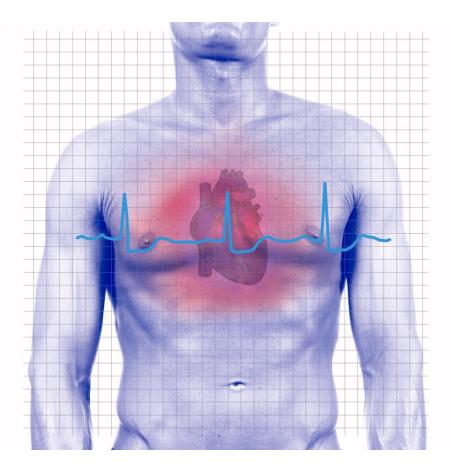
Basics of HEART RATE VARIABILITY

Applied to Psychophysiology

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Introduction

This guide was written to instruct clinicians on the use of heart rate variability (HRV) in clinical biofeedback, as a form of **applied psychophysiology**.

Contrary to the common assumption, a healthy heart doesn't beat regularly as a metronome. The heart's moment by moment beating rate is naturally influenced by many physiological factors and varies constantly. The most obvious of these factors is physical activity. At rest, for example, your heart might beat in the neighborhood of 60 times per minute. When you're running for the bus or swimming lengths, it might accelerate to well over 90 times a minute. In fact, the range defined by your minimum to maximum heart rate capacity is a key factor defining your level of health. As a person's physical condition deteriorates, this range can actually become narrowed to the point of limiting their ability to adapt to the stresses of daily life. From that point on, any unexpected stressful condition could push the individual to the limit of their adaptability and put them at risk for cardiovascular accidents and possibly death.

The idea of exercising the cardiovascular system to maintain as wide as possible a range of heart rate adaptability is not new. Medical practitioners have prescribed physical exercise and stress management techniques, such as meditation or yoga, for generations. In recent years, a novel biofeedback method has gained serious recognition from practitioners of many health care professions. A set of standard signal processing and analysis methods, proposed in 1996, has been widely accepted by practitioners and manufacturers of equipment. A number of heart rate variability (HRV) biofeedback protocols have been developed and tested for various clinical conditions. The research shows sufficient evidence of success that it places HRV biofeedback ahead of many other self-regulation methods in terms of acceptance by mainstream medicine.

This manual will teach you the basic concepts of HRV biofeedback and help you learn how to apply them within your clinical practice. After you have become familiar with the key concepts, it is strongly recommended that you do some hands-on training, before using them on a real examinee.

As simple as this method may sound, it still requires practice.

HRV biofeedback is clinically indicated for:

- Relaxation
- Stress control
- Exercising the respiratory and cardiovascular systems

Note: This document is not intended to replace scientific and clinical literature (a bibliography of references is provided on page 31).

Detection of Heartbeat Signals

The principal component of successful HRV biofeedback is the ability to detect heartbeats reliably and precisely. Two types of physiological sensors can be used:

- Electrocardiography (EKG/ECG)
- Blood Volume Pulse (BVP, also called photoplethysmography)

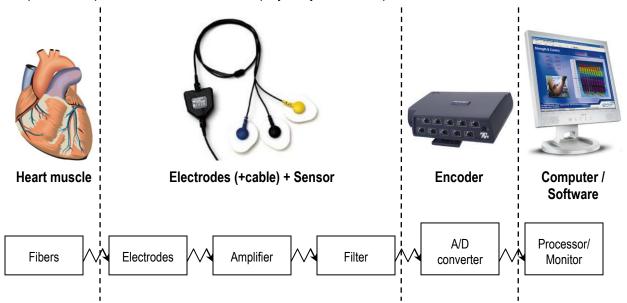
Both sensors are noninvasive and detect a specific physiological change associated with the heart's activity, but their functions are based on very different principles.

In either case, the sensor's output shows a recognizable beat pattern that is used to calculate the time between consecutive beats, or interbeat interval (IBI), and the corresponding heart rate (HR, in beats per minute). From a series of such IBI values, a number of mathematical processes can be applied to extract the standard metrics used for HRV biofeedback.

When doing HRV training, it is also common to use a respiration sensor for practicing breathing exercises and to train to increase respiration sinus arrhythmia (RSA). The respiration sensor detects the expansion and contraction of the chest as a person breathes.

Electrocardiography (EKG)

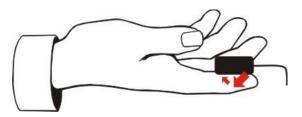
The EKG sensor detects the electrical signal that is generated by the heart muscle each time it contracts. This electrical signal propagates throughout the body and is captured by the electrodes, then amplified and filtered by the sensor before being converted to a digital signal by the encoder. It is then sent to the computer to be processed, recorded and displayed by the BioGraph Infiniti software.



Blood volume pulse (BVP)

The processing sequence for the BVP sensor is similar, except for the type of physiological change that is being measured. The BVP sensor shines infrared light through the finger and measures the amount of light reflected by the skin.

The amount of reflected light varies during each heart beat as more or less blood rushes through the capillaries. The sensor converts the reflected light into an electrical signal that is then sent to the computer to be processed.



Respiration

The respiration sensor is strapped around the client's chest. It detects breathing by monitoring the expansion and contraction of the rib cage during inhalation and exhalation.

The resulting signal looks like a wave that moves up and down with each breath. The software uses the wave pattern to calculate the respiration period (duration), rate and amplitude.

- **Note:** For clarity this graphic shows sensors strapped around a bare chest. In practice, you would normally strap respiration sensors over a client's clothing.

A/D converter (Encoder)

Thought Technology's analog to digital (A/D) converters are called "encoders".

- **ProComp2** has 2 channels (A and B) sampling at 256 samples per second and 2 channels (C and D) sampling at 32 samples per second. Because channel A contains an integrated EEG sensor, it cannot be used to measure other types of physiological data. (Only two channels at a time can be used to record data, either A and B, or C and D.)
- **ProComp5 Infiniti** has 2 channels (A and B) sampling at 2048 samples per second and 3 channels (C to E) sampling at 256 samples/second.
- **ProComp Infiniti** has 2 channels (A and B) sampling at 2048 samples per second and 6 channels (C to H) sampling at 256 samples/second.
- FlexComp Infiniti has 10 channels (A to J) sampling at 2048 samples per second. (This encoder was designed primarily for research data acquisition, hence the high sampling rate on all channels.)









ProComp Infiniti

FlexComp Infiniti

The **sample rate** (for instance, 2048 samples per second) is the number of measures (samples) per second taken from the continuous signal (analog signal). In this case, the analog signal is the EKG, BVP or respiration signal captured by the sensor. The series of samples constitutes the digital signal.

Although EKG or BVP signals can be sampled at 256 samples per second, it is generally recommended to use a higher sampling rate (2048) because this allows for a higher precision in the detection of heartbeats and, subsequently, in the HRV calculations (<u>Gary G. et al</u>).

Note: For research data acquisition, use of the higher sampling rate (2048) is crucial. For biofeedback purposes, such as paced breathing training, a lower sampling rate may be adequate.

Because it is a slow signal with no high-frequency component, the respiration signal can be sampled at 256 samples per second with no loss of precision. A higher sampling rate can be used, but provides no additional benefit.

Amplifier (Sensor)

Thought Technology's HRV sensors are:

- EKG-Flex/Pro (SA9306M): Detects heart beats by measuring the heart's electrical activity. The unit of measure is in millivolts.
- **BVP-Flex/Pro (SA9308M):** Detects heart beats by measuring the pulse in a finger tip. The unit of measure is relative (%).
- **Resp-Flex/Pro (SA9311M):** Detects breathing by measuring the chest's expansion and contraction. The unit of measure is relative (%).



EKG-Flex/Pro (SA9306M)



BVP-Flex/Pro (SA9308M)



Resp-Flex/Pro (SA9311M)

Note: Thought Technology also manufactures a receiver for the Polar[™] transmitter belts. The receiver works principally with two Polar[™] products, the T31 coded[™] transmitter and the T31 transmitter. Thought Technology does not provide the belt. More information about these products is provided on the Polar[™] web site: www.polar.fi/en/products/accessories.

Recommended encoder inputs (Channels)

When you purchase a specialized application suite from Thought Technology (such as the Physiology Suite or the EEG Suite) each sensor is preassigned to a specific encoder input so you don't have to worry about selecting the right sampling rate.

If you design your own channel sets, follow these simple rules:

The EKG and BVP sensors can both be used on channel B of the ProComp 2 but not on channels A, C or D. They can be used on all channels of the ProComp 5 Infiniti and ProComp Infiniti encoders but channels A and B are preferred because they allow a higher sampling rate. They can be used on all channels of the FlexComp Infiniti.

Since the respiration sensor can be used with any sampling rate, it can be connected to any input of any encoder (with the exception of channel A of the ProComp 2). Generally, however, it is connected to an input with a lower sampling rate.

In the following tables:

Green check marks indicate the proper sensor inputs for each encoder type.

✓ Yellow check marks show connections that are possible but not recommended.

Red X marks show which input cannot be used for that sensor.

ProComp2	Α	В	С	D						
EKG-Flex/Pro	×		×	×						
BVP-Flex/Pro	×	\checkmark	×	×						
Resp-Flex/Pro	×									
ProComp5	Α	В	С	D	E					
EKG-Flex/Pro	\checkmark	\checkmark								
BVP-Flex/Pro										
Resp-Flex/Pro										
r										
ProComp	Α	В	С	D	Е	F	G	н		
ProComp EKG-Flex/Pro	A V	B ✓	C V	D V	E	F	G V	H V		
· · ·			-							
EKG-Flex/Pro										
EKG-Flex/Pro BVP-Flex/Pro	∑ ∑								1	J
EKG-Flex/Pro BVP-Flex/Pro Resp-Flex/Pro									I	J
EKG-Flex/Pro BVP-Flex/Pro Resp-Flex/Pro FlexComp	▼ ▼ ▼	✓ ✓ ✓ B	 ✓ ✓ ✓ ✓ C 	✓ ✓ ✓ D		▼ ▼ F	✓ ✓ ✓ G	✓ ✓ ✓ H		

Note: When you use a FlexComp Infiniti encoder with a specialized application suite from Thought Technology, such as the Physiology Suite, you must set its DIP switches to the ProComp Infiniti protocol settings. (This changes the sampling rates of the FlexComp Infiniti inputs to match those of the ProComp Infiniti encoder.) Instructions for changing DIP switch settings are found in the FlexComp Infiniti Hardware Manual.

Using the EKG sensor

Although it is possible to use the EKG sensor with dry electrodes and no skin preparation, this setup raises the probability of artifacts distorting the signal and causing calculation errors. As a general rule, skin preparation enhances the signal quality, reduces the probability of artifacts and minimizes the need for post-recording artifact rejection.

Surface electrodes and cables

The EKG sensor requires the use of silver-silver chloride electrodes to make electrical contact between the skin and the sensor. In order to optimize the signal quality, a wide electrode placement is recommended, usually on the chest or on the forearms, so the electrodes should be indirectly connected to the sensor via an extender cable. To further enhance the signal quality, we recommend using pre-gelled electrodes like Thought Technology's UniGel electrodes (T3425M). Dry single strip electrodes can be used but Triode electrodes (T3402M) should be avoided because the triangular configuration is not appropriate for detecting EKG.



T3425 – UniGel electrodes (single use): The UniGel electrode should be the first choice if signal quality is important (i.e., for data acquisition).

Three UniGels can be snapped to the yellow, blue and black leads of the extender cable and applied to the client's chest or forearms, depending on the preferred electrode configuration.



T3404 – Single strip electrodes (single use): The single strip electrodes are the perfect choice for biofeedback sessions where ease of use matters and signal quality is not crucial.

The strip can be cut into single electrodes and snapped to the yellow, blue and black leads of the extender cable to be applied to the client's chest or forearms, depending on the preferred electrode configuration.

Skin preparation



For any recording where signal quality matters, good skin preparation is important to get a clean signal and avoid artifacts. Before applying the EKG electrodes, make sure the skin surface is clean and dry by rubbing it with an alcohol pad. Abrading the skin with an abrasive cream, such as NuPrep, can also help. If necessary, shaving excess body hair can be required.

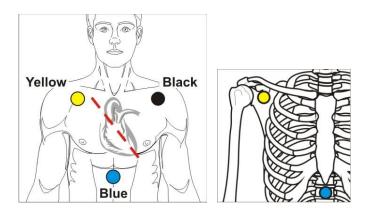
Enhancing electrode contact



For optimal contact between the electrode and the skin, it is recommended to use conductive gel. Pre-gelled electrodes (UniGel) are easiest but it is possible to apply a bit of conductive paste or gel to the center of the single strip electrodes (grey area only) before applying them to the skin. Make sure the electrodes are placed firmly on the skin and that there is good contact between the skin and the electrode.

Chest placement

The ideal electrode placement for detecting EKG is a triangular configuration on the chest where the yellow and blue electrodes are parallel with the heart's main axis (see illustration). The yellow and black electrodes should be placed over the right and left coracoid processes, respectively, and the blue electrode over the xiphoid process.



Purpose: Establishing a tight triangular area over the heart to minimize the risk of artifacts from interference from chest and arm muscle activity.

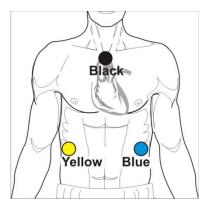
Commonly used in psychophysiology for: Running stress assessments, when working with male clients or when arm placements lead to too many artifacts.

Location: Coracoid and xiphoid processes. See above pictures.

Abdominal placement

Since some clients may find the idea of exposing their chest area uncomfortable, an acceptable alternative is to place the electrodes on the abdomen (<u>Baker, Peper & Shaffer</u>).

Ask the client to lift their shirt and place the yellow electrode below the ribs on the right and the blue electrode at the same level on the left. The black electrode can go anywhere, but a good location is the upper sternum area.



Purpose: Establishing a triangular area around the heart that maintains a good signal quality but less invasive to the client.

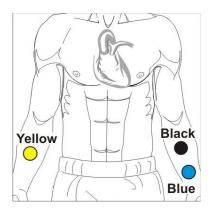
Commonly used in psychophysiology for: Running stress assessments, when working with female clients or when arm placements lead to too many artifacts.

Location: Just below the ribs, on either side, and on the upper part of the sternum. See above picture.

Arm placement

An easier electrode placement uses the forearms, as indicated in the illustration. The yellow electrode is on the right arm and the other two are on the left arm.

Ideally, an area with little or no hair is preferred. The arm placement is more susceptible to artifacts, particularly interference caused by arm and chest muscle activity.



Purpose: Extending the triangular area from the chest to forearm positions allows a more comfortable setup.

Commonly used in psychophysiology for: Biofeedback training sessions where the client can sit and stay relaxed or working with female clients who are uncomfortable with the chest or abdominal placement.

Location: Medial aspect of the forearms. See above picture.

Wrist placement

The forearm electrode placement requires the use of an extender cable with longer leads like the one that is sold with the EKG Wrist straps (SA9325). The wrist straps provide the easiest placement method for EKG signal detection. For best results, it is recommended to use BioGraph Infiniti's **Advanced IBI from EKG** beat detection algorithm when working with the Wrist Straps.



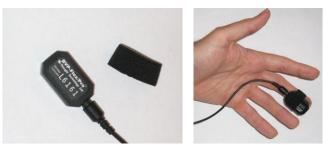
Purpose: Providing the easiest electrode placement method for working with the EKG sensor.

Commonly used in psychophysiology for: Quickest placement for HRV biofeedback.

Location: Left and right palmar aspect of the wrist. See above picture.

Using the BVP sensor

The BVP sensor does not require skin preparation as it is placed directly in contact with the skin. Place the sensor against the fleshy part of the first joint of any finger and hold it in position using the elastic strap.



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Optimizing placement

The BVP sensor is sensitive to light, movement and pressure. To prevent artifacts caused by light interference, be careful to adjust the two small openings on the sensor's front so they are firmly pressed against the finger pad and there is no gap between the black edges of the sensor and the skin. If the client's fingers are too small, it is recommended to place the sensor on the thumb pad.

Pressing the sensor too firmly against the finger pad will decrease the sensitivity of the signal because excessive pressure reduces blood circulation in the finger tip. Look at the raw signal when adjusting the sensor against the finger pad and try to maximize the signal amplitude.

While using the BVP sensor, instruct the client to keep their arms and hands as immobile as possible. This is made easier by having them rest their hands on their thighs, palm side up.

Using the respiration sensor

To place the respiration sensor, unravel the strap and attach it around the client's abdomen so that the sensor is in the front. The fit should be snug enough that the strap stays fixed when the subject is relaxed.

Ask the client to breathe out fully and tighten the sensor slightly at full expiration. This should provide enough slack in the rubber strap to allow it to stretch without being overextended when the abdomen expands.

Optimizing placement

Before strapping the sensor around the client's chest, observe the client's breathing pattern and determine what level of their rib cage expands and contracts the most during respiration. Placing the sensor around the level of widest expansion will produce a better signal. Since the software is looking for a sinusoidal signal that goes up when breathing in and down when breathing out, it is important to instruct the client to breathe as regularly as possible to avoid artifacts.

If artifacts are detected, you can modify the software settings to tell the software whether to expect slow or fast breaths ("Slow" indicating fewer than 10 breaths per minute). On the slow setting, long respiration cycles are expected and any short waveforms will be ignored. In the BioGraph Infiniti software, this setting is found by accessing the *Edit Virtual Channel Settings* option in the *Edit* menu. For more information, please refer to the on-line help manual (Press F1).



The rubber strap stretches when the abdomen expands during breathing.

The HRV Signals

Physiological basis of EKG

The heart is a muscular organ, about the size of a closed fist, located in the left side of the chest. With every beat, it pushes blood through the body's arteries and veins, delivering oxygen and nutrients to the organs and muscles. Its rhythmic contractions are controlled by natural pacemakers called the sinoatrial (SA) and atrioventricular nodes. These two nodes sequence the pumping action of the heart's atrial and ventricular cavities and set an average rate between 60 to 100 contractions per minute. A heartbeat is a fairly complicated series of electrical events, involving depolarization and repolarization of muscle fibers, triggering the contraction, first in the atria, then in the ventricles. The electrical waveform propagates throughout the body and can be picked up by placing electrodes on the chest or on the arms and legs.



Physiological basis of BVP

With every beat, the heart pushes a volume of blood through the body's arteries and veins, causing a wave which travels from the heart to the peripheral regions of the body (arms and legs) and eventually returns to the heart. The body's circulation system distributes the oxygen-rich blood through the tissues through a finer and finer series of arteries, arterioles and capillaries. It then gathers up deoxygenated blood via the capillaries, venules and veins. As the blood reaches a specific area of skin, the pulse wave causes a sudden increase in engorgement in the tissues, which is rapidly followed by a return to normal tissue perfusion.

The BVP sensor detects this process by shining infrared light through the tip of a finger and measuring how much light is reflected. (Blood is red because it reflects the red frequencies of the light spectrum.) As the amount of blood increases, more light is reflected, creating a rise in the signal. As the surge of blood dissipates, the signal falls back down. This waveform is slightly complicated by the fact that the direct pulse wave caused by the systolic contraction is bounced back from the lower body and causes a secondary "reflected" wave, which appears as a secondary rise in the signal. The gap between the direct and reflected waves is called the dicrotic notch.

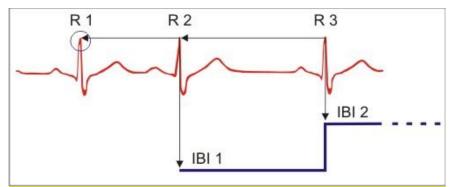


Signal processing

Using either the EKG or the BVP signals, it is possible to detect the regular pattern of beats using specially designed software algorithms. Such an algorithm is able to process the recording signal and determine with some precision when each beat is occurring. One fact worth noting is that using the EKG signal allows for much more precision in the detection of beats than using BVP because the EKG wave has a sharper definition than the rounded pulse waveform (<u>Gary G. et al</u>).

As it detects a new beat, the algorithm outputs the time between this beat and the previous one, which is called the interbeat interval (IBI). When computed specifically from the EKG signal, the interbeat interval is often called R-to-R (or RR) interval. BioGraph Infiniti includes one **IBI from BVP** and two **IBI from EKG** algorithms that are able to detect heartbeats.

When a session starts recording, the algorithm scans the incoming sampled data and tries to recognize specific patterns, like the R-spike in the EKG signal or the point of maximum deviation in the BVP signal. (Some published articles refer to this key point in either waveform as the fiducial point.) As soon as the first beat is detected, the program starts counting time and waits for the next beat. As soon as the second beat is detected, the interbeat interval (the time between beats) is calculated (in milliseconds). Figure 1 shows this process: R 1 is the first R-spike. As soon as R 2 is detected, IBI 1 is calculated. The program keeps doing this until the end of the session. The IBI data is the source of most HRV computations.



The standards of measurements, physiological interpretation and clinical use for HRV that are most often used were described in an article published by the <u>Task Force of The European Society of Cardiology and</u> <u>The North American Society of Pacing and Electrophysiology</u> in 1996. They included a number of time domain and frequency domain methods for analyzing short and long term recordings of IBI data. One key factor to analyzing IBI data for HRV is that the data should be artifact-free.

HRV data artifacts

General considerations

Artifact is unwanted information contained within a signal, most often originating from other sources. It can drown out or distort the real signal so much that a meaningful analysis becomes difficult or impossible. Analyzing data for HRV is somewhat different from analyzing other physiological data because it is not so much concerned by the raw EKG or BVP signals as it is concerned by the IBI values which are extracted from the raw data. Thus, the types of artifacts that matter most are those that cause errors in IBI values.

The EKG signal is measured in microvolts (1 μ V = one millionth of a Volt), which means it is a fairly small signal. Because artifact can be many times larger than the actual signal, some care has to be taken to minimize all possible sources of signal distortion. This is often illustrated with the GIGO principle: Garbage in, garbage out. If the raw signal you are recording contains too much artifact, the IBI output will be wrong and the analysis that can be generated from it will probably be bad as well.

This section presents the different artifacts, how to recognize them and how to prevent them.

HRV artifacts

When processing signals for HRV analysis, two kinds of artifacts can occur: **Missed beats** and **Extra beats**. Both types are most often caused by signal distortion. A missed beat, for example, can occur when the signal is so distorted that the software is unable to identify the beat pattern and only picks up on the next good beat. The end result is an artificially long IBI value. An extra beat, on the other hand, occurs when the program confuses a distortion in the signal for a beat and "sees" two beats, or more, when there should be only one. Extra beats cause the appearance of abnormally short IBI values. Both types of artifacts are easily seen on a graph which plots the IBI values over time as sudden very high rises or very low drops.



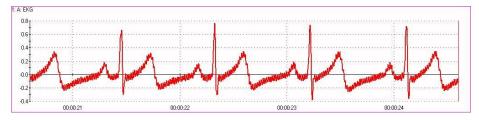
It is important to mention that there are also real natural physiological events (as opposed to artifacts, which aren't) that can cause unwanted distortions in the IBI values. Such occurrences as an ectopic premature atrial or ventricular contraction (PAC or PVC) will engender oddball IBI values which disturb the frequency domain calculations and should be corrected.

Note: Sometimes these events will also be referred to as "missed beats" because the heart flutter sensation they cause feels like the heart "just skipped a beat".

EKG artifacts

Line interference (50/60Hz noise)

This is the most common artifact. It comes from the power line and is transmitted by electrical devices (such as the computer) placed near the data acquisition device (such as your Infiniti encoder). Generally, this is seen as a high frequency oscillation riding on top of the EKG signal. On the screen, it looks like the trace is fuzzy:



Line interference is not usually a problem with the EKG signal because the R-spike is very often much wider than the noise and still detectable. To remove line interference noise, use the **Notch Filters** setting in the main screen *Options* menu of BioGraph Infiniti. The EKG notch filter filters out the selected frequency (50 or 60 Hertz) and lets the rest of the signal come through. Using proper skin preparation and clean gelled electrodes also helps reduce the effect of line interference.

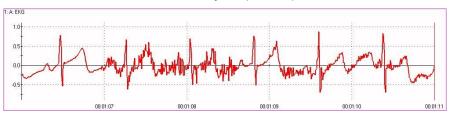
Electronic devices also generate their own frequencies that will not be removed by the Notch filter.

Additional precautions must be taken, such as keeping the device 3 feet (1 meter) away from any electronic equipment and 10 feet (3 meters) away from any radio transmitting devices.

Disconnect all the unused sensors from the encoder. If not connected to the examinee, they may act as antennas and capture unwanted signals that would corrupt the signal.

Muscle contraction artifacts

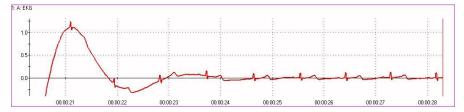
Since the heart is a muscle, its electrical activity is not very different from the electrical activity of other muscles. It is easy for the EKG signal to be drowned out by nearby muscle contractions, particularly from chest and arm muscles. This type of artifact can cause lots of extra beats. The occurrence of muscle contraction artifacts is reduced by using a chest electrode placement and by instructing the client to relax their muscles, rest their arms on their knees and stay as quiet as possible.



Direct current (DC) offset artifacts

The EKG signal normally oscillates around a zero baseline. A direct current offset can make the signal drift up or down on the scale when the impedance between the skin and the electrodes is too high. In some circumstances this can cause missed beats.

This is generally seen when the contact between the skin and the electrode is bad (which can happen when applying electrodes on greasy or dirty skin) or by reusing electrodes too many times (which deteriorates the quality of the conductive gel). Cleaning the skin prior to applying the electrode and using fresh electrodes would prevent this artifact.



Electrode movement artifacts

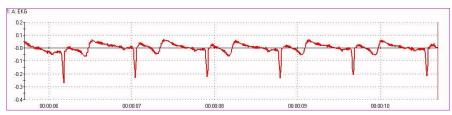
If the client moves enough to tug on the extender cable and pull one of the electrodes off the skin you will see very wide signal deviations as the ability to capture the small electric signal is lost. These very wide distortions completely confuse the beat detection algorithm and cause many extra beats.



Artifacts caused by pulling can be avoided by making sure that the cables are loose enough to provide some freedom of motion. Also, using gelled electrodes, such as the UniGel (T3425) and the EKG Wrist straps (SA9325) can improve the strength of the electrode contact and minimize the occurrence of this sort of artifact.

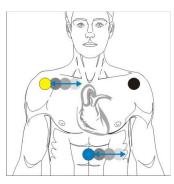
Electrode polarity artifacts

The direction (up or down) as well as the relative amplitude of the R spike is dependent on the position of the yellow and blue electrodes in reference to the heart's main axis.



If the R-spike appears to be going down, then you may have reversed their positions. Be sure to place the yellow electrode on the right shoulder and the blue electrode over the xyphoid process.

Adjusting the position of the electrodes on the client's chest or arms can help maximize the R-spike's amplitude.



BVP artifacts

Sensor movement

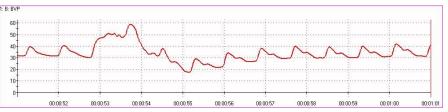
Because its operating principle is not electrical but based on measuring reflected infrared (IR) light, the BVP sensor is susceptible to different sources of artifact from EKG. Sensor movement is the largest cause of artifact in the BVP signal because it creates very wide deviations which can drown out the signal completely and cause missed or extra beats.



Tugging on the sensor cable can also lift the sensor off the finger and create tiny gaps through which ambient light can seep and cause the signal to jump wildly and rapidly. This problem can be minimized by attaching the sensor as firmly as possible to the client's finger (but not so tight as to cut off the circulation) and instructing the client to rest his hands on his knees and keep as still as possible. Some clinicians will wrap a dark cloth hood over the finger and sensor to prevent light seepage.

Client movement

Client movement can be a bit more difficult to troubleshoot because it can cause artifact of many shapes. **Sudden changes in blood pressure** can occur when the client stands up or sits down while being monitored. When this occurs, the signal can appear to drift down or up as the client's base blood pressure adjusts to the change of position. Moving ones arms up or down in relationship to the heart level can also cause drifting.

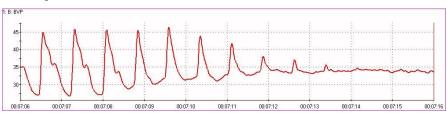


Nervous fidgeting will cause recurring patterns in the signal that can look like a strange physiological response. Finger tapping, for example, creates regular odd notches that look like multiple dicrotic notches. It is a good idea to always keep a discreet eye on the client when attempting to troubleshoot artifact in a signal.



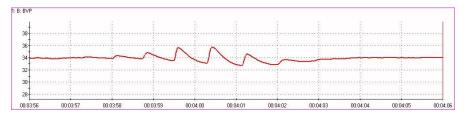
Sensor on too tight

If the sensor is strapped on the finger too tightly, the pressure against the finger pad can cut off the circulation and dampen or flatten the pulse wave completely. The BVP sensor has to be tight enough to hold firmly but not so tight as to cut off the circulation.



Cold fingers

If the client is very nervous, their stress reaction can cause the circulation in the hands and finger to be drastically reduced. This can be seen as a very weak pulse or no pulse at all.



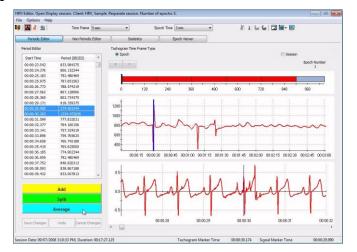
To minimize the effect of cold fingers from nervousness, allow the client a few minutes to relax and become acclimatized to your office environment. You can ask them to rub their hands together or put their hands in a sink full of warm water for a couple of minutes. The causes for cold fingers can also be pathological, as in Raynaud's disease. If this is the case, you can try recording BVP from the client's toes (if it's OK with him) or you might have to use the EKG sensor instead. In some cases, placing the sensor on the thumb pad will help, because the thumb doesn't get as cold as the other fingers.

Normalizing IBI data

Despite the best methods for skin preparation, electrode placement and client instruction, it is very difficult to record absolutely clean, artifact-free data. Any statistical analysis performed on a recording that includes artifact will produce measures of dubious value. That is why it is good practice to review the session and carefully eliminate any artifact before generating statistics. The process of reviewing data to eliminate artifacts for HRV analysis is called **normalizing** and is quite different from the usual methods of artifact rejection which consist of cutting out artifact from a physiological recording to process only the good bits. For most physiological signals, like skin conductance or temperature, this method is acceptable but not for HRV analysis because it requires that some care be taken to maintain the time distribution of IBI values.

As it is detected, each new beat is recorded with a duration and a time of occurrence, from the beginning of the session. Since HRV analysis is concerned with changes (variability) of heart rate over time, any editing of the data should not create shifts or gaps in the time sequence of heartbeats. There are a few methods for doing this (<u>Gary G. et al</u>).

The **Cardiopro Infiniti HRV Analysis module** provides IBI editing functions which allows users to **Split** abnormally large IBI values (caused by missed beats) into two or more smaller values, **Sum** two or more abnormally small IBI values (caused by extra beats) into one larger one and **Average** pairs of consecutive IBI values where one is too large and the other too small (usually caused by ectopic beats from premature atrial or ventricular contractions – PAC or PVC). These operations provide a standard method for correcting artifacts without causing shifts in the time distribution of IBIs.



The above screen shot from the Cardiopro Infiniti HRV module shows the IBI editing function. The software allows users to locate and edit artifacts, either manually or automatically, prior to generating the statistical analysis and printing out reports. This tool is useful for clinicians who need great accuracy in their assessments or researchers.

Standards of measurement

As previously mentioned, in 1996, the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology published an article called: <u>Guidelines – Heart rate</u> variability, Standards of measurement, physiological interpretation, and clinical use. This article defined norms for analyzing HRV that are still in use today. Two broad categories of metrics were defined, time domain (changes over time) and frequency domain (spectrum of oscillatory components). This section discusses the most commonly used metrics from both categories.

Time domain

SDNN

The majority of time domain metrics are **statistical methods**. These include basic calculations, such as mean, minimum and maximum, of course, but one metric of particular interest is the standard deviation (SD) of the interbeat intervals (IBI). Because the IBI is sometimes called the R-to-R interval ("R" as in "R-spike"), this calculation is referred to as **SDRR** or, if the calculation is done on *normalized* IBI data (edited to remove artifacts, see page 16), it will often be called **SDNN**. In this case, the NN refers to Normal-to-Normal interval. One important aspect of this metric is that it should be calculated over a specific and fixed period of time, or **epoch**, to carry any significance. The two epoch "windows" most often used are 24 hours (long term) and 5 minutes (short term).

Significant results are achieved only by comparing SDNN values calculated over epochs of identical durations. Trying to compare SDNN values calculated for sessions of different durations is not appropriate. Also note that SDNN values may be unreliable if they are calculated over epochs that are too short.

SDANN is the standard deviation of averaged NN intervals, calculated from a series of epochs of identical durations. This value represents the total recording period (session).

RMSSD is the square root of the mean squared differences of successive NN intervals.

NN50 is the total number of pairs of consecutive normalized IBI values that differ by more than 50 milliseconds. 50 ms is the usual period that is used but some published articles have been looking at values as low as 10 ms (<u>Mietus, Peng et al</u>). This value is user-defined and can be modified from the channel set settings.

pNN50 is the NN50 expressed as a proportion, i.e., dividing NN50 by the total number of NN intervals for the epoch.

HR Max-Min is the peak to trough difference in heart rate that occurs during a full breath cycle. This metric is affected by respiratory sinus arrhythmia (RSA) and is generally described as a measure of vagal tone (vagus nerve activity). High HR Max-Min values represent high vagal tone. Low values which remain relatively steady during breathing exercises generally indicate poor vagal tone.

Geometrical Methods are also used, such as the sample density distribution or Lorenz plot of NN intervals, but, since many of the metrics correlate closely together, it is not necessary to use them all. The few time domain metrics described above are most commonly used.

Frequency domain

Power Spectrum

The frequency domain methods require that a series of normalized IBI values be processed through a mathematical operation, such as a fast Fourier transform (FFT), to analyze the frequency information contained in the recording. The result is shown on a **power spectrum**, which shows a breakdown of all the frequencies (oscillations) contained in each epoch. Generally, the spectrum is calculated over the same epoch duration as the SDNN metric (long or short term) and the **relative power** or **total power** for selected sub-bands of the whole spectrum is computed.

VLF, LF & HF: Within the whole HRV frequency spectrum, three sub-bands are of particular interest because of the type of heart rate pacing activity that can be observed within them: The Very Low Frequency (VLF) band is composed of frequencies less than 0.04 Hz (Hertz), the Low Frequency (LF) band considers the frequencies between 0.04 and 0.15 Hz and the High Frequency (HF) band looks at frequencies between 0.15 and 0.4 Hz. When analyzing long term recordings (24 h), the Ultra Low Frequency band (ULF, less than 0.003 Hz) becomes relevant but its clinical significance is not clearly defined. There are many physiological factors that influence the power distribution within the HRV bands. For a detailed explanation of this, please refer to the manual *Principles and Practice of Stress Management* by Dr. Paul Lehrer et al.

As a quick rule of thumb:

- VLF activity is said to reflect mainly sympathetic system activity but also the vascular tone loop of the baroreflex system, thermal regulation and activity of the renin-angiotensin system.
- LF frequencies show activity of the baroreflex function (blood pressure maintenance) and of the parasympathetic system.
- **HF** power is mostly influenced by processes modulating gas exchange efficiency, respiratory sinus arrhythmia (RSA) and activity from the Vagus nerve.

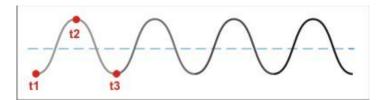
LF/HF: The relative amounts of LF and HF power, expressed as a ratio, is generally understood as a measure of the balance between sympathetic and parasympathetic nervous system activity.

These are complex relationships, though, and some care must be taken when interpreting this type of metric. Generally, it can be said that the principal purpose of HRV biofeedback is to train the regulatory aspects of HRV more than the autonomic balance.

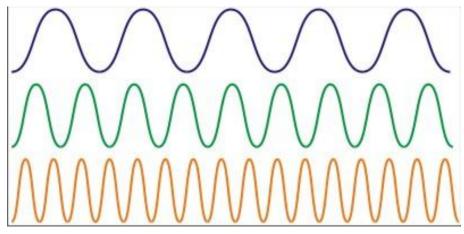
Clinical rationale

As previously mentioned, a healthy heart doesn't beat with absolute regularly. In fact, a certain amount of variability is more desirable than clockwork regularity because it is physiologically associated with a greater capacity to adapt to life's everyday challenges. Some published research shows that dramatically reduced heart rate variability is an indicator of possible cardiovascular problems (Kors, Swenne and Greiser, Galinier M. et al). It also reflects the effects of stress, depression, anxiety, inflammation, neural dysfunction, and other disease processes.

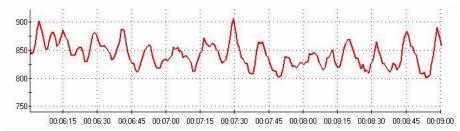
In reality, the heart's rhythm is constantly being adjusted by a number of physiological factors, such as blood oxygen and CO₂ levels, blood pressure, body temperature, respiration rate and depth, etc. Each of these factors is regulated by a homeostatic process which *tends* to maintain a stable, constant condition but ends up entraining the system in an oscillatory pattern:



The above illustration shows a simplified homeostatic pattern, such as the blood oxygen level regulation system. Ideally, the oxygen level should be maintained at a constant level represented by the blue dotted line. At point t1, the oxygen level is determined to be too low. This triggers a physiological reaction to increase it. When it reaches the target level, it can't stop rapidly, so it overshoots and reaches point t2, where the oxygen level reaches another threshold which is determined to be too high and triggers an opposite physiological reaction to decrease it. At t3, it is low enough to trigger another increase, and so on. This example is, of course, extremely simplified but it gives the idea: Most physiological regulation systems are inherently oscillatory in nature. In addition, each of these regulation systems runs at its own cycle period. Some are very slow. Some are slow. Some are fast.



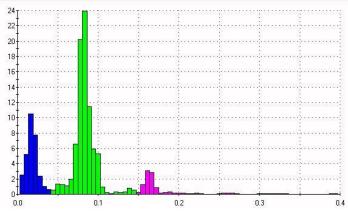
Although the example shows the three frequencies separately, in a real physiological signal, all frequencies would be folded together into a single wavy line. The graph below shows a 5 minute recording of IBI values where oscillations of many frequencies can be seen, superimposed over one another.



The power spectrum graph converts this time domain sequence into a frequency domain distribution of the oscillatory components that are present in the recording. The graph below represents each frequency as a vertical bar (bin). Tall bins indicate the presence of more waves of that particular frequency. In the example, you can see a few high bins in the VLF (dark blue) band and in the LF (green) band, below 0.1 Hz. There is also a little bit of HF (pink) activity in this epoch.

The primary clinical proposition underlying HRV biofeedback training is that increasing heart rate variability (as reflected by the amount of power in the HRV spectrum) brings positive health benefits. HRV training generally aims at achieving two specific goals:

- 1) Increase the total power overall, and
- 2) Focus as much of that power as possible within the LF band.



Breathing: The physiological process which influences heart rate most directly is breathing. As you breathe in, your heart rate tends to increase. As you breathe out, it tends to decrease. This entrainment effect is called **respiratory sinus arrhythmia (RSA)**. The average, everyday, irregular breathing doesn't have a very notable influence on HRV power but slow, deep and regular breathing does. The most basic exercise practiced during HRV training is slow regular abdominal breathing.

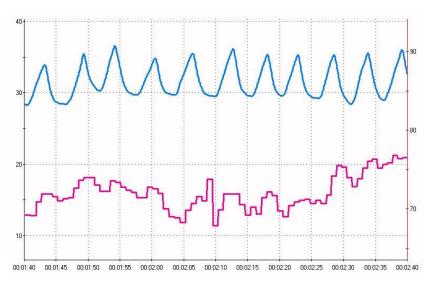
Paced breathing: To help the client learn how to breathe slowly and regularly, it is often useful to provide timed guidance for breathing in and breathing out. A respiration pacer is a software tool which gives visual or audio cues for the client to follow as closely as possible: Breathe in, hold, breathe out and hold again. With paced breathing, it becomes relatively easy to slow the client's breathing rate down to below 9 breaths per minute, which shifts the RSA component of HRV down towards the LF band.

Resonance frequency training: Another observation that was made by HRV researchers is that paced breathing at a specific rate of about 6 breaths per minute can significantly increase the power in the LF band in the HRV spectrum. At that breathing rate, it is proposed that the effect of multiple physiological heart rate pacers combines to create a form of resonance within the cardiovascular system. This causes significantly more power to be expanded in the HRV spectrum than the levels seen at any other breathing rate, specifically in the LF band. More power in the HRV spectrum means more heart rate variability and, in turn, more health benefits. There are published articles suggesting that **resonance frequency training** can be beneficial to people who suffer from a number of health problems (Vaschillo, Vaschillo and Lehrer).

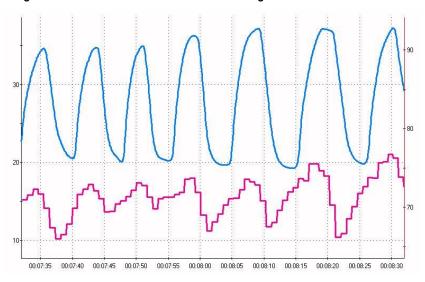
Examples of use

RSA biofeedback

As suggested above, HRV biofeedback training generally involves some form of breathing practice. The most often used training protocol shows the respiration signal and the heart rate signal moving together across the screen. As the client breathes in, the respiration signal goes up and as he breathes out, the signal goes down. As the client learns to breathe slowly and regularly, the signal starts looking like a sine wave. The illustration below shows the respiration signal as a blue line and the heart rate as a pink line. You can count about 11 bumps on the blue line. Because the time scale across the screen is set to one minute, this indicates a breathing rate of around 11 breaths per minute. At this rate, you can see bumps on the heart rate signal, but the correspondence between both signals is not tight.



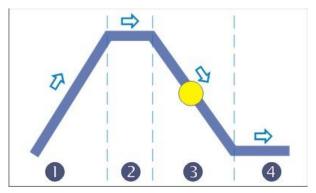
As the client slows down his breathing, you will count less and less breathing waves across the screen. At some point, the red line becomes entrained in the pattern and the heart rate starts increasing with the in breath and decreasing with the out breath. This is RSA training.



When the heart rate signal is in synchrony with the respiration signal, meaning that they move up and down at the same time, this is called heart rate coherence. If the client starts straining or breathing at a rate that is not natural for his/her physiology, you might observe a shift in phase where the two waveforms lose their togetherness or even start going in opposite directions. If this happens, try slightly increasing or decreasing the breathing rate to bring the waves back in synch.

Paced breathing

Sometimes it helps the client learn to breathe more regularly if he can follow the lead given by a respiration pacer. A respiration pacer allows a clinician to set a target breathing rate, say 8 breaths per minute and adjust the duration of each component of the breathing cycle: (1) inhale, (2) hold in, (3) exhale and (4) hold out. Many breathing exercises, for example, require the inhalation duration to be longer than the exhalation duration. During the exercise, the colored dot moves along the blue line from left to right and the client attempts to follow the dot, breathing in when it goes up, holding their breath in while it goes across the top, and so on.



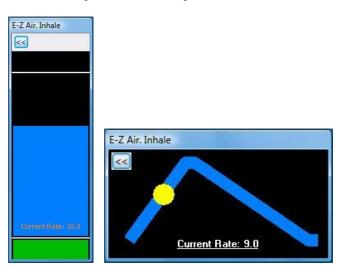
BioGraph Infiniti provides a respiration pacer which has three modes:

Constant, which maintains a single target breathing rate throughout the exercise.

Dynamic, which leads the client from a clinician-defined start rate (i.e., 10 breaths/min) towards a target rate (i.e., 6 breaths/min) over a period of time. Once the target rate is reached, it is maintained until the end of the exercise.

The third mode is called **Adaptive** because it uses the client's actual breathing rate to set the start rate. From that rate, the pacer will slowly adjust its pacing towards the target rate unless the client is unable to follow. Then it will readjust itself based on the client's breathing rate and start again.

The Biofeedback Foundation of Europe (www.bfe.org) offers a simple respiration pacer program, EZ-Air (\$19.95), which has a lot of interesting features, including a dynamic training mode, a bar graph mode and a breathing exercise mode during which the pacer will pop up at prescribed intervals to allow the user to perform short breathing exercises. After the brief exercise, the pacer hides itself again.



Resonance frequency training

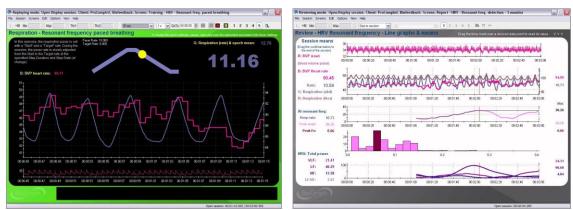
"Resonance" frequency is referred to in earlier research as "resonant" frequency.

Resonance frequency training is a little more involved than simply breathing 6 times per minute because a person's precise resonance frequency is dependent on factors such as height and gender (<u>Lehrer et al</u>). (Research is showing that the resonance frequency is affected by differences in the relative size of the circulatory system and the volume of blood that it contains.) In order to be able to train someone to breathe at the resonance frequency, it must first be determined. The proper method for assessing a person's resonance frequency is described in detail in chapter 10 of the manual <u>Principles and Practice of Stress</u> <u>Management</u>, by Dr. Paul Lehrer and his team.

The BioGraph Infiniti EEG Suite provides a category of screens which integrate physiology measures, including HRV, with EEG measures. The HRV measures incorporated in these screens function very similarly to the screens described below for the Physiology Suite.

The BioGraph Infiniti Physiology Suite incorporates a number of assessment and training screens for HRV. The screen **Training – Resonant freq. paced breathing** uses the respiration pacer's dynamic mode to lead the client, from a start rate of 10 breaths per minute, to a target rate of 6 breaths per minute and decreases its pacing rate by 0.5 breath/minute, every two minutes. While doing so, the program tracks the magnitude of the power spectrum's peak frequency to identify the moment at which the power is maximized. At that moment, the breathing rate is noted. That is the rate at which this client should breathe when doing resonance frequency training.

The review screen shows a line graph with the moment by moment changes in power, at the peak frequency. At the resonance frequency, that line reaches its maximum. By moving the time marker to that point, you can see the breathing rate which generated that amount of power.



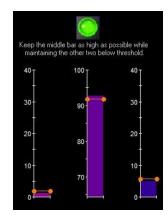
If you would like to determine the client's resonance frequency with higher precision, you can easily modify the respiration pacer's settings to run the same exercise but varying the breathing rate from 7 to 4.5 breaths per minute and decrementing the rate by smaller values, like 0.1 or 0.2 breaths per minute.

Other HRV training options

The Physiology Suite also includes training screens which use other HRV metrics for biofeedback, such as Total Power, Percentage of Total Power (% power), HR Max-Min, SDRR, and others.

The Total Power and Percentage of Total Power

training screens show three bar graphs, one for each of the HRV sub-bands, VLF, LF and HF. As the client practices the prescribed breathing exercise, the bars grow and shrink to reflect shifts in HRV power. The training goal for the client is to allow the LF frequency band – shown in the middle – to grow as much as possible while keeping the other two bars, on the side, as small as possible. When this occurs, audiovisual feedback is given to encourage the client to sustain this condition.



The **HR Max-Min** protocol is based on RSA training. After each breath, a proportional tone is heard indicating if the HR Max-Min value has increased or decreased and the feedback screen rewards higher values. The goal is to try to produce tones that are as high as possible.

The **SDRR** feedback screen trains clients to increase the standard deviation of the IBI value. Since the standard deviation represents the spread of values in a given set of numbers, its value is expected to increase as HRV increases.

A Typical Procedure

Preparing the examining room

Electromagnetic interferences

The Infiniti encoder and the EKG sensor are capable of detecting very tiny electrical signals (millionths of a Volt) generated by the human body. Therefore the system is very sensitive to electromagnetic fields generated by other devices in the room or nearby, such as radio transmitting devices, computer monitors, medical devices (for example x-ray machines) and fluorescent lights. It is a good idea to turn such devices off if they are not needed during the session.

It is also recommended to make sure that your biofeedback instrumentation is kept at least 10 feet away from any radio transmitting devices and 3 feet away from electronic devices (including monitors) and fluorescent, halogen or neon lights.

Finally, disconnect any unused sensors from the encoder (unless they are connected to the client) because otherwise they may act as antennas and capture unwanted signals that would corrupt the signal.

Electrostatic discharges

To prevent static discharge from damaging the sensor and/or encoder, use antistatic mats or sprays in your working area. A humidifier may also be used to help prevent static environments by conditioning hot, dry air.

Preparing the examinee

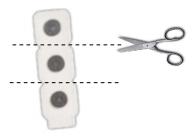
- Explain the procedure to the examinee.
- Identify the physiology you want to monitor (EKG, BVP and respiration) and explain how you will connect and place the sensors.
- If you are using EKG, it is recommended to clean the skin with an alcohol pad so the skin is clean and dry, before you apply the electrodes. If necessary, shave excess body hair. (See *Skin preparation* and *Enhancing electrode contact* in the section *Using the EKG sensor* on page 6.)
- Apply sensors to the examinee as follows:

Connecting the EKG sensor electrodes



As described in the section **Using the EKG sensor** (page 6), we recommend pre-gelled UniGel electrodes (T3425) if signal quality is important (i.e., for data acquisition).

Three UniGels can be snapped to the yellow, blue and black leads of the extender cable and applied to the client's chest or forearms, depending on the preferred electrode configuration.



Single strip electrodes (T3404) can also be used with a little bit of conductive paste. Use them dry for biofeedback sessions where ease of use matters and signal quality is not crucial.

You can cut the strip into single electrodes and snap them to the yellow, blue and black leads of the extender cable. Apply the electrodes to the client's chest or forearms, depending on the preferred electrode configuration.

- Snap the selected electrodes to the leads of the extender cable, as shown in the following illustration.
- Connect the EKG extender cable to the EKG-Flex/Pro sensor, as shown below. Ensure that it is correctly oriented to the encoder.



Correct orientation of the EKG sensor

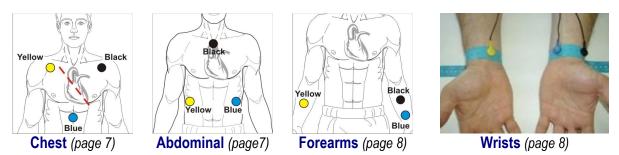
The EKG sensor has removable cables and two input entrances. It is important to orient it correctly to the encoder so that the physiological signal is properly received.

The EKG sensor is correctly oriented to the encoder when the cable joining them is connected to the bottom input of the sensor. The extender cable with electrodes is connected to the top input of the EKG sensor.



Connect to encoder

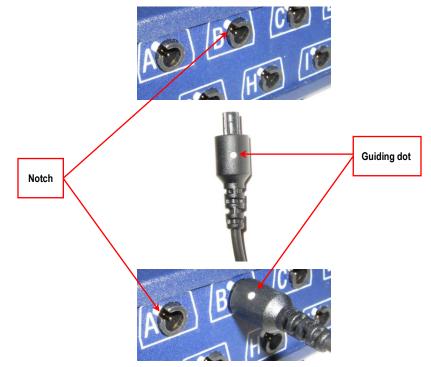
• The electrodes can be applied to the client in any one of four placements:



- If you are using a BVP sensor instead, place it on one of the client's fingers, as shown on page 9.
- Strap the respiration sensor around the client's chest, as shown on page 9.
- Instruct the examinee to relax and to follow the instructions for each task as it is presented.

Connecting the sensors to the encoder

• When connecting a Thought Technology sensor to the encoder, make sure to properly line up the guiding dot on the top of the plug with the notch in the device's input socket.



Be careful: Forcing the plug into the jack in any other position may damage your equipment.

When used with Thought Technology's Physiology Suite or EEG Suite, the **EKG-Flex/Pro** sensor must be connected to **Input A**. The **BVP-Flex/Pro** sensor is always connected to **Input B**. The **Resp-Flex/Pro** sensor strapped around the abdominal area is plugged into **Input G**. If you are using an optional secondary respiration sensor around the chest, it plugs into **Input H**.

Note: These inputs are standardized for the ProComp Infiniti, ProComp5 Infiniti, and FlexComp Infiniti encoders. They don't apply to the ProComp 2 encoder, for which the Suite predefines different inputs.

Starting a session

The first step to recording a session is to launch BioGraph Infiniti.

You can do this by double clicking either on the BioGraph Infiniti desktop icon or on one of the Physiology Suite Favorites shortcuts, which were installed with the suite.



The process of recording and reviewing/reporting sessions is described in the manual *Getting Started with BioGraph Infiniti* (SA7913), located in the Manuals folder which you can find by double-clicking on the BioGraph Infiniti Docs & Editors desktop icon.

When using BioGraph Infiniti, you can also find contextual information in the on-line help documentation. Press the F1 key on your keyboard to open Help from any dialog box in the program.

Before you start

Open BioGraph Infiniti, and select your client and your screen/script.

When you are getting ready to launch a recording, you may want to perform one or more of the following functions:

- Edit a display screen with the Screen Editor: Screens menu
- Verify the battery level: Options menu
- Predefine the statistics for an open display session: Options menu

Recording

From the Main Frame Screen, you can then start recording a session by clicking on the Start button:



Anatomy of an HRV screen

HRV screens in the Physiology Suite are built from a number of common screen components and follow the same overall screen arrangement structure to simplify their use.

A typical screen is shown on the next page. It has been divided into sections corresponding to various screen components. For each section there is also a description, explaining the purpose and function of the screen instruments in that section, and associating them with the HRV concepts contained in this guide.



"Training – HRV - % power, pacer & anim" screen from Thought Technology Physiology Suite

- 1) VLF, LF and HF power bars: These bar graphs show either the total power or the percentage of total power values for the three bands of the HRV frequency spectrum, very low frequency, low frequency and high frequency. The automatic thresholds are set to reward increasing the amplitude of the middle bar (LF) while decreasing the amplitude of the two side bars. The green light turns bright when these three conditions are met.
- 2) Raw signal graph: This graph shows the raw EKG or BVP signal. Even if the graph is fairly small, you can easily see the regular pattern of each beat and detect any occurrence of loss of signal (flat line) or artifact.
- 3) **Respiration pacer:** The pacer can be set to lead the client through breathing exercises. You can adjust the pacer by right-clicking over the instrument and selecting the **Edit Pacer Settings** option.
- 4) RSA Graph: This graph shows 1 or 2 respiration signals (abdominal and, optionally, thoracic) with the heart rate signal. The client can use this display to practice coherence breathing, which is another term for respiratory sinus arrhythmia training where the respiration and heart rate signals are in phase.
- 5) Media instrument: Some feedback screens display an animation while others play a DVD. The media instrument generally provides the client with immediate feedback, by playing the media content when all the success conditions are met (for example, increasing LF while decreasing VLF and HF).

- 6) 2D Spectrum: The spectrum graph shows a two dimensional view of the frequency distribution in the moment by moment HRV power spectrum. When doing resonance frequency training you should expect to see a very tall light blue bar growing in the LF band (dark purple), usually around 0.1 Hz, which is the frequency that corresponds to 6 breaths per minute. The light blue bar indicates the peak frequency, i.e. the tallest bar in the whole spectrum, at any moment. As the client's breathing rate changes, you will see the bar shift along the spectrum.
- 7) Trend instrument: This graph shows long term changes in the session by plotting a periodic average (epoch mean) of the trained signals (in this case, VLF, LF and HF). The trend graph is useful for seeing the client's overall success in achieving the training goals. The same type of graph is usually shown in the review screen so you can see changes across the whole session.

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- 9. EKG placement for artifact free HRV training: Clinical note. Niels Baker, Erik Peper, & Fred Shaffer. The Netherlands, San Francisco State University, USA, Truman University, USA.

Suggested Additional Resources

- 1. Biofeedback: A practitioner's guide, 3rd Edition. Schwartz, Mark S. & Andrasik Frank. (2003). New York: Guilford Press.
- 2. Handbook of Mind-Body Medicine for Primary Care. Moss, D., McGrady, A., Davies, T., Wickramasekera, I. (2003). SAGE Publications.
- Biofeedback Mastery-An Experiential Teaching and Self-Training Manual. Peper, E., Tylova, H., Gibney, K.H., Harvey, R., & Combatalade, D. (2008). Wheat Ridge, CO: AAPB. ISBN 978-1-60702-419-4.
- 4. Biofeedback Tutor (2010). Shaffer, F., Ph.D. Kirksville, MO. Biosource Software.

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