Auditory Brain Stem Response IMPC_ABR_002

Purpose

To estimate hearing sensitivity and other physiological parameters, the sound-evoked potentials known as auditory brainstem response (ABR) are measured under anesthesia and analyzed.

Ontological description: MP:0004738 - abnormal brainstem auditory evoked potential.

Experimental Design

Minimum number of mutant animals: 4 mice.

Age of animals at test: 14 weeks (±3 days).

Sexual dimorphism: Gender unimportant (sexual dimorphism is not expected to be a factor).

Equipment

1. Audio signal generators, amplifiers and high-performance speakers

2. Calibration equipment (microphone, etc)

3. EEG Needle electrodes

4. Biological amplifier & headstage

5. Data acquisition hardware

6. Software to control signal presentation and data acquisition

7. Data Analysis software/database application

8. Sound Attenuating room

9. Hot plate

Procedure

1. Test mouse with click box. Is the Preyer Reflex present? Optional.

2. Anesthetize the mouse.

3. Insert sub dermal needle electrodes; ground electrode on vertex; reference electrode overlying left bulla; active electrode overlying right bulla. Place mouse on restrainer plate in a prone position inside a sound attenuating room.

4. Put the speaker sound transmission duct on the mouse external auditory foramen and insert tip of the duct to make certain they are connected closely.

5. Record a click-evoked ABR (70dB SPL) to ensure a good ABR is present (in non-impaired mice). Optional.

6. ABRs are recorded to clicks (1µs duration, positive transient) presented from 0-85 dB SPL in 5dB steps, presented 256 times at 42.6/sec.

7. ABRs are recorded to the following frequencies and levels; 6kHz (0-85dB SPL), 12kHz (0-85dB SPL), 18kHz (0-85dB SPL), 24kHz (0-85dB SPL) and 30kHz (0-85dB SPL), presented in 5dB intervals. Tone pips are 1ms in duration, with a 0.2 ms rise/fall time, presented 512 times at 42.6/sec (optional values). Tone stimuli are presented in decreasing frequency order for a

particular sound level and from low to high stimulus level.

8. If deafness/hearing impairment is suspected for a particular mutant line (e.g. by elevated thresholds or absence of ABR waveforms at any stimulus level), all stimulus presentation levels should be, instrument permitting, extended to 95dB SPL.

9. Record a final click-evoked ABR (70dB SPL), to check for any deterioration of the click-evoked response during recordings. Optional.

Notes

If other tests are being performed under the same anesthetic regime, it is advised to perform the ABR first.

Raw data is uploaded to a database for display of waveforms and threshold allocation for each mouse and for display of plots of threshold for each frequency and click for each individual mouse and for each mutant line.

Detailed methodology can be found in Ingham, Pearson & Steel (2011) Current Protocols in Mouse Biology 1: 279-287.

Threshold Analysis (Phase 1). Thresholds are defined for each mouse and for each stimulus as the lowest intensity at which any part of the ABR waveform can be visually recognized by a trained operator. Calls are made on data from each line based on a set of rules. For each stimulus, over 60% or more of thresholds must fall outside of a 95% reference range (based on a large population of wildtype data) for that result to be classed as significant. A manual call option can be used by an experienced operator to include or not include a particular dataset, to override the rules-based call. A line is called as significant if any one of the click or frequency stimuli are called as significant.

Waveform analysis (Phase 2 - Optional). Overlay plots of click-evoked waveforms recorded at threshold +20dB and +50dB are viewed by experienced operators to determine if there are any obvious abnormalities in waveform shape.

Sound System Calibration. Optionally a calibration curve is recorded at the start of each experimental day. These can be used to check the consistency of the sound delivery system over time. Once or twice annually, the calibration of this microphone used for sound delivery should be checked using a calibration instrumentation suitable for use with high frequencies.

Test Click ABR. Optionally the amplitude of the responses to the 70dB SPL click recorded at the start and end of the protocol can be compared to ensure there is no significant physiological deterioration of response.

ABR thresholds. Thresholds are allocated to each stimulus for each mouse by a trained & skilled operator recording the data. A random selection of thresholds is checked by a second skilled operator.

Parameters

	Version	Type	Increment	Option	Derived	Unit	Data Type
Body weight IMPC_ABR_001_001	1.4	simpleParameter				g	FLOAT
Click-evoked ABR threshold IMPC_ABR_002_001	1.3	simpleParameter				dB SPL	FLOAT
Click-evoked ABR waveforms (numerical format) IMPC_ABR_003_001	1.0	seriesParameter					FLOAT
6kHz-evoked ABR Threshold	1.3	simpleParameter				dB SPL	FLOAT

	Version	Type	Increment	Option	Derived	Unit	Data Type
IMPC_ABR_004_001							
6kHz-evoked ABR waveforms (numerical format) IMPC_ABR_005_001	1.0	seriesParameter		No			FLOAT
12kHz-evoked ABR Threshold IMPC_ABR_006_001	1.3	simpleParameter				dB SPL	FLOAT
12kHz-evoked ABR waveforms (numerical format) IMPC_ABR_007_001	1.0	seriesParameter		No			FLOAT
18kHz-evoked ABR Threshold IMPC_ABR_008_001	1.3	simpleParameter				dB SPL	FLOAT
18kHz-evoked ABR waveforms (numerical format) IMPC_ABR_009_001	1.0	seriesParameter		No			FLOAT
24kHz-evoked ABR Threshold IMPC_ABR_010_001	1.3	simpleParameter				dB SPL	FLOAT
24kHz-evoked ABR waveforms (numerical format) IMPC_ABR_011_001	1.0	seriesParameter		No			FLOAT
30kHz-evoked ABR Threshold IMPC_ABR_012_001	1.3	simpleParameter				dB SPL	FLOAT
30kHz-evoked ABR waveforms (numerical format) IMPC_ABR_013_001	1.0	seriesParameter		No			FLOAT
Click-evoked + 6 to 30kHz tone waveforms (pdf format) IMPC_ABR_014_001	1.2	mediaParameter		No			IMAGE
Audiograms IMPC_ABR_015_001	1.2	seriesParameter				dB SPL	FLOAT
Preyer Reflex IMPC_ABR_016_001	1.0	simpleParameter		Yes / No			TEXT
Click +20dB waveforms (numerical format) IMPC_ABR_017_001	1.0	seriesParameter		No			FLOAT
Click +50dB waveforms (numerical format) IMPC_ABR_018_001	1.0	seriesParameter		No			FLOAT
Click +20dB+50dB waveforms (pdf format) IMPC_ABR_019_001	1.2	mediaParameter		No			IMAGE
P1-N1 amplitude (at each stimulus level) IMPC_ABR_020_001	1.1	seriesParameter		No		uV	FLOAT
P3-N3 amplitude (at each stimulus level) IMPC_ABR_021_001	1.1	seriesParameter		No		uV	FLOAT
P1 Latency (at each stimulus level)	1.1	seriesParameter		No		ms	FLOAT

	Version	Type	Increment	Option	Derived	Unit	Data Type
IMPC_ABR_022_001							
N1 Latency (at each stimulus level) IMPC_ABR_023_001	1.1	seriesParameter		No		ms	FLOAT
P3 Latency (at each stimulus level) IMPC_ABR_024_001	1.1	seriesParameter		No		ms	FLOAT
N3 Latency (at each stimulus level) IMPC_ABR_025_001	1.1	seriesParameter		No		ms	FLOAT
P1-P3 Interval (at each stimulus level) IMPC_ABR_026_001	1.1	seriesParameter		No		ms	FLOAT
N1-N3 Interval (at each stimulus level) IMPC_ABR_027_001	1.1	seriesParameter		No		ms	FLOAT

Metadata

	Version	Type	Increment	Option	Derived	Unit	Data Type
Range of test stimuli used IMPC_ABR_028_001	1.1	procedureMetadata		Click,6,12,18,24,30 kHz			TEXT
Range of stimulus levels used - Click IMPC_ABR_029_001	1.1	procedureMetadata		0-88		dB SPL	TEXT
Range of stimulus levels used - 6kHz IMPC_ABR_030_001	1.1	procedureMetadata		0-88		dB SPL	TEXT
Range of stimulus levels used - 12kHz IMPC_ABR_031_001	1.1	procedureMetadata		-6.8-88		dB SPL	TEXT
Range of stimulus levels used - 18kHz IMPC_ABR_032_001	1.1	procedureMetadata		0-80		dB SPL	TEXT
Range of stimulus levels used - 24kHz IMPC_ABR_033_001	1.1	procedureMetadata		0-90		dB SPL	TEXT
Range of stimulus levels used - 30kHz IMPC_ABR_034_001	1.1	procedureMetadata		0-85		dB SPL	TEXT
Extension of stimulus levels IMPC_ABR_035_001	1.0	procedureMetadata		10		dB SPL	INT
Stimulus level step size IMPC_ABR_036_001	1.1	procedureMetadata		5		dB SPL	INT
Tone Pip Duration IMPC_ABR_037_001	1.0	procedureMetadata		1		ms	INT

	Version	Type	Increment	Option	Derived	Unit	Data Type
Tone Pip rise/fall IMPC_ABR_038_001	1.0	procedureMetadata		0.2		ms	INT
Repetition Rate IMPC_ABR_039_001	1.0	procedureMetadata		10		s^-1	FLOAT
Number averages IMPC_ABR_040_001	1.0	procedureMetadata		512			INT
Recording Environment IMPC_ABR_041_001	1.0	procedureMetadata		Sound proof room			TEXT
Anesthetic agent 1 IMPC_ABR_042_001	1.0	procedureMetadata		Pentobarbital			TEXT
Anesthetic agent 2 IMPC_ABR_043_001	1.0	procedureMetadata		None			TEXT
Anesthetic agent 1 dosage IMPC_ABR_044_001	1.0	procedureMetadata		0.08		mg/g	FLOAT
Anesthetic agent 2 dosage IMPC_ABR_045_001	1.0	procedureMetadata		0		mg/g	FLOAT
Anesthetic administration route IMPC_ABR_046_001	1.1	procedureMetadata		Intraperitoneal			TEXT
Time of injection IMPC_ABR_048_001	1.2	procedureMetadata					TIME
Equipment ID IMPC_ABR_049_001	1.0	procedureMetadata					TEXT
Equipment manufacturer IMPC_ABR_050_001	1.0	procedureMetadata		TDT (Tucker Davis Technologies)			TEXT
Equipment model IMPC_ABR_051_001	1.0	procedureMetadata		RP2.1 based system, RA4PA Medusa Preamplifier			TEXT
Software IMPC_ABR_052_001	1.0	procedureMetadata		Sanger bespoke Tucker Davis Technologies			TEXT
Experimenter ID IMPC_ABR_053_001	1.0	procedureMetadata					TEXT
Date equipment last calibrated IMPC_ABR_054_001	1.2	procedureMetadata					DATE