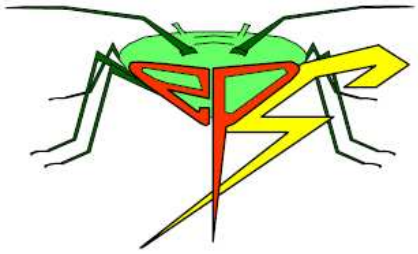
July 2014

MANUAL

Giga-4/8

EPG system



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Appendix 1
Making a Faraday cage

1. GIGA AMPLIFIER

Introduction

The Giga amplifier series has been developed for recording Electrical Penetration Graph (EPG) signals. The amplifier is especially suitable for aphids and other Homoptera, but other piercing-sucking insects/arthropods might be used as well (thrips, mosquitoes, bugs, ticks, etc.). Chewing insects are not suitable. The amplifier allows simultaneous recording from maximally four or eight insects with the *Giga-4* and *Giga-8* model, respectively. The name 'Giga' refers to the 1 Giga-Ohm (10^9 Ω) input resistance. Together with the extremely low input bias current (< 0.1 pA) this forms the most important specification of the measuring principle in the *full EPG recording system* (Tjallingii, 2010) earlier called *DC system* (Tjallingii, 1985; 1988). The specifications (Table 1) allow recording more details and biologically important information (see Tjallingii, 2000; 2010) than the AC based EPG-systems (McLean & Weigt, 1968) or later derived mixed systems (Backus, 2010).

By incorporating plant and insect in the electrical circuit of the EPG system electrical resistance and potentials generated by the insects activities in the plant, such as bio-potentials can be recorded. The EPG therefore reflects differences between specific genotypes of insect and/or plant related plant suitability or resistance. The latter one suggest a ´versatile´ use but in fact only the DC part of it (a copy of the Giga systems) produces reliable signals.

Data acquisition needs an analogue/digital (AD) signal conversion device, which has been build-in the Giga systems since 2011 and a USB connector should be used to get data files on your PC hard disk using the ***STYLET***$^+$ software, operating under Windows (XP-W7). The signal is monitored during acquisition. A separate EPG analysis module is adapted to handling and analysis of EPG signals. Further data processing can be done in any spreadsheet program and some Macros have been developed (see www.epgsystems.eu page Data Processing).

Table 1. Technical specifications

amplifier (EPG probe)	
- amplification (gain)	50x
- input resistance	1 Giga Ohm
- input bias current	~ 1 pA
control unit	
- 2nd stage amplification (gain)	1x (calibrated) to 2x, resulting in 50x to ca. 100x
- calibration pulse	-50 mVolt (circa)
- max. Vs adjustment	± 2 Volt
- max. output	± 6 Volt
- panel meter safe area indication	± 5 Volt
- 2nd stage amplification (gain)	1x (calibrated) to 2x, resulting in 50x to ca. 100x
- calibration pulse	-50 mVolt (circa)
power supply	220 or 110* Volt AC / ± 8Volt DC (300 mA)

* should be ordered

Unpacking and inspection

Check the shipped *PACKING LIST*, compare it to Table 2, and check that all parts that are included. Claim any missing or damaged article within one month.

Table 2.

Packing lists of items of the complete Giga system

	Giga-4	**Giga-8**
Control unit (main box)	1	1
build-in AD device (USB)		
4 ch 12 bit (Dataq Di155)	(1)	
4 ch 14 bit (Dataq Di710)		(1)
Power supply, 220 or 110V*AC / ± 8V DC	1	1
EPG probes	4	8
Swivel clamps for probe mounting	4	8
Mounting rod Ø 10mm	1	1
Cabling		
wired plant electrodes	4	8
test cable (‘insect pin’ to red banana)	1	1
grounding lead (black banana to alligator)	1	1
Wiring kit:		
coil with 30 m gold wire (Ø 12.5* or 18 µm)	1	1
vial with silver glue, ca. 2 ml , 25% Ag	2	2
insect electrodes (connector-pegs/nails, Ø 1.2mm)	15	30
bunch of insect electrode extension wire (Ø 0.2mm)	2	2
USB cable	1	1

* should be ordered

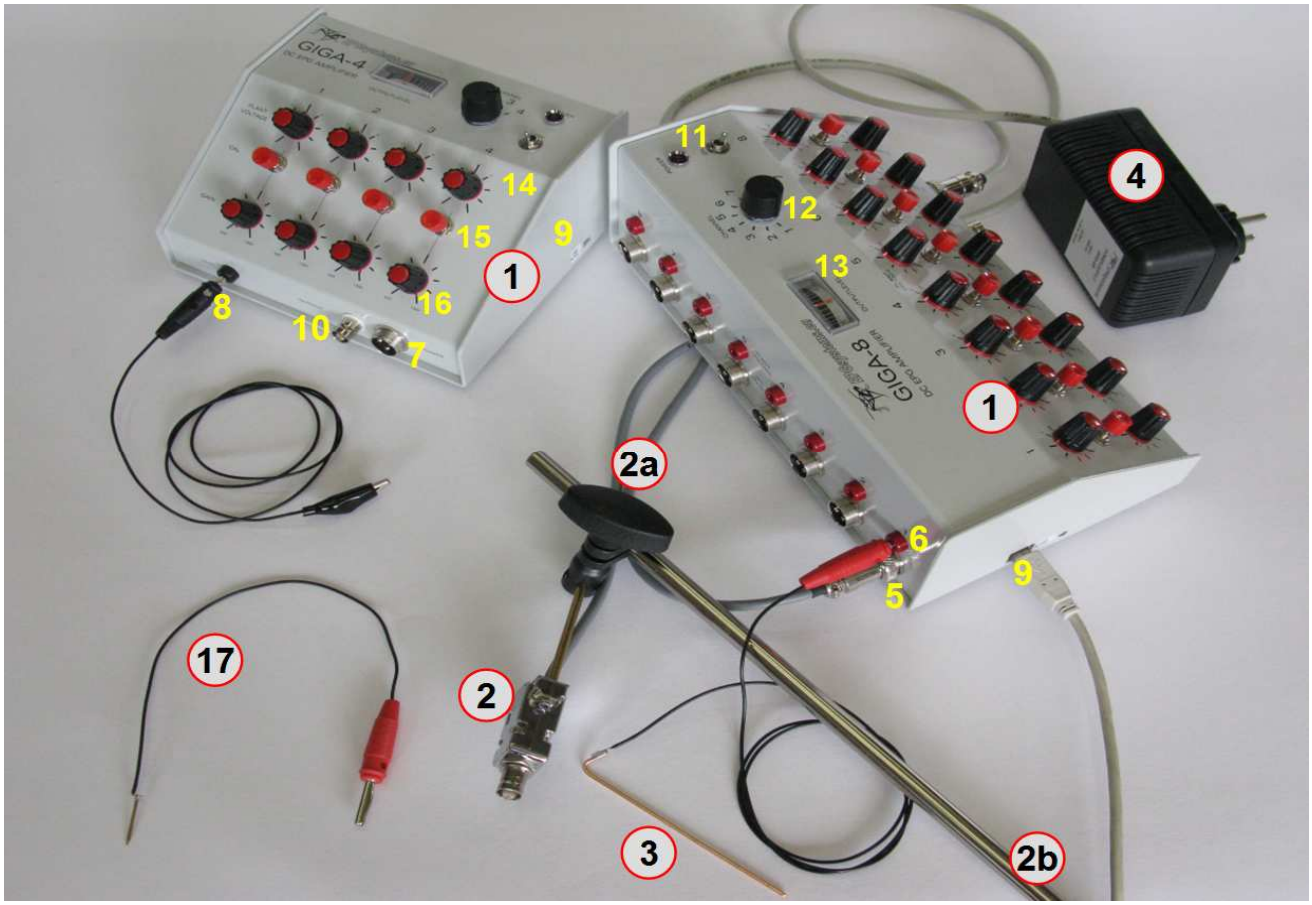
Fig. 1. Main Giga features

① ***Control Box,*** Giga-4 or -8 to adjust for each channel the plant voltage and gain; with calibration pulse.

② ***EPG Probes***, the small elementary amplifiers, one for each channel (insect)

③ ***Plant electrode***, one for each channel (plant)

④ ***Power Supply***, 110 or 220V AC:±8V DC



EPG Giga-4 and -8 with all items connected

① Giga-8 (rear view), Giga-4 (front view)
② EPG probe: primary amplifier*
2a Swivel clamp* for rod/stand attachment
2b rod (ø10mm) to mount EPG probes
③ power supply, 220V (110V opt.)AC/±8V DC
④ plant electrode*
5 probe connector inserted*
6 plant electrode connected*
7 power input
8 ground cable inserted in GND connector to Faraday cage by its alligator clip
9 USB cable in AD converter output
10 single output for selected channel (see 12)
11 power switch with lamp indicator
12 channel selection button (see 10 and 13)
13 panel meter showing output voltage of selected channel
14 plant voltage adjustment knob*
15 calibration pulse push button (ca. -50 mV)*
16 gain adjustment button (50-100x)*
⑰ test cable (separate, see tests on p. 6)

* one per channel

2. INSTALLATION, PUTTING UNITS TOGETHER

Data acquisition

1. Install the AD device (internal in Giga) and ***Stylet***$^{+}$ software (see instructions from download package)
2. Connect the Giga-USB device and follow the instructions in the Stylet+ software manual.

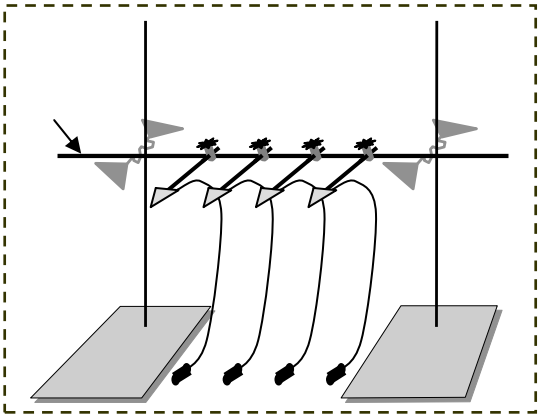


Fig. 2 Stands with 4 probes

Giga experimental set up

1. Set up a proper Faraday cage (for construction, see appendix) on a table or place without shock distortions during EPG recording.
2. Do not mount a lamp or any other AC powered device inside the cage!
3. Fix the mounting rod with the swivel clamp attached EPG probes horizontally, in the example (Figure 2) shown between two ordinary (chemical) stands with standard clamps (not provided by EPG systems). For the Giga-8, screw the two rods together to make one rod with 8 EPG probes. Place probes in the Faraday cage as far to the rear as possible to reduce noise.
4. Put the Giga device (while power switched **off**, see warning) in front of the Faraday and plug in each 4-pin connector of an EPG probe into the sockets at the rear of the device (Fig. 1, (5)). Only one position is possible for these plugs to insert, rotate the plug until it fits properly. Screw the ring on the socket's border. Do not use force to screw. When it does not go easy, first turn the ring counter clock wise until the thread fits.
5. Connect the plant electrodes with their connectors (red bananas) of into the red sockets at the rear of the Giga (Fig. 1 (6)), above the probe sockets.
6. Similarly, insert the power supply plug into the 3-pin socket on the front face of the control unit (7). Wait to plug in the power supply unit into the electricity net. Also check that the power switch on top of the control unit is **not '**ON'.
7. Connect the USB cable between the USB sockets on the Giga and the computer.
   Old situation (devices before 2012): Insert the 9-pin plug of the AD cable into the 9-pin output socket on the front face of the Giga (next to (10)) and fix its screws and connect the other end of the AD cable to your AD USB device.
8. Finally, connect the equipment to the Faraday cage using the ground lead, insert the black banana plug into the ground socket of the Giga (8) and connect the alligator clip end of the cable to a blank metal part of the Faraday cage. It will be good to check ground connections between all parts of the set up using a multimeter, resistance between all cage faces and the Giga's gnd. should be 0 Ohm on the multimeter.

Now the set up is ready for testing. First, you can test it without computer connection (installation check, next chapter), using the panel meter only. Then it should be tested with computer, using the screen display of ***Stylet***$^{+}$ on the monitor screen during (test) acquisition in the mean while. This requires the software to be installed first, see *install-6.pdf* (website, page Downloads/installation).

3. EQUIPMENT TESTS AND NOISE

WARNING:

1. ***Do not connect probes to the Giga device while switched on!***
2. ***Static electrical charge is a danger for the EPG probe input; for precautions see next chapter***

Before the first use, but also at any time later, when there is any doubt about proper functioning, it is good to test the equipment according to one or both of the following test procedures. Mounting an insect is not required yet.

3.1 Giga control unit & EPG probe tests

This is a test for proper operation of the Giga4 or -8 device and the EPG probes (Fig.1, item (2)).
It is recommended to test inside the Faraday cage.

Test without using Stylet+ software (no computer connection needed)

Equipment preparations will be as on previous page. Thus, power switch on Giga off, power adapter (transformer ±8V DC) to connected the grid (110V or 220V AC), and EPG probes to the Giga.

Open input test

1. No electrode or cable should be connected to any of the EPG probes' inputs.
2. Switch ON the power of Giga so that the red control lamp is on.
3. Turn the CHANNEL selection button to channel 1 and check the needle of the panel meter:
   - Needle in the 0 Volt (middle) position, or very close to it, means that this EPG probe is OK.
   - Needle in left minimum or right maximum position indicates a damaged probe (!).
4. Turn the selection button to all other channels subsequently and check the panel meter positions.
5. Switch Giga's power OFF.

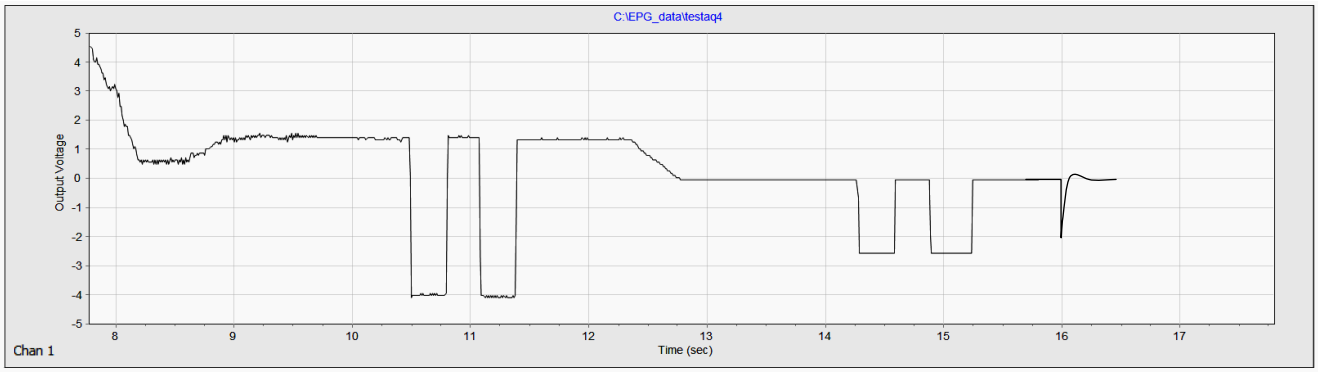
Test cable test (item 17)

6. Giga's power OFF
7. Connect the red banana plug of the test cable (other end of cable has a brass insect pin) into the red plant voltage socket (6) on the Giga
8. Turn the CHANNEL section knob (12) to the channel 1 position
9. *IMPORTANT*: Now first discharge any static electricity of your body before the next step. Touch with both hands any blank metal part on the Giga. For example, the outside of the channel connectors (5) or the brass attachment bars of the EPG probes.
10. Switch power ON now.
11. The panel meter should reflecting the output voltage of channel 1 should indicate 0 Volt
12. Now insert the insect pin of the test cable into the input of the EPG probe of channel 1. Use the fingers of your discharged hands (see step 9). Use one hand to contact the outside of the BNC socket of the probe input and the fingers of your other hand to insert the test cable pin carefully.
13. With the GAIN knob turned counter clockwise, turn the PLANT VOLTAGE knob of ch.1 (14)) to about the 0 volt position, and check the needle in the panel meter, which should go in the + or - direction with small movements of the plant voltage knob in + and - direction, respectively.

14. With the needle in a positive position now press the CAL (calibration) button on the Giga a watch the needle for the pulse response.
15. Repeat these procedures with the GAIN knob turned clock wise. If the needle goes off scale now turn the PLANT VOLTAGE knob negatively to get it lower and within the scale area. The CAL button response will be twice as strong now. Turn the gain knob back counter clockwise after this.
16. Leave the needle in a positive position and remove the brass insect pin of the test lead. Now the needle should return to the 0V position (or very near to 0V; see 6.).
17. Check the probes of the other channels successively.

Test with Stylet+ software (or other data acquisition software)

18. Giga's power OFF
19. Connect EPG probes to all Giga channels.
20. With the USB cable connected between Giga and your computer start a 1h EPG recording session (see Stylet+ manual)
21. Once the recording has started switch the Giga power ON and follow the signal traces in the display panels.
22. Follow the instruction for **open input** and **test cable test**s 1. - 15. above, neglecting Giga's channel selection knob and panel meter. Now push for each channel the respective chan. button to observe its signal trace in the top panel.



Screen display of channel 1 during a test recording. Starting (left) with a PLANT VOLTAGE knob adjustment was adjusted from some positive voltage to about +0.5 and then to +1.3 V. Then the GAIN knob was here first in the 100x (clockwise) position and 2 times with the CAL button a calibration pulse (at about 10.5 and 11.1 sec) was given. Then the GAIN knob is turned counter clockwise to 50x at 12.5 sec and starting at about 14.2 sec, 2 more calibration pulses were given. At 16 sec the test cable is removed from the EPG probe and the signal returned to 0 Volt.

3.2 Noise, noise, noise, . . .

Electrical noise, better called '50 Hz noise' (60Hz for USA) is a nasty phenomenon. We can distinguish two noise sources in our set up, *environmental* and *system noise,* although they cannot be separated strictly.

Environmental noise comes from the local electricity situation in lab and depends on the location and distance of 220 (110)V AC power lines and cables. This noise is likely to be reduced it by removing power cords from the Faraday cage vicinity and proper cage shielding and grounding. System noise, on the other hand, comes from the power or ground leads and is more difficult to get rid of.

Equipment preparations

1. Test should be done with the EPG probe inside the Faraday cage. The Giga main unit can be put outside, in front or on one of side the cage. It is important to know the noise level with open and closed cage but first, leave the cage half open (hanging cage, with a grounding wire between cage and bottom plate) or with an open front side (standing cage).
2. Connect USB cable from the Giga (or separate AD device) to the computer and start a 1 h EPG recording session (see ***Stylet***$^{+}$ manual).
3. *IMPORTANT*: Now first discharge any static electricity of your body before the next step. Touch with both hands any blank metal part on the Giga. For example, the outside of the channel connectors (5) or the brass attachment bars of the EPG probes.
4. Insert carefully an 'empty' brass insect pin with only a copper wire into the EPG probe input.
5. Switch Giga ON

Noise test procedure

1. Presumably there will be noise on the display, which will appear as a sinus or a thick line. Often the noise will vary regularly over time, i.e. the width of noise line will get smaller and wider regularly.
2. If some noise is shown, you can close the cage (lower the hanging cage, close its doors, or hook the front screen on the cage to reduce the noise further. Hopefully, this will work but if the amplitude is not affected, go to system noise.
3. Open the cage half or remove the front screen and move one hand into the cage close to the electrode of the displayed channel but do not touch it. Stand or sit without touching with your hands, neither the hand moved into cage nor your other hand or arm to any metal part of the cage or equipment.
4. You will see a noise increase when your hand approaches the electrode and a decrease when it is removed.
5. While observing the noise amplitude from one hand close to the electrode, touch with your other hand the Faraday cage. The noise should abruptly be reduced or at least back to the level shown in point 1.
6. Reducing environmental noise is mainly a matter of shielding and grounding. The Faraday cage is crucial but if the cage is not properly grounded, i.e. connected to the equipment it will not work. Thus:
   a. Check again the main ground connections of the Faraday cage and Giga system by using an Ohm meter (low resistance with multimeter in Ohm position, or with sound signal).
   b. Verify the electrical connections between all Faraday cage faces, including the metal cage bottom and (removable) front face. The multimeter should display a close 0 Ohm value when touching one face with one lead of the meter and the other face with the other lead. The electrical contact between the EPG probe and the outside of the BNC input connector with the cage should also be tested.
   c. All (chemical) stands and other items should be grounded to cage as well (show 0 Ohm connection).
   d. No AC (110V or 220V) powered lamps or instruments should be used inside the cage or close to the (half) open cage! Leave them outside and illuminate insects and plants through the cage mesh.
   e. After having improved connections and grounding, check the noise level again on the display (points 1-6).

7. When the front side of the cage is open the noise level may be low enough for EPG recording. The advantage of an open front is that the positions of the insects can be inspected easy. By performing any inspection always keep one hand in touch with the Faraday cage to avoid noise (see point 5 above). If noise cannot be reduced by better connections or grounding, you should add a front screen during recording.

System noise is a persistent noise and mostly all 6. a-e measures above will not have an effect. This 50Hz noise comes from the mains, the grid connections and enters by recording equipment leads. It enters by the power (brown), return (blue), or safety ground (yellow/green coded) leads directly or inductively. Also, return and safety ground noise may be generated by any electric devices outside the Faraday cage, in the lab room, or elsewhere in the building. To get rid of this noise you may try one of the following points or a combination:

a. Check whether the computer has a 3 pin power plug (or 2 pins + a safety ground; countries are using different plugs). If somewhere a 2 pin connector occurs between power outlet and PC (extension cord, for example), replace it with a 3 pin one.
b. Connect the computer or laptop (with a power adaptor connected) externally, using an alligator clip lead to connect one of the outside connector screws or nuts to the Faraday cage.
c. 'Ground' the system – the Faraday cage is a good point – to external ground such as the metal water pipe or central heating system.
d. Check for loose contacts.
e. Alternatively, if after making all these connections properly there is no noise reduction, try the opposite.
   - If all power connections are 3-pin (with safety ground) replace one with only 2 pins or plug a 2 pin extension cord (power and return only) between power outlet and computer.
   - Remove grounding leads to water or heating systems. Sometimes 'grounding' makes things 'worse'.
   - Noise generated by other devices can be checked by switching these off temporary. Such noise may be absent for some time and then, all at a sudden, it may pop up very nasty again. If such a device has been found, try to connect it to a grid socket of a different group or move it to a different location.
   - Alternatively, move your EPG set up to a grid socket of different group or to a different location.

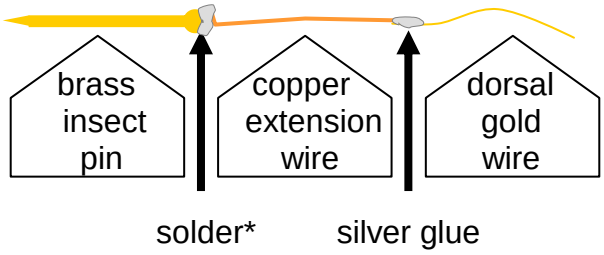
4. GETTING EPG RECORDING STARTED

Making insect electrodes

Before insect attachment make more than just enough complete insect electrodes. Use some good method to store them individually inserted in polystyrene foam in a box, for example.

1. Take pieces of copper extension wire (Ø 0.2mm) of about 2 cm long and solder one to each brass insect pin (Fig. 3). Use core wires from a spare piece of a cord, for example (some are supplied). Only use uncoated wires from ordinary 110/220V leads!

Fig. 3. Complete insect electrode



**** Note: apply soldering fluid before soldering !***

2. Then pieces of gold wire should be prepared using a stereo microscope. Handle the coil of gold wire and handle it with care (see 4).
3. Pieces of gold wire of about 2 cm long are appropriate for aphids. Cicadellidae or Delphacidae may need about 5 cm whereas whiteflies need 0.5 to 1 cm (and prefer a thinner wire).
4. Good illumination is important. Use only the green tag to loosen the wire on the coil, and fix the loose end with that tag afterwards. Mostly more wire will get lost by inappropriate coil handling than by the actual use. Cut a single piece of 10-12cm of wire, good for 5 or 6 pieces maximally. Do not make many separate 2 cm pieces in advance, they will get lost.
5. Now apply silver glue to the copper extension wire. Gold wire cannot be soldered but should be attached by the silver glue.

   Note on silver glue: *silver glue needs special care! The silver particles are heavy and sink to the bottom of the container. Thorough shaking before use is needed. Mostly, after shaking there will be enough glue for use at the inside of the lid of the opened vial. Close the vial after use immediately! Also, create/use any support to keep the opened container upright!!! The silver glue is water based. So, when it might dry, it can be diluted by clean tap water but once completely dried it will be difficult to restore its original shape (see also instructions about silver glue and gold wire on* http://www.epgsystems.eu/downloads.php). *One vial of 2ml should be enough for hundreds of insect attachments. Store not used vials cool. Decay by micro-organisms is at risk.*

   Insert the copper extension wire - soldered to the brass insect pin - for about 2-3 mm in the opened lid of the silver glue vial. Move the glue containing extension wire to the loose end of the gold wire and make contact with some overlap. Then after letting the glue dry for about one minute cut the 2 cm piece of the gold wire. Pin the completed electrode in some foam plastic support and take the next insect pin + extension wire to complete an electrode from the same gold wire.

Insect attachment

This seems the most difficult and delicate part of EPG recording for outsiders but it is rather simple. Nevertheless, the attachment of an insect to a gold wire with silver glue should be carried out quietly. Bad contacts give poor EPGs. Fixing the insect during the manipulations is easy but not strictly needed. It depends on the size and the mobility of the insect. For fixation, a vacuum ´needle´ or suction device may be helpful to handle the insect. We use (Eppendorf) pipette tips, valve-connected to a vacuum system and mounted pointing upward under the stereo microscope. Do not use CO_2 or cooling if you can manage without but smaller and active insects such as whiteflies or bigger hoppers may need it.

1. If insects having wax on their abdomen or thorax should be cleaned first. Use a fine brush wetted with water and some detergent.
2. Applying silver glue to the insect first is recommended. Use a thin insect pin or needle to put a small droplet of glue on the abdomen or thorax and let it dry.
3. Then put a smaller second droplet on the dorsal dot and insert the gold wire (of the prepared insect electrode, previous section) in the wet glue so that the wire points back- and upwards from the insect. Let the glue dry, then disconnect the vacuum/stop sucking and gently lift the insect with wire. Leave the electrode with the hooked up insect, hanging in the air on a safe place. For example, insert the electrode in some polystyrene foam in an oblique position, so that the insect cannot climb in the electrode.
4. Prepare a few more wired insects that strictly needed.

5. Select the leaf or plant part that is relevant to purpose of the experiment. If a leaf is used prevent (natural) leaf movements by fixing the leaf. Also, prevent an insect position that will lead to a hanging without possible contact restoration.
6. Mount the first wired insect by inserting its electrode into the input connector of the EPG probe of ch.1 and watch that it becomes in a proper position. Legs should be in walking position to the plant surface. Then lift or move the attached insect a bit the get it hanging a few mm from plant's recording site and mount the next insect.
7. After all insects are properly positioned but still hanging a few mm from the plant surface, start the computer for data acquisition. Now all hanging insects are moved one by one onto their recording site. Using this protocol will entertains that always the beginning of the first probe (stylet penetration) will be recorded.
8. Wait for started probing by each insect and then adjust the PLANTS VOLTAGE and GAIN buttons' positions on the Giga control unit (see next section).
4. During this adjustment procedure - **after** penetration has started - you can also check for the quality of the silver paint contacts. When pressing the calibration button (CAL) on the control unit the pulse should be visible in the output signal as a proper square pulse (Fig. 4, good). If only the edges are seen without a clear level of the pulse, the electrical quality of the contact is poor. Try to experience this technique first with aphids (the best known insects for EPGs) if you doubt that you master the technique. Also, aphids have their own 'square pulses', i.e. the potential drops (pds).

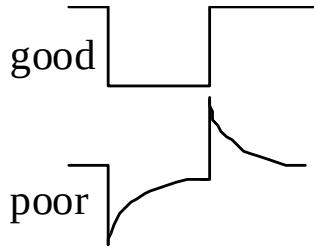


Figure 4

Proper Amplifier Adjustment

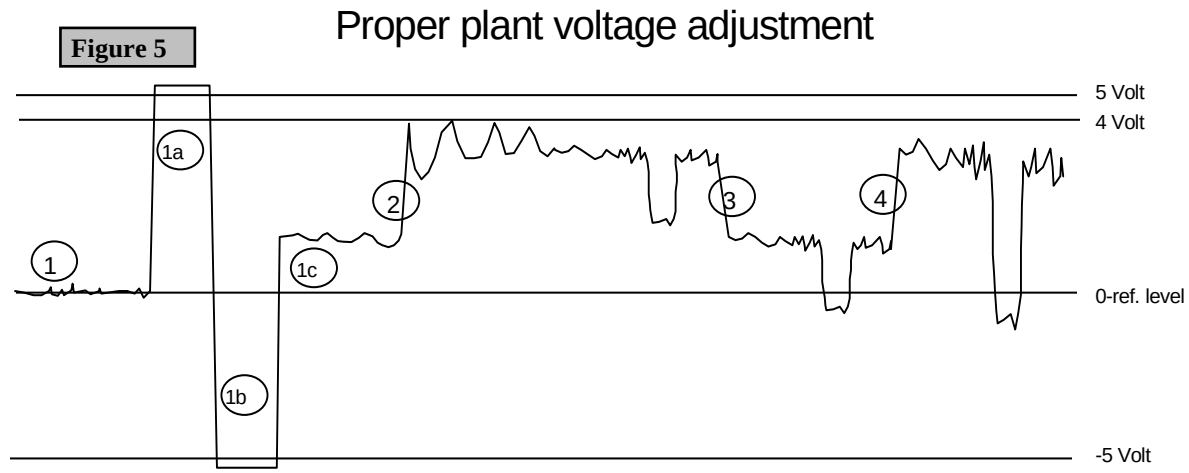
Proper amplifier adjustment is very important for getting nice signals and proper waveform analysis. Every EPG recording and each insect (operating channel) should be adjusted individually for an optimal output signal. This adjustment should be made after recording has started and during stylet penetration. Thus the highest resolution and the best signal to noise ratio will be recorded.
Adjust each channel only once and leave all signal changes then unaltered. The signal will certainly change in during the recording time and deviate from the ideal adjustment. But adjusting often will cause difficulties in the EPG interpretation; one will get confused when the signal changes again and again, not being able to distinguish between changes by the insect or by the operator. Only if the signal will run out of scale readjustment will be needed.

Adjustment procedure for each channel (Fig. 5).

1. Connect an aphid and wait for the first penetration while the adjustment knob (3) for the PLANT VOLTAGE is in the middle position. The GAIN knob (5) should be in the most counter clock wise position (50x). Watch the panel meter. During non-penetration the needle of the panel meter will be in the middle (0 Volts) about, the base line at 0 volt on the screen display.
2. Adjust the plant voltage at the first EPG waveforms so that the highest peaks reach +4 to +5 Volt, right on the panel meter. Somewhat later, when the EPG reaches B and C waveforms successively, the signal should be maximally at a level of about + 4 Volts.
3. Now press the calibration button (4) and look what the lower level of the pulse is. If the level goes just through the 0 volt reference level (base line level) the adjustment is perfect. Often this will not be the case, however. So, readjust the plant potential with pressed calibration button until this is true. The pulse button has about the same voltage as a pd waveform (-50 mV) so that when the calibration pulses go down just trough the 0 reference level, the pd's will do so as well.

4. To get back the maximal 4 Volt output level, adjusted in point 2, now not the plant voltage button but the gain button should be used so that the pd- or calibration pulse level will stay just below 0 Volt.

Figure 5



1. Wait until penetration starts. Then, the signal can be either
   1a out of range positive
   1b out of range negative
   1c rather weak, near O Volt
2. adjust the plant potential until the output is max. 4 Volt during waveform C
3. readjust to get the output just below 0 Volt when the "cal" pulse button is pressed
4. now adjust GAIN to the output to max. 4 Volt again, when the “cal” button is released

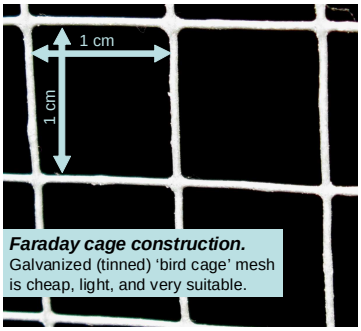
This adjustment is optimal for EPG recording. Although the positive recording is just a convention, the other adjustments do provide an optimal resolution, using the AD converter 4096 of the 12 bits as good as possible. A sub-optimal adjustment may lead to difficulties in waveform identification. Also, when the Y axis in the analysis panel is magnified for looking at details, the noise (50 or 60 Hz) will be amplified as well and thus disturb the signal image more than when no amplification is needed due to optimal recording.

For the use of EPG recording software , see data acquisition software manuals or instructions.

Appendix 1

Constructing a Faraday cage

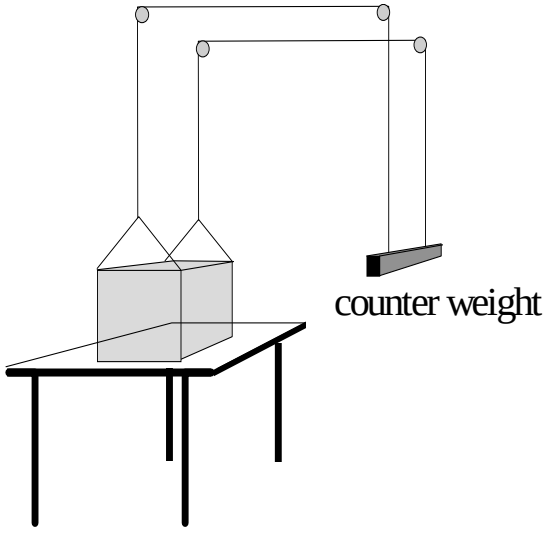
A simple and efficient Faraday cage can be constructed by using metal mosquito or (even more simple) bird mesh. A galvanised (zinc coated) or blank metal quality should be used, not a plastic coated or painted quality. If only painted mesh is available, the paint should partly be removed at the edges (use sand paper) in order to ensure electrical contact between the cage faces. The mesh width (holes of the netting) should never exceed 1 cm (!) and smaller than 2 mm is exaggerated, expensive, and will keep light out that is needed for photosynthesis by the plants. Mosquito mesh needs to be supported by a metal or wooden frame.



Faraday cage construction.
Galvanized (tinned) 'bird cage' mesh is cheap, light, and very suitable.

Constructing a **hanging cage**, supported by cords to the ceiling and pulleys (wheels) to a counter weight (see figure), is an elegant low budget solution. This cage can be elevated and pulled down in one movement, leaving the set up on an 'open table', accessible from all sides to change the plants, insects´ adjustments, etc. Alternatively, a **standing cage** on a table could be made. To access the cage's interior, an open front side is needed. To avoid noise (see noise section) the cage should be screened during recording with hinged mesh 'doors', or by a separate removable mesh screen to be hooked to the cage during recording. Whatever type of cage you are using; always take care that all cage faces - front screen and bottom plate included - should be electrically interconnected to each other, which is especially critical with wooden frames. Occasionally, the cage front may be left open during recording if the noise level is very low.

The **size** of the cage should be big enough to contain the expected size of the (potted) plants and large enough for the stands with the EPG probes. The control unit can remain outside the cage so that any voltage adjustments can easily be made during EPG recording.



The **bottom** plate must be an intrinsic part of the cage and should make good electrical contact with the other cage parts. It can be made of the same netting but easier it can be a metal plate of damage protected foil. Using a thick (0.8-1cm) iron plate will enable the use of magnetic stands supporting the probes. These can be fixed firmly and electrically connected to the plate, if not painted (!). A cheap solution is to use aluminium foil but that is damaged easily. Of course it can be covered by a plastic or glass plate but will cause electrical insulation of stands from the bottom. This should be re-established by separate wire contacts then.

Never let the plants or soil make electrical contact to the bottom during EPG recording. Always place the pots on an insulating dish. If a stereo microscope is used for visual inspection the cage should either be large enough to put it inside or small enough to remain outside leaving space to move in the front face.