ABR full HAS_ABR_002

Purpose

Auditory brainstem response test determines hearing sensitivity and other physiological parameters using evoked potential recordings in anesthetized mice.

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

Experimental Design

Age of animals at test: 14 and 39 weeks.

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

Equipment

1.Audio signal generators, amplifiers and loudspeakers

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 chamber

9.Heating blanket

Procedure

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

3. Place mouse on heating blanket in sound chamber and insert sub dermal needle electrodes; active electrode on vertex; reference electrode overlying left bulla; ground electrode overlying right bulla (See Figure 1).
4. Place mouse unrestrained in a prone position, nose forward, at the calibrated distance from the leading edge of the speaker to the mouse's interaural axis, on a thermostatically controlled blanket, inside a sound attenuating booth.
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 (10μs 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 5ms in duration, with a 1ms rise/fall time, presented 256 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.
10.

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 (See Figure 2) 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.

The information about the date of the experiment, that is the date when the measurement is performed, is an important parameter which is to be submitted in the Experiment xml file (dateOfExperiment="2013-02-28").

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.

Input-Output functions (Phase 3 - Optional). If the waveforms appear abnormal, plots of P1-N1 and P3-N3 amplitude, P1, N1, P3 and N3 latency and P1-P3 and N1-N3 interpeak interval for click stimuli against dB sensation level (dB above click threshold) are generated. For each parameter, if 60% or more of values fall outside a 95% reference range for 5 adjacent sensation levels, the parameter is called as significant. Again, a manual override option allows operator discretion in judging calls made by these fixed rules.

Data QC

1. **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 Bruel & Kjaer PULSE system with a Type 4231 calibrator calibrator or other equivalent calibration instrumentation suitable for use with high frequencies.

2. **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.
3. **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 and Metadata

Body weight HAS_ABR_001_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: g

Description: body_weight

Threshold measurement HAS_ABR_002_001 | v1.3

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: dB SPL

Description: 9040_db_threshold

Increments: broadband click, 8 khz, 16 khz, 32 khz,

General comments HAS_ABR_003_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Description: general_comments_about the mouse

Equipment ID HAS_ABR_004_001 | v1.2

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Description: equipment_name

Equipment manufacturer HAS_ABR_005_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: equipment_manufacturer

Options: TDT, IHS,

Equipment model HAS_ABR_006_001 | v1.1

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Description: equipment_model

Options: RP (OLD), RZ (NEW), SMART EP,

Anaesthetic reversal HAS_ABR_008_001 | v1.1

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: anaesthetic_reversing_agent

Options: Yes, No,

Date of procedure HAS_ABR_009_001 | v1.2

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: date_of_measurement

PIL number HAS_ABR_010_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

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Tone pips repetition rate HAS_ABR_012_001 | v1.2

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Unit Measured: s^-1

Description: repetition_rate

Options: 42.6,

Date equipment last calibrated HAS_ABR_015_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Description: date_equipment_last_calibrated

QC user HAS_ABR_016_001 | v1.1

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

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Anesthetic agent 1 HAS_ABR_017_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Description: anesthetic_agent_1

Options: Ketamine,

Anesthetic agent 2 HAS_ABR_018_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Description: anesthetic_agent_2

Options: Xylazine,

Anesthetic agent 1 dosage HAS_ABR_019_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Unit Measured: mg/g

Description: anesthetic_agent_1_dosage

Options: 1,

Anesthetic agent 2 dosage HAS_ABR_020_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Unit Measured: mg/g

Description: anesthetic_agent_2_dosage

Options: 0.01,

Click repetition rate HAS_ABR_021_001 | v1.1

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Options: 21.1,

Software HAS_ABR_013_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: Biosig RP, Biosig RT, IHS smart EP,

Procedural comments HAS_ABR_014_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false