Auditory Brain Stem Response IMPC_ABR_002

Purpose

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


Experimental Design

- Minimum number of animals: 4 mutant animals of the same zygosity but of any sex
- Age at test: Week 14
- Sex: We do not expect the results of this test to show sexual dimorphism

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.

Figure 1. To indicate positioning of sub-dermal needle electrodes for ABR recording.

a. Active electrode in position on the vertex. b. Reference/Earth electrode positioned behind the ear.

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.


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.
**Figure 2. Click-evoked ABRs.** In this case, threshold was determined to be 25dB SPL. The points on the waveform defined as P1, N1, P3 and N3 are indicated by arrows.

**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 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.

**Metadata Parameters examples**

<table>
<thead>
<tr>
<th>Metadata</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of test stimuli used</td>
<td>The array of stimuli used to record ABRs. E.g. Click; 6,12,18,24,30kHz.</td>
</tr>
<tr>
<td>Range of stimulus levels used - Click</td>
<td>The range of stimulus levels used for click. E.g. 0-85dB SPL.</td>
</tr>
<tr>
<td>Range of stimulus levels used - 6kHz</td>
<td>The range of stimulus levels used for tone at 6kHz. E.g. 20-85dB SPL.</td>
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<tr>
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<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Range of stimulus levels used - 12kHz</td>
<td>The range of stimulus levels used for tone at 12kHz. E.g. 0-85dB SPL.</td>
</tr>
<tr>
<td>Range of stimulus levels used - 18kHz</td>
<td>The range of stimulus levels used for tone at 18kHz. E.g. 0-85dB SPL.</td>
</tr>
<tr>
<td>Range of stimulus levels used - 24kHz</td>
<td>The range of stimulus levels used for tone at 24kHz. E.g. 10-85dB SPL.</td>
</tr>
<tr>
<td>Range of stimulus levels used - 30kHz</td>
<td>The range of stimulus levels used for tone at 30kHz. E.g. 20-85dB SPL.</td>
</tr>
<tr>
<td>Extension of stimulus levels</td>
<td>The extension of the stimulus level used if any, due to suspected hearing impairment. E.g. 95 dB SPL.</td>
</tr>
<tr>
<td>Stimulus level step size</td>
<td>The frequency increase step size of the stimulus level. E.g. 5dB.</td>
</tr>
<tr>
<td>Tone Pip Duration</td>
<td>The duration of the tone pip. E.g. 5ms.</td>
</tr>
<tr>
<td>Tone Pip rise/fall</td>
<td>The rise/fall time of the tone pip. E.g. 1ms.</td>
</tr>
<tr>
<td>Repetition Rate</td>
<td>The rate of the stimuli presentation. E.g. 42.6/s</td>
</tr>
<tr>
<td>Number averages</td>
<td>The number of times the tone is presented. E.g. 256.</td>
</tr>
<tr>
<td>Recording Environment</td>
<td>The mouse environment during the recording. E.g. In sound attenuating booth on thermo-statically controlled heating pad (@ 37°C).</td>
</tr>
<tr>
<td>Anesthetic agent 1</td>
<td>Anesthetic 1 used to sedate the mouse. E.g. Ketamine.</td>
</tr>
<tr>
<td>Anesthetic agent 2</td>
<td>Anesthetic 2 used to sedate the mouse. E.g. Xylazine.</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Anesthetic agent 1 dosage</td>
<td>Dosage for anesthetic 1. E.g. 1mg/g.</td>
</tr>
<tr>
<td>Anesthetic agent 2 dosage</td>
<td>Dosage for anesthetic 2. E.g. 0.01mg/g</td>
</tr>
<tr>
<td>Anesthetic administration route</td>
<td>The route of anesthetic administration. E.g. Intraperitoneal (i.p.).</td>
</tr>
<tr>
<td>Date of Test</td>
<td>Date of test execution.</td>
</tr>
<tr>
<td>Time of injection</td>
<td>Time of anesthetic injection.</td>
</tr>
<tr>
<td>Equipment ID</td>
<td>When more than 1 machine having same model and manufacturer is used, e.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc.</td>
</tr>
<tr>
<td>Equipment manufacturer</td>
<td>Manufacturer of the equipment. E.g. TDT (Tucker Davis Technologies).</td>
</tr>
<tr>
<td>Equipment model</td>
<td>Model of the equipment. E.g. RP2.1 based system, RA4PA Medusa Preamplifier.</td>
</tr>
<tr>
<td>Software</td>
<td>The software used to control signal presentation and data acquisition. E.g. Sanger bespoke averager software.</td>
</tr>
<tr>
<td>Experimenter ID</td>
<td>An ID of any format to be used coherently both inside the same procedure and for all procedures. E.g. Harw_001, or 1/2/3.</td>
</tr>
<tr>
<td>Date equipment last calibrated</td>
<td>Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.</td>
</tr>
</tbody>
</table>

**Parameters and Metadata**
**Body weight** IMPC_ABR_001_001 | v1.4

simpleParameter


Unit Measured: g

Description: body_weight

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**Click-evoked ABR threshold** IMPC_ABR_002_001 | v1.3

simpleParameter


Unit Measured: dB SPL

Description: click_evoked_abr_threshold

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**Click-evoked ABR waveforms (numerical format)** IMPC_ABR_003_001 | v1.0

seriesParameter


Description: click_evoked_abr_waveforms_numerical_format_
6kHz-evoked ABR Threshold  IMPC_ABR_004_001 | v1.3

**simpleParameter**

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

**Unit Measured:** dB SPL

**Description:** 6khz_evoked_abr_threshold

---

6kHz-evoked ABR waveforms (numerical format)  IMPC_ABR_005_001 | v1.0

**seriesParameter**

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

**Description:** 6khz_evoked_abr_waveforms_numerical_format

---

12kHz-evoked ABR Threshold  IMPC_ABR_006_001 | v1.3

**simpleParameter**

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

**Unit Measured:** dB SPL

**Description:** 12khz_evoked_abr_threshold
**12kHz-evoked ABR waveforms (numerical format)**  IMPC_ABR_007_001 | v1.0

*seriesParameter*


Description: 12khz_evoked_abr_waveforms_numerical_format_

---

**18kHz-evoked ABR Threshold**  IMPC_ABR_008_001 | v1.3

*simpleParameter*


Unit Measured: dB SPL

Description: 18khz_evoked_abr_threshold

---

**18kHz-evoked ABR waveforms (numerical format)**  IMPC_ABR_009_001 | v1.0

*seriesParameter*


Description: 18khz_evoked_abr_waveforms_numerical_format_
**24kHz-evoked ABR Threshold**  IMPC_ABR_010_001 | v1.3

**simpleParameter**


Unit Measured: dB SPL

Description: 24khz_evoked_abr_threshold

---

**24kHz-evoked ABR waveforms (numerical format)** IMPC_ABR_011_001 | v1.0

**seriesParameter**


Description: 24khz_evoked_abr_waveforms_numerical_format_

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**30kHz-evoked ABR Threshold** IMPC_ABR_012_001 | v1.3

**simpleParameter**


Unit Measured: dB SPL

Description: 30khz_evoked_abr_threshold

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30kHz-evoked ABR waveforms (numerical format) IMPC_ABR_013_001 | v1.0


Description: 30khz_evoked_abr_waveforms_numerical_format_

Click-evoked + 6 to 30kHz tone waveforms (pdf format) IMPC_ABR_014_001 | v1.2


Description: click_evoked_6_to_30khz_tone_waveforms_pdf_format_

Audiograms IMPC_ABR_015_001 | v1.2


Unit Measured: dB SPL

Description: audiograms
**Preyer Reflex** IMPC_ABR_016_001 | v1.0

*simpleParameter*

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

Description: preyer_reflex

Options: Yes, No,

---

**Click +20dB waveforms (numerical format)** IMPC_ABR_017_001 | v1.0

*seriesParameter*

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

Description: click_20db_waveforms_numerical_format_

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**Click +50dB waveforms (numerical format)** IMPC_ABR_018_001 | v1.0

*seriesParameter*

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

Description: click_50db_waveforms_numerical_format_
Click +20dB+50dB waveforms (pdf format) IMPC_ABR_019_001 | v1.2
mediaParameter


Description: click_20db_50db_waveforms_pdf_format_

P1-N1 amplitude (at each stimulus level) IMPC_ABR_020_001 | v1.1
seriesParameter


Unit Measured: uV

Description: p1_n1_amplitude_at_each_stimulus_level_

P3-N3 amplitude (at each stimulus level) IMPC_ABR_021_001 | v1.1
seriesParameter


Unit Measured: uV

Description: p3_n3_amplitude_at_each_stimulus_level_
P1 Latency (at each stimulus level)  IMPC_ABR_022_001 | v1.1
seriesParameter


Unit Measured: ms

Description: p1_latency_at_each_stimulus_level_

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N1 Latency (at each stimulus level)  IMPC_ABR_023_001 | v1.1
seriesParameter


Unit Measured: ms

Description: n1_latency_at_each_stimulus_level_

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P3 Latency (at each stimulus level)  IMPC_ABR_024_001 | v1.1
seriesParameter


Unit Measured: ms

Description: p3_latency_at_each_stimulus_level_
N3 Latency (at each stimulus level) IMPC_ABR_025_001 | v1.1

seriesParameter


Unit Measured: ms

Description: n3_latency_at_each_stimulus_level_

P1-P3 Interval (at each stimulus level) IMPC_ABR_026_001 | v1.1

seriesParameter


Unit Measured: ms

Description: p1_p3_interval_at_each_stimulus_level_

N1-N3 Interval (at each stimulus level) IMPC_ABR_027_001 | v1.1

seriesParameter


Unit Measured: ms

Description: n1_n3_interval_at_each_stimulus_level_
Range of test stimuli used  IMPC_ABR_028_001 | v1.1

procedureMetadata


Description: range_of_test_stimuli_used

Options: Click, 6,12,18,24,30 kHz, Tone,6,12,18,24,30 kHz, Click,6,12,18,24,30 kHz,

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Range of stimulus levels used - Click  IMPC_ABR_029_001 | v1.1

procedureMetadata


Unit Measured: dB SPL

Description: range_of_stimulus_levels_used_click

Options: 0-85, 0-75, 0-88,

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Range of stimulus levels used - 6kHz  IMPC_ABR_030_001 | v1.1

procedureMetadata


Unit Measured: dB SPL
Range of stimulus levels used - 6kHz

**Description:** range_of_stimulus_levels_used_6khz

**Options:** 20-85, 0-85, 0-95, 20-90, 0-88,

---

Range of stimulus levels used - 12kHz

**IMPC_ABR_031_001 | v1.1**

**Description:** range_of_stimulus_levels_used_12khz

**Options:** 0-85, 20-85, 0-95, 0-90,

---

Range of stimulus levels used - 18kHz

**IMPC_ABR_032_001 | v1.1**

**Description:** range_of_stimulus_levels_used_18khz

**Options:** 0-85, 20-85, 0-95, 0-90,

---

Range of stimulus levels used - 24kHz

**IMPC_ABR_033_001 | v1.1**

---
**Range of stimulus levels used - 30kHz** IMPC_ABR_034_001 | v1.1

**Unit Measured:** dB SPL

**Description:** range_of_stimulus_levels_used_30khz

**Options:** 0-85, 20-85, 0-95, 20-90,

---

**Extension of stimulus levels** IMPC_ABR_035_001 | v1.0

**Unit Measured:** dB SPL

**Description:** extension_of_stimulus_levels

**Options:** 95, 90,
Stimulus level step size  IMPC_ABR_036_001 | v1.1


Unit Measured: dB SPL

Description: stimulus_level_step_size

Options: 5,

---------------------------------------------------------------

Tone Pip Duration  IMPC_ABR_037_001 | v1.0


Unit Measured: ms

Description: tone_pip_duration

Options: 5, 7, 1,

---------------------------------------------------------------

Tone Pip rise/fall  IMPC_ABR_038_001 | v1.0


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Unit Measured: ms
Description: tone_pip_rise_fall
Options: 1, 0.2,

Repetition Rate IMPC_ABR_039_001 | v1.0
procedureMetadata


Unit Measured: s^-1
Description: repetition_rate
Options: 42.6, 20, 10,

Number averages IMPC_ABR_040_001 | v1.0
procedureMetadata


Description: number_averages
Options: 256, 300, 512, 542,

Recording Environment IMPC_ABR_041_001 | v1.0
procedureMetadata

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

**Description**: recording_environment

**Options**: In sound attenuating booth on thermo-statically controlled heating pad (@ 37°C), Sound proof room, Sound proof booth, Med Associates PVC sound attenuated chamber,

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**Anesthetic agent 1** IMPC_ABR_042_001 | v1.0

procedureMetadata

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

**Description**: anesthetic_agent_1

**Options**: Ketamine, Avertin, Pentobarbital,

---

**Anesthetic agent 2** IMPC_ABR_043_001 | v1.0

procedureMetadata

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

**Description**: anesthetic_agent_2

**Options**: Xylazine, Avertin, None,
Anesthetic agent 1 dosage  IMPC_ABR_044_001 | v1.0

**Procedure Metadata**

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

**Unit Measured:** mg/g

**Description:** anesthetic_agent_1_dosage

**Options:** 1, 0.1, 0.8, 0.11, 0.011, 10, 0.2, 0.08,

---

Anesthetic agent 2 dosage  IMPC_ABR_045_001 | v1.0

**Procedure Metadata**

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

**Unit Measured:** mg/g

**Description:** anesthetic_agent_2_dosage

**Options:** 0.01, 0.016, 0.1, 1, 0.011, 0.02, 0,

---

Anesthetic administration route  IMPC_ABR_046_001 | v1.1

**Procedure Metadata**

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

**Description:** anesthetic_administration_route

**Options:** Intraperitoneal, Intramuscular, Subcutaneous,
Time of injection  IMPC_ABR_048_001 | v1.2

Description: time_of_injection

---

Equipment ID  IMPC_ABR_049_001 | v1.0

Description: equipment_id

---

Equipment manufacturer  IMPC_ABR_050_001 | v1.0

Description: equipment_manufacturer

Options: TDT (Tucker Davis Technologies), Intelligent Hearing Systems,
Equipment model IMPC_ABR_051_001 | v1.0

**Description**: equipment_model

**Options**: RP2.1 based system, RA4PA Medusa Preamplifier, III, Smart EP, RZ6-A-P1 bioacoustic system, RA4PA pre-amp / Digitizer, RA4LI headstage, MF1-S multi field speaker, RZ6, Z-series 2-DSP bioacoustic system, Medusa PreAmp/Digitizer, Medusa LI headstage, Multi field speaker/Stereo, Medusa4Z.

Software IMPC_ABR_052_001 | v1.0

**Description**: software

**Options**: Sanger bespoke averager software, Cambridge Electronic Design, HIS Smart, BioSigRZ, SmartEP, Tucker Davis Technologies.

Experimenter ID IMPC_ABR_053_001 | v1.0

**Description**: experiment
**Description:** experimenter_id

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**Date equipment last calibrated** IMPC_ABR_054_001 | v1.2

procedureMetadata

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

**Description:** date_equipment_last_calibrated

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