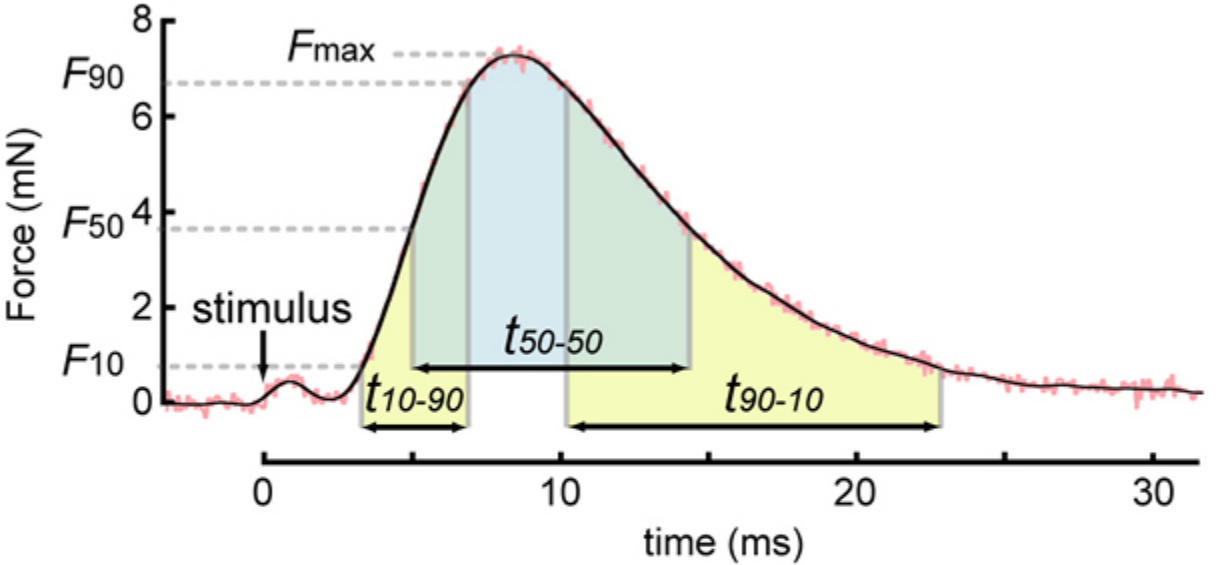
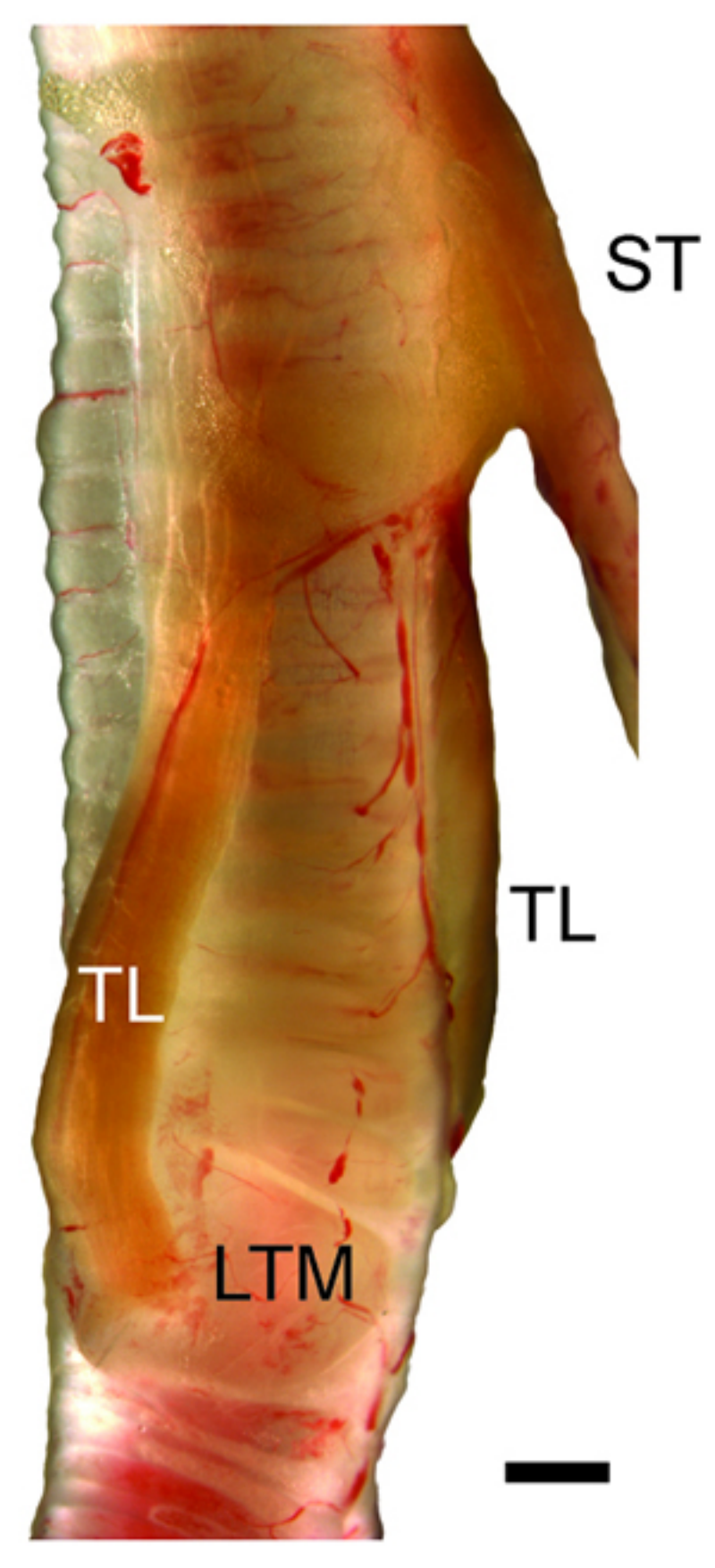
Figure S1 Elemans et al.



Single twitch contraction of TL preparation. F_{10}, F_{50} and F_{90} were defined as 10%, 50% and 90% of the maximum force F_{max}. $t_{10\text{-}90}$, time to ascend from F_{10} to F_{90}, $t_{90\text{-}10}$, time to descent from F_{90} to F_{10}. $t_{50\text{-}50}$, half-twitch time. Within each twitch, force rapidly developed ($t_{10\text{-}90}$; TL, 3.5±0.4 ms and ST, 4.7±1.2 ms) and decreased ($t_{90\text{-}10}$; TL, 10.6±1.7 ms and ST, 10.4±2.2 ms).


ST
TL
TL
LTM

Supplementary Methods

***In vivo* recordings.** Sound and electromyographic (EMG) activity of musculus tracheolateralis (TL) and m. sternotrachealis (ST) were recorded of six spontaneously vocalizing male ring doves (Salt Lake City, Utah, USA). Teflon-coated copper wire electrodes (65 µm diameter) with 1 mm tips of Nickel wire (25 µm diameter) were inserted about 2 mm apart in the muscle body to avoid the movement artefacts that we encountered in our initial measurements with the electrodes placed closely together[1]. Separated electrodes provided signals from several motor-units at low impedance. The electrodes were glued to the fascia with cyanoacrylate tissue adhesive, routed out of the interclavicular airsac and led subcutaneously to a backpack[2]. Spontaneous vocalizations started 1-2 days after surgery. The caged bird was placed in the centre of a cubic-meter box, open at the front, with sound-insulating foam to suppress reflections. Sound was recorded at 20-30 cm from the cage using two microphones (Audiotechnica AT835b and a 1/4" Brüel & Kjær omni-directional condenser microphone model 4939 for calibrations). We obtained signals from two healthy, spontaneously vocalizing males. We analysed five coos of one animal, in which both EMG and sound recordings showed distinct pulses in the trill. The other animal showed similar EMG patterns, but the sound amplitude was too weak to analyse individual pulses in the trill. Experiments followed federal regulations and approval for animal experimentation. We conducted standard correlations to determine the relationship between binarized EMG and sound signals. We compared correlations using a Wilcoxon Signed Rank test[3].

***In vitro* muscle performance.** Seven ring doves (Rotterdam, The Netherlands) were anaesthetized and euthanized using CO_2/O_2. Whole-muscle preparations of the left TL (5.4±0.8 mg, n=7) and left ST (5.7±1.4 mg, n=7) were dissected within 1 hour, whilst continuously flushing the muscles with oxygenated Ringers solution[4]. Finished preparations were stored up to five hours at room temperature on Sylgard-covered Petri

dishes in oxygenated Ringers solutions. Muscle preparations were mounted on both ends in a test chamber filled with oxygenated Ringers' solution at 39±0.2°C. One end of the muscle was mounted to the arm of a dual-mode servo system (Aurora Scientific model 300b). After a rest period of 60 minutes, several tests were conducted at 2-minute intervals to optimise resting length and twitch parameters[4]. Optimal TL resting length was 7.29±0.76 mm, measured from insertion on lateral tympaniform membranes (LTM) to tracheal ring *T11*, optimal ST resting length was 14.60±0.73 mm. Optimal tetanic stimulation was defined as the stimulus frequency at which the highest isometric stress was measured that did not decline during 100 ms of stimulation. Maximal isometric stress (MIS) was calculated as F_{max} /A_{cr} of the muscle, where the cross-sectional area A_{cr} was estimated from the resting length and weight of the muscle fibres (assuming a constant density[5] of 1060kg/m^3). After testing, non-contractile and dead material was removed before weighing the preparations (Mettler Toledo type AG204; ±0.1mg). Muscle fibres were stimulated with external-field platinum wire electrodes. Twitch stimulations were applied using MuscleWork Software developed for Labview 6.0 (National Instruments), kindly provided by R.J. Josephson and J. Malamud. The twitch characteristics are shown in Figure S1. Figure S2 shows the sound-generating syringeal membranes, or LTM, suspended between the tracheal rings; their tension and position is controlled by two antagonistic muscle pairs, TL and ST (partly shown).We used Matlab 6.5 (The Mathworks) to analyse our data and to construct the playback signal in Figure 1**b** by averaging the time signature of ten coos recorded (Brüel & Kjær condenser microphone, model 4939) from one animal in a semi-anechoic room at Wageningen University, The Netherlands. The resulting trill block pattern was played back with the optimal[4] stimulus amplitude and tetanic frequency for each preparation. The animal testing committee of Wageningen University approved all experiments.

References:

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