wild-type *Drosophila* and the mutant. Therefore, it cannot be attributed to the effect of the mutation.
15. For quantitative verification of this statement, autocovariance functions of the bump noise were calculated for both the wild type and the mutant, $norpA^{H52}$. The autocovariance function $C(\tau)$ gives a picture of how the signal correlates to its future (or past) at an interval τ. If the occurrence of bumps is totally uncorrelated, the autocovariance function would describe the time course of individual bumps. In the above calculation, the autocovariance functions of bump noise for the wild type and the mutant were found to have very similar dependence on τ, suggesting that the time course of individual bumps is similar.
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Prenatal Experience and Avian Development: Brief Auditory Stimulation Accelerates the Hatching of Japanese Quail

Abstract. *A single 2-hour exposure to auditory stimulation at any point during the final 3 days of incubation accelerates the hatching of Japanese quail. The 3-day sensitive period includes both prenatal and perinatal stages of incubation. So far as is known these results provide the first unequivocal evidence that short-term prenatal sensory stimulation can affect the development of an avian embryo.*

Brief (*1*) periods of sensory experience can profoundly affect a developing organism. The evidence for this in vertebrates has been limited to studies of the visual system after birth (*2*). We now report that brief stimulation of the auditory system can also affect vertebrate development. Moreover, this sensitivity exists during the prenatal period of development. Our results indicate that brief prenatal exposure of an avian embryo to auditory stimulation can accelerate the time of hatching, reducing the normal incubation time by as much as 10 percent.

Many species of precocial ground-nesting birds brood egg clutches whose eggs hatch within a short time of one another (*3*). This synchronization of hatching is of putative adaptive advantage in that it reduces the period during which the parents must choose between care for unhatched eggs and newly hatched young. That is, during incubation the eggs must be maintained within a narrow range of environmental conditions (*4*). The behavior required of the parents to provide these incubation conditions is in conflict with the behavior required later for the care of the highly mobile, precocial brood. After the eggs hatch the parents must utilize new tactics to protect the young from predators and must introduce the young to available food resources before their embryonic reserves are exhausted. The strategy of synchronization of hatching allows the most efficient division of the demands on the parents for care of the young before and after hatching.

Synchronization of hatching is mediated by acoustic communication between the individual members within a clutch. Audible clicks, which are correlated with the embryo's respiratory cycle (*5*), provide the basis for the inter-egg communication (*6*). Acoustic presentation of synthetic clicks (such as brief, broad-band bursts of noise) has shown that the hatching of embryos following acoustic stimulation is accelerated or retarded depending on the click repetition rate (*7*), the duration of the stimulating period, and gestational age of the embryo.

Under normal incubation conditions, Japanese quail (*Coturnix coturnix japonica*) hatch on day 17 of incubation. Continuous exposure during the final 2 days of incubation to synthetic clicks at stimulation rates of approximately three per second optimally advances the hatching of Japanese quail, while rates above or below the optimum produce less significant hatching advancement or even hatching retardation, as compared to unstimulated controls (*7*).

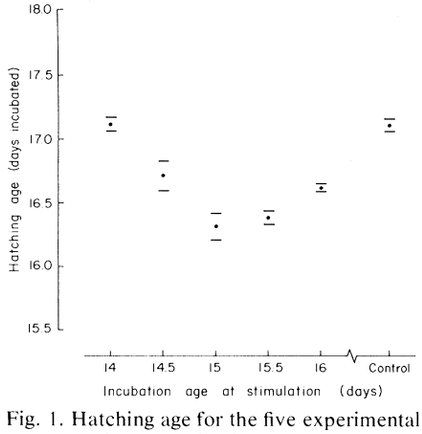

Fig. 1. Hatching age for the five experimental groups and the unstimulated control group. Experimental groups were exposed to auditory stimulation for 2 hours at the incubation age indicated (*9*). Means and standard errors are shown. Sample sizes for experimental groups days 14 through 16 and controls are 35, 46, 41, 39, 39, and 96, respectively.

We first studied the developmental stages at which exposure of the embryo to synthetic clicks at the optimal repetition rate would accelerate the hatching of Japanese quail, and found that the hatching time could be advanced only if the embryos were stimulated during the final 3 days of incubation. We then systematically examined the effects of stimulation during this interval and report here that very brief exposures to synthetic clicks at any point within this 3-day sensitive period significantly advance the hatching time.

Japanese quail eggs (Cornell University, Department of Poultry Science) were incubated in Jamesway forced-draft incubators (37.5°C, 60 percent relative humidity), in which the eggs were turned automatically every 3 hours (*8*). Before day 14 the eggs were checked for viability (candled), and the viable embryos were randomly grouped into clutches of six eggs. The eggs within a clutch were formed into a 2 by 3 array, with each egg in contact with its nearest neighbors.

Experimental clutches were stimulated for 2 hours at 14, 14.5, 15, 15.5, or 16 days of incubation (*9*). In the procedure for sound presentation, the tray containing the clutch was rapidly transferred from the control incubator to a second incubator that was equipped with a KLH model 11 loudspeaker. The temperature and relative humidity of both incubators were identical. The speaker was centered 12.5 cm above the clutch and the stimuli were presented at 80-db sound pressure level peak (General Radio model 33 sound level meter) at the optimal rate of three per second (*7*). The stimuli were 37-msec, broad-band (0.1 to 8 khz) bursts of noise generated by a unijunction relaxation oscillator, whose resistance-capacitance values were chosen to maximize the spectral flatness of the speaker output. Control clutches were treated identically but not exposed to the 2-hour stimulation period.

After 16 days of incubation the eggs were placed on an automated device that recorded the individual hatching times. Each egg was placed on a separate balanced spoon that held down a microswitch. When the animal hatched, the mi-

croswitch was released and the time was recorded by an event recorder. The eggs were separated by at least 8.5 cm to reduce acoustic communication between neighboring eggs during this final period of incubation.

Exposure to 2 hours of auditory stimulation at any point after day 14 of incubation shortened the total incubation period required for hatching (Fig. 1). Paired comparisons (t-tests) between the experimental and control groups showed that only the day-14 comparison was nonsignificant, while all of the others were highly significant ($P < .001$).

The U-shaped distribution in Fig. 1 does not necessarily indicate a decline in the effectiveness of stimulation after 15 days of incubation. Instead, because the acceleration of hatching must occur during the interval between stimulation and the mean hatching time of the controls, the hatching time of the stimulated animals must approach that of the controls as the interval from stimulation to the mean hatching time of the controls approaches zero. However, concomitant with this tendency for the difference between experimental and control means to decrease with stimulation at successively later stages of incubation, there is a tendency for the relative acceleration of hatching to increase (Table 1). Therefore, rather than emphasize that the experimental group stimulated on day 15 required the minimum period of incubation, we conclude that the entire interval from day 14 to hatching constitutes a sensitive period, during which time brief bouts of acoustic stimulation can accelerate the Japanese quail embryo's rate of development. A more inclusive measure than hatching, one that examines specific substages of development, will be required before any of the experimental groups can be singled out as showing an exceptional effect.

Before pipping (first cracking of the shell), the embryo is isolated from the environment by a continuous shell, and consequently can be described as being in a prenatal state. The interval from pipping through hatching is described as the perinatal period. Under control conditions, Japanese quail do not pip their shells until after 15.5 days of incubation; 83 percent pip their shells after 16 days of incubation. Thus, the groups stimulated on days 14.5, 15, or 15.5 demonstrate prenatal sensitivity to auditory stimulation. The group stimulated on day 16 demonstrates prenatal or perinatal effects, depending on whether or not the shells were pipped at the time of stimulation.

The dramatic difference in the effects of stimulation on day 14.5 of incubation, compared with day 14 or earlier, may depend on maturation of the developing auditory system. While no information is available on the sequence of auditory maturation in Japanese quail, comparative studies of single neuron responses in the cochlear nuclei in other species of precocial birds indicate that the last 20 percent of incubation is characterized by a rapid increase in frequency range and sensitivity (*10*). Such an increase in sensitivity could account for the click stimuli exceeding threshold for the first time between day 14 and day 14.5 of incubation.

Table 1. Relative hatching acceleration of each experimental group after 2 hours of acoustic stimulation. Relative acceleration equals hatching acceleration divided by the interval available for hatching acceleration; that is, relative acceleration equals $(C - E)/(C - S)$, where C is mean control hatch time, E is mean experimental hatch time, and S is incubation age at stimulation.

Incubation age at stimulation (day)	Relative acceleration (%)
14	0
14.5	15
15	32
15.5	45
16	44

The development of an avian embryo normally continues until shortly before hatching, and must be complete for successful hatching (*11*). If an embryo is prematurely removed from its shell before it is ready to hatch, it will not survive (*12*). Since the time of hatching can only be changed if the developmental sequence leading to hatching is also changed (*11*), it follows that the acceleration of hatching time is the result of accelerated embryogenesis. This report provides, to our knowledge, the first unequivocal evidence that the rate of development of a vertebrate embryo can be affected by brief exposure to sensory stimulation during the prenatal period. The acceleration of embryogenesis reported here is analogous, in some features, to the plasticity reported in studies of the visual system after birth. Plasticity refers to two aspects of developmental lability: (i) the organization of the system is changed, and (ii) the rate of maturation of the system is changed as a result of experience or physiological stimulation. In the first case, organizational plasticity, the elements of the system acquire novel characteristics following selective experience. In the second case, maturational plasticity, the time course of ontogenetic development is altered, so that the relative durations of the various developmental stages are changed following selective experience. It is the second aspect of plasticity which is relevant to our study. The accelerated embryos are not premature; they do not differ from normal hatchlings in any of the physiological or behavioral measures examined (*13*).

Maturational plasticity and organizational plasticity are not mutually exclusive processes. We do not know whether or not the maturational plasticity initiated by auditory stimulation is correlated with organizational plasticity in the auditory system. The auditory system represents only one of several potential inputs that can affect the time course of embryogenesis. Other sensory stimuli that can accelerate the development of oviparous species are light (*14*), temperature (*4*), and vibration (*6*). However, only auditory stimulation has been shown to initiate maturational plasticity following such brief prenatal periods of sensory experience.

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References and Notes

1. The term "brief" in this report refers to intervals of 3 hours or less.
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