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Changes in multiple brain regions underlie species differences in a complex, congenital behavior

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ABSTRACT **The evolutionary brain modifications that produce any complex, congenital behavioral difference between two species have never been identified. Evolutionary processes may (*i*) alter a single, "higher" brain area that generates and/or coordinates the diverse motor components of a complex act; (*ii*) separately change independent, "lower" brain areas that modulate the fine motor control of the individual components; or (*iii*) modify both types of areas. This study explores the brain localization of a species difference in one such behavior, the crowing of chickens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*). Two major subcomponents of the behavioral difference can be independently transferred with interspecies transplantation of separate brain regions, despite the fact that these components, sound and patterned head movement, occur together in a highly integrated fashion. To our knowledge, this is the first experimental demonstration that species differences in a complex behavior are built up from separate changes to distinct cell groups in different parts of the brain and that these cell groups have independent effects on individual behavioral components.**

Congenital species differences in behavior are those that persist when different species are reared in similar environments. Despite recent progress in understanding both the mechanisms of vertebrate neural development (1–4) and changes in developmental processes that could yield major morphological differences in brain size and the organization of brain areas (5–11), evolutionary changes in more subtle features underlying the striking differences seen in congenital behaviors among species with similar brain architecture remain to be explained.

Species differences in complex behavioral acts could result from several alternative mechanisms. Most simply, they could be produced by changing the features of cells within a single, higher brain area that generates motor patterns or coordinates the activity of various behavioral components into a unified whole. Alternatively, there could be independent changes to different, lower brain areas more involved with modulating the fine details of the different components of a complex motor act. This latter possibility seems more difficult to achieve because it requires independent changes at different brain locations. Finally, behavioral differences could result from a combination of evolutionary changes to both types of brain areas.

Recent techniques for creating surgical brain chimeras between avian species that can hatch and behave normally (12–19) have made it possible to study this question empirically, using a vocal behavior called crowing. Crowing is a complex but relatively stereotyped hormone-dependent vocalization delivered by adult male gallinaceous birds (20–29). Crowing and other patterns of adult male sexual behavior can be induced in juvenile males and females within a few days of hatching by administration of the steroid hormone testosterone (30–36). The structure of juvenile crows is stable within individuals, and although each individual has a unique crow, there is a great resemblance among the crows of different animals within a species (21, 25–28, 33). Single chicken and quail crows differ reliably in two parameters: their sound pattern and the pattern of head movement given during their delivery (Fig. 1).

Chicken crows generally have a single part (some individuals have an interruption of airflow in this single part, which disappears with age), and except for a tendency to dip their head slightly at the beginning of sound production, chickens do not have any consistent movement of the head in the vertical plane at frequencies >4 Hz during crowing. Quail crows have two or three parts with very distinctive temporal relationships among them. They also have a distinctive pattern of amplitude and frequency modulations in the final part of the crow. Quail rapidly bob their heads up and down at frequencies of 4–20 Hz during crowing, in synchrony with these amplitude and frequency modulations. Both quail and chickens have a large amplitude deflection of the head up and forward preparatory to crowing that has varying kinetics within and between individuals; the quail head bobs are superimposed on this larger amplitude head movement. Quail do not produce such head bobs when giving other vocalizations in their vocal repertoire. Species differences in acoustical and gestural aspects of crowing do not appear to be influenced by imitative learning (ref. 37 and unpublished data).

In a previous study, it was found that the acoustical temporal pattern characteristic of quail crowing can be transplanted into chickens when the quail donor portion includes the primordium of the midbrain (14). The present study began by examining videotaped records of two of these animals to ascertain their pattern of head movement.

As a control for general behavioral abnormalities in the head movement of chimeras, yawning (38, 39), part of the normal behavioral repertoire of both chicks and Japanese quail, was recorded. During yawning in both species, the neck is stretched vertically and the upper mandible is raised upward; the head follows the same overall trajectory as the low frequency, high amplitude head movement preparatory to crowing in both chickens and quail (Figs. 1 and 2). This is followed by swallowing and closing the bill. Yawning is not usually accompanied by any sound in either species.

As an additional surgical control, chicken–chicken transplants were carried out to assess the effects of surgical intervention on head movement. None of the chicken–chicken chimeras showed any differences in crowing, head movement, yawning, or any other obvious behavior from unoperated chickens. Thus, the behavioral effects described below are not attributable to surgical procedures.

MATERIALS AND METHODS

Video Analysis. For the data in Table 1, 15 videotaped crows were examined for the Mes–Pro chimera, and four were

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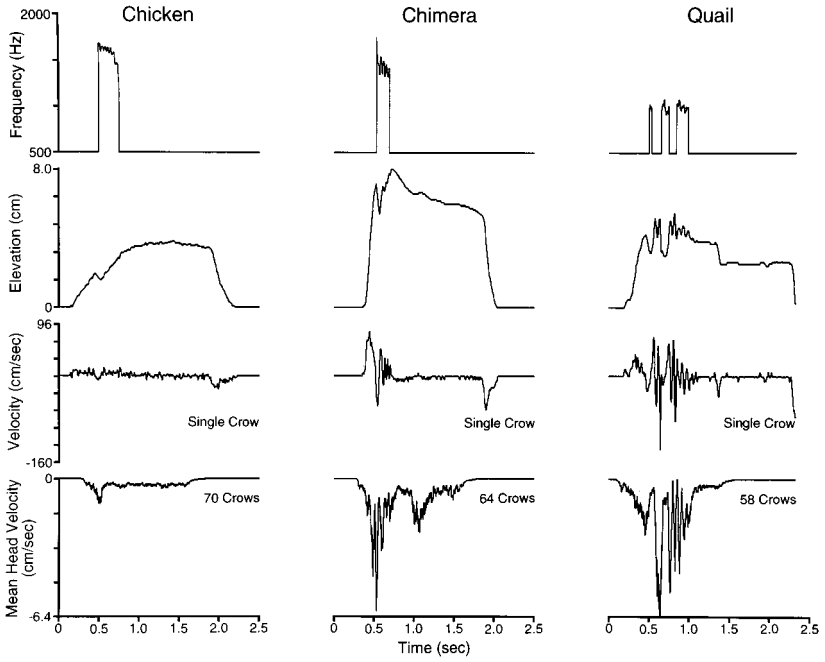

FIG. 1. Representative crows and head movements for a chicken, a caudal brainstem chimera, and a quail. (*Top three lines*) Sound [Frequency (Hz)], vertical head position [Elevation (cm)], and instantaneous vertical head velocity [Velocity (cm/sec)] profiles of a single crow for a chicken, a chimera of somites 4–5, and a quail. Note the difference in sound patterning and head movement between the chicken and the quail and the resemblance of the chimera's sound to the former and head movement to the latter. (*Bottom line*) Mean head velocity profile for all crows given by these three subjects. Chickens have relatively flat movement profiles because they move their heads slowly in the vertical direction during crowing and lack a consistent pattern of head movement during the crow. Quail and chimeras make faster, phasic head movements with consistent temporal patterning.

examined for the Met–Di chimera. All crows were recorded on a Panasonic (Secaucus, NJ) WV32500/8AF Color Video Camera connected to a Panasonic AG6400 Portable Video Recorder. The difference between chicken and quail head movement and the number of quail head bobs during the crow are easily visualized on standard videotapes played at one-half speed. As a part of this study, seven chickens and six quail were examined using both standard videotaping and head movement measurement. For all seven chickens and four of the quail, 20 of the crows measured in the head movement apparatus from each animal were simultaneously videotaped, and the videotapes were visually scored before the head movement analysis using a Panasonic AG-7510 Video Player (the remaining two quail had five crows each compared in this way). There was perfect agreement in all cases between the judgment of whether an animal bobbed its head on the videotape and the measured head movement. There was also perfect agreement between the judgment from the videotape of the number of head bobs individual quail performed in their crow and the number measured.

Table 1. Characteristics of subjects used in this study

Subject	*n*	Crow type*	Head movement type†
Quail–chicken chimera			
Rostral 1/3 Mes + Di	2	C	C§
Caudal 1/3 Mes + Met	2	C	C§
Somites 2-4	2	C	C§
Somites 4-5	8	C	Q§
Somites 5-7	5	C	Q§
Met–Di and Mes–Pro‡	2‡	Q‡	C¶
Chicken–chicken chimera			
Somites 4-5 and 5-7	3	C	C§
Unoperated animals			
Chickens	11	C	C¶
Chickens	7	C	C§¶
Quail	12	Q	Q¶
Quail	6	Q	Q§¶

Pro, prosencephalon; Di, diencephalon; Mes, mesencephalon; Met, metencephalon.

*Crow type assignment was based on the morphology of fundamental frequency–time contours and the temporal pattern of energy distribution across all crows given by each individual. C, Those individuals whose frequency–time contours and temporal distribution of energy match those of normal chickens; Q, individuals whose frequency–time contours and temporal distribution of energy match those of normal quail.

†Head movement type assignment was based on whether the individual showed greater head movement than normal chickens and on the morphology of this head movement pattern as described in the text. C, Those individuals that showed no greater head movement during crowing than normal chickens; Q, those individuals that had head movement greater than normal chickens that matched the pattern features of normal quail head movements.

‡Data from Balaban *et al.* (14).

§Head movement measured directly.

¶Head movement scored from videotape.

Surgical Procedures. Surgical procedures were in accordance with institutional guidelines as described (14). Domestic chicken eggs and Japanese quail eggs were obtained from commercial sources within 24 h of laying. All surgeries were isochronic and isotopic. Control transplant operations (chicken–chicken) were carried out in an identical manner between two different chicken embryos.

Recording Sounds and Head Movement. Experiments were conducted in a heated Acoustic Systems (Austin, TX) sound attenuation chamber; its inner walls were covered with 2-in thick Illbruck (Minneapolis, MN) acoustic foam insulation. Video recordings were made with a Panasonic WV32500/8AF Color Video Camera connected to a Panasonic AG6400 Portable Video Recorder. Sound was recorded using a Shure (Evanston, IL) Prologue 16L Lo-Z condenser microphone connected to a Rane (Everett, WA) MS-1 microphone stage preamplifier. Head movement was simultaneously recorded using two ISCAN (Burlington, MA) RK-446R Video Movement Tracking Systems operating in parallel. One of these systems measured movement of the bird's head from above, and the other measured from the side. Each system supplied a two-channel output voltage every 8 ms, representing the position of the brightest object in the x and y dimensions of a 255 × 511-pixel video field. The upper mandible of the bird was reliably made the brightest object by painting it with nontoxic fluorescent orange t-shirt paint (DEKA PERMAIR 592, Decart, Morrisville, VT) and recording data under black light fluorescent lamps. In gallinaceous birds, the upper mandible is rigidly fixed to the skull; this provides a reliable measure of the movement of the head. Subjects were allowed to move freely inside a clear Plexiglas cylinder during recording. The

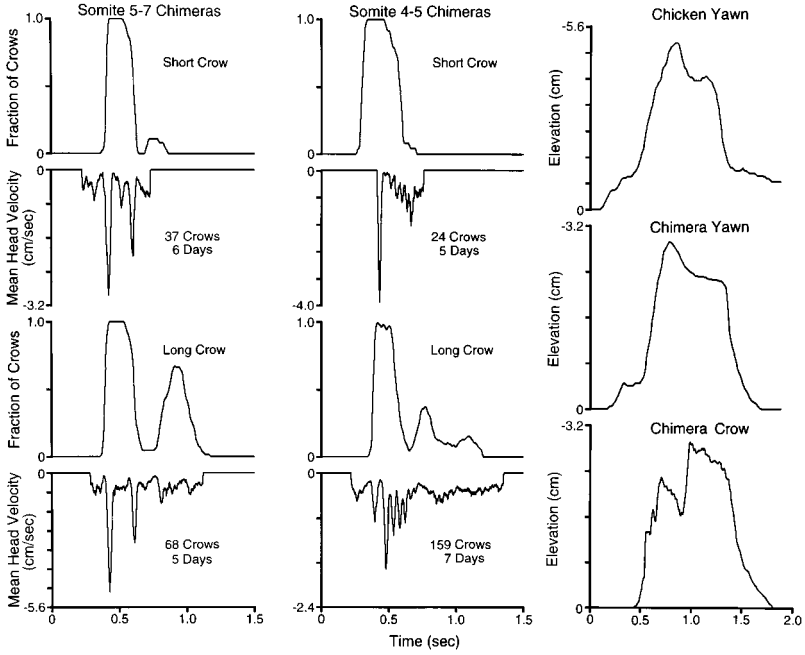

FIG. 2. The relationship between head movement and sound production in caudal brainstem chimeras. (*Left* and *Center*) The correlation between crow length and head movement in caudal brainstem chimeras. Crow length was defined by the length of time from the start of sound energy until 90% of all sound energy had been accumulated; head movement duration was defined by the duration of mean head velocity changes greater than those shown by normal chickens. For each type of caudal brainstem transplant, an animal with a shorter (*Top*) and longer (*Bottom*) crow are shown. A histogram of the proportion of all time-aligned crows in which sound energy occurred at each point in time (Fraction of Crows) and a profile representing mean head velocities greater than those shown by chickens from 500 ms before crow onset to 1000 ms after crow onset are shown. (*Right*) A comparison of the vertical head position profiles of a single chicken yawn (*Top*: compare with chicken crow in Fig. 1), a single caudal brainstem chimera yawn (*Middle*: transplant of somites 4–5), and a single crow (*Bottom*) given by the same chimeric animal on the same day as the yawn.

preamplified acoustic waveform and the x and y outputs from both video movement tracking systems were routed directly to an analog-to-digital converter [Data Translation, Burlington, MA, DT-2821G, 12-bit resolution, 40-kHz sampling rate; the audio signal was first low pass-filtered at 16 kHz using a Frequency Devices (Haverhill, MA) 901 12-pole Butterworth filter] and stored on a Gateway 486 or 586 computer using programs written in the SIGNAL programming language (Engineering Design, Belmont, MA). In some cases, data were stored on a TEAC (Montebello, CA) RD-180T PCM Data Recorder before computer storage. The head movement x and y coordinates from the two tracking systems were combined using a program written in SIGNAL that triangulated the position of the animal's head in three dimensions. The operation of the program was tested with several geometries of LED lamps a known distance apart and had resolution and accuracy both >0.5 mm. The system was calibrated at the beginning of each recording session. For this study, only the change in the vertical position (elevation) of the subject's head was used.

Experimental Procedures. Animal handling and experimental and killing procedures were in accordance with institutional guidelines. Within 6–12 h of hatching, chimeric and unoperated animals were implanted s.c. with either Silastic medical tubing capsules (Dow–Corning; 0.635-mm inner diameter, 1.19-mm outer diameter) packed with crystalline testosterone propionate (Sigma) or with sterile 5-mg slow release testosterone propionate pellets (Innovative Research of America) after receiving a topical application of 2% lidocaine on the skin overlying the area to be implanted. Animals were kept in mixed groups in a commercial gamebird brooder. For recordings of crowing and head movement, animals were removed singly from their brooder and treated as described above. At the end of a recording session, subjects were replaced in the brooder. Recordings were carried out for the first week posthatching to insure that none of the chimeras would begin to reject their grafted quail tissue (40, 41). At this time, animals were killed with an overdose of Metofane (Pitman–Moore Inc., Mundelein, IL) and were perfused transcardially with Carnoy's fixative. When the brain was completely fixed, it was dissected free along with the adjacent cervical spinal cord and processed for paraffin sectioning. Brains were cut into serial, transverse, 10-μm sections, mounted onto slides, and stained with cresyl violet to reveal the chick–quail cell marker (42). Statistical analyses were carried out on a Macintosh Quadra 630 computer using either the STATVIEW/SUPER ANOVA (Abacus Concepts, Berkeley, CA) or SYSTAT (Systat, Evanston, IL) statistical packages.

Acoustic Analysis. Crows and other vocalizations were analyzed using programs written in the SIGNAL programming language (Engineering Design). Sounds were turned into digital spectrograms (frequency resolution, 156 Hz; time resolution, 6.4 ms; time increment between successive fast Fourier transforms, 0.4 ms) (43) and the fundamental frequency–time contour was calculated by band-limited energy tracking (44). The fundamental frequency–time contour was used to derive amplitude–time contours for each harmonic of the fundamental, thus allowing a total synthetic reconstruction of the crow (ref. 44 and E. B. & Beeman, K., unpublished data). The fundamental frequency–time contours from each day of recording for each subject were time-aligned using cross-correlation, and the time-aligned frequency contours were summed and then divided by the number of crows to give a mean frequency–time contour for each subject on each day of recording. The average coefficients of variation for these contours were below 5% for each subject. To remove the effects of body size and maturation of the vocal tract, the average frequency of the contour for each day was measured and a grand mean was calculated for all recording days of each subject. The deviation of the average frequency of the contour for each day from this grand mean was multiplied by -1 and then added to the daily contours to bring their average frequencies to the same value.

Head Movement Analysis. The following manipulations were automatically performed by programs written in the SIGNAL programming language (Engineering Design). The instantaneous vertical velocity waveform for each crow within a subject was smoothed with an 8-ms time window and differentiated, and peaks in velocity were detected using zero-crossing. A plot of the location of the negative velocity peak of each oscillatory movement and its magnitude (difference between the magnitude of the preceding positive peak and the negative peak) was stored for each crow; these plots were then summed up for all of the crows after they had been aligned according to their acoustic features. This sum histogram was then divided by the histogram of the number of head movements at each position in time and smoothed with a 4-ms time window to make it continuous. The head movement plots of all seven chickens in which head movement was directly measured were aligned using cross-correlation and then each

corresponding point in time was compared in their plots; a separate plot was constructed containing the maxima at each point in time. The SD of this plot was calculated, and this value was added to each time point in the plot. This "chicken maximum + 1 SD" plot was used to determine when quail and chimeras had greater head movement than chickens by comparing the two plots aligned according to when the sound started. To be considered greater, the quail or chimera values had to exceed the chicken values for at least 40 ms. All quail and chimera plots were gated in this way to produce the waveforms shown in Figs. 2 and 3. These gated plots represent the changes in mean head velocity in the vertical direction greater than that shown by normal chickens.

RESULTS

Midbrain Transplants Change Species Crow Acoustics but Not Head Movement. Inspection of videotaped records of two quail donor, chicken host midbrain chimeras from a previous study (14) revealed that they moved their heads like chickens when crowing, without any visible vertical oscillations of the head (Table 1). These subjects gave crows with the quail temporal pattern but without the pattern of amplitude and frequency modulation characteristic of quail crows. In quail crows, the head movements are correlated with amplitude and frequency modulations in the acoustic signal (Fig. 1).

Head Movement Is Specifically Altered in Caudal Brainstem Chimeras. Two types of quail donor, chicken host transplants (both involving the caudal brainstem) produced animals that moved their heads differently than normal chickens during crowing (transplant of somites 5–7 and 4–5). Brainstem transplants immediately rostral to these, transplants involving the mesencephalon, and chicken–chicken transplants of the same regions all had no effect on head movement during yawning or crowing. Table 1 summarizes the subjects examined in this study.

The rest of this report examines the characteristics of the crowing and head movement of chimeras of somites 4–5 and 5–7 [one chimeric animal from each of these groups gave <10 crows over the course of recordings (both gave quail-like head bobs), and data from these two birds were not used in subsequent statistical analyses]. Fig. 1 illustrates simultaneous recordings of vertical head movement and sound production from an unoperated chicken, a caudal brainstem chimera, and an unoperated quail.

Head movements in chimeras of somites 4–5 and 5–7 had a specific relationship with sound production (Fig. 2). When considered as a group, caudal brainstem chimeras exhibited a significant positive correlation between the duration of their crows and the duration of their head movements ($r = 0.85$; $n = 11$; $P < 0.0005$). Chimeras of somites 4–5 and 5–7 did not differ in the lengths of the their crows [somites 5–7 ($n = 4$): 1199 ± 879 ms; somites 4–5 ($n = 7$): 1189 ± 780 ms] (Mann–Whitney U test: $U = 13$, $z = -0.189$, $P = 0.85$) or in the duration of their head movements [somites 5–7 ($n = 4$): 767 ± 179 ms; somites 4–5 ($n = 7$): 786 ± 271 ms] (Mann–Whitney U test: $U = 13$, $z = 0.189$, $P = 0.85$). Fig. 2 shows an example of an individual with a longer and a shorter crow from each group.

For all of these chimeric individuals, at least two yawns were recorded (and at least five were visually witnessed), and no yawn was ever seen to involve the quail-like vertical oscillations of the head seen during crowing (Fig. 2). Many instances of other calls in the normal vocal repertoire of chicks were also recorded from each animal (especially loud contact calls, contentment calls, and alarm calls to moving objects), and quail crow-like head bobbing was not seen for any of the other vocalizations. These observations suggest that, to trigger the quail-like head movement seen during crowing, the chimeric animal must be giving the crow vocalization.

The quail head movement pattern is characterized by the presence of two clear phases: an initial period of slower head velocity changes (5–15 Hz), whose exact position varies slightly from crow to crow, and a terminal period of faster head velocity changes (15–20 Hz) that tend to occupy a more precise temporal position from crow to crow (Fig. 3). Chimeras of somites 4–5 and 5–7 were classified with respect to the quail pattern based on the temporal patterning and frequency content of their head velocity profiles.

The patterns of chimera and quail head movements were compared in several ways. An ANOVA of the time interval between the start of the crow and the first appearance of the fast phase of head movement was conducted among quail, chimeras of somites 5–7, and chimeras of somites 4–5. There was significant variation among the three groups [quail ($n = 6$): 489 ± 86 ms; somites 5–7 ($n = 3$): 514 ± 40 ms; somites 4–5 ($n = 7$): 234 ± 154 ms ($F = 9.85$, $P = .0025$). Values of quail and of chimeras of somites 5–7 were not different from each other ($P > 0.7602$, Bonferroni test), and both of these groups showed longer time intervals than chimeras of somites 4–5 (both $P < 0.02$, Bonferroni tests). The relative rms amplitudes of the fast head bobs given by the chimeras of somites 4–5

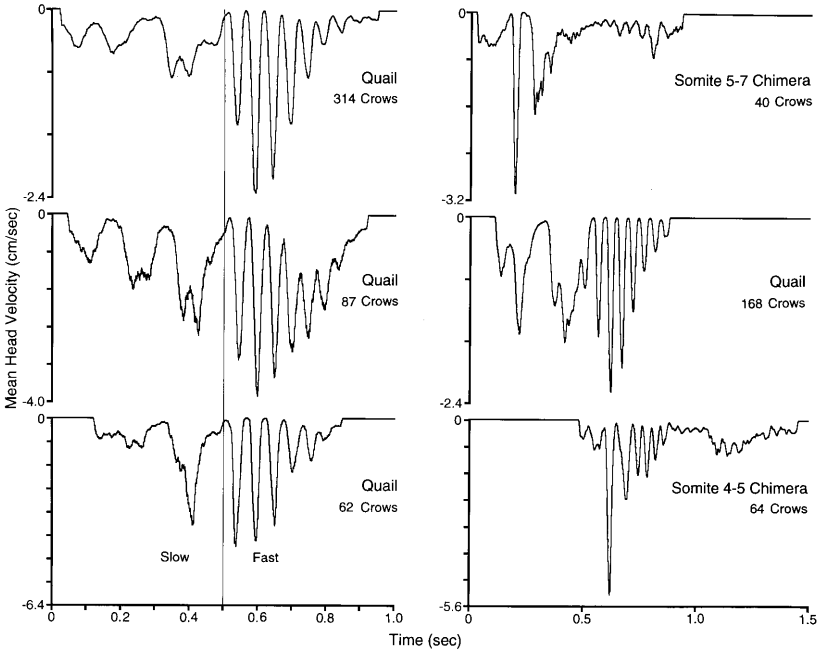

FIG. 3. Head movement patterning in quail and caudal brainstem chimeras. (*Left*) Profiles representing mean head velocities greater than those shown by chickens from 500 ms before crow onset to 1000 ms after crow onset are shown for three different individual quail. Note the general conservation of pattern from individual to individual and the two-part structure consisting of an initial slow phase (given during the first two short notes at the beginning of the crow) and a fast phase (given during the longer amplitude- and frequency-modulated trill at the end of the crow). (*Right*) Comparison of the morphology of head movement profiles in one chimera of somites 5–7 (*Top*), one quail (*Middle*), and one chimera of somites 4–5 (*Bottom*).

(0.288 ± 0.075) were greater than those shown by chimeras of somites 5–7 (0.110 ± 0.012) (Mann–Whitney U test: $U = 0$, z = −2.39, $P < 0.02$), but both were below quail values (0.392 ± 0.036) (Kruskal–Wallis test with post hoc tests: H = 10.38, $P < 0.01$; both post hoc comparisons of chimeras to quail, $P < 0.05$). The durations of the fast phases of quail and of chimeras of somites 4–5 and 5–7 did not show any significant statistical variation among groups (Kruskal–Wallis test: H = 2.31, $P = 0.33$). In all chimeras with longer crows, the head movements given late in the crow were of lower amplitude than those given early in the crow (Figs. 2 and 3).

Thus, both groups of caudal brainstem chimeras reliably reproduced normal aspects of different temporal portions of the quail head movement sequence. The more caudal (somites 5–7) chimeras started with slow head velocity components in the normal quail frequency range; individuals whose crows were long enough (three of the four) gave a degraded version of the quail fast phase at times that were no different than those seen in normal quail (Figs. 2 and 3). The more rostral (somites 4–5) transplant gave only the final fast phase part of the sequence.

Species Crow Acoustics Are Not Altered in Caudal Brainstem Chimeras. The acoustic morphology of the crows of all chimeras of somites 4–5 and 5–7 could in every case be matched up with those of normal chickens (Fig. 4). Chicks and caudal brainstem chimeras (transplants of somites 4–5 and 5–7 combined into one group) showed a significant positive correlation between crow duration and age in days since the start of incubation (chicks: r = 0.375, $n = 101$, $P < 0.0001$; chimeras: r = 0.251, $n = 61$, $P = 0.05$). A nested ANOVA showed that the chimeras had consistently shorter crows [day ($df = 6$), $F = 2.725$, $P = 0.0154$; chimera vs. normal chick nested within day ($df = 7$), $F = 3.816$, $P = 0.0008$]. Post hoc tests suggested that the crow durations for the first 3 days posthatching were not significantly different but that the chimera crow durations for the succeeding 4 days were shorter than their chicken counterparts. This difference may be due to the quail head movement of chimeras interrupting the sound production of their chicken-like crow. Many of the earliest crows that chimeras gave were interrupted for short periods (20–30 ms) irregularly during the movement of the head, and all exhibited a sharp drop-off in sound production in the initial part of their crows when the most intensive head movement was occurring throughout the recording period (Fig. 2).

Localization of Transplanted Material. Rostrocaudal migration of cells in the brainstem during development (44–48) made it difficult to delineate the exact extent of the transplants due to chick–quail cell mixing at transplant boundaries. Transplants of somites 4–5 had the largest concentration of quail cells contained in the medulla, beginning at levels slightly rostral to the start of nucleus XII and nucleus supraspinalis and extending caudally to a position about one–third of the way through each of these nuclei. This region corresponds to rostrocaudal coordinates P 2.4-P 3.6 in the stereotaxic chicken brain atlas of Kuenzel and Masson (49). Transplants of somites 5–7 had a scattering of cells in the rostral portions of these nuclei with the largest concentration at levels containing the main bodies of both nucleus XII and nucleus supraspinalis, at medullary levels P 3.2-P 4.2 (49). Previous work has shown several areas in this region that are thought to be important for breathing, vocalization, and head movement in birds (50–55). The transplants contained material from the entire circumference of the neural tube, so the quail cell composition of many structural areas covaried. More exact delineation of the area(s) responsible for the behavioral effect will require transplants with smaller rostrocaudal and dorsoventral extents.

DISCUSSION

The experiments reported here are not primarily concerned with elucidating the involvement of separate brain areas in the different, coordinated components of a single behavior. This is a well documented phenomenon for many behaviors, including bird song (55). The focus is rather on the localization of functional differences in the brains of these two species that affect the components of a complex, congenital behavior.

Brain regions that function the same way in these two species will not yield any behavioral effect when transplanted between them, regardless of whether their "output" affects one component or many components of a behavior. The chimera will still behave like a normal member of the host species. Transplantation will identify only those brain regions that function differently with regard to behavioral performance. Such functional differences could theoretically occur at any level of brain organization. The work reported here and previously (14), using transplants covering all areas of the brain, has found two regions that affect the species difference in crowing performance. The degree to which the functional differences in these regions influence many components or only a single component of this complex behavior is

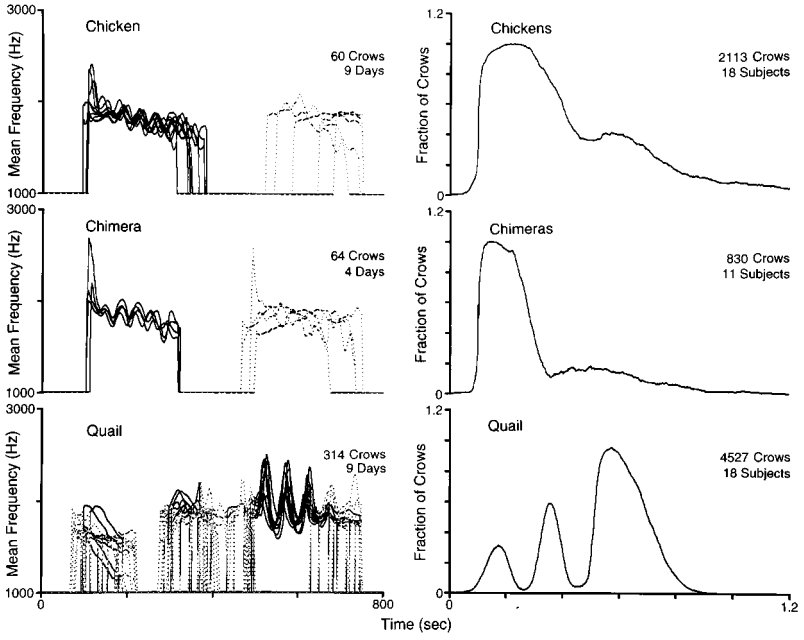

FIG. 4. Structural morphology and temporal patterning in the crowing sounds of chicks, caudal brainstem chimeras, and quail. (*Left*) Superimposed plot of daily mean frequency contours of one chicken, the chimera of somites 4–5 shown in Fig. 1, and one quail. Solid lines represent crow components that are present in >50% of the crows on each day; dotted lines represent crow components present in <50% of the crows on each day. Note the conservation of overall structure in the crows of the chicken and the chimera from day to day, as well as the strong resemblance between the crows of this particular chicken and chimera. Similar matches were found between the morphology of the crows of all other chimeras and normal chickens. There is structural conservation of the three major components of the quail call despite variation of when each component starts and ends from day to day. (*Right*) The temporal patterning of crowing in 18 chickens (*Top*), 11 caudal brainstem chimeras (*Middle*), and 18 quail (*Bottom*). These histograms were constructed by aligning the crowing sound histograms of the animals in each group using cross-correlation and summing the aligned curves for each group. Note the temporal morphology of the quail and the similarity in the basic shape of the chicken and chimera patterns despite the fact that the chimeras tend to have shorter crows.

of particular interest for understanding how evolution changes brains to change behavior. Although previous work in the fruit fly *Drosophila melanogaster* has separately examined the number of genes involved in interspecies reproductive isolation, including behavioral attributes (56, 57), and the anatomical localization of sex differences in mating behavior within a species using mosaic individuals (58–66), this is the first study to examine the functional localization of cell groups that confer species differences in the subcomponents of a single homologous behavior.

There are three particularly striking aspects of the results presented here. First, the fact that quail head movements were so well integrated into the chicken crowing performance is significant because it implies that the quail cells in the transplant had a well coordinated functional relationship with the other chicken parts of the brain that orchestrate crowing. The head movement may have a quail phenotype because the actual motor pattern is autonomously generated in the caudal brainstem and the quail cells there simply receive an activating signal from the chicken cells that communicate with them or because the motor pattern is generated by a more distributed group of cells and quail cells in the brainstem exert developmental effects on the functional phenotype of chicken cells in other parts of the brain.

A second aspect of interest stems from the fact that at least one of the brain regions affected by the transplants was the nucleus supraspinalis, a column of motor cells that innervate the major extrinsic neck muscles (67, 68) used in the generation of head movements. It is noteworthy that the chimeric animals only gave the quail head movement pattern when crowing, despite the fact that, when the head is moved during yawning and noncrowing vocalizations, animals presumably use some of the same quail motor cells to activate the neck musculature. The transplanted cells seem to function "normally" in several different modes in chickens just as they do in quail; whatever the signals are that decide whether these cells do or do not produce the quail head movement pattern on a particular occasion, the chicken host brain clearly has the capacity to generate them. Sound production and head movement may be independently produced, but they clearly interact. If the pattern of sound production is not well matched to the pattern of head movement, as in the caudal brainstem chimeras studied here, the interaction may be a disruptive one. It will be instructive to see what happens in "double" chimeras of the midbrain and brainstem, in which sound production and head movement patterns are well matched, particularly with regard to whether the head movements induce quail-like amplitude and frequency modulations in the sound.

The third aspect of interest is the change in the portion of the quail head movement pattern that one obtains in the chimeras with a change in the rostrocaudal position of the transplant. This implies that there is some underlying structure in the anatomy of the cell groups in the quail caudal brainstem that reliably generates different portions of the temporal head movement sequence at different rostrocaudal positions.

The results suggest that species differences in this complex behavior are produced by alterations in the phenotypes of different, regionally separated groups of cells in the brain that independently affect particular behavioral subcomponents. A simple model in which crowing differences are due to evolutionary changes in a single higher brain area is not tenable. Whether the quail cell differences that produce the behavioral change in the chimeras have effects that are autonomous to these lower brain areas or have a developmental impact on the phenotypes of chicken cells in higher brain regions will be addressed in future experiments.

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