Physiology & Behavior 135 (2014) 232–236

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

The effects of Eph–ephrin mutations on pre-pulse inhibition in mice

Andrea Liuzzo [a,*], Lincoln Gray [a,1], Matthew Wallace [b,2], Mark Gabriele [b,2]

[a] Dept. of Communication Sciences and Disorders, James Madison University, MSC 4304, 801 Carrier Dr., Harrisonburg, VA 22807, United States
[b] Dept. of Biology, James Madison University, MSC 7801, 951 Carrier Dr., Harrisonburg, VA 22807, United States

HIGHLIGHTS

- Eph-ephrin mutations affect pre-pulse inhibition (PPI) when compared to controls.
- Heterozygous EphA4$^{lacZ/+}$ mice showed a normal startle response and normal PPI. Ephrin-B3^{null} mice showed a normal startle response, but little PPI. Homozygous EphA4$^{lacZ/lacZ}$ mice showed a diminished startle response and diminished PPI.
- The pattern of responses to the pre-pulse and to the startling stimulus is different in various mutations suggesting complex alterations of the psychometric function due to Eph-ephrin mutations.
- Previously published data in control and wild-type mice support present findings.

ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 9 April 2014
Accepted 29 May 2014
Available online 17 June 2014

Keywords:
Acoustic startle response
PPI
Pre-pulse inhibition
Eph/ephrin
EphA4
Ephrin-B3

ABSTRACT

Eph–ephrin signaling is known to be important in directing topographic projections in the afferent auditory pathway, including connections to various subdivisions of the inferior colliculus (IC). The acoustic startle-response (ASR) is a reliable reflexive behavioral response in mammals elicited by an unexpected intense acoustic startle-eliciting stimulus (ES). It is mediated by a sub-cortical pathway that includes the IC. The ASR amplitude can be measured with an accelerometer under the subject and can be decreased in amplitude by presenting a less intense, non-startling stimulus 5–300 ms before the ES. This reflexive decrement in ASR is called pre-pulse inhibition (PPI) and indicates that the relatively soft pre-pulse was heard. PPI is a general trait among mammals. Mice have been used recently to study this response and to reveal how genetic mutations affect neural circuits and hence the ASR and PPI. In this experiment, we measured the effect of Eph–ephrin mutations using control mice (C57BL/6 J), mice with compromised EphA4 signaling (EphA4$^{lacZ/+}$, EphA4$^{lacZ/lacZ}$), and knockout ephrin-B3 mice (ephrin-B3 $^{+/-,\ -/-}$). Control and EphA4$^{lacZ/+s}$trains showed robust PPI (up to 75% decrement in ASR) to an offset of a 70 dB SPL background noise at 50 ms before the ES. Ephrin-B3 knockout mice and EphA4 homozygous mutants were only marginally significant in PPI (<25% decrement and <33% decrement, respectively) to the same conditions. This decrement in PPI highlights the importance of ephrin-B3 and EphA4 interactions in ordering auditory behavioral circuits. Thus, different mutations in certain members of the signaling family produce a full range of changes in PPI, from minimal to nearly maximal. This technique can be easily adapted to study other aspects of hearing in a wider range of mutations. Along with ongoing neuroanatomical studies, this allows careful quantification of how the auditory anatomical, physiological and now behavioral phenotype is affected by changes in Eph–ephrin expression and functionality.

1. Introduction

1.1. Eph–ephrin signaling

The Eph receptors and their ligands, the ephrins, play a strong role in the development of the auditory system in mice by patterning the tonotopic structure from the auditory periphery to the brainstem and up to the auditory cortex [1–3]. Eph–ephrin interactions involve two subfamilies: As and Bs. Binding affinities are strongest within subfamilies; a noteworthy exception is the strong interaction between EphA4 and ephrin-B2, -B3 [4–6]. The mouse organ of Corti and spiral ganglion cells have strong expression of EphA4, EphB1, B2, and B3, ephrin-A2, -A5, and ephrin-B1, and -B2 [7–9]. Recent findings underscore the importance of Eph–ephrin signaling in the mammalian inferior colliculus (IC) prior to hearing onset [3,10]. Graded and modular expression patterns of EphA4 and ephrin-B2 correlate with developing projections to various IC subdivisions, suggesting their involvement in guiding tonotopic and patterned arrangements in the mouse midbrain [10].

* Corresponding author. Tel.: +1 603 560 2156.
[1] Tel.: +1 540 568 8154.
[2] Tel.: +1 540 568 6333.

http://dx.doi.org/10.1016/j.physbeh.2014.05.044
0031-9384/

Furthermore, accurate topographic mapping of terminal fields from the lateral superior olive to the IC is lost in ephrin-B2 mutants with compromised signaling [3]. Given the involvement of these subcortical auditory circuits in the ASR and the influences of Eph–ephrins on their development and organization, we hypothesize that Eph–ephrin mutants will display altered pre-pulse inhibition (PPI) when compared to controls and wild-types (WTs).

1.2. Acoustic startle reflex and pre-pulse inhibition

The acoustic startle response (ASR) is a motor response elicited by and directly following the presentation of an unexpected intense acoustic stimulus [11,12]. It is a rapid contraction of skeletal muscles that is considered a defensive response [13]. This behavioral response can be measured with the use of an accelerometer placed directly beneath the subject [14]. The magnitude of this response can be altered by a variety of factors including the addition of a non-startling stimulus (pre-pulse) presented before the startle-eliciting stimulus (ES) [15]. To detect ASR and its attenuation by the addition of a pre-pulse stimulus, reflex modification audiometry (RMA) is rapid and efficient [14,16].

Pre-pulse inhibition (PPI) can occur when a stimulus softer than the ES, called the pre-pulse, is presented ~ 5–300 ms before the ES. The perception of this pre-pulse stimulus reduces ASR amplitude. The PPI paradigm has been used in various research efforts as it is sensitive to manipulations in many parameters, is reliable across time, is easily quantified, and is controlled by a simple neural circuit that is conserved across mammalian species [13]. It has advantages over operant conditioning paradigms in that it does not require training or reinforcement efforts [17].

Allen & Ison [14] studied the effects of inter-stimulus intervals (ISIs) of the pre-pulse stimulus in CBA/CaJ inbred mice via an offset paradigm, an onset paradigm, and a speaker swap of 180° azimuth. The most robust PPI was elicited by the offset of a 70 dB SPL broad band noise (filtered from 1 kHz to 50 kHz) with an ISI of 50 ms.

1.3. Critical auditory structures

In the mammalian ASR, stimuli are transduced in the cochlea, and subsequently transmitted to the auditory nerve, cochlear nuclei (CN), nuclei of the lateral lemniscus (NLL), nucleus reticularis pontis caudalis (PnC - located at the head of the reticulospinal tract), and ultimately to the spinal motor neurons, which then innervate flexor and extensor muscles of the body [16,17]. The inhibitory modulation of mouse ASR is influenced by the IC, most notably its lateral and dorsal cortex subdivisions (LCIC and DCIC) [18]. The addition of a pre-pulse stimulus inhibits the ASR by interfering with the neural circuit at the level of the IC where excitatory input is sent to the pedunculopontine tegmental nucleus, which in turn inhibits the PnC of the ASR neural pathway [17].

1.4. Goal of experiment

Experiments addressing the effects of Eph–ephrin mutations on behavioral pre-pulse inhibition in mice are currently lacking. Therefore, the aim of this study is to better understand the behavioral effects of Eph–ephrin signaling by comparing Eph–ephrin mutant mice to controls using Allen & Ison's PPI procedure [14]. The following is a behavioral evaluation of mutations that have been studied genetically and histologically.

2. Materials and methods

2.1. Subjects

Mice (n = 20) of two different Eph–ephrin mutations and a control group were used. The control group consisted of seven C57BL/6 J mice and two WT offspring of heterozygous EphA4^{lacZ} parents. Two strains of mutant mice were tested: ephrin-B3^{null} (n = 4, 2 homozygous, 2 heterozygous) and EphA4^{lacZ} (n = 7, 4 heterozygous and 3 homozygous). Mice varied between the ages of 31 days and 75 days and were tested twice. The average age at the first test was 37 (+/− 8.2) days. The average time between the first and second test was 15.5 (+/− 4.4) days. All mice were tested before the expected onset of age-related hearing loss of 8 months in the C57BL/6 J strain [19]. All mice were group-housed (4–6 mice per cage) in a BioZone MiniSmart Rack System in a controlled constant climate. All testing was done during the daylight hours. Food and water were always available except during testing which lasted approximately 60 min. The James Madison University Institutional Animal Care and Use Committee (IACUC) approved all procedures prior to experimentation.

2.1.1. Genotyping procedures

Breeding pairs to establish the EphA4 colony were obtained through Mutant Mouse Regional Resource Center (MMRRC, NCRR-NIH). Ephrin-B3 breeding pairs were acquired from Dr. Mark Henkemeyer (UT Southwestern Medical Center). Tail samples of EphA4 and ephrin-B3 mice were processed for genotyping utilizing an Easy-DNA kit (Invitrogen, Carlsbad, CA). EphA4 primer (EphA4-forward 5′ GTTTCCGCTCTGAGCT TATACTGC-3′, EphA4-reverse 5′ ACAGTGAGTGGACAAAGAGACAGG-3′, lacZ 5′-CGCTCTTACCAAAGGGCAAACC-3′) and ephrin-B3 primer (EB3-forward 5′-GACGGCGGGCCAAGCCTTCGGAGAG -3′, EB3-reverse 5′-ATAGCCAGGAGGAGCCAAAGAG-3′, lacZ 5′-AGGCGATTAAGTTGGGTA ACG-3′) were used for PCR amplification [20,21]. Gel electrophoresis of PCR product resulted in EphA4 WT (639-bp) and/or mutant (800-bp) allele bands, and ephrin-B3 WT (401-bp) and/or mutant (142-bp) allele bands.

2.2. Apparatus and stimuli

Mice were tested in a 5 cm inside-diameter by 12.5 cm long San Diego instruments Plexiglas tube attached to an accelerometer taken from the SR-LAB mouse-testing chamber. This tube was placed in the middle of a 7′ × 7′ (2.13 m × 2.13 m) Industrial Acoustic double-walled, double-floored, sound-attenuating booth. The chamber was 18″ (45.7 cm) beneath a Ross Audio Systems TW 30 compression tweeter. The pre-pulse stimulus was presented via a Tucker Davis Technology ES1 compression tweeter 15 cm to one side of the testing chamber. Startle eliciting stimuli (ES) were 110 dB, 15 ms broad-band noise, high-pass filtered at 8 kHz, and rapidly gated. Calibration showed significant energy up to 50 kHz, 110 dB SPLrms in a 768 Hz to 50 kHz band. The ES noise was generated using a Tucker Davis Technology Real-Time Processor, TDT RP2.1, and was amplified by a Crown XLS202 amplifier. The pre-pulse stimulus was a continuous high-pass noise filtered at 4 kHz (1 kHz to 100 kHz bandwidth = 70 dB SPLrms +/− 1 dB SPLrms). The offset was approximately instantaneous (on to off in one 50 μs cycle of the DAC). Calibrations of the stimuli were done with an Agilent 35670A Spectrum Analyzer, ¼″ microphone (Bruel & Kjaer 4939) placed in the center of the Plexiglas tube, amplified by a Listen, Inc. Sound Connect amplifier.

The force of the startle reflex was transduced by an accelerometer beneath the testing tube. The voltage from the accelerometer was low-pass filtered at 1 kHz and amplified times 100 (20 dB + 20 dB) by a Krohn-Hite model 3343 filter and input to a TDT-RP2.1. This input was digitized at 200 kHz for 100 ms starting at the same time that the startling stimulus began. Test trials began 2 min after the mouse was placed in the testing chamber (2 min acclimation period), and testing continued for about 60 min. All subjects were run with the lights off.

2.3. General procedures

The pre-pulse in this experiment was an offset of the 70 dB stimulus at 90° azimuth to the mouse. Sixteen different conditions were repeated in 11 different blocks. There were 13 different inter-stimulus intervals (ISI: time between offset of carrier stimulus and presentation of