

May 2015 TechTip



### May 2015 Spotlight

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## TechSpot

### Referencing with the Digital Lynx SX and Cheetah 5.6.3

Referencing Flexibility is of key importance when recording extracellular single units and/or LFP/EEG in chronically implanted animals. The software-controlled analog reference selection facilities of the Digital Lynx SX system allow use of multiple references and post-implant reference selection and optimization. It is a feature not possible on systems with a single fixed reference, such as Intan-based headstages.

#### Flexible Referencing of the Digital Lynx SX System

Our Digital Lynx SX with the Digital Reference Selector (DRS-36) board offers many options for referencing. Each DRS-36 Board allows 8 different references to be active at one time for the associated 32 input channels, and also provides the option to drive 8 shared global references across all input boards. Reference options include system ground, animal ground, multiple dedicated references and any recording input channel.

#### Skull Screw Ground Implant during Surgery

A Skull Screw must always be implanted on every subject. The skull screw wire is connected to one of the dedicated ground pinholes on the Neuralynx Electrode Interface Boards (EIB).

This connects the animal's body to the acquisition system ground - and MUST be made to keep the subject and recording electrodes at the same voltage potential as the system.

FNS: French  
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### System Ground (PGnd) and Animal Ground (AGnd) as Starting References

System ground (PGnd) is often a good first choice as a reference for a newly implanted animal before all recording electrodes and references are driven to their “normal” depth. This gives a good initial indication of electrode signals and location.

Using the system ground (PGnd) may not be the best noise reduction reference because any noise signal present on the tether ground wire will result in a common mode noise signal on all channels. This ground wire noise signal is the result of coupled external noise sources plus the voltage drop of the headstage pre-amplifier power supply differential current (~20 amp between +/- 5V amplifier power lines) times the resistance of the ground wire (<10 ohms). It is possible to see a noise voltage of 10 to 80 micro-volts between the system ground and the animal's EIB ground.

### Animal Ground (AGnd) Reference Tether Wire Signal

Most Neuralynx tethers and headstages have a separate “non current carrying ground wire” called Animal Ground (AGnd). AGnd is connected to the headstage ground (and thus the EIB and animal's skull screw) and a high impedance buffer amplifier on the DRS-36 to eliminate any current flow in the wire (< 1nA). Since virtually no current flows through this wire, there is no voltage drop and is therefore an accurate representation of the true voltage potential of the subject's skull screw for improved signal referencing purposes.

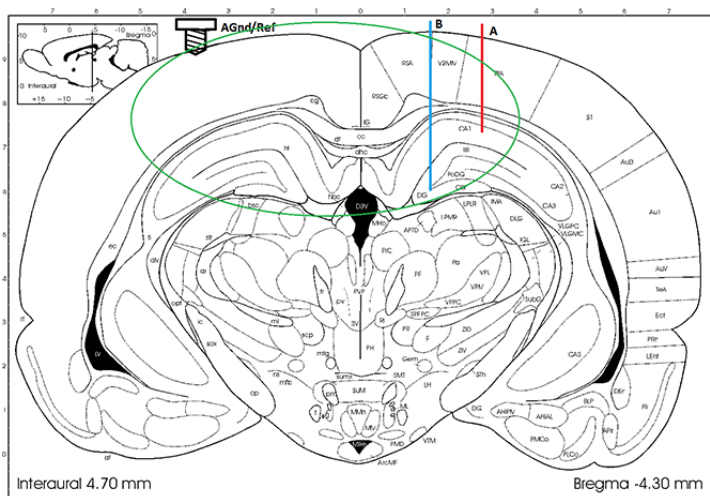


Figure 1

### Dedicated Reference Wires

When recording from very distant brain areas, it is usually possible to improve the quality of the recorded signal (signal-to-noise ratio) by using a dedicated reference electrode. One way to achieve such a result is to use references across the

electrodes. This method consists of selecting a channel with very low neural activity (a quiet channel) to be used as the reference for other channels (cross-referencing), providing a significant reduction of the local background noise and an improved prominence of the neural activity. The two signals will be similar to each other being generated from the same neural network. The reduced noise will improve Spike Sorting results.

As mentioned above, Cheetah acquisition software allows for each type of acquisition entity (Continuous Sampled Channel, "CSC" and Spike, "SE" "ST" "TT") to select references individually, up to a maximum of 8 for each DRS board. This provides a large range of options to select a quiet channel (Figure 2).

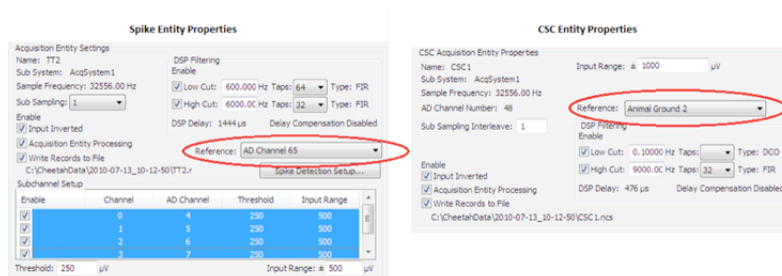


Figure 2

In case of multi-site recordings where electrodes are placed in distinct and anatomically separated brain regions, the use of dedicated reference electrodes may become very important, especially if researchers are interested in examining the relationships between firing units and the LFP oscillations of the underlying network. In these cases, the cross-referencing method is still applicable. As an alternative, additional reference signals obtained from wires added to the implant for the electrode can be used to further specify the reference source location so that the oscillatory waves and the firing activities can be directly analyzed to highlight localized patterns of activation (Figure 3).

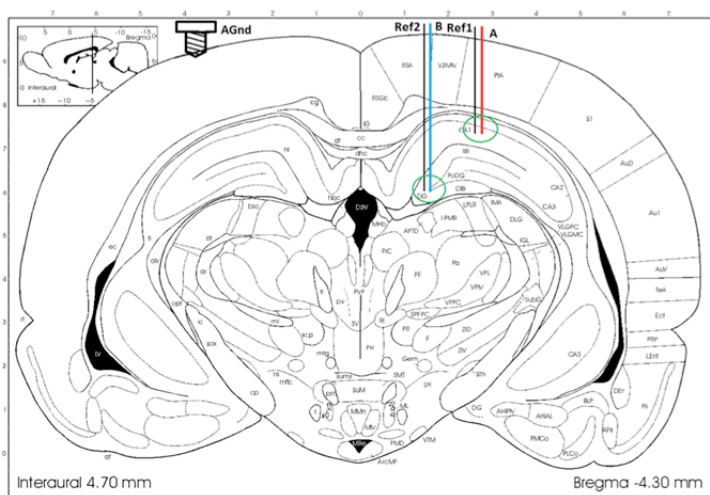


Figure 3

The addition of dedicated reference electrodes used as local references is quite straight forward using the Neuralynx hardware: EIBs and Headstages are designed to carry additional reference channels for this function (Figure 4).

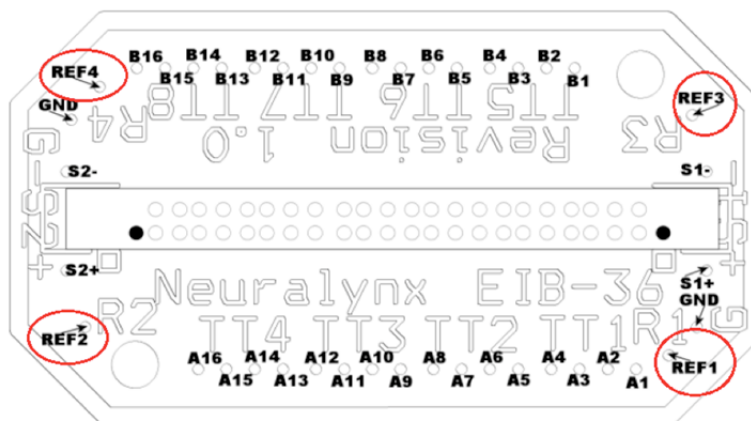


Figure 4

The activities recorded using local references are obtained independently from the other brain areas which allows for a direct comparison between electrode groups to show patterns of synchronous or asynchronous oscillations that may represent higher levels of neural processing.

Using these techniques provides easy “tuning” of references to obtain better signals and controlled recording of desired signals directly without the need for complex software reference subtraction processing. Additionally, the controllable reference selection can avoid catastrophic results from reference or ground electrode degradation or failure.



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