Supplemental Figure 1. Complete Area X lesions leave song variability intact. Data are plotted as in Figure 2A-E for four subsong birds, three of which received 100% Area X lesions (A-Q) and one of which received a 90% lesion (S-V). While large Area X lesions left variability intact, in most cases the detailed shape of the syllable and gap distributions was affected by the lesion. (A,F,L,R) Neu-N staining of left and right hemispheres confirmed bilateral elimination of Area X in the four birds. (B-E) Representative example of a case where Area X lesion resulted in a reduced occurrence of short syllables (B-C). (F-K, L-Q) Representative examples of two birds where Area X lesion had the opposite effect: causing an increase in the production of short syllables. (R-V) Example of yet another bird that preferentially lost syllables of intermediate (~100 ms) duration following Area X lesion. Importantly, across birds, even though Area X lesions could cause changes in song structure, syllable and gap durations, as well as syllable phonology remained highly variable.

Supplemental Figure 2. Methods for histological verification of lesions. (A-H) DLM lesions. (A-B) Schematics of verification of DLM lesions. (A) In control birds, injection of tracer in LMAN results in anterograde labeling of LMAN terminals in RA, and retrogradely labeled cell bodies in DLM. (B) In DLM-lesioned birds, terminals in RA confirm the successful injection of the tracer into LMAN and the preservation of the LMAN→RA pathway, but there are no retrogradely labeled LMAN-projecting DLM neurons. (C-E) In a bird with DLM intact, Neu-N antibody stains cell bodies in DLM (see methods) (C), and DLM neurons retrogradely labeled by injection of tracer into LMAN are readily visible (D). (E) Anterogradely labeled terminals in RA confirm that tracer was

injected into LMAN. (F-H) Images are plotted as in (C-E) for a bird with a complete DLM lesion. (F) Only background fluorescence, and no cell bodies, is visible within boundaries of DLM after Neu-N labeling. Note that auditory thalamic nucleus Ovoidalis (red arrow) remained intact following lesions. (G) Note the absence of retrogradely labeled DLM neurons despite injection of tracer into LMAN, demonstrated by terminals in RA (H). The song of this DLM-lesioned bird is shown in figure 3G-K. (I-K) HVC lesions. HVC and RA are readily visible in Neu-N stain (I), but HVC and its surrounding tissue are clearly eliminated bilaterally by electrolytic burns (M-N, see methods). The song of this HVC lesioned bird is shown in Figure 6C.

Supplemental Figure 3. Area X lesions resulted in poor song imitation. Representative song development in two brothers: one that received a sham lesion at dph 41 (left) and one that received bilateral lesions to Area X (right). Note that while the songs of both brothers were highly variable at dph 55, the song of the Area X lesioned bird failed to incorporate acoustic elements of the tutor song. By young adulthood (dph 80, bottom), the control bird had learned the tutor motif (‘abc’, labeled in red), while the song of the lesioned bird remained highly variable and did not resemble the tutor song. The observed effects of juvenile Area X lesions on the later development of adult song are consistent with previously studies (Scharff and Nottebohm 1991; Sohrabji et al. 1990).