EVOLOCUS Advertising Feature

Neurologger 3 and its history

Neurologger has been originally designed to record EEG, local field potentials and neuronal activity in freely moving animals in their natural environments. Later its spectrum of applicability was extended to EMG and ECG recording. Recently Neurologger has been applied to study of auditory communication in animals and, thanks to increased up to 200 kHz sampling rate in single-channel mode, to study of ultrasonic echolocation in bats. Today Neurologger is represented by two versions: Neurologger 2A/2B and Neurologger 3. Neurologger 2A/2B remains our lightest version (1.3 g without battery) and it is capable to record up to 4 channels at 33.3 kHz, 10-bit into soldered on board memory (1 or 2 GB). Neurologger 3 is slightly heavier (1.7 g without battery), it records up to 32 channels at 20.8 kHz, 16-bit into microSDXC card (4-256 GB) and it has remote control and data access through BlueTooth communication with Windows 10 machines.

The first version of the device engineered in 2002 was capable to record up to 8 EEG channels at 500 Hz or up to 2 neuronal channels at 10 kHz (Vyssotski *et al.*, 2006¹). The data were stored at Secure Digital (SD) memory card with the capacity up to 32 GB. However, because of its size $(66 \times 36 \times 10 \text{ mm})$ and weight (22 g) the logging unit was attached at the back of the animal and was connected to the head with the cable. The last was not really convenient. For this reason the first Neurologger version is currently used only with the large animals attached to the head (Lesku *et al.*, 2011⁶; Lyamin *et al.*, 2012¹⁰).

To have easy recording of EEG and neuronal activity in small animals, the second miniature version of Neurologger has been designed in 2005. Significant decrease of size $(22 \times 15 \times 5 \text{ mm})$ and weight (2 g) allowed us to attach the unit directly to the head of laboratory mice and flying homing pigeons (Vyssotski *et al.*, 2009³). Neurologger 2 was capable to record up to 4 channels at sampling rate up to 9.6 kHz in its soldered 256 MB memory. This version has been successfully used in the set of studies (Rattenborg *et al.*, 2008²; Pang *et al.*, 2009⁴; Brankack *et al.*, 2010⁵). Starting from 2009 the next modification of the logger called Neurologger 2A has been developed. Standing on successful concept of Neurologger 2, the novel version has got a set of new features. One of them is precise real time infrared (IR) synchronization of the record in the logger with the external

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events. Synchronizing labels can be sent manually by an operator or automatically by a computer. Specialized unit for sending these labels is called "Neurologger Synchronizer". Its features are described in separate documentation (see **Supplementary Information**). The second feature is recording of 3-D acceleration. The default sampling rate of accelerometer is 400 Hz – it has been found sufficient for most cases. However, the sampling rate can be increased up to 1 kHz if needed. In



Figure 1 | Neurologger 3 (**a**) and Neurologger 2A/2B (**b**). Shown Neurologger 3 (**a**) has 32 channels and 128 GB Micro SDXC card (Samsung). Both Neurologger 3 and Neurologger 2A/2B have infrared (IR) sensor to receive information from external equipment, sent by infrared (IR) emitter (e.g. processed signal from a video camera, for example – animal "track"). Two black "eyes" on the top of Neurologger 2A/2B (**b**) are optical elements of IR sensor. Neurologger 2B differs from Neurologger 2A by increased sampling rate (33.3 kHz vs. 19.2 kHz) and several added modes, including single-channel 200 kHz mode.



Figure 2 | Neurologger 3, an example of its memory (Samsung 128 GB Micro SDXC) and two examples of its battery. Batteries of different capacities can be used depending on desired record duration. Two examples of batteries of different weight/capacity are shown. The logger needs only one battery. Note that the duration of record is usually limited by the battery and not by the memory size.

addition, memory capacity has been increased up to 1 GB (2 GB by request) and maximal electrophysiological data sampling rate was increased up to 19.2 kHz, 4 channels. The last allowed us to use this unit for studying vocal communication in birds (Anisimov *et al.*, 2014^{22}). Version 2A is the most popular Neurologger version that was used in 20 publications including publication in *Science*⁹.

The next version Neurologger 2B form 2015 is enhanced version of Neurologger 2A. The maximal sampling rate of all four channels was increased up to 33.3 kHz (from to 19.2 kHz in the Neurologger 2A). This was done for recording of vocal communication in some animals whose vocalization spectrum exceeds 9.6 kHz. In addition, special modification of Neurologger 2B records single channel data with frequencies up to 200 kHz. This feature was added for studying echolocation in bats, but it also can be used for investigation of ultrasonic communication in rodents (mice and rats).

The necessity to record multichannel neuronal data leaded to manufacturing the third version of Neurologger in 2016. Neurologger 3 has been designed to record 16 or 32 neuronal channels having size and weight similar to the second version. However, neuronal activity usually should be correlated with animal behavior. To record vocalization of the animal, an audio cascade with a microphone capable of recording frequencies up to 100 kHz (200 kHz sampling rate) has been added.

Basically, an idea to record ultrasonic vocalizations has been inherited from the previous version of the Neurologger 2B. However, contrary to the previous model, the novel version allows us to record ultrasound simultaneously with 32-channel neuronal activity.

In addition, animal behavior can be tracked by an array of inertial sensors (3-D accelerometer and 3-D gyroscope) complemented by 3-D magnetic compass. All sensors can be polled with the frequencies up to 600 Hz, simultaneously with recording of neuronal and ultrasonic data.

The Neurologger 3 is designed to record neuronal activity, LFPs or EEG from up to 32 electrodes. The maximal sampling rate is 20.833 kHz per channel in 32-channel version and 25 kHz in 16-channel. The frequency band of electrophysiological activity recording is freely configurable in 32-channel version and factory configurable in 16-channel version. Neuro-recording part is based on Intan RHD2132 and RHA2116 chips (32- and 16-channel versions respectively). These chips are known to be the best in the market. Sound recording is normally realized by 12-bit 200 ksps ADC of the microcontroller of the Neurologger. However, one also can use one channel of 16-bit Intan chip to record sound, but its frequency band will be limited by Intan settings common for all channels. One should note that if maximal number of channels (32) and maximal neuronal sampling rate 20.833 kHz are used, sampling rate of audio channel can't exceed 125 kHz. To sample the microphone channel with the maximal frequency 200 kHz one has to decrease neuronal sampling rate to 15.625 kHz (in 32-channel mode). The inertial sensors and magnetometer (3-D accelerometer + 3-D gyroscope + 3-D compass = "9-D" motion sensors) can be sampled in the background of all these modes with the sampling rate about 600 Hz. Resolution of all sensors is 16 bit. Ranges are software configurable. Resuming, the following two modes can be recommended:

- 32 neuronal channels 20.833 kHz, sound 125 kHz, motion sensors 579 Hz. Dataflow to memory: 1.77 megabytes per second.
- 32 neuronal channels 15.625 kHz, sound 200 kHz, motion sensors 625 Hz. Dataflow to memory: 1.60 megabytes per second.

The logger consumes about 25 mA from 3.7 V Lithiumpolymeric battery in these modes.

Weights of the logger parts without neuronal recording board are shown in the **Fig. 2**. The scale is in centimeters.



Figure 3 Neurologger 3 with its headstage and memory. To record neuronal activity the neuronal headstage should be attached to the processor module how it is shown in this picture (**a**). The 32-channel headstage with Hirose connector is placed at the bottom side (invisible). Then, the memory card is placed above the headstage (**b**). This combination weighs 1.73 g.



Figure 4 | Four neuronal headstages, available for Neurologger 3. From the left to the right: 16-channel Hirose, 32-channel Hirose, 16-channel Omnetics and 32-channel Omnetics. Both Omnetics and Hirose types are pin compatible with Neuronexus silicon probes. Please see Neuronexus Internet side for the pin layouts. Omnetics connectors are larger and they are more widespread. Many companies produce electrodes with this type of connectors. Their benefit is the mechanical strength; no additional fixation of the logger on the head is needed if Omnetics connectors are used. Hirose connectors are smaller, but they need a special clamp that would push two opposite parts to each other for reliable fixation.

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One of the most important parameters of neuronal recording system is internal noise of the amplification cascade that should be as small as possible. Also, no disturbances should penetrate to the high-impedance electrode inputs. The following sample of record shows signal recorded by the Neurologger 3 with 32-channel headstage. In this test all channels except one were connected to the signal generator producing 10 μ V rectangular pulses with frequency 5 Hz. The last top-most channel was connected to the microphone to record environmental noise. The recording cascade was configured for the frequency band 1-7500 Hz (band-pass filter). As one can see, 10 μ V steps are clearly visible (**Fig. 5**). The internal noise of the amplification cascade is about 2 μ V RMS.

However, the system also should be capable to record signals from the high-impedance sources. To test this capability of our recording system, we provided 10 μ V signal from the signal generator to the logger though resistors of nominal 10 k Ω , 100 k Ω and 1 M Ω . The following three charts show examples of records obtained with the listed above impedances of neuronal recording electrodes (**Fig. 6**). One can see that the record with 10 k Ω resistor is practically indistinguishable from the signal recorded from the low-impedance signal source directly. An increase of the source impedance up to 100 k Ω and 1M Ω increases the background noise as expected. However, even in the case of 1 M Ω source the 10 μ V steps are clearly visible in the recording, because typical spike size recorded from 1 M Ω electrodes is usually about 100 μ V or more.



Figure 5 Noise and electromagnetic disturbances in the Neurologger 3. Neurologger 3 has extremely low noise and electromagnetic disturbances in its frequency range F = 1-3300 Hz. The original signal recorded in the frequency band 1-7500 Hz was additionally low-pass filtered at 3300 Hz frequency. The duration of shown fragment is 1 second. A sequence of 10 μ V peak-to-peak rectangular pulses was given to the input of the logger either directly (chart above) or through 10 K Ω , 100 K Ω or 1 M Ω resistors (**Fig. 6**). The last (top) channel was connected to the microphone to record environmental sounds. The 10 μ V step is clearly visible in all channels, except the last (top) one.



Figure 6 Noise and electromagnetic disturbances in the Neurologger 3 when a sequence of 10 μ V peak-to-peak rectangular pulses was given to the input of the logger through 10 K Ω (**a**), 100 K Ω (**b**) or 1 M Ω (**c**) resistors. All other conditions are identical to the conditions of the record in the **Fig. 5**.

	Neurologger 1	Neurologger 2A/2B	Neurologger 3
Primary usage	EEG, EMG and LFPs recording in large animals: marine mammals, ruminants	EEG, EMG and LFPs recording in mice and larger animals; ECG, vocalization recording including ultrasonic	Multichannel neuronal recording in mice and larger animals; vocalization recording including ultrasonic
Number of channels	8 differential channels	4 channels freely assigned to two referent electrodes	16 or 32 channels with one referent electrode; or 16 differential channels
ADC resolution	10 bit; 2x oversampling in low-frequency modes	10 bit; 2x-8x oversampling in low- frequency modes	16 bit
Sampling rate	8 channels up to 800 Hz; or 2 channels up to 10 kHz; or 1 channel 20 kHz; higher sampling rates by request	Version 2A: 4 channels up to 19.2 kHz Version 2B: 4 channels up to 33.3 kHz; or 1 channel up to 200 kHz	 32-channel version: 32 channels up to 20.8 kHz; free selection of channel sequence; selected channels can be sampled more often than others 16-channel version: Fixed sequence of 16 channels up to 25 kHz
Locomotion recording	Optional analog 3-D accelerometer occupies three channels	Optional 3-D accelerometer	3-D accelerometer, 3-D gyroscope, 3-D magnetic compass
Vocalization recording	-	Optional microphone and contact microphone are connected to neurophysiological channels; optional dynamic range expansion	Dedicated 12-bit 200 ksps microphone ADC works simultaneously with neuronal 16-bit ADC; optional dynamic range expansion; attachment of a microphone to one 16-bit channel is also possible
Expansion possibilities	Asynchronous serial bus up to 1.5 Mbps (UART) and digital input/output lines at the main CPU can be custom programmed by request.	Dedicated communication controller with different peripheral interfaces is connected to 8 Mbps synchronous bus (SPI). It can be custom programmed by request.	Inter-integrated circuit (I2C) communication bus 400 kbps makes possible chained connection of multiple custom-developed peripheral devices. Development of the following peripheral units is planned or will be done by request: optical and electrical brain stimulators, motorized microdrive, GPS. 32-ch version has 3 auxiliary analog inputs and one digital output.
Data memory	Micro-SD high-capacity (4-32 GB) memory card	Soldered memory chip 1-2 GB	Micro SD high-capacity (4-32 GB) or extended capacity (64-256 GB Micro SDXC) memory card
Maximal memory filling speed	30 kBps (2 channels, 10 kHz)	300 kBps (1 channel, 200 kHz)	1.77 MBps (32 channels 20.8 kHz, sound 125 kHz, motion sensors 580 Hz)
Maximal recording duration	Limited by the battery	1 GB will be filled when 4 channels are sampled with the frequency: 100 Hz: 20 days 17 h 400 Hz: 5 days 4 h 1600 Hz: 1 day 7 h 9.6 kHz: 5 h 10 min 19.2 kHz: 2 h 35 min 33.3 kHz: 1 h 29 min One channel at 200 kHz: 59 min 39 s 3D accelerometer increases volume by 50% in low-frequency modes.	In most cases limited by the battery. 128 GB card is sufficient for recording during 20 hours in the highest data rate mode listed above. If only 32 channels are sampled with the frequency 15.625 kHz, 128 GB is sufficient for 31 hours.
Current consumption	~5.5 mA in EEG mode	Version 2A: 1.5 - 4.3 mA, mode- dependent; In EEG mode with 3D accelerometer. 2.0 mA Version 2B: in high-frequency modes (≥33.3 kHz): 6.0 - 11.7 mA, mode- dependent	11-25 mA, mode-dependent; All neuronal modes ~25 mA.
Logger size (w/o battery)	36 x 31 x 6 mm	From 18 x 15 x 3 to 22 x 15 x 8 mm	From 20 x 15 x 6 mm to 24 x 15 x 8 mm
Logger weight (w/o battery)	5.31 g	0.95 - 1.71 g, version-dependent	1.29 – 1.96 g, version-dependent
Recommended batteries and their weights	Lithium-polymeric 3.7 V 240 mAh 9.0 g rechargeable battery will be sufficient for 1 day 19 h. Non-rechargeable 3.6 V 1200 mAh 8.9 g LS14250 will be sufficient for 9 days.	A couple of non-rechargeable Zn-Air 1.4 V batteries ZA 10 (75 mAh), ZA312 (175 mAh), ZA13 (305 mAh) with the weights per pair 0.635, 1.02, 1.66 g respectively will be sufficient for 1, 2 and 4 days of EEG recording. Lithium-polymeric batteries, for instance 3.7 V 12 mAh 0.38 g GM300910, also can be used.	Lithium-polymeric 3,7 V 20 mAh, 0.63 g 40 mAh, 1.05 g 50 mAh, 1.58 g Will provide duration of neuronal recording of 15 min, 1 h 15 min and 2 h 15 min respectively.

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Additional information

Supplementary Information accompanies this advertising feature at http://www.evolocus.com/evolocus/v1/evolocus-01-I-s.pdf

Competing financial interests: Neurologger is a trademark, registered in the USA, #3776356. Neurologger 2A/2B and Neurologger 3 are protected by U.S. patents #8,160,688 and #9,492,085 (both patents are applicable to Neurologger 2A/2B and to Neurologger 3). Other patents are pending.

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