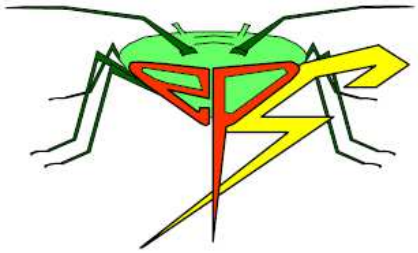
MANUAL

Giga 8d

EPG recording systems



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1. The Giga-8d EPG device

Introduction. The present Giga-8d model and former models Giga-4 and Giga-8 have been developed to record the Electrical Penetration Graph (EPG) signals from aphids and other hemipterans, especially. But other insects with piercing mouthparts such as thrips, and spider mites, or blood feeding mosquitoes, ticks have been used as well. Although the main interest comes from plant resistance, plant pathogen transmission, and insecticide research, EPGs have recently been used in plant electrophysiology studies as well. Chewing insects, on the other hand, are not suitable for EPG recording since fluid contacts during plant feeding is short-circuiting signals from specific feeding or salivation activities. Simultaneous recording from maximally eight insects can be accomplished with the *Giga-8d* model, each on a separate plant but also, recording from several insects one plant is possible, although proper recording adjustments for each insect are more difficult then.

"Giga" refers to the standard* 1 Giga-Ohm (10^9 Ω) input resistance of the amplifier. Together with the extremely low input bias current (< 0.1 pA) this is the most important specification of the *EPG recording system* (Tjallingii, 2010). For a complete list of specifications, see Table 1. A more complete overview of this and earlier EPG systems can be found on www.epgsystems.eu page *Measuring systems*.

Older Giga types. Look on p.

STYLET$^+$ software is a special free EPG data acquisition program for the Giga-8d. A separate EPG analysis module of software is adapted to handling and analysis of EPG signals. Update the last version! In case of doubt mailto:info@epgsystems.eu

Further data processing can be done in any spreadsheet program and some Macros have been developed (see www.epgsystems.eu *page* Downloads, section Data Processing).

System requirements. The Giga-8d has an integrated analog-digital (AD) converter on board with USB output, allowing data acquisition mediated by ***STYLET***$^+$ software, which displays the signal on screen simultaneously. All Windows platform versions (-XP, -7, -8, & -10) are compatible but Macintosh computers can be used on as well ***if*** there is a ***bootable*** Windows environment installed on your Mac (virtual Windows within a Mac background does not work!).

Table 1. Technical specifications

EPG probe (pre-amplifier)	
- amplification (gain)	50x
- input resistance	1 GΩ*
- input bias current	~ 1 pA
Giga8d control unit	
- 2nd stage amplification (gain)	1x to 2x (resulting in 50-100x total gain)
- calibration pulse	-50 mVolt (circa)
- max. Vplant adjustment	± 0.5 Volt
- max. output	± 5.85 Volt
- *Stylet*$^+$ scale range limits	± 5 Volt
- AD converter	resolution 14 bit; sample rate 100Hz (default)
Power supply	220 or 110* Volt AC / ± 8Volt DC (300 mA)

* *A special probe option with a switchable 1GΩ/1TΩ (10^{-12}Ω) input resistance. See Appendix 5*

2. 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. Giga8d Order packages contents

Basic EPG system

Item	number
Giga-8*d* control unit (main box) with build-in AD device	1
Power supply, 220 or 110V*AC / ± 8V DC	1
EPG probes	8
Swivel clamps for probe mounting	8
Mounting rod 40 cm; Ø 10mm	2
Cabling	
wired plant electrodes	8
test cable (‘insect pin’ to red banana)	1
grounding lead (black banana to alligator)	1
USB cable (micro USB on one side)	1
Wiring kit:	
vial with silver glue (water based non toxic), ca. 2 ml , 25% Ag	2
insect electrodes (connector-pegs/nails, Ø 1.2mm)	30
bunch of insect electrode extension wire (Ø 0.2mm)	2

Complete EPG system

This will contain presumably one of the following items:
- coil with 30 m gold wire (Ø 18 µm)
- coil with 30 m gold wire (Ø 12.5 µm)

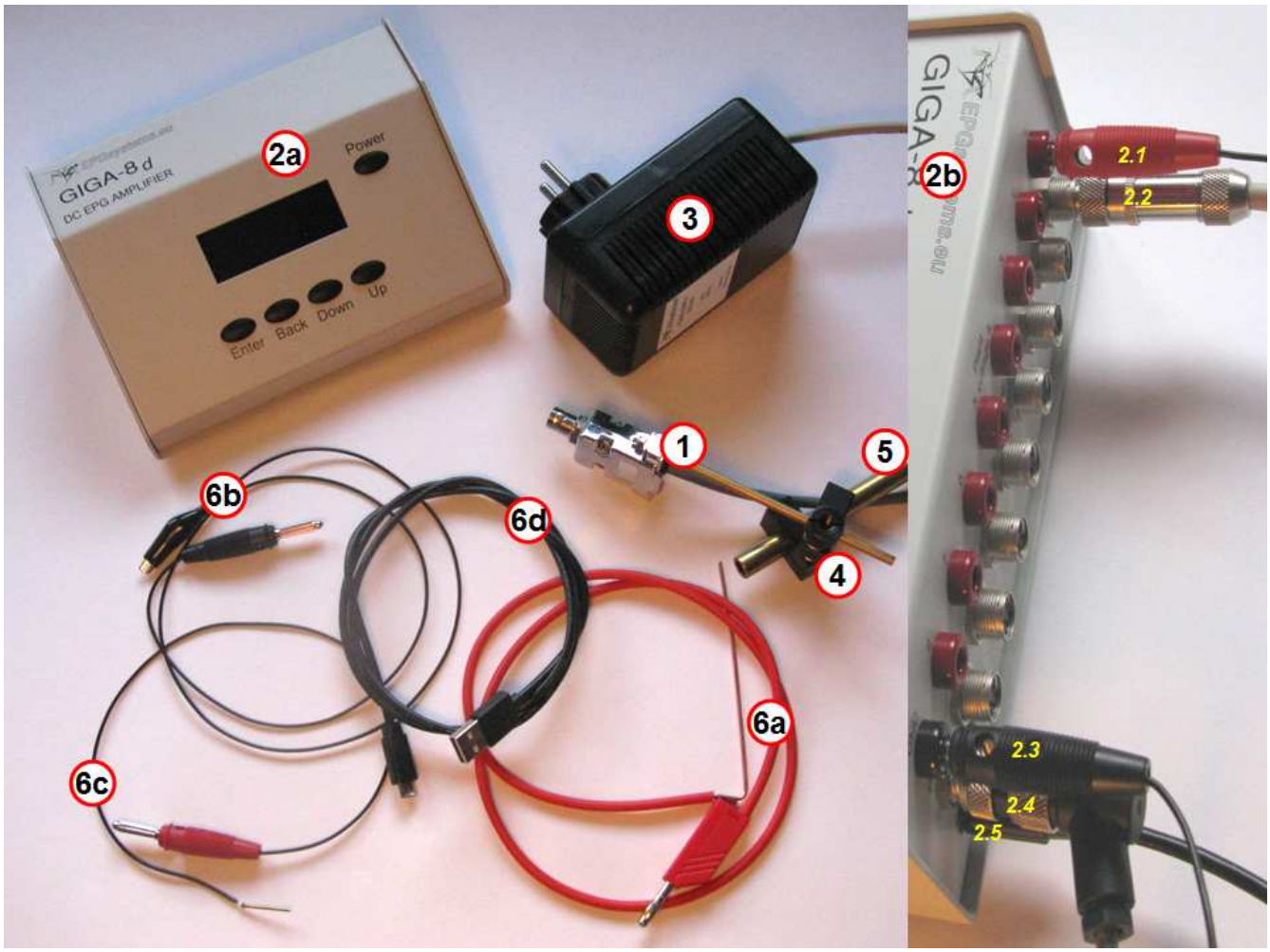
Wire diameter (Ø) depends on the insect (see website information)

3. Giga-8d device and all separate (basic system) parts

Figure 1 (next page). EPG Giga-8d with hardware parts (right side: rear of Giga-8d device)

(1) Probe: primary (pre-)amplifier*	(3) power supply, 220V(110V opt.)AC/±8V DC
(2a) Giga-8d control unit, front view	(4) Swivel clamp* for flexible rod attachment
(2b) Rear view; with cables for:	(5) rod (ø10mm) to mount the probes
2.1 plant voltage*	(6a) cable with plant (soil) electrode *
2.2 probe*	(6b) gnd cable to Faraday cage by alligator clip
2.3 ground (gnd)	(6c) test cable with insect pin (fitting in probe input)
2.4 power	(6d) micro-USB cable
2.5 micro USB	

* one per channel



Power supply voltage

The AC voltage from the mains can be 110 or 220 VAC and the label on the supply should (!) indicate the internally adjusted input voltage. You may switch the device to the other voltage. Unscrew the 4 crews of the power supply cap and open the device. You will see this (Fig. 2):

Figure 2 Power supply interior

Here the slider shows '230' indicating the maximum AC voltage allowed and it is suitable for 220 VAC. When slit to the other position you will see '115' indicating the suitability for 110 VAC. So, you can adjust the input voltage to your appropriate voltage.

WARNING: A 115 V adjusted power supply should **never** be connected to 220 VAC !

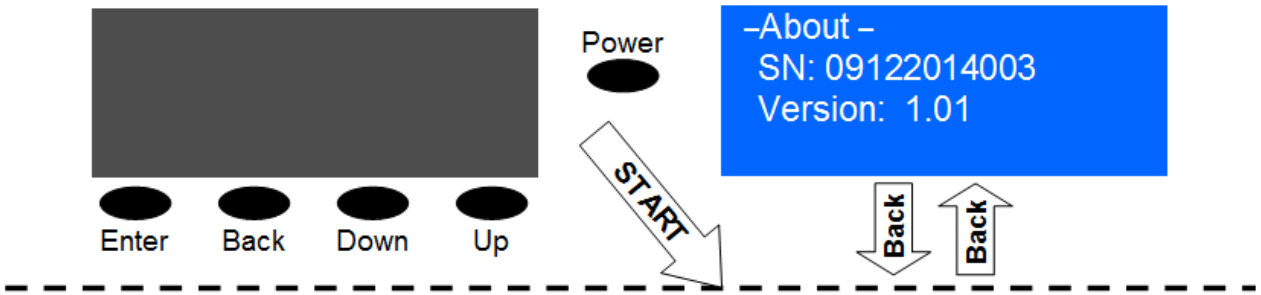
Therefore, if you change the voltage slider always indicated the new voltage on the label with a marker pencil (permanent type) !

4. Digital Giga-8d operation

4.1 Digital Pushbutton Operation. Control panel menus

Exercise this first, no software (***Stylet***$^{+}$) and Giga-8d installation are needed yet.

Start



Operation Displayed control panels are numbered *1* to *8*: description on next page.

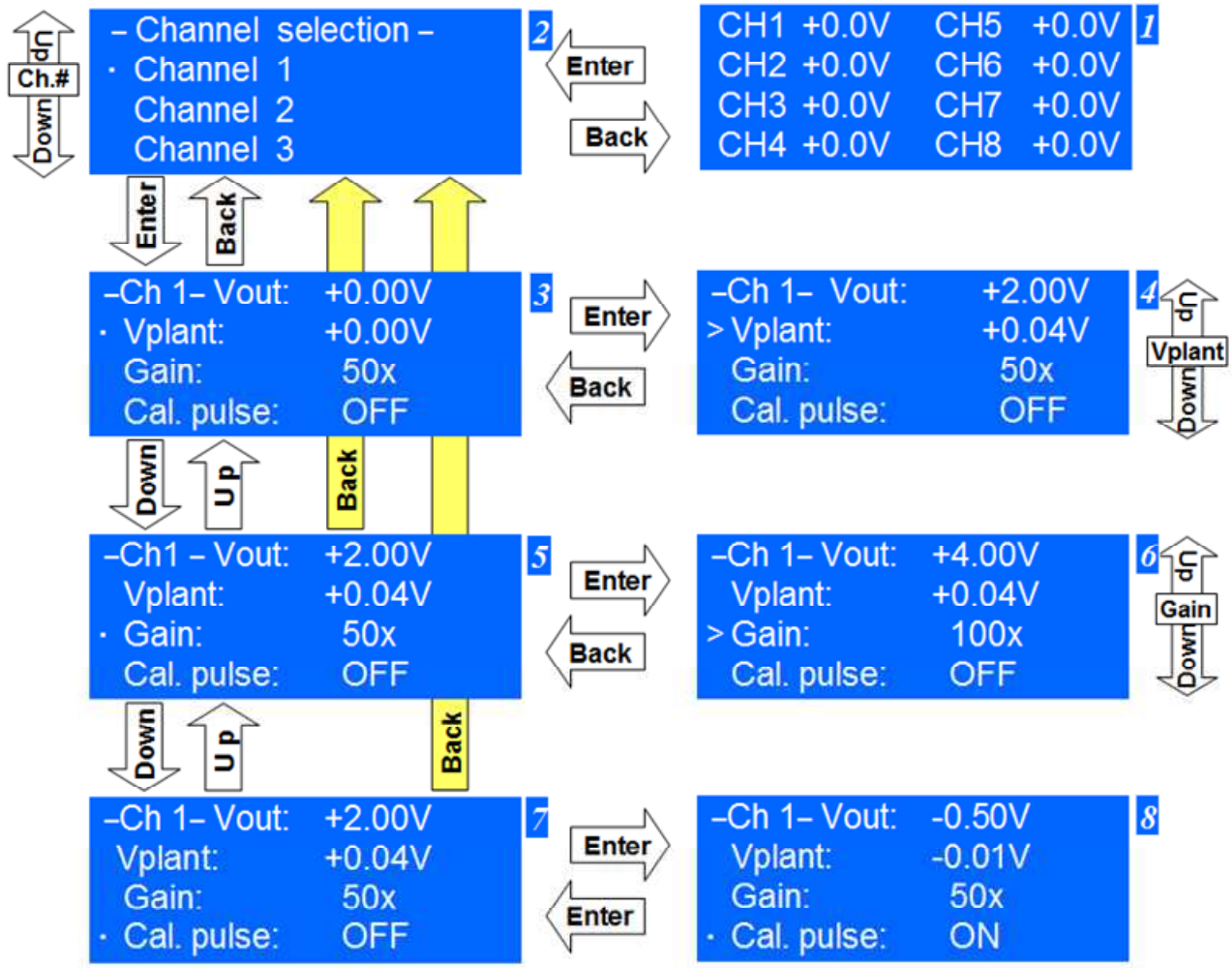


Figure 3 Schematic mode of pushbutton operations with their displayed results.

4.2 Description of Pushbutton functions Enter, Back, Down, UP in Power, Giga-8*d* menu

Start

Power switches the control box on and shows the blue display panel *1* with all 8 channels on 4 lines
shortly shows a panel with text "EPG GIGA8d"

Back shows the - About – panel with serial number and firmware version number

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Operation

Enter will then show panel 2, the Channel selection screen

Down will move you to the one channel number higher

UP will move you to one channel number lower

Enter will move you to adjustment menu of the selected channel#: panel 3 and the ▪ Vplant line

Enter activates Vplant adjustment by showing panel *4* (and ▪ Vplant changes to > Vplant)

UP Increases the Vplant (value shown on same line right). A very small step on a single stroke, increasing steps by keeping UP pressed and stopping when releasing the UP button.

Down As with UP but with opposite sign.

Back brings you back to panel 3 with inactivated ▪ Vplant

Down brings you to panel 5 and the ▪ Gain adjustment line

Enter activates Gain adjustment by showing panel *6* (and ▪ Gain changes to > Gain)

UP Increases the Gain (value shown on same line right). Starting at 50x, increase by very small step on single strokes, and increasing steps by keeping UP pressed; stopping when releasing the UP button: 100x is maximum value.

Down As with UP but in opposite direction.

Back brings you back to panel 5 and the inactivated ▪ Gain line

Down brings you to panel *7*, the Ca. pulse adjustment line ▪ Ca. pulse: OFF

Enter activates the pulse and shows panel *8* with ▪ Ca. pulse: ON. A pulse of about -50mV is then added to VPlant, and becomes visible on line

Enter (again) inactivates the pulse and will show panel *7* again.

UP moves you to the ▪ Gain line (panel 3)

UP moves you to the ▪ Vplant line (panel 5)

Back moves you to the Channel selection panel 2

Back moves you to the 8 channel panel *1*

Power switches the Giga-8d control box off.

5 Installation

5.1 *Stylet*+ software installation

Download ***Stylet***+ setup.exe and the install.pdf from www.EPGsystems.eu, page Downloads, section installation and read the pdf's instructions. Regularly look for newer versions.

5.2 Connecting Giga-8d parts

WARNING

1. ***Do not connect or disconnect probes to the Giga device while switched on!***
2. ***Static electrical charge is a danger for the probe input: Touch both hands to the Faraday cage (GND connected) to discharge yourself before inserting any pin (test cable or insect electrode) into the input of a probe***

First insert and screw (Fig 4, top) the connectors of all probes to the Giga-8d device. Start at channel 1 and work to channel 8. Leave your probes connected to the Giga-8d as indicated in the above *WARNING* in order to avoid damaged probes.

Then insert and screw the power supply connector into its Giga-8d socket; screw only the indicated ring (Fig. 4, bottom) Finally insert the micro-USB connector of the USB cable into the socket below the power socket of the Giga-8*d* and connect the other end of the cable to the computer.

Finally, insert the power supply into the wall socket of the main grid (220/110 VAC) but be sure the label on your power supply matches the correct (maximum) voltage: 230 or 115 VAC but do **not** push the Power button on the Giga-8d yet.

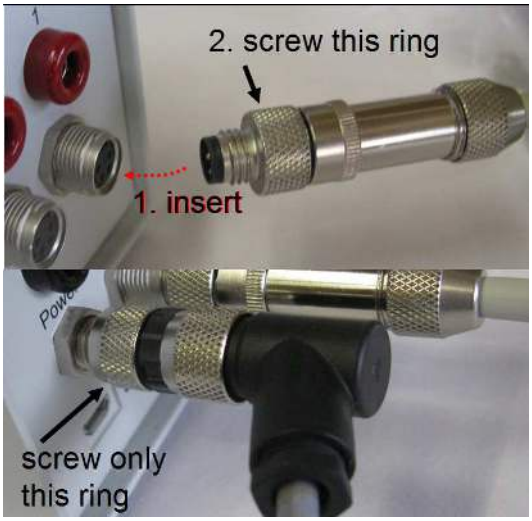


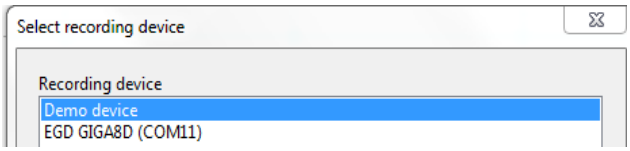
Figure 4
Plugging and screwing connectors

5.3 Giga-8*d* installation

When all part are connected your computer should be on line (internet connected). Now push the Power button on the Giga-8d. Your computer will find the new hardware (AD converter) inside your Giga-8d and will automatically search and install the hardware driver (Windows-7 & -8). There are two things to install, 1) the USB Serial Converter (AD device) and 2) the USB Serial Port (Com port), which may take a few minutes and you will get the message that the device is ready to be used. For Windows-XP the hardware wizard will pop up. Allow searching on the internet to install the AD device and the Com port. Once installed, this procedure not further needed and internet access should be switched off during EPG data acquisition sessions.

Open stylet+d.exe (shortcut) to run a data acquisition session (EPG recording). In the R bottom corner the device EGD GIGA8D (COM16)/Std should be shown (comport # may differ).

5.4 Run a demo recording (see also *Stylet*+ manual)



Before starting a real EPG recording session it is good to run a demo session. With a L mouse click on EGD GIGA8D (COM16)/Std you will get the Select recording device panel. Click on Demo device and OK to get a 4 channel demo screen. After typing a file name (e.g. "test") and some text in the comment 2 space (e.g. "demo") you can click the [START] button and an sine wave will appear running fro L to R and the scrolling left. After the pre-set 0.01h running time the session will finish.

6 Experimental EPG Setup

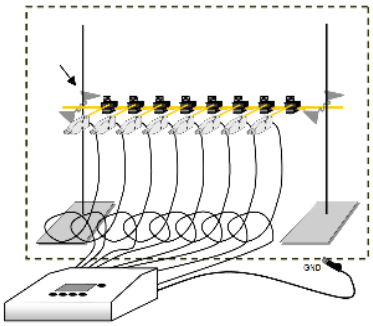


Figure 5
Stands with 8 probes

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 lamps or any other AC powered device inside the cage!
3. Screw the two rods together to make one 80cm rod for all 8 probes and fix the rod, for example (Fig. 5), between two ordinary (chemical) stands with standard clamps (not provided by EPG systems). Leave all probes connected to the Giga-8d device.
4. Mount the probes in the Faraday cage as far to the rear as possible to reduce noise but put the Giga device outside in front, or at the L or R side of the Faraday cage.
5. Connect the USB cable between Giga-8d and your computer.
6. Insert the ground cable plug (black banana; Fig. 1, 6b) into the black GND socket on the Giga-8d use the alligator clip to connect it to the Faraday cage. It should be a blank metal part of the cage.
   It may 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.
7. Wait connecting of the plant electrodes (red bananas) into the red sockets at the rear of the Giga until plants are put in the cage. Plant electrodes should not contact to each other or to any metal part of the Faraday cage at any time while the power is switched on.

The set up is ready for testing now.

Before the first real use, but also at any time later if there is doubt about proper set up or device functioning, it is good to test the equipment according to the following test procedures, using the three channel adjustments: 1) plant voltage, 2) gain, and 3) calibration pulse application (see panel 2). Equipment preparations should be as on previous page. Insect or plant electrodes are not yet required.

Test with open input of probe (no wire connection to the probe)

1. Switch the Giga power on, and wait with illuminated blue display for about half a minute
2. Then start ***Stylet***$^+$ a 1h acquisition session (see manual *Stylet*$^+$). Signals of all 8 channels will be visible in the bottom panel and every channel can selected to be displayed in detail in the top panel, initially this is ch.1 by default.
3. On the computer screen all channels should give a 0 Volt line for all channels and in Giga's panel *1* the voltages next to all CH numbers should be near about 0 volt as well.
   However, noise may be shown as well. If high, close the front of the Faraday cage (or other measures; see next chapter: 5.2 Noise, noise, noise, . . .).
4. Select channel 1 in control panel 2 and check the values of Vplant and Vout. They should still show the value close to 0 Volt
   ***NOTE**: <u>The probe is **damaged** when</u> - with open input only! - Vout is either + or –5.85 V or out of range in the Stylet$^+$ screen display.*
5. Also in the ***Stylet***$^+$ display of ch.1 on the computer screen the signal should be at a 0 Volt level, indicating that the EPG probe is OK. But if the signal is either + or – 5V (out of scale) this indicates that the probe is damaged (!).
6. Select all 8 channels subsequently and check the Giga control panel values as well as the ***Stylet***$^+$ signals on the computer screen.
7. Switch Giga Power off.

Continued test with test cable (Fig. 1 6c) connected to probe input

8. Insert the red banana plug of the test cable into the red plant voltage socket of ch.1 (Fig. 6 (2.1)) on the rear of the Giga and the brass pin carefully into the probe input (BNC connector; Fig. 6). Note WARNING on p. 7.
9. Switch the Giga power on, illuminating the blue display and start a 1 hour ***Stylet***$^{+}$ acquisition session (see ***Stylet***$^{+}$ manual).
10. Often connecting the test cable may show a voltage offset up to 2V, initially (Fig. 7, beginning); differing per channel.
11. In control panel 2 activate the Vplant adjustment (see section 4.1) then (panel 3) increase Vplant with the Up button, then decrease Vplant with Down subsequently, and finally return Vplant to about 1V (Fig. 7).
12. Move Back to panel 2 and Down to Cal. Pulse.
13. Push Enter several times to switch the pulse ON and OFF and observe the pulse response (Fig. 7). The pulse values should become visible in Vplant (decrease by about 0.05V), Vout (step down by about 2.5V) on the control panel.
14. Switch the pulse OFF and move in panel 2 to Gain, activate it, and increase the Gain (Up) from 50x to 100x
15. Go Back and move in to Cal. Pulse and push the pulse.
16. Switch (Enter) the pulse ON and OFF several times again
    The pulse response in the ***Stylet***$^{+}$ display should be twice as large now and the, Vout value should be so too± a decrease by 5V (100x-50mV).
17. Switch the pulse OFF

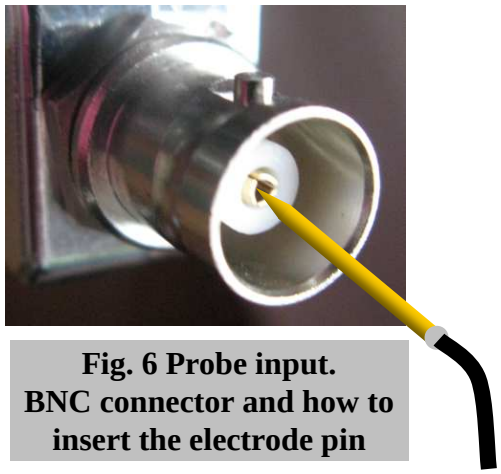


Fig. 6 Probe input. BNC connector and how to insert the electrode pin

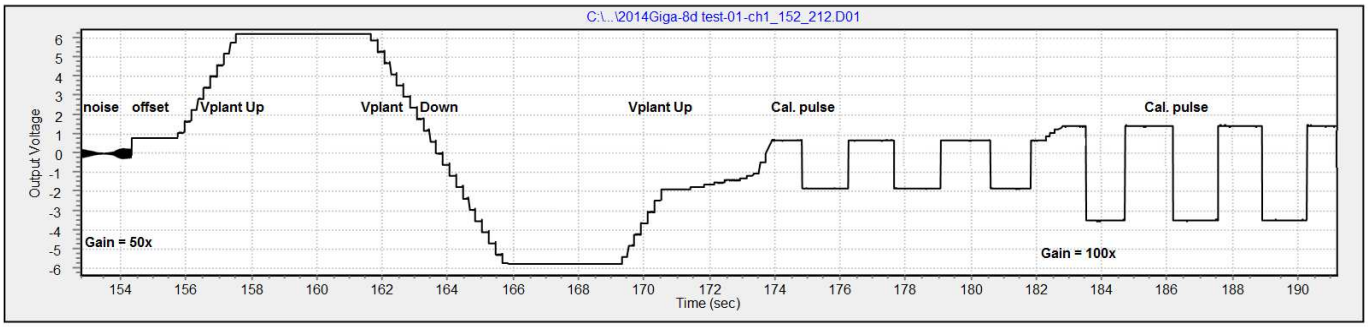


Fig. 7 *Stylet*$^{+}$ panel showing (L to R): Vplant increase and decrease, Cal.pulse 50x, and 100x

18. Repeat points 11-17 for all other channels

8. 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.
Always:

Environmental noise comes from the local electricity grid and depends on the location of and distance to 110 or 220 Volt (AC) power lines and cables to the probe input (Fig. 5). This noise is likely to be reduced it by moving any power cords from the Faraday cage vicinity (the open front, especially) and proper cage shielding and grounding.

Grounding equipment parts

a. Probes should be inside the Faraday cage but put the Giga unit itself outside.
b. Faraday cage and Giga should be connected by the alligator clip of the ground cable. A check by using a multimeter (Ohm range) for low resistance (about 0 Ohm) between the probe supporting rod as well as the outside of the BNC input connector and stands, and between these and the cage and Giga ground. One lead of the meter the multimeter should touch one part and the other lead another part.
c. All Faraday cage faces, including the metal cage bottom and (removable) front face should be connected electrically. The electrical contact between the EPG with the cage should also be tested.
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 or by DC powered led lamps.

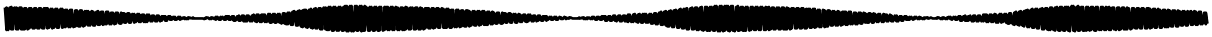
Measuring noise

e. Close the cage and start a 1 h EPG recording session (see ***Stylet***$^{+}$ manual)
f. Open the cage and close it again and observe the noise level in both conditions
   - In the ***Stylet***$^{+}$ top panel starting with ch.1 push the [Inspect] button to look at a detail during recording. You can look closer when pressing [inspection] and then the + for the Y axis 
   - Noise should be less than 0.2 Volt peak-peak
   - If there is a considerable noise left, check for all Giga-ground and Giga-cage connections. If all connections seem OK, then look further at the *system noise* section.

Noise test procedure

To proceed with the following test, carefully insert an insect pin (brass nail) with only a copper extension wire (see insect electrode, p.11) into the EPG probe input but remind WARNING 2, and first discharge your body's static electricity by touching both hands to the cage before the next steps.

1. Continue with or start a new ***Stylet***$^{+}$ recording session.
2. When the cage is (partly) open, you will see some noise in the ***Stylet***$^{+}$ display panel, which will often appear as a sinus wave with a slow regularly increasing / decreasing amplitude over time.



3. Closing the cage will reduce the noise further but if not go to next section's system noise.
4. Now open the cage half way and move one hand into the cage close to the empty insect electrode in the probe's input of the displayed channel but do not touch it without touching the cage or a grounded Giga part.
5. You will see a noise increase when your hand approaches the electrode and a decrease when it is removed.
6. While observing the noise amplitude from your hand close to the electrode, touch with your other hand the Faraday cage. The noise should abruptly be reduced then or at least go back to the level shown in point 2.
7. 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 Giga or other equipment it will not work.

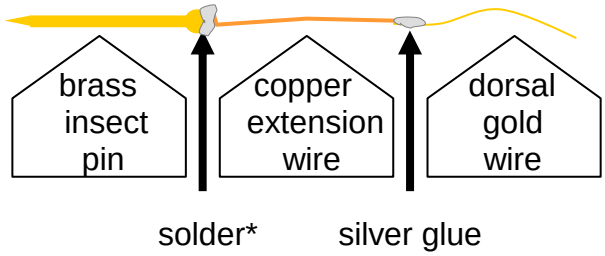
Note: If the front side of the cage can be left open with an acceptable noise the advantage is that the positions of the insects can be inspected and restored easily, if needed. But when the noise level does not allow so, the front screen should be used during recording.

System noise is a persistent noise occurring in all channels equally and all grounding and shielding measures mentioned above will have no effect. This 50Hz noise comes by the power connection leads from the local grid. It enters by the power (brown), return (blue), or safety ground (yellow/green coded) lead directly or inductively. Also, return and safety ground noise may be generated by other 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:

1. Check for loose grounding contacts.
2. Check whether the computer has a 3 pin power plug (i.e. pin 1 and 2 for power and return, pin 3 for the safety ground. You may either disable the pin 3 safety ground to your computer, or – the opposite – replace a 2 pin connection by a 3 pin one, thus enabling safety grounding.
3. You can connect the computer externally to a grounding point. For example, between a water or central heating pipe and the Faraday cage by using alligator clip leads.
4. Alternatively, if any of such grounding exists already, remove grounding leads to water or heating systems. Sometimes 'grounding' makes things 'worse'.
5. 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.
6. Alternatively, move your EPG set up to a grid socket of different group or to a different location.

9 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.



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

Fig. 8. Complete insect electrode

1. Take pieces of copper extension wire (Ø 0.2mm) of about 2 cm long and solder one to each brass insect pin (Fig. 8). Use core wires from a spare piece of a cord, for example (some are supplied). Only use uncoated wires from ordinary 110/220V leads!
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 will soon sink to the bottom of the vial. Thorough shaking before use is always 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). 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.

10 Insect attachment (wiring) and plant access

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.

Wiring

1. If insects have wax particles on their abdomen or thorax should be cleaned first. Use a fine dry brush first and then wet it with water and some detergent.
2. Shake the vial with silver glue thoroughly; if available, use vortex device!
3. Apply a small droplet of silver glue to the insect's abdomen (or thorax) using a thin insect pin or needle. For good electrical contact, let this first droplet dry and then put a second droplet on top of the dried first one.
4. 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 and let the glue dry for a few minutes.
5. Disconnect the vacuum/stop sucking and gently lift the insect with wire and assist the release with a small brush if legs are sucked into the vacuum pipette hole.
6. Insert the electrode into a piece of styropor to store the wired insect hanging in the air on a safe place and avoid climbing in the electrode.
7. Prepare a few more wired insects than strictly needed.

Plant access

8. 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, avoid an insect position that may lead to a hanging without possible access resumption.
9. 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 to get it hanging a few mm above the recording site on the plant and mount the next insect.
10. After all insects are properly positioned but still hanging a few mm above the plant surface, start the computer for data acquisition.
11. Now move all hanging insects onto their recording site. Using this protocol will uncertain that the first probe (stylet penetration) will be recorded for all insects.
12. Wait for each insect separately until probing has started and then adjust the amplifier settings (next section).

11. Proper Amplifier Adjustment

There are 2 adjustments needed: 1) Plant voltage, and 2) Gain. Moreover, there is a calibration pulse button, a -50 mV pulse on top of the plant voltage (see Menu, p. 8).

Proper amplifier adjustment is very important for getting good signals, suitable for proper waveform analysis. Every EPG recording and each insect (channel) should be adjusted individually for an optimal output signal. This adjustment should be made after recording has started and can only be done in a channel during stylet penetration by the insect. The adjustment aims recording the highest resolution and the best signal to noise ratio.

Adjust each channel only in the beginning, say the first 30 minutes, and only re-adjust if a signal may get out of scale due to natural changes. Small deviations from the ideal adjustment need no new adjustments. Only adjust again if the signal goes off scale because it will often cause interpretation difficulties because confusion between natural and operator caused signal changes.

Channel adjustment procedure

1. Start your ***Stylet***$^{+}$ session (***Stylet***$^{+}$ manual); use latest version http://www.epgsystems.eu/downloads
2. Lower all wired insects onto the plant and wait for the first penetration in any channel. Select that channel (see: Digital Giga-8*d* operation, p.5 & 6) Adjust Vplant from any position (e.g. Fig. 9 signal levels 1a, 1b, or 1c) so that it will become in position after 2, i.e. maximally at about 4-5 Volt output voltage in the computer panel or Vout on the Giga-8*d* display. The Gain should be 50x first. When not probing voltage on both devices should be close 0 Volt base on both displays.
3. Now go to calibration (Cal. Pulse) and push the pulse ON for about 1 second and then OFF. Observe the lower level of the pulse response. Readjust Vplant then until the level goes just through the 0 volt reference level (base line level); then the adjustment is perfect.
4. Now go to Gain and adjust it until the signal reaches a level of about 4 Volt again, which will not always be possible but go as close as possible.

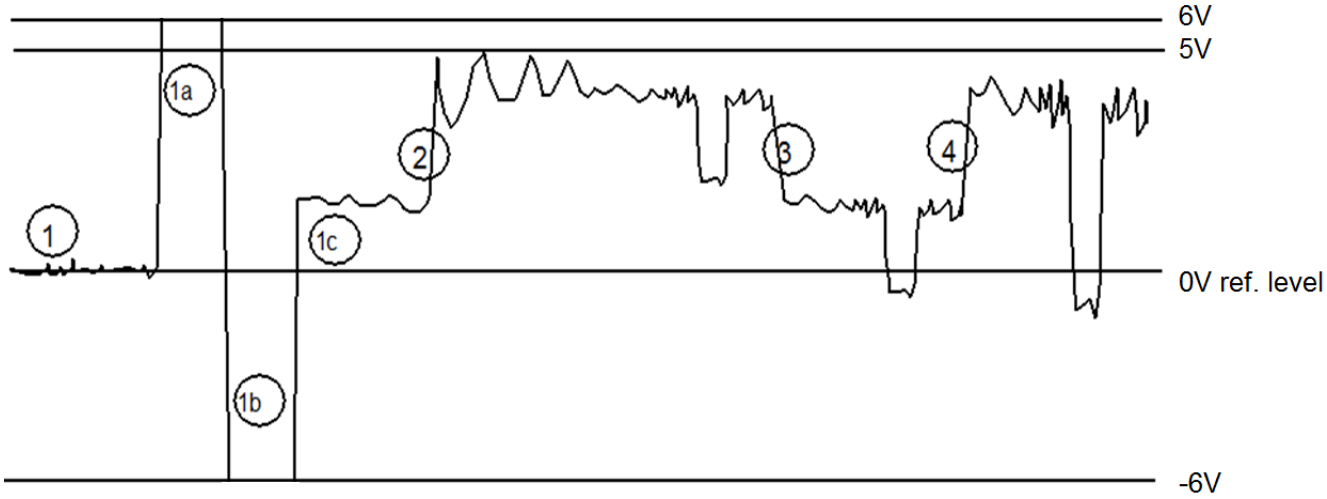


Figure 9. Optimal plant voltage adjustment for EPG recording

This adjustment is optimal for EPG recording, although positive recording is just a convention; the other adjustments aspects provide an optimal resolution, using the AD converter resolution as good as possible. A sub-optimal adjustment may lead to difficulties in waveform identification. Later zooming

in of the Y axis during analysis will often not allow showing details due to amplifying noise (50 or 60 Hz) as well and thus disturb the signal image more than when no amplification is needed due to optimal recording.

In spite of adjustment efforts, a poor may be recorded. This mostly is due to too high insect electrode resistance by a poor silver glue contact. The cal. pulse response during probing will look then like the lower trace in Fig. 10. Although not always preclude signal analysis completely and one may tolerate a somewhat lower signal quality. The only way to avoid a poor silver contact is shaking the glue thoroughly before use! A good contact should show a nice square calibration pulse response in the EPG signal (upper trace). The aphid's own 'square pulses', the potential drops will be disturbed in the same way as the cal. pulse response.

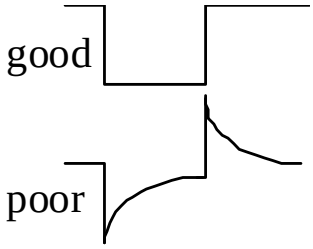


Figure 10.
Cal. pulse responses

12. Previous Giga models

In the preceding text the present Giga8d has been focused especially. However the former Giga-4 and Giga-8 models are not different in their essential properties. The STYLET+ software supports all former models and all models can be used for 2 and 4 channel acquisition. Only the digital operation is in fact new for the Giga-8d and replaces the 2 knobs and 1 button for each channel of the previous Giga models. The plant voltage, calibration pulse, and gain functions are the same and in this manual these functions as indicated by control panel numbers (such as 2) use the corresponding buttons with the same function for each channel, just neglecting the other information on p.5 & 6. Channel selection, plant voltage, gain and calibration pulse are all manually of course but for the tests and acquisition adjustments the functions are exactly the same as in the new model. For old models the installation on new computers there is and old installation guide (old_install_guide.pdf) with the 3 appropriate drivers for the Dataq AD converters used in these devices, either as a separate device, or build-in in the Giga-4 or Giga-8.

Appendix 1

Constructing a Faraday cage

A simple and efficient Faraday cage can be constructed by using metal mosquito netting or (even more simple) bird cage wire (Fig. A1). 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 not 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 screen will needs to be supported by a metal or wooden frame.

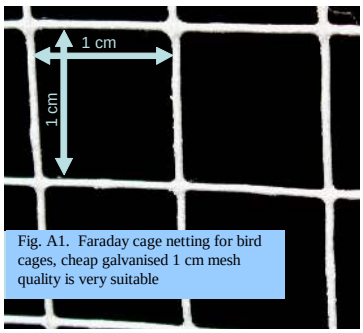


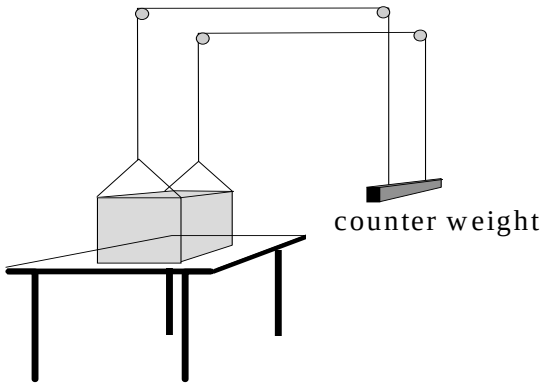
Fig. A1. Faraday cage netting for bird cages, cheap galvanised 1 cm mesh quality is very suitable

Constructing a **hanging cage**, supported by cords to the ceiling and pulleys (wheels) to a counter weight (see figure) needs no supporting construction and 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, insect 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 open front should be closed during recording with hinged screen, or by a separate removable screen, 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 critical when a wooden frame support is used. 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 aluminum 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.

Appendix 2

Self made SILVER GLUE for EPG recording

Preparing your own glue may be an option but remind that drying out is the main risk. So, make small vials for use (2 ml) and close the lid always after use! Save the stock solution at about 5° Celsius in a tight (!) container. With 2 ml you should be able to wire 500 aphids, or more!

INGREDIENTS:

1. Water

2. Paper glue:
   - This is critical.
   - I use transparent glue as sold in 'glue pens' (see 2 different samples here).
   - Rather ordinary stuff, but NOT all transparent glue is suitable!
   - Try first a mall amount on a microscope slide glass and look whether it mixes well with the silver powder
   - If no smooth suspension is achieved, try another glue source.

3. Detergent
   - Triton X100 or any other
   - 1 droplet / 100 ml is more than enough

4. Silver powder (fine grain, about 2 micrometer or less)
   - WARNING: this is expensive and one should consider that a minimum amount is sold by some suppliers
   - Look at the internet for a supplier of silver powder (grains or flakes < 10µm)

PREPARATION:
for about 10 ml glue (use an electronic balance).

1. weigh subsequently in one vial (≥ 20 ml, to be closed tightly)
   - 4 g water
   - 4 g paper glue
   - 4 g silver powder

2. add ingredients should be mixed thoroughly, also each time when new small vials for direct use are filled

* make a stock solution: water + detergent, 100 ml water + 1-2 droplets detergent

---

WARNING: I cannot guarantee this recipe for all samples of glue. I have had some difficulties to find the good glue. The 'Glue pens' seem most appropriate.

Appendix 3

Tip to handle a gold wire spool

to prevent an entangled mass of wire

First use:

1 Prepare a small piece of adhesive tape (ca. 2x2 mm)

2 Use a scalpel to cut the wire at the red spot side of the spool to get a free end

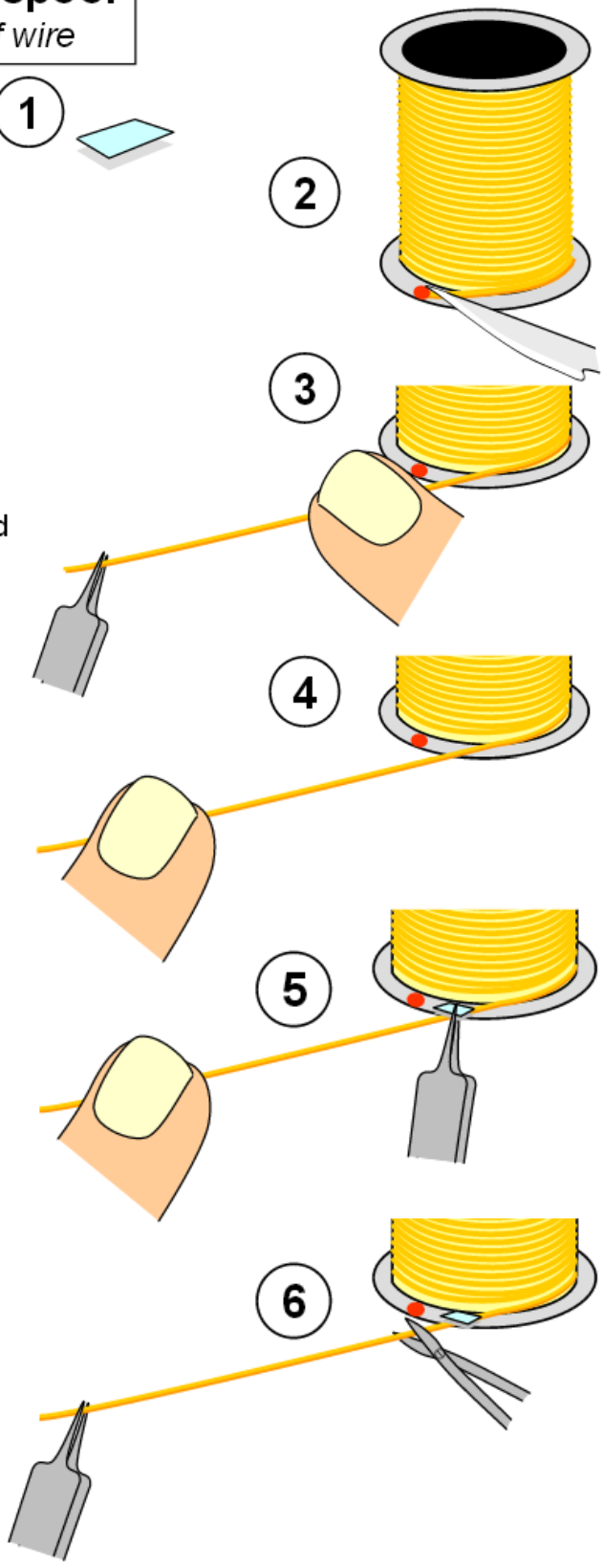
All next use:

3 Take the free end with forceps and unwind the wire a few windings (not more than ca. 10cm) and put your R finger* on the wire while keeping it stretched

4 Then remove the forceps and put your L finger on the end of the wire

5 Keep the wire stretched and fix it on the spool rim with the prepared piece of adhesive tape (see 1)

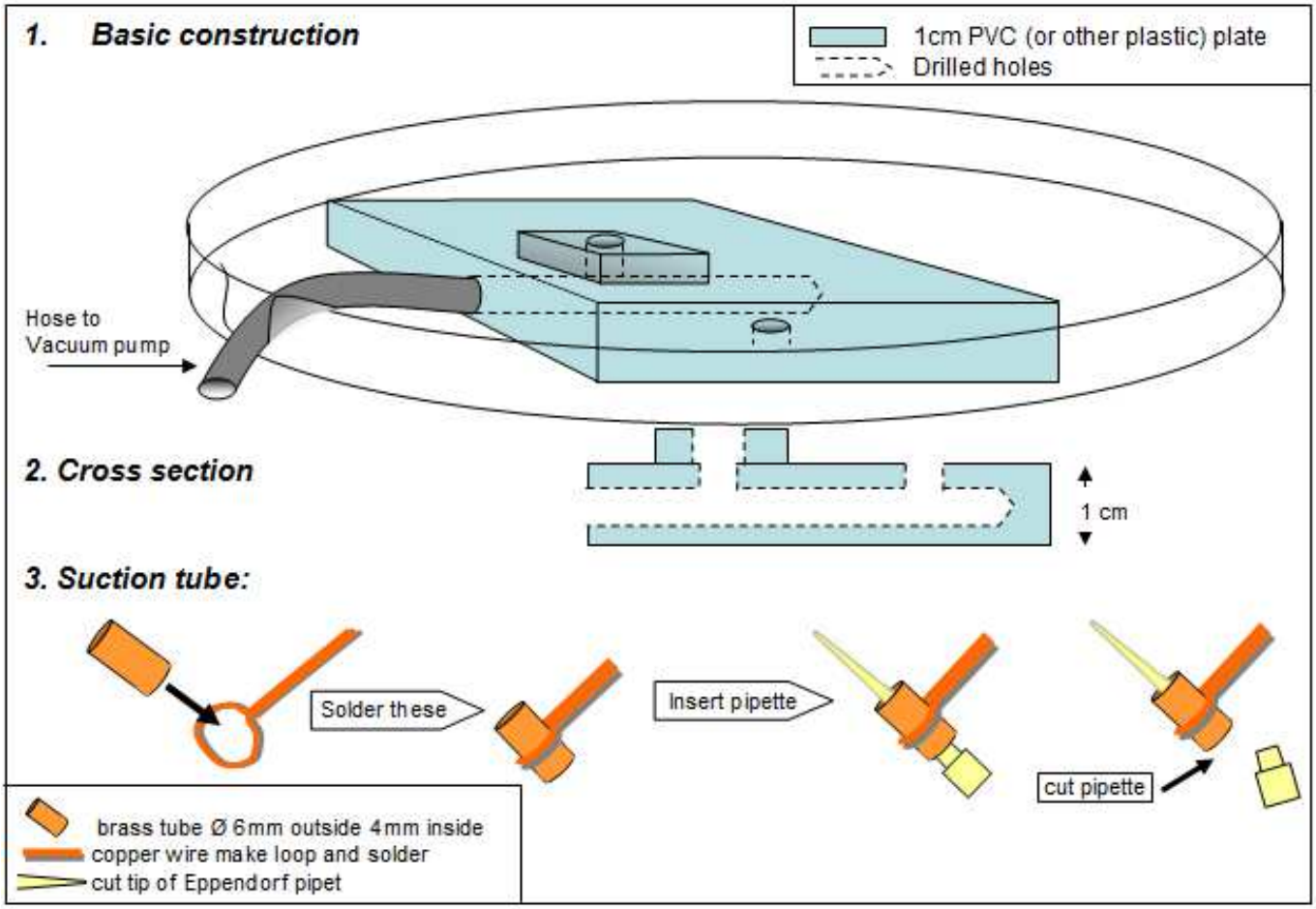
6 Get the free end of the wire with forceps again and cut 10 cm piece by small scissors or a scalpel the to prepare electrodes of shorter length: 1 – 2 cm, depending on how much movement you will allow the insect.

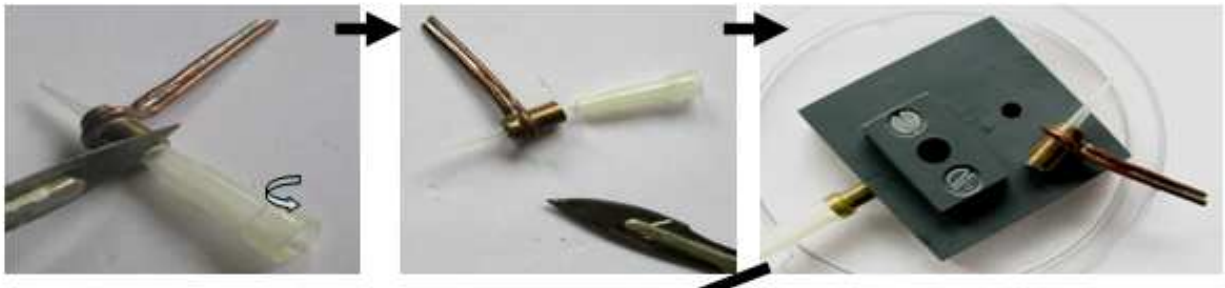


** if left handed, change R and L*

Appendix 4

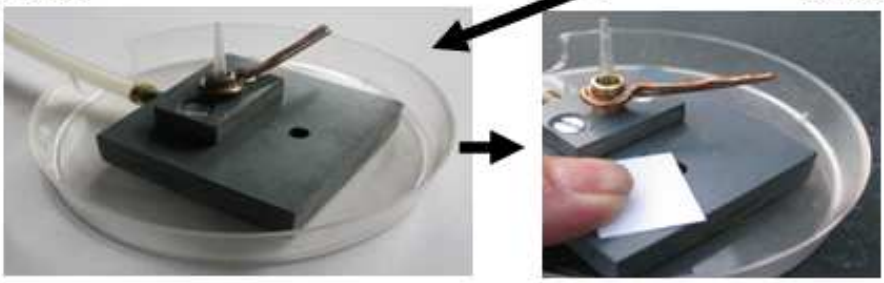
Vacuum suction device construction:





cutting the pipet

mounting the holder with pipet



Petri dish attachment (double sided tape) allows moving under the microscope

conecting to the vacuum (pump or vacuum system)

sliding a piece of paper

Under the stereomicroscope:

1. *mount aphid on the pipet tip (adjust hole size to aphid size)*
2. *adjusting suction force with finger*
3. *use handle to turn it in a covenient position*
4. *first apply silver glue on the insect's dorsum, the insert gold wire*
5. *first apply silver glue on the insect's dorsum*
6. *then insert gold wire into the glue*
7. *let it dry*
8. *Remove paper, then wired insect*

Appendix 5

The emf switch probe (Giga 8d 2017 series *[& *2018]*): Mounting and operating.

*A special 'emf switch' probe (2017 series – to be ordered as 'Special item') has been developed with a 'normal-EPG' to 'emf-EPG mode' switch: see http://www.epgsystems.eu/epg/measuring-systems and [*2018] http://www.epgsystems.eu/phocadownload/Availability.pdf*

Using the emf switch probe

Features The emf (dip)switch is supported on a small board with the switch and a 2 pin socket to be connected to the connector at the end of the smaller cable coming from the probe.
Mounting the small emf switch board See Fig. 11.

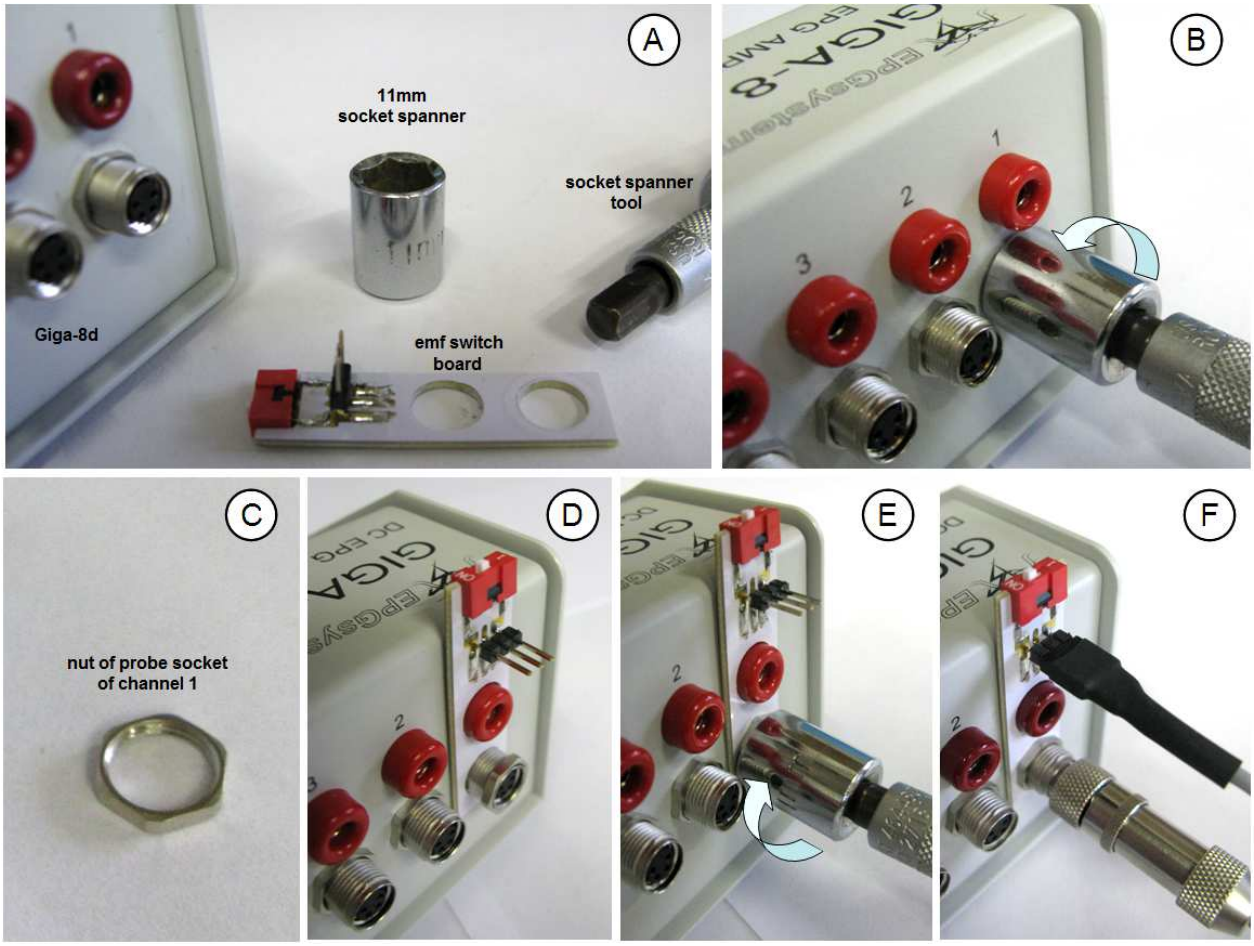


Figure 11. Mounting and connecting the emf switch board. A. The 11mm socket spanner with tool to (B) loosen the nut (C) of the ch.1 probe socket. D. Slip the board over the probe socket and the plant red voltage socket and screw and fasten (E) the nut to fix the switch board. Connect the special probe with emf switch cable and slip the small connector over the 3 pins of the switch contact position is not important. The ON position of the switch is the normal mode (1 GΩ) of the probe the other position is emf-mode (Ri=1.5 TΩ).

Operation The easiest way to begin emf-EPG recording with this type of probe is to start in normal mode; i.e. switch in ON position. As soon as the insect starts probing, the plant voltage should adjusted according the instructions in the Channel adjustment procedure (previous page). Then change the dipswitch to the other position (opposite to ON) and readjust the plant voltage if needed. Mostly, you will not experience a great change in the signal features other than that the potential drops that will be larger. However, during non/probing (np) when the current probing ends a big change will be seen. Then the non probing signal will be shown as a very unstable low frequency period, only ending when

a stylet insertion starts a new probing period (Fig. 12). This instability is due to the very high input resistance in emf mode allowing small voltage sources from the insect without electrical contact to the plant voltage source but also from the amplifier chip (input bias current from the OpAmp) cannot release their charge and affect the amplifier input. As soon as the stylets are inserted the much lower aphid-plant resistance restores the normal signal. The non-probing signal in normal mode at about 0 Volt will not be recorded, but during probing the voltages will be accurate.

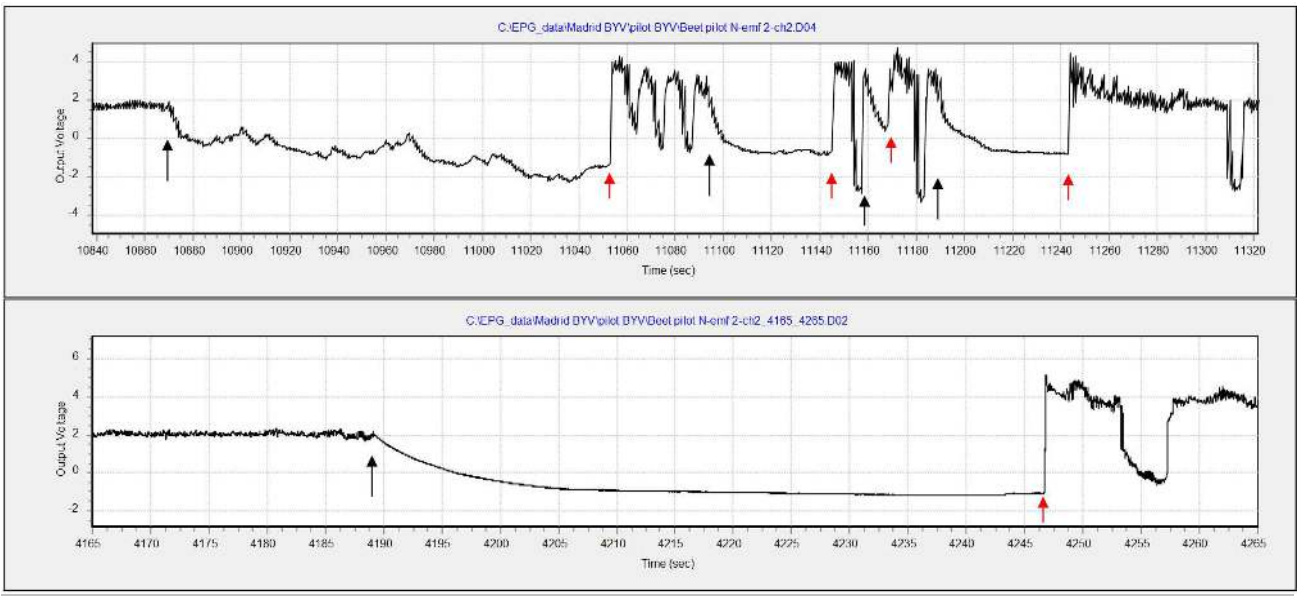


Figure 12. Special EPG signal features during non-probing while in epg-mode. Red arrows start pf new probes; black arrows, start of non-probing, reflecting a high instability. Top, overview; bottom, detail (not from top signal).