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Classification of discomfort-related ultrasonic calls in pup and adult fat-tailed gerbils *Pachyuromys duprasi*

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Classification of discomfort-related ultrasonic calls (UCs) is an important prerequisite for their applicability as indicators of emotional arousal in laboratory rodents. We classified spectrographically the UCs collected from 40 individual pups (17 males and 23 females from 11 litters) and 20 adult (10 males and 10 females) fat-tailed gerbils *Pachyuromys duprasi* during 420-s isolation-and-handling tests. As ultrasound emerges in fat-tailed gerbils at 6th day of life, we used recordings of 6–10-day-old pups. The UCs were provided by 22 of 40 pups (782 calls in total) and by 7 (3 male, 4 female) of 20 adults (248 calls in total). The UCs differed in the fundamental frequency contour: Flat, Chevron, Downward, Upward, Short (within 4 ms) and Complex (up-down many times), and in the number of notes (1, 2, 3, many) resulting from up or down frequency jumps over 10 kHz. This variation resulted in the total of 42 UC types. In pups, most frequent were single-note UCs (600 of 782 calls), with either Flat (299 calls) or Chevron (193 calls) contour. Another widespread call type was the three-note UC (in total 96 calls with down-and-up frequency jumps) with Chevron contour. In adults, as in pups, most UCs were single-note (193 of 243 calls) with contours Chevron (57 calls), Flat (40 calls), or Short (35 calls). Compared to adult UCs, the pup UCs were longer (50.0 ± 30.9 ms vs 28.1 ± 97.1 ms) and lower in frequency, with the peak frequency of 47.8 ± 5.8 kHz vs 58.8 ± 8.7 kHz; the maximum fundamental frequency of 52.0 ± 4.8 kHz vs 64.2 ± 11.0 kHz, and the minimum fundamental frequency of 41.8 ± 6.4 kHz vs 50.9 ± 8.8 kHz. Consistent differences between pups and adults were found also for most widespread single-note Flat and single-note Chevron UCs, taken separately from other call types. The ontogenetic pathway of fat-tailed gerbils UCs (towards shorter and higher-frequency calls) resembles those of bats and domestic mice. Support: the Russian Science Foundation, grant 14-14-00237.