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Research report

Sound exposure accelerates reflex emergence and development in young rats

Edmund Cheung So[a,b,c], Ying-Hui Chen[a], Chieh-Yu Huang[d], Jen-Yin Chen[a], Bu-Miin Huang[e], Paul Wai-Fung Poon[d,*]

[a] Department of Anaesthesia, Chi Mei Medical Center (Yong Kang & Loui Ying), 901, Chung Hwa Road, Yong Kang City, Tainan, Taiwan
[b] Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan
[c] Department of Cosmetic Science, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
[d] Department of Physiology, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan
[e] Department of Cell biology & Anatomy, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan

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ABSTRACT

Early sensory experience affects brain development. In rats, most somatic reflexes are not expressed at birth but may take as long as 2 weeks to emerge. Whether sensory enrichment during this early period affects reflex maturation remains unknown. Here, we exposed rat pups to a pure tone (4 kHz, 65 dB SPL, 8 h/day) with their nursing mother during the first 3 postnatal weeks and measured the times when reflexes appeared on the basis of video recordings. Sound exposure accelerated by about 15% the appearance of all reflexes assessed (righting, cliff avoidance, vibrissa placing, negative geotaxis and auditory startle, $p < 0.001$). In addition, sound exposure accelerated the appearance of developmental characteristics: incisor eruption, ear unfolding and eye opening. These changes occurred concomitantly with an increase in pups' body and brain weights, together with a dramatic increase in fluid intake of the nursing mother. These findings are the first evidence that early sound exposure, even before opening of ear canals, accelerates reflex development. We speculate that the observed changes could involve the nursing mother.

1. Introduction

Brain development is affected by environmental factors such as sensory over-stimulation [16], handling [9], enriched environment [8,20,24], dietary intake [15] and maternal care [4]. For example, dark rearing young rats reduces vascular density and thickness of their visual cortices [2]. Exposure to tone remodels tonotopic maps at the auditory midbrain [25] and the primary auditory cortex [23]. Raising rats in an enriched environment also increases cortical thickness [10] and neurogenesis in the hippocampus [20]. Experience acquired during the 'critical periods' of young animals leads to marked changes in neural sensitivity, for example improved response strength, threshold, selectivity, and latency of cortical neurons [13]. The morphology, chemistry and physiology of the brain are markedly altered by environmental stimuli [26]. Other studies provided further details of similar changes ranging from molecular to anatomical and functional levels [36,27]. The exact mechanisms of these changes are however not fully understood, but the response selectivity of neurons, remodeling of neural circuits [34], genetic programming [24] and the regulation of protein expression [38] are likely involved [28].

In the case of rats, pups are born with immature sensory systems including non-functional cochleae during the first week [18]. Sound stimuli delivered during this early stage could in theory exert no effects on neural development. For this reason, experiments with early sound exposure typically start around postnatal day 8, or after the opening of ear canals [15]. However, a study on the responses of central auditory neurons of young rats following sound exposures suggested that sound-driven effects could extend to periods even before opening of ear canals [5].

If sound stimulation during this early stage does affect brain development, it may change the time when somatic reflexes emerge. Since these reflexes normally appear in a specific temporal order during the first 14 days [6], their appearance is commonly used to assess the speed of neural development during early postnatal periods [12,14,15,30,37]. Altered times of reflex appearance could reflect developmental changes, presumably involving the spinal cord, brainstem and perhaps higher centers [14].

These reflex appearances are known to be strongly influenced by maternal inputs. For instance, food deprivation of the nursing mother delays reflex appearance in her pups [33]. Depriving pups of maternal care or dietary intake, albeit partially, retards their gross development (e.g., body weight reduction, delayed eye opening and walking), leads to cell death in the hippocampus [4], slows down

* Corresponding author at: Department of Physiology, College of Medicine, National Cheng Kung University, 1, University Road, Tainan 70101, Taiwan.
Tel.: +886 6 2353535x5458; fax: +886 6 236 2780.
E-mail address: ppoon@mail.ncku.edu.tw (P.W.-F. Poon).

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central neural transmission [15] and prolongs response latency of the geotactic reflex [12]. During the neonatal period, the developing brain is also vulnerable to the suppressive actions of drugs [14].

Here, we determined using reflex appearance as the main index whether sound exposure (4 kHz, 65 dB SPL, 8 h/day) during this early postnatal stage (P1–P21, traversing the time ear canals open around P10–P12) affects brain development. Specifically, we studied sound effects on rat pups in the development of five somatic reflexes: righting, cliff avoidance, vibrissa placing, negative geotaxis and auditory startle. We hypothesized that during this early stage, if maternal inputs are altered after prolonged acoustic stimulation, reflex emergence of pups would be affected.

2. Methods

All experimental procedures were approved by the Ethics Committee, Laboratory Animal Center, National Cheng Kung University and followed National Institute of Health "Guide for the Use and Care of Laboratory Animals".

One pregnant rat was experimented at any one time. The pregnant rat (Sprague–Dawley; obtained from our Laboratory Animal Center; original source: Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA, USA) was reared (water and food *ad libitum*) until the day of delivery inside a standard rat cage (Acrylic, $W \times L \times H$: 25 cm × 46 cm × 20 cm, with a metallic grill-top for the insertion of water feeding-bottle and the placement of chow pellets). The cage was placed on the floor of a wooden chamber ($W \times L \times H$: 0.6 m × 0.6 m × 1.3 m). Walls of the chamber were lined with sound-absorbing materials (corrugated sponge) except one side, which was open with a curtain for the purposes of animal caring and for ventilation. Ambient room temperature was controlled between 24 and 26 °C using an air-conditioner and relative humidity kept between 50 and 70% with a dehumidifier. Saw dust bedding was manually changed every day. A normal light/dark period of 12 h/12 h was controlled by switching the room lights with an automatic timer. No particular environmental enrichment was provided. Litter size was kept at 10 pups (male:female = 1:1); surplus animals, by random selection, were sacrificed for another experiment.

Animals were exposed during their active night times to a continuous tone (4 kHz, 8 h/day, 23:00–07:00 h) from postnatal day 1 (P1) to day 21 (P21). Sounds were presented through a free-field speaker (Pioneer S77, Pioneer Electronic, Shindian City, Taipei, Taiwan) placed on the ceiling. The sound intensity was 65 dB sound pressure level (SPL), as calibrated at the site of the animals (Bruel & Kjaer 4191 microphone, Skodsborgvej, Nærum, Denmark) [4]. Equal numbers of control animals were reared in the same environment but without the sound. Background sound levels (measured 30 cm above the cage) either with or without pups during 19:00–7:00 h were between 17.68 and 17.67 dB SPL, reflecting basically ambient room noise. During this 3-week period, we video recorded reflex responses of individual pups on a daily basis (between 14:00 and 16:00 h). The five reflex tests were done in random order each day until P21. Reflex assessments basically followed the method described by Heyser [18]. A digital camera (T-N610, Sharp cooperation, section 2, Jianguo N. Road, Chung Shan District, Taipei, Taiwan) was used to image the body movements (30 image/s). For a given pup, a minimum interval of 10 min was allowed between consecutive reflex tests. An experimenter blindfolded to the exposure option manually analyzed the recorded images. Specifically, body movements were analyzed image-by-image in slow motion playback of the video (Ulead 10 software, Corel, Taipei, Taiwan). To study the reflex latencies, 3 rats from each litter were randomly selected for the measurements.

2.1. Behavioral tests

2.1.1. Righting reflex

We first removed a pup from the mother and manually laid it on its back to induce the righting reflex. The observation time window was 1 min per trial. A successful righting reflex was counted when four limbs had all returned to the ground (i.e., from supine to prone). A clean soft cloth on a table-top and a warming light source provided the testing environment for this and some other reflexes (Fig. 1A).

2.1.2. Cliff avoidance

The pup was manually placed with its nose and forefeet hanging over a table edge to induce a withdrawal response. The table was 30 cm above ground with a clean cotton pad (2 cm thick) positioned 5 cm below the table surface to prevent injury to the pup in case of an accidental fall off the table. The observation time was 30 s. Withdrawal of the head and forelimbs back onto the table was counted as a positive response (Fig. 1B).

2.1.3. Negative geotaxis

The pup was placed prone on a horizontal wooden board, which was then tilted 25° off horizontal with its tail pointing up the slope and its head facing down. Its ability to turn the body axis up the slope for an angular excursion of 45°, 90°, 135° or 180° within 1 min was given a reflex score of 1–4 [14] (Fig. 1C).

2.1.4. Vibrissa placing

The pup was manually held by the tail and its vibrissae were brought gently in contact with a vertical wooden board. Uplifting of both head and forelimbs towards the board surface was taken as a positive response (Fig. 1D).

2.1.5. Auditory startle

We used a hand-held mousetrap to produce the sound stimulus due to its greater efficacy in inducing startle response and being less likely to be adapted. The pup was placed prone on the table-top approximately 1 m away from a hand-held mousetrap which on manual release generated a loud sound as the spring-loaded metal frame snapped the wooden base (115 ± 5 dB SPL as determined with a Bruel & Kjaer 4191 microphone system). Caution was taken to avoid physical contact between the mousetrap and the observation table to suppress non-airborne mechanical stimulation to the animal. This sudden loud sound consistently induced an acoustic startle reflex in adult rats. A sudden retraction of the pup's head or limbs in response to the sound was taken as a positive startle reflex [15]. For timing purpose, the voltage output of a microphone was displayed on an oscilloscope to mark the onset of the snap and the oscilloscope output was captured on the images (Fig. 1E).

2.2. Assessments of development

2.2.1. Water consumption

Since pups do not drink from the feeding bottle before weaning but rely solely on maternal milk for fluid intake, water consumption of the dam (or more specifically of the nursing mother) would reflect sound-driven maternal changes. Water consumption of each dam was therefore measured daily in the morning based on the water left in the feeding bottle.

2.2.2. Body weight

Individual pups were weighed on P1, P7, P14 and P21 using a digital balance (A&D EK-1200, 0.1 gm resolution, A & D instrument, Unit 24/26 Blacklands Way Abingdon Business Park, Abingdon, Oxon OX14 1DY, UK).

2.2.3. Brain weight

On P21, after euthanasia (Sodium Pentobarbital, 50 mg/kg, i.p.) and transcardial perfusion with saline and 4% paraformaldehyde solutions, brains were carefully removed and weighed on the digital balance by an experimenter blindfolded to the exposure option.

2.2.4. Body development

The times of emergence of three standard development landmarks for each pup: incisor eruption, ear unfolding and eye opening, were determined visually by two experimenters on a daily basis. A positive result was recorded when both experimenters agreed on the observation.

2.3. Statistical analysis

Results (mean ± SEM) from body and brain weight, developmental landmarks (incisor eruption, ear unfolding and eye opening) and reflex emergence (righting, cliff avoidance, negative geotaxis, vibrissa placing and auditory startle) were compared between the exposed and control groups using two tailed unpaired Student's *t*-test. Repeated measurement ANOVA test was used to compare water intake of the nursing mother (exposure vs. control), overall body weight of the pups and latency of negative geotaxis. Inter-litter variances were checked by Kruskal–Wallis *H* test. Mann–Whitney *U* test was used for reflex latency comparison between exposed and control groups. Significant differences were set at $p < 0.05$.

3. Results

3.1. Water consumption

The nursing mothers of the sound-exposed group showed increased daily water intake from P2 to P21 (repeated measurement ANOVA: $F(1,8) = 26.71, p < 0.002$). The departure from control peaked around P9 (Fig. 2A and B).

3.2. Body and brain weights

On P1, there was no statistical difference in body weight between the two groups (unpaired *t*-test, $t = 1.8$, $n = 50$, $p > 0.05$). Sound exposure increased body weight in exposed rats on P7 (12.9 ± 0.14 g vs. 12.3 ± 0.13 g, unpaired *t*-test, $t = 3.3$, $p < 0.002$,