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BRAIN RESEARCH

Research Report

Maternal approaches to pup ultrasonic vocalizations produced by a nanocrystalline silicon thermo-acoustic emitter

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ARTICLE INFO

Article history:
Accepted 9 May 2007
Available online 12 June 2007

Keywords:
Maternal behavior
Mus musculus
Nanocrystalline silicon
Thermo-acoustic emitter
Ultrasonic vocalization

ABSTRACT

When infant rodents are isolated from their mother and littermates, they cool rapidly and emit ultrasonic vocalizations (USVs). The effect of pup USVs on the mother has been investigated using models of pup USVs from ultrasonic speakers. We used a nanocrystalline silicon thermo-acoustic emitter (nc-Si emitter) to investigate mothers' responses to digitally reproduced pup USVs in mice. The nc-Si emitter could reproduce ultrasonic sounds more accurately than conventional emitters. We compared the sound properties of pup USVs and reproduced USVs. We then investigated maternal responses to hypothermic pups, which emit USVs, and anesthetized pups, which are silent, as well as maternal responses to pup USVs reproduced by the nc-Si emitter and a silent mode. The nc-Si emitter can reproduce pup USVs very accurately in terms of duration, frequency, and sound pressure level. Mothers approached reproduced digitally recorded pup USVs from the nc-Si emitter and their behavior was similar to their behavior toward hypothermic pups. In contrast, mothers did not approach other synthesized ultrasounds, such as double-duration USVs, double-silence domain ultrasounds nor double-ultrasonic domain ultrasounds, indicating that they approach the specific profiles of pup USVs. These results indicate that the nc-Si emitter can be useful to elucidate the role of ultrasonic acoustic communication in rodents.

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Abbreviations: USVs, ultrasonic vocalizations; SPL, sound pressure level; nc-Si emitter, nanocrystalline silicon thermo-acoustic emitter; nc-Si, nanocrystalline silicon

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doi:10.1016/j.brainres.2007.05.056

1. Introduction

During the first 2 weeks postpartum, rodent pups have limited thermoregulatory capabilities and depend on their mother and littermates to maintain thermal homeostasis. When infant pups are isolated from their mother and littermates, they cool rapidly and emit ultrasonic vocalizations (USVs), including whistles and clicks (Branchi et al., 2001; Ehret, 2005; Hofer, 1996). Whistles are pure ultrasonic sounds characterized by frequencies between 30 and 80 kHz, a duration of 10–100 ms, and sound pressure level (SPL) of 60–100 dB (Bell et al., 1972; Liu et al., 2003). Clicks, sometimes produced together with whistles, are short broad-band sounds that contain both sonic and ultrasonic components (Bell et al., 1972; Haack et al., 1983), and the calling rate of these sounds varies developmentally (Elwood and Keeling, 1982). Pup USVs are modulated by several factors such as cold, tactile, and olfactory stimuli (Bell et al., 1971, 1972; Branchi et al., 1998; Conely and Bell, 1978; Okon, 1970a,b).

Pup USVs play an important role in the pups' survival because they stimulate maternal behaviors such as orienting, searching, and retrieving (Ehret, 1992; Farrell and Alberts, 2002a,b; Noirot, 1972; Smotherman et al., 1974; Zimmerberg et al., 2003). Several studies have shown direct evidence that pup USVs elicit maternal orienting and searching behavior by using models of pup USVs from ultrasonic speakers (Allin and Banks, 1972; Ehret and Haack, 1982; Farrell and Alberts, 2002b; Sewell, 1970; Smith, 1976; Smotherman et al., 1974). Hofer and his colleagues have explored how social conditions affect rat pups USVs, and suggested that pup USVs have communicative role for pup retrieval (Hofer et al., 1993, 1994). As to mouse pup USVs, reproduced and synthesized USVs have been tested if the dam proximately approaches to the sound source, and it was suggested that specific profile was necessary for dam's perception of USVs (Ehret, 1992, 2005). The most common mechanism for reproducing ultrasound is via a piezoelectric transducer. However, the frequency bandwidth of most piezoelectric transducers is limited, and commercially available ultrasound speakers have narrower range or larger SPL (e.g., Avisoft ultrasound speaker, range 20–80 kHz, ±6 dB SPL: Ultrasound Advice, range 20–100 kHz, ±6 dB SPL). More than that, these commercially available emitters are larger in size of vibration body (Avisoft ScanSpeak, 80 mm × 80 mm: Ultrasond Advice, 50 mm × 50 mm) than the new nc-Sci emitter (less than 2 mm × 2 mm), which is crucially important for the studies of localization of acoustic source. Therefore, it is still under disputation if the maternal approach to pup USVs occurs because the mother recognizes properties of pup USVs or because the mother is curious about sounds.

We have recently demonstrated that a nanocrystalline silicon thermo-acoustic emitter (nc-Si emitter) can reproduce digitally recorded pup USVs very accurately in terms of call duration, frequency, and SPL (Kihara et al., 2006). Here, we used an nc-Si emitter that exhibits an almost completely flat frequency response over a wide range without any mechanical vibration.

Our aim was to determine whether highly reproducible ultrasounds from the nc-Si emitter elicited maternal behavior, as in previous playback experiments. We first compared the properties of pup USVs with those of pup USVs reproduced by an nc-Si emitter. Second, we compared the mother's response to vocalizing and non-vocalizing pups and the mother's response to reproduced pup USVs. We then presented another synthesized ultrasound to control for the effect of ultrasound stimulus. If mothers specifically approach ultrasound profiles matching pup USVs, the synthesized ultrasound should not induce an approach to the emitter. We assessed the legitimacy of the nc-Si emitter as an experimental tool for researching ultrasonic communication and pup USVs.

2. Results

2.1. *Frequency response of the nc-Si emitter*

The frequency response of the nc-Si emitter is shown in Fig. 1. The measured SPL was approximately 64 dB between 25 and 80 kHz. The deviation of SPL from the average was less than ±5 dB SPL.

2.2. *Comparison of waveform, spectrogram, and mapping SPL: pup USVs vs. reproduced pup USVs*

Representative ultrasonic waveforms and spectrograms from a real pup USV and from a reproduced pup USV are shown in Figs. 2A and B, respectively. The first segment of the spectrogram was mapped to the SPL (Figs. 2C, D). The maximum SPL is denoted by 0 dB in this diagram, and the SPL interval between the contour planes is 12 dB. The similarity between the two spectrograms, measured as the correlation coefficient for pattern recognition, was as high as 0.84 (Cohen, 1988).

2.3. *Maternal response to pup USVs*

The mean durations of stay in a tube and of search the mesh were significantly longer on the hypothermic pup side than on the anesthetized pup side (Fig. 3A; stay: $z=-2.240$,

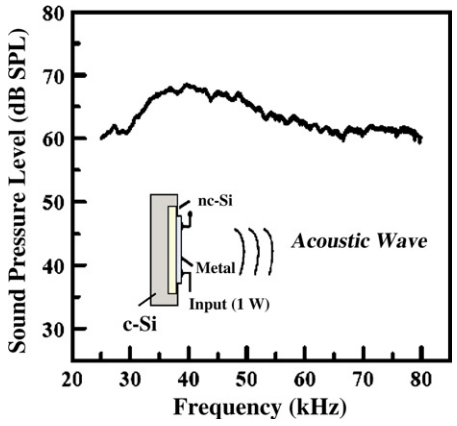

Fig. 1 – Measured sound pressure level (SPL) generated by the nc-Si emitter as a function of frequency. An almost constant SPL is observed between 25 and 80 kHz. The SPL level was approximately 64 dB, between 25 and 80 kHz, with the deviation of SPL level less than ±5 dB.