Giga-8dd manual

and instructions for recording EPGs

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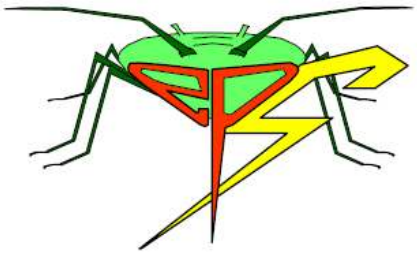
EPG recording system

(Firmware Version 2.09)



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CONTENTS

*The Giga-8dd EPG system**

1 Introduction

1.1 The Giga-8dd model

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 spider mites have been used. Blood feeding feeders, mosquitoes and ticks and insect parasitic mites have been used as well.

Although the main applied interest comes from host 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. Up to eight insects maximally can be recorded simultaneously, each on a separate plant or on shared plants (see p. 12).

The device name **Giga** refers to the default input resistance (Ri) of 1 Giga-Ohm (10^9 Ω) of the primary amplifier probe (head stage amplifier) for normal EPG recording; **-8** refers to the maximal number of insects (device channels) for simultaneously recording; **dd** refers to the **d**igital output signal and the **d**ifferential configuration of the primary amplifier (Appendix 5). The 1GΩ Ri value (normal mode) can be switched to 10TΩ (emf mode) that can be used – among others - for correct measurements of (pure) emf-components such as extra- versus intracellularly plant voltages at the stylet tips.

All new *Giga-8dd* model properties since 2020 are. (Table 1):

- Power supplied by the computer USB connector (no external power supply)
- Field operation of insects using a laptop (and laptop battery)
- Mouse control from PC screen of channel selection with voltage supply (Vs) adjustment
- Remote switching of input resistance between 1GΩ (normal mode) and 10TΩ (emf mode)

Table 1. Technical Giga-8*dd* specifications

EPG probe 8 Primary amplifiers with a cable to control unit.	
- amplification	primary gain 50x
- input resistances	default 1 GΩ (10^9Ω), switch to 10 TΩ (10^{13}Ω)
- input bias current	30 fA (10^{-15}A)
Giga-8*dd* control unit	
- 2nd stage amplification (gain)	up to 2x (50-100x total gain)
- calibration pulse	-50 mVolt (circa)
- Vsupply (DC offset)	± 0.5 Volt
- max output	± 5.85 Volt (~, Stylet+ analysis display)
- *Stylet*$^+$ input scale range limits	± 5 Volt (recording display);
- AD converter	resolution 14 bit; sample rate 100Hz (default)
- Power supply	5V DC from computer USB socket (internally converted to ± 5.85 Volt DC)

1.2 Stylet$^+$ software.

Free EPG software (Downloads) for data acquisition (module Stylet+d.exe) for all Giga devices to date and also, for EPG signal analysis of acquired data files (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.

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

1.3 Computer requirements

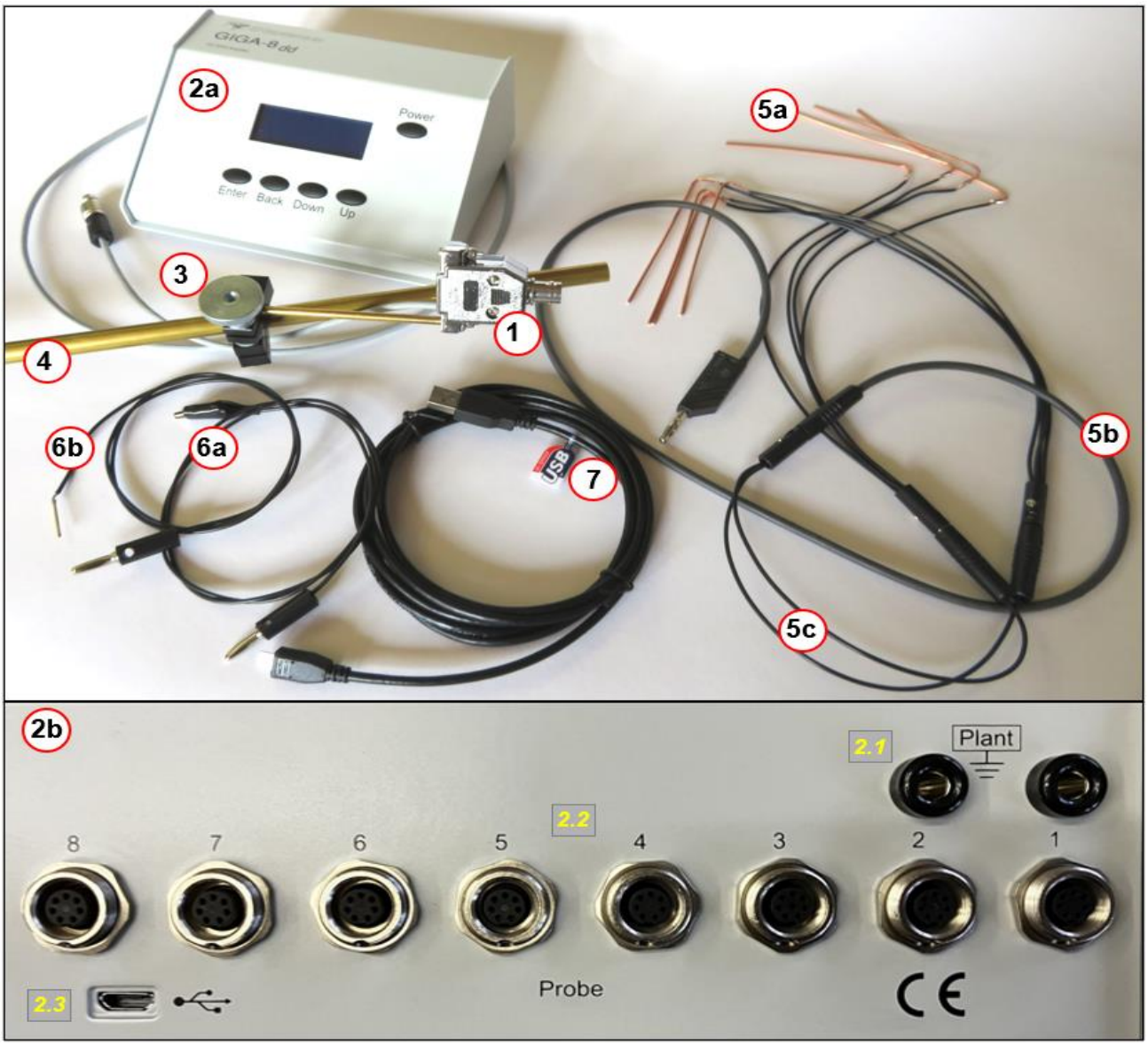
The Giga-8*dd* has an integrated analog-digital (AD) converter on board with USB output, allowing data acquisition mediated by ***STYLET***$^{+}$ software, which displays the signal on screen simultaneously. All ***Windows*** platform versions (-XP, -7, up to 11) are compatible. All Macintosh computers operating in *virtual Windows environment* can be used for the signal analysis part Stylet+a.exe, However. for the data acquisition (recording) part Stylet+d.exe only Mac computers with a ***bootable*** Windows environment; IOS/Windows choice at start up can be used.

2 Basic EPG system parts

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

Table 2. One 'Basic EPG system' package contents

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 nontoxic), 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
Separately ordered items	
- Gold wire, Ø depends on insect size (see website)	*(optional)*
coil with 30 m gold wire (Ø 18 µm)	
coil with 30 m gold wire (Ø 12.5 µm)	
- Extra silver glue	
- Special items on request	



1	Probe: primary amplifier	4	rod (38 cm, ø10mm) to mount probes, 2x
2a	Giga-8*dd* control unit, front/top view	5a	plant/soil electrode, 8x*
2b	Rear view; with sockets for:	5b	**main plant-soil cable (75cm)***
2.1	**ground socket; to plant(s) and Faraday cage** 2x	5c	intermediate 2 branch plant/soil cable*
2.2	EPG probe socket 8x	6a	ground-Faraday cage cable (alligator clip)
2.3	micro USB socket (±8V DC power and Data) 1x	6b	test cable with insect pin (fitting in probe input)
3	Swivel clamp* for flexible rod attachment	7	micro-USB cable

Figure 1 (next page). All device parts (top), device rear view (middle), and legend (bottom)

3. Installation of hardware and software

1. Wait to connect the Giga device to the computer (USB) and the cables of the 8 probes (1)
2. Go to https://www.epgsystems.eu/downloads-install-files-manuals/category/3-installation to download the *Installation guide 2020.pdf*, the (neglect warnings for **.exe** files), and *uninstall.exe*
3. Run *setup 2020.exe* and put shortcuts on desktop or taskbar. For older models than the Giga-8dd model use one of the other *Installation guides*. Running the setup is finished by an opened Stylet+ data acquisition screen in Demo mode.
4. Close the application (right top **x**).

4. Device operation

4.1 Images of device panels

- Use the USB cable (7) to connect the device to your computer 2.3 socket but **wait** to connect the 8 probes .
- Press the **Power** button to switch on the device and show panel 1. Then open (run) the data acquisition program ***Stylet+d.exe***
- The other 4 pushbuttons on the device **Enter**, **Back**, **Down**, and **Up** determine and change the recording settings and move you through the panels up to 11 shown here and explained on next page. Exercise to get used set and change the recording conditions.

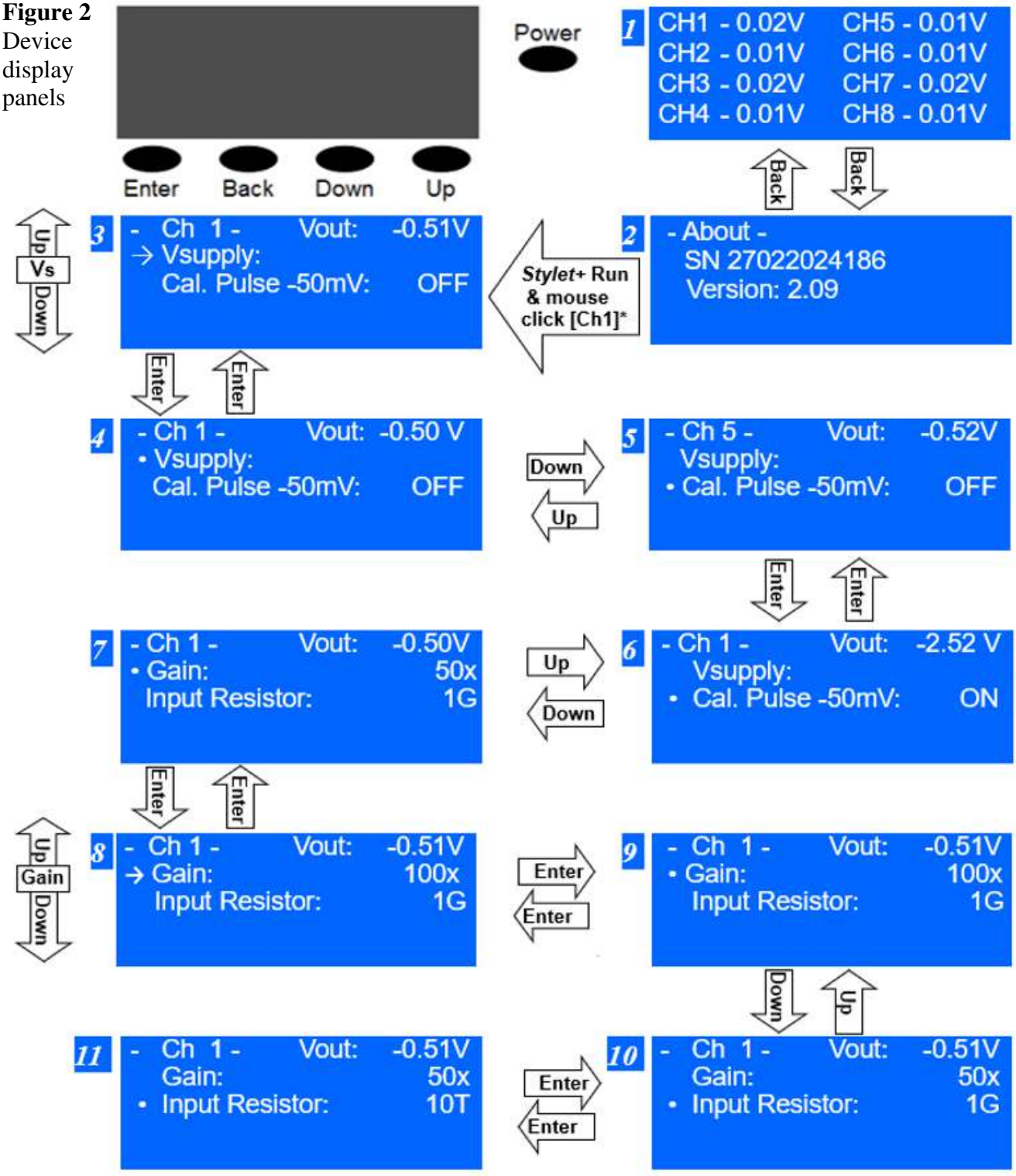


Figure 2 Device display panels

4.2 Device panel control

- Start the data acquisition program ***Stylet+d.exe*** always **before** pushing the **Power** button on the device**.**

- Pushing the other buttons: **Enter**, **Back**, **Down** and **Up** (labeled below inside arrows) enables setting and changing the recording settings and moves between the 11 panels.
- **Back** will show the About–panel 2 with serial (SN) and firmware version (2.09) number
- **Back** again then will show *1* again

Figure 3

Though control now can be continued is the push buttons it is easier and faster to use a *mouse click* on the Stylet+ computer *screen shortcut*. A click on the ch2 screen button (Fig.3) for example, will display the channel 2 recorded signal on the large top screen and also, panel 3 on the device will be show → Vsupply of ch2.

By using the **Up** and **Down** pushbuttons on the device the supplied voltage can now be increased or decreased, respectively. Fast access to a specific channel and changing the voltage supply is important during recording (chapter 5.6).

- **Enter** will move to panel *4* showing the inactivated • Vsupply status of →Vsupply (another **Enter** returns to its active status).
- A **Down** push will show to panel 5 • Cal.pulse -50mV: OFF and **Enter** now adds a -50mV pulse to the signal in the selected channel as indicated by • Cal.pulse -50mV: ON in panel *6* which is ended by **Enter** (back to the panel 5) Thus the pulse duration is determined by the ON – OFF interval.
- **Down** will now show panel *6* the inactive • Gain 50x panel *7* that is activated by **Enter**: → Gain 50x in panel *8*, which allows a gain increase by **Up** (maximum is 100x) and a gain decrease by **Down** (minimum is 50x).
- **Enter** will move to panel *9* (in fact identical to panel *7*) and **Down** will move to the • Input resistor 1G panel *10* that provides a switch from the 1GΩ default input resistance value for normal EPG recording to 10TΩ by **Enter** and panel *11* • Input resistor 10T. The switch 10TΩ input resistance to abandon R-components. Then only emf-components will be recorded and 10TΩ input resistance is referred to as the 'emf-mode' (see https://www.epgsystems.eu/epg-measuring; Appendix 1). Another **Enter** push returns the system to 'normal 1GΩ mode' (panel *10*).

- Back to panel 3 is always possible by a mouse click on any ch screen button.

4.3 Connecting device system parts

WARNING

1. ***Do only connect or disconnect EPG probes while the Giga device switched off!***
2. ***Use alligator clip cable to connect Giga8 (black sockets) to Faraday cage.***
3. ***Before inserting any pin (test cable or insect electrode) into the input of an EPG probe (by hand or forceps), first touch both hands to the GND connected cage to discharge yourself. Static electricity is dangerous for the probe input circuit.***

- Connect the 8 probes by inserting and screwing each cable connector into sockets 2.2 on the back of the device (mind *Warning*).
- Use a properly constructed Faraday cage (Appendix 3) and place it on a stable table or lab-bench to prevent shock distortions during recording.
- No 110/220V AC lamps, devices, or cables inside the Faraday cage, only outside the cage or DC-LED lamps inside.
- Connect the Giga to the Faraday cage by the alligator clip ground cable to one of the black sockets (Fig.1, 2.1). Be sure there is good electrical contact between cage bottom and all its sides. A separate removable front side should make electrical contact to the other cage faces when put in place (use multimeter).
- Four probes have been shipped on two 38 cm brass mounting rods. Mount either two separate rods or screw them together. Fix the rod(s) in the Faraday cage, e.g. between two ordinary (chemical) stands using standard clamps (Fig.3, arrow; not provided by EPG systems).
- Position the probes (and later the plants!) far back in the Faraday cage to reduce noise picked up by the 'open' probe input when the front of the cage is not closed. Place the Giga device outside the cage, either in front, or at the left or right side.
- Connect the USB cable between Giga and your computer.
- Your set up is ready for recording now.

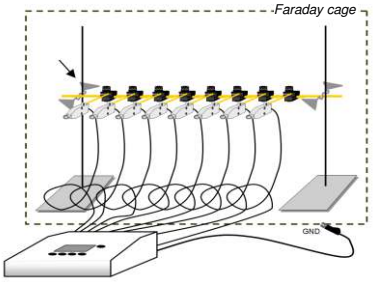


Figure 3. Mounting 8 probes using 2 chemical stands with clamps (arrow).

5. EPG recording

5.1 Run a demo recording (recommended)

Do not push the Power button; keep the device *switched off* and open/run Stylet+d. Briefly a message Check for supported devices will appear and at the bottom Demo device will be shown.

Type a file name (Fig.4A, File (direct....) (e.g. demo file), any text in the Comments 2: field, for now use the pre-set 0.01h recording time

Click the green START button and 8 program generated signals will appear, running from left to right in each channel: sine wave signals in odd channels, random noise signals in even channels. Compressed signals are shown bottom left (Fig.4B) and by default ch1 is shown enlarged at top (not shown in Fig,4). A mouse click on another ch number will put its signal enlarged on top, set to a 10 sec or 1 min time frame. After the default 0.01 hour (=36s) recording time is completed the session will be finished, the message Recording Done appears in the File Format Converter window (Fig.4C). One intermediate file C:\EPG_data\demo.aq8 will be stored as well as 8 automatically converted separate channel files in the same folder (e.g. for ch1: C:\EPG_data\ demo-ch1.D01). After file conversion is finished click [Close]. For further explanation and instructions see Stylet+ manual.

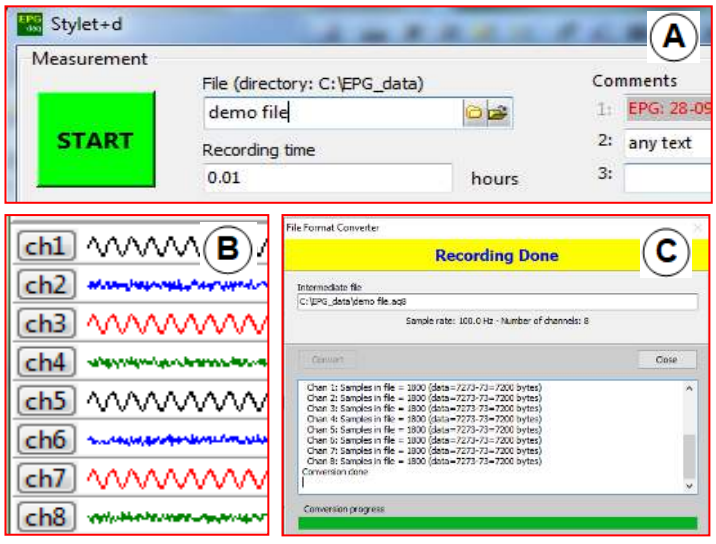


Figure 4. Parts of the Stylet+ demo recording screen. **A**, Fill out file name, comment, and recording time. **B**. compressed signals of 8 channels. **C**. saved data files

5.2 Plants and insect conditions

Standardization of the plant genotype, plant rearing conditions (regular watering!) is important. Also, regular refreshment of the insect rearing is essential for reliable recording data. For the experiments. select the insect instar and plant growth stage and plant part that will be most representative for the research aims. Use within an insect instar use a safe age to prevent molting during recording.

To compare experimental treatments (plant genotypes, disease infection, pesticide sprayed, rearing history, etc.), it is advisable to randomly assign individuals with different treatments to recording sessions; rather than running separate sessions with single treatments.

5.3 Wiring conditions

Water based (non-toxic) silver glue is used for most insects. Note that the glue dries out quickly; close the glue vial soon after use and prevent knocking it over (Appendix 1). Wiring is often regarded as the most difficult and delicate part of EPG recording for outsiders but once mastered, it's routine. Bad glue contacts give poor signals.

Insects fixation is mostly needed to apply silver glue to the insect it is done under a stereo microscope in general, although some insects can be wired while in rest and will not be disturbed by a glue application depending on their size and mobility. Using a head magnifier will then be sufficient.

For insect fixation, a vacuum suction device (Appendix 3) is a simple and useful tool in combination with a vacuum pump. A vacuum pump mostly belongs the standard lab equipment but smaller (and more silent) devices are commercial available (pressure ≤ 0.5Bar). A water jet suction device also works well but aquarium pumps are mostly not strong enough.

Do not use CO_2 for insect fixation. Cooling by small cooling unit under the microscope is especially useful for insects smaller than aphids (Whitefly, thrips, etc.).

If insects have wax particles on their abdomen or thorax they should be cleaned first. Use a fine dry brush first and then a wet brush with water and detergent.

5.4 Silver glue application

1. Shake the vial with silver glue thoroughly; if available, use vortex device.
2. 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.
3. Insert the gold wire of the prepared insect electrode (Appendix X) in the wet glue so that the wire points back- and upwards from the insect and let the glue dry for a few minutes.
4. Stop vacuum suction and gently lift the insect with wire and assist the release with a small brush if needed.
5. Insert the electrode insect pin into a piece of styrofoam to store the wired insect hanging in the air on a safe place and avoid climbing it in the electrode wire.
6. Prepare a few more wired insects than strictly needed.

5.5 Start of recording and plant access

Mount the insects to the probes and position them close to the selected leaf or plant part, relevant to your experimental research objectives. Be aware that plant parts – leaves especially – are moving over time and may drag the insect out of place. Attaching the leaf to a mounted plastic or wooden strip with a hair clip will prevent this (Appendix 3; Fig.B, arrow). Now move each hanging insect to the recording location and check that it lands in a suitable penetration position on the plant, but then lift the insect back a few mm. Now [Start] the Stylet+d session on the computer thus preventing that the beginning of plant penetration will not be recorded for some insects.

5.6. Optimal channel adjustment (Ri=1GΩ)

After start of recording carefully observe the compressed signals and when the first insect (channel) starts a plant penetration click the channel button on the left (Fig. 4B) of the signal to adjust its proper voltage level in the enlarged top window. In Fig.6 the signals related to the adjustments are shown.

1. Initially a non-probing baseline with some noise is shown at (1).
2. At the first plant penetration (probing period) the signal can be shown on a very different voltage levels:
   - a partly out of scale positive level (1a)
   - a partly out of scale negative level (1b)
   - a moderate negative level (1c)
   - a moderate positive level (1d)
   - or any intermediate level
3. The voltage level should be optimized.
   - First at (Fig.6 (2)) the voltage level is adjusted by →Vsupply **Up** or **Down** until the maximal peaks in the signal reach a voltage of about +5V (Fig.2, panel 3).
   - At (3) a calibration pulse is given by •Cal.Pulse-50mV and **Enter** ON and **Enter** OFF
   - Then (4) the →Vsupply is lowered by **Down** until the pulse minimum level passes 0V.
   - And next, at (5) the →Gain: 50x is increased by **Up** until the voltage will reach the previous voltage (2) level of about 4-5 Volt again. The calibration pulse will then remain passing 0V.
4. The voltage levels (1a) - (1d) are caused by *electrode potentials* in the primary circuit that differ for each channel (see https://www.epgsystems.eu/epg-measuring).
5. The optimal voltage level adjustment is now complete.
6. If the voltage level may show large changes later during the recording some readjustments my be needed but mostly changes are small and need no further actions.

The optimal adjustment procedure provides an optimal signal, using the AD converter resolution as good as possible and a good balance between R- and emf-components (see same link as in 4).

Poor adjustments will lead to difficulties in waveform identification. Zooming in on the y-axis offline later during signal analysis will not provide sufficient detail. Optimal recording adjustment is very important and should be repeated in every new recording and in each channel again.

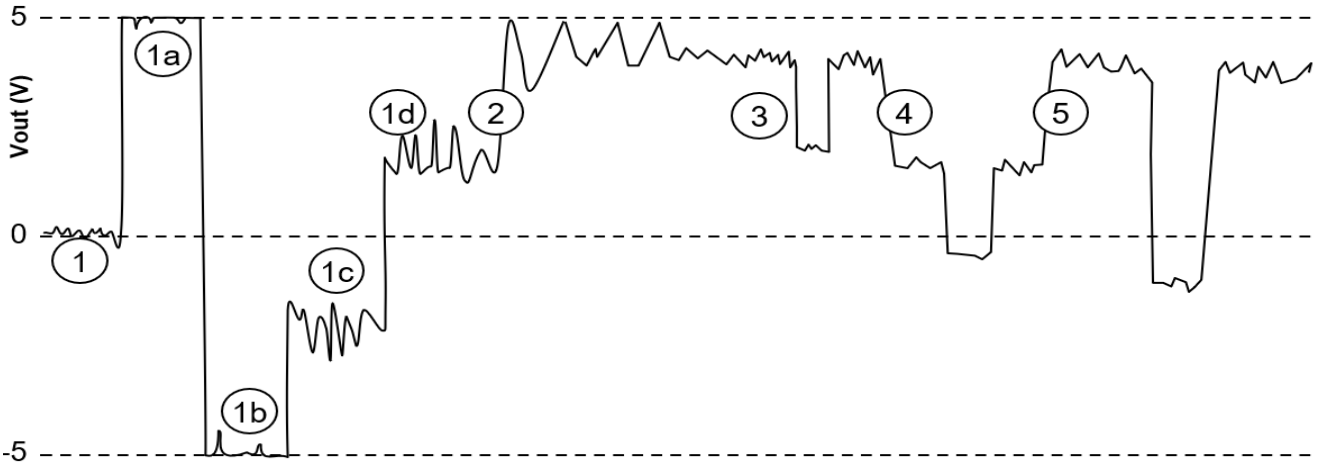


Figure 6. Optimal voltage level adjustment that is recommended for each channel (insect). The signal appearances are shown when starting a Stylet+d recording with subsequent plant access of an insect. Explanation see text.

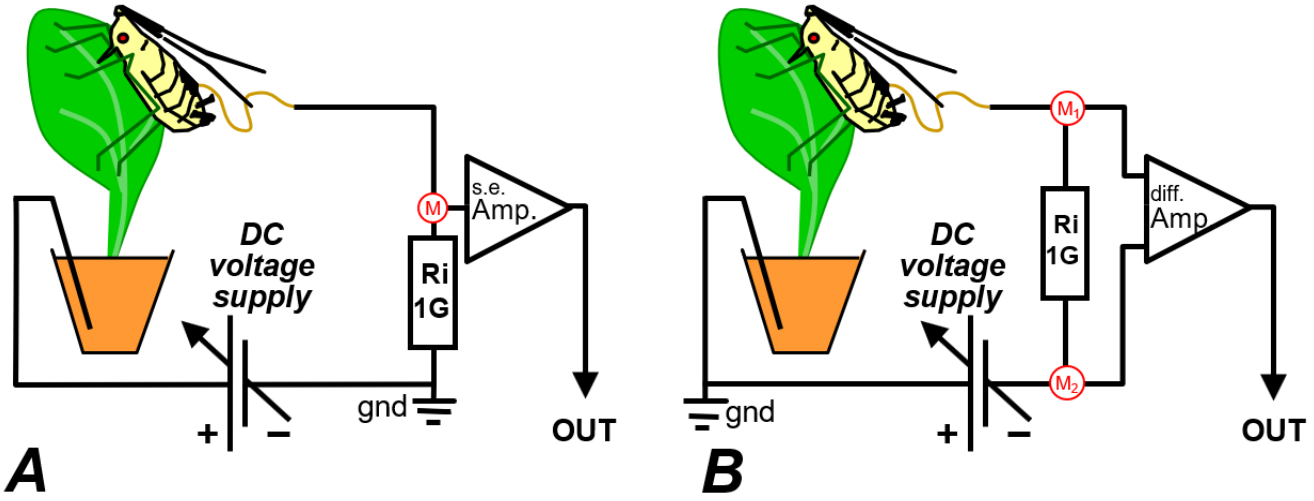
For EPG analysis see https://www.epgsystems.eu/downloads-install-files-manuals/file/137-last-update-2-oct-20

Appendix 1

Giga-8dd device properties

Differential circuit configuration

All earlier Giga models used a 'single' ended configuration of their head stage amplifiers (Fig.A). In this more simple configuration voltages are measured across the 1GΩ input resistor (Ri), between the measuring point M and ground. The plant is connected to the Vsupply source of about ±0.5V. The drawback is that each plant has its own Vsupply voltage and therefore, should not touch each other and the Faraday cage to avoid short circuiting the signals. In the differential configuration the signal is measured across Ri too between two measuring points M_1 and M_2, independent of the circuit ground. All plants share the common ground and therefore they may touch each other as well as the Faraday cage. Also, this enables EPG recording in the field: the soil (=planet earth) and rooted plants are grounded. Also, this allows recording from more than one insect on the same plant with individual (channel, insect) control of voltage supply and gain.



Circuit configurations in head stage amplifiers. **A.** The earlier used single ended (s.e,) configuration with plant connected to the Vsupply source. **B**. The newer *Giga-8dd* differential (diff.) configuration with plant connected to ground.

Recording in 'emf-mode'

A switch in each head stage amplifier changes its input resistance (Ri) from 1GΩ (normal mode) to 10TΩ (emf-mode). The latter refers to the sensitivity of the head stage amplifier to emf-components exclusively. Resistance fluctuations of the insect will not change the signal when Ri = 10TΩ and the signal therefore, only reflects the voltages generated by the plant and insect in the amplifier circuit (https://www.epgsystems.eu/epg-measuring). This means that e.g. the potential drop voltage measured reflects the real membrane potential of the plant cell punctured. Also, brief voltage changes during long lasting punctures of phloem sieve elements reflect internal plant signals (Salvador et al., 2014).

However recording in emf-mode only reflects good signals during stylet penetration periods. During non-probing periods the signals are unreliable because huge fluctuations due to small electric field changes and (very small) circuit instabilities. Once the stylets make contact with the plant, this circuit instability no longer has an effect. Switching to emf-mode will only provide reliable signals when done during stylet penetration periods.

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 identical to all other the signal voltage values as long as the insect resistance remains the same. However, it is often noted that the response at the leading edge of a calibration pulse differs from the response at the trailing edge of the pulse. Nevertheless, the pulse edge responses provide some indication of insect resistance at that time (see *https://www.epgsystems.eu/measuring-systems*). Plant resistance is usually relatively low and will not play a role in such calculations.

Appendix 2

Consumables & own made tools

Faraday cage

A simple and efficient Faraday cage can be constructed by using metal mosquito netting or (even more simple) bird cage wire (Fig.A). A galvanized (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.

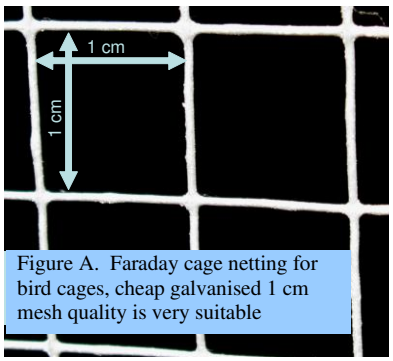


Figure A. Faraday cage netting for bird cages, cheap galvanised 1 cm mesh quality is very suitable

Constructing a **hanging cage**, supported by cords to the ceiling and pulleys (wheels) to a counter weight (Fig.B) 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 cage permanently set up on a table could be made. To access the cage's interior, an open front side is needed. To avoid noise (Appendix 4) the open front should be closed during recording,

Whatever type of cage you are using; always take care that all cage faces - front screen and bottom plate included - should be of electrically conductive material and interconnected to each other. When a wooden frame is used make wire connections between sides. The cage front may be left open during recording if the noise level allows so.

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** must be an intrinsic part of the cage, electrically in contact with the other cage sides. 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.
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.

***Using old Giga models*: 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 newer ***Giga-8dd*** and successors no such precautions are needed (Appendix 1).

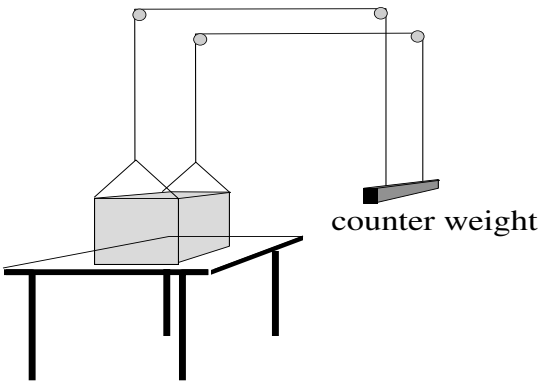


Figure B

Silver glue

Vial support Silver glue dries out easily, thus always close the vial soon after use. One vial of 2ml should be enough for hundreds of insect attachments but drying out and knocking over a vial are the main risks. A **support block** to prevent this is made from hard wood or plastic (e.g. PVC plate) with a drilled hole to fit the bottle (Fig.A). The block should not be made of light weight material.

Silver glue is water based. So, when somewhat dried, it can be diluted by clean tap water. But once completely dried it will be difficult to restore its original composition.

Store spare vials of glue in the refrigerator (5ºC). Some decay by micro-organisms is at risk.

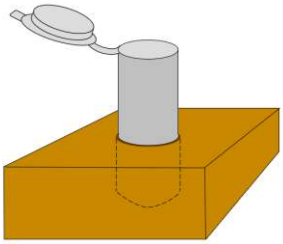


Figure A

Own made glue

INGREDIENTS:

1 Cooled boiled water
2 Water based glue. The water based paper glue supplied by EPG Systems uses cheap transparent paper glue as sold in 'glue pens'; two different samples here (Fig.B). However, NOT all ordinary kids glue is suitable! Try first a small 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. Hhousehold cleaning product or Triton X100 is suitable. Use a few droplets for 10ml.
4 Silver powder, of about 3-5µm flake or grain. This is the most expensive ingredient. Look at the internet for suppliers.

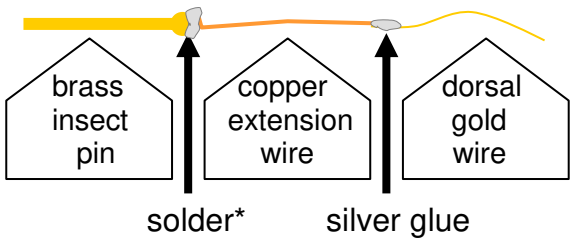
PREPARATION: for 12g glue stock (about 10 ml)
Use a 0.01g resolution balance).

1. weigh subsequently in one stock vial (≥ 20 ml, to be closed tightly)
   - 4 g water
   - 4 g paper glue
   - 4 g silver powder
   - 2-3 droplets of detergent.
2. Mix thoroughly (Vortex) and fill 2ml vials for wiring use

Figure B. Two samples .

Making insect electrodes

1. Take a piece of copper extension wire (about 2cm) and solder it to a brass insect pin (Fig.C). More extension wire can be made of stripped common 110/220V power cord.
2. Prepare pieces of 18µm gold wire using a stereo microscope: aphids need about 2cm and Cicadellids about 5cm pieces. Whiteflies need 0.5 to 1 cm pieces of or 12.5µm wire (or 1µm Platinum wire).
3. Gold wire cannot be soldered and should be silver glue connected to the extension wire. Use some overlap to glue the two wires.
4. Let the glue dry for about one minute. and insert the completed insect electrode with insect pin into a piece of Styrofoam.
5. Prepare some spare insect electrodes than needed for the recording session.



Note: apply soldering fluid (flux) before soldering !

Figure C. Complete insect electrode

Gold wire

treatment to prevent wire entanglement

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

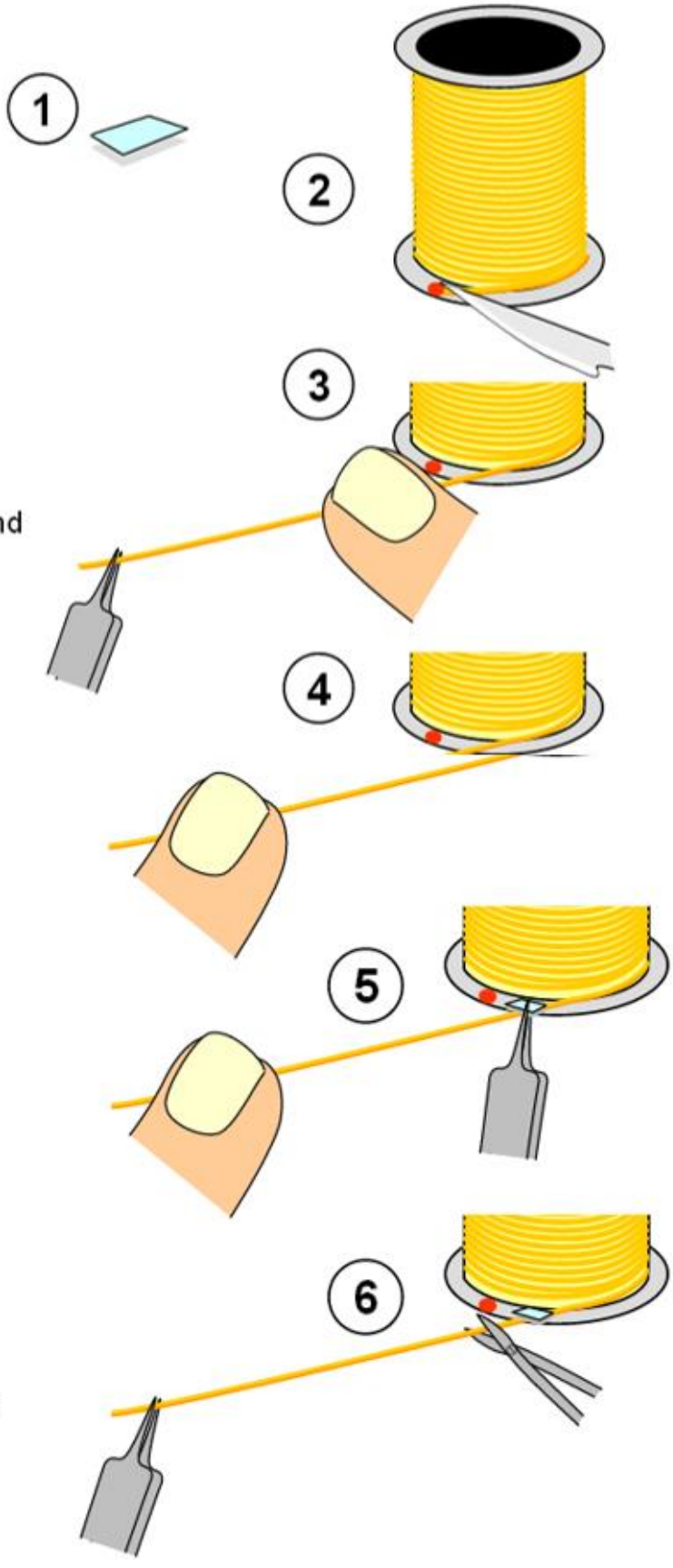
All next use:

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)

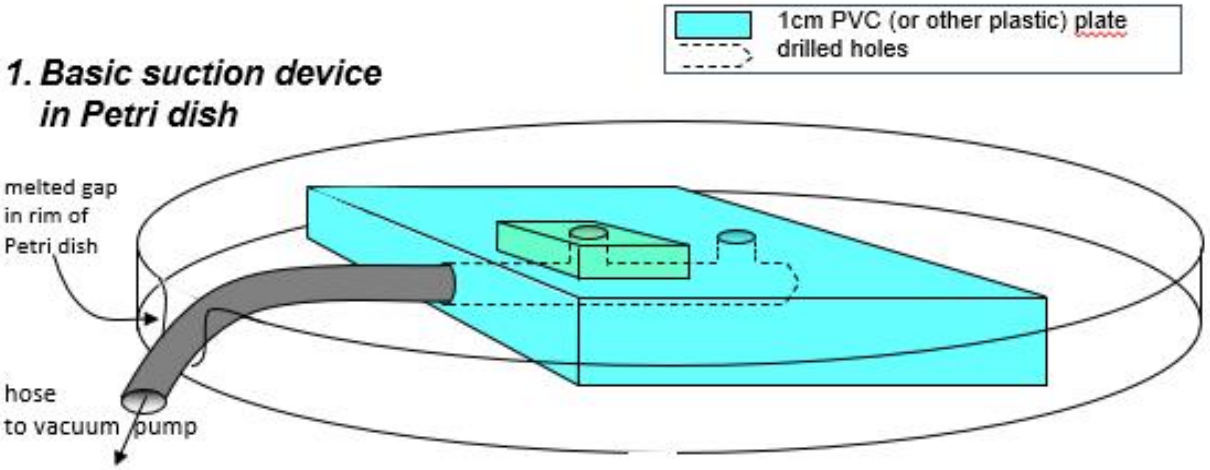
6 Get the free end of the wire with forceps again and cut 10 cm piece by small scissors or a scalpel the to prepare electrodes of shorter length: 1 – 2 cm, depending on how much movement you will allow the insect.

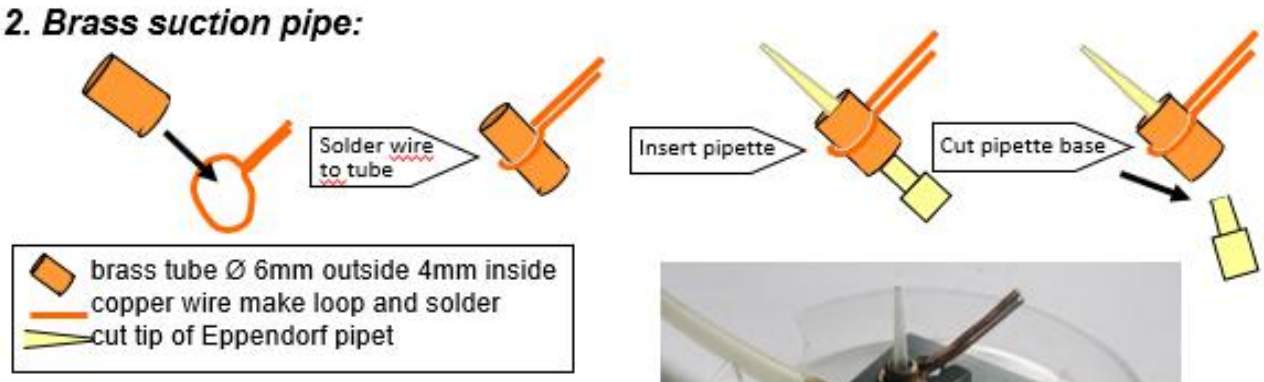


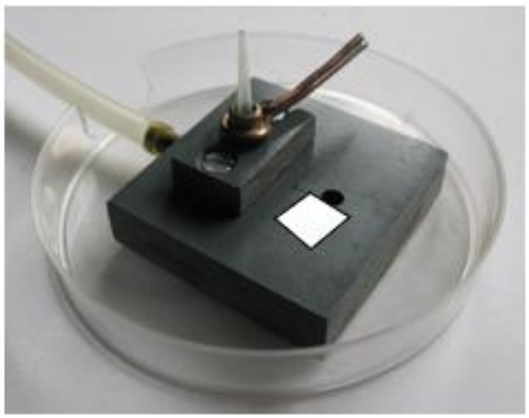
** if left handed, change R and L*

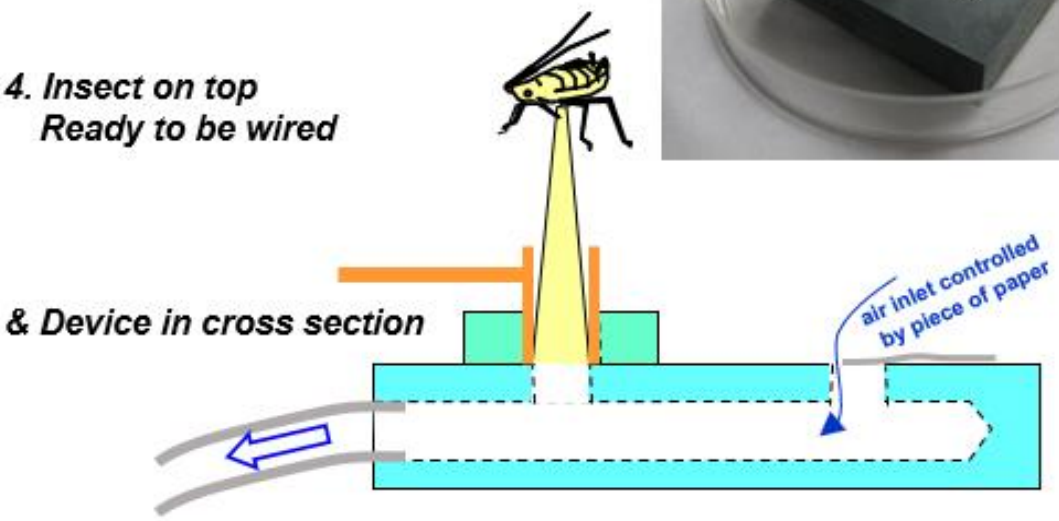
Vacuum suction

Making a simple suction device









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 3

Arranging plants & insects

Use of supplied cabling. Plants and insects can be used in different arrangements. Their numbers may be equal: 2, 4, or 8 of each (number of channels set in [Options] on Stylet+d taskbar) or alternatively, more than 1 insect can be recorded on one plant (see below). Two electrode branches with 4 plant electrodes are supplied. Each plant should always have one plant electrode connected to one of the two Plant device sockets (Fig.A). Also the Faraday cage should be grounded using the alligator clip ground cable (Fig.1 6a) to one of these sockets directly (or indirectly dashed).

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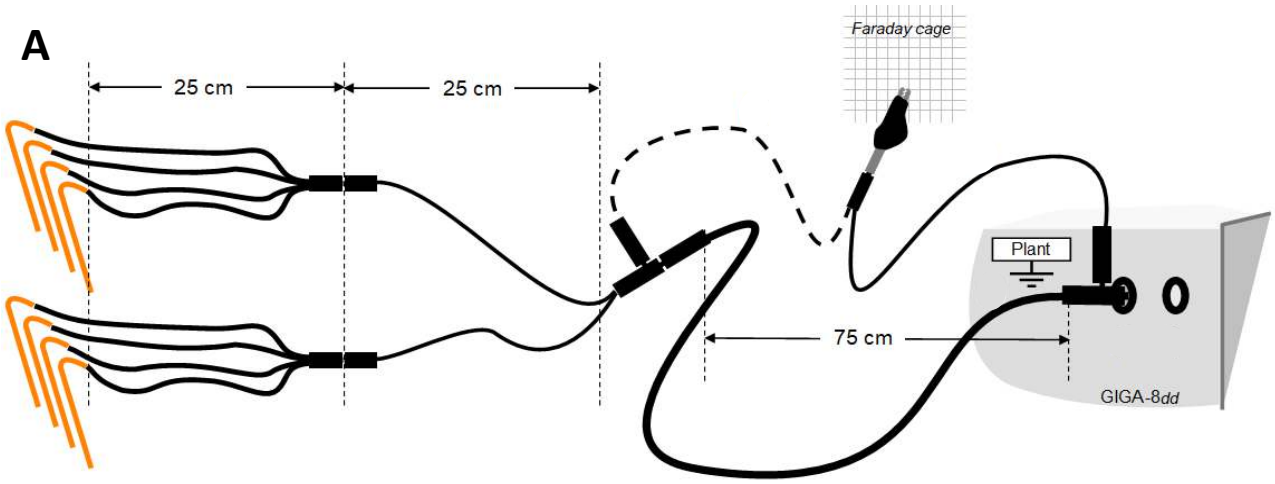

Figure A. Wire configuration of plant electrodes. All plant electrodes are interconnected and connected to one of the two (black) ground-plant sockets on the device back side, and in contact with the Faraday cage.

The most common arrangement is to record from one insect per plant (Fig.B), However, some research objectives may require recording more insects on one plant. This is not a problem due to the differential design of the Giga-8dd (Appendix 1; unlike previous Giga models).

In case of field recordings, it may often be the only option to use one plant or tree.

For field and lab recordings it is wise to prevent plant movements by securing the leaf or branch. In the lab a strip (wood or plastic) under the leaves (Fig.B, black arrow) and a hair clip for each leaf is sufficient. In the field, a tripod can be used to secure the plant and an additional strip to support a leaf or stem.

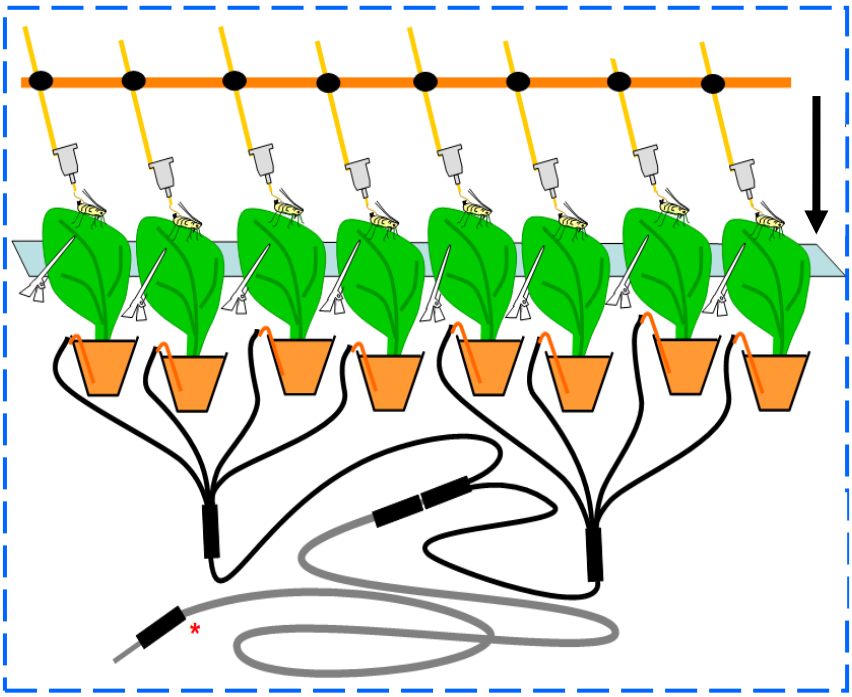

Figure B. Wire configuration of plant electrodes. All plant electrodes are interconnected and connected to one of the two (black) ground-plant sockets on the device back side, and in contact

Appendix 4

Trouble shooting

Noise, noise, noise, . .

Electrical noise, better called '50 Hz noise' (USA 60Hz) cab be 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 lab electricity grid and depends on the location of and distance to 110 or 220 Volt (AC) power lines and cables to the probe input. 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.

Interconnections of equipment parts including the Faraday cage and Giga device is very important. If the signal is noisy, check for 0 Ohm resistance – use multimeter; Ohm range – between the cages faces (bottom too), and the Giga ground socket (Appendix 3, Fig.B). Use multimeter and ask a technician if you are not familiar to that.

No AC (110V or 220V) powered lamps or instruments should be used inside the cage or close to the its open front. Leave lamps outside and illuminate insects and plants through the mesh of the Faraday cage or use DC powered led lamps inside.

To proceed with the following test, insert an empty insect electrode into one channel of an EPG probe input (remind WARNING p.7), 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.

Note: For arranging plant and insect connections using the provided cabling, see appendix 1

Signal distortion

A poor signal quality may be recorded 1) as a general poor signal amplitude due to sub-optimal Vsupply adjustment (section 7), or 2) as a distorted potential drop (e.g. in aphids) by a poor silver glue contact, mostly caused by wax secretion between insect and silver glue. The higher insect electrode resistance with a small intrinsic capacitance will affect some waveform properties. Especially the edges of calibration pulses and potential drops (Fig.) will show 'overshoots'; i.e. they will act then as a high pass filter enabling fast signal components and disabling slow components.

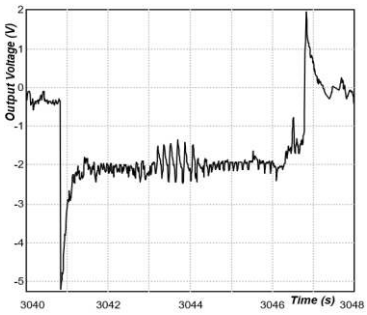

Figure. pd overshoots .

Equipment tests

If you suspect that the giga device or some of your head stage amplifiers (EPG probes) are not working properly, perform the following tests.

***Test 1* with open input**

The test needs all probes mounted inside the Faraday cage and connected to the Giga-8dd, the USB cable connected to the computer. No insects, no plants, so far.

1 Push Power button on the Giga device. The display should light blue and show panel *1*
2 Then start Stylet+d , give a file name, set Recording time to 1 h, and type any text (or just one character) in Comments space 2.
3 Click START and – as with the demo test (chapter 5.1) – and compressed signals of all 8 channels at left and ch1 signal enlarged in the top window (default at start).
4 All compressed channel signals should show a (near to) 0Volt line.

5 In Giga panel *1* the voltages next to all ch numbers should be near 0. Give a mouse click on ch1 and panel 3 will be shown. Check that the same Vout value will not be larger than + or –1V.
6 A *damaged probe* will show an out of range voltage of – or +6.14 V Vout.
7 On the Stylet+ computer screen this is + or – out of range. Check this and contact *EPG systems* for repair !
8 In some cases a single channel will show a high very noise level. Interchange the head stage amplifier with a different device channel. If the same amplifier shows the high noise : contact *EPG systems* Check all 8 channels subsequently on the computer screen.
9 Test completed.

***Test 2* with test cable** (Fig.1 6b)

Figure A. EPG probe input .

With Giga on and 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.

1. Carefully (see ***WARNING*** on p.7) insert the brass pin of the test cable into the BNC connector, i.e. first into the input of the ch1 EPG probe (Fig. A).
2. Start a new 1 hour EPG recording using Stylet+d acquisition session by START.
3. Upon test cable connection the small voltage offset (Fig.B) differing somewhat per channel will not affect the following test steps.
4. Give a mouse click on the [ch1] button of the *Stylet+* screen. The Giga control panel will jump to panel 3 ch1.
5. Keep pressing the **Up** button until the scale maximum Vsupply on the computer screen is reached (Fig.B, time 156-158s). 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.B) 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 response difference output voltage scale, Fig.B).
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

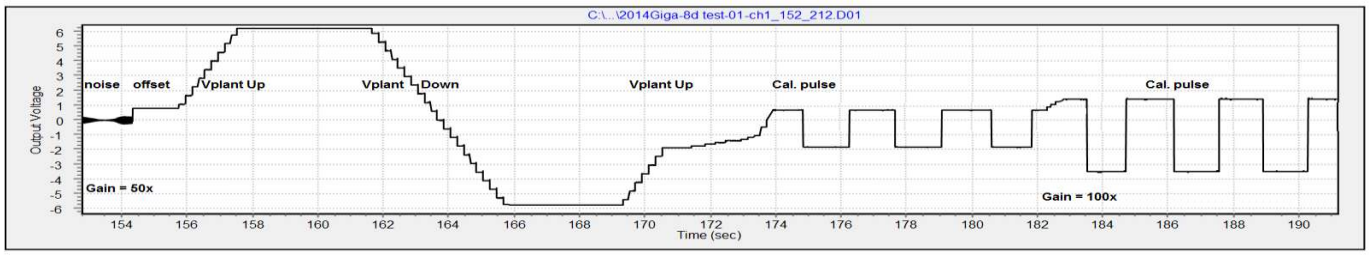

Figure B. *Stylet+* screen. From L to R: open input (noise), test cable insertion (at 145s showing a small positive offset), Vplant Up, Vplant Down, Vplant Up, Cal. pulse 50x gain, and (at 183s) Cal. pulse 100x