

# Sex and Parental Experience Determine the Onset of an Instinctive Behavior in Mice



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Ultrasonic calls of young mouse pups (*Mus musculus*) release parental care (retrieving pups, licking, nest-building) in adults of either sex [1]. Here, we demonstrate that female and male mice need different periods of contact with pups both for the performance of pup retrieving and for the acquisition of the preference for its ultrasonic releaser. The results show a sex-dependent difference in the occurrence of an instinctive behavior and in the speed of learning to associate a stimulus with this behavior.

Behavioral tests were conducted with 2-5-month-old *Mus musculus* (outbred strain NMRI) including mothers and fathers with caretaking experience for one total broodcaring period (24 days), fathers and females co-caring for a litter over 1-5 days postpartum of the mother and males and females without any breeding experience and without pup contact (naive animals). The same setup and procedures as described elsewhere [2] were used. In short, experimental animals were adapted at least for 5 h before tests started to a sound-proof room and a running board (length 110 cm, width 8 cm) with a central nest depression. Experienced mothers and fathers had their whole

second litter, co-caring fathers and females at least seven pups in the nest depression. Tests were conducted in dim red ( $\ll 1$  lx) illumination either between 0700 and 1100 hours or 1700 and 2100 hours. Experimental animals had to retrieve five pups of their own (mothers and fathers) or of a donor-litter (2-5 days old) which were thoroughly embrocated with the nest material of the animal in test. Pups were placed 30 cm from the nest depression on either side of the running board. If the first pup was not retrieved within 10 min, animals were scored unresponsive.

Table 1 shows the results of the retrieving tests. Naive males and fathers co-caring for their pups for only one day are clearly non-retrieving. More than half of the naive females and a significant portion ( $p < 0.05$ ) of the females co-caring for one day retrieve. Animals of either sex with pup experience for five days or more all show retrieving behavior ( $p < 0.01$ ). Pup-retrieving behavior thus can occur in totally unexperienced females and becomes fully established after one day of sensitization with pups. Males (fathers) need more than one day sensitization with pups to develop this behavior at all.

Next, pup-retrieving animals were tested for their ability to recognize ultrasounds as releasers (key stimuli) for retrieving behavior. Previous tests [2] have shown that ultrasounds of pups as well as tone bursts with frequencies between 40 and 60 kHz are recognized as preferred stimuli for initiating pup retrieving while 20-kHz-tone bursts are not (neutral stimuli). In a two-alternative choice test (for details see [2]) the animals had to leave the nest depression of the running board within 10 min of stimulus onset and approach for at least 30 cm one or the other ultrasonic speaker which were fixed 65 cm from the nest beyond the ends of the running board. One speaker emitted 50-kHz the other, alternatively, 20-kHz-tone bursts (80 ms flat top duration, 10 ms ramps, 3/s repetition rate, all 71 dB above the average auditory threshold of the animals [3]). The animals had a maximum of six runs, always starting in the nest, in which 50 and 20 kHz were assigned randomly to the speakers.

Table 1 clearly shows that females co-caring for pups over 5 days significantly prefer 50-kHz ultrasounds as do experienced mothers and fathers. Animals of all the other groups do not discriminate 50- and 20-kHz-tone bursts. Naive females, females co-caring for 1 day and fathers co-caring for up to 5 days, although responsive to retrieve pups, do obviously not recognize 50-kHz ultrasounds as a preferred releaser for retrieving behavior. It seems that they do not learn the specificity of the key stimulus, i.e. to discriminate meaningful from irrelevant sound within the given periods of broodcaring experience.

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Table 1. Pup retrieving and ultrasound discrimination in the various experimental groups of animals. Experienced: one full brood cycle; 5 (1) day: five (one) days experience by co-caring for pups; naive: no experience with pups; ns - non-significant

	Retrieving			Discrimination		
	Number of animals		<i>p</i> (binomial two-tailed)	Number of runs towards		<i>p</i> (binomial two-tailed)
	retrieving	not retrieving		50 kHz	20 kHz	
Experienced mothers	32	5	<0.01	68	32	<0.01
Experienced fathers	27	10	<0.01	72	33	<0.01
5 day ♀	13	2	<0.01	42	19	<0.01
5 day ♂	13	2	<0.01	37	30	ns
1 day ♀	12	3	<0.05	30	22	ns
1 day ♂	0	15	<0.001	—	—	—
naive ♀	8	7	ns	42	38	ns
naive ♂	0	15	<0.001	—	—	—