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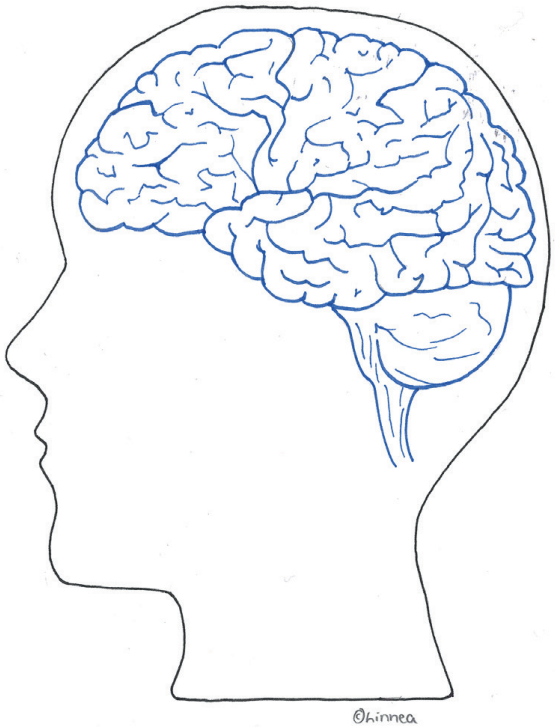
Jari Kurkela

Auditory Perceptual Learning

Evidence from Electrophysiological Recordings in Rodents and Humans



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ABSTRACT

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The acoustic environment contains important cues for survival, making their prompt detection behaviourally relevant. Auditory perceptual detection of changes can be enhanced via behavioural training, which results in the formation of new memory representations of learned sounds. In early development, during the sensitive period, the emergence of new memory representations of sounds, such as speech sounds in humans, is automatic and can happen passively without attentive training. It is traditionally thought that, after the sensitive period, only attentive training can enhance change detection and induce long-term memory representations. However, whether passive exposure to sounds can develop long-term memory representations in adulthood is not yet fully resolved. Auditory change detection can be studied by measuring the brain's electrical activity. Study I demonstrated that change detection in mice, as measured with the brain's local-field potentials (LFPs) from the auditory cortex, was dependent on sensory memory and reflected detection of regularity violations, as has been found previously in humans. Study II, conducted on adult rats, showed that passive exposure to novel speech sounds for three consecutive days, 12 hours per day, resulted in the formation of long-term memory traces as indexed by LFPs in the auditory cortex. Furthermore, in Study III, conducted on adult humans, passive exposure to novel speech sounds for four consecutive days, two hours per day, modulated the brain's event-related potentials (ERPs), reflecting the development of new memory representations. Overall, the results from Studies I and II suggest that auditory memory and perceptual learning are reflected by automatic brain responses in rodents, indicating that these animal models are feasible for studying the neural underpinnings of auditory cognition. Studies II and III demonstrate that passive exposure in adulthood can induce the formation of new memory traces. These results require re-evaluation of the prevailing theories of perceptual learning.

Keywords: perceptual learning, passive exposure to sounds, event-related potentials (ERP), change detection, speech sounds

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TIIVISTELMÄ (FINNISH ABSTRACT)

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Yllättävät muutokset äänimaailmassa viestittävät yleensä selviytymisen kannalta tärkeistä vihjeistä, joten niiden nopea ja automaattinen havaitseminen on tärkeää. Tietoinen harjoittelu mahdollistaa havainto-oppimisen ja oppimisen seurauksena hermostoon muodostuu uusia muistijälkiä. Varhaisessa kehityksessä herkkyyskauden aikana muistijälkien muodostuminen, ihmisellä erityisesti puheäänille, on automaattista, eikä siihen vaadita tietoista harjoittelua. Toisaalta perinteisesti on ajateltu, että herkkyyskauden jälkeen vain aktiivinen harjoittelu johtaa uusien muistijälkien muodostumiseen. Muutoksen havaitsemisen hermostollista perustaa voidaan tutkia mittaamalla aivojen sähköisiä jännitevasteita. Ensimmäinen osatutkimus osoitti, että hiirten kuuloaivokuorelta mitatut sähköiset jännitevasteet muutoksiin äänen taajuudessa heijastivat lyhytkestoisen muistin toimintaa samoin kun aiemmin on havaittu ihmisillä ja rotilla. Toisessa osakokeessa aikuisille rotille esitettiin ihmisen puheääniä kolmen perättäisen päivän ajan 12 tuntia päivässä. Jännitevastemittaukset rottien kuuloaivokuorelta osoittivat, että altistaminen äänille paransi muutoksen havaitsemista puheäänten piirteissä ja sai aikaan ääniin liittyviä uusia muistijälkiä. Kolmannessa osatutkimuksessa aikuisille ihmisille esitettiin muutoksia vieraan kielen puheäänten piirteissä neljän perättäisen päivän ajan, kaksi tuntia päivässä. Aivovastemittaukset päänahan pinnalta aikuisilla ihmisillä osoittivat, että altistaminen uusille puheäänille johti muistijälkien muodostumiseen samoin kuin havaittiin rotilla osatutkimuksessa II. Kokonaisuudessaan osatutkimukset I ja II osoittivat, että automaattiset aivovasteet hiirillä ja rotilla ilmentävät muistin toimintaa ja havainto-oppimista kuulojärjestelmässä tehden näistä eläinmalleista hyödyllisiä kuuloon liittyvän tiedonkäsittelyn tutkimuksessa. II ja III osatutkimus osoittivat, että myös aikuisuudessa pelkkä passiivinen altistuminen äänille johtaa uusien muistijälkien syntymiseen. Nämä tulokset vaativat havainto-oppimiseen liittyvien teorioiden uudelleenarviointia.

Avainsanat: havainto-oppiminen, herätevaste, passiivinen altistuminen, aistimuisti, elektrofysiologia, herkkyyskausi, muutoksen havaitseminen

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Taking into account the instructions given and comments made by the co-authors, the author of this thesis contributed to the original publications as follows: In Study I, he participated in data analysis and writing the manuscript. In Study II, he participated in designing the experiments, data collection, data analysis and writing the manuscript. In Study III, he participated in data collection, data analysis and writing the manuscript.

FIGURES

CONTENTS

1 INTRODUCTION

Even though the perception of sounds feels effortless, the auditory neural system is regarded as the most complex of all sensory pathways (Møller, 2006). Auditory perception is temporally dependent, and it is always present, which distinguishes it from other senses, such as vision.

During our daily lives, we are constantly under the influence of multiple sources of sound, which carry fundamental information that influences behaviour. Our surroundings are full of acoustics cues, such as the sound of an approaching car when we are planning to cross the street or a cellphone ringing in our pocket. Accurate auditory perception is also necessary for daily communication since we mainly deliver our thoughts and ideas through spoken language.

In infancy, during the so-called sensitive period, the auditory system is highly plastic for environmental influences. The sensitive period seems to be universal across different mammalian species, like humans and rats (de Villers-Sidani, Chang, Bao, & Merzenich, 2007). During the sensitive period, the human brain can change to encode and discriminate different speech sounds from a new language automatically and without training, even during nocturnal sleep (Cheour et al., 1998; 2002; Kuhl, 2004). The auditory neural system remains plastic in adulthood, but it is commonly thought that effortful training with behaviourally meaningful stimuli is needed to elicit plastic changes. The aim of this dissertation is to investigate the basic properties of auditory change detection across different species, namely in mice, rats and humans. Furthermore, this study tests whether passive exposure in adulthood, in both rats and humans, can promote plastic changes in the central auditory system.

1.1 Auditory sensory system and change detection

Sounds are pressure waves that are transmitted through a medium. Sound characteristics are described by frequency, amplitude and timbre. Frequency

determines how high or low we hear the pitch of a sound; it is usually measured in Hertz (Hz). Amplitude determines how intensive, i.e. loud, the sound feels; it is usually reported in decibels (dB). Lastly, timbre describes the complexity of a sound that is comprised of combination of different frequencies. The auditory sensory system converts these characteristics into action potentials, which are interpreted as the perception of sound.

The auditory sensory system starts from the ear. The cochlea resides in the inner ear and the basilar membrane is embedded inside the cochlea. Along the basilar membrane, auditory sensory receptors called hair cells are organized so that the cells near the cochlear base respond most strongly to higher frequency sounds, while the hair cells near the apex respond to lower sound frequencies (Møller, 2006). From the cochlear nerve, the auditory signal follows an ascending auditory pathway. The signal is transmitted through three main relay nuclei—the ventral cochlear nucleus (CN), inferior colliculus (ICC) and medial geniculate body (MGB)—before it reaches the auditory cerebral cortex (Møller, 2006). Single-cell recordings from these nuclei show that distinct cell groups only respond maximally to a certain frequency, similar to what occurs in the basilar membrane (Malmierca et al., 2008; Saenz & Langers, 2014). This tonotopic representation of frequencies also applies to the auditory cortex (Møller, 2006; Saenz & Langers, 2014). The auditory cortex then constructs perceptual representations of the sounds, and we are able to hear different sounds as music or speech (Goldstein & Naglieri, 2011).

1.1.1 Auditory change detection

When something unexpected happens in the auditory environment it may carry information that is fundamental for survival. Therefore, rapid and automatic detection of these unexpected events is essential. Humans and animals alike have developed the capacity to promptly detect changes in sounds (e.g. Astikainen et al., 2011; Ruusuvirta, Penttonen, & Korhonen, 1998; Sams, Paavilainen, Alho, & Näätänen, 1985; Schröger, 1996).

Neural responses to sounds can be recorded with an electroencephalography (EEG). Sounds presented to a participant elicit specific changes in EEG amplitude. When EEG is recorded time-locked to the sound presentation, these amplitude changes are called event-related potentials (ERPs).

Auditory change detection can be experimentally studied by presenting sounds in a so-called oddball condition, while event-related potentials (ERPs) are recorded. In the oddball condition, a repetitive 'standard' sound is randomly replaced with a different, rare 'deviant' sound. The repetitive standard sound creates an expected auditory regularity, which the rare deviant sound violates.

These violations elicit the mismatch negativity (MMN) (Näätänen, Gaillard, & Mäntysalo, 1978). The MMN is observed from the difference wave calculated by subtracting ERPs to standard sounds from ERPs to deviant sounds; it occurs 150–250 ms after stimulus onset in fronto-central scalp locations in adult humans. No attention is required for MMN elicitation; the

MMN is observed even in sleeping participants, comatose patients and humans under anaesthesia (Fischer, Luauté, Adeleine, & Morlet, 2004; Heinke et al., 2004; Sallinen, Kaartinen, & Lyytinen, 1994). In fact, the MMN is best observed when the subject's attention is directed away from the auditory stimuli to avoid elicitation of attention-dependent ERP components, such as N2b and P3b (Polich, 2007; Sams et al., 1985).

The MMN is elicited by changes in simple sound features, such as changes in frequency (Sams et al., 1985), intensity (Schröger, 1996) and duration (Joutsiniemi et al., 1998; Michie et al., 2000). Also, deviations in more complex tones or repetitive tone patterns (Atienza & Cantero, 2001; Näätänen, Schröger, Karakas, Tervaniemi, & Paavilainen, 1993) and changes in abstract rules in sound series (Carral et al., 2005) elicit the MMN. Furthermore, changes in naturalistic stimuli, such as changes in speech sounds and words, elicit the MMN (Alho et al., 1998; Shtyrov et al., 1998).

The MMN has been shown to be a good index of behavioural discrimination ability (Tiitinen, May, Reinikainen, & Näätänen, 1994). The physical difference between the deviant stimuli and standard stimuli needs to exceed a certain limit to elicit the MMN (Sams et al., 1985). If the sounds are too similar and the auditory system cannot discriminate the sounds, the MMN is not found (Sams et al., 1985). The physical difference between sounds that are behaviourally discriminable seems to correlate with MMN amplitude (for review see Näätänen, Paavilainen, Rinne, & Alho, 2007).

The MMN is not observed exclusively in human subjects. An animal model for the MMN was first demonstrated in recordings in cats (Csépe et al., 1987), and later, in other animals, such as monkeys, rabbits and guinea pigs (e.g. Javitt, Schroeder, Steinschneider, Arezzo, & Vaughan, 1992; Kraus, McGee, Littman, Nicol, & King, 1994; Ruusuvirta, Korhonen, Arikoski, & Kivirikko, 1996). More recently, epidural recordings of the local field potentials (LFPs) of the auditory cortex have demonstrated mismatch response (MMR) in rats (e.g. Ahmed et al., 2011; Astikainen et al., 2011; Jung et al., 2013; Nakamura et al., 2011; Ruusuvirta, Penttonen, & Korhonen, 1998) and few studies in mice (Parras et al., 2017; Umbricht, Vyssotki, Latanov, Nitsch, & Lipp, 2005).

1.1.2 The deviance detection interpretation of the MMN

There are two main hypotheses for the neural mechanisms behind MMN elicitation (Harms, Michie, & Näätänen, 2016). The first is usually called the deviance detection hypothesis, or more recently, the predictive coding hypothesis (Garrido, Kilner, Stephan, & Friston, 2009; Winkler, Denham, & Nelken, 2009). The second is referred to as the adaptation or refractoriness hypothesis (Jääskeläinen et al., 2004; May & Tiitinen, 2010; Winkler, 2007). The predictive coding hypothesis states that the repetitive presentation of standard sounds creates a predictable model of the sound environment. Sudden presentation of a deviant sound violates this prediction and a 'prediction error' signal is generated, which is reflected by the MMN (Harms et al., 2016). In contrast, according to the adaptation hypothesis, the MMN reflects mere

differences in the adaptation of neural elements tuned to standard stimuli compared to those responding to rare deviant stimuli (Jääskeläinen et al., 2004; May & Tiitinen, 2010).

Single cells adapt to frequently occurring sounds yet respond strongly to rare stimuli (Escera & Malmierca, 2014; Ulanovsky, Las, & Nelken, 2003). The adaptation of neural responses to repeated sounds while maintaining their responsiveness to uncommon ones is known as stimulus-specific adaptation (SSA), and it is thought to be one of the mechanisms that allows the detection of regularity violations. SSA was originally demonstrated in the auditory cortex (Ulanovsky et al., 2003), but it has also been found along the auditory pathway in the inferior colliculus (e.g. Malmierca, Cristaudo, Pérez-González, & Covey, 2009) and the medial geniculate body (Anderson, Christianson, & Linden, 2009; Antunes, Nelken, Covey, & Malmierca, 2010). SSA has been suggested to be a contributing factor in MMN generation, even though the onset latency of the SSA (~20 ms after stimulus onset) is considerably earlier than the typical MMN latency (Ulanovsky et al., 2003; for a review see Escera & Malmierca, 2014).

In humans, the MMN is observed at 150-250 ms latency, which partly overlaps with N1 latency (Näätänen et al., 2007). N1 is elicited by all hearable auditory stimuli and adapts when the same stimulus is repeated (Näätänen & Picton, 1987). The adaptation hypothesis claims that the MMN is not a separate component from N1, but is a suppressed response to standard stimuli, and that N1 and the MMN are generated by the same neural populations (Jääskeläinen et al., 2004; May & Tiitinen, 2010).

Several experimental control conditions are developed to determine whether the MMN is a reflection of deviance detection, independent from adaptation. Probably, the most used control condition is the many-standards control condition, also called the equiprobable control condition (Jacobsen & Schröger, 2001; Jacobsen, Schröger, Horenkamp, & Winkler, 2003; Schröger, 1996). In this control condition, sounds used as deviants in the oddball condition (control-deviant sound) are presented amongst several different sounds, all with the same probability. The control-deviant sound also has the same probability as the deviant sound in the oddball condition; since, in the equiprobable condition multiple, stimuli are presented in random order, no predictions can be established. Importantly, the responses to the control-deviant sound have the same level of adaptation as the responses to the deviant sound in the oddball condition. If the response to the deviant sound in the oddball condition is larger than the response to the same sound as a control, one can argue that the oddball deviant response reflects 'the genuine MMN', indicating the detection of regularity violations, not mere adaptation.

The MMN specific to regularity violations has been demonstrated in humans (Jacobsen & Schröger, 2001; Schröger, 1996) but also in awake and anaesthetized rats (Ahmed et al., 2011; Astikainen et. al, 2011; Harms et al., 2014; Jung et al., 2013; Nakamura et al., 2011; Shiramatsu, Kanzaki, & Takahashi, 2013). In mice, only one study has demonstrated change detection specific to regularity violations using LFP recordings (Parras et al., 2017). In addition,

patch recordings have found some evidence of subthreshold deviance detection (Chen, Helmchen, & Lu, 2015).

The MMN reflects sensory memory function. In humans, when the length of a silent gap between the sounds in the oddball condition is lengthened from two seconds to four seconds, MMN amplitude decreases (Mäntysalo & Näätänen, 1987). This is comparable to the time span of the human echoic memory (Bötter-Gandor & Ullsperger, 1992; Darwin, Turvey, & Crowder, 1972). The decrease in MMN amplitude due to the lengthening of the silent gap between stimuli has been interpreted as a decay of the memory trace of the standard sound, making its comparison to the deviant sound impossible. In rats, frequency changes elicited the MMR when the inter-stimulus interval (ISI) was 375 ms, but it disappeared with 600 ms ISI (Astikainen et al., 2011). However, previous studies have not investigated the dependence of the MMR on sensory memory in mice.

1.2 Auditory perceptual learning

Perceptual learning refers to an improvement in a sensory task as the result of practice (Gilbert, Sigman, & Crist, 2001; Goldstone, 1998; Tsodyks & Gilbert, 2011). Improvement develops progressively over many trials; this distinguishes it from other, more explicit types of learning, which may require only a single exposure to a stimulus. The improvement tends to persist over weeks, months, or even a lifetime, and so distinguishes it from adaptation and priming (Fahle, 2005). It is also thought that perceptual learning requires attention to the task at hand. Perceptual learning can be enhanced with feedback, although that is not a prerequisite. Improvement is specific to the learned stimulus features and generalization rarely occurs (Fahle, 2005).

Naturally, perceptual learning is reflected by plastic changes at the neural level. For example, classical conditioning, operant conditioning and positive or negative reinforcement result in enlarged cortical representations of the trained stimuli in animals. These changes have been shown for different types of stimuli, such as pure tone frequencies, sound intensities and temporal modulation rates (Bao, Chang, Woods, & Merzenich, 2004; Engineer et al., 2008; Polley, Heiser, Blake, Schreiner, & Merzenich, 2004; Recanzone, Schreiner, & Merzenich, 1993; Rutkowski & Weinberger, 2005). In humans, discrimination training and identification training have been shown to enhance MMN amplitude and shorten MMN latency when training to discriminate complex frequencies or learning Morse code (Atienza & Cantero, 2001; Kraus et al., 1995; Kujala et al., 2003; Menning, Roberts, & Pantev, 2000; Näätänen et al., 1993; Tremblay, Kraus, Carrell, & McGee, 1997).

Training effects have been shown, not only for simple stimulus features, but also for speech sounds in humans and rodents. Active training of foreign language features enhances discrimination ability, resulting in enhanced MMN (Kraus et al., 1995; Tamminen, Peltola, Kujala, & Näätänen, 2015; Tremblay et

al., 1997). Discrimination training of speech sounds also enhances the cortical responses of these sounds in rats (Engineer et al., 2008). The behavioural ability of rats to discriminate between consonant sounds correlates with precise spatiotemporal activity patterns in the primary auditory cortex (A1) in rats (Engineer et al., 2008). Furthermore, training reorganizes the representation of the sounds in the auditory cortex (Engineer et al., 2014). It seems that speech sounds that evoke very similar neural patterns of activity are difficult for rats to discriminate, while speech sounds that evoke very distinct neural patterns of activity are easier to discriminate (Engineer et al., 2014).

The changes in MMN amplitude and latency observed after behavioural training have been interpreted as the development of memory traces for the learned stimuli. This was first suggested in a study where subtle changes in native language features elicited the MMN, while foreign language features did not (Näätänen et al., 1997). Furthermore, acquiring fluent command of a previously foreign language develops cortical memory representations specific to that language. Hungarians who had acquired fluent command of Finnish could discriminate Finnish phonemes as indexed by MMN elicitation, but Hungarians naive to the Finnish language did not (Winkler et al., 1999). Furthermore, the MMN reflects memory consolidation of new memory traces. The MMN response recorded 48 hours after the end of training was significantly larger in amplitude than that recorded 24 hours after the end of training (Atienza, Cantero, & Stickgold, 2004).

In addition to the MMN, other human ERP components like N2b, P2, P3a and P3b show learning related enhancements. In the passive oddball condition, deviant stimuli can also elicit a P3a component following the MMN. The P3a typically peaks at latency of 200-400 ms for deviant sound and is thought to reflect involuntary attention orientation towards novel or deviant stimuli in the oddball condition (Polich, 2007). Training with Morse code enhanced the P3a amplitude (Uther, Kujala, Huotilainen, Shtyrov, & Näätänen, 2006). Also, active training in discriminating pitch (Seppänen, Pesonen, & Tervaniemi, 2012) and tone sequences (Atienza et al., 2004) have led to increased P3a amplitude.

Contrary to the MMN and P3a, the N2b and P3b are elicited by deviant sounds when subjects selectively attend to them. The N2b reflects attentional deviance detection from the mentally-stored expectation of the standard sound in the working memory (Näätänen, Simpson, & Loveless, 1982; Patel & Azzam, 2005). The N2b is observed in central scalp locations and typically peaks at 100-300 ms after the stimulus onset (Patel & Azzam, 2005; Sams et al., 1985). The P3b peaks around 250-500 ms latency and has posterior scalp topography (Polich, 2007). It is suggested that the P3b reflects attentional demands and working memory update processing (Polich, 2007).

The context updating theory of the P3b component suggests that a memory comparison process is engaged to evaluate if the upcoming stimuli is either the same as the previous stimulus or different (Polich, 2007). If the incoming stimuli is recognized as different from the memory representation of the previous stimulus then the neural representation of the stimulus

environment is updated in the working memory and the P3b component is elicited (Polich, 2007). In a training study participants discriminated speech sounds presented in an active oddball task at three consecutive time points over a two-week interval, (Giroud, Lemke, Reich, Matthes, & Meyer, 2017). This training enhanced the N2b and P3b microstates and was accompanied by improvements in reaction times.

It is somewhat surprising, taking it into account that P3b was discovered over 40 years ago (Polich, 2007), that only one previous study has investigated the effects of perceptual learning and exposure to P3b (Giroud et al., 2017). Since P3b is associated with frontal lobe and temporal lobe function, it might be a feasible tool to investigate the role of attentional demand and working memory load in auditory perceptual learning.

Lastly, also the P2 component has shown learning related enhancements. P2 is regarded as an exogenous response that is elicited by both attended and non-attended stimuli and has latency of approximately 150 - 250 ms after stimulus onset (Crowley & Colrain, 2003). P2 amplitude to repeatedly presented single sound increases after behavioral discrimination or identification training (Ross, Jamali, & Tremblay, 2013; Sheehan et al., 2005; Tremblay, 2007; Tremblay, Inoue, McClannahan, & Ross, 2010). Enhancements in P2 amplitude has been thought to reflect better sound feature encoding (Crowley & Colrain, 2003).

1.3 Perceptual learning in different stages of development

In infancy, during the sensitive period, the cerebral cortex is highly adaptive to the acoustic environment, which has a strong effect on cortical representations of sounds in animals and humans (Chang & Merzenich, 2003; Cheour et al., 2002; Keuroghlian & Knudsen, 2007; Zhang, Bao, & Merzenich, 2001). When rat pups were passively exposed to a single sound for 10-16 hours per day throughout the sensitive period, the results were dramatic. The tonotopic representation of frequencies were influenced so that the frequency of the sound used in the exposure was over represented and had larger than normal receptive fields (Zhang et al., 2001). On the other hand, very noisy environmental exposure can hinder and postpone the formation of tonotopic maps. As soon as a rat pup is returned to an environment that has a normal auditory scene, the development of the cortex continues normally (Chang & Merzenich, 2003).

In four-month-old human infants, exposure to melodies of either guitar or marimba timbre over the course of a week enhanced the MMN amplitude of the exposed feature, but not the unexposed feature (Trainor, Lee, & Bosnyak, 2011). In addition, exposure to temporally modulated non-speech sounds in humans aged four to seven months impacted the acoustic mapping measured with EEG (Benasich, Choudhury, Realpe-Bonilla, & Roesler, 2014). Interestingly, speech sound exposure during sleep was sufficient to enhance the MMN for the exposed sounds in newborns at ages of one to seven days (Cheour et al., 2002).

Contrary to infancy, passive acoustic exposure alone in adulthood has not been sufficient to elicit plastic changes as indexed by the MMN (Elmer, Hausheer, Albrecht, & Kühnis, 2017; Näätänen et al., 1993; Sheehan, McArthur, & Bishop, 2005) or behavioral responses (Wright et al, 2010, 2015). Same has been found in adult rats. Simply exposing an animal repetitively to a single sound does not drive plasticity in the adult cortex, as measured by cortical representation for the exposed sounds (Bao, Chan, & Merzenich, 2001; Bao, Chan, Zhang, & Merzenich, 2003; Keuroghlian & Knudsen, 2007; Zhang et al., 2001).

However, in adulthood, behavioral training that is combined with sessions of mere passive exposure is more efficient than training sessions alone (Wright et al. 2015). Furthermore, passive exposure to single repeated sound increases the P2 amplitude, which reflects better sound feature encoding (Ross, Jamali, & Tremblay, 2013; Sheehan et al., 2005; Tremblay, 2007; Tremblay, Inoue, McClannahan, & Ross, 2010). In addition, in adult mice, passive exposure to simple tones causes adaptation in single cell responses measured from the auditory cortex (Kato, Gillet, & Isaacson, 2015).

From here, it seems that, after the sensitive period, the emergence of memory traces related to change detection can be observed only if the discrimination is actively trained. Yet, in humans, only short passive exposure to sounds (1 to 2 hours) has been applied (Elmer et al., 2017; Näätänen et al., 1993; Sheehan et al., 2005). In the present studies, passive exposure was administered during a single day and the effects of exposure were measured during the same day. Therefore, memory consolidation during nocturnal sleep, which has been shown to be crucial for learning (Atienza et al., 2004; Wright et al., 2010, 2015), was not involved. In one previous study, were both active training and passive test sessions were applied for three consecutive days, authors speculated that the mere exposure to the stimuli during the testing could have been enough to elicit the changes in MMN (Tamminen et al. 2015). This has not been tested exclusively, however. There is, therefore, a need for further studies on the effect of mere passive exposure on auditory change detection.

1.4 Aims of the research

This dissertation aims to answer three fundamental questions. First, do mice exhibit MMN specific to regularity violations, as has been found in humans and rats? Second, can rats discriminate between complex speech sounds, and is it possible to induce long-term memory representation of speech sounds via passive exposure in adult rats? Lastly, is it possible to induce long-term memory representations to foreign speech sounds via passive exposure in adult humans?

The aim of **Study I** was to determine to what extent the MMR in mice is similar to the MMN in humans. Two fundamental properties related to human

MMN were investigated: specificity to regularity violations and dependency on sensory memory. In previous studies, the MMR was elicited in mice (Ehrlichman et al., 2009; Ehrlichman, Maxwell, Majumdar, & Siegel, 2008). Still, only one previous study applied proper control conditions to exclude the adaption hypothesis (Parras et al., 2017). Furthermore, no studies have investigated the role of sensory memory in mice by varying the length of the ISI. We hypothesized that changes in sound frequencies would elicit the MMR, which reflects regularity violations, and that lengthening the ISI would diminish the MMR amplitude.

The aim of **Study II** was to test whether passive exposure to speech sounds can form long-term memory representations in adult rats. Earlier studies have shown that environmental exposure enhances the cortical representations of sounds during the sensitive period in infancy, while in adulthood, training with behaviourally meaningful stimuli is needed to cause plastic changes in cortical maps (Bao, Chan, & Merzenich, 2001; Bao, Chan, Zhang, & Merzenich, 2003; Keuroghlian & Knudsen, 2007; Zhang et al., 2001). Here, we hypothesized that the change detection mechanism could be enhanced with passive exposure, which would be reflected as an increased MMR, caused by the sounds used during the exposure.

The aim of **Study III** was to investigate whether passive exposure to foreign speech sounds can enhance long-term memory representations in adult humans. In early infancy, the learning of speech sounds and memory trace formation for these sounds occur relatively automatically and without direct attention, even during nocturnal sleep (Cheour et al., 2002; 1998). However, previous attempts to demonstrate auditory discrimination learning due to brief, single-session, passive exposure in adults have failed (Elmer et al., 2017; Näätänen et al., 1993; Sheehan et al., 2005). We hypothesized that a two-hour passive exposure in humans for four consecutive days would result in increased amplitudes and shortened latencies of the ERP components that reflect change detection (MMN) and attention switching (P3a). In addition, increases in amplitude and shortening of latencies in attention driven N2b and P3b components were expected.

2 METHODS

2.1 Subjects

In **Study I**, adult male mice (C57BIJ, n = 13) were used, while in **Study II**, adult male rats (Wistar, n = 15) were used. Thirty-nine Finnish speaking young adult human participants volunteered for **Study III**. Procedures of the animal studies were approved by the Finnish National Animal Experiment Board (ESAVI/10646/04.10.07/2014) and the experiments were carried out in accordance with the guidelines of the European Community Council Directive 2010/63/EU. In **Study III**, written informed consent was obtained from each participant before inclusion in the study. **Study III**, conducted in humans, was undertaken in accordance with the Declaration of Helsinki, and the ethical committee of the University of Jyväskylä approved the research protocol.

2.2 Stimuli and procedures

In **Study I,** oddball and equiprobable conditions were used to present the stimuli (Figure 1). In two separate oddball conditions, different ISI (offset to onset), 375 ms or 600 ms, were used. In both oddball conditions, two deviant sounds (probability for each = 0.0625), 3.5 kHz and 4.5 kHz in frequency, were interspersed with frequently occurring standard sounds of 4.0 kHz (probability = 0.875), with the restriction that at least two standard sounds were played between the deviant sounds. The equiprobable control condition had 16 different sounds with frequencies ranging from 3.3 kHz to 4.8 kHz in 0.1 kHz steps (Figure 1). The sounds were presented with an offset to onset ISI of 375 ms and each sound had the same probability as the deviant sounds in the oddball condition (probability for each = 0.0625). All sounds were 50 ms in length. During the sound presentation, LFPs were recorded from the auditory cortex.

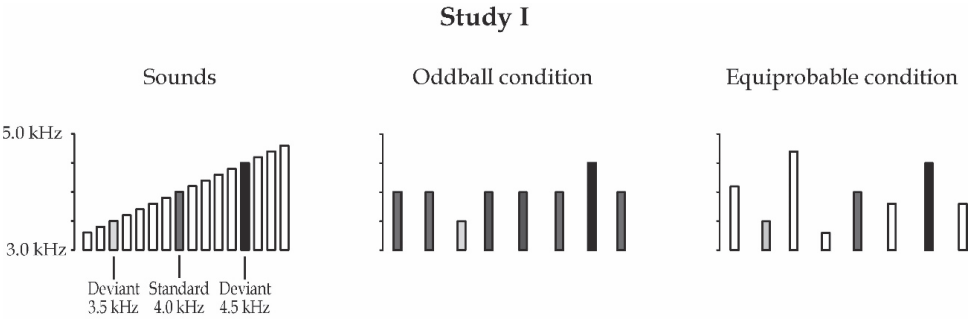

FIGURE 1 Sounds and stimulus conditions in Study I. In the oddball condition, two deviant sounds were interspersed with a standard sound. In the equiprobable condition, the same sounds that were used in the oddball condition were presented in random order with several other sounds. Importantly, the probability of the deviant sounds was the same in the oddball and equiprobable conditions allowing comparison of the responses to these sounds with the same level of adaption.

In **Study II**, animals were divided randomly into two groups. One group of animals was exposed to tonal changes (n = 7) and the other to spectrotemporal changes (n = 8) in speech sound /a/ (Figure 2). The exposure lasted three consecutive days, 12 hours per day, resulting in 36 total hours of exposure. The animals were exposed to the sounds in the oddball condition while in their home cages. Both oddball conditions consisted of standard sounds (p = 0.80) and two deviants (small and large changes to the standard sound, p = 0.10 each) and were presented pseudo-randomly so that at least two standards were played between two deviant sounds. The sounds were presented with an offset to onset ISI of 335 ms.

After the exposure, LFPs from the auditory cortex were measured using the same speech sounds applied in the exposure; in this case, both types of changes were presented to both animal groups, but in separate oddball stimulus blocks.

In **Study III**, one group of participants (n = 18) were passively exposed to tonal changes (Figure 2) for four consecutive days, 2 hours per day, while they watched silent movies. Another group of participants (n = 21) served as a control group and no exposure was given. During the exposure, sounds were presented in an oddball condition where two deviant sounds, with large or small changes to the standard sound (p = 0.10 each), were interspersed amongst a frequently played standard sound (p = 0.80), with a restriction that at least two standard sounds were played before any deviant sound. The sounds were presented with ISI that varied randomly between 440 and 520 ms.

Before the passive exposure, all participants had an EEG-measurement where tonal changes were presented in ignore and active oddball conditions. During the ignore condition, participants were instructed to focus on a silent

movie, and the sounds were played in the background via loudspeaker. In the active oddball condition, participants were instructed to press a button if they noticed differences in the sound series. The day after the last exposure session, all participants were measured again with an EEG, using an identical protocol as before the passive exposure.

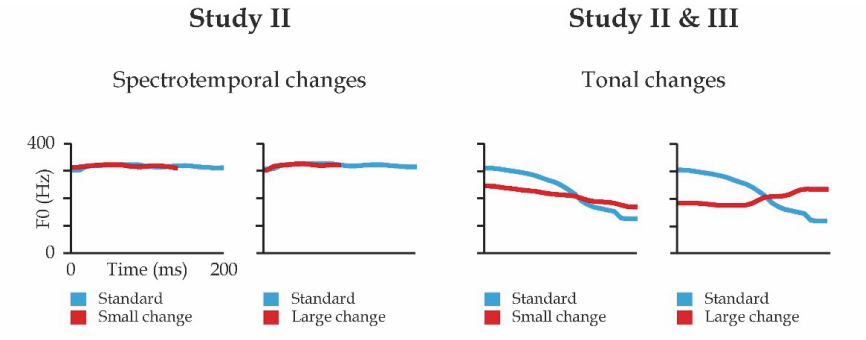

FIGURE 2 Fundamental frequencies (F0) of the speech sounds used in Study II & III. Note that the method for shortening the spectrotemporal sounds caused differences between the standard (blue) and deviant (red) sounds immediately at the beginning of the sound.

2.3 Electrophysiological recordings

In **Studies I** and **II**, continuous local field potentials (LFPs) were recorded from the left auditory cortex under urethane anesthesia. Teflon-insulated silver wire (diameter 200 μm) was placed on the surface of the dura.

In **Study III**, raw EEGs were recorded with a 128-channel sensor net using Ag-AgCl electrodes arranged according to the extended international 10–20 system and referenced to Cz during the recording.

2.4 Statistical analyses

In **Study I**, the mean amplitude values were calculated from three regions of interest (ROI) (30-70 ms, 80-120 ms and 140-180 ms after stimulus onset). For the mean values, repeated measures of variance (ANOVA) were applied to stimulus type (standard vs. deviant), deviant type (4.5 kHz, 3.5 kHz), ISI (375 ms, 600 ms) and ROI (30-70 ms, 80-120 ms, 140-180 ms). Post-hoc tests for statistically significant interactions in ANOVA were carried out using two-tailed paired samples t-tests. To test if the responses to oddball deviant sounds and the responses to control sounds differed in amplitude, two-tailed paired

samples t-tests were applied to mean values separately for each ROI. All t-tests were carried out using bootstrapping with 1000 permutations.

In **Study II**, the mean amplitude values were calculated from a time window of 100-150 ms from the stimulus onset. Then, for the mean values, ANOVA was applied to stimulus type (standard, deviant) and deviant type (small, large change) and between subjects factor was group (exposed, naive). Two-tailed one-sample t-tests were used as post-hoc tests.

In **Study III**, mean amplitude values were calculated separately for each ERP component from a time window after the stimulus onset. Time windows of 190-240 ms for the MMN and 250-300 ms for the P3a in the ignore condition were chosen. Time windows of 210-260 ms for the N2b and 360-410 ms for the P3b in the active condition were chosen. The mean values for the MMN and the P3a were calculated as the mean from three separate electrode clusters roughly corresponding to F3, Fz and F4, respectively. For the N2b, the electrode clusters corresponded to C3, Fz and C4. For the P3b, the electrode clusters corresponded to P3, Pz and P4. Separately for each component, the mean values were analyzed with ANOVA with within-subjects factors stimulus type (standard, deviant), deviant type (small, large change), electrode cluster (left, mid, right) and session (pre, post exposure) and between subjects factor was group (exposed, naive).

In **Study III** the latencies were analyzed from deviant responses. The electrode clusters for the latency analyses were the same as those applied for the amplitude analysis.

In **Study III** two-tailed paired samples t-tests were carried out for post-hoc comparisons. All t-tests were carried out using bootstrapping with 1000 permutations. A criterion for significance was CI 97% to correct for multiple comparisons.

When the interaction effect of session x group was found to be significant, two-tailed Pearson correlation coefficients were calculated between behavioural responses (reaction times and the corresponding ERP-amplitude and ERP-latency).

3 RESULTS

3.1 Study I: Auditory change detection in mice is dependent on sensory memory

Study I investigated whether the MMR in mice reflects memory-based encoding of regularity violations and if it is dependent on sensory memory. This was investigated by recording LFPs from the auditory cortex of anesthetized mice. We recorded responses to frequency changes in an oddball condition, where occasional changes in frequency were interspersed amongst a standard sound. The ISIs between stimuli were manipulated so that, in one oddball condition, it was 375 ms, and in another, 600 ms. Lastly, an equiprobable condition was applied with 375 ms ISI as the control for the adaptation effect.

It was found that the frequency changes elicited differential responses (deviant - standard). At ROIs of 80-120 ms and 140-180 ms, responses to both 4.5 kHz and 3.5 kHz deviant sounds elicited larger responses than standard sounds. Responses elicited by the deviant sounds did not differ from each other in these ROIs. However, in the earlier 30-70 ms ROI, only the responses to 4.5 kHz deviant sounds were larger than the responses to standard sounds. Furthermore, the 4.5 kHz deviant sound elicited larger responses than the 3.5 kHz deviant sound (Figure 3 A, B).

Additionally, the differential response was larger when the ISI was 375 ms than when it was 600 ms. The decrease in the differential response amplitude was due to the observation that the responses to standard sound enlarged when the ISI was prolonged from 375 ms to 600 ms, while the deviant responses were unaffected (Figure 3 A, B).

To test the underlying mechanism of the differential response, the responses to deviant sound in the oddball condition were compared to responses to physically identical sounds presented in the equiprobable control condition. In 80-120 and 140-180 ms ROIs, both deviant types elicited larger responses than the same sound in the control condition. In the earlier 30-70 ms

ROI, there were no significant differences between the deviant and control responses (Figure 3A).

In conclusion, the results from **Study I** demonstrate that the MMR to frequency changes in mice are specific to regularity violations and dependent on the sensory memory.

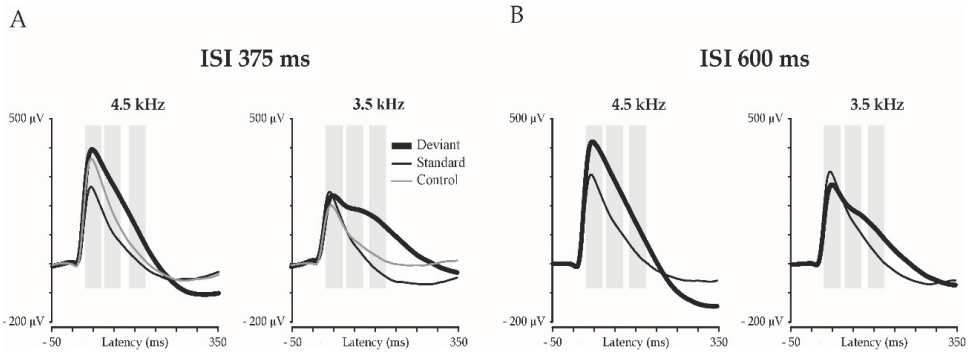

FIGURE 3 Study I: Frequency changes reflects genuine change detection, which was dependent on sensory memory. Grand averaged waveforms for the responses to deviant (thick black), standard (thin black) and control sounds (gray). Light grey rectangles indicate ROI at 30-70 ms, 80-120 ms and 140-180 ms after stimulus onset, respectively. The control condition was applied only in the 375 ms ISI condition, since the adaptation must explain the responses with the 375 ms ISI in order to explain the responses obtained with longer ISIs.

3.2 Study II: Passive exposure induces memory traces to speech sounds in adult rats

Study II investigated whether passive exposure to human speech sounds can form long-term memory representations in the rat brain. One group of animals was exposed to spectrotemporal changes and other group to tonal changes in speech sound /a/ for three consecutive days, 12 hours per day. Exposure was delivered in an oddball condition where one deviant had a large difference and the other a small difference, as compared to the standard sound. The MMR amplitude was used as an index of the memory representation. This was investigated by recording LFPs from the auditory cortex of anesthetized rats after exposure to both spectrotemporal and tonal changes in separate oddball conditions.

Large changes in spectrotemporal features elicited the MMR in both groups. Small changes elicited the MMR only in the group exposed to these stimuli (Figure 4A). Tonal changes did not elicit the MMR in either group, and no exposure effect was observed (Figure 4B).

Results from **Study II** demonstrate that passive exposure to speech sound changes can, indeed, induce long-term memory representations in the adult rat brain. This was demonstrated for spectrotemporal features in speech sounds, but not for tonal features.

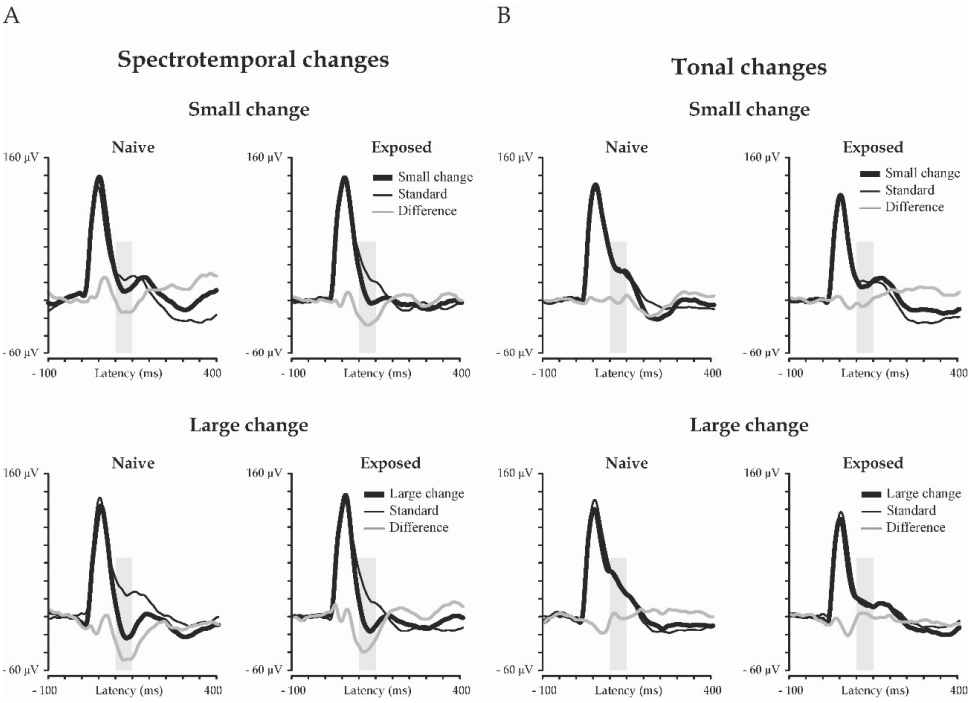

FIGURE 4 Study II: Passive exposure to spectrotemporal changes induces plastic changes in cortical responses. Grand-averaged waveforms for the responses to deviant sounds (thick black), standard sounds (thin black), and differential (gray) responses. Light gray rectangles represent ROI of 100-150 ms after the stimulus onset.

3.3 Study III: Passive exposure induces memory traces to foreign speech sounds in adult humans

Study III examined the possibility that passive exposure to foreign speech sounds in adulthood could enhance neural discrimination of these sounds. One group of participants was exposed to tonal changes in vowel /a/ for four consecutive days, two hours per day, while they concentrated on silent movies. Another group of participants served as controls and no exposure was delivered. Before and after the exposure, both groups of participants had an EEG measurement where tonal changes were presented in ignore and active oddball paradigms.

Reaction times shortened from pre-measurement to post-measurement for both large and small changes. Furthermore, the change in reaction times was greater for the small change than for the large change. The accuracy for the large change was significantly better than the accuracy for the small change. However, the exposure did not have any effect on reaction times or accuracy.

Results from the ignore test condition showed that, after the exposure, MMN latency got significantly shorter compared to the pre-measurement in the exposure group, while no changes were observed in the control group. On the other hand, no amplitude changes were observed in either group.

The P3a latency for the large change shortened after the exposure. In the control group, no latency changes were observed. For the P3a amplitude, both standard and deviant response amplitudes got significantly more positive after the exposure. In contrast, in the control group, the amplitude of the deviant response got more negative from pre-measurement to post-measurement.

In the active test condition, the P3b component was enhanced due to passive exposure. In participants who were exposed to speech sounds, the small change elicited larger P3b components after the exposure compared to the pre-measurement; this enhancement correlated negatively with reaction times to the same stimuli in post-measurement (Figure 5 A, C). No changes in amplitude were observed in the control group (Figure 5 B, D) or in the latencies in either group.

Study III concluded that passive exposure for four consecutive days, two hours per day, enhanced the participants' change detection for foreign speech sounds. These results show that passive exposure to speech sounds can induce plastic changes related to change detection in the adult brain, which were previously observed only in infancy during the sensitive period.

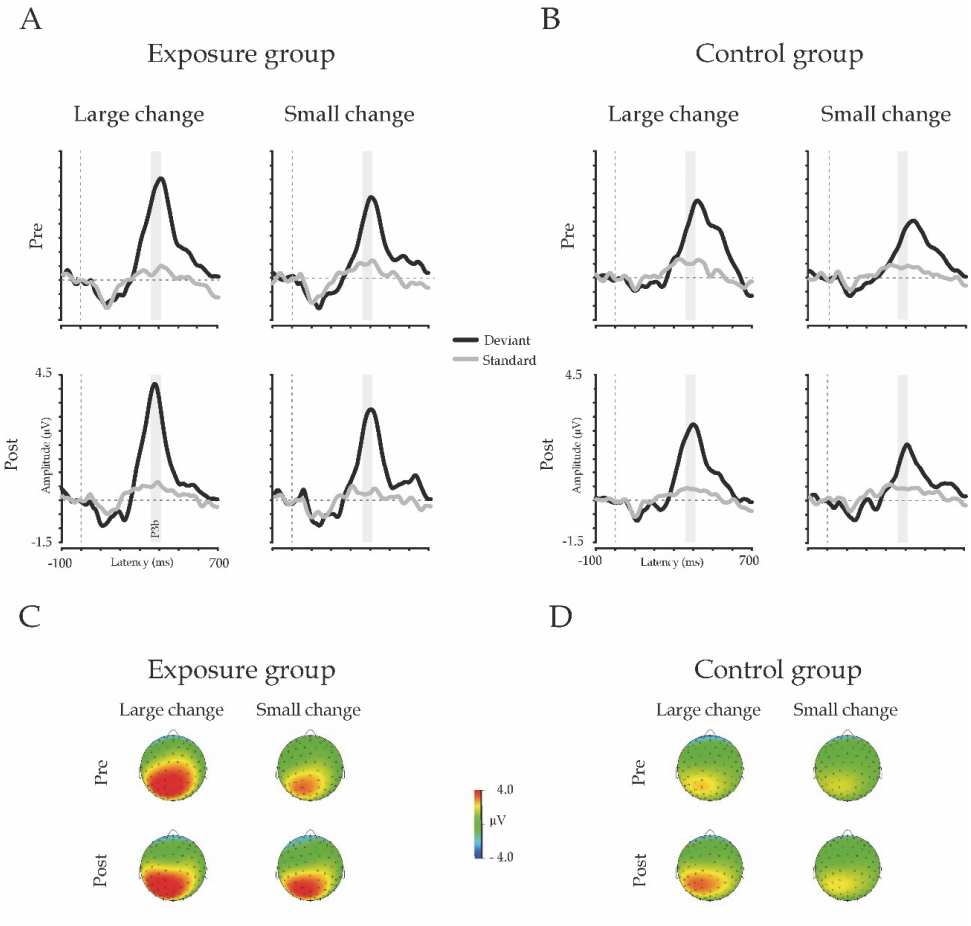

FIGURE 5 Study III: Passive exposure enhances the P3b component for the small tonal change. (**A**, **B**) Grand averaged waveforms for responses to deviant (black) and standard sounds (gray). The averaged amplitude values are presented from the collapsed electrode clusters (left, middle and right parietal) from the time window of 360-410 ms after the stimulus onset. (**C**, **D**) Scalp topographies for the differential responses (deviant - standard) from the 128 electrodes for the P3b at 360-410 ms for the exposure group (**C**) and for the control group (**D**).

4 DISCUSSION

4.1 Change detection in mice

The main finding in **Study I** was that the MMR in mice showed two of the same properties as the MMN in humans. Namely, the MMR in mice reflected genuine change detection, and not a mere adaptation, which was dependent on sensory memory. This was investigated by measuring LFPs from the auditory cortex of urethane-anesthetized mice.

It was found that deviant sounds elicited larger responses than standard sounds in all investigated time ROIs, pointing to the occurrence of change detection. Further, the responses to oddball deviant sounds were larger than the responses to physically identical control sounds at ROIs of 80-120 ms and 140-180 ms after stimulus onset, but not at the earlier 30-70 ms ROI. It can thus be interpreted that, at these later ROIs, the MMR in mice reflects genuine deviance detection, similar to humans and anesthetized (Astikainen et al., 2011; Jacobsen & Schröger, 2001; Nakamura et al., 2011; Näätänen, Jacobsen, & Winkler, 2005; Parras et al., 2017; Shiramatsu et al., 2013) and awake rats (Harms et al., 2014). This is in line with prior results in awake mice, which showed detection of regularity violations with recording of LFPs and single cell responses from the subcortical and cortical auditory areas (Parras et al., 2017). That study demonstrated that the genuine deviance detection was more evident in the cortical than subcortical areas and in non-lemniscal than lemniscal regions. Together with the findings by Parras et al. (2017), results from the present study support the notion that the MMR in mice is not explained by mere neural adaptation but reflects memory-based detection of regularity violations.

However, the earliest ROI (30-70 ms) did not show differential response specific to regularity violations. Even though the ascending (4.5 kHz) deviant elicited a larger response than the standard sound (4.0 kHz), the oddball-deviant response did not differ from the control sound response amplitudes. These results could indicate that the earliest part of the MMR to the 4.5 kHz deviant sound is explained by a mechanism related to neural adaptation.

Furthermore, in the earliest ROI, the responses could be associated with the human N1, which encodes stimulus energy and is thus larger for high frequency sounds than low frequency sounds (Näätänen & Picton, 1987).

The MMR in mice was also dependent on sensory memory. When the ISI was extended from 375 ms to 600 ms, the MMR amplitude diminished, similarly as in previous human studies (Bartha-Doering, Deuster, Giordano, Am Zehnhoff-Dinnesen, & Dobel, 2015). Here, the MMR amplitude was diminished due to the fact that the responses to standard sounds got larger when the ISI was prolonged, but the responses to deviant sounds did not change. This suggests that the memory trace for the standard sound is critical for the change detection process.

According to results from **Study I**, the sensory memory span in mice appears to be at least 600 ms. However, since longer ISIs were not applied, the limit of the sensory memory duration could not be determined. Furthermore, only frequency changes in sounds were applied, but it is known that different stimulus features have different sensory memory decay times (Bartha-Doering et al., 2015). In future, different combinations of stimulus features and ISI should be used to more precisely determine the sensory memory duration in mice.

The MMR was not more sensitive to ascending (4.5 kHz) deviant sounds compared to descending (3.5 kHz) deviant sounds, with standard sound being 4.0 kHz. However, in a previous study in rats, which applied the same frequencies as **Study I**, the MMR was observed only for ascending deviant sounds (Astikainen et al., 2011). In humans, both ascending and descending deviants elicit the MMN, but ascending deviants elicit larger MMN compared to descending deviants (Peter, McArthur, & Thompson, 2010; Ruusuvirta & Astikainen, 2012). It seems that change detection in mice is not as sensitive to ascending deviations in frequency as in rats, at least when these relatively low frequencies to mice optimal hearing range are used (Heffner & Heffner, 2007).

Here in the equiprobable control condition frequencies from 3.3 kHz o 4.8 kHz in 0.1 kHz steps were used, so consecutive tones had 2.1 - 2.9 % difference in frequency. Mice are able to behaviorally discriminate tones when their difference in frequency is 4 - 7 % (de Hoz & Nelken, 2014). In light of this, it could have been that mice perceived some of the consecutive tones in the equiprobable control condition as one stimulus. Furthermore, neurons in the auditory cortex adapt to tone frequency that is presented repeatedly but this may have happened also to tones with closely adjacent frequencies - a phenomenom called cross-frequency adaptation (Taaseh et al., 2011). Therefore, the equiprobable control condition applied in Study I was not entirely optimal to control for adaptation effects on the MMR.

4.2 Passive exposure in adult rats

Study II revealed that passive exposure to speech sounds for three consecutive days, 12 hours per day, is sufficient to induce long-term memory representations for these sounds in adult animals. The animal group exposed to changes in spectrotemporal features in speech sound /a/ demonstrated a statistically significant MMR at 100-150 ms latency for the small changes in spectrotemporal features after the exposure, while animals exposed to tonal changes did not. Previously, plastic changes in the auditory system have been demonstrated for behaviourally relevant stimuli only (Bao, Chan, & Merzenich, 2001; Bao, Chan, Zhang, & Merzenich, 2003; Keuroghlian & Knudsen, 2007; Zhang et al., 2001).

In our study, the exposure was delivered in the oddball condition, which is the so-called statistical learning paradigm, and LFPs were recoded to speech sounds. In contrast, previous studies have applied stimulus conditions, including only repetition of a single stimulus, such as pure tone or temporal modulation rates in pure tone, and single-cell responses were measured (Bao et al., 2004; de Villers-Sidani et al., 2007; Zhang et al., 2001). In these previous studies, passive exposure did not result in reorganization of the cortical maps of the adult animals. It is difficult to estimate whether our findings were related to the type of stimuli, stimulus condition or measurement. Here we used speech sounds that contained several frequencies, which probably activated larger cell populations on the cortex than simple tones. Furthermore, the change detection mechanism was different from the mechanism related to detection of a single sound. Lastly, we measured LFPs instead of single cell responses, which captured the activity of a relatively large population of cells from the cortex.

In our study, the exposure lasted for three consecutive days, 12 hours per day. This exposure time is less than in previous studies in rats in which exposure had been three days, 24 hours per day (de Villers-Sidani et al., 2007), 19 days, 1-16 hours per day (Zhang et al., 2001) or up to 2 months, two hours per day (Bao et al., 2004), but no exposure effects were observed when single cell responses were recorded. However, in one study, exposure of five consecutive days, 20 minutes per day, was used in mice; single cell responses showed increased inhibition to exposed tone (Kato et al., 2015). This leaves it inconclusive what amount of exposure time is needed to elicit changes in the neural level. This is most probably also different for different stimulus types and animal species.

In addition to the exposure time duration, the amount of stimulus repetitions can be one factor influencing the exposure effect. In our study, > 250,000 stimuli were presented within the 36-hour exposure period. It is plausible that the relatively large number of stimuli repetitions administered in a statistical learning paradigm enabled observation of the effect of passive exposure.

In general, the finding that rats can discriminate spectrotemporal changes in speech sounds is in line with previous studies showing that the auditory system of a rat can discriminate speech sounds (Ahmed et al., 2011; Engineer et al., 2008). However, the tonal changes were not discriminable for the rats. This is somewhat surprising, since, in a previous study, rats demonstrated the MMR to raising and falling frequencies in sinusoidal tone pairs (Ruusuvirta et al., 2007). It can be speculated that those tonal changes that occur gradually (over a 200 ms period) are more difficult for the rat brain to discriminate than faster changes in sound frequency (i.e. within 50 ms, as in Ruusuvirta et al., 2007). In addition, it has been shown that speech sounds that evoke very similar neural patterns, measured with single cell recordings, are difficult for the rat to discriminate (Engineer et al., 2008, 2014). It might be that the relatively small changes in frequency in tonal speech sounds used in **Study II** evoked similar neural patterns making them non-discriminable for the rat brain.

4.3 Passive exposure in adult humans

In **Study III**, passive exposure to foreign speech sounds for four consecutive days, two hours per day, enhanced the discrimination of the speech sounds at a neural level in adult humans. Enhancement in auditory discrimination, including learning to discriminate speech sounds, was previously thought to occur passively only during the sensitive period in infancy (Cheour et al., 1998; Kuhl, 2004).

In **Study III**, MMN latency shortened after the exposure, while no changes were observed in the control group between pre-measurement and post-measurement. Previously, only behavioural training had been shown to modulate MMN latency (Kraus et al., 1995; Tremblay et al., 1997, 1998), reflecting enhanced discrimination ability of the sounds used during the training.

Even though MMM latency was shortened due to the passive exposure, MMN amplitude did not change. Behavioral training usually increases MMN amplitude (Kraus et al., 1995; Näätänen et al., 1993; Tremblay et al., 1997). It is possible that the amount of passive exposure applied in **Study III** was not sufficient to elicit changes in MMN amplitude.

Even if the amplitude of the MMN response was not changed due to passive exposure, the subsequent P3a amplitude was changed. Passive exposure to speech sounds enhanced the P3a amplitude and shortened its latency. Previously, active discrimination training of pitch (Seppänen et al., 2012), tone sequences (Atienza et al., 2004) or learning to use Morse code (Uther et al., 2006) have led to increased P3a amplitude. **Study III** thus demonstrates that mere passive exposure is sufficient to change the involuntary attention-shifting mechanism, to which the P3a is typically linked.

It is also possible that the exposure related changes in P3a amplitude hindered the observability of the MMN amplitude changes due to passive

exposure. Namely since P3a amplitude increased and latency shortened, it can be assumed that the MMN and the P3a were more overlapped in the post than pre measurement.

In the active test condition, in the N2b time window, responses to standard and deviant sounds became larger, towards positive polarity in the exposure group, but also, to some extent, in the control group. Namely, while the changes in the N2b were observed in all electrode sites in the exposure group, the change was found only in a single electrode cluster in the control group. Therefore, there seems to be some exposure effect in the N2b time window. However, since the responses to standard and deviant sounds became more positive in amplitude, it might be that these responses do not purely reflect the N2b, but instead the P2 component, which is usually observed to peak around the same latency (here the time window was 210-260 ms post stimulus onset). Indeed, enhancement for the P2 has previously been observed to standard sound or a single repetitive sound after passive exposure (Sheehan et al., 2005; Tremblay et al., 2010). In **Study III**, during the exposure, the standard sound was repeated many times, but the deviant sounds were also played over 10,000 times, which could also explain the P2 enhancement for the deviant responses. However, it remains inconclusive whether passive exposure affected the exogenous stimulus classification (P2) or the attention driven deviance detection from a mentally-stored representation of the standard (N2b).

The passive exposure enhanced the attention driven P3b component for small tonal changes. Previously, it has been shown that when the perceptual task becomes easier, the P3b is enhanced (Isreal, Chesney, Wickens, & Donchin, 1980; Kramer, Wickens, & Donchin, 1985). Furthermore, one previous study has shown that active training to discriminate speech sounds enhances the microstates related to the P3b component accompanied by improvements in behavioural reaction times (Giroud et al., 2017). In light of the context-updating model (Polich, 2007), passive exposure seems to ease conscious discrimination between the representation of standard sound in working memory and the deviant sound input.

In **Study III**, exposure expanded over four consecutive days, resulting in a total of eight hours of exposure. Previously, passive exposure had been applied only for short periods of time (1-2 hours) during a single day, with no exposure effects in ERPs, reflecting discrimination have been demonstrated (Elmer et al., 2017; Näätänen et al., 1993; Sheehan et al., 2005). Studies applying behavioural training have also spread the training sessions over several days (1-7 days) (Kraus et al., 1995; Tremblay et al., 1997). Furthermore, sleep deprivation or measurements taken on the same day have prevented the emergence or enhancement of the MMN and the P3a, when behavioural training has been applied (Alain, Zhu, He, & Ross, 2015; Atienza et al., 2004). Thus, nocturnal sleep seems to be a crucial factor for the emergence of learning-related enhancement in the MMN and the P3a, probably due to memory consolidation. In **Study III**, the exposure expanded over four consecutive days and the post-EEG measurement was a day after the last exposure session, so nocturnal sleep

could have facilitated memory consolidation of exposed sounds, allowing the observation of the exposure effect.

4.4 General discussion

This dissertation research shows that rodent models of auditory change detection can be feasible to study neural mechanisms of auditory cognition. MMR was demonstrated to frequency changes in mice (**Study I**) and spectrotemporal changes in speech sounds in rats (**Study II**). In mice, MMR reflected genuine change detection and was dependent on sensory memory, similar to the MMN in humans. From the theoretical point of view, results from **Study I** support the deviance detection hypothesis (Garrido, Kilner, Stephan, & Friston, 2009; Winkler, Denham, & Nelken, 2009) as the neural mechanism behind MMN generation in mice. In summary, these results, together with previous results, support the notion that the MMR can be reliably observed across rodent species (Astikainen et al., 2011; Harms et al., 2014; Parras et al., 2017; Ruusuvirta, Penttonen, & Korhonen, 1998). Furthermore, these results indicate that rodent models are feasible for studying the neural underpinnings of auditory change detection, which are also related to speech perception.

In addition, the results from this dissertation verify that mere passive exposure to novel sounds can provoke enhanced neural discrimination in adulthood in both humans and rats. The results suggest that experience-dependent plasticity, which can have a long-lasting impact on neural representations, is preserved in adulthood across at least these two mammalian species.

Furthermore, even though human speech sounds are behaviourally less important for rats than their own vocalizations (Bao, 2015), passive exposure still enhanced their neural representation. In humans, the speech sounds applied in the exposure contained tonal features, which are absent in the native language of the Finnish participants, and, for that reason, were difficult for them to discriminate. Even though the tonal changes contained no behavioural meaning for the participants, passive exposure enhanced the neural discrimination of these changes. It seems that sensory exposure during adulthood readies the nervous system to discriminate stimuli that might become behaviourally relevant, which is similar to previous findings on passive exposure during the sensitive period (Bao, 2015; Kuhl, 2004).

In **Study III**, participants were not informed about the characteristics of the used stimuli or the changes in them. Taking that into account, participants could not form linguistic meaning for the speech sounds. Similar sounds in native Chinese are distinguished as members of different categories of speech sounds as indexed by the difference in the MMN (Xi, Zhang, Shu, Zhang, & Li, 2010), N2b and P3b components (Zhang, Xi, Wu, Shu, & Li, 2012). Furthermore, this could have also hindered the appearance of improvement in behavioural level.

In humans, the exposure effect was observed most prominently for the P3a and P3b components. These components are thought to have their sources in the large brain areas within the frontal and temporal lobes (Polich, 2007), which result in larger response amplitudes in ERPs. On the other hand, the source of the MMN is in the temporo-frontal network (Garrido et al., 2009; Näätänen et al., 2007), which is a relatively small region and results in smaller response amplitudes in scalp-recorded ERPs. The better signal-to-noise ratio for the P3 components than for the MMN component might have contributed to the observed result pattern, which demonstrated the exposure effect in the P3 components, but not in the MMN components.

The results from this dissertation could be implemented in foreign language learning programs. Passive exposure could be utilized in classroom teaching of languages or in self-learning. Courses teaching a new language could have sessions of mere passive exposure in addition to attentive learning, which could ease a student's perception of the crucial features of the new language. Also, smart phone applications could be developed where passive exposure, in combination with behavioural training, could ease learning. In particular, passive exposure before any behavioural demands could possibly ease the stress and anxiety related to beginning to learn a new language (Hashemi, 2011).

The methods of this dissertation have some limitations that need to be discussed. I investigated automatic change detection with anesthetized animals. The present experiments as well as several previous ones have applied urethane as an anesthetic agent and successfully demonstrated genuine mismatch response and dependence on sensory memory (Ahmed et al., 2011; Astikainen, Ruusuvirta, Wikgren, & Penttonen, 2006; Astikainen et al., 2011). Furthermore, Parras et al (2017) demonstrated genuine change detection in both urethane-anesthetized and awake rodents. Thus, urethane-induced anesthesia seems to leave the sensory memory change detection functions in rodents mostly unaffected. Studies with conscious animals would, however, allow better comparability to ERP studies in humans.

Here the effect of passive exposure was observed in spectrotemporal changes in rats and tonal changes in humans in the vowel /a/. In this thesis, the aim was to demonstrate the effect of passive exposure in adult subjects to change detection as indexed by the brain responses. Even if the stimuli were speech sounds, we cannot conclude that the effects were specific to speech sounds or spectrotemporally complex sounds in general. Neither have I claimed that change detection in these speech sound features reflects linguistic processing in humans. In future studies, the effects of passive exposure to words and different linguistic features should be investigated.

Participants watched silent movies during the exposure in **Study III**. Questions about the plot of the movie were asked after every 30 minutes to ensure that the participants were directing their attention to the movie. However, we cannot exclusively argue that the participants did not pay any attention to the sounds during the two-hour sessions. In **Study II**, the rats were

exposed in their home cages while they performed normal daily behaviour. Again, however, we cannot exclusively argue that the rats did not direct their attention to the sounds at any point during the exposure.

From a statistical point of view, in **Study I** and **Study II** more strict controls for multiple comparisons could have been applied. In **Study I**, we had clear hypothesis that responses to deviant sounds should be larger than responses to standard sounds or responses to control sounds. However, we did not have specific hypothesis of the different ROIs. Again, in **Study II**, we had clear hypothesis for enhanced MMN in response to the specific type of sound exposure. However, we did not have specific hypothesis that the exposure effect would be different for large or small change. In those cases where we did not have specific hypothesis, correction for multiple comparisons could have been applied. Even though corrected P-values are not reported in the original publications of **Study I** and **II**, majority of the post-hoc test would have survived the conservative Bonferroni correction. Moreover, the effect sizes suggest that the observed effects were quite large (all Cohen's $d > 0.7$) in both Study I and Study II.

Previous studies in animals have focused mainly on the function of the cortical areas or the nuclei along the ascending auditory pathway, partly dismissing the possible role of other brain regions in auditory change detection. Namely, the MMN-like responses can be observed, for example, in the hippocampus, even though these responses were not controlled for possible adaptation and they might have been volume conducted from the auditory cortical areas (Astikainen, Ruusuvirta, & Korhonen, 2005; Ruusuvirta, Astikainen, Wikgren, & Nokia, 2010; Ruusuvirta, Lipponen, Pellinen, Penttonen, & Astikainen, 2013; Witten et al., 2014). In the future, the role of the hippocampus and its interaction with cortical areas in change detection and perceptual learning could be studied in animal models more carefully.

In conclusion, the results from this dissertation demonstrate genuine change detection in mice, which is dependent on sensory memory. Furthermore, in humans and rats, passive exposure to novel sounds resulted in the development of long-term memory traces to these sounds, pointing to perceptual learning. Thus, the auditory change detection mechanism seems to share some of the same fundamental properties in mice, rats and humans, and perceptual learning occurs even without effortful training in adult subjects.

YHTEENVETO

Kuulonvarainen havainto-oppiminen: Tuloksia elektrofysiologisista mittauksista jyrsijöiltä ja ihmisiltä

Yllättävät muutokset äänimaailmassa ympärillämme viestittävät yleensä selviytymisen kannalta tärkeistä vihjeistä, joten niiden nopea ja automaattinen havaitseminen on tärkeää. Kyky havaita näitä käyttäytymiselle tärkeitä muutoksia äänissä parantuu harjoittelun avulla ja tällaista oppimista kutsutaan havainto-oppimiseksi. Havainto-oppimisen seurauksena hermostoon muodostuu uusia muistijälkiä. Kuitenkin varhaisessa kehityksessä herkkyyskauden aikana muistijälkien muodostuminen, jota ihmisellä tapahtuu erityisesti puheääniin liittyen, on automaattista, eikä siihen vaadita tietoista harjoittelua. Toisaalta perinteisesti on ajateltu, että herkkyyskauden jälkeen vain aktiivinen harjoittelu johtaa uusien muistijälkien muodostumiseen.

Kokeellisesti muutoksen havaitsemiseen liittyvää hermostollista perustaa voidaan tutkia mittaamalla aivojen sähköisiä jännitevasteita ärsyketilanteessa, jossa esitetään toistuvan äänen joukossa silloin tällöin satunnaisesti poikkeava ääni. Ihmisillä toistuva ääni muodostaa hermostollisen muistimallin, johon poikkeavaa ääntä verrataan aivoissa. Mikäli poikkeava ääni ei sovi toistuvan äänen muodostamaan muistimalliin, aiheuttaa se muutoksen havaitsemista ilmentävän aivovasteen. Lisäksi vaihtelemalla äänten esitysvälin pituutta voidaan tutkia myös kaikumuistin eli lyhytkestoisen muistin merkitystä muutoksen havaitsemisessa.

Väitöskirjan ensimmäisessä osatutkimuksessa tarkasteltiin, onko hiiren muutoksen havaitsemiseen liittyvä aivovaste toiminnallisesti samankaltainen kuin ihmisellä. Hiirille esitettiin muutoksia äänen taajuudessa. Ensin äänet esitettiin lyhyellä ja sitten pidemmällä esitysvälillä. Tällä tavoin voitiin tutkia muutoksen havaitsemista ilmentävän aivovasteen riippuvaisuutta lyhytkestoisen muistin toiminnasta. Lisäksi muutoksia äänen taajuudessa esitettiin ärsyketilanteessa, jossa kaikilla äänillä oli sama esiintymistodennäköisyys. Tällä kontrollikokeella voitiin varmistaa, että muutoksen havaitsemisvaste esiintyy vain harvoin esitettyihin poikkeaviin ääniin. Äänien esityksen aikana mitattiin aivojen sähköisiä jännitevasteita hiiren kuuloaivokuorelta. Aivovasteet äänen muutoksiin osoittivat, että hiiren muutoksen havaitseminen pohjautuu toistuvan äänen luomaan muistimalliin ja se on riippuvainen lyhytkestoisen muistin toiminnasta.

Toisessa osakokeessa tutkittiin voiko pelkkä passiivinen altistuminen puheäänille saada aikaan uusia pitkäkestoisia muistijälkiä aikuisilla rotilla. Aikuisille rotille esitettiin muutoksia ihmisen puheäänissä kolmen perättäisen päivän ajan 12 tuntia päivässä. Ensimmäiselle ryhmälle eläimiä esitettiin spektrotemporaalisia muutoksia ja toiselle ryhmälle eläimiä tonaalisia muutoksia vokaalissa /a/. Kenttäpotentiaalimittaukset rottien kuuloaivokuorelta osoittivat, että altistaminen äänille paransi muutoksen havaitsemista puheäänten piirteissä ja sai aikaan ääniin liittyviä uusia muistijälkiä. Tämä havaittiin spektrotemporaalisille muutoksille mutta ei tonaalisille muutoksille. Tulokset osoittavat, että pelkkä

passiivinen altistuminen saa aikaan havainto-oppimiseen liittyviä muistijälkiä myös aikuisilla eläimillä.

Kolmannessa osakokeessa tutkittiin, saako pelkkä passiivinen altistuminen vieraan kielen puheäänteille aikaan uusia pitkäkestoisia muistijälkiä aikuisilla ihmisillä. Ryhmälle aikuisia koehenkilöitä esitettiin tonaalisia muutoksia neljän perättäisen päivän ajan kaksi tuntia päivässä samalla kun he keskittyivät katselemaan mykkäelokuvia. Ennen ja jälkeen altistuksen mitattiin aivojen sähköisiä jännitevasteita samoihin ääniin, joita käytettiin altistuksessa. Toinen ryhmä koehenkilöitä ei saanut vastaavaa äänialtistusta, vaan he osallistuivat pelkästään aivovastemittauksiin. Aivovastemittaukset päänahan pinnalta osoittivat, että altistaminen vieraan kielen puheäänille johti muistijälkien muodostumiseen. Vastaavia muutoksia ei havaittu koehenkilöillä, jotka eivät altistuneet puheäänille. Tulokset osoittavat, että pelkkä passiivinen altistuminen saa aikaan pitkäkestoisia muistijälkiä myös aikuisilla ihmisillä.

Kokonaisuudessaan tämän väitöskirjan tulokset viittaavat siihen, että muutoksen havaitsemisen pohjautuu samankaltaisiin periaatteisiin hiirellä, rotalla ja ihmisellä. Hiirillä muutoksen havaitseminen osoitettiin olevan riippuvainen lyhytkestoisen muistin toiminnasta, samalla tavalla kuin ihmisillä. Lisäksi havaittiin ihmisillä ja rotilla, että myös aikuisuudessa pelkkä passiivinen altistuminen äänille johtaa uusien muistijälkien syntymiseen. Tämän on aikaisemmin oletettu olevan mahdollista vain varhaisen kehityksen herkkyyskauden aikana. Väitöskirjan tulosten valossa havainto-oppimiseen liittyvät vallitsevat teoriat vaativat uudelleen arviointia.

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ORIGINAL PAPERS

I

ELECTROPHYSIOLOGICAL EVIDENCE OF MEMORY-BASED DETECTION OF AUDITORY REGULARITY VIOLATIONS IN ANESTHETIZED MICE

by

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Electrophysiological evidence of memory-based detection of auditory regularity violations in anesthetized mice

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In humans, automatic change detection is reflected by an electrical brain response called mismatch negativity (MMN). Mismatch response is also elicited in mice, but it is unclear to what extent it is functionally similar to human MMN. We investigated this possible similarity by recording local field potentials from the auditory cortex of anesthetized mice. First, we tested whether the response to stimulus changes reflected the detection of regularity violations or adaptation to standard stimuli. Responses obtained from an oddball condition, where occasional changes in frequency were presented amongst of a standard sound, were compared to responses obtained from a control condition, where no regularities existed. To test whether the differential response to the deviant sounds in the oddball condition is dependent on sensory memory, responses from the oddball condition using 375 ms and 600 ms inter-stimulus intervals (ISI) were compared. We found a differential response to deviant sounds which was larger with the shorter than the longer ISI. Furthermore, the oddball deviant sound elicited larger response than the same sound in the control condition. These results demonstrate that the mismatch response in mice reflects detection of regularity violations and sensory memory function, as the human MMN.

Detection of sudden changes in the auditory environment is an important task for the brain, as these changes may signal behaviourally relevant information. In the auditory modality, automatic change detection can be studied in humans by recording the event-related potential (ERP) called mismatch negativity (MMN)[1]. MMN is an important tool for preclinical research since it reflects cognitive dysfunction in several neuropsychiatric and neurological diseases[2,3], and MMN also has the potential to be utilized as a clinical tool in the future[4].

A response analogous to the human MMN, usually called mismatch response (MMR), is elicited in animals in local field potential (LFP) recordings[5]. Many of the existing animal studies have been conducted in rats e.g.[6–8], but there are also studies in mice, guinea pigs, cats, monkeys, and rabbits[9–14].

MMN is experimentally elicited by using an oddball stimulus condition in which rarely presented 'deviant' sounds are randomly interspersed with frequently presented 'standard' sounds. MMN is usually defined as a difference between the responses to standard and deviant sounds, and has been suggested as a method to index change detection based on the comparison process between memory traces formed by the repetitive standard sounds and the incoming input of the deviant sounds[15].

When MMN is recorded in a stimulus condition with a single standard stimulus, it is also possible that deviant sounds generate larger responses than standard sounds because the cell population responding to the frequently occurring sound is less adapted than the distinct cell population responding to the rare sound[16]. The impact of this adaptation effect can be estimated by using a 'many standards' control condition, also called an 'equiprobable' control condition[17–21]. In this condition, the same stimulus used as a deviant sound in the oddball sequence is presented among several different stimuli, all having the same probability within the series. This allows for the comparison of the responses to two physically identical stimuli presented under both the oddball condition and the equiprobable condition. Since the presentation rate for the oddball deviant sound and for the control sound

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is the same, the obtained responses have similar levels of adaptation. Larger amplitude responses to the oddball deviant sound than to the control sound can thus be interpreted as responsiveness specific to regularity violations in the oddball condition.

MMN studies in humans have demonstrated larger responses to oddball deviant sounds when compared to control sounds (same sound in equiprobable condition)[18] (for a review, see[21]). Equiprobable condition has been applied similarly in awake rats[7,8,22], in rats under anaesthesia[7,20,23,24], and these results have demonstrated larger amplitude responses to deviant sounds than control sounds. Only one earlier study has applied equiprobable condition in combination with LFP recording in mice[25]. This study applied also so called cascade control condition[26]. Both of the control conditions showed larger responses to deviant than control sounds, reflecting memory-based encoding of regularity violations in mice[25]. However, the finding was based on small number of mice, and replication of the result is thus important.

In addition to its specificity to regularity violations, another key feature of the human MMN is its dependency on the sensory memory span. This has been demonstrated by manipulating the length of a silent gap, known as the inter-stimulus interval (ISI), between the sounds in the oddball series. If the ISI between the stimuli in the series is too long, the MMN is not elicited[21], likely because the deviant sound input is compared to the transient memory trace for the standards, and decay in this memory trace does not allow for the comparison and consequent deviance detection. In humans, MMN amplitude decreases when lengthening the ISI from two seconds to four seconds[27,28], comparable to the time span of the human echoic memory[29]. In rats, frequency changes elicited the MMR (4.0 kHz vs. 4.2 kHz) when the ISI was 375 ms, but it faded with a 600 ms ISI[20]. When the frequency change was larger (4.0 kHz vs. 4.5 kHz), the MMR was found also at a 600 ms ISI, but the response faded again when ISI was prolonged to 1000 ms[20]. However, previous studies have not determined the span of the sensory memory in mice.

We reasoned that, similarly to humans and rats, auditory MMR in mice would reflect memory-based encoding of regularity violations. Accordingly, we expected that the MMR would be dependent on the sensory memory span.

We recorded LFP responses to frequency changes from the auditory cortex of urethane-anesthetized animals. To elicit a MMR, sounds were presented in the oddball condition containing two deviant sounds, one at 3.5 kHz and the other at 4.5 kHz in frequency, interspersed with a 4.0 kHz standard sound. In separate stimulus blocks, ISIs were 375 ms and 600 ms. The equiprobable control condition was applied to investigate whether the differential response to the oddball condition, if elicited, reflected detection of regularity violations or merely different levels of neural adaptation to the standard and deviant stimuli.

Methods

Subjects and surgery. Adult male mice (n = 13, C57BlJ, age 16.5 ± 1 weeks, weight 28.7 g ± 1.3, mean ± SD) acquired from the Lab Animal Centre at University of Eastern Finland (Kuopio, Finland) were used in the experiment. The mice were housed in group cages and maintained under a normal 12 h light/12 h dark cycle with constant temperature (22 ± 1 °C) and humidity (50–60%). Food and water were available ad libitum. All animal procedures were approved by the Finnish National Animal Experiment Board (ESAVI/10646/04.10.07/2014) and carried out in accordance with the guidelines of the European Community Council Directive 2010/63/EU. After the experiment, anesthetized animals were sacrificed by cervical dislocation.

First, the mice were pre-anaesthetised with 5% isoflurane and positioned in a stereotaxic frame with non-rupture ear bars (David Kopf Instruments, Tujunga, CA). Following this, a single dose of the main anaesthetic, urethane (Sigma-Aldrich, St. Louis, MO, USA; 7.5%), was administered via intraperitoneal injection (1.2 g/kg), and administration of isoflurane was gradually diminished over 5 minutes to 2%[30]. The administration of isoflurane was terminated before any surgical procedures. The level of anaesthesia was tested before the surgery and between the recording sessions using pedal withdrawal reflex, and extra doses of urethane were given (0.1–0.2 ml) if any response was observed. The skull was exposed, and carefully cleaned and dried. For the reference electrode, a hole was drilled in the skull over the right side of the cerebellum and a small insulin needle (BD Lo-Dose syringe, USA) was inserted in the cerebellum (AP: −5.8 mm, ML: 1–2 mm, and DV: 2 mm). The same type of needle inserted subcutaneously into the neck served as the ground electrode. In order to record LFPs from the left primary auditory cortex, overlying muscle and skull were carefully removed (2 × 2 mm region) to expose the dura on the primary auditory cortex (AP: 2.6–3.6 mm, ML: 3.5–4.5 mm from the bregma).

Electrophysiological recording. The continuous LFP measurement was first amplified tenfold using a low-noise preamplifier (MPA8I, MultiChannel Systems MCS, GmbH, Reutlingen, Germany). The signal was then fed to a filter amplifier (FA64I, filter: 1–3000 Hz, MultiChannel Systems MCS, GmbH, Reutlingen, Germany). Signal were digitized (USBME-64 System, tenfold) and recorded with MC_Rack software (MultiChannel Systems MCS, GmbH, Reutlingen, Germany) using a 10 kHz sampling rate, low-pass filtered at 500 Hz with a second order Bessel filter, and finally downsampled to 2 kHz.

Before beginning stimulation, the ear bar from the right ear was removed, allowing for normal hearing of the stimuli. External support for head fixation was attached to the skull. A tip of a Teflon-insulated silver wire (diameter 200 μm, A-M Systems, Carlsberg, WA, USA) was placed on the surface of the dura above the left auditory cortex where on-line recorded epidural potentials to sound stimuli had the highest amplitude.

Stimulation. Sinusoidal sounds of 50 ms in duration, including 5 ms rise and fall times, were used as stimuli. The sounds were created using Adobe Audition software (Adobe Systems Incorporated, CA, USA) and presented electronically using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA, USA) via an active loudspeaker system (Studiopro 3, N-audio, Irwindale, CA, USA). The stimulation was presented with the passive part of

the loudspeaker system directed towards the right ear of the animal at a distance of 20 cm with a sound pressure level (SPL) of 70 dB, as measured with a sound level meter using a C-weighted scale (Sound level meter Type 2240 - Brüel & Kjær Sound & Vibration, DK-2850, Nærum, Denmark).

Three different stimulus blocks, two oddball stimulus blocks and one equiprobable control stimulus block, were presented in a counterbalanced order between the subjects. In each stimulus block, 3200 stimuli were presented. In the two oddball blocks, ISIs (offset to onset) of either 375 ms or 600 ms were used. In both oddball conditions, two deviant sounds (probability for each = 0.0625) at 3.5 kHz and 4.5 kHz in frequency respectively, were interspersed with a frequently occurring standard of 4.0 kHz (probability = 0.875). The sounds were well within a mouse's hearing threshold[31]. The sounds in the oddball condition were delivered in a pseudorandom order, with the restriction that consecutive deviant sounds were separated by at least two standard sounds.

The equiprobable condition had 16 different sounds with frequencies ranging from 3.3 kHz to 4.8 kHz in 0.1 kHz steps. The sounds were presented with an offset to onset ISI of 375 ms and each sound had the same probability as the deviant sounds in the oddball condition (probability for each = 0.0625). The equiprobable control condition was applied only for the shorter 375 ms ISI, since the adaptation must explain the responses with the 375 ms ISI in order to explain the responses obtained with longer ISIs[5,32].

Data analysis. The offline data analysis was performed using the Brain Vision Analyzer 2.1. (Brain Products GmbH, Gilching Germany). The data were filtered offline at 1–30 Hz (24 dB/octave). Segments from 50 ms before and 350 ms after stimulus onset were averaged for each animal and each stimulus type independently. These segments were baseline-corrected against the mean of their 50 ms before stimulus onset. Averaging for the oddball condition was done separately for both types of deviant responses and for the standard responses immediately preceding the deviants. For the equiprobable control condition, segments were averaged separately for the two control-deviant responses at 3.5 kHz and 4.5 kHz.

Statistics. Statistical analyses were carried out by using IBM SPSS Statistics for Windows, version 24.0 (Armonk, NY: IBM Corporated). Mean amplitude values from three different regions of interest (ROIs), 30–70 ms, 80–120 ms, and 140–180 ms after the stimulus onset, were selected for analysis on the basis of visual inspection of the waveforms and previous literature[6,20,33,34]. A repeated measure analysis of variance (ANOVA) was applied with stimulus type (standard, deviant), deviant type (4.5 kHz, 3.5 kHz), ISI (375 ms, 600 ms) and region of interest (ROI: 30–70 ms, 80–120 ms, 140–180 ms) as the within-subject factors. Huynh-Feldt-adjusted degrees of freedom were used whenever the sphericity assumption was violated. A partial eta square (η_p^2) is reported for the effect size. *P*-value smaller than 0.05 was applied as a criterion for statistical significance in ANOVA.

Post-hoc analyses for the ANOVA were carried out by using two-tailed paired samples t-test with the bootstrapping method (1000 permutations) as implemented in IBM SPSS Statistics for Windows, version 24.0. Confidence interval (CI) of 95% was applied as a criterion for statistical significance.

In order to test whether the responses to oddball deviant and control sounds differed in amplitude, two-tailed paired samples t-tests were applied. Those were carried out by using bootstrapping with 1000 permutations, and CIs of 95% were applied as a criterion for statistical significance.

Even if the ANOVA is a useful method for investigating main and interaction effects here, it cannot determine the latency of the significant differential responses accurately. To this end, the averaged responses to standard and deviant sounds were compared timepoint-by-timepoint with paired t-tests separately for each ISI condition. Whenever the responses to standard and deviant sounds differed in the oddball condition, deviant and control sound responses were also compared using the same method. Paired two-tailed t-tests were carried out using bootstrapping with 1000 permutations. If the 95% CI indicated a significant difference at least in 10 consecutive data points, the effect was considered as significant.

All the paired samples t-tests were two-tailed, and Cohen's d (d) with pooled standard deviations is reported for the effect size for them.

Data availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Results

We studied whether a differential response to frequency changes in the oddball condition was elicited in anesthetized mice, and how it was affected by ISI and deviance frequency direction in different regions of interest. Whenever the MMR was elicited, the underlying mechanism of the response was investigated (genuine deviance detection or mere adaptation) by comparing responses between oddball-deviant and control sounds. Last, the latencies of the differential response and the latency of the differential response reflecting genuine deviance detection were determined using timepoint-by-timepoint comparisons.

Differential response. Table 1 shows significant main effects and interaction effects for the ANOVA model.

The stimulus type main effect indicated that responses to the deviant sounds were larger (219.6 μV ± 100.6) compared to responses to the standard sounds (124.9 μV ± 78.2) (Fig. 1).

In addition, there was an interaction effect of stimulus type x ISI. Post hoc tests showed that the deviant sounds elicited larger responses (214.5 μV ± 103.3) than the standard sounds (100.2 μV ± 76.8) when the ISI was 375 ms, $P = 0.003$, 95% CI [68.0, 160.5], d = 1.3, as well as when the ISI was 600 ms (deviant 224.7 μV ± 113.1, standard 149.7 μV ± 91.1, $P = 0.015$, 95% CI [36.6, 116.5], d = 0.7), (Fig. 1d). The differential response (Fig. 1c), calculated

Effect	df	F	*P*	η_p^2
Stimulus type	1,12	19.1	0.001	0.61
ROI	2,11	52.1	<0.0001	0.90
Stimulus type x ISI	1,12	4.9	0.047	0.29
Deviant type x ROI	2,11	8.2	0.007	0.60
Stimulus type x Deviant type x ROI	2,11	14.1	0.001	0.72

Table 1. Summary of the significant effects in the repeated measures of ANOVA. Degrees of freedom (df), F-values (F), *P*-values (*P*), and parietal eta squared (η_p^2) for effect sizes.

by subtracting the standard response from deviant response, was larger when the ISI was 375 ms (114.3 μV ± 89.0) than when it was 600 ms (75.0 μV ± 79.5), $P = 0.042$, 95% CI [7.7, 71.7], d = 0.5.

Next, we investigated whether the ISI had effect on the response to the standard stimuli, deviant stimuli, or both. Paired samples t-tests showed that the responses to the standard sounds were larger in the 600 ms ISI (149.7 μV ± 91.1) than in the 375 ms ISI condition (100.2 μV ± 76.8), $P = 0.038$, 95% CI [−82.3, −19.0], d = 0.6, while the responses to the deviant sounds were unchanged when the ISI was prolonged (Fig. 1d).

There was also an interaction effect of stimulus type x deviant type x ROI. Post hoc tests showed that at 30–70 ms ROI the responses were larger to the 4.5 kHz deviant sound than to the standard sound. However, the 3.5 kHz deviant sound did not elicit a larger response compared to the standard sound (Fig. 1e, Table 2). Comparison between the deviant sounds showed that the 4.5 kHz deviant sound (381.8 μV ± 149.6) elicited larger response than the 3.5 kHz deviant sound (232.4 μV ± 93.0), $P = 0.001$, 95% CI [75.5, 228.3], d = 1.2. At ROIs of 80–120 ms and 140–180 ms responses to both deviant types were larger than responses to standard sound, but there were no differences between the responses to the 3.5 kHz and 4.5 kHz deviant sounds (Fig. 1e, Table 2).

Genuine deviance detection. In order to test the underlying mechanism of the differential response in the oddball condition, a control condition was applied in which 16 sounds were presented with the same probability (equiprobable condition). Figure 2 shows the grand averaged responses to oddball-deviant and physically identical control sounds. Paired t-tests (bootstrapping with 1000 iterations) were used to compare the responses elicited by the deviant sounds in the oddball condition to the corresponding sounds in the control condition.

In 30–70 ms ROI, there was no significant difference between the oddball-deviant and control sound responses. In 80–120 ms and 140–180 ms ROIs, both deviant types elicited larger responses than the same sound in the control condition (Fig. 2b, Table 3).

The latency of the MMR. Lastly, we defined the latency range of the significant differential response and that for the genuine MMR response with timepoint-by-timepoint comparisons (bootstrapping with 1000 permutations).

Table 4 and shows the latencies for the differential response (deviant - standard) and genuine MMR (deviant - control), and the Figs 1a,b and 2a, respectively, corresponding significant effects (Cohen's d). The differential responses reflected partly genuine MMR. Notably, the earliest latency (before 53.5. ms) of the response to the 4.5 kHz sound and the latest part of the response to the 3.5 kHz sound did not differ between the deviant and control sound.

Discussion

We found a robust differential response to sound frequency changes in electrophysiological recordings from the auditory cortex of anaesthetized mice. Importantly, in light of the control condition, the response starting at the latency of 53.5 ms or 71.0 ms, depending on the deviant stimulus frequency (Table 4), reflected detection of regularity violations instead of mere adaptation similarly to the human MMN (for a review, see[21]). The ISI manipulation also affected the MMR, reflecting that the change detection in sound frequency is dependent upon the sensory memory.

Here, the equiprobable condition was applied as a control condition together with the recording of auditory cortical LFPs in mice. The ANOVA model showed, that differential responses were elicited in the oddball condition in the all ROIs, but the responses to oddball-deviant sounds were larger than those to physically identical control sounds, at ROIs of 80–120 ms and 140–180 ms. This result demonstrates genuine deviance detection that is in line with previous MMN studies in humans[18,21], and with LFP measurements in anesthetized rats[7,20,23,24]. Our result is also similar to that in awake mice, showing detection of regularity violations with LFPs and single cell responses recorded from different subcortical and auditory cortical areas[25]. Genuine deviance detection was more evident in cortical than subcortical areas (inferior colliculus, medial geniculate body) and in non-lemniscal than lemniscal regions[25]. Together with the previous findings[25], the present results suggest that the MMR in mice cannot be explained by mere neural adaptation, or refractoriness, which is more profound for repetitive standard sounds than for rare sounds.

Also another study which applied recordings of single cell responses from mice's auditory cortex provided evidence of detection of regularity violations[35]. It found that especially late responses of the subthreshold membrane potentials were specific to oddball condition. LFPs are also sensitive to sub-threshold neural processes, linking our finding closely to the previously mentioned finding obtained with sub-threshold membrane potentials[35].

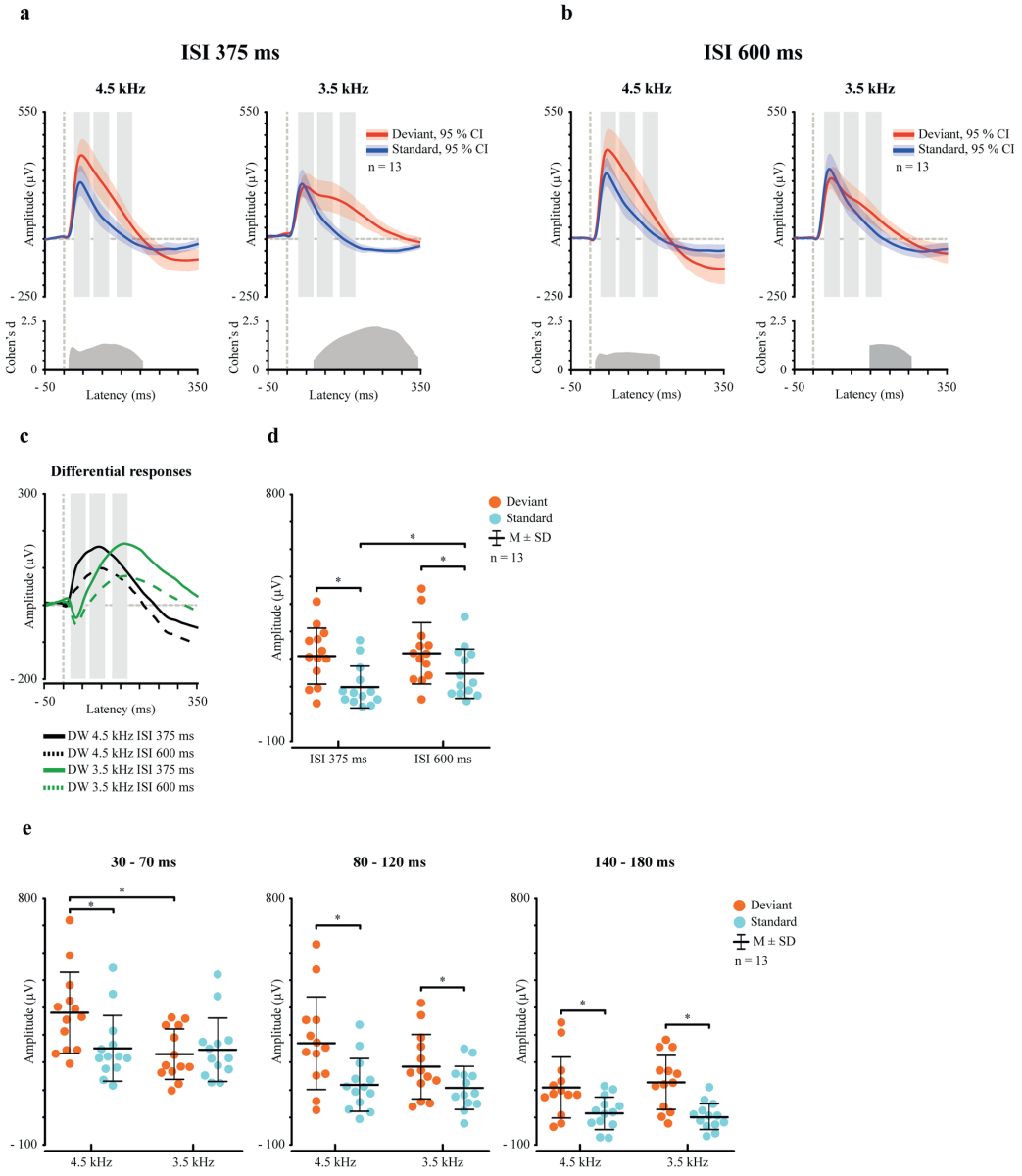

Figure 1. Differential response elicited by frequency changes is diminished by the lengthening of the ISI. (**a,b**) Grand averaged waveforms for the deviant (red) and standard (blue) sounds with 95% CIs. Effect size (Cohen's d) is reported for the latency range of the significant difference. Light grey rectangles represent regions of interest (ROI) at 30–70 ms, 80–120 ms, and 140–180 ms used in analysis conducted with ANOVA. (**c**) Differential responses (DW, deviant - standard). Note that the differential responses are here shown for illustrative purposes separately for the two deviant types, but do not represent the found interaction effect (stimulus type x ISI). (**d,e**) Point plots indicate individual values and are presented with mean and standard deviation. *Indicates a statistically significant difference as defined by the 95% CI. (**d**) The deviant sounds elicited larger responses compared to responses elicited by the standard sounds when the ISI was 375 ms, and same was observed when the ISI was 600 ms. Furthermore, responses to standard sound were larger when the ISI was 600 ms compared to responses to standard sound when the ISI was 375 ms, while deviant responses did not change. (**e**) The 4.5 kHz deviant sound elicited a larger response compared to responses elicited by the standard sound at the 30–70 ms ROI. Furthermore, the 4.5 kHz deviant sound elicited larger responses compared to responses elicited by the 3.5 kHz deviant sound. In the 80–120 ms and 140–180 ms ROIs, both deviant types elicited larger responses compared to responses elicited by the standard sound.

ROI	Deviant type	Deviant	Standard	*P*	d	95% CI
30–70 ms	4.5 kHz	381.8 ± 149.6	253.9 ± 121.1	0.009	0.9	[68.4, 192.7]
	3.5 kHz	232.4 ± 93.0	248.6 ± 116.9	0.650	0.2	[−79.2, 44.4]
80–120 ms	4.5 kHz	274.5 ± 170.7	120.8 ± 97.5	0.007	1.1	[27.9, 136.9]
	3.5 kHz	187.8 ± 118.9	109.1 ± 79.7	0.026	0.8	[47.2, 140.6]
140–180 ms	4.5 kHz	111.1 ± 112.5	15.7 ± 59.9	0.042	1.1	[54.3, 150.6]
	3.5 kHz	129.8 ± 99.8	1.6 ± 49.8	0.001	1.6	[82.4, 176.0]

Table 2. Mean amplitude values (μV) and standard deviations (SD) for 3.5 kHz and 4.5 kHz deviant responses and standard responses in three regions of interests (ROIs). *P*-values (*P*), Cohen's d and 95% confidence interval (CI) for paired sample t-tests comparing the deviant responses to the standard responses (1000 permutations in bootstrapping).

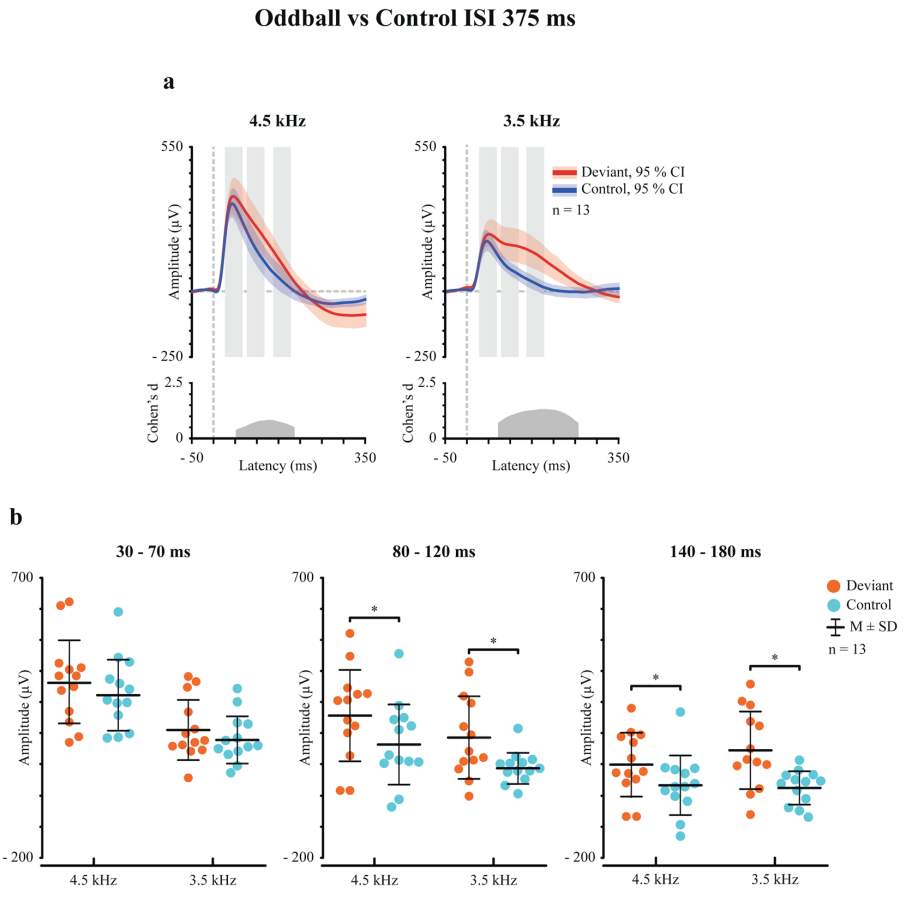

Figure 2. Frequency changes elicit genuine deviance detection. **(a)** Grand averaged waveforms for the deviant (red) and control (blue) sounds with 95% CIs. Effect size (Cohen's d) is reported for the latency range of the significant difference. Light grey rectangles represent ROIs of 30–70 ms, 80–120 ms, and 140–180 ms, used in the paired samples t-tests. (**b**) Point plots indicate individual values and are presented with mean and standard deviation. *Indicates statistically significant difference as defined by the 95% CI. (**b**) There were no differences between the oddball-deviant and control sound responses in the 30–70 ms ROI. In both, 80–120 ms and 140–180 ms ROI, the deviant sounds elicited a larger response than the same sound in the control condition.

On the contrary to results in the two latest ROIs, the ANOVA analysis showed that the differential response in the first ROI (30–70 ms after stimulus onset) was at least partly related to adaptation, since the oddball-deviant response did not differ from the control sound response in amplitude. At that early latency, the ascending

ROI	Deviant type	Deviant	Control	*P*	d	95% CI
30–70 ms	4.5 kHz	368.2 ± 138.2	328.0 ± 115.2	0.113	0.3	[−4.0, 80.4]
	3.5 kHz	214.9 ± 97.7	182.8 ± 76.6	0.225	0.4	[−17.0, 75.0]
80–120 ms	4.5 kHz	261.8 ± 148.3	167.8 ± 130.3	0.007	0.7	[52.1, 139.0]
	3.5 kHz	190.3 ± 134.1	90.2 ± 50.6	0.011	1.0	[46.8, 156.7]
140–180 ms	4.5 kHz	102.6 ± 103.6	35.5 ± 96.8	0.007	0.7	[33.5, 106.8]
	3.5 kHz	149.0 ± 126.0	26.9 ± 54.3	0.002	1.3	[69.2, 180.1]

Table 3. Mean amplitude values (μV) and standard deviations (SD) for 3.5 kHz and 4.5 kHz deviant responses and corresponding control responses in three regions of interest (ROIs). *P*-values (*P*), Cohen's d and 95% confidence interval (CI) for paired samples t-tests comparing the deviant responses to the control responses (1000 permutations in bootstrapping).

ISI	Deviant type	Differential response	Genuine MMR
375 ms	4.5 kHz	14.0–204.5	53.5–185.0
	3.5 kHz	71.0–341.5	71.0–255.5
600 ms	4.5 kHz	15.0–182.0	N.A.
	3.5 kHz	149.0–264.5	N.A.

Table 4. Summary of the significant results from the timepoint-by-timepoint paired samples t-tests between the deviant and standard responses (differential response) and between the deviant and control responses (genuine MMR). Units are in milliseconds (ms). The equiprobable control condition with inter-stimulus interval (ISI) of 375 ms was applied.

(4.5 kHz) deviant sound elicited a differential response in comparison to the standard sound response, but the descending (3.5 kHz) deviant sound did not. In contrast, at the later ROIs, which were found to be specific to regularity violations, deviant responses did not show similar sensitivity to the ascending frequency changes. This pattern of findings may indicate that the early latency of the response associates to the human N1 response, which mostly encodes stimulus energy[15] and is thus larger for high frequency sounds than low frequency sounds.

The claim that this early response associates to the human N1 gained further support when the latency for the MMR was determined with timepoint-by-timepoint comparisons. Differential responses to the 4.5 kHz deviant sound started at 14 ms after the stimulus onset. However, the latency range for the genuine MMR took place at 53.5–185 ms after the stimulus onset. The differential response to the 3.5 kHz deviant sound started later, at 71 ms after the stimulus onset, but it reflected genuine MMR from the beginning (71–255.5 ms after stimulus onset). Therefore, it can be concluded that the earliest part of the MMR to a 4.5 kHz deviant sound can be explained by a mechanism related to neural adaptation.

The MMR in mice was also dependent on the decay of the sensory memory. Extending the ISI from 375 ms to 600 ms diminished the MMR amplitude, as previously found in humans (for a review see[32]). Here, the MMR amplitude was diminished due to the fact that the responses to standard sounds got larger when the ISI was prolonged, while the deviant responses did not change. To the best of our knowledge, the effect of ISI manipulations on standard and deviant sound responses has not been investigated separately in either human or animal studies. However, in our previous study in rabbits, hippocampal responses to auditory and visual changes showed that the ISI manipulation mostly affected the standard responses, not the deviant responses[36].

The sensory memory duration in mice appears to be at least 600 ms, but since no ISI of longer duration was applied, we were not able to define the upper limit of the sensory memory duration in mice. Here, we used only one type of stimulus change (frequency), and it is known that different stimulus features have different sensory memory decay times[32]. In addition, the amount of physical difference between the standard and deviant sounds also has an effect on memory decay time[20]. In future studies, the limit for the sensory memory storage time, as well as its dependency on the type of stimulus change and the amount of physical difference between standard and deviant stimuli, could be studied in mice.

Like many of the previous studies investigating automatic change detection with the MMR or corresponding single cell responses in animals, this study was conducted with anesthetized animals[6,7,11,14,20,23,24,33,34,37]. Even if studies in conscious animals would allow better comparability to event-related potential studies in humans, it is plausible that the current study provides reliable evidence of deviance detection in mice. This is also supported by a recent study where LFP reflecting the genuine MMR to frequency changes was found similarly in urethane-anesthetized rats and in awake mice[25]. Urethane-induced anaesthesia seems to leave the sensory memory and change detection functions of the rodent auditory system mostly unaffected, as the mismatch response and its dependence on the sensory memory has been demonstrated in several previous studies[6,20,23,33,38].

In conclusion, our data demonstrates that the MMR to frequency changes in mice shares two key properties with the human MMN: specificity to regularity violations and dependence on the sensory memory. Results presented here open up new opportunities to use, for instance, optogenetic manipulations in order to further study sensory-cognitive functions with mice models.

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Author Contributions

J.L.O.K., A.L. and P.A. designed the study. A.L. collected the data. J.L.O.K. and I.K. analysed the data. J.L.O.K. and P.A. drafted the manuscript. All authors contributed to the writing of the manuscript and accepted the final version.

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II

PASSIVE EXPOSURE TO SPEECH SOUNDS INDUCES LONG-TERM MEMORY REPRESENTATIONS IN THE AUDITORY CORTEX OF ADULT RATS

by

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Passive exposure to speech sounds induces long-term memory representations in the auditory cortex of adult rats

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Experience-induced changes in the functioning of the auditory cortex are prominent in early life, especially during a critical period. Although auditory perceptual learning takes place automatically during this critical period, it is thought to require active training in later life. Previous studies demonstrated rapid changes in single-cell responses of anesthetized adult animals while exposed to sounds presented in a statistical learning paradigm. However, whether passive exposure to sounds can form long-term memory representations remains to be demonstrated. To investigate this issue, we first exposed adult rats to human speech sounds for 3 consecutive days, 12 h/d. Two groups of rats exposed to either spectrotemporal or tonal changes in speech sounds served as controls for each other. Then, electrophysiological brain responses from the auditory cortex were recorded to the same stimuli. In both the exposure and test phase statistical learning paradigm, was applied. The exposure effect was found for the spectrotemporal sounds, but not for the tonal sounds. Only the animals exposed to spectrotemporal sounds differentiated subtle changes in these stimuli as indexed by the mismatch negativity response. The results point to the occurrence of long-term memory traces for the speech sounds due to passive exposure in adult animals.

The cerebral cortex is highly adaptive to the sensory environment, and the acoustic environment has a strong effect on cortical representations of sounds in animals[1,2] and humans[3–5], especially within a critical period during early development. In human infants, auditory perceptual learning, including learning of speech sound discrimination, occurs automatically without direct attention, even during nocturnal sleep[3].

After the critical period, auditory perceptual learning in humans is thought to be efficient only when supported by attentive training[4,5]. There is also a critical period for auditory learning in young animals[6,7], after which behaviourally relevant stimuli are required for perceptual learning (for a review, see[6]). The application of behaviourally meaningful stimuli in adult animals has been shown to induce learning-related plastic changes in cortical maps in response to different types of stimulation, such as pure tone frequencies[8,9], sound intensities[10], temporal modulation rates[11], and speech sounds[12].

In humans, cortical map changes have seldom been investigated because this would involve the use of an invasive method. However, electrophysiological recordings of so-called mismatch negativity (MMN)[13] (for a review, see[14]) can be done non-invasively, and these revealed perceptual learning-related plasticity in cortical sound representations of adult humans due to active training[15–17]. In those studies, a few hours of training, usually for 3–6 d, caused long-term memory traces for the trained sounds.

A few studies have examined the effects of passive exposure on animals and humans. Studies of passive sound exposure in animals focused on the formation of sound representations (cortical maps) in the auditory cortex and did not detect observable effects, even after several days of exposure[1,6,11]. In a human study, passive listening to vowels for approximately of one h did not improve sound discrimination ability or produce any changes in ERPs[18].

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In anaesthetized animals, MMN, known also as the mismatch response (MMR), in local-field potentials (LFPs) and stimulus-specific adaptation (SSA) in single-cell responses have been observed, pointing to rapid perceptual learning within a single recording session (for reviews, see[19–22]). Modulation of MMN[23–26] and SSA[27–29] in response to changes in pure tone frequency, syllables, and other complex stimuli are reported in the auditory cortex, as well as in subcortical structures in the auditory pathway (MMN[30] and SSA[31,32], for a review, see[33]).

The aforementioned findings on MMN and SSA in animals are intriguing, as they demonstrate learning-related functional changes in the brain activity of adult animals, in the absence of behavioural relevance of the stimuli. Thus far, these responses have been shown only during a single session of passive sound exposure, indicating that short-term memory representations of the sounds are sufficient for their elicitation. It is not known, however, if the effect of passive exposure can be long-lasting, leading to formation of long-term memory representations.

To test whether the passive exposure can form long-term memory representations, we first exposed adult rats to behaviourally irrelevant speech sounds for 36 h on three consecutive nights for 12 h/d. We applied a statistical learning paradigm, i.e. an oddball condition, with a large number of stimuli presented to the animals (total of approximately 250,000 sounds). Since the exposure was delivered during three consecutive nights, the consolidation of memory traces for speech sounds was expected to occur[15,16]. In a between-group design, two groups of freely moving animals were exposed to two different continuous sequences of speech sounds. For one group, the sequence consisted of repetitions of a vowel/a/spoken by a Finnish speaker with changes in its spectrotemporal features. For the other group, tonal changes were presented in repetitions of a vowel/a/spoken by a Chinese speaker. Both the standard and deviant stimuli in the sound series were different between the animal groups, allowing the groups to serve as controls for each other. The sounds were presented in the oddball condition, in which a rarely presented deviant sound was occasionally interspersed with a standard sound, with an inter-stimulus interval (offset to onset) of 335 ms. Two different deviant sounds were interspersed with standard sounds: one with a large and the other with a small physical difference from the standard.

To reveal possible long-term memory traces for the speech sounds, LFPs in the auditory cortex of anesthetized rats were measured after the exposure ended. The same sound series that were applied in the exposure phase were also used in the test phase, but both the spectrotemporal and tonal changes were now presented to both animal groups, allowing comparison of the naive and exposed animals' responses. In humans, MMN is a reliable indicator of auditory discrimination ability (for a review, see[14]), including speech sound discrimination ability[15,34]. An increase in the MMN amplitude[15,17] and a decrease in latency[35,36] reflects enhanced sound discrimination ability. Therefore, in the present study, we expected to observe increased MMN amplitudes and decreased latencies in response to the specific type of sound exposure.

Results

All the stimuli elicited an auditory response, which peaked approximately 50 ms after the stimulus onset (Figs 1 and 2). Analyses of the mean amplitude values 100–150 ms after the onset of the stimulus was chosen a priori, based on earlier studies of MMN latency in rats[37,38] and grand-averaged waveforms. The ANOVA and post-hoc tests revealed that large spectrotemporal changes elicited MMN in both animal groups, whereas small spectrotemporal changes elicited MMN only in the group of animals exposed to these stimuli (for details, see Fig. 1). Tonal changes did not elicit MMN in either animal group, and no exposure effect was observed (Fig. 2).

To determine the effect of the sound exposure on neural discrimination ability we compared the differential responses (the deviant minus the standard) by applying time-point-by-time-point t-tests (permutation tests) not only on the MMN latency but also on the whole waveform. First, the differential response to both spectrotemporal and tonal changes was compared against zero within each animal group separately. Second, if both animal groups showed a statistically significant differential response, the differential responses of the groups were compared to determine possible differences in latency.

With regard to the spectrotemporal sounds, large changes elicited a statistically significant differential response (MMN) in the exposure group 91–119 ms after the onset of the stimulus (smallest $P = 0.034$, 95% CI [−62.82–(−18.50)]; largest significant $P = 0.049$, 95% CI [−53.50–(−2.91)]). In the animal group naive to these sounds, MMN occurred 86–170 ms after the stimulus onset (smallest $P = 0.003$, 95% CI [−64.37–(−25.96)]; largest significant $P = 0.049$, 95% CI [−32.06–(−5.32)]). The between-group comparisons of the differential responses (deviant vs. standard) revealed no differences.

The small spectrotemporal changes elicited differential responses only in the group exposed to these stimuli. A significant differential response was observed 90–156 ms after the onset of the stimulus (smallest $P = 0.003$, 95% CI [−64.37–(−25.96)]; largest $P = 0.049$, 95% CI [−32.06–(−5.32)]).

With regard to the tonal changes, differential responses were not found in either group.

Discussion

Herein, by applying an electrophysiological method, we demonstrated that passive auditory exposure can induce plastic changes in the function of the auditory cortex, even in adult animals. Passive exposure to speech sound changes for 3 d for a total of 36 h was sufficient to induce long-term memory representations of the sounds. This was observed as emergence of the MMN response for the small changes in spectrotemporal sounds in the animal group exposed to these sounds but not in the group of animals exposed to different sounds.

Previous studies failed to show effects of passive exposure on reorganization of the cortical maps of the auditory cortex in adult animals[1,11,39]. It is not clear whether the positive finding in the present study of the effectiveness of passive exposure on auditory cortex plasticity is related to the type of measurement (LFPs vs. single-cell responses), stimulus condition (oddball condition vs. repetition of one stimulus) or other methodological differences between the present and previous experiments.

We used LFPs to measure the co-activity of local neural networks, whereas previous studies employed single-cell responses[1,11,39]. LFPs capture synchronized synaptic potentials, afterpotentials of somatodendritic

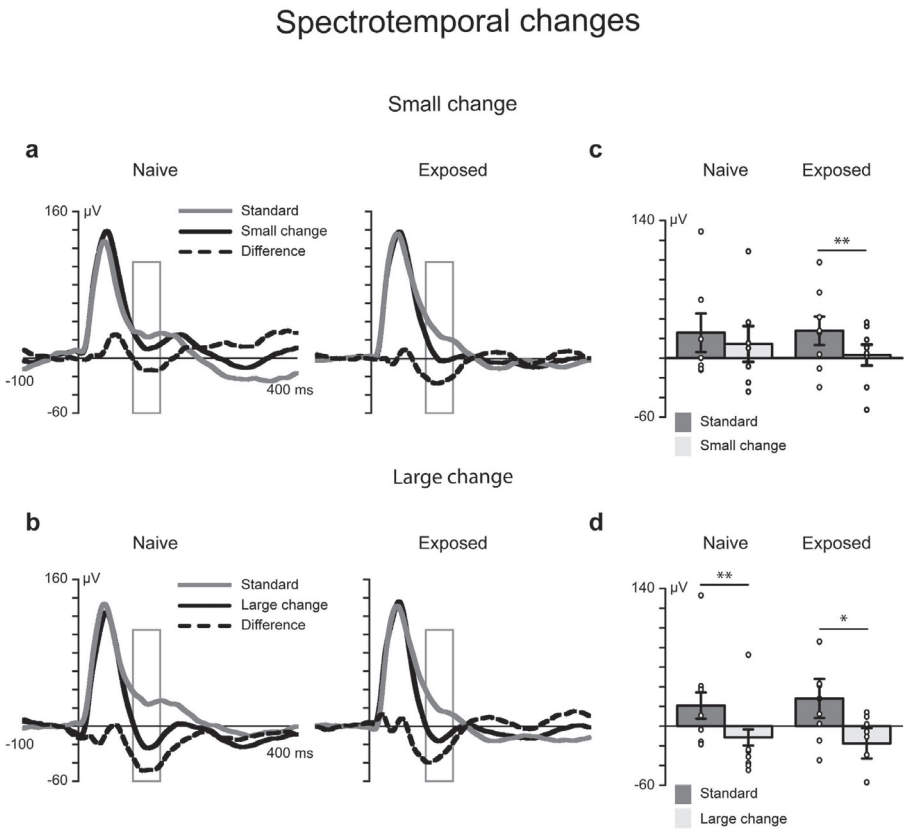

Figure 1. Passive exposure to spectrotemporal changes enhanced cortical responses to the same sounds presented later. (a,b) The grey line denotes responses to frequently presented standard sounds, and the black line denotes the responses to large or small changes in sound (deviant). The black dashed line represents the differential response (MMN), which was calculated by subtracting the response to the standard sound from the response to the deviant sound. The rectangles show the region of interest (mean amplitude values 100–150 ms after the stimulus onset). A repeated measures analysis of variance (ANOVA) of the factors 'stimulus' (standard vs. deviant), 'deviant type' (small vs. large change) and 'group' (exposed, $n=8$ vs. naive, $n=7$) revealed a three-way interaction effect: $F_{1,13}=4.85$, $P=0.046$, $\eta^2_p=0.272$. (**c,d**) Response amplitudes for each animal (marked with circles) and the error bars indicating the mean and standard error of the mean values, $^*P<0.05$, $^{**}P<0.01$. (**c**) The small change in spectrotemporal features elicited the MMN response (M = −32.36 μV, SEM = ±10.49) in the exposed animals ($t[7]=3.08$, $P<0.009$, $d=1.258$) but not in the naive animals (M = −11.28 μV, SEM = ±9.16) ($t[6]=1.232$, $P=0.264$, $d=0.465$). (**d**) The large change in spectrotemporal features elicited the MMN in both the naive (M = −45.61 μV, SEM = ±9.80) and exposed animal (M = −32.36 μV, SEM = ±10.49) groups ($t[6]=4.66$, $P<0.003$, $d=1.760$ and $t[7]=3.08$ $P<0.018$, $d=1.090$, respectively).

spikes and voltage-gated membrane oscillations[40–42]. As a result, LFPs are sensitive to sub-threshold neural processes and carry information about the state of the neural networks.

Another novel aspect of the present study was the use of a statistical learning paradigm (i.e. oddball condition), both in the exposure and test phases, in which rare changes in sounds are presented. Changes in stimulus environment can signal threat to animal and their biological significance is thus high. Previous studies applied only one stimulus type (pure tone[1,35] or temporal modulation rates in pure tones[11]).

Previous studies that failed to show an effect of passive exposure on cortical sound representations in adult animals used a longer exposure time than that employed in the present experiment. For example, De Villers-Sidani *et al.*[35] exposed rats for 3 d, 24 h/d. In other studies, the exposure times were 19 d, 1–16 h/d[1] and up to 2 mon, 2 h/d[11]. Thus, the exposure time in our study compared to that of previous studies seems not to explain the different findings.

The number of stimulus presentations during the exposure period could also be important. We used a short inter-stimulus interval between the stimuli (335 ms, offset-to onset). Thus, approximately two stimuli per second and more than 250,000 stimuli within 36 h were presented to the animals. The large amount of repetition of the

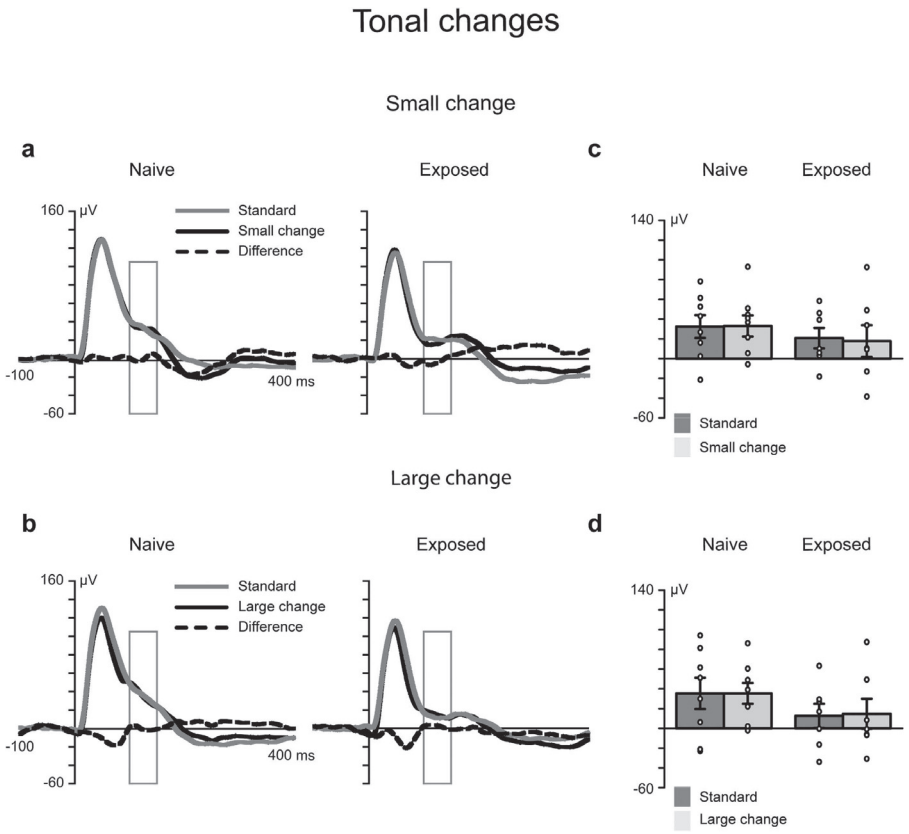

Figure 2. Tonal changes elicited neither an MMR response nor an exposure effect. (a,b) The grey line denotes the responses to frequently presented standard sounds, and the black line represents the responses to large or small changes in the deviating sound. The black dashed line represents the differential response (MMR), which was calculated by subtracting the response to the standard sound from the response to the deviant sound. The rectangles show the region of interest (mean amplitude values 100–150 ms after the stimulus onset). A repeated measures analysis of variance (ANOVA) of the factors 'stimulus type' (standard vs. deviant), 'deviant type' (small vs. large change) and 'group' (exposed, $n = 7$ vs. naive, $n = 8$) revealed no statistically significant effects. (**c,d**) Response amplitudes for each animal (marked with circles) and the error bars indicating the mean and standard error of the mean values.

stimuli, together with the statistical learning paradigm and neural network-level electrophysiological recording, may have enabled us to observe the effect of passive exposure.

A recent study by Kato *et al.*[43] that utilized two-photon calcium imaging in mice demonstrated that passive exposure to simple tones for 5 d (20 min/d) caused a progressive increase in the number of cells (layer 2/3 pyramidal cells of A1) showing inhibition by the tone presentation. Our data cannot provide conclusive evidence on the neural mechanism of the observed perceptual learning. Since the state of the brain and also the level of anaesthesia fluctuate temporarily distant responses cannot be reliably compared. This prevented direct comparison of standard and deviant responses between animal groups and instead group differences were investigated by comparing the differential responses. However, the finding by Kato *et al.*[43] is in line with that of studies of SSA[27–29]. In those studies, SSA occurred in response to standard sounds (habituation), enabling the detection of the deviant sounds. It can be speculated that in our present experiment, the formation of the memory trace to the standard stimulus during the exposure period enabled better deviance detection, as indexed by the emergence of MMN for small spectrotemporal change only in the exposure group but not that in the group of animals exposed to different sounds.

In the present study, only exposure to spectrotemporal, not tonal, changes was effective in modulating MMN. The absence of MMN to tonal changes in both the exposed and naive animal groups indicated that the rat brain did not differentiate between the tonal features. This finding was somewhat surprising, as MMN in rats is elicited by rising and falling sinusoidal tones[44]. It can be assumed that tonal changes that occur gradually (i.e. over a period of 200 ms) are more difficult for the rat brain to differentiate than fast changes in sound frequency.

As demonstrated by the response latency, the MMN was sensitive to subtle changes in spectrotemporal features of human speech sounds at the beginning of the sounds but not to decrements in the duration of these sounds (Fig. 1). The latency of the MMN in the present experiment was somewhat longer than that of the MMN to frequency changes in pure tones (60–100 ms post-stimulus[23]). This is in line with the idea that the MMN latency reflects the complexity of the underlying cognitive process[14]. Indeed, we observed even longer latency responses (217 ms after the onset of the change) in rats to changes in abstract rules in syllables[45]. However, in the present study, the response latency was not shorter in the exposure group than in the naive group for the large spectrotemporal changes which elicited the MMN response in both groups. Similarly in a previous study in humans, active training modulated amplitude, but not latency, of the MMN response[46].

Future studies should investigate the time required for passive exposure to induce plastic changes. As we measured the neural discrimination ability after only 3 d of exposure, not for example, after each night, it is not possible to conclude whether a smaller amount of exposure would also induce plastic changes in the function of the auditory cortex.

In sum, we found that rats could detect subtle changes in spectrotemporal features of human speech sounds, as indicated by electrophysiological responses recorded from the auditory cortex. Further, after exposure for 36 h, the change detection response of the group passively exposed to these sounds was enhanced but not that of the group exposed to different sounds. Our findings thus demonstrate that passive exposure to speech sound changes can induce long-term memory representations.

Methods

Animals. The study consisted of 15 adult male Wistar rats aged 24.5 ± 1.8 wk weighing 490.5 ± 44.4 g (mean $\pm$ SD). The estimation of the number of animals required for the experiment was based on a previous rat study of the detection of changes in responses to speech sounds[45]. The animals were individually housed and maintained in a 12-h light/dark cycle (lights on at 7.00 a.m.). All the experimental procedures and animal care protocols were approved by the National Experiment Board in Finland (licence ESAVI/10646/04.10.07/2014) and were in accordance with the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures. After the experiment, the animals first received an overdose of urethane, and they were then sacrificed by cervical dislocation.

Exposure. The animals were randomly divided into two groups. One group was exposed to tonal changes ($n = 7$), and the other was exposed to spectrotemporal changes ($n = 8$). Both groups were exposed to the sounds for three consecutive nights (12 h/night from 7 p.m. to 7 a.m.), giving a total exposure time of 36 h. The animals were exposed to the sound stimuli in their home cages from a passive loudspeaker system (StudioPro 3, M-Audio Inc., Cumberland, USA), which was directed towards the cages. The sound pressure level for each tone was 70 dB, as measured using a sound-level meter (type 2235, Bruel & Kjaer, Nærum Denmark), with C-weighting (optimized for 40–100 dB measurement).

Stimuli and procedure. In the tonal stimulus series, the animals were exposed to different tones of the speech sound/a/. The sounds were prepared so that first phoneme/a/was spoken by a female native Chinese speaker with rising (i.e. Chinese lexical tone 2) and falling (i.e. Chinese lexical tone 4) lexical tones, and they were recorded at a sampling rate of 44.1 kHz. The sounds were then digitally edited using SoundForge software (SoundForge 9, Sony Corporation, Japan) to ensure they had a constant duration of 200 ms. To isolate the lexical tones and keep the rest of the acoustic features identical, pitch tier transfer was performed using Praat software (Praat v5.4.06, University of Amsterdam). Pitch tier transfer generated a rising tone and a falling tone, which were identical to each other, except for a pitch contour difference in fundamental frequency (F0). These two tones were taken as the endpoint stimuli to create a continuum of lexical tones with 10 interval steps. A morphing technique was performed in Matlab (The MathWorks, Inc., MA, US), with a STRAIGHT tool[47] to create the three tones applied in the experiment. The repeatedly presented standard sound was the falling tone, and deviant sounds were a slightly falling tone (small change) and a rising tone (large change) (Fig. 3), corresponding to the tone continua 11, 7 and 3, respectively, as reported previously in detail elsewhere[48]. All the stimuli were normalized to have the same root mean square intensity.

In the spectrotemporal stimulus series, changes in the speech sound/a/were presented. They were prepared so, that phoneme/a/was first spoken by a female native Finnish speaker, and it was recorded at a sampling rate of 44.1 kHz. The sound was then digitally edited using SoundForge software (SoundForge 9, Sony Corporation, Japan) to ensure it had a constant duration of 200 ms. A morphing technique was performed in Matlab (The MathWorks, Inc., MA, US), using a STRAIGHT tool to modify the length of the stimuli and keep the fundamental frequency (F0) constant (Fig. 3). The repeatedly presented standard sound was the vowel/a/, which was 200 ms in duration. The deviant sounds were a 150-ms sound (small change) and 100-ms sound (large change) (Fig. 3). The method for shortening the sounds caused differences between the standard and deviant sounds immediately at the beginning of the sound.

Surgery and LFP recording. After the exposure to the sounds, on the same day the exposure ended (2–7 h after the exposure), the animals were anesthetized with intraperitoneal injections (1.2 g/kg dose, 0.24 g/ml concentration) of urethane (Sigma-Aldrich, St. Louis, MO, US). Supplemental doses were injected if the required level of anaesthesia was not obtained. The level of anaesthesia was monitored by testing the withdrawal reflexes. The animals were rehydrated with a 2 ml saline injection (subcutaneous) every 2 h.

The head of the animal was attached to a stereotaxic instrument (David Kopf Instruments, Model 962, Tujunga, CA, US). Under local anaesthesia (lidocaine 20%, Orion Pharma, Espoo, Finland), the skin and underlying muscles were removed, and a unilateral craniotomy was performed to expose a 2×2 mm region of dura

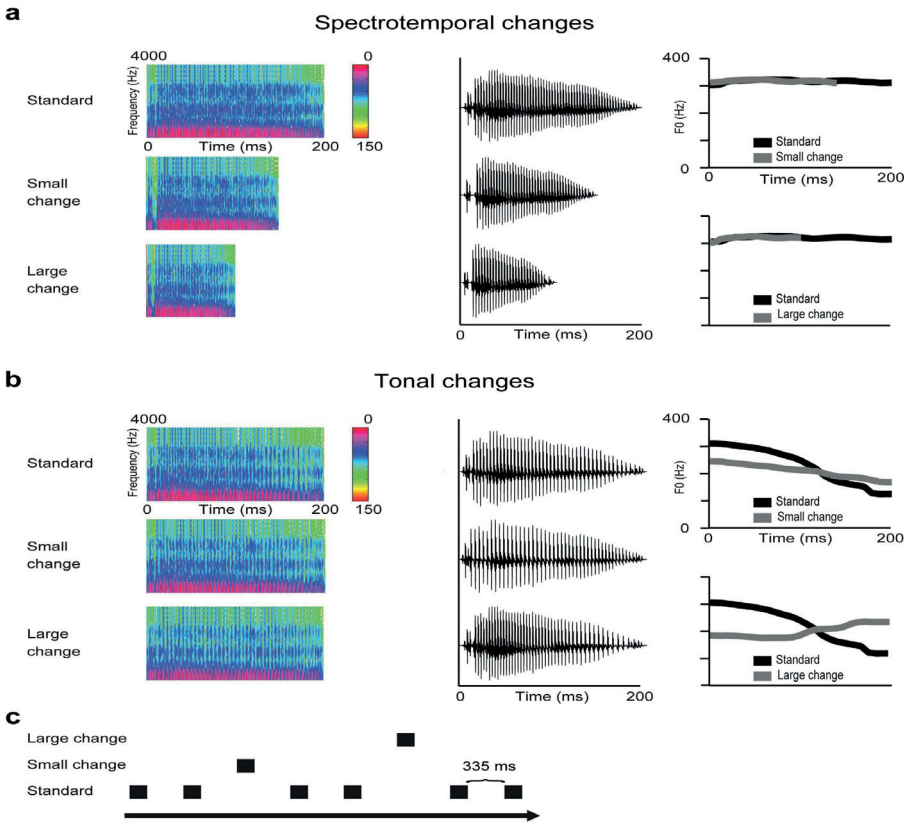

Figure 3. Sounds and the stimulus condition applied in the exposure and test phases. (a,b) Spectrograms, waveforms and fundamental frequencies of the sounds. The animals were exposed to either spectrotemporal (**a**) or tonal changes (**b**) in the vowel/a/. (**c**) The sounds were presented in an oddball series, where frequently presented standard sounds were interspersed with a large or small change in the sound. After the exposure, the LFPs in response to both stimulus types were measured in all the animals. The sounds were presented in the oddball condition, where frequently occurring standard stimuli (probability of 0.80) were interspersed with two deviant sounds (large or small change, probability of 0.10 each), using E-prime 1.2. software (Psychology Software Tools Inc., Sharpsburg, US). The inter-stimulus interval was 335 ms (offset to onset). The stimuli were delivered in a pseudorandom fashion, with the restriction that consecutive deviant sounds were separated by at least two standard sounds.

over the auditory cortex in the left hemisphere (4.5–6.5 mm posterior and 6–8 mm ventral to bregma). The tip of a PFA-insulated silver wire (A-M Systems, Chantilly, VA, US), 200 μm in diameter, was positioned on the surface of the dura. Although the coordinates for the craniotomy refer to the primary auditory cortex, some activity from the higher sensory areas may also have been captured. The latter is due to individual differences in the organization of the primary auditory cortex and the relatively large size of the tip of the electrode, allowing the signal to be conducted from adjacent areas.

A 29 G injection needle (Terumo, Leuven, Belgium) in the cerebellum served as the reference point, and a similar injection needle located under the neck skin provided the grounding point. Before recording the LFP, a headstage, composed of a screw and dental acrylic, was attached to the right prefrontal part of the skull to hold the head in place and allow removal of the right ear bar.

The auditory evoked potentials of all the animals to both the spectrotemporal and tonal changes were measured in separate blocks in a counterbalanced order between the animals. Both the spectrotemporal sound series and tonal sound series consisted of 1600 standard sounds and 400 deviant sounds (200 small and 200 large changes). The properties of the stimuli and presentation conditions were the same as in the exposure phase.

A continuous electrocorticogram was first 10-fold amplified using a low-noise MPA8I pre-amplifier (MultiChannel Systems MCS GmbH, Reutlingen, Germany). The signal was further fed to a filter amplifier (FA64I, filter: 1–5000 Hz, MCS). All the signals were digitized (USBME-64 System, MCS) and recorded with

McRack software (MCS), using a 2000-Hz sampling rate. Finally, all the signals were digitally band-pass filtered between 1 and 500 Hz (high-pass: low-pass: fourth-order Bessel).

Data analysis. The data were off-line filtered at 0.1–30 Hz (24 dB/octave). Sweeps from 100 ms before to 400 ms after the stimulus onset were averaged separately for both types of the deviant sounds and standard sounds that immediately preceded the deviant sounds. The averaged waveforms were baseline-corrected against the mean of a 100-ms pre-stimulus period.

Statistics. The mean amplitude values from a time window of 100–150 ms from the stimulus onset were included in a repeated measures analysis of variance (ANOVA), with the within-subjects factors 'stimulus type' (standard vs. deviant) and 'deviant type' (small vs. large change) and a between-subjects factor 'group' (exposed vs. naive). Separate ANOVAs were performed for spectrotemporal and tonal sound series. The averaged data was normally distributed (Shapiro-Wilk test all P-values > 0.05) enabling to use parametric statistical tests.

Two-tailed one-sample *t*-tests were used as post-hoc tests to further investigate the interaction effects found in the ANOVA. Huynh–Feldt-corrected degrees of freedom were used whenever the sphericity assumption was violated. The corrected *P* values are reported, but the degrees of freedom are reported as uncorrected. Partial eta squared (η^2_p) was used as an index of the effect size estimates for the ANOVA, and Cohen's *d* of that for the *t*-tests. *P* values smaller than 0.05 were considered significant.

We also compared the differential responses (deviant response minus the standard response) against zero (two-tailed one-sample *t*-test, permutation statistics as implemented in IBM Statistics, SPSS 24) at consecutive time points to determine the latency of a significant MMN response and detect possible differences in other than the MMN latency. To reveal only robust differences, 20 consecutive time points (corresponding to a 10-ms time segment) were required to show a significant difference. The *P*-values ($P < 0.05$) after bootstrapping with 1000 permutations are reported, together with their 95% confidence intervals.

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Author Contributions

J.L.O.K., A.L., J.A.H., R.N. and P.A. designed the study. J.L.O.K. and A.L. collected the data. J.L.O.K. analyzed the data and drafted the manuscript. All the authors contributed to the writing of the manuscript and accepted the final version of it.

Additional Information

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III

PASSIVE EXPOSURE TO FOREIGN SPEECH SOUNDS ENHANCES MEMORY TRACE FORMATION IN ADULT HUMANS

by

Jari L. O. Kurkela, Jarmo A. Hämäläinen, Paavo H. T. Leppänen, Hua Shu & Piia Astikainen, 2018

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