MANUAL

Giga-8dd

Firmware Version 2.09

EPG recording system*

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Appendices

* For older Giga types use downloads *manual_Giga-8d.pdf* or *manual_old_Gigas.pdf*.

1. The Giga-8dd EPG system

1.1 Giga-8dd device. The *Giga8-dd* and its precursors (models Giga-8d, Giga-8 and Giga-4) have been developed to record Electrical Penetration Graph (EPG) signals from plant feeding insects with piercing mouthparts, mostly Hemipterans: Heteroptera and Homoptera (Sternorrhyncha: aphids, whiteflies, etc., and Auchenorrhyncha: Delphacids, Cicadellidae etc.) but also thrips, and even spider mites: Blood feeding mosquitoes and ticks and insect parasitic mites have been used as well. Although the main applied interest comes from plant resistance, plant pathogen transmission, and insecticide research, fundamental research interest in plant-insect interactions, especially plant electrophysiology aspects have recently been studied using EPGs as well. Chewing insects – on the other hand – are not suitable for EPG recording. Simultaneous recording from maximally eight insects can be accomplished; either on one or on a separate plant for each individual (see p. 12).
The device name "Giga" refers to the default input resistance of 1 Giga-Ohm (10^9 Ω) of the amplifier for normal EPG recording. Together with the extremely low input bias current (< 30 fA) this is the most important specification (Table 1) of the *EPG recording system* (Tjallingii, 2010).

The 2018 and later *Giga-8dd* models have the following properties (Table 1):

- Power supplied now by the computer USB connector
- Field operation (power supplied by laptop)
- Proper recording adjustments of each individual insect on the same or on a different plant
- Digital function operation among which input resistance switch from 1GΩ to 10TΩ for correct plant potential measurements. *More information, see www.epgsystems.eu/measuring_systems*

Table 1. Technical Giga 8dd specifications

EPG probe	primary amplifier (cable to control unit)
- primary amplification (gain)	50x
- input resistances	default 1 GΩ (10^9Ω), switch to 10 TΩ (10^{13}Ω)
- input bias current	30 fA (10^{-15A})
Giga8dd control unit	
- 2nd stage amplification (gain)	up to 2x (50-100x total gain)
- calibration pulse	-50 mV (circa)
- max Vsupply adjustment	± 0.5 Volt
- max output	± 5.85 Volt (~, Stylet+ analysis display)
- *Stylet*$^+$ scale range limits	± 5 Volt (recording display);
- AD converter	resolution 14 bit; sample rate 100Hz (default)
- Power supply	5V from computer USB socket (converted to ± 5.85 VDC)

1.2 Stylet$^+$ software. This free EPG software has a data acquisition module (Stylet+d.exe) designed for all Giga devices to date, and an EPG signal analysis module (Stylet+a.exe). To keep your system updated, go to https://www.epgsystems.eu/downloads-install-files-manuals/category/3-installation and read the installation guides. In case of doubt, mail to 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).

1.3 System requirements. The Giga-8*dd* 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. For data acquisition only ***if*** there is a ***bootable*** Windows environment installed on your Mac. With a virtual Windows within the Mac OS background only Stylet+ analysis will work. data acquisition will not!

1.4 Installation. System requirements, hard and software installation procedure in ***Manual Stylet+ v1.34, chapter 2***

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, please.

Table 2. Order package contents

Basic EPG system

Item	number
Giga-8*dd* control unit (main box) with build-in AD device	1
micro USB cable Giga8dd-PC	1
EPG probes	8
Swivel clamps for probe mounting	8
Mounting rod 40 cm; Ø 10mm (parts can be screwed to one rod)	2
Cabling	
main Giga-plant cable (75 cm)	1
2 branches intermediate cable (25 cm)	1
4 branches cables with a soil electrodes of each branch	2
ground lead with alligator clip for Faraday cage connection (75 cm)	1
ground test cable with brass connector pin (75 cm)	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

Additionally ordered other items *(optional)*
one or more 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-8dd device and all separate (basic system) parts

1 Probe: primary amplifier*

2a Giga-8*dd* control unit, front/top view

2b Rear view; with sockets for:

2.1 2 black ground* sockets; to plant(s) and Faraday cage

2.2 EPG probe 8x

2.3 micro USB (power and data) 1x

3 Swivel clamp* for flexible rod attachment

4 rod (38 cm, ø10mm) to mount probes, 2x

5a plant/soil electrode, 8x*

5b main plant-soil cable (75cm)*

5c intermediate 2 branch plant/soil cable*

6a ground-Faraday cage cable (alligator clip)

6b test cable with insect pin (fitting in probe input)

7 micro-USB cable

* for use, see Fig. 10, p.14

Figure 1 EPG Giga-8ddwith hardware parts (bottom: rear of Giga-8*dd* device)

4. *Giga-8dd operation*

New 2020 innovation: ***Stylet***[+] control during recording; firmware Version 2.09 needed, see **4.3**

4.1 Digital Pushbutton Operation without running *Stylet*[+] software: Control panel menus

Giga-8dd operation control panels numbered *1* to *10*; function description on next page. To exercise, connect your Giga-8*dd* to your computer USB port (probes connected[*] or not).

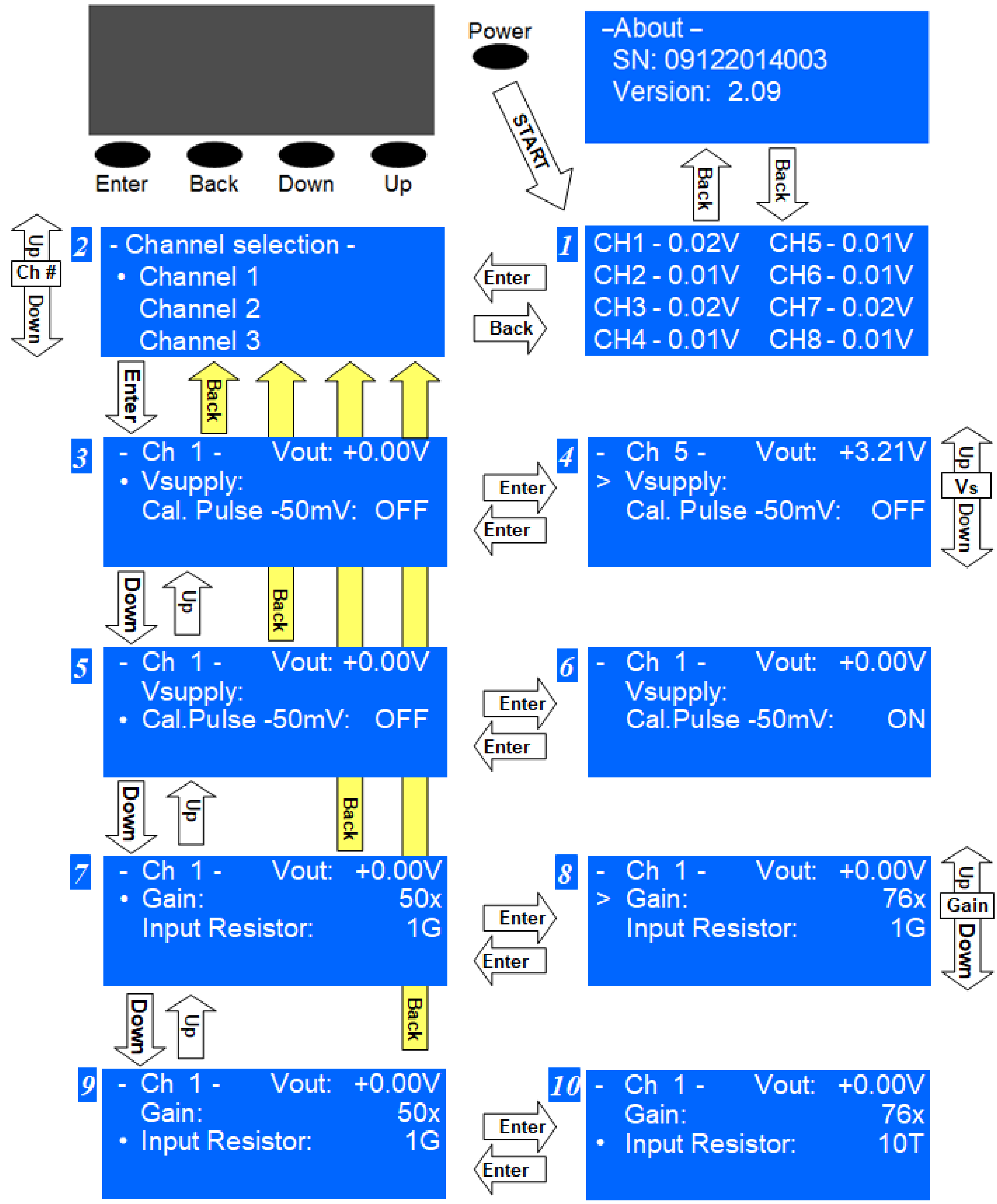


Figure 3 Digital operation panels displayed after button pushes (next page).

[*] *WARNING 1.* page 8

4.2 Pushbutton functions

Start Black panel, be sure the USB cable is inserted and your computer is on (*Stylet*⁺ open or not).

(Power) Push button to switch the Giga on. Blue display panel *1* is shown with all 8 channels on 4 lines with the actual output voltage of each channel

Back shows the - About – panel with serial number (SN) and firmware version; **Back** again to *1*.

Operation functions:

Enter will show panel 2, – Channel selection –

Down will scroll you to one channel higher	*the dot* (•) *before* "Channel"
UP will scroll you to one channel lower	indicates the selected channel

Enter moves you to panel 3, the ▪ Vsupply adjustment line of the selected Ch number

Enter activates voltage supply adjustment: panel 3 , ▪ Vsupply changes to panel 4 , > Vsupply

UP A single stroke increases the voltage supply with a very small, by keeping UP pressed the voltage increases more and stops upon button release

Down As with UP but in opposite direction.

Back or **Enter** brings you back to the inactivated ▪ Vsupply panel 3

Down moves you to panel 5 and the Calibration pulse line: ▪ Cal. Pulse -50mV OFF

Enter activates the pulse and shows panel 6 with ▪ Cal. pulse -50mV ON.
A pulse of about -50mV is then added to the supplied voltage

Enter (again) inactivates the pulse and brings you back to panel 5

Down moves you to panel 7 , the ▪ Gain adjustment line (50x; default minimum value)

Enter activates Gain adjustment: panel 7 , ▪ Gain changes to panel 8 , > Gain

UP Increases the Gain (value shown on same line) from default 50x. Gain increases by small steps upon single strokes, or increasing more by keeping UP pressed and stopping upon button release. 100x is the default maximum value.

Down As with UP but in opposite direction.

Back or **Enter** brings you back to panel 7 and the inactivated ▪ Gain line

Down moves you one line down to • Input Resistor 1G (Ri= 1GΩ; normal mode)

Enter changes to • Input Resistor 10T So from 1GΩ to 10TΩ (=10^{13}Ω; emf-mode)

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

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

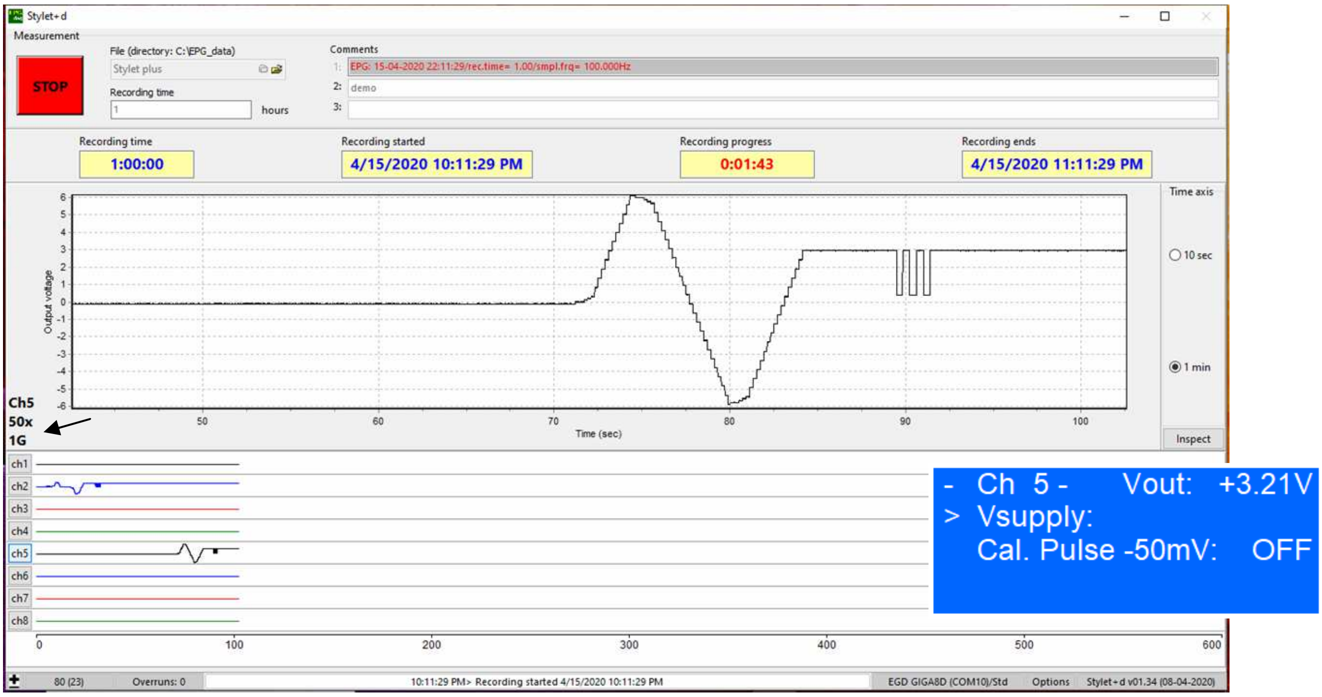
Back in panels 3 5 7 and 9 brings you back the Channel selection panel 2

Back (again) moves you to the 8 channel panel 1

Power switches the Giga-8*dd* off.

4.3 Special *Stylet*+ controlled Giga-8dd operation during data acquisition (*new 2020*)

During EPG recording (data acquisition by running stylet+d.exe) direct actions are often required for optimal channel adjustment (chapter 11). To choose the channel (insect) on basis of what is visible on the screen display of the computer is made available by Stylet+ control of the > Vsupply function of the



Giga-8dd. The screen here shows what happened when ch 2 was selected and then a mouse click was given on the screen pushbutton [ch5] at time 40. The detailed signal of ch 5 is displayed on top (as usual) and now the current gain and input resistor adjustment (50x and 1G) of ch 5 are shown too (arrow). The main thing is that the Giga-8dd digital display (blue panel inset) now jumped to Ch 5 and activated the > Vsupply function enabling immediate Vs adjustment by using the (Up) and (Down) pushbutton on the device, which is much faster than all device pushbutton control steps (Back), (Up)/(Down) for the new channel choice, (Enter), and (Enter) to achieve the same. So this is assumed to make a frequently used sequence of control steps easier.

5. Preparing EPG recording with Stylet+

5.1 Hard and software installation

Download and read first the *Installation_Guide_2018.pdf* from www.EPGsystems.eu, page Downloads section installation. Then implement the instruction to download and run *Stylet setup_ 000000.exe* (*version #*). This will put the AD drivers as well as the Stylet+ desktop shortcuts for its two applications on your hard disk.

Disconnect your computer from internet (stand alone). Connect your computer to the Giga (micro USB cable) and press its power button. As soon as is this is done for the 1st time, the computer will identify the AD device (inside the Giga) and install the drivers in two steps (USB device and COM port). Wait until finished as will become visible in the right lower corner of your screen.

Regularly look for newer *Stylet_setup* versions.

1. Connect the Giga-8*dd* to the USB port of your computer but don't push the Power button. This will automatically start installing the hardware (AD converter) inside the device.

Depending on your Windows version this will show a message "New USB device" (Windows-XP, & -7) and you should wait then for a "ready to use" message, or no messages at all (Windows-10), and just wait for about 30 seconds then,

2. Push the Power button now and wa it for about 30 seconds. If no screen messages are shown continue with 5.1.4.
3. If a message "USB devise not recognised" appears go to 6.
4. Open (run) Stylet+d.exe by clicking on , the desktop shortcut.
5. Inspect now what is displayed in the right bottom area of the window. If all requirements are matched you should see here EGD GIGA8D (COM25)/Std (the COM number may differ).
6. If Demo device is shown here instead, improper USB device recognition and functioning of the Giga-8dd device may be have occurred. The "USB device not recognised" screen message will be shown, and if the correct EGD GIGA8D (COM25)/Std is displayed running Stylet+d results in a huge noise in all channels. This problem is caused when the USB does not provide enough current to support the Giga-8*dd* can device. It can be solved by using a "powered USB HUB"; i.e. a HUB powered by 220/110VAC to be inserted between your computer and the Giga-8dd. You will be able to buy this item in most computer shops (about 15-20 €/US$). Contact https://www.info.epgsystems.eu for support, if needed.

5.2 Run a demo recording

Stylet+d will run in demo mode when no device is connected.

Then: Type any file name in the 'File (directory . . .)' space (e.g. "demo file") and any text should be added in the Comments space 2: (e.g. "any text"), recording time default. Now click on the green [START] button (Fig. 5A). A sine wave will appear running from left to right in the large **Ch1** top panel. Also, in the 8 compresses bottom traces (Fig. 5B) the odd channels will show a sine wave whereas the even channels will show a noise signal. While running, click the [ch2] button at the left and the ch 2 noise signal will be displayed in the detailed top screen, etc. After the default 0.01 hour (= 36s) recording time of 0.01h (36 s) is completed the session will be finished, the [Recording Done] window (Fig. 5C) appears and the file will automatically be converted into 8 separate files, one for each channel. After file conversion is finished click [Close]. For further explanation and instructions see Stylet+ manual.

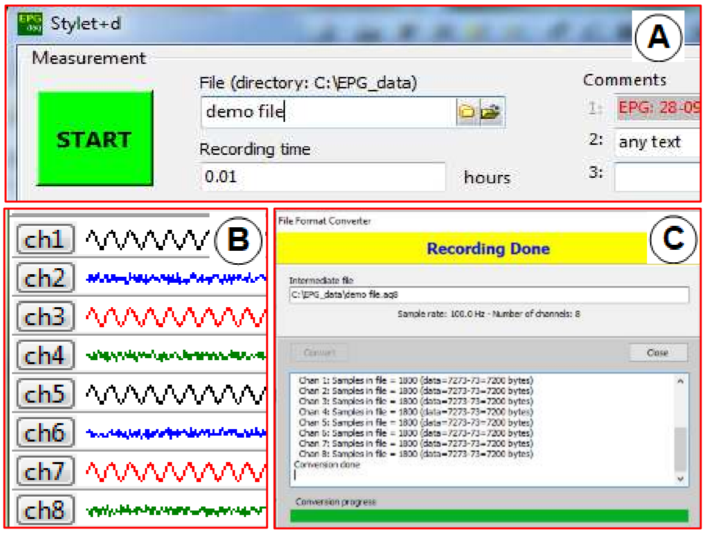


Figure 5. Stylet+ demo recording screen.

5.3 Connecting Giga-8*dd* 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.

3. When inserting a wired insect or the test cable (by hand or forceps) do so only after touching with the (same or other) hand the outside of the probe (metal plated and grounded) or the grounded Faraday cage (check!).

- With power off (no blue lighted Giga display) insert and screw the connector of each of the 8 probes at the rear of the Giga device and leave them connected to the Giga-8dd to avoid damage risk (see Warning).
- Use a properly constructed Faraday cage (see Appendix 1) and place it on a stable table or lab-bench without shock distortions during EPG recording. Do not mount lamps or any other AC powered devices or cables inside the cage!
- Connect the Giga to the Faraday cage using the alligator clip cable and one of the black ground sockets on de Giga rear side. Be sure there is good electrical contact between all cage sides. If a separate removable front side is used, be sure that it makes electrical contact to the other cage faces when put in place.
- The probes have been shipped with 4 on each of the two 38 cm brass mounting rods. Mount either two separate rods or screw them together. To screw the two together, loosen the swivel clamps of one rod while lying this on a table – so that you can turn the rod without turning the probes – and screw this rod into the threaded end of the other rod. Then fasten the 4 clamps again. Fix the rod(s) in the Faraday cage – for example - between two ordinary (chemical) stands (Fig. 6) using standard clamps (not provided by EPG systems).
- Position the stands with probes in the Faraday cage as far to the rear as possible to reduce noise picked up by the probes. Put the Giga device outside the cage, either in front, at the left or right side.
- Prepare Giga-plant connections using the cables mentioned in Table 2 (see also Chpt.9, p. 13/14) .
- Connect the USB cable between Giga and your computer.
- Your set up is ready for testing now.

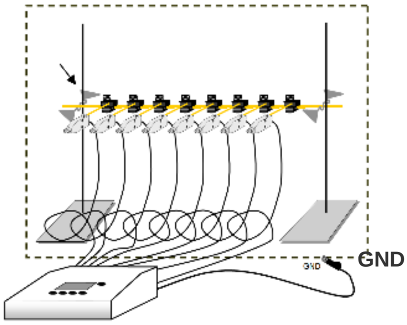


Figure 6
Stands with 8 mounted probes

6. *Equipment tests*

6.1 Test 1 with open input of probe (no wire connection to the probe)

1 Push the Power on the Giga. The display should light blue and show panel *1*
2 Then start Stylet+d, give a file name, adjust Recording time to 1 hours, and type any text (or just one character) in comment required text space 2 (!): see manual *Stylet*$^+$
3 Click [START] and – as with the demo test – signals of all 8 channels will be visible in the bottom panel and the ch1 signal is displayed in detail in the top panel (default at start)
4 All channels should show a 0 Volt line, although some noise will be shown if not in a closed Faraday cage (or other if noise comes from cables: see next chapter 7)
5 In Giga panel *1* the voltages next to all ch numbers should be near 0. Push Enter 2 times to select channel 1 as shown on the Giga panel 3 and check for about the same Vout value again.

NOTE: A ***damaged probe*** - open input only! - will show Vout of + or – 6.14 V. On the Stylet$^+$ computer screen this is + or – out of range. Check this and contact EPG systems for repair.

6 Select all 8 channels subsequently and check the values on the Giga control panel as well as on the computer screen.

Test completed.

6.2 Test 2 with test cable (Fig. 1 6c)

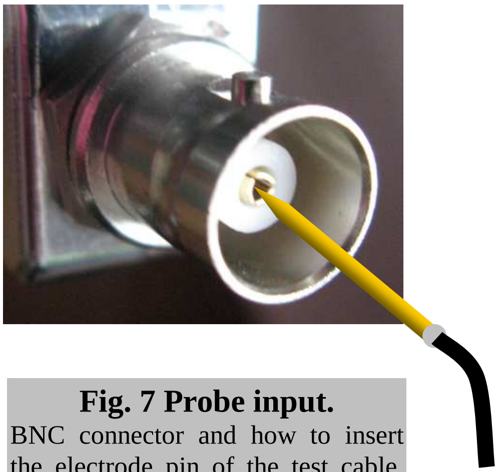


Fig. 7 Probe input. BNC connector and how to insert the electrode pin of the test cable, noise tests (p. 10), or a wired insect.

1. With Giga connected as in Test 1, insert the banana plug of the test cable into one of the black plant/ground sockets (Fig. 1 *2.1*) on the rear of the Giga. Both sockets are interconnected and equal.
2. Carefully (see ***WARNING*** on p. 7.) insert the brass pin at the other end of the test cable into the BNC connector, i.e. the input of the ch1 probe (Fig. 7).
3. Press the power button on the Giga. Then start a new 1 hour EPG recording using Stylet+d acquisition session (see manual *Stylet*$^{+}$) by a click on [START].
4. Often upon connection of the test cable a small voltage offset (Fig. 8) differing somewhat per channel may be shown. This is normal (see section 6.3 below) and will not affect the following test steps.
5. Now the next two steps have an identical effect, thus:
   Either Give a mouse click on the [ch 1] button in the lower part of the *Stylet*$^{+}$ screen (see section 4.3, p.7). The Giga control panel will jump to 4 (of ch 1 now) and the Giga UP and Down pushbuttons are available for control directly.
   Or Push the Enter button to activate · Vsupply (3) to > Vsupply (4) and use the Giga UP and Down pushbuttons.
   Then keep pressing the Up button until the scale maximum on the computer screen (Fig. 8). Then decrease Vsupply with the Down button subsequently until the scale minimum, and finally increase the Vsupply again up to about +1V.
6. Press Enter then Down to · Cal.Pulse -50mV OFF line, panel 5.
7. Push Enter to switch the pulse ON and Enter again to switch it OFF. Repeat this several times and observe the pulse response (Fig. 8) on the computer screen. This should accomplish a negative output voltage step of -2.5V (-2500mV) lower than before the pulse at 50x gain.
8. With Cal.Pulse -50mV OFF press Down one line move to · Gain: 50x, panel 7, press Enter to > GAIN 50x , and increase the Gain (keep Up pressed) from 50x to 100x
   Press Enter, then UP to the Cal. Pulse line and push the Enter button (pulse ON) and again (pulse OFF) several times and watch the computer screen for the two times larger pulse response shown now (5V output voltage, Fig. 8).
9. Push the Back button or click the [ch 2] screen button to repeat all Test 2 steps for channel 2.
10. Do so for all other channels

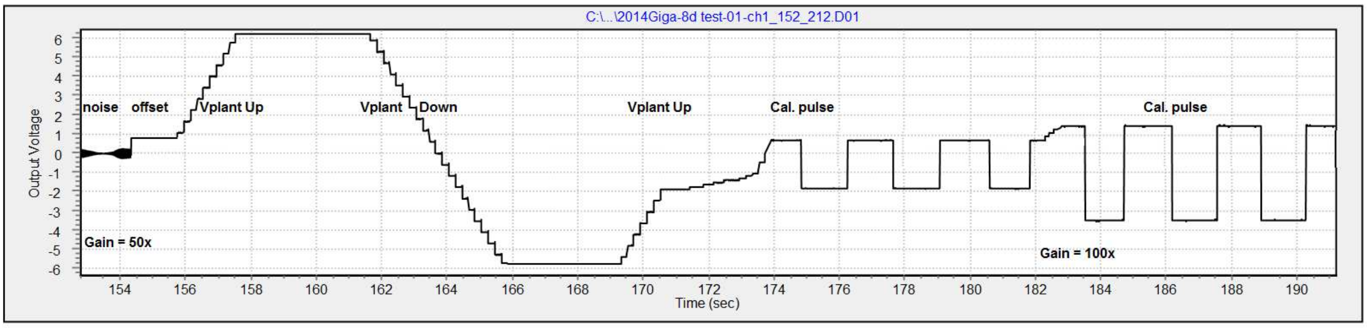


Fig. 8 *Stylet*$^{+}$ screen. From L to R: open input (noise), test cable insertion with offset, Vplant Up and Down decrease, Cal.pulse 50x, then 100x

6.3. Supplied voltage control

The actual value of the supplied voltage (Vsupply in 3) is may deviate from 0 Volt (Fig 8, offset) which is a normal property of the Giga device. In fact this offset is not relevant. The function of the supplied voltage is to compensate for other intrinsic voltages in the primary circuit, the electrode potentials especially, which can be up to ±100 mV (!) and also some stable voltages are generated in the plant. The supplied voltage effect on the intrinsic voltages is shown in Vout as seen in the computer screen and should be adjusted by the user for each channel at start of each new recording: see chapter 11. For a more complete discussion see: *https://www.epgsystems.eu/measuring-systems*

7. Noise, noise, noise, . . .

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

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. 7). When the probe inputs are open (no wires inserted) it will be lower then when an insect electrode is inserted (with or without insect). This noise will be reduced (shielded) by the Faraday cage, which should be ‘grounded’ to one of the Giga ground (black banana plug) sockets. If the front side of the Faraday cage is open, close it to see the effect. It may be reduced by moving any power cords from the Faraday cage vicinity (of an open front, especially) and proper cage shielding and grounding. As an open front is often convenient, move the mounted probes as far as possible to the cage back side.

Equipment part connections

a. Although the probes should be inside the Faraday cage, the Giga unit itself can be outside.
b. Faraday cage and Giga should be connected by using the alligator clip of the ground cable. If the signal is noisy, check – by using a multimeter (Ohm range) – for low resistance (about 0 Ohm) between the cages faces (bottom too), and the Giga (ground socket), the probe’s BNC input connector outer surface (Fig. 7), the mounting rod, chemical stands. Contact one lead of the multimeter should touch one part and the other lead another part (some multimeters beep in case of good contact; ask a technician if you are not familiar).
c. 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 mesh of the Faraday cage or use DC powered led lamps inside.

Noise test procedure

To proceed with the following test, carefully insert an insect pin (brass nail) with only a copper extension wire (empty insect electrode, p.11) into the EPG probe input but remind WARNING 2 and 3, thus first discharge your body’s static electricity by touching both hands to the cage before the next steps.

1. Start a ***Stylet***+ recording session.
2. When the cage is (partly) open, you may 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 ‘system noise’ below.

4. Now open the cage half way. Move one hand without touching this or your other hand into the cage close to the empty insect electrode in the probe's input (of the displayed channel) but do not touch it.
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, now 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 (electrical connection of setup parts). The Faraday cage is crucial but if the cage is not properly connected to the Giga and the other equipment parts 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 placed back during recording.

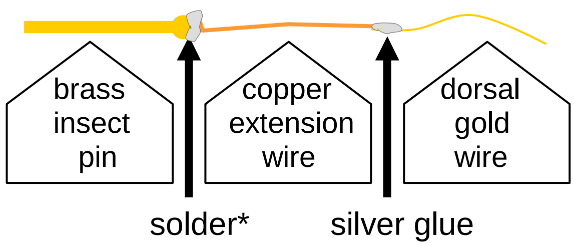
System noise is a persistent noise occurring in all channels equally and all grounding and shielding measures mentioned above will have no effect. This 50Hz noise comes – directly or inductively – by the power (mostly brown or black), return (mostly blue), or safety ground (mostly green-yellow) leads from the local grid. This noise may be generated by other electrical 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 such 'grounding' makes noise '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 and is very nasty. 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, plug in your computer to a grid socket of different group or try a different location in the building.

8. *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. 9). Use core wires from a spare piece of a cord, for example (some are supplied). Only use uncoated wires from ordinary 110/220V leads!



Note: apply soldering fluid (flux) before soldering !

Figure 9. Complete insect electrode

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 2cm long for aphids; Cicadellids may need about 5 cm, whereas whiteflies need 0.5 to 1 cm and 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.
   - Insert the copper extension wire - soldered to the brass insect pin - for about 2-3 mm into the silver glue present in open lid of the vial.
   - Move the glue containing extension wire to the loose end of the gold wire and make contact with some overlap.
   - Let the glue dry for about one minute.
   - Cut a 2 cm piece from of the longer gold wire and pin the completed electrode in a piece of styropor.
   - Take the next insect pin + extension wire to complete another electrode from the same gold wire, etc.

<u>Note on silver glue</u>: *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. <u>Close the vial soon after use!</u> Also, make/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 composition. Instructions about silver glue and gold wire in Appendix 2.*
One vial of 2ml should be enough for hundreds of insect attachments. Store not used vials cool. Decay by micro-organisms is at risk.

9. Arranging Plant and Insects and their connections to the Giga

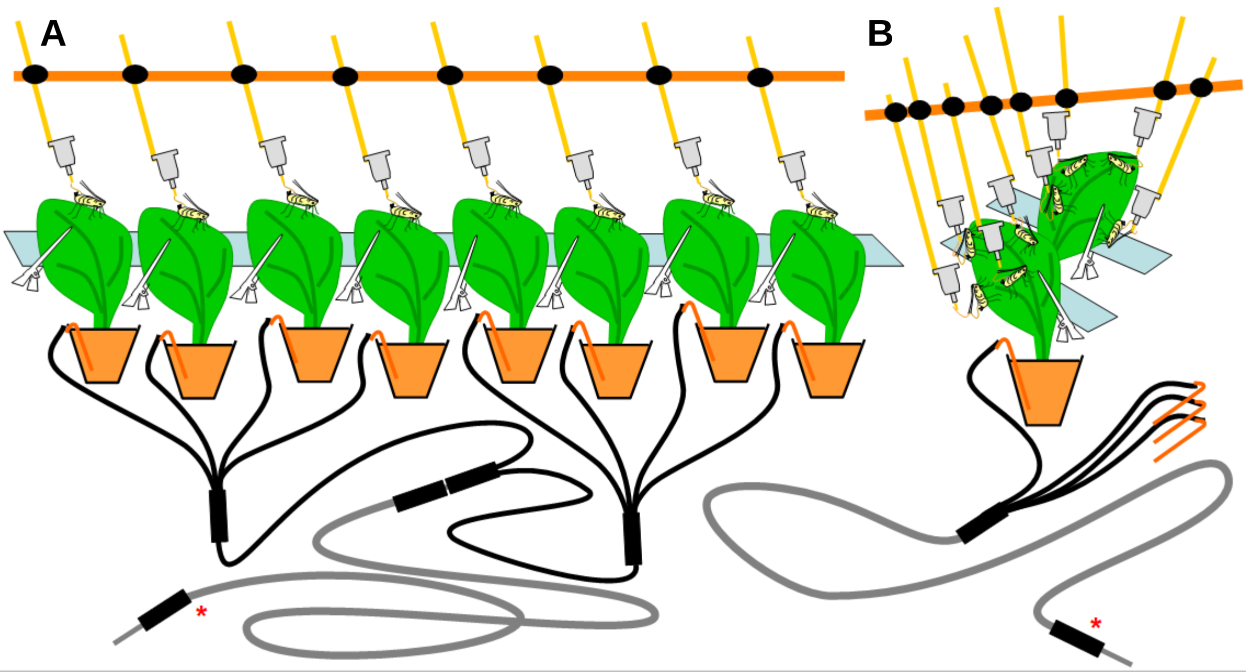


Figure 10. Connection cables and arrangements of plants, insects, and electrodes.
Leaves fixed with hair clips to a plastic strip (blue) behind. Main cable* with **A**, 8 separate insect-plants; **B**, one plant (the 2 branch intermediate cable and 3 soil electrodes not unused) with up to 8 insects on one plant.

Plants and insects can be arranged in different manners: from 8 separate insect-plant sets (Fig. 10A) to 1 plant with 8 insects (Fig. 10B), and all combinations in between. Moreover not all 8 channels need to be used: limiting the number to using 2 or 4 channels can be set in Stylet+ (see that manual). In contrast to previous Giga-8d models the Giga-8*dd* has only 2 identical black (plant/ground Plant) ground sockets on its rear side. Channel adjustments (chapter 11) are made on the insect side now, no longer on the plant side as in the past (Appendix 5). Thus all plants are sharing the same ground as the Faraday cage and the computer (USB connector). The advantage is that plants may touch the Faraday cage and plants can be put on the cage bottom (and make electrical contact) without any problem. This new feature also allows **application outside in the field** with a laptop computer, although this will need mounting the probes and rod or tripod construction, which also fixes the plant part to prevent disturbance by wind. Outside one should care about low enough electrical conductivity. Moisten the soil near the recording plant, or use a screw in the bark of a tree or shrub to connect to the plant/ground.

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.

Insect 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 by e.g. a hair clip to a mounted strip (Perspex or so). Also, avoid an insect position that may lead to a hanging without access resumption possibility.
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 any insect (channel) that has started probing and adjust its amplifier properly (next section).

11. Proper Amplifier Adjustment. ***ESSENTIAL FOR ALL EPG RECORDING!***

There are 2 adjustments needed: 1) Supplied voltage, and 2) Gain. Moreover, there is a calibration pulse button, a -50 mV pulse on top of the supplied 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 (electrode potential changes and insect resistance increase). 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.

Optimal channel adjustment procedure (to perform for every new recording session!)

1. First start the ***Stylet***$^+$ recording session on your computer.
   Then lower all wired insects onto the plant - in as short a time as possible - and wait for the first penetration in any channel. Select that channel by a mouse click on the [Ch#] screen button (see: ***Stylet***$^+$ controlled Giga-8dd operation, p.7) of the computer. The voltage level of that channel may be at level 1, 1a, 1a, 1b, 1c, or 1d.
2. With gain at 50x first (default) adjust Vsupply from any of these initially displayed voltages to a signal level 2; the maximal signal peaks going up to about 5 Volt on the computer screen.
3. Now go to calibration pulse (display 5 line Cal. Pulse-50mV: OFF) and push (Enter) the pulse ON for about 1 second and then OFF. Observe the lower level of the pulse response.
4. Readjust supply now until the level goes just through the 0 Volt level: the supply voltage adjustment is now perfect
5. Then go to Gain and adjust the signal level to about 4V, the high peaks of the signal reaching 5 V. The adjustment of this channel is completed. Go to the next channel that shows a penetration until all channels are done.

This adjustments procedure provides an optimal resolution, using the AD converter resolution as good as possible and a good balance between R- and emf-components (see *https://www.epgsystems.eu/measuring-systems*). Poor adjustments will lead to difficulties in waveform identification. Later digital Y axis zooming during analysis may often not allow showing details due to amplifying noise (50 or 60 Hz) as well as resolution limits. Optimal recording adjustment is very important and should be repeated in every new recording and in each channel again.

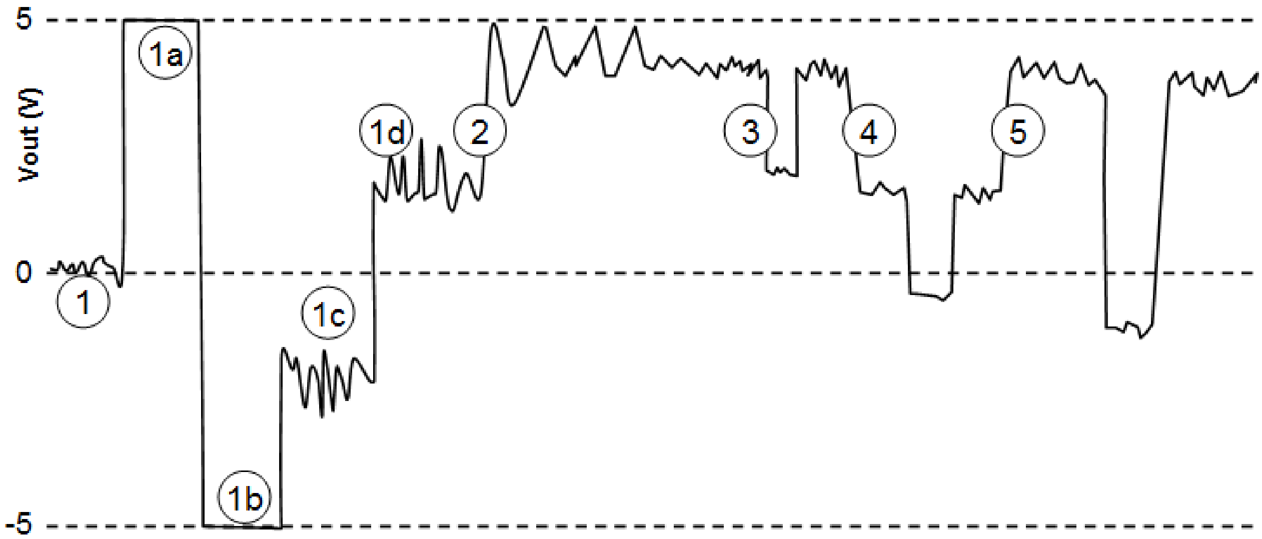


Figure 11. Optimal plant voltage adjustment for EPG recording.
At start of a stylet penetration a possible signal voltage level may be 1, 1a, 1b, 1c or 1d. At Vsupply 2 adjustment to maximal peak voltage up to about 5 Volt (Vout); at 3 a -50 mV calibration pulse is given; at 4 Vsupply adjustment to a level allowing the calibration pulse to go trough the 0 Volt reference level; at 5 increasing the gain until the maximal peak voltage of about 4-5 Volt again: the calibration pulse remains passing the 0 Volt level then.

In spite of appropriate adjustment efforts, a poor signal quality may be recorded due to a poor silver glue contact, which causes a high insect electrode resistance and allows fast signal components like the edges of the calibration pulse or the edges of potential drops (intracellular punctures in a number of Homopterans). The edges then show an 'overshoot' during will as in the lower trace of Fig. 12. Although not always preclude signal analysis completely and one may often tolerate a somewhat lower signal quality. The only way to avoid a poor wire contact is shaking the glue thoroughly before use to get enough silver in the applied glue droplet. A good contact should show a nice square calibration pulse response in the EPG signal (upper trace).

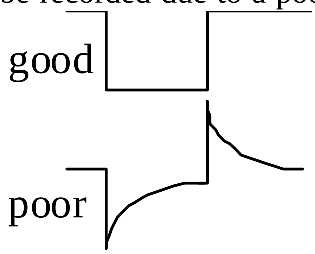


Figure 12. Calibration pulse

12. Electromotive force (emf) mode (Input resistor = 1G /10TΩ; panel 9 / *10*)

The normal input resistance of the probe amplifier is 1GΩ (normal mode) and this function allows an input resistor change from one value to the other. With the 10TΩ input resistor no insect resistance changes will bias the recorded output voltage value. Thus correct voltage values changes on top of the voltage level of the signal can be measured now. The 10TΩ input option (emf-mode recording) is especially useful in plant electrophysiology related studies. The wired insect can be used now as a 'self penetrating micro-electrode' in studying membrane potentials of plant cells. Of course the emf-components in the EPG signal will still 'interfere' with the measured plant potentials but during long lasting stable voltage levels of the emf-components the plant potentials can be measured quite accurate. For example, during the phloem ingestion waveform E2 in aphids the membrane potential of a sieve element can be measured very well and its fluctuations due to plant damage or other plant disturbances have been measured, indeed. EPG recording in emf-mode therefore, is a nice new opportunity. One can remotely operate the switch before or during an EPG recording from normal mode to emf-mode and back without disturbing the insect. It is wise to insert some 'marks' in the signal in order to recognize later when mode switches occurred. An easy way to do so is to give – for example – 3 calibration pulses shortly after a switch to normal mode, and 5 pulses after a switch to emf-mode.

Routine recordings should use the 1GΩ input normal-mode recording. The insect-plant resistance fluctuates from infinite (non-probing) to a minimum level that appears to be specific for each insect group and is presumably mainly determined by the diameter of the stylet canals. For aphids this minimum resistance is about 1GΩ and for Auchenorryncha and small Heteropterans about 10-50 MΩ. During waveforms with a strong R-component (actual fluctuations are mostly not known so far) the R/emf balance is important too (see p. 15: *Proper Amplifier Adjustment)*. It has been argued that the input resistance should match the minimum insect resistance – for Auchenorrhycha with a lower minimum insect resistance, especially – but this would imply a higher electrical current trough the insect. For the time being that seems not a good option. As long as there are no indications that waveform identification will become difficult, there is no reason to do so. More research is needed to study insect and these waveform resistance aspects. Recent suggestions to match minimum insect resistance with amplifier input resistance are rather speculative (see *https://www.epgsystems.eu/measuring-systems* for a more detailed discussion).

13. Calibration and electrical resistance of the insect

Pushing the calibration pulse button (panel 5 and 6) is about -50mV. This means that when used during a recording the pulse response is proportionally reflecting all other signal voltage values as well. However this is only be true if the insect resistance (in fact the total resistance of insect, plant and electrode interfaces) would not change. One often observes that at the leading edge of the calibration pulse the response difference is not exactly the same as at the end edge of the pulse. Nevertheless, the pulse responses give some indication and can be used to calculate the insect resistance too (see *https://www.epgsystems.eu/measuring-systems*).

14. Previous Giga models

In the preceding text the present Giga8dd has been focused especially. However the former Giga-4 and Giga-8 models are not different in their working principles. ***Stylet**+* software supports all former models and all models can be used for 2 and 4 channel acquisition. Only the digital operation and the differential preamplifier (probe) are in fact new for the Giga-8dd. The installation and user manuals of older types can be downloaded from *https://www.epgsystems.eu/downloads-install-files-manuals*.

---oo---

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. 1). 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.

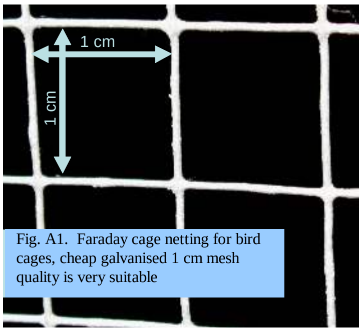


Fig. A1. Faraday cage netting for bird cages, cheap galvanised 1 cm mesh quality is very suitable

Figure 1

Constructing a **hanging cage**, supported by cords to the ceiling and pulleys (wheels) to a counter weight (Fig. 2) 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 s eparate 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.

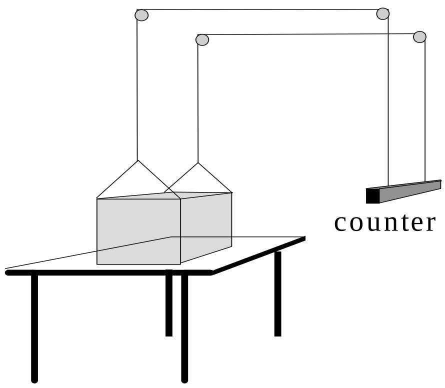


Figure 2

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. Ground the microscope too.

Old Gigas*: Never** let the plants or soil make electrical contact to the bottom during EPG recording. Always place the pots on an insulating dish. For the new ***Giga-8dd no such precautions are needed.

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

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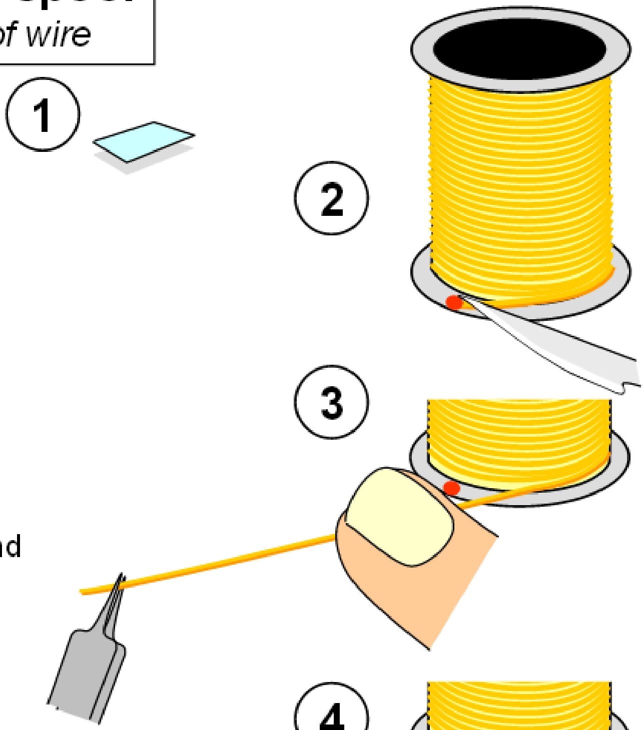
WARNING: I cannot guarantee this recipe for all samples of glue. I have had some difficulties to find the good glue. The 'Glue pens' seem most appropriate.

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

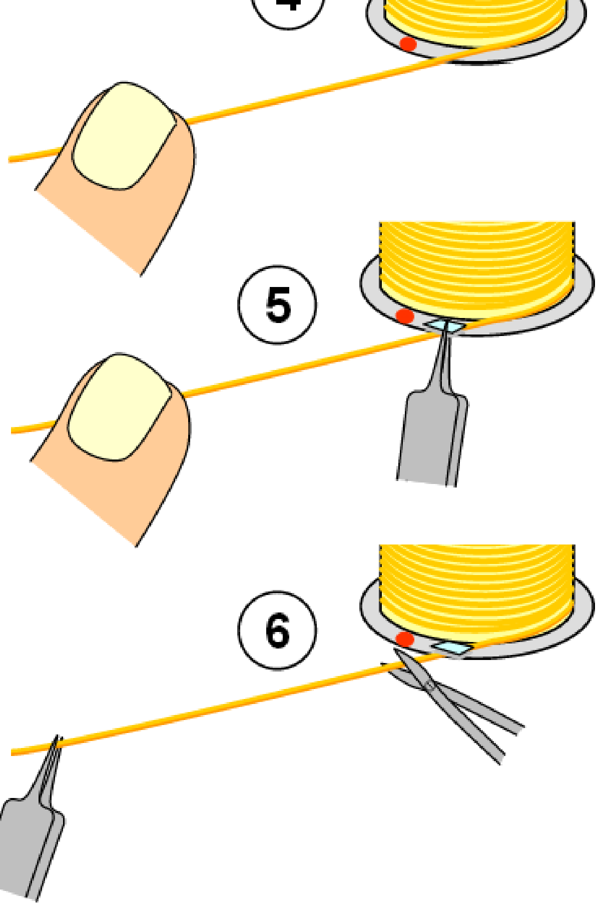
All next use:

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)

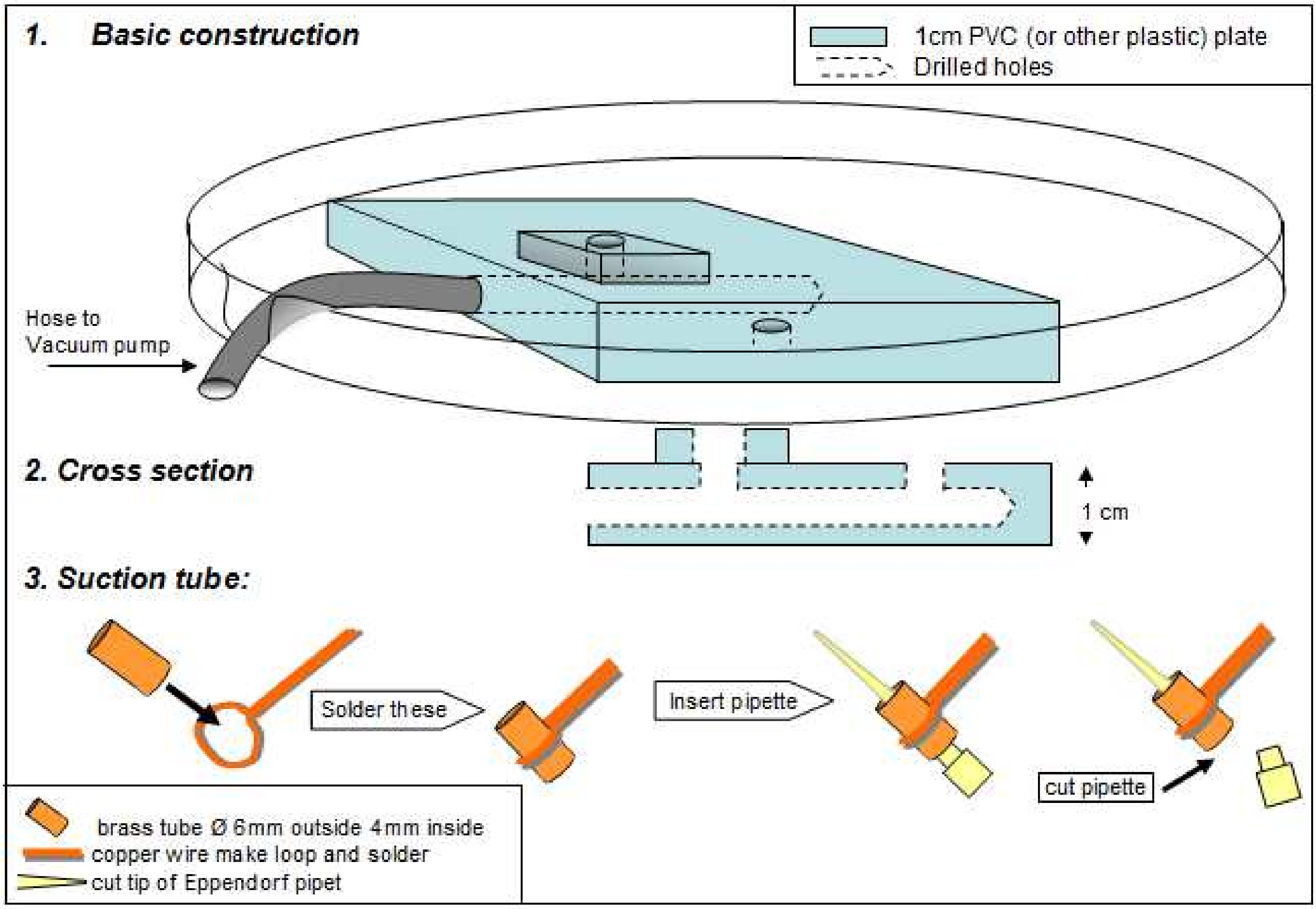
6 Get the free end of the wire with forceps again and cut 10 cm piece by small scissors or a scalpel the to prepare electrodes of shorter length: 1 – 2 cm, depending on how much movement you will allow the insect.



** if left handed, change R and L*

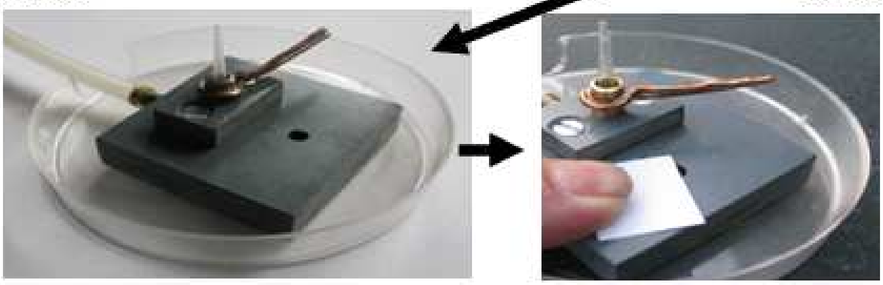
Appendix 4

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

Electrical circuits of EPG probes

So far the usual probe circuit was single ended (Fig. 13A): the signal input at + of the amplifier (centre triangle), i.e. the measuring point of the primary circuit: ground – plant voltage – plant/insect – 1GΩ - ground. With a –/+ 0.5V voltage source supplied to the plant. The advantage is the simplicity of the circuit and lab conditions it is adequate. The drawback is that it cannot be used in the field: the planet earth voltage cannot be controlled by the supplied voltage without system grounding problems. Also, with more than one insect on the same plant the supplied voltage/gain adjustments cannot be adjusted optimally for each insects: (see section 12).

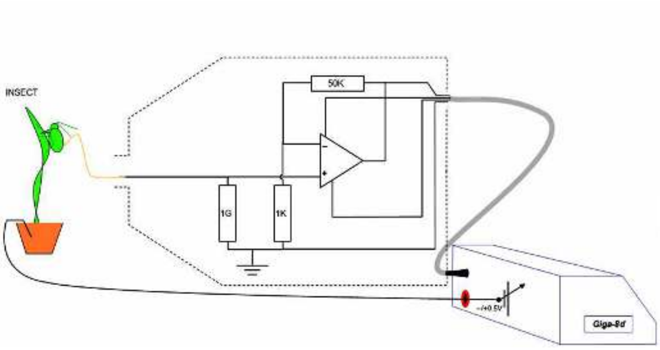


Figure 13A. Single ended probe

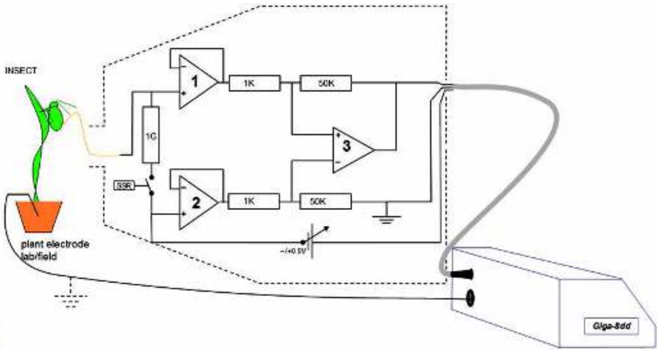


Figure 13B. Differential probe circuit

In the new Giga-8*dd* the probe has been changed to differential circuit (Fig. 13 B.) in which the plant is directly connected to ground (earth in the field) and the supplied voltage is now supplied to the former 'grounded' side of the input resistor, thus in the circuit the connection sequence is: ground – soil/plant/insect – input resistor – supplied voltage – ground. Now there are in fact two measuring points, one is the + input of amplifier 1, and the other is the + input of amplifier 2 (Fig. 13B). Both voltages are subtracted by the + and – input of amplifier 3, which then results in exactly the same signal voltage as in the single ended circuit. In the single ended circuit the (by convention) positive voltage at the measuring point is compared with the ground (0V) voltage. In the differential circuit the ground (0V) + all additional voltages in the primary circuit at the input of amplifier 1 are compared with the negative supplied voltage (inverted supplied voltage to get the conventional positive signal voltage) at the input of amplifier 2. In order to get the same signal at the probe output amplifier 3 is inverting the signal again. The voltage differences between + inputs of amplifiers 1 and 2 are measured and the input resistor is either 1GΩ (closed switch) or 10TΩ (open switch, resistance of amplifier 1 and 2 to ground).