

FIG. 5. Call stimuli evoke differential responses in mated females. In (A) awake as well as in (B) anesthetized mated females, presentation of either the mate's call (Mate) or the familiar call (Familiar) evoked significantly greater responses (*, all $P < 0.05$) than presentation of the unfamiliar call (Unfamiliar). (C) The same call stimuli did not evoke differential responses in anesthetized control females that had not heard them previously. Each bar represents the mean \pm SEM of the response strength index (RS).

neurons did not distinguish between the two call stimuli. Concordantly, the overall d' value did not reveal any clear preference for either of the two calls (mean \pm SEM, -0.67 ± 0.79 ; $t_{1,13} = 0.6$, $P = 0.55$). However, the focus on the number of highly selective cells yielded another picture. Most recording sites (12/14) exhibited a d' value of either $> +1$ or < -1 . Some exhibited a preference for the mate's call ($d' > +1$; seven of 14) while others preferred the familiar call ($d' < -1$; five of 14). Therefore, these last results suggest a bias toward either the mate's call or the familiar call rather than a lack of discrimination between the two calls.

Similar habituation rates to the different call stimuli

One of the main characteristics of the auditory responses of NCM neurons is a decline in their magnitude with song or call repetition (Chew *et al.*, 1995). This also occurred in our study. By the tenth repetition, on average, the RS had declined to 70–80% of its initial value (Fig. 6). Based on the mean spike rate during call presentation (trial 1 vs. trial 45–50), repetition significantly affected the magnitude of the responses, regardless of the identity of the male that produced the call stimulus (mate's call, $F_{1,13} = 8.5$, $P = 0.007$; familiar call, $F_{1,13} = 6.17$, $P = 0.019$; unfamiliar call, $F_{1,13} = 4.94$, $P = 0.035$).

A previous study reported that, in males, the rate of habituation of NCM auditory responses differed between a novel song stimulus and a song heard early in development, the tutor's song; the habituation rate

was higher for novel than for familiar song stimuli (Phan *et al.*, 2006). As the presentation of either the mate's call or the familiar call elicited a stronger response than the unfamiliar call, it was of importance to examine whether a mechanism of plasticity that relied on stimulus exposure, such as habituation, could have contributed to the difference in response magnitude between call stimuli. No significant interaction was found between call repetition (trial 1 vs. trial 45–50) and call identity, indicating that the habituation rate did not differ between call stimuli ($P > 0.37$ for all comparisons). Also, the habituation rate of each multiunit site to a given call stimulus was estimated by computing the linear regression (Phan *et al.*, 2006). The slope of the response curve did not differ between call stimuli, indicating that habituation was not slower for the mate's call or the familiar call than for the unfamiliar call (repeated-measures ANOVA, $F_{2,26} = 0.78$, $P = 0.48$). Taken together, these results rule out the contribution of any habituation-dependent mechanism of plasticity to explain the observed differences in response magnitude between call stimuli in mated females.

Anesthetized mated vs. control females

To further examine how calls varying in familiarity are represented in the dorsorostral NCM of females, the auditory responses of single units were recorded in anesthetized mated females ($n = 12$). The preference of the population of NCM neurons for either the mate's call or the familiar call over the unfamiliar call could result from memorization, but it could also be due to another process, such as a more pronounced sensitivity to certain acoustic features of vocalizations. To address this issue, we examined auditory responses driven by the three call stimuli in control females that had never heard them before ($n = 5$). To do so, we quantitatively analysed the call-evoked responses of 68 well-isolated single units in mated females and 69 single units in control females.

As reported by Stripling *et al.* (1997), NCM single units in our study varied widely (up to 30-fold) in their rate of spontaneous activity. Also, there was considerable unit-to-unit variability in the pattern of responses of NCM responses to call stimuli, as previously observed (Stripling *et al.*, 1997, 2001; Terleph *et al.*, 2006). Figure 7 gives examples of different call-evoked responses that were driven by the same familiar and unfamiliar call stimuli. Some cells responded to the playback of a call stimulus with sustained activation for the duration of the stimulus while others showed maximal firing soon after stimulus onset (with a latency of 20–30 ms) followed by a more-or-less abrupt decline in activity to the baseline level throughout the rest of the stimulus. Quantitatively, the presentation of call stimuli caused,

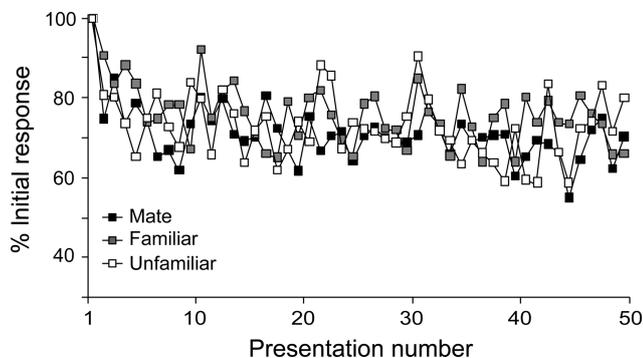


FIG. 6. Response habituation with repeated-call stimulus presentation. Trial-by-trial responses are shown for the mate's call (dark gray squares), the familiar call (light gray squares) and the unfamiliar call (white squares). Responses are expressed as a percentage of the response at trial 1. While response magnitude differed between either the mate's call or the familiar call on the one hand and the unfamiliar call on the other, similar modulation rates were observed.