

Supplementary Information for

Auditory representation of learned sound sequences in motor regions of the macaque brain

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Other supplementary materials for this manuscript include the following:

Movie S1

Supplementary Text

Role of premotor and prefrontal areas

The participation of premotor and prefrontal areas in auditory-motor sequence learning is well known (for examples from piano playing in humans see (1–4)).

The contribution of different frontal regions in the current paradigm is discussed here.

Premotor versus primary motor cortex

Within premotor cortex, the relative contributions of premotor and primary motor areas to learned auditory-motor behavior may depend on the proficiency of that behavior. This was demonstrated in one of our monkeys, which was scanned at different stages of piano training: Activation of premotor area F2 but not of primary motor cortex F1 was found when the monkey was still in the early stages of training. By contrast, F1 activation became more prevalent at a later stage, when the animal was more proficient in its performance (**Fig. S7** and **Fig. 1**).

High proficiency with playing a piece on a musical instrument after extensive training is sometimes described as involving “muscle memory”. Instead, this state of overtraining may reflect the degree of involvement of primary motor cortex.

Orbital medial prefrontal cortex for somatosensory feedback?

Areas 12m and 12r in the orbital medial prefrontal cortex (OMPFC) were activated in both early and late phases of training. The OMPFC is connected to ventral premotor cortex in area 6 (areas 6va and 6vb), Area 12m, in particular, receives somatosensory input from face, digit, and forelimb regions in the

opercular part of area 1-2, in area 7b, in the second somatosensory area (SII), and in the anterior intraparietal area (AIP) (5). This suggests that somatosensory feedback and its integration with premotor activity plays an important role in the present form of auditory-motor learning and forelimb behavior, as it does in speech production (6).

Supplementary motor areas

One set of premotor areas often associated with auditory-motor behavior (7, 8) and sequence learning (2, 9) was conspicuously absent from the activated regions found in this study: the supplementary motor areas (SMA and pre-SMA). SMA and pre-SMA are responsible for the coding of precise time intervals and rhythms (SMA) and for the initiation of movement (pre-SMA) (10, 11). As temporal precision was not an enforced aspect of task training in this experiment, the task-associated neuronal assemblies formed through training may not have tightly incorporated SMA and pre-SMA neurons.

Mirror neuron area F5

Also absent was activation of area F5, which is regarded as the center piece of the mirror neuron system in the frontal cortex. However, the classical definition of mirror neurons in the visual domain is that they fire when monkeys observe (and understand) the action of another monkey. The same definition applies, by analogy, to mirror neurons in the auditory domain: Neurons respond to the tearing of a piece of paper, because they associate the sound with a particular action (12). In the present case, the activation of motor-related cortex results from a sound sequence that the same monkey has learned to produce, which

presumably constitutes neither the observation nor the understanding of an action. Activation of such a sensorimotor event, therefore, remains limited to primary and early premotor areas and does not extend to area F5.

When discussing visual mirror neurons, it needs to be considered that LED-based assistance was provided in the initial stages of training. However, this was only the case during a very early phase. In the later stages of the study, the monkeys played almost completely without visual cues. During the actual fMRI experiment, no lights were presented at all. Therefore, it is unlikely that any memories of initial LED assistance would have exerted a major influence on the monkeys' behavior or on fMRI activation. The piano apparatus was never present or visible during fMRI acquisition, further excluding hypothetical contribution of visuo-motor mirror neurons. While our results, therefore, speak against the involvement of a visuo-motor mirror system, it is possible that the internal model system we postulate is in fact part of an analogous "auditory-motor mirror" system. Whether such a system includes area F5 may best be tested with single-unit electrophysiology.

Insula as a potential site for a command apparatus

Left precentral gyrus of the insula, a cortical area in the lateral sulcus beneath the frontal, parietal and temporal lobes (13), has been associated with apraxia of speech (14), an inability to coordinate complex articulatory movements, in human stroke patients. In the macaque, the caudal part of the insula receives reciprocal projections from auditory cortical areas in the superior temporal gyrus

and sulcus and from secondary somatosensory cortex (15). Correspondingly, the region shows activation by somatosensory and auditory stimuli, including conspecific vocalizations (16, 17). The caudal insula is further connected with the putamen (16), especially with its dorsal, sensorimotor part, which was also activated in our study. Furthermore, it is connected with the inferior frontal cortex (Broca's region in humans), but its precise connectivity with ventral sensorimotor cortex (vSMC), containing both motor and sensory representations of speech sounds in humans (18, 19), to our knowledge, has yet to be determined.

Handedness and laterality of auditory-motor activation

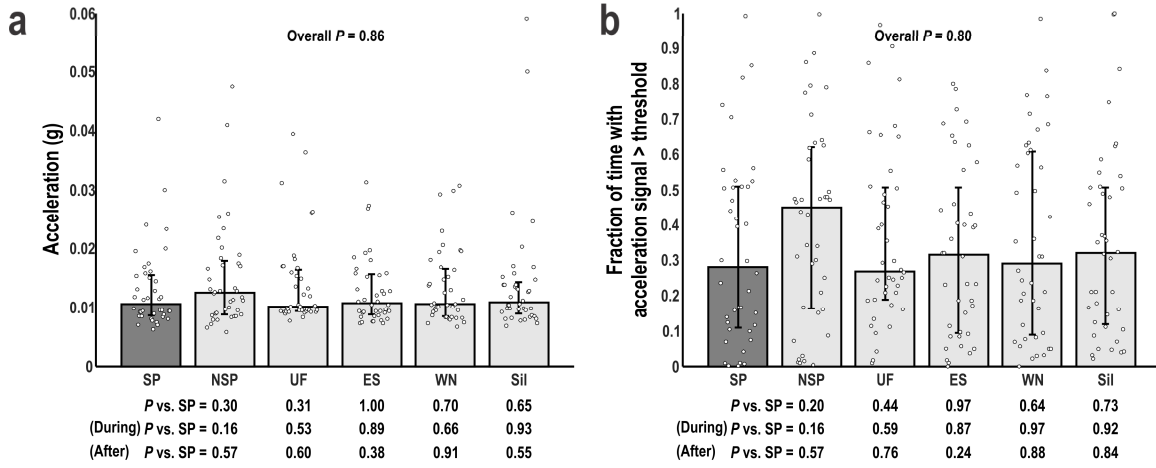
All three monkeys used primarily their left hand for playing the piano, but this was due to the way the apparatus was set up, whereby they used the right hand to hold on to the cage (see **Movie S1**). Studies of handedness in rhesus monkeys (e.g. (20)) found that there was an almost even distribution of right- and left-handers. Among our own monkeys, Monkey Ra appeared to be predominantly left-handed, monkey Ch right-handed, and monkey Do used both hands. (We have videos that show monkey Do attempting to play with both hands.)

Brain activation is expected to differ between playing piano and listening to piano (as it differs between speaking and listening to speech (19)), since, for instance, the monkey doesn't move its arm/hand (not even subliminally, as shown in **Fig. S1**) while listening. Although one obviously expects activation of auditory cortex in both situations, it is unclear whether there is hemispheric asymmetry: In playback studies of species-specific vocalizations, different groups

have reported inconsistent results, e.g. Poremba et al. (2004) report left asymmetry of auditory activation; Petkov et al. (2008) right asymmetry; Joly et al. (2012) left asymmetry; Ortiz-Rios et al. (2015) right asymmetry (21–24). It is uncertain, therefore, that the left lateralization of the M1 response, when the monkeys listen to piano sequences, is an early precursor of language lateralization, although it could be a correlate of ear dominance.

Supplementary Figures

Movement during and after stimulus



Electrical muscle activity (EMG) during and after stimulus

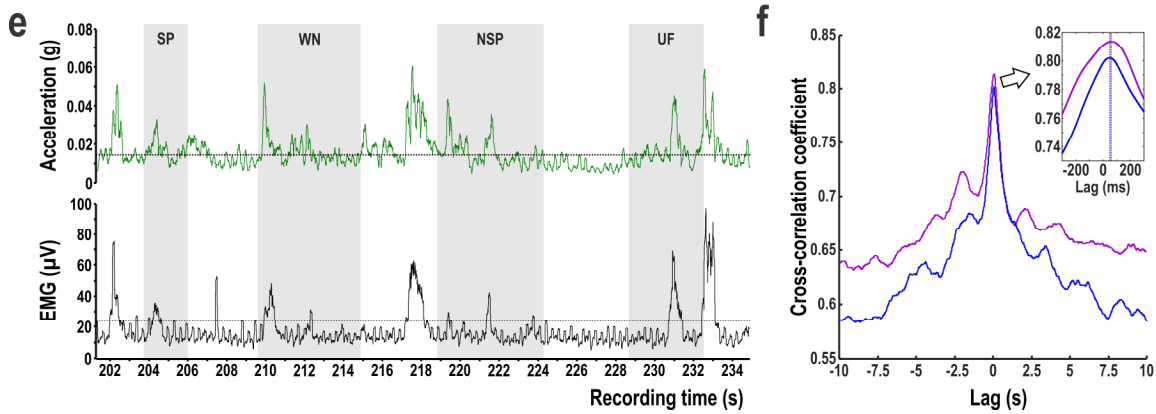
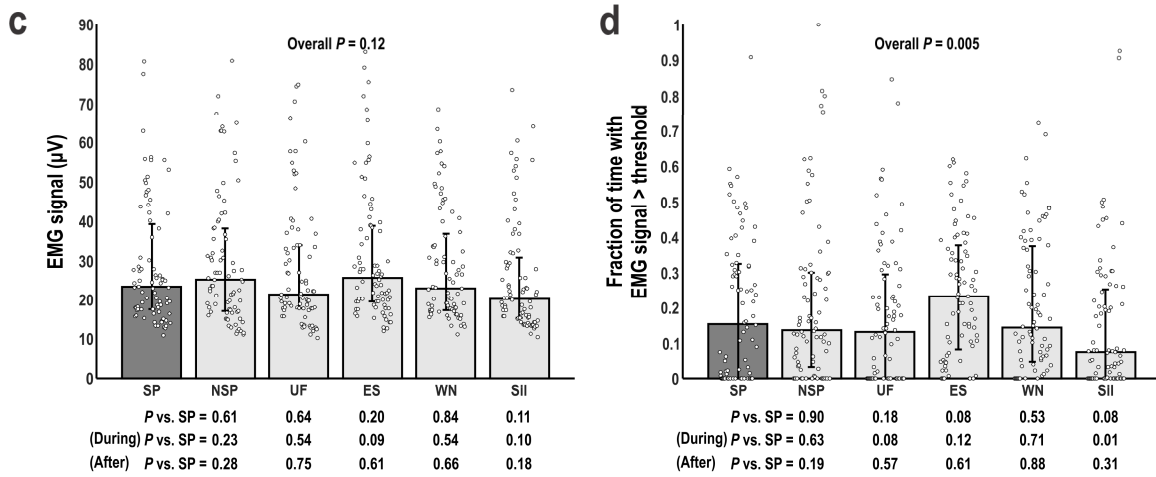


Figure S1. Recording of movement and electrical muscle activity (EMG) was performed in monkey Ra. Since the animal used exclusively his left arm when playing the monkey piano, EMG/movement recordings were performed in this arm. The EMG showed no increased motor activity evoked by the SP sequence compared to control stimuli, indicating that the SP-specific activity in F1 and the putamen (**Fig 3 and 4**) was not motor-, but auditory-driven. During recordings, monkey Ra was listening to six stimulus types: SP, NSP, and UF sequences, environmental sounds (ES), white noise bursts (WN), and silence (Sil). **(a)** Arm movements determined with RMS-averaged accelerometer signal per stimulus type, from stimulus onset until 3 s after stimulus end. Bars: median signal level across 40 stimulus presentations (two sessions), error bars: upper/ lower quartile, circles: signal level during individual presentations. Signal levels are compared across all stimulus types with Kruskal-Wallis test (Overall P), and between SP sequences and each of other stimulus types with Mann-Whitney test, not corrected for multiple comparisons (P values below the graph). Additionally, P values are provided for Mann-Whitney tests performed only over the stimulus duration (During) and only over 3 seconds after stimulus end (After). **(b)** Fraction of time with the arm-movement signal above threshold (see **Methods**). Data presentation and statistics in **b**, **c**, and **d** same as in **a**. **(c)** EMG activity. Panels **c** and **d** include data from 80 presentations (four sessions). **(d)** Fraction of time with the EMG signal above threshold. **(e)** Example recording snippet. Upper (green) trace: RMS-averaged accelerometer (arm movement) signal. Lower (black) trace: RMS-averaged EMG signal. Horizontal lines show

visually established thresholds used to determine results in **b** and **d**. Gray rectangles: stimulus presentation periods. The ~3-Hz periodic component visible in the EMG trace is caused by the pulse and is consistent with published heart rates for chaired macaques (e.g., (25, 26)). **(f)** Cross-correlation analysis. The accelerometer and EMG signals were correlated, with the movement lagging 50-60 ms behind EMG (inset). Two plotted lines show results from two recording sessions involving movement measurement.

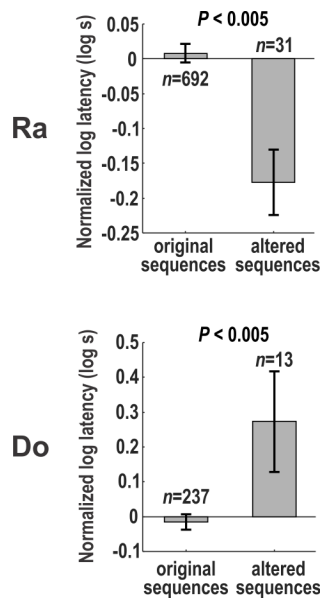


Figure S2. Catch trials using silencing of the entire sequence. In a subset of sessions for monkeys Ra and Do, lever presses were programmed to produce no sound in about 5% of the sequences. Latencies of lever presses immediately following the silent presses were significantly altered compared to regular sequences in both monkeys (Ra showed faster responses, while Do showed significantly slower responses). Error bars show +/- SEM. See **Methods** for details, and **Fig. 1d** for catch trials using alteration of a single note.

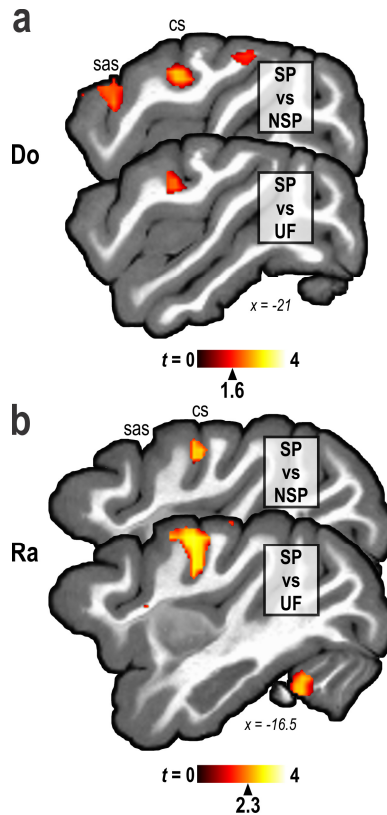


Figure S3. Activation of motor cortex by listening to self-produced (SP) sound sequences vs. non-self-produced (NSP) and unfamiliar (UF) sound sequences compared individually. In monkey Do, comparison of BOLD responses evoked by a sound sequence that monkey Do had been trained to produce (SP) vs. one that she was familiar with but never produced (NSP) or vs. unfamiliar sound sequences (UF) found activation in the anterior bank of the central sulcus **(a)** ($p < 0.05$ uncorrected, $k \geq 15$ voxels). In monkey Ra, the SP vs. NSP and SP vs. UF contrasts revealed activation in the same region **(b)** ($p < 0.01$ uncorrected; $k \geq 25$ voxels). Compare to **Fig. 3**.

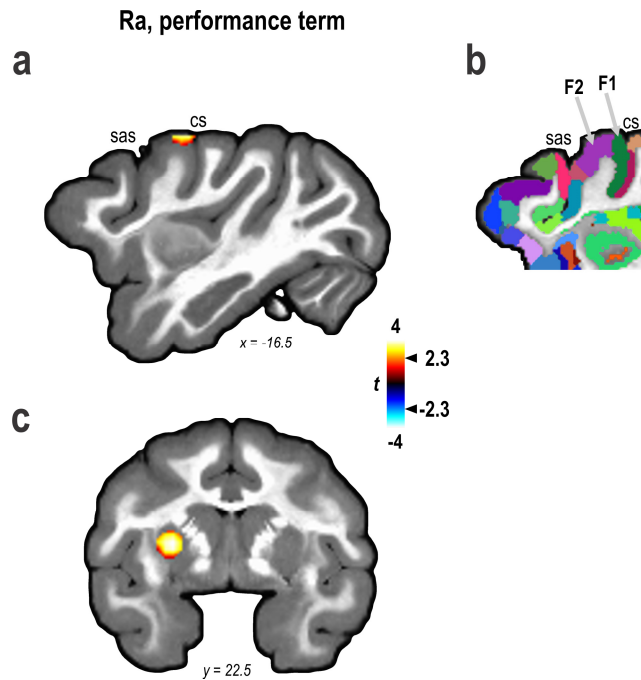


Figure S4. Correlation of behavioral performance with the BOLD signal. In monkey Ra, the behavioral performance term (see **Methods**) was significant in areas F1/F2 (**a**) and in the putamen (**c**) ($p < 0.01$ uncorrected; $k \geq 25$ voxels). These foci were similar to foci revealed by comparison of activation by SP vs NSP or UF sequences (compare to **Figs. 3** and **S3** for F1/F2, and to **Figs. 4** and **S5** for the putamen). (**b**) D99 atlas segmentation of the same sagittal slice of the same brain (27). cs, central sulcus, sas, superior arcuate sulcus.

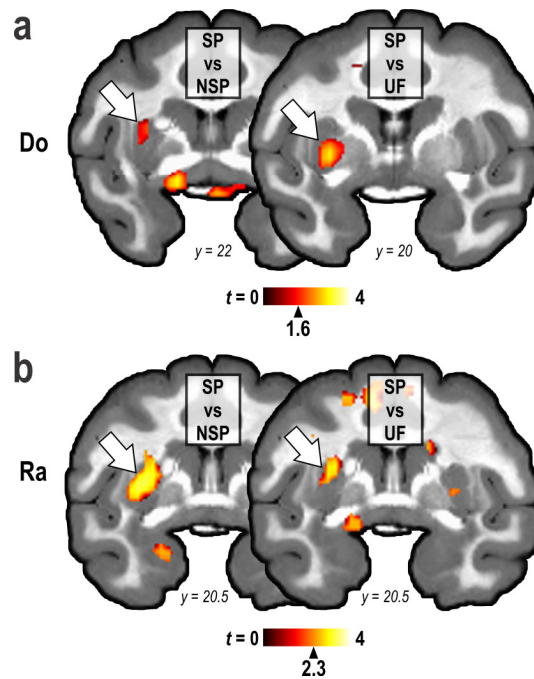


Figure S5. Activation of the putamen by listening to self-produced sound sequences (SP) compared separately to non-self-produced (NSP) and unfamiliar (UF) sound sequences. In monkey Do, comparison of BOLD responses evoked by a sound sequence that monkey Do had been trained to produce (SP) vs. one that she was familiar with but never produced (NSP) or vs. unfamiliar sound sequences (UF) found activation in the putamen **(a)** ($p < 0.05$ uncorrected, $k \geq 15$ voxels). In monkey Ra, SP vs. NSP and SP vs. UF contrasts revealed activation in the same structure **(b)** ($p < 0.01$ uncorrected; $k \geq 25$ voxels). Compare to **Fig. 4**.

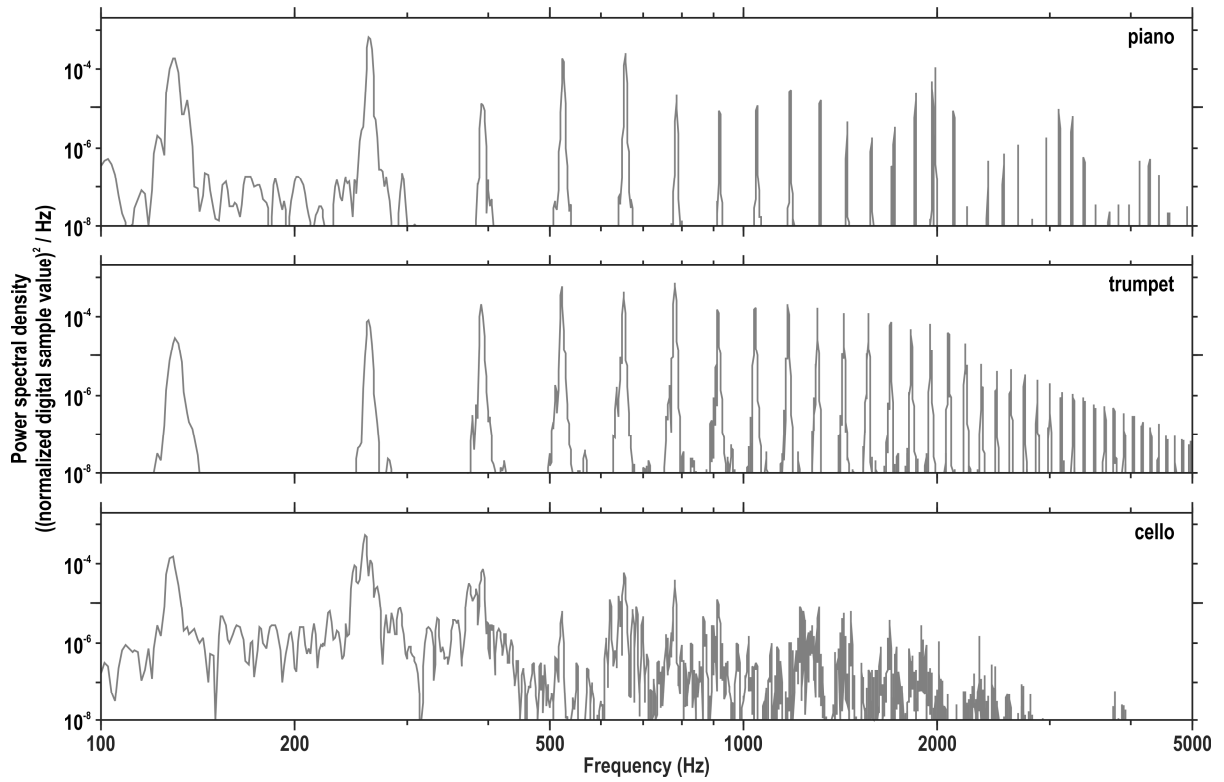


Figure S6. Power spectral density of C3 notes of the three timbres used in the study. The overtones cover generally similar frequency ranges.

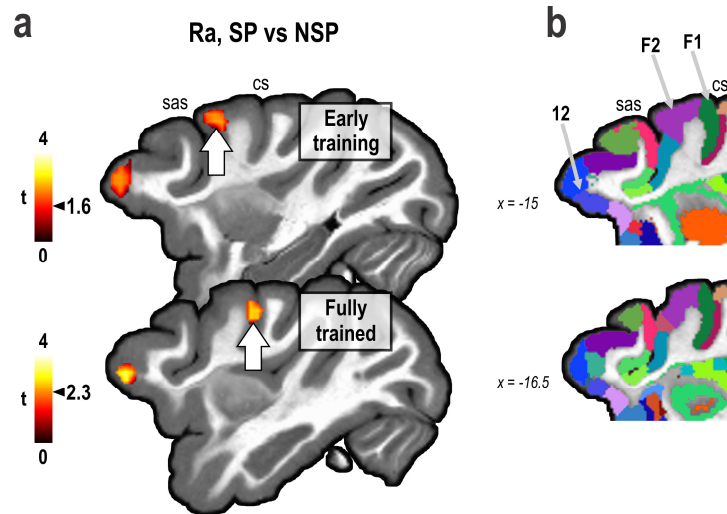


Figure S7. Effect of training. (a) As a result of training, activation for SP sequences (as compared to activation for NSP sequences) appears to shift from premotor to motor areas (arrows). Top: During early training with visual guidance (black circles in **Fig. 1c**) activation was found in left premotor area F2 ($p < 0.05$ uncorrected, $k \geq 15$ voxels). Bottom: In a fully trained animal (red circles in **Fig. 1c**), activation was found in area F1 ($p < 0.01$ uncorrected, $k \geq 15$ voxels, same data as in **Fig. 3**). Early data were only available for monkey Ra and for the SP vs NSP contrast. (b) D99 atlas segmentation of the same brain (27). The most frontal area of activation that was active in both early and late phases of training was within area 12 (5, 28), which is part of orbital medial prefrontal cortex.

Movie S1 (separate file)

Video of monkey Ra performing his self-produced (SP) sequence on the 'monkey piano'. A catch trial is included, whereby a normal piano sound is substituted with a trumpet note.

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