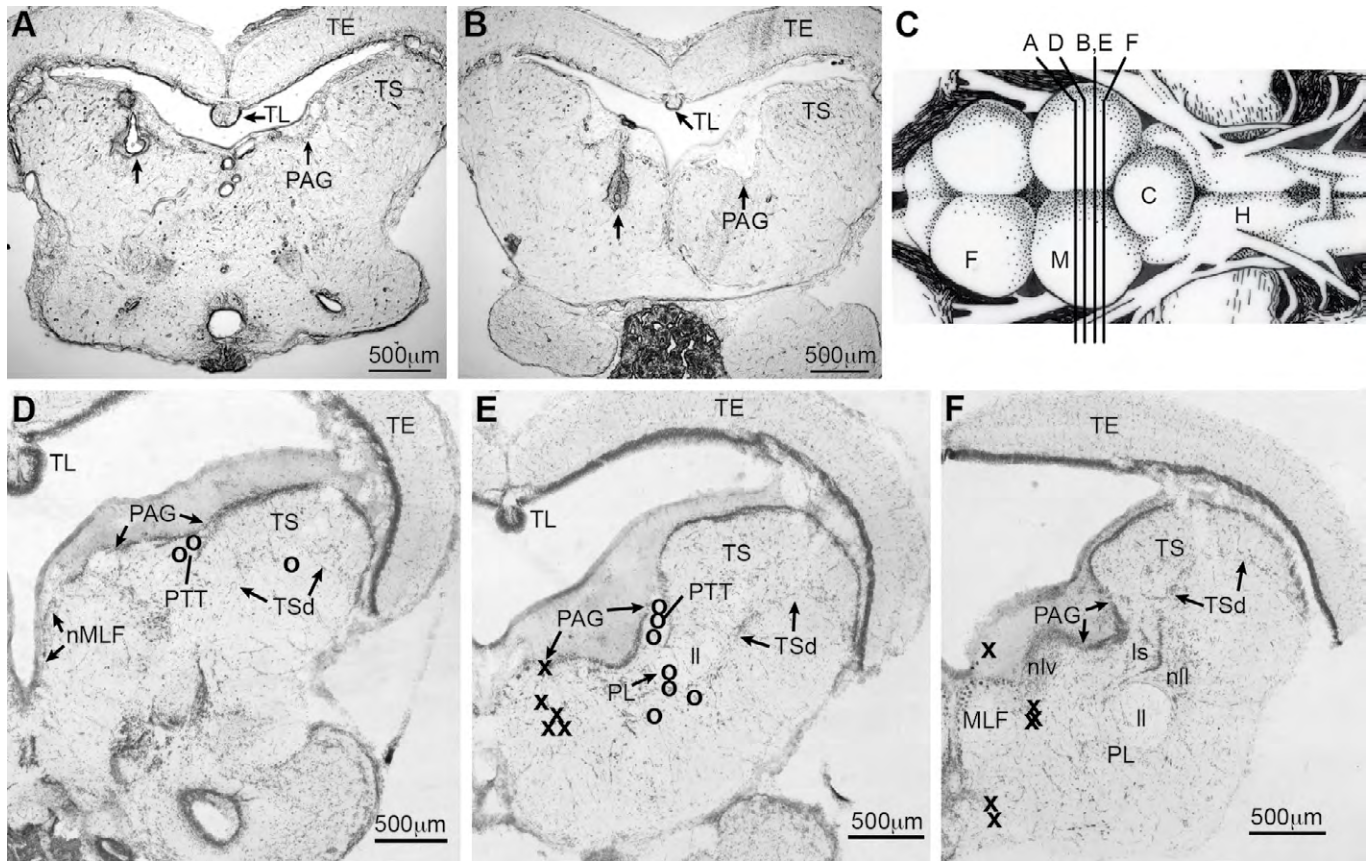


**Fig. S1. Medial sites evoked shorter fictive calls, even after lateral stimulation.** Three males held in normal 15 h light:9 h dark cycle were tested at the lateral site first for a 120 min session and a SRC at the same site, followed by recording a second SRC at the medial site. (A) Fictive call duration recorded over 120 min from the lateral site. (B) Fictive call latency recorded over 120 min from the medial site. (C) Threshold stimulus required to elicit fictive calls over 120 min sessions. (D) Fictive calls elicited during lateral SRC are significantly longer than medial SRC, demonstrating that regardless of stimulus order, lateral midbrain stimulation reliably elicits longer duration calls. P value is shown for the fixed effect of stimulus-site. Data are presented as mean±standard error.



**Fig. S2. Localization of lateral and medial midbrain lesion sites.** Sample lateral (A) and medial (B) lesions are indicated by arrows. (C) Dorsal view of the midshipman brain, showing relative midbrain levels at which coronal sections in D-F were taken. Abbreviations: F, forebrain; M, midbrain; C, cerebellum; H, hindbrain. (D-F) The relative positions of medial (x) and lateral (o) lesion sites were mapped onto midbrain sections at three transverse levels adapted from Kittelberger and Bass (2013). Of the medial lesions, seven fell within the midbrain tegmentum adjacent to the medial longitudinal fasciculus (MLF) that connects the vocal midbrain with the hindbrain vocal pattern generator (E,F), one was on the medial edge of the PAG (E), one was within the MLF (F), and two were below the MLF (F). Of the lateral lesions, five were in the paratotal tegmentum (PTT) that lies below the lateral periaqueductal gray (PAG) (D,E), one within the deep layer of the torus semicircularis (TSd) (D), and four just below or along ventral aspect of the paralemniscal tegmentum (PL) that borders the lateral lemniscus (ll) (E). Abbreviations: Is, isthmal nucleus; nll, nucleus of the ll; nMLF, nucleus of the MLF; nlv, nucleus lateralis valvulae; TE, midbrain tectum; TL, torus longitudinalis.