Hearing Research 313 (2014) 67–74

Contents lists available at ScienceDirect

Hearing Research

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

Research paper

Behavioral responses of deafened guinea pigs to intracochlear electrical stimulation: a new rapid psychophysical procedure

Martijn J.H. Agterberg [a,b,*], Huib Versnel [a]

[a] Department of Otorhinolaryngology and Head & Neck Surgery, Brain Center Rudolf Magnus, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

[b] Department of Biophysics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 25 May 2013
Received in revised form
21 April 2014
Accepted 23 April 2014
Available online 5 May 2014

ABSTRACT

In auditory research the guinea pig is often preferred above rats and mice because of the easily accessible cochlea and because the frequency range of its hearing is more comparable to that of humans. Studies of the guinea-pig auditory system primarily apply histological and electrophysiological measures. Behavioral animal paradigms, in particular in combination with these histological and electrophysiological methods, are necessary in the development of new therapeutic interventions. However, the guinea pig is not considered an attractive animal for behavioral experiments. Therefore, the purpose of this study was to develop a behavioral task suitable for guinea pigs, that can be utilized in cochlear-implant related research. Guinea pigs were trained in a modified shuttle-box in which a stream of air was used as unconditioned stimulus (UCS). A stream of air was preferred over conventionally used methods as electric foot-shocks since it produces less stress, which is a confounding factor in behavioral experiments. Hearing guinea pigs were trained to respond to acoustic stimuli. They responded correctly within only five sessions of ten minutes. The animals maintained their performance four weeks after the right cochlea was implanted with an electrode array. After systemic deafening, the animals responded in the first session immediately to intracochlear electrical stimulation. These responses were not affected by daily chronic electrical stimulation (CES). In conclusion, the present study demonstrates that guinea pigs can be trained relatively fast to respond to acoustic stimuli, and that the training has a lasting effect, which generalizes to intracochlear electrical stimulation after deafening. Furthermore, it demonstrates that bilaterally deafened guinea pigs with substantial (~50%) loss of spiral ganglion cells (SGCs), detect intracochlear electrical stimulation.

1. Introduction

The guinea pig is one of the most frequently used species in auditory research. An attractive advantage of the guinea pig is the surgical accessibility of the cochlea. The guinea pig is also often favored over rat and mouse because its hearing extends to lower frequencies, and is therefore more comparable to that of humans. Numerous histological and electrophysiological methods have been applied to study structure and function of the cochlea. Recent examples of such studies are protection from noise trauma and ototoxicity (Alagic et al., 2011; Berglin et al., 2011), tinnitus (Berger et al., 2013; Eggermont, 2013), repair of hair cells by gene therapy (Izumikawa et al., 2005), and rescue of spiral ganglion cells (SGCs) by neurotrophic treatment (Ernfors et al., 1996; Agterberg et al., 2009; Fransson et al., 2010; Pettingill et al., 2011). The functional gain of survival of SGCs is commonly tested by electrically evoked auditory brainstem responses (ABRs). In order to make the translation to deaf humans with a cochlear implant, an important step would be a behavioral test from which the results could be correlated to the histological and electrophysiological measures in the same animals. With a behavioral animal model, it can be examined, whether more and better functioning SGCs result in a better performance with a cochlear implant. Numerous other fundamental questions related to deafness and cochlear implantation can be

Abbreviations: ABR, auditory brainstem response; CAR, correct avoidance response; CES, chronic electrical stimulation; CS, conditioned stimulus; CI, cochlear implant; SGCs, spiral ganglion cells; UCS, unconditioned stimulus

* Corresponding author. Department of Biophysics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands. Tel.: +31 24 3614238; fax: +31 24 3653450.
E-mail address: m.agterberg@donders.ru.nl (M.J.H. Agterberg).

http://dx.doi.org/10.1016/j.heares.2014.04.011
0378-5955/

addressed within this model. For example, the consequences of cortical reorganization for behavioral performance, and the importance of the behavioral relevance of electrical stimulation for central auditory reorganization (Klinke et al., 1999; Fallon et al., 2009; Sharma et al., 2009; Vollmer and Beitel, 2011; Kral and Sharma, 2012), could be investigated.

The aim of the present study is to develop a behavioral animal model that can be used to test the detection of electric stimuli delivered through a cochlear implant. Compared to other species, guinea pigs are hard to train on operant behavioral tasks due to both their erratic behavior and sensitivity to stress. Exposure to stress often results in passive behavior by the guinea pig, which is not conducive to operant behavioral training. Nevertheless, several behavioral models have been presented (Nicol et al., 1992; Mulders et al., 2011; Dehmel et al., 2012; Berger et al., 2013), and also a behavioral guinea pig model for studies of electrical stimulation of the cochlea has been successfully developed by Pfingst and colleagues (Miller et al., 1995; Chikar et al., 2008; Kang et al., 2010; Pfingst et al., 2011). However, their model is time consuming (3–6 months with 90-min training sessions per day, for 5 days/week, to obtain stable detection thresholds), and prior to testing the behavioral responses to electric stimulation of the cochlea, the guinea pigs are deafened unilaterally instead of bilaterally. In the present behavioral model guinea pigs are bilaterally deafened because that mimics the common clinical situation of cochlear implant patients.

Pilot experiments in our laboratory in guinea pigs with a behavioral operant paradigm, using food as a positive reinforcement, were time consuming and not successful. Aiming for a relatively fast method, we adopted a method described by Philippens et al. (1992). To reduce stress, a stream of air is applied as aversive unconditioned stimulus (UCS), because this is thought to be milder than conventionally used electric foot shocks. With this model we have previously demonstrated that guinea pigs could be trained easily to respond to acoustic stimuli (Agterberg et al., 2010a). In the present study we examine whether guinea pigs trained to acoustic stimuli, can be trained to respond to electric stimuli after a period without training and with interventions including cochlear implantation and deafening.

2. Materials & methods

2.1. Animals

Ten female albino guinea pigs (strain: Dunkin Hartley; weighing 250–350 g) were purchased from Harlan Laboratories (Horst, The Netherlands) and housed in the animal care facility of the Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht. All animals had free access to food and water, and were kept under standard laboratory conditions. Part of the present study (training to acoustical stimuli) has been described for 15 normal-hearing guinea pigs in a short report (Agterberg et al., 2010a). Ten of those 15 animals were used in the present behavioral study. These animals were also used to investigate the effect of chronic electrical stimulation (CES) on SGC survival (see Agterberg et al., 2010b). All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC # 03.04.036).

2.2. Shuttle-box study design

Animals were acclimatized to the laboratory conditions for a one week period. During this week the animals were handled daily by transporting them to the sound-attenuated room in which the behavioral tests were performed. Animals were first trained to respond to an acoustic stimulus. The behavioral test procedure was adapted from Philippens et al. (1992), and has been described previously with respect to detection of acoustic stimuli (Agterberg et al., 2010a). The guinea pigs were trained to avoid an unpleasant stream of air (6250 cm^3/s), the UCS, presented from above and directed on the whole body. The animals could avoid the stream of air by moving (shuttling) into the other compartment within 15 s after the conditioned stimulus (CS, narrow-band noise) had been turned on. The shuttle box, placed in a sound-attenuated chamber, consisted of two equal compartments of 23 × 23 × 23 cm (Fig. 1). Infrared beams (OMRON, type E3ZM-T63) on each side of the partition detected shuttles. Shuttle events were registered through an I/O card (National Instruments, USB-6008) and a personal computer. Air streams above each compartment were delivered through tubes (diameter 1 cm) positioned above the shuttle box and they were controlled through magnetic valves (M&M international, type D266DVU230) connected to the I/O card. Labview software (version 8.2, National Instruments) was applied to control the stimuli and to store the shuttle events.

The CS was a narrow-band noise centered around 10 kHz (slope −6 dB per octave). The noise stimuli were generated at a sample frequency of 44.1 kHz, and presented through a PC sound card and an amplifier (Tucker–Davis Technologies, type SA1) with a Fane tweeter (J-104) positioned at 30 cm above the center of the shuttle box. The sound level was calibrated by a sound level meter (Bruël & Kjaer, 2203) and a 1-in. condenser microphone (Bruël & Kjaer, 4132).

Fig. 2 shows a schematic illustration of the different intervals and associated responses. A shuttle response to the CS within 15 s was classified as a correct avoidance response (CAR). If the animal did not respond to the CS, the CS was terminated and the stream of air (UCS) was turned on. A shuttle response to the UCS within 20 s was classified as an escape response. CARs terminated the CS and escape responses terminated the UCS. A failure to respond to CS and UCS was classified as 'no response'. A shuttle before the first trial or during an inter-trial interval, was classified as 'spontaneous shuttle'. The inter-trial interval was randomly varied between 20 and 30 s. To compare the spontaneous shuttle rate to the responses

Fig. 1. Photograph of the shuttle-box from front and top view. Sizes of the shuttle-box are indicated. Black arrows indicate the copper tubes (diameter 1 cm) which delivered the stream of air (6250 cm^3/s). White arrowheads indicate the infrared beam emitter and receiver on either side of the partition.