appropriate page. View each presentation and delete all presentations that had both stimuli. Save this file as “UCR”.
12. Open the file “Conditioning” again. View each presentation and delete all presentations that delivered the startling stimulus. Save this file as “CR”
13. Open the file “UCR”. Compare the first presentation to the last. The last presentation should be markedly reduced in amplitude compared to first presentation. This is because the subject has adapted to the tone. The cursor will give time and amplitude of its position in the “Cursor” box in the top right-hand corner of the Scope program.
14. Open the file “CR”. View the various presentations, then view the averaged waveform (X page). Notice that a blink waveform is now present in response to this normally “non-startling” stimulus. This is because your subject has associated the soft stimulus to the occurrence of the loud startling stimulus. Such a response in known as a Conditioned Response (CR). See Figure 5.

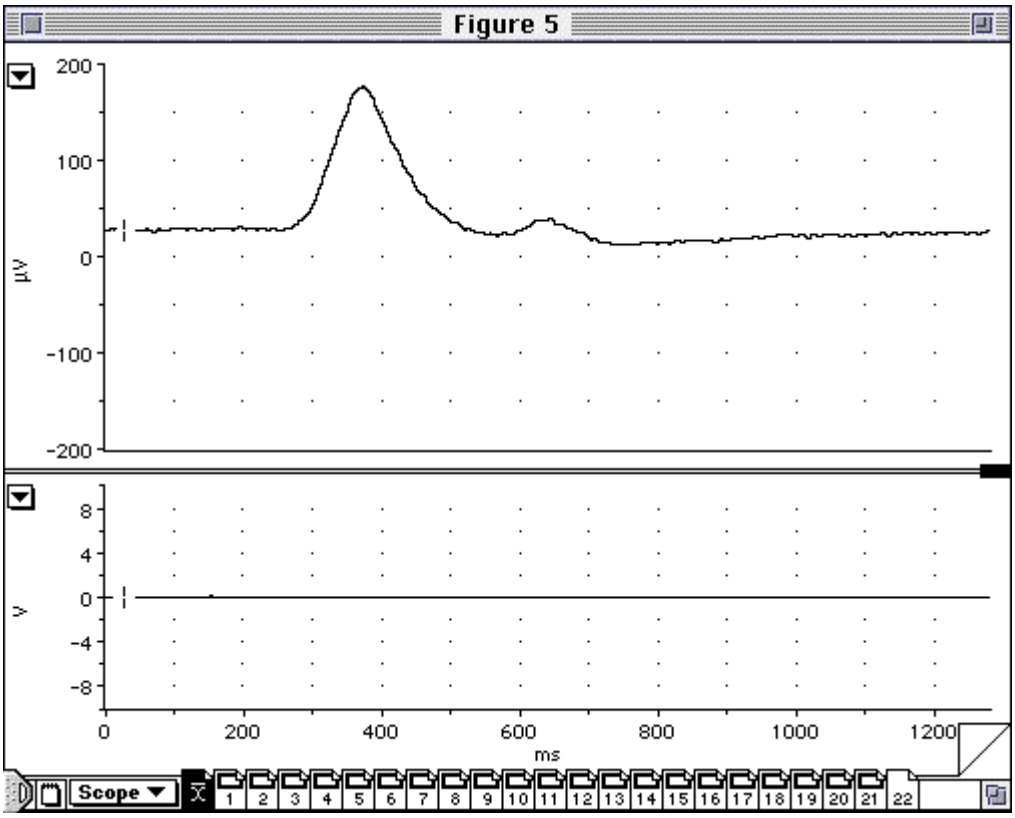

Figure 5. A Conditioned Response.

Further Investigation

1. Have the CRs in Exercise 3 (point 45) also shown similar adaptation effects to that of the UCRs (point 44)? What role does the inter-stimulus interval have on the experimental effects observed in Exercises 2 & 3? Are these intervals different for a reason?

2. From what you've learned from Exercise 2, you could try manipulating attention to startling stimuli by observing subjects' responses while they are completing a complex task. For example, the experimenter could hold up a card with a number and ask each subject to count backward from that number in lots of 13. A few tones could be presented during this task. As a control condition, a few tones could be presented while the subject counts forwards, in ones, from a number presented by the experimenter. These two tasks could be repeated several times until about 20-30 tone presentations from each condition are collected for comparisons. When task demands are high, what would you expect from the eye-blink startle response?
3. From what you've learned from Exercise 2, you could try investigating startle eye-blink amplitude and latencies under different subject-affect conditions. For example, one could contrast SEB when viewing randomly presented pictures of sexually attractive people and pictures of car accidents. What would you expect in terms of SEB amplitude and/or latency?

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