dishabituated to a three-dot array having the same density as the six-dot array (R. G. Cooper, P. Starkey, J. Dannemiller, in preparation). In two other experiments (P. Starkey, E. Spelke, R. Gelman, in preparation) infants discriminated three- versus four-dot arrays and two- versus three-object arrays when slides of heterogeneous arrays of common household objects rather than dots were used as stimuli. This study is important since total contour varied considerably from slide to slide.

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Associative Learning in Premature Hydranencephalic and Normal Twins

Abstract. *A hydranencephalic infant lacking cerebral hemispheres and a normal twin were tested for associative learning. After repeated trials in which two stimuli were temporally paired, test trials were given in which the second stimulus was omitted. Cardiac orienting responses to stimulus omission indicated that learning had taken place in both infants.*

Continued research into the neural mechanisms of behavior has greatly expanded our appreciation of the capacity of lower brain structures to subserve many complex behavioral processes—processes which historically have been considered to be the domain of the cerebral hemispheres. Decerebrated animals retain a vast array of behavioral functions, including the capacities for habituation and learning (*1*). The breadth of the capability of brainstem mechanisms has recently been punctuated by Norman *et al.* (*2*), who demonstrated a classically conditioned eyelid response in cats with upper brainstem transections and further documented discriminative conditioning between auditory stimuli in the same animals. In addition, Huston and Borbely (*3*) have provided evidence of operant conditioning in the decerebrated rat, using brain stimulation as the reinforcer.

As impressive as these data may be, their implications concerning the functional organization of the human brainstem are clouded by the fact that the behavioral capacity of animals with upper brainstem transections diminishes dramatically with the increasing phylogenetic level of the organism (*1*). The tragic conditions of anencephaly and hydranencephaly in the human infant, which are characterized by the absence of the cerebral hemispheres, provide human clinical parallels to the animal brainstem preparation. Although generally short-lived, anencephalic and hydranencephalic infants retain many vegetative and reflexive capacities of the normal neonate (*4, 5*). Consonant with the experimental findings in animals, accumulating reports on hydranencephalic and anencephalic infants tend to confirm the retention of habituation processes (*4, 6*). The capacity of these infants to develop learned associations, however, has not, to our knowledge, been documented. In order to further examine the learning capabilities of subcortical networks in the human, we tested for associative learning in a premature twin pair, one member of which was hydranencephalic, the other apparently normal. We here report evidence of stimulus-stimulus association in both infants.

The subjects were dizygotic twins, one male and one female, born 2 months prematurely at an estimated gestational age of 32 weeks. The male showed no clinical abnormality, but examination of the female revealed widespread transillumination of the skull and hyperactive Moro and deep tendon reflexes. Air encephalography confirmed the tentative diagnosis of hydranencephaly, a condition of variable etiology in which the cerebral hemispheres are replaced by a thin meningeal and ependymal membrane (*7*). The air study (Fig. 1) revealed the virtual absence of the cerebral hemispheres. These findings were further confirmed by computerized axial tomography, which also revealed grossly abnormal basal ganglia and dorsal thalami.

The learning capability of the infants was evaluated through tests for the development of conditioned associations between two simple stimuli. Ethical considerations precluded the use of the strong stimuli typical of conditioning studies; therefore, we used an adaptation of the perceptual disparity procedure (*8*) to evaluate stimulus-stimulus association through the establishment of stimulus "expectancy." Two innocuous stimuli were paired in close temporal contiguity and repeatedly presented to the subject.

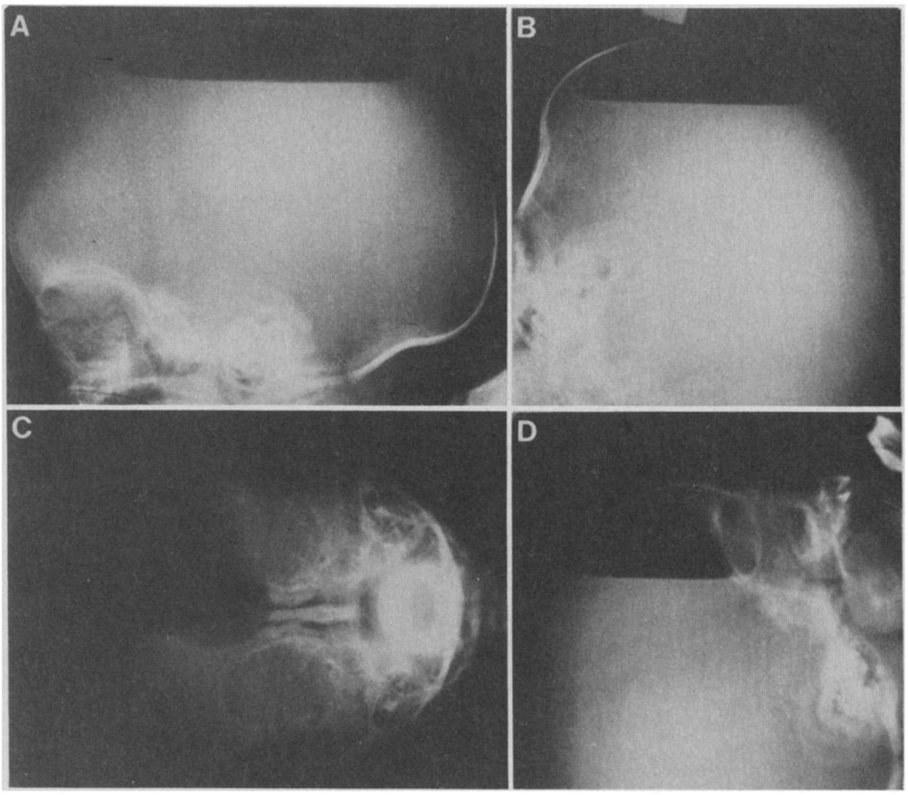

Fig. 1. Pneumonencephalograms demonstrating the condition of hydranencephaly in the female infant. (A) Upright lateral view; note dorsal locus of air in the cranial cavity. (B) Brow down lateral view. (C) Left side down view. (D) Brow up view.

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Interspersed within these conditioning trials were occasional test trials in which the first stimulus was presented alone. Under this procedure, if an association develops between the two stimuli such that the subject learns that the first stimulus predicts the second, a disparity is introduced in the test trials when the second stimulus is omitted, resulting in a generalized reaction such as an orienting response (*9*). The reaction in test trials occurs to the omission of a stimulus—an omission that could only be detected if some conditioned expectancy, or stimulus association, had been formed during the stimulus pairings.

Both the hydranencephalic and normal infants were tested for stimulus association during four separate sessions carried out in a dimly illuminated and quiet room adjacent to the nursery care unit of the hospital (*10*). The infants were born an estimated 2 months prematurely and were tested on days 40, 50, 57, and 62 after birth. The stimuli consisted of a tone (1 kHz, 76 dB referred to 0.0002 μbar) and a light (reflector-type flashlight directed toward the infant's face and yielding an illuminance of 90 foot-candles ($\sim$ 1000 lumen/m^2) at the subject plane). Both stimuli were under the control of a solid-state programming apparatus. A typical delayed conditioning procedure was followed such that the tone, lasting 1.5 seconds, was terminated concurrently with the onset of the light stimulus which was 0.5 second long. A total of 162 paired-stimulus trials were given over the four sessions and a total of 18 test trials with the tone alone were interspersed within the paired trials (one test trial each nine paired trials). In addition, two test trials with the tone alone were given before each session, and five extinction trials with the tone alone were given at the end of each session. An ongoing electrocardiogram was recorded for 5 seconds before and 15 seconds after each stimulus presentation (*11*). Intertrial intervals averaged 30 seconds (15, 30, and 45 seconds block randomized). However, when the infant moved or cried, testing was delayed until heart rate had returned to a stable baseline. The pattern of results was virtually identical across all four sessions, and no systematic trends were apparent over time. Consequently, the data from all four sessions were pooled for illustrations and analysis.

The results provide evidence for stimulus association in both the normal and hydranencephalic infants. Heart rate responses to the tone stimulus before tone-light pairings were negligible in the normal infant and deceleratory in the hydranencephalic infant (Fig. 2A). Responses in the first two paired-stimulus trials were slightly deceleratory for both infants (Fig. 2B). After tone-light pairings, however, presentation of the tone alone on test trials resulted in a notable long-latency cardiac acceleration to stimulus omission, while responses on pretest paired-stimulus trials continued to show the typical deceleratory pattern (Fig. 2C) (*12*).

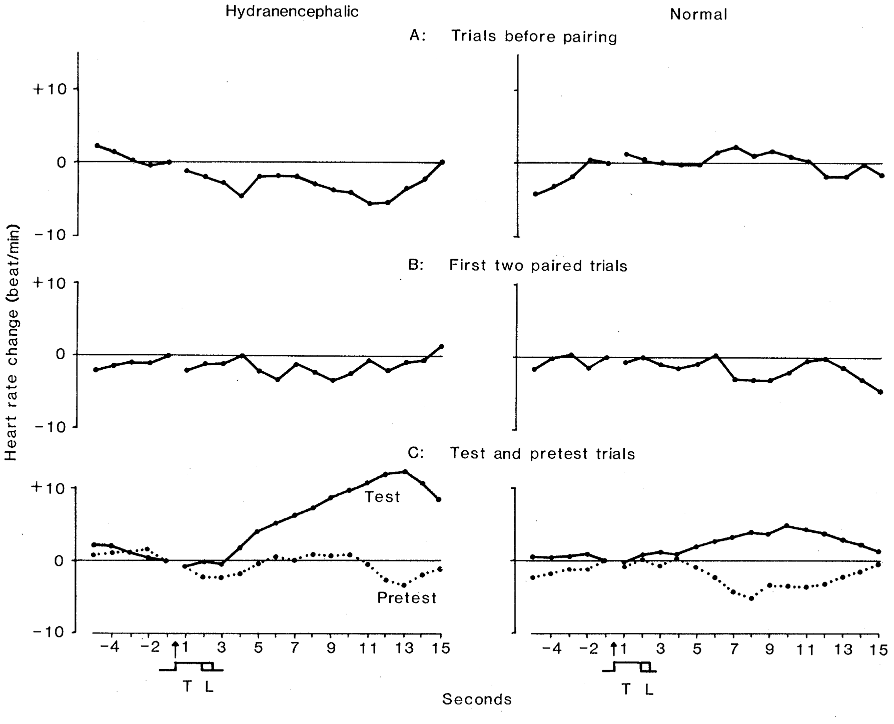

Fig. 2. Summary of heart rate changes during the tone-light stimulus association sessions. (A) Changes in heart rate for 5 seconds before and 15 seconds after the tone stimulus, presented alone prior to tone-light pairings. (B) Responses on the first two tone-light pairing trials of each session. (C) Heart rate responses on the test trials with tone alone compared with responses on the immediately preceding paired tone-light pretest trials.

Statistical analyses of the results (Wilcoxon matched-pairs signed-ranks tests) revealed that there were no significant differences in prestimulus baseline heart rates either between infants or between test trials and pretest trials (median prestimulus heart rate for the hydranencephalic infant on test trials, 148.4 beat/min, on pretest trials, 152.9; for the normal infant on test trials, 150, on pretest trials, 151.9). On the other hand, the poststimulus heart rate on test trials was significantly greater than on pretest trials (the median difference for the hydranencephalic infant, 5 beat/min, $T = 14.5$, $P < .01$; for the normal infant, 5.8 beat/min, $T = 8$, $P < .01$). Additional analyses revealed that this effect was due to a significant late cardiac acceleration on test trials. No significant rate changes from prestimulus baselines were seen on either test or pretest trials for the first half of the poststimulus period. However, in the second half of the poststimulus period, heart rates on test trials for both infants were significantly elevated over their respective baseline levels and over the corresponding rates on pretest trials (median increase over baseline levels for the hydranencephalic infant, 7.6 beat/min, $T = 11$, $P < .01$; over the pretest trials, 9.3 beat/min, $T = 4$, $P < .01$; median increase for the normal infant, 4.1 beat/min, $T = 29$, $P < .05$; over the pretest trials, 7.0 beat/min, $T = 25.5$, $P < .05$). Extinction trials run after each session revealed a rapid decay of the cardiac acceleration in response to the test tone. Within three trials, no evidence of acceleratory responses remained.

The consistent cardiac responses observed in response to stimulus omission in both the hydranencephalic and normal infants clearly document the existence in the premature human infant of associative processes relatively independent of the cerebral hemispheres. Classical conditioning in normal full-term infants has previously been demonstrated, although it is often difficult to establish (*13, 14*). In contrast, the expectancy procedure we used yielded rapid and consistent learning in both the normal and hydranencephalic infants. A significant advantage of the expectancy procedure is that it does not require strong unconditioned stimuli, which can alter the behavioral state of the subject and lead to confounding with the nonassociative processes of sensitization and pseudoconditioning (*14, 15*). Moreover, previous findings (*16*) suggest that the expectancy procedure may provide a more sensitive index of association than classical conditioning. In view of these considerations, the expectancy procedure may prove valuable to the study of associative processes in normal and brain-damaged infants.

Our results, providing what we believe to be the first evidence of associative learning in the hydranencephalic infant, bolster the growing view that subcortical networks are capable of mediating complex behavioral processes. The progressive expansion of cerebral cortical systems through phylogenetic development has undoubtedly contributed to the elaboration of behavioral capacities. Nevertheless, complex psychological processes, such as learning, do not appear to be exclusively within the domain of the cerebral hemispheres.

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17. We dedicate this report to Neal E. Miller, a pioneer in the study of brain-behavioral function and learning processes. This research was cleared by the Human Subjects Committee of Children's Hospital and Ohio State University. Parental permission was obtained before testing. We gratefully acknowledge the cooperation of the attending physician, G. Morrow, and the counsel of D. D. Wickens and C. D. Wickens.

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Limulus Brain Modulates the Structure and Function of the Lateral Eyes

Abstract. *At night efferent optic nerve activity generated by a circadian clock in the* Limulus *brain changes the structure of the photoreceptor and surrounding pigment cells in the animal's lateral eyes. The structural changes allow each ommatidium to gather light from a wider area at night than during the day. Visual sensitivity is thereby increased, but spatial resolution is diminished. At daybreak efferent activity from the clock stops, the structural changes reverse, and the field of view of each ommatidium decreases. The cyclic changes are endogenous and continue in the dark. Thus, under the control of a circadian clock, the* Limulus *eye exchanges its daytime acuity for greater sensitivity at night.*

Visual sensitivity exhibits a circadian rhythm in *Limulus*, the horseshoe crab (*1*). At night a clock in the brain transmits nerve impulses via efferent fibers to the photoreceptor cells of the lateral compound eyes. The efferent input increases the response and decreases the spontaneous activity of the photoreceptors (*2*) and optic nerve fibers (*1*). In addition, the efferent input is essential for the daily turnover of the rhabdom structures of the photoreceptors (*3*). We report here that the efferent input also changes the morphology of the ommatidial cells (*4, 5*). The circadian changes in morphology increase the light quantum catch and the field of view of single ommatidia. At night the acceptance angle of an ommatidium doubles, allowing the photoreceptors to view a larger region in visual space.

An ommatidium in the *Limulus* eye is composed of 8 to 12 photoreceptors (retinular cells) clustered tightly around the dendrite of an eccentric cell. Light collected by the corneal lens passes through an aperture formed by surrounding pigment cells and enters the photosensitive rhabdom of the retinular cells. The rhabdom is composed of microvilli, which contain a rhodopsin-like visual pigment. The responses of the retinular cells to light are conducted to the eccentric cell by electrical coupling with its dendrite. The eccentric cell, in turn, generates optic nerve impulses and transmits them to the brain.

During the day the aperture formed by the distal pigment cells is small (17 μm in diameter) and the retinular cells are separated from the lens by about 30 μm (Fig. 1). The light entering the retinular cells is thereby restricted. As shown in the cross-sectional view, processes of proximal pigment cells containing large granules run between adjacent retinular cells at the ends of the rhabdomeral rays. Smaller pigment granules in the cytoplasm of the retinular cells are concentrated near the edge of the rhabdom.

At night, during the period of peak efferent optic nerve activity, the ommatidial structure changes (Fig. 1). The pigment cell processes move radially away from the ommatidial axis, increasing the diameter of the aperture to 60 μm (*5*). The retinular cells shift position and lie within 4 μm of the corneal lens. Also, they appear to be compressed against the base of the lens. The rhabdom is shortened by 36 percent and widened by 34 percent. In cross section the individual rays of the rhabdom appear folded; in the longitudinal reconstruction they are seen as loops. The deep folds in the rhabdom appear to induce bends in the distal portion of the eccentric cell dendrite.

These morphological changes continue in complete darkness, following the circadian rhythm of efferent optic nerve activity (*6*). Cutting the optic nerve abolishes the cyclic changes and leaves the morphology of ommatidia in the daytime state. Delivering pulses of current to the distal end of the cut optic nerve during the day changes the morphology to the nighttime state. We conclude that efferent fibers in the optic nerve mediate

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