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Rhythmic Spontaneous Activity in the Developing Avian Auditory System

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Microelectrode recordings of spontaneous multiple unit activity were made from nucleus magnocellularis (NM) and nucleus laminaris (NL), second- and third-order nuclei in the chick auditory system, between 14 and 19 d of incubation (E14–E19). Spontaneous firing in E14–E18 embryos occurred in synchronous bursts at periodic intervals. A rhythmic pattern of spontaneous firing was also observed in the auditory nerve but not in nonauditory regions of the brainstem. The mean interburst interval in NM and NL decreased from 4.9 sec at E14–E15 to 2.1 sec at E18. By E19, 2 d prior to hatching, synchronous bursting was replaced by an unpatterned, steady level of firing comparable to the background discharge that is present in NM and NL of hatchling birds. Bursting was not correlated with heart beat or respiration and was not affected by removal of the middle-ear ossicle. Rhythmic bursting could be reset, blocked, or induced by sound stimulation. Cochlea removal or pharmacological blockade of auditory nerve activity with TTX eliminated bursting. These results indicate that the synchrony and rhythmicity of impulse firing reflect normal physiological activity, most likely of cochlear origin.

The present findings show that spontaneous activity in the embryonic avian auditory system, like that in the immature mammalian visual pathway (Maffei and Galli-Resta, 1990; Meister et al., 1991), occurs in a synchronously rhythmic pattern. This similarity raises the possibility that such activity may be a general feature of early sensory system development. Patterned spontaneous firing in the chick takes place during a period of embryogenesis when auditory thresholds are high and when it is unlikely that physiological function *in ovo* is influenced significantly by normally occurring levels of airborne sound. Brainstem auditory neurons undergo substantial changes in structure and innervation during this same period. It is speculated that the temporal pattern of spontaneous discharge may provide cues that contribute to these developmental events.

[Key words: development, chicken, hearing, cochlear nucleus, auditory nerve, nucleus magnocellularis, nucleus laminaris]

Sensory neurons exhibit two components in the discharge of action potentials. Presentation of a suprathreshold stimulus alters the frequency or pattern of neural firing. In addition, many neurons discharge "spontaneously" in the absence of any intentional sensory stimulation. Studies of spontaneous activity in the auditory system indicate that the ongoing firing of first- and some second-order neurons likely results from a basal release of neurotransmitter at the hair cell–auditory nerve fiber synapse (Koerber et al., 1966; for references, see Guth et al., 1991).

Action potential discharge in afferent sensory fibers plays an important role in the development of the nervous system. Much of the evidence for this conclusion comes from the finding that manipulations that alter the amount or pattern of sensory input produce abnormalities in neural structure, electrophysiological response properties, and sensory-guided behavior (Hubel and Wiesel, 1965; Tees, 1976; Silverman and Clopton, 1977; Feng and Rogowski, 1980; Conlee and Parks, 1981; Sherman and Spear, 1982; Smith et al., 1983; Knudsen et al., 1984; Brugge et al., 1985; Meisami and Noushinfar, 1986). Such studies, in which afferent activity is altered by peripheral manipulations, primarily implicate evoked activity as contributing to neural development. However, recent experiments in which action potential discharge has been totally eliminated with the sodium channel blocker TTX show that spontaneous firing also plays an important role. For example, elimination of all retinal ganglion cell firing produces abnormalities in cortical ocular dominance columns and neuronal response properties that differ from those that occur following the selective elimination of evoked activity (Dubin et al., 1986; Stryker and Harris, 1986). In the visual system of the fetal kitten, where only spontaneously generated action potentials are likely to occur, blockade of retinal ganglion cell discharge prevents the normal segregation of retinogeniculate afferents from taking place (Shatz and Stryker, 1988). Several lines of evidence further suggest that the temporal correlation of firing among neurons, not merely the occurrence of spontaneous discharge per se, is a particularly important cue, especially in the formation of spatially ordered projections (Willshaw and von der Malsburg, 1976; Fawcett and O'Leary, 1985; Sanes and Constantine-Paton, 1985a; Stryker and Harris, 1986). The most compelling support for this comes from finding that altering the timing of impulse firing while still maintaining nor-

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mal levels of neural activity can produce differences in connectivity. This includes the regeneration of the retinotectal projection in goldfish exposed to stroboscopic illumination and the development of ocular dominance columns in kittens in which the timing of impulse activity is controlled by electrical stimulation of the optic nerves (Stryker and Strickland, 1984; Schmidt and Eisele, 1985; Cook and Rankin, 1986).

The idea that the temporal pattern of spontaneous activity plays an important role in brain development has led me to examine the characteristics of spontaneous firing in the brainstem auditory nuclei of the chick during the embryonic development of hearing. Nucleus magnocellularis (NM) and nucleus laminaris (NL), second- and third-order auditory nuclei, are topographically and tonotopically organized. The basilar papilla (avian cochlea) projects onto the ipsilateral NM via the auditory nerve. NM, in turn, sends a bilateral projection to NL. The anteromedial portions of NM and NL receive input from the proximal (high-frequency) end of the papilla and respond to high frequency sounds while more posterolateral regions of the nuclei receive input from successively more distal (low frequency) locations along the papilla and are tuned to progressively lower frequencies (Boord and Rasmussen, 1963; Boord, 1969; Parks and Rubel, 1975; Lippe and Rubel, 1985).

Chicks, like humans, are precocial with respect to the development of hearing, and most of the structural and functional development of the avian brainstem auditory system occurs *in ovo*. An adult number of cochlear hair cells is present by 10 d of incubation (E10) (Tilney et al., 1986; Katayama and Corwin, 1989). Functional synaptogenesis in NM and NL takes place between E11 and E13, approximately the same time that evoked responses to intense auditory stimulation can first be recorded from the brainstem (Saunders et al., 1973; Jackson et al., 1982). Embryos are first exposed to normal intensities of airborne sound on E19, when they penetrate into the airspace. By hatching on E21 auditory structure and function are adult-like in most respects (for review and references, see Rubel and Parks, 1988; Manley et al., 1991).

In the present experiment, microelectrode recordings of spontaneous multiple-unit activity were made in NM and NL between E14 and E19. The results show that the temporal pattern of spontaneous discharge changes during the embryonic development of hearing. Between E14 and E18 spontaneous firing occurs in synchronous bursts at periodic intervals. By E19 the rhythmic pattern of synchronous discharge is replaced by an unpatterned, relatively steady level of firing similar to the background discharge that occurs in NM and NL after hatching.

Materials and Methods

Subjects. White Leghorn and Hubbard × Hubbard chicken embryos between E14 and E19 were used. Embryos were obtained from fertile eggs that were purchased from a commercial supplier and incubated in the laboratory in a forced-draft incubator at 37.5°C and 50–60% humidity. Data on spontaneous firing in hatchlings were obtained from recordings that had been made from NM and NL of 1–2-week-old birds during two prior studies (Lippe and Rubel, 1985; Lippe, 1987).

Surgical preparation. The techniques for recording from embryos were similar to those described previously (Lippe and Rubel, 1985; Lippe, 1987). Embryos were exposed for recording by gently lifting their head out through a small hole that was made in the shell overlying the air space. The embryos were anesthetized with a single intramuscular injection of 4 mg of ketamine hydrochloride (Ketalar) and spontaneous movements that normally occur *in ovo* were eliminated with 1 mg of gallamine triethiodide (Flaxedil, i.m.). The tissue surrounding the right ear was dissected away so that the tympanic membrane could be clearly seen.

Embryos in which the effects of various experimental manipulations on spontaneous activity were to be examined underwent a prior surgical removal of the left basilar papilla. This ensured that any spontaneous discharges that might be recorded in the brainstem auditory nuclei following these manipulations (performed on the right ear) did not originate from activity generated in the left papilla. To remove the papilla, a large incision was first made in the left tympanic membrane. The columella was visualized through the incision, grasped with a jeweler's forceps, and removed. A glass pipette was then inserted through the oval window and the basilar papilla was removed by aspiration. Any bleeding was stopped by placing a small piece of Gelfoam into the oval window.

For recording, the egg and embryo were placed inside a humidified and temperature-controlled (38°C) Plexiglas chamber, and the embryo's head was supported by inserting its beak into a speculum filled with dental impression compound. The skull and dura overlying the cerebellum were removed with forceps. Embryos in which recordings were to be made from the auditory nerve underwent cerebellar aspiration. Removal of the cerebellum exposes the floor of the fourth ventricle and allows the fibers of the auditory nerve to be seen as they pass over NM on the dorsal surface of the medulla.

Heart rate was monitored throughout the experiment with Grass platinum subdermal electrodes that were inserted through two small holes made in the shell on either side of the embryo. Any arrythmia indicates that the physiological condition of the embryo is deteriorating. Data from embryos judged to be in poor physiological condition are not included in the quantitative analyses presented in Results.

Sound delivery and calibration. Sound was presented to the right ear through a brass tube sealed over the ear canal with stopcock grease. A Beyer DT48 headphone was coupled to the delivery tube and used for the presentation of sound. The sound pressure at the ear opening was measured with a calibrated probe tube inserted down the center of the delivery tube and connected to a Bruel and Kjaer 0.5-inch condenser microphone. The output of the microphone was led to a Hewlett Packard 3561A Dynamic Signal Analyzer for calibration of the system.

Pure tones were produced by a Wavetek function generator, amplified, and then passed through a Coulbourn electronic switch and a Hewlett Packard 350-D attenuator. Acoustic stimuli were either continuous tones or tone bursts (50–100 msec in duration, 5 msec rise/fall times).

Recording procedures. Multiple-unit activity was recorded with glass-insulated tungsten microelectrodes having impedances of 0.5–5.0 MΩ. Potentials from the electrode were amplified, band-pass filtered (0.5–10 kHz), viewed on an oscilloscope, monitored over a loudspeaker, and recorded on magnetic tape for off-line analysis. The amplified unit activity was also led to a window discriminator that generated standard size pulses for all action potentials whose amplitude exceeded the voltage level set on the discriminator. The pulses were fed to a computer that counted the number of spikes in sequential 100 msec duration time bins.

Physiological recordings were carried out inside a single- or double-walled sound-attenuated chamber (Industrial Acoustics Corp.). The electrode was lowered through the cerebellum on the right side of the brain while 90–100 dB SPL tone bursts were presented. Once the electrode had entered NM or NL, as indicated by a large increase in spontaneous firing and the appearance of sound-evoked activity, the movement of the electrode was stopped and recording was begun. After some experience the brainstem auditory nuclei can be located within two or three electrode penetrations by using surface landmarks. Recording began approximately 1 hr following the induction of anesthesia and typically lasted 2–7 hr.

Observations in normal embryos. Ongoing multiple unit activity was recorded from NM and NL in embryos between E14 and E19 ($n = 150$). These embryos did not undergo any experimental treatments. Recordings were made with the sound delivery tube sealed to the side of the head over the right ear canal but with audio amplifier turned off. In several of these experiments the effect of acoustic stimulation on the pattern of spontaneous firing was examined by presenting tone bursts or continuous tones ($n = 10$). In these studies the characteristic frequency of the largest-amplitude units at the recording site was estimated using audiovisual criteria as described previously (Lippe and Rubel, 1985). The pulses generated by the window discriminator for the largest amplitude units in the multiple unit activity were monitored on a loudspeaker and on the oscilloscope screen. The characteristic frequency was estimated by determining the frequency at which the lowest excitatory response threshold occurred.

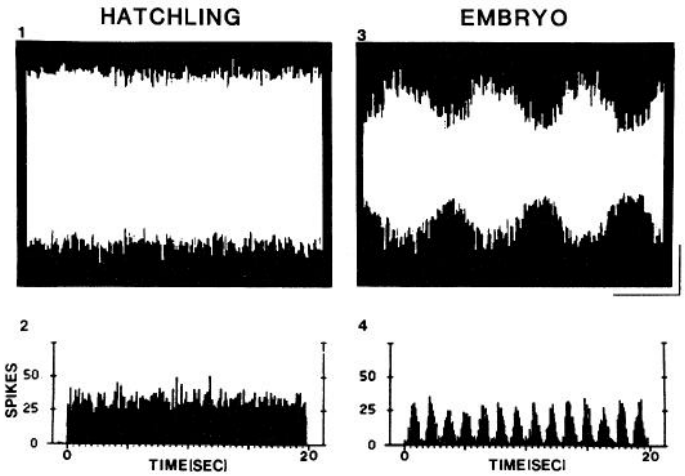


Figure 1. Spontaneous multiple-unit activity in NM of a 2-week-old chicken (*HATCHLING*) and an E18 embryo (*EMBRYO*). *Panels 1* and *3* are photographs of multiple-unit activity from the oscilloscope screen. *Panels 2* and *4* show counts of the number of spikes in sequential 100 msec time intervals during these recordings. The spike counts in this and all subsequent figures are for single sweeps of activity. The voltage level of the window discriminator was set so that only the largest amplitude units were counted. Because the same voltage level was not always used, absolute spike counts cannot be meaningfully compared between records. Calibration: 2 sec, 150 μV.

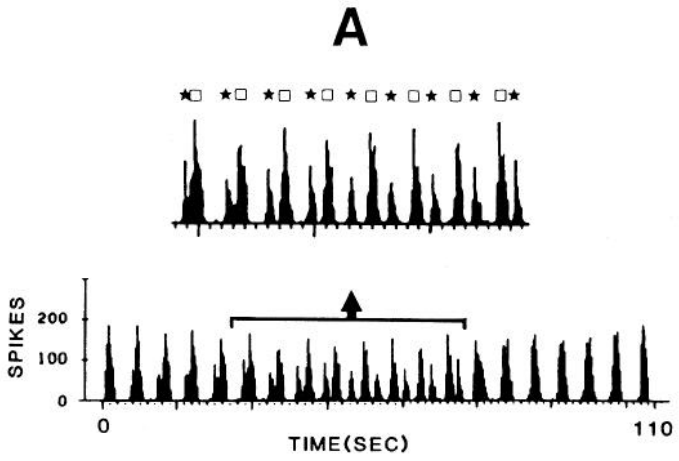
Recordings of ongoing multiple-unit activity were made from the auditory nerve in a separate group of E17 embryos ($n = 5$). Removal of the cerebellum in these embryos allowed the recording electrode to be visually placed on the auditory nerve where it passes over the dorsal surface of NM.

Columella and basilar papilla removal. E17 embryos were used. These embryos had previously undergone removal of the left basilar papilla. Ongoing multiple-unit activity was recorded from NM and NL on the right side of the brain prior to and following removal of the right columella ($n = 6$) or combined removal of the right columella and basilar papilla ($n = 5$). The surgical procedures were similar to those used on the left ear but were performed with the embryo supported inside the Plexiglas recording chamber and while the recording electrode was positioned in NM or NL.

Tetrodotoxin. E17 embryos in which the effect of columella removal had been examined were used (see above; $n = 5$). Following removal of the right columella, 1 μl of a 0.14 mM solution of tetrodotoxin (TTX) was placed onto the right oval window with a Hamilton syringe. Ongoing multiple-unit activity in the brainstem auditory nuclei on the right side of the brain was monitored prior to and for 3–12 hr following the application of TTX. A solution of 0.22 mM TTX was first prepared by dissolving lyophilized TTX with citrate (Sigma) in 0.9% sodium chloride. This was then diluted with 0.1% fast green in 0.9% sodium chloride to make a final working solution of 0.14 mM TTX. In three experiments, a control solution of 1 μl of vehicle (0.1% fast green in 0.9% sodium chloride) was placed on the oval window prior to application of TTX.

Histological procedures. At the termination of the experiment the embryo was killed with an overdose of sodium pentobarbital. In approximately half the experiments, small marking lesions were made by passing an 8 μA cathodal current for 10 sec through the tip of the recording electrode. The marking lesions were later used to reconstruct the electrode tracts and determine the recording sites. The brain was blocked *in situ* at the same angle as the electrode penetration and fixed by immersion in Bouins solution. All embryos were staged according to Hamburger and Hamilton (1951).

Ears in which the basilar papilla had been aspirated were dissected under a surgical microscope to verify the completeness of papilla removal. Brainstems that contained marking lesions were dissected from the brain, dehydrated, embedded in paraffin, and sectioned serially at 10 μm in the coronal plane. Sections were mounted on glass slides, stained with thionin, and examined under the light microscope to determine the location of the electrode tip.



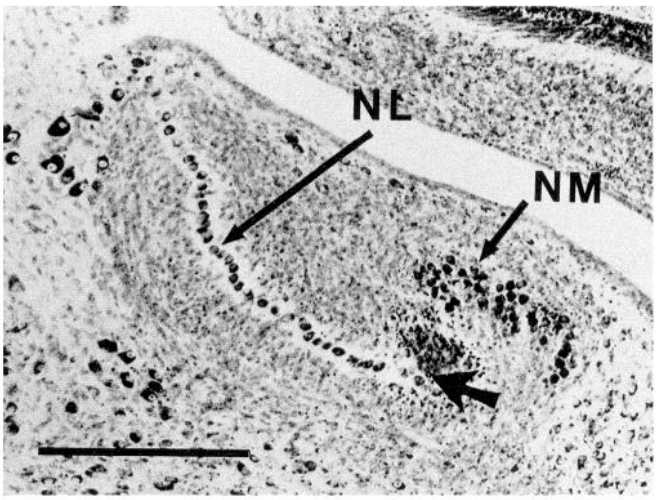


Figure 2. *A*, Two different rates of bursting activity recorded simultaneously from the same electrode. The spike count in the *lower panel* shows what appears to be a single burst gradually separating into two components that merge again. The *upper panel* shows a portion of the spike count on an expanded time scale. The two populations of units are indicated, respectively, by the *star* and *square*. The progressive shift in the temporal relationship between the two populations of units reflects a difference in their bursting rates. NL, E15. *B*, Photomicrograph of a transverse section through the brainstem showing the location in NL where the "dual" rhythm in *A* was recorded. The electrode tip was located near the medial border of NL at the ventral margin of the marking lesion (*arrow*). *NM*, nucleus magnocellularis; *NL*, nucleus laminaris. Scale bar, 250 μm.

Results

The results are based upon successful experiments in 166 embryos between E14 and E19, the majority of experiments being conducted in E16–E18 embryos. Embryos younger than E16 are difficult to handle and maintain in good physiological condition for long periods during recording. For this reason, only 10 E14–E15 embryos were successfully studied.

Spontaneous multiple-unit activity was present in NM and NL at all ages that were examined, including E14. This is 1–3 d after the onset of functional synaptogenesis in these regions (Jackson et al., 1982). Because no attempt was made to record

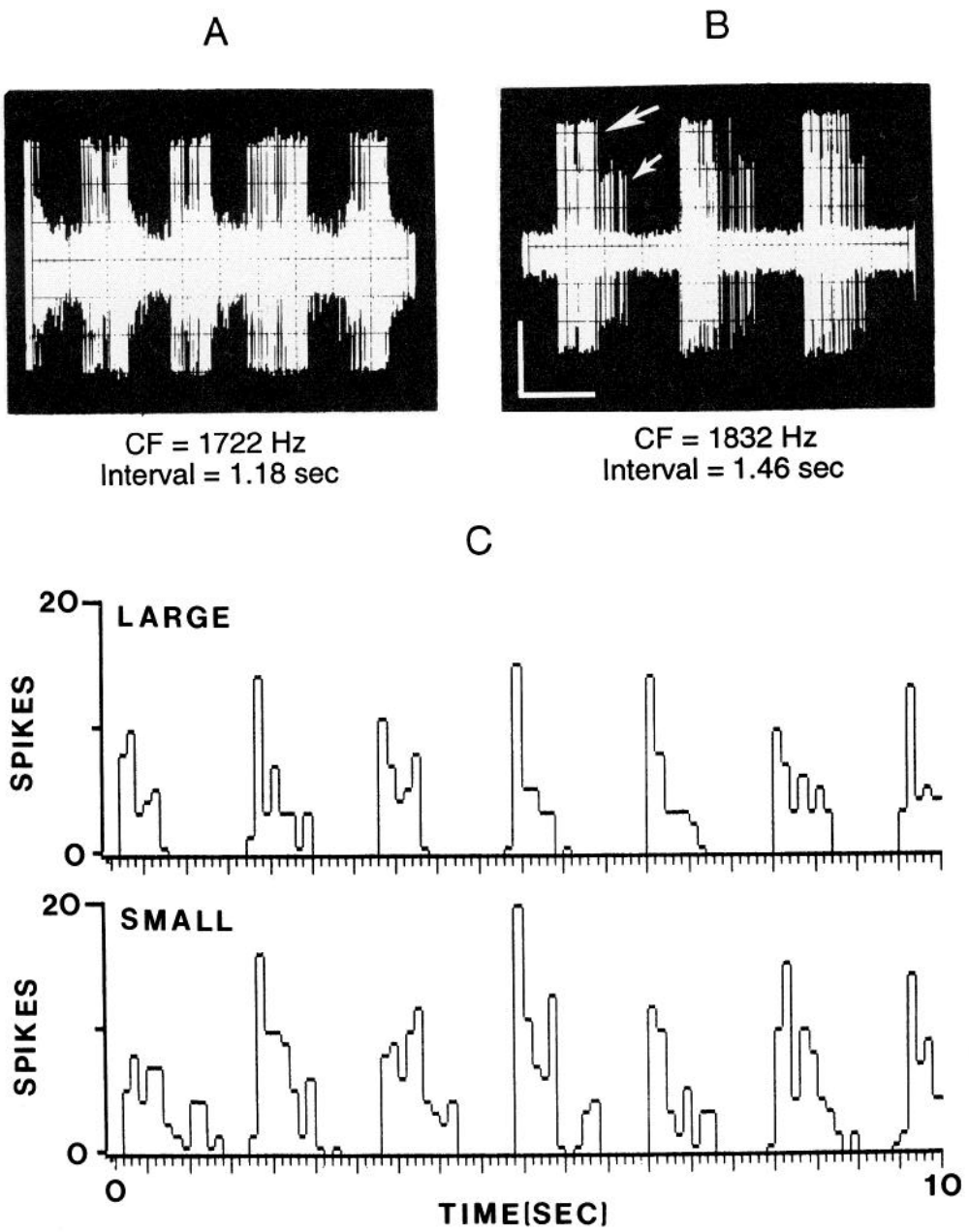


Figure 3. Spontaneous firing of single magnocellular neurons. *A*, A single unit discharges in periodic bursts. E18. *B*, Two single units, indicated by the *large* and *small arrows*, were recorded simultaneously by the same electrode. Each unit discharges in bursts at periodic intervals. The discharges of the two units overlap in time. E17. The characteristic frequency (*CF*) and mean interburst interval are indicated (for the larger-amplitude unit in *B*). Calibration: 1 sec, 250 μV. *C*, Spike counts of the two different amplitude units (*LARGE, SMALL*) shown in *B*.

from embryos younger than E14, it is not known if spontaneous firing occurs at earlier ages.

General characteristics of spontaneous activity. The temporal pattern of spontaneous multiple-unit discharge changed during development. Spontaneous firing in E19 embryos was similar to that in posthatch birds and occurred at a relatively steady level without any detectable temporal pattern. In contrast, between E14 and E18 spontaneous firing occurred in synchronous bursts at periodic intervals (Fig. 1). The transition at E19 from a rhythmic to a steady, adult-like pattern of discharge occurs before embryos have penetrated into the air space and begun pulmonary respiration.

During some recordings the pattern of periodic bursting occurred continuously. In others, the rhythmic pattern was interrupted for periods of approximately 1–8 sec when spontaneous firing continued at a relatively steady level but bursts were absent. Infrequently, what sounded like two separate populations of units with slightly different rates of bursting were heard over the loudspeaker. Although these dual rhythms were difficult to see in the traces of multiple-unit activity on the oscilloscope screen, they could be detected in the spike counts. An example from an E15 embryo is shown in Figure 2*A*. What appears to be a unitary burst of multiple-unit activity separates into two components. The temporal relationship between the two components shifts gradually over time until the components again merge into a single burst. Dual rhythms were seen only in embryos younger than approximately mid-E17. The anatomical locations from which dual rhythms were recorded were not studied systematically. However, in three experiments in which marking lesions were made following the recording of dual rhythms, the electrode tip was located in NL (Fig. 2*B*).

Rhythmic spontaneous firing was also recorded from the regions surrounding NM and NL, where the afferent and efferent fibers of these nuclei pass, and from nucleus angularis, another

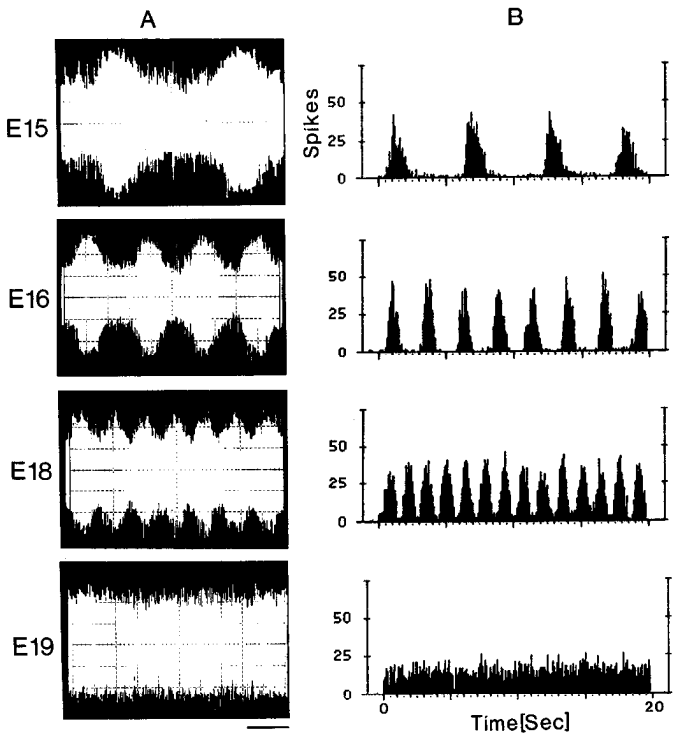


Figure 4. Spontaneous multiple-unit activity and corresponding spike counts from the brainstem auditory nuclei of E15, E16, E18, and E19 embryos. The intervals between bursts of activity become progressively shorter in older embryos. Because the traces were photographed with different vertical gains on the oscilloscope, the amplitude of the multiple-unit activity cannot be meaningfully compared across ages. Calibration, 2 sec.

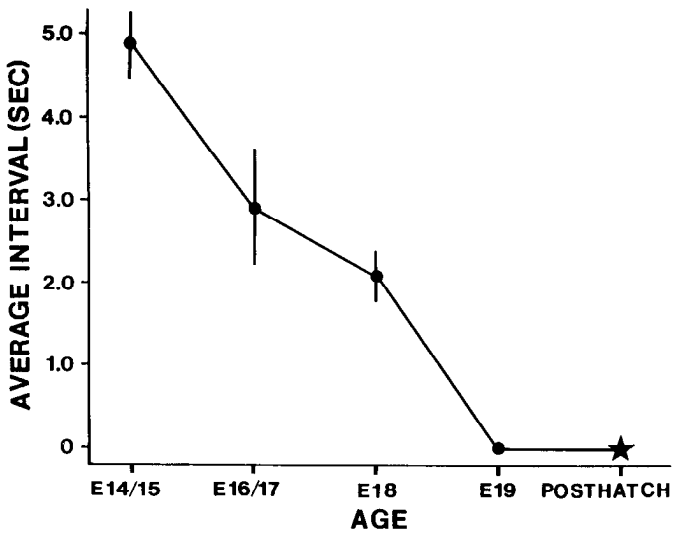


Figure 5. The mean interval between bursts of spontaneous activity as a function of age. The mean interburst interval in individual embryos was calculated from 2–3 min of spontaneous activity recorded continuously at a single location. The time bin containing the maximum number of counts during each burst of activity was identified, and the mean time interval between sequential bins was measured. Periods of time when bursting was absent were not included in the analysis. Number of embryos upon which each mean is based: E14/E15, $n = 6$; E16/17, $n = 12$; E18, $n = 10$; E19, $n = 12$. The data point for hatchlings is based on recordings of spontaneous multiple-unit activity made in 1–2-week-old chickens during two previous studies (Lippe and Rubel, 1985; Lippe, 1987). Error bars = SD.

second-order auditory nucleus that is innervated by the auditory nerve. Rhythmic bursting was never observed in the cerebellum or in nonauditory regions of the brainstem located anterior, posterior or ventral to NM and NL.

All E14–E18 embryos did not exhibit a rhythmic pattern of spontaneous activity. In some embryos spontaneous firing occurred at a steady level rather than in synchronous bursts. In others, rhythmicity was present at the beginning of recording. However, the interburst interval became progressively longer during the recording session until bursting no longer occurred and only a steady level of discharge remained. It is likely that the absence of bursting reflected a deteriorated physiological condition, since heart rates in these embryos were commonly abnormal, auditory thresholds were elevated, and the embryo frequently died shortly following the disappearance of bursting activity.

Single units. Thirteen well-isolated individual units plus two pairs of units were recorded during the course of the multiple-unit experiments (Fig. 3). Of the 13 units, 11 discharged in bursts at periodic intervals while two units fired irregularly. The spontaneous firing of 10 of the rhythmic units was measured over an interval of 1–2 min. Overall discharge rates of individual units ranged from 3.3 to 49.9 spikes/sec (mean ± SD = 22.5 ± 15.6). Firing rates during the bursts considered alone ranged from 9.0 to 101 spikes/sec (mean ± SD = 36.6 ± 28.5). The individual bursts ranged from 0.54 to 2.12 sec in duration (mean ± SD = 1.18 ± 0.54). Some neurons were quiescent between bursts while others discharged intermittently during the interburst intervals.

The two pairs of simultaneously recorded units also discharged in bursts at periodic intervals. The alternating periods of firing and inactivity were highly coincident between the two units of each pair. An example of the temporal correlation in firing between two of the simultaneously recorded units is shown in Figure 3, *B* and *C*. Comparison of the spike counts shows that the firing of the two units overlapped in time, with the mean duration of burst discharges being longer for the smaller amplitude unit (975 vs 535 msec). These observations on the spontaneous firing of single units indicate that the temporal pattern of multiple-unit activity reflects the synchronous discharge of many nearby neurons, each of which fires in bursts at periodic intervals.

Change in rate of rhythmic bursting. It was apparent both from listening to the multiple-unit activity over the loudspeaker and from viewing the activity on the oscilloscope that the rate of rhythmic bursting increases during development (Fig. 4). To quantify this, the mean interval between bursts of spontaneous activity was measured in 6–12 individual embryos at different ages. Figure 5 shows that the mean interburst interval decreased from 4.9 sec at E14–E15 (burst frequency = 0.20 Hz) to 2.1 sec by E18 (burst frequency = 0.48 Hz).

Manipulations: columella removal, cochlea removal, and TTX-induced blockade of auditory nerve activity. These manipulations were performed to confirm that bursting activity reflects the discharge of auditory neurons and is and not an artifact of either the recording methods or uncontrolled background sound and to determine if bursting is generated within the cochlea. All these experiments were performed in E16–E18 embryos that had previously undergone removal of the basilar papilla from the left ear.

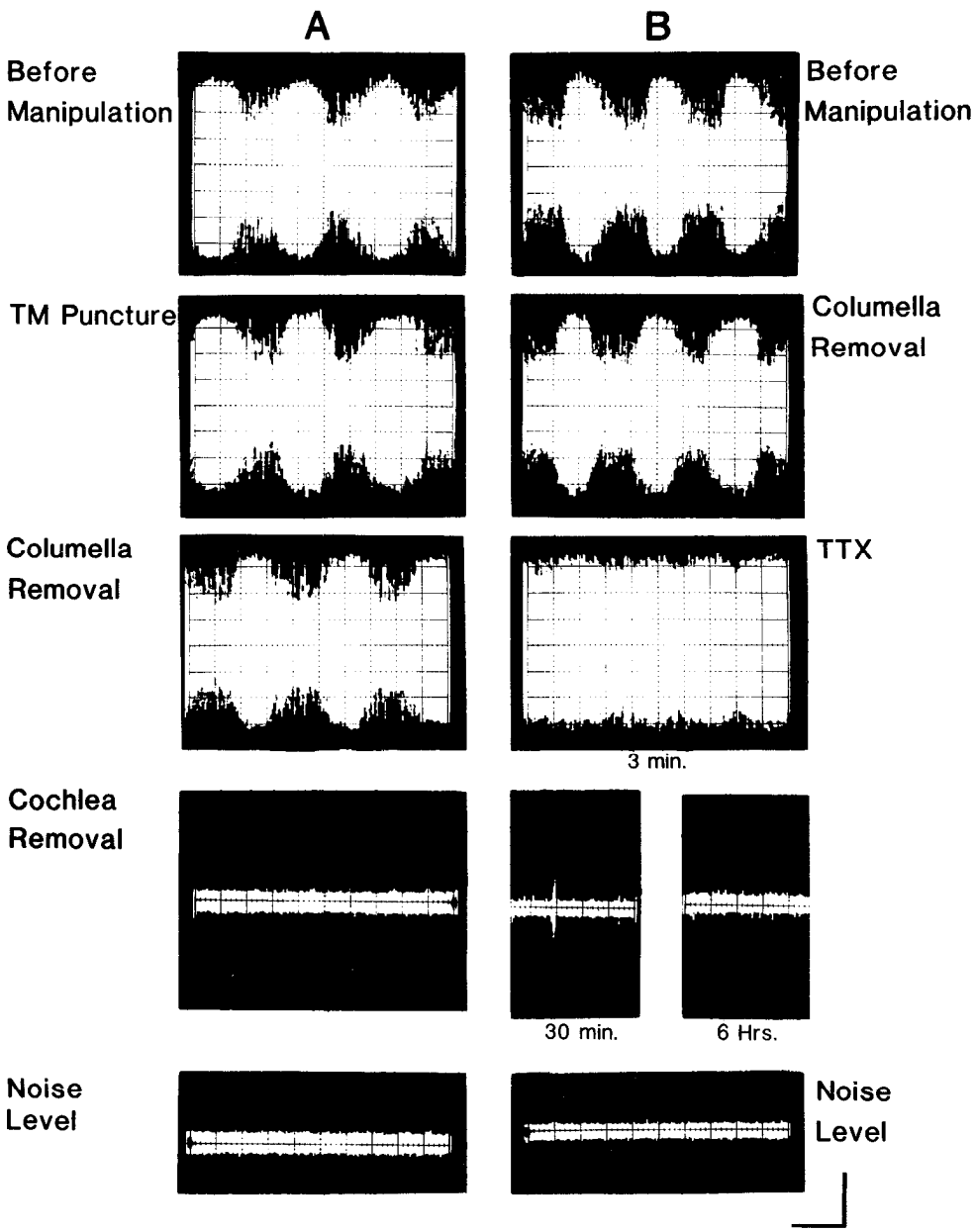
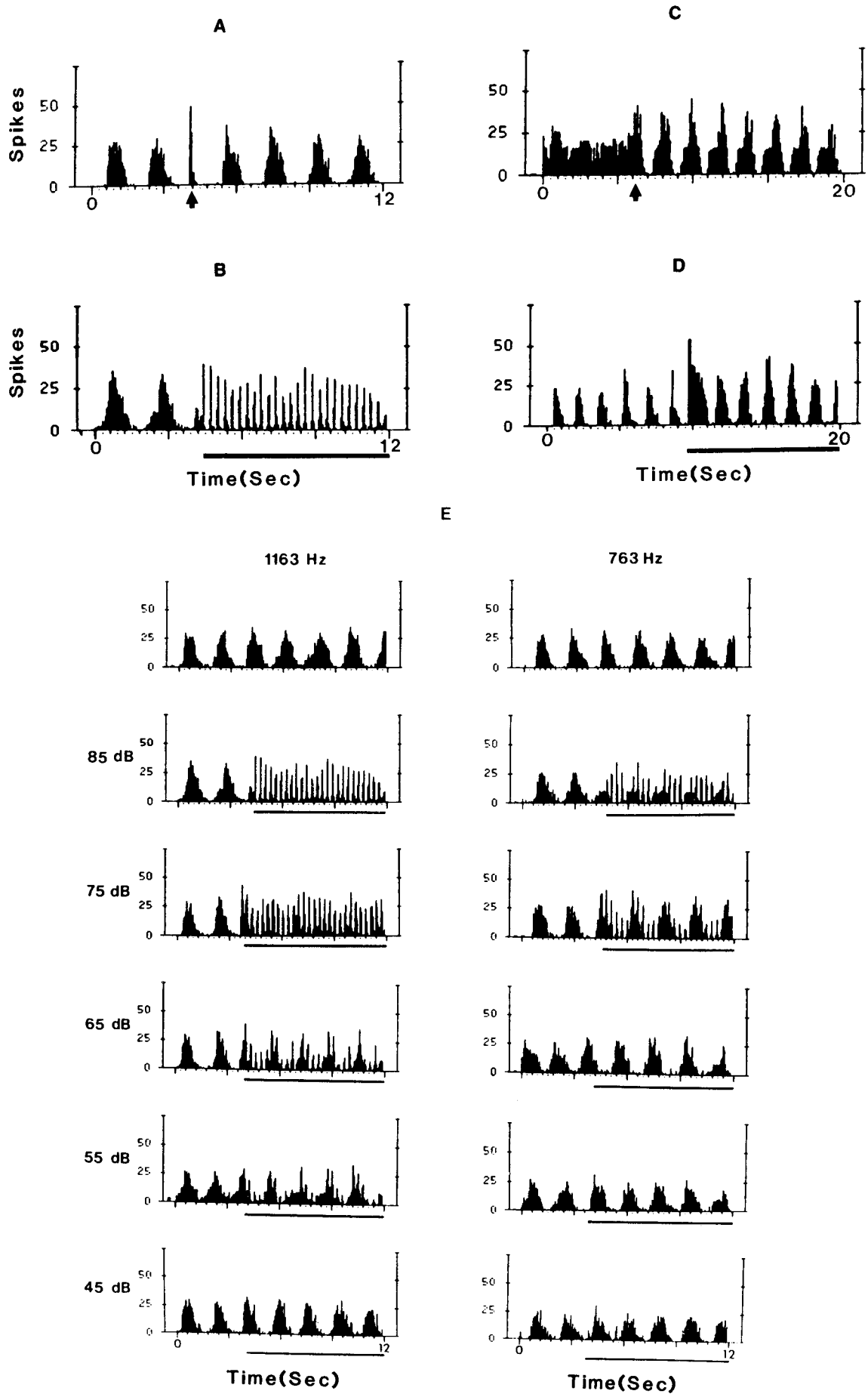


Figure 6. Effect of tympanic membrane puncture (*TM*), columella removal, cochlea removal, and TTX on rhythmic spontaneous multiple-unit activity in two E17 embryos (*A, B*). The manipulations on each embryo were performed in the order shown in the respective columns. The *right column* shows spontaneous activity at 3 min, 30 min, and 6 hr following application of TTX to the oval window. The *lower panels* show the electrical activity that was recorded in the dead embryo. This reflects the electrical noise level of the recording system. Calibration: 100 μm, 2 sec.

Figure 6 shows representative records from two E17 embryos, one in which columella removal was followed by aspiration of the basilar papilla (*A*), and the other in which columella removal was followed by application of TTX to the oval window (*B*). Neither puncture of the tympanic membrane (labeled TM) nor removal of the columella had any detectable effect upon rhythmic bursting. These manipulations sometimes caused small changes in the overall amplitude of multiple-unit activity, most likely because of small displacements in the position of recording electrode. However, rhythmic bursting always continued, indicating that rhythmicity does not result from stimulation by uncontrolled background sound or contraction of the middle-ear muscle.

Both cochlea removal and application of TTX to the oval window abolished rhythmic bursting and decreased the amplitude of multiple-unit activity to the noise level of the recording system. Application of a control solution to the oval window had no effect. These results are similar to findings in hatchlings that cochlea removal and TTX-induced blockade of auditory nerve activity abolish spontaneous firing in NM (Born and Rubel, 1988; Born et al., 1991). In the present study, rhythmicity and spontaneous firing were eliminated almost immediately following basilar papilla removal and gradually, over a period of 2–5 min, following TTX. Following the application of TTX, bursting was always abolished before the overall amplitude of spontaneous activity decreased significantly. Spontaneous firing never returned within the 2–14 hr period that brainstem activity was monitored following the application of TTX. The elimination of bursting following both TTX and cochlear removal indicates that bursting activity reflects the discharge of auditory neurons and supports the idea that rhythmicity and synchrony are generated within the cochlea.

Acoustic stimulation. The effect of sound stimulation on the rhythmic pattern of spontaneous multiple-unit activity was ex-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amined in 10 E17–E18 embryos. Sound stimulation altered the rhythmicity of spontaneous discharge in several predictable ways (Fig. 7*A–D*). Presentation of a single tone burst during a burst of spontaneous activity blocked the remainder of the burst of activity and reset the rhythm. Rhythmic bursting was completely or partially blocked during a train of tone bursts. Sound stimulation could also trigger the occurrence of bursting. Presentation of a tone burst during a period when bursting was temporally absent caused the rhythmic pattern to reappear. Furthermore, in some embryos in which rhythmicity was otherwise absent, a high-intensity sound sometimes induced a brief period of periodic bursting. All these effects of acoustic stimulation varied systematically as a function of intensity and frequency, being greater at higher intensities and at the characteristic frequency of the recorded neurons (Fig. 7*E*).

Auditory nerve. The portion of the auditory nerve that passes over NM on the dorsal surface of the brainstem was exposed for recording by aspirating the cerebellum. Embryos do not tolerate cerebellar removal well because the large amount of bleeding that results is difficult to control at these early ages. Spontaneous multiple-unit activity was present in the auditory nerves of the five embryos studied. In two embryos, spontaneous activity in the nerve as well as in the underlying brainstem nuclei occurred at a steady level and bursting was absent. The absence of bursting may have been due to trauma caused by the cerebellar removal. Spontaneous firing in the auditory nerves of the three other embryos occurred in a rhythmic pattern similar to that observed in NM and NL. The frequency of auditory nerve bursting in these three embryos ranged from 0.20 to 0.38 Hz, values that fall within the range of brainstem rhythmic rates at the same stage of development.

Discussion

The results show that spontaneous activity is present in NM and NL of chickens as early as E14, 1–3 d after the onset of functional synaptogenesis in these nuclei (Jackson et al., 1982). It is currently not known if spontaneous activity is present at younger ages. Spontaneous firing in E14–E15 embryos occurs in synchronous bursts at periodic intervals. The interval between bursts decreases progressively during development until E19, when rhythmic bursting is replaced by an unpatterned, steady level of discharge similar to the ongoing firing that occurs in the brainstem nuclei of hatchling chicks. Recordings from individual neurons indicate that the temporal pattern of multiple-unit activity reflects the synchronous firing of many neighboring units, each of which discharges in bursts at periodic intervals. The present data provide no information about the number of NM and NL neurons that discharge spontaneously in periodic bursts. It is also not known how the relative synchrony of firing between neurons varies as a function of intercellular distance. This is of particular interest because of several lines of evidence that suggest that spatiotemporal correlations in neuronal discharge contribute to the formation of spatially segregated and topographically ordered projections (for reviews and references, see Fawcett and O'Leary, 1985; Schmidt and Tieman, 1985; Frank, 1987; Constantine-Paton et al., 1990; Shatz, 1990).

The present results are similar to findings in the mammalian visual pathway that electrical activity in the form of synchronous spontaneous activity is generated prenatally, before visual input is capable of influencing physiological function (Maffei and Galli-Resta, 1990; Meister et al., 1991). Although physiological responses to acoustic input in birds occur initially on E11–E12 of incubation, auditory thresholds at this time are very high (110–130 dB SPL) and remain elevated until just a few days before hatching (E19), when the embryo penetrates into the airspace and is first exposed to normal intensities of airborne sound (Saunders et al., 1973; Rebillard and Rubel, 1981). Thus, it seems likely that throughout most of embryonic development neural activity in the avian brainstem auditory pathway is dominated by correlated spontaneous action potential firing. The occurrence of synchronous spontaneous firing in both the immature avian auditory and mammalian visual pathways raises the possibility that such activity may be a general feature of early sensory system development.

Mechanism of generation. Several observations indicate that the rhythmicity and synchrony of firing result from normal physiological processes and are not an artifact of uncontrolled background sound, movement or an abnormal physiological condition. Stimulation by ambient sound is unlikely to be responsible since auditory thresholds in E14–E17 embryos are 30–70 dB higher than adult values (Saunders et al., 1973; Rebillard and Rubel, 1981). In addition, columella removal, which produces a 40 dB conductive hearing loss in hatchling birds, does not affect the temporal pattern of firing. The absence of an effect of columella removal also indicates that periodic bursts are not produced by contractions of the middle-ear muscle. Movements or brain pulsations that might result from respiration or heart beat also do not play a role. Pulmonary respiration in the chick does not begin until E19–E20, by which time rhythmic bursts no longer occur. The embryonic heart rate (200–300 beats/min) is considerably faster than the rate of rhythmic bursting, and simultaneous recordings of neural and cardiac activity show that bursts of spontaneous activity and heartbeats are not correlated (data not presented). Finally, it is unlikely that rhythmic bursting results from an abnormal physiological state. Spontaneous firing occurs in periodic bursts when heart rate and auditory thresholds are normal, but bursting disappears when the condition of the embryo deteriorates, as indicated by arrhythmias in heartbeat and elevations in auditory thresholds.

The present findings provide a few clues regarding the location of the rhythm generating mechanism(s). The observation that the synchronous bursting can be induced and reset by auditory stimulation implies that the temporal pattern is produced by

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Figure 7. Effects of acoustic stimulation on the temporal pattern of spontaneous multiple-unit activity. Presentation of sound is indicated by the *arrows* and *bars* beneath the spike counts. Spike counts are for a single repetition. The frequency of the tonal stimuli in *A–D* was at the characteristic frequency of the largest amplitude units in the recordings (see Materials and Methods). *A*, Single tone burst blocks the remainder of the burst of spontaneous activity and resets the rhythm. Stimulus: 1162 Hz, 50 msec, 85 dB. E17. *B*, A train of tone bursts evokes responses and blocks the bursts of spontaneous firing. Stimulus: 1200 Hz, 50 msec, 3.5 tone bursts/sec, 85 dB. E17. *C*, A single tone burst presented when rhythmic bursting was temporarily absent evokes a response and causes rhythmic bursting to reappear. Stimulus: 1440 Hz, 100 msec, 80 dB. E18. *D*, The rhythmic pattern of spontaneous firing is maintained during a long duration tone. Stimulus: 1160 Hz, 85 dB. E17. *E*, The blocking effect of a train of tone bursts on rhythmic bursting varies as a function of stimulus intensity and frequency. The largest-amplitude units in the multiple-unit activity were tuned to 1163 Hz. Stimulus: 3.5 tone bursts/sec. E17.

processes within the auditory pathway and not by the modulation of auditory neurons by nonauditory regions of the brain. The present results do not exclude the possibility that processes intrinsic to the brainstem nuclei may contribute to or, conceivably, be responsible for the rhythmicity and synchrony of discharge. For example, a tonic excitatory input to magnocellular neurons could trigger an intrinsic electrical autorhythmicity. However, the finding that the auditory nerve also discharges spontaneously in synchronous bursts suggests that rhythmicity and synchrony originate peripherally, most probably within the cochlea.

Comparison with mammals. Spontaneous firing in several different regions of the mammalian brain occurs in synchronous bursts during pre- as well as postnatal development. (Huttenlocher, 1967; Rapisardi et al., 1975; Corner and Mirmiran, 1990; Maffei and Galli-Resta, 1990; Meister et al., 1991). Auditory neurons in developing kittens respond to sound stimulation in periodic bursts (Pujol, 1972; Carlier et al., 1975; Walsh and McGee, 1988). However, spontaneous discharge rates of immature mammalian auditory neurons are very low. To my knowledge, periodicities or synchrony in spontaneous firing have not been observed (Romand and Marty, 1975; Brugge et al., 1978; Woolf and Ryan, 1985; Walsh and McGee, 1987).

It is possible that the mechanisms generating the rhythmicity and synchrony of spontaneous firing in avian embryos are not present in mammals. Although avian and mammalian cochleas exhibit many structural and functional parallels, several differences do exist, including the pattern of efferent innervation, hair cell–tectorial membrane coupling and mechanisms of tuning (Rebillard and Pujol, 1983; Fuchs et al., 1988; Manley et al., 1988). Alternatively, the failure to date to detect patterned spontaneous firing in mammals may reflect the recording conditions. Electrophysiological studies of auditory system development in mammals have typically been conducted after the onset of hearing and using barbiturate anesthesia. It is quite possible that neurons in the developing mammalian auditory system discharge spontaneously in synchronous bursts, but only at relatively early stages of development. Barbiturates may selectively suppress "bursty" spontaneous firing of auditory neurons and can greatly decrease the frequency of spontaneous discharge (Bock and Webster, 1974; Anastasio et al., 1985). Low levels of neural discharge might make any periodicity or synchrony in ongoing firing difficult to detect, especially when recording from individual units rather than groups of neurons. Given these considerations, it will be important to examine the characteristics of spontaneous activity in the developing mammalian auditory system using a nonbarbiturate anesthesia, particularly at times prior to the onset of hearing.

Functional importance. Presently, one can only speculate regarding what, if any, role the temporal pattern of spontaneous firing might play in the developing avian auditory system. Some developmental events such as the formation of specialized dendritic organization occur normally in the absence of cochlear afferents and presumably do not involve presynaptic activity (Parks and Jackson, 1984; Parks et al., 1987). However, other processes can be considered. For example, spontaneous firing contributes to the growth and survival of auditory neurons in hatchlings and, most likely, in embryos as well (Levi-Montalcini, 1949; Parks, 1979; Parks and Jackson, 1984; Tucci and Rubel, 1985; Parks et al., 1987). Given that immature sensory neurons are difficult to drive synaptically (e.g., Sanes and Constantine-Paton, 1985b), it is possible that the intermittent, synchronous pattern of spontaneous discharge would be particularly effective in activating auditory neurons during embryogenesis and ensuring that neuronal activity necessary for normal development is transmitted throughout the auditory pathway.

An alternative possibility is that the temporal pattern of spontaneous firing may play an instructive role in the formation of specific synaptic connections. Temporal correlations in neural firing provide cues that contribute to the refinement of spatially ordered projections in the developing mammalian visual pathway (for reviews and references, see Fawcett and O'Leary, 1985; Schmidt and Tieman, 1985; Frank, 1987; Constantine-Paton et al., 1990; Shatz, 1990). Synchrony in the spontaneous discharge of visual neurons may be particularly important during periods of fetal or early postnatal development when physiological functioning is not affected by sensory input (Shatz and Stryker, 1988; Meister et al., 1991). It is possible that similar mechanisms might also operate during the embryogenesis of the avian auditory system and contribute to processes such as the selection and alignment of auditory projections (Jackson and Parks, 1982; Jhaveri and Morest, 1982; Young and Rubel, 1986).

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