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Rhesus Monkeys with Late-Onset Hydrocephalus Differ From Non-impaired Animals During Neonatal Neurobehavioral Assessments: Six-Year Retrospective Analysis

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Background and Purpose: **A recent case study indicated that a hydrocephalic rhesus monkey had abnormal response patterns in a standardized neonatal primate assessment. We conducted a retrospective study to determine whether this assessment could also differentiate neonatal rhesus monkeys that appeared normal but developed signs of hydrocephalus later in life from neonates with normal development and no evidence of hydrocephalus.**

Methods: **One-hundred eighty-two rhesus monkeys were assessed on postnatal days 7, 14, 21, and 30. As neonates, clinical signs of hydrocephalus or other illnesses were not evident in any animal. Six monkeys developed signs of hydrocephalus between 5 months and 5 years of age, and each received confirmed diagnoses of hydrocephalus at necropsy.**

Results: **Compared with colony norms, the monkeys that developed hydrocephalus had diminished orientation abilities, more muscle tension, less behavioral evidence of distress, and more pronounced responses to some reflex-evoking stimuli, and difficulty in self-righting (day 7 only). Discriminant function analysis comparing the hydrocephalic animals with a matched control group provided a high probability of correct group assignment at days 7, 14, and 21.**

Conclusions: **Some as yet undetermined factor may predispose some monkeys to develop hydrocephalus, which may also be reflected in different scores on neurodevelopmental test items during early infancy.**

Hydrocephalus, although most commonly observed in nonhuman primates in the pre- and immediately postnatal period (1–3), occasionally develops in older animals. Hydrocephalus can be either congenital (i.e., present at birth) or acquired (4). In many reported cases of older individuals, the hydrocephalus appears to be acquired (*Papio papio*: tumor origin, 5; A*otus nancymae*: parasitic origin, 6; *Ateles* sp.: traumatic origin, 7), although this is not always the case (*Gorilla gorilla*: congenital origin, 7). In any case, the disorder is characterized by buildup of cerebrospinal fluid in the cerebral ventricles or subarachnoid spaces. This buildup could be due to structural defects that limit the amount of space in the ventricles, therefore impeding the free flow of cerebrospinal fluid (CSF); this type of hydrocephalus is most likely congenital, or hereditary, in origin. An alternative pathway to hydrocephalus is via the presence of excess amounts of cerebrospinal fluid. This could result from excess production of CSF or from decreased drainage or resorption of CSF (8, 9). Acquired causes of hydrocephalus can include bacterial or viral infection, tumor, trauma, parasitic infestation, and nutrition disorders (4, 8, 9).

The behavioral and neuropsychological concomitants of hydrocephalus in animals and humans are well documented. Reported behavioral manifestations of hydrocephalus in animals include sleepiness or lethargy, low activity levels, weakness, depression, ataxia, and blindness (4, 6, 10–12). In humans, the causes and consequences of hydrocephalus have been well characterized. In particular, specific dysfunctions, such as delayed motor development, difficulty orienting to and following a visual stimulus, and abnormally brisk reflexes, are common in hydrocephalic humans. Disorders of state regulation, such as excess lethargy and irritability, also appear to characterize hydrocephalic individuals (13).

In a previous report, we documented that hydrocephalus in a rhesus macaque neonate was associated with compromised functioning on a standardized neurodevelopmental battery (14). The hydrocephalic infant of that case study had low scores for orientation and motor maturity items and high levels of assessed irritability. In the study reported here, we retrospectively compared animals that developed hydrocephalus later in life (late infancy through early adulthood) with non-impaired animals, using the same infant assessment procedure. Because all infants appeared clinically normal at the time of assessment, we did not anticipate profound deficits in the animals that later developed hydrocephalus.

With few exceptions, neither human nor animal literature contains many instances in which deficits are realized or assessed prior to development of the clinical syndrome of hydrocephalus. One series of studies documented deficits in motor activity, including abnormal postures and motor patterns, in human neonates prior to development of neurologic impairment (15–17). On the basis of these studies, the aforementioned case report, and documented characteristics of behavior in hydrocephalic individuals, we predicted deficiencies in visual orientation and tracking abilities, irritable temperament, excess drowsiness, poor muscle tone, less motor coordination, and less

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mature motor responses in normal-appearing rhesus infants that later developed hydrocephalus.

Materials and Methods

Subjects and housing: Subjects were 104 nursery-reared (NR) and 78 mother-reared (MR) rhesus macaques born at the National Institutes of Health Laboratory of Comparative Ethology captive colony between 1991 and 1996. Animals from the six yearly cohorts were combined for the analyses because all procedures were identical across the six cohorts. At the time of assessment, all animals appeared to be clinically normal, so that none manifested any of the signs associated with hydrocephalus, including enlarged skull, weakness, ataxia, or inability to locomote or self-feed. Further, animals were not under treatment for any other condition (e.g., diarrhea, respiratory illness, or parasites).

The NR infants were separated from their mothers on postpartum days 1 to 3 and reared according to previously published procedures (18). From days 1 to 15 of life, animals were individually housed in 51 x 38 x 43-cm plastic cages. Each cage contained a 25-cm-high inanimate "surrogate mother" composed of a 16.5-cm circumference polypropylene cylinder attached to an 11.5-cm-wide circular metal base by a flexible metal component. The surrogate was covered with an electric heating pad, which was itself covered with fleece fabric. Loose pieces of fleece fabric covered the floor of the cage. The internal temperature was maintained at approximately 27°C. Infants could see and hear, but not physically contact other infants. At 15 days of age, infants were moved to another, larger room into individual wire mesh cages measuring 64 x 61 x 76 cm. The animals retained their surrogates covered with fleece, albeit minus the heating pad. As under the earlier housing condition, animals were in visual, auditory, and olfactory, but not tactile contact with other infants. Lights were on from 7 AM to 8 PM. Room temperature was kept between 22 and 26°C, and humidity was maintained at 50 to 55%. The NR infants were placed in social groups of 4 to 5 animals when the youngest group member reached 37 days of age and were housed in 71 x 81 x 152-cm cages.

Nursery-reared animals were provided with a 50:50 mixture of Similac (Ross Laboratories, Columbus, OH) and Primilac (Bio-Serv, Inc., Frenchtown, NJ) formulas. They were hand-fed until they were old enough to independently feed themselves. Formula was administered ad libitum until the age of 4 months, when the animals were fed a ration of 200 ml of formula/d, at 5 months, they received 100 ml/d, and at 6 months, they were entirely weaned from formula. Purina High Protein monkey chow (No. 5038) and water were provided ad libitum when NR animals reached the age of one month.

Mother-reared infants lived with either biological or adoptive mothers in social groups containing 2 adult males, 6 to 8 adult females, and other infant offspring of the adult females. Each social group also contained 2 to 5 infants. These animals lived in indoor-outdoor pens, composed of welded galvanized steel mesh, connected by guillotine doors. The floor of the pens was covered with wood chips. The indoor pen measured 2.44 x 3.05 x 2.21 m; the dimensions of the outdoor pen were 2.44 x 3.0 x 2.44 m. Animals were allowed access between indoor and outdoor portions except when confined to one half for cleaning, or during inclement weather (e.g., temperature below freezing). Inside the pens, the lights were maintained on a 12:12-hour light:dark cycle (7 AM to 7 PM). Animals in these social groups were fed the aforementioned high-protein monkey chow and received water ad libitum. Supplemental fruit was provided 3 times each week; sunflower seeds were presented daily.

Neonatal assessment: A 30-minute developmental assessment battery was administered to all monkeys on days 7, 14, 21, and 30 of life. This test was derived from the Brazelton Neonatal Assessment Scale used in human newborns (19), and has been described in detail elsewhere (20). The test was administered between 11 AM and 1 PM. The NR infants were hand-caught from the home cage; MR infants were separated from the mother's ventrum via hand- or net-catching of the mothers and chemical immobilization of the mothers with ketamine hydrochloride (10 mg/kg of body weight). As long as the infant remains on the mother's ventrum, this procedure does not activate the stress-reactive hypothalamic-pituitary-adrenal system (21). All infants were hand-carried by the experimenter to a testing room and given five minutes to adapt before testing began. The test items were administered in predetermined sequence. Initially, orientation abilities and attention to visual and auditory stimuli were assessed. This was followed by measurement of a variety of reflex and sensorimotor functions, including tactile responsiveness, postural adjustment capabilities, and muscle tone. In addition, the response to a brief challenge was assessed during a 5-minute session in which the animal was placed in a small, empty novel cage. Temperament characteristics were rated after administration of the orienting and neuromotor items, on the basis of the infant's behavior throughout the test period. These measures included the tester's impressions of the animal's fearfulness, tendency to struggle, consolability, irritability, ability to self-soothe, cuddliness, and overall state of arousal. Table 1 lists the behavioral definitions of specific test items in their order of administration.

Post-neonatal history: Noninvasive behavioral observations were conducted for all animals in their home cages twice weekly, from birth through six months of age. In addition, blood and CSF samples were obtained on postnatal days 14, 30, 60, 90, 120, and 150. These samples were collected after either neonatal assessment (days 14 and 30) or after a 20-minute period in which monkeys were removed from the home cage and placed alone in an unfamiliar environment (days 60 to 150). Cerebrospinal fluid and blood samples were also collected weekly for eight consecutive weeks when monkeys were approximately six months old. This series of physiologic samples was collected in conjunction with a four-week series of separation from social partners. At completion of the separation series, all MR and NR animals from each birth cohort were placed together in an indoor-outdoor pen (identical to the pen in which MR animals had been housed). Between 1991 and 1996, six animals (four MR, two NR) developed hydrocephalus; observed clinical signs included lethargy (n = 1), ataxia (n = 2), seizure activity (n = 2), visual difficulties (n = 2), nystagmus (n = 1), pupil dilatation (n = 2), and clonic neck extension (n = 1). Diagnosis of hydrocephalus was determined at necropsy. In all but one (undetermined) case, hydrocephalus was considered to be of congenital origin (i.e., there was no evidence of trauma, other illness, or parasitic load). These animals will be referred to as late-onset hydrocephalic (HY) infants, as differentiated from non-impaired (NI) infants.

Statistical analysis: Following the described procedure (20), several individual neonatal test items were condensed into four

Table 1. Neonatal assessment item definitions

	Item	Definition
Orientation cluster		
	Visual orientation	Eyes oriented toward toy (Mickey Mouse face) held in four positions in infant's periphery
	Visual following	Eyes following moving toy (same as above) in horizontal and vertical directions
	Duration of looking	Examiner rating of duration of looks on orienting items
	Attention	Examiner rating of attention on orienting items
State control cluster		
	Irritability	Amount of distress noted during the entire examination
	Consolability	Ease of consoling infant after distress
	Predominant state	State of infant during examination
	Struggle	Amount of squirming during examination
Motor maturity cluster		
	Coordination	Quality of motor activity rated during the 5-minute observation period
	Head posture prone	Ability to hold head up when held in air prone
	Head posture supine	Ability to hold head up when held in air supine
	Labyrinthian righting	Realignment of head when body is tilted 45° sideways
	Response speed	Examiner rating of speed of responding
Activity cluster		
	Passive	Duration spent inactive during the 5-minute observation period
	Coordination	Quality of motor activity rated during the 5-minute observation period
	Motor activity	Observation of amount of motor activity during the 5-minute observation period
	Spontaneous locomotion	Quality of locomotion rated during the 5-minute observation period
Individual test items		
	Reach and grasp	Attempts to grab visual orient/follow toy
	Auditory startle	Response to sudden noise (metal object against metal bowl 2 cm from back of head
	Auditory orient	Eyes oriented toward lipsmacking sound (examiner simulation of monkey sound) made in infant's periphery
	Distractibility	Examiner rating of infant distractibility during orienting items
	Tactile response	Response to tactile stimulus (wooden end of Q-tip distal to proximal) to four extremities
	Galant's response	Response to cephalocaudal tactile stimulus lateral to vertebral column
	Palmar and plantar grasp	Response to examiner's index finger placed in palm or sole of foot
	Inversion	Response to briefly being held upside down
	Body righting	Time noted for infant to turn from supine to prone
	Aversion on back	Vocal response to lying supine
	Traction	Infant pulled from supine to sitting and head lag noted
	Response intensity	Examiner rating of quality of vocal reactions
	Soothability	Examiner rating of frequency of interventions necessary to calm infant
	Cuddliness	Infant's response to cuddling or flexing the infant toward examiner
	Tremulousness	Examiner rating of tremulousness
	Vocalization count	Number of vocalizations in a 60-second period in novel cage
	Calming self	Infant's behavior when placed in an enclosed area for 5 minutes
	Fine motor manipulation	Duration of time engaged in manipulation of environment during 5-minute test
	Fearfulness	Fear grimaces and/or trembling
	Self-mouthing	Inserting hands or feet in mouth
	Maintenance of balance	Infant is held in the sitting position and support is withdrawn
	Passive resistance	Degree of resistance to passive flexion and extension of limbs
	Active power	Strength of muscles while actively contracting
	Placing response	Infant places hand or foot on table after tactile stimulus (table edge) on dorsum of hand or foot
	Parachute response	Upper extremity limb extension following headfirst descent toward surface
	Rotation reflex	Degree to which head and/or eyes turn into the direction of rotation, with head free and with head restrained
	Restrain	Duration of struggle or vocalization to 10-second restraint in supine position
	Persistence	Frequency of resistance attempts while restrained in supine position
	Rooting reflex	Infant's response (head turning toward stimulus) to light tactile stimulus at the corner of the mouth

clusters: orienting, state control, motor maturity, and activity for analysis (Table 1). An initial analysis of variance (ANOVA) procedure was performed, comparing the scores of the HY animals with the colony norms, for clusters and all test items that were not part of a cluster. This procedure allowed us to determine for which items the HY infants deviated from colony averages, and provided information for use in further analyses. In accordance with standard procedure in our laboratory, items that were not components of clusters also were analyzed statistically. Analyses were conducted, using three-way univariate analyses of variance with independent factors of rearing (MR; NR) and outcome (HY; NI) with day of testing (day 7; day 14; day 21; day 30) as a repeated measure (SuperANOVA: Abacus Concepts, Berkeley, CA). Significance was set at $P < 0.05$ for all analyses.

In addition, discriminant function analyses were performed to determine the probability of correct assignment of animals to the HY and NI groups. To determine whether we could identify certain variables as early predictors of late-onset hydrocephalus, we used multivariate discriminant function analyses (SAS 6.12, SAS Institute, Inc., Cary, NC) to obtain classifications of the six late-onset HY infants, compared with a control group of six NI infants at each age. The control group was selected by matching each HY infant with the closest-age NI infant from the same rearing condition. We chose non-parametric discriminant function analysis because our small dataset violated the multivariate normality assumption, and we adjusted each analysis for prior probabilities to compensate for missing data from two HY infants at days 7 and 21. Because of constraints imposed by the small sample size (22), we limited our input variables to the eight variables (total number of cases minus two) that best discriminated the HY and NI infants by use of the Mann-Whitney U tests at minimal significance of $P < 0.10$ (Table 2). At day 30, only two variables reached this criterion.

Finally, we used the cross-validation classification procedure, which excludes each case from the calculation, and examined the percentage of correctly classified cases.

Table 2. Variables used in all discriminant function analyses.

Behavior	Group	Age (days) 7	14	21
Vocal 60 seconds	HY NI	ND	14.3 (2.00)** 25.7 (4.00)	ND
Aud. orient right	HY NI	ND	ND	1.88 (0.12)** 1.17 (0.21)
Aversion on back	HY NI	0.88 (0.25)** 1.75 (0.17)	ND	ND
Calm self	HY NI	0.38 (0.24)*** 1.92 (0.08)	ND	0.62 (0.38)* 1.50 (0.26)
Consolability	HY NI	2.00 (0.00)** 0.75 (0.36)	1.42 (0.20)** 0.67 (0.31)	1.50 (0.29)** 0.33 (0.33)
Cuddliness	HY NI	ND	1.67 (0.25)** 0.58 (0.33)	1.62 (0.24)** 0.42 (0.33)
Irritability	HY NI	1.38 (0.24)** 0.50 (0.18)	ND	0.88 (0.24)** 0.17 (0.17)
Placing upper	HY NI	1.75 (0.25)** 0.42 (0.20)	ND	ND
Plantar grasp left	HY NI	ND	1.17 (0.40)** 0.08 (0.08)	ND
Predominant state	HY NI	ND	0.67 (0.21)** 1.42 (0.33)	0.62 (0.38)** 1.58 (0.33)
Response intensity	HY NI	ND	ND	1.00 (0.35)* 1.67 (0.33)
Root right	HY NI	ND	0.67 (0.42)* 1.58 (0.27)	ND
Soothability	HY NI	0.12 (0.12)** 1.33 (0.33)	0.75 (0.21)* 1.50 (0.34)	ND
Auditory startle	HY NI	1.50 (0.29)** 0.50 (0.22)	ND	ND
Struggle	HY NI	ND	0.50 (0.22)** 1.42 (0.33)	0.62 (0.38)** 1.67 (0.33)
Vis follow horizontal	HY NI	1.12 (0.12)** 0.33 (0.21)	ND	ND

HY = late-onset hydrocephalic; NI = non-impaired. Values represent means, with SEM in parentheses. Asterisks indicate significant differences between HY and NI groups (*$P < 0.10$, ** $P < 0.05$, *** $P < 0.001$). Mann-Whitney P values adjusted for ties.

Results

Analysis of variance: Only one item had a significant main effect of outcome. The HY infants had higher than average muscle tension while their limbs were being manipulated through their range of motion by the experimenter than did NI infants (**passive resistance**, F [1, 178] = 4.53, $P < 0.05$; HY mean = 1.43 ± 0.01; NI mean = 1.07 ± 0.02: for this test item a score of 1.0 indicates average resistance). Three significant outcome-by-test-day interaction effects are depicted in Table 3. In two instances, differences between outcome conditions were mainly observed on day 7. For the test items, **mean placing response** (F [3, 438] = 3.43, $P < 0.05$) and **startle to auditory** (F [3, 440] = 2.61, $P = 0.05$), HY monkeys demonstrated higher scores than did NI animals, indicating more pronounced response to stimulation, on day 7. For the **orientation cluster** (F [3, 439] = 2.80, $P < 0.05$), on day 7, scores from both groups were similar, but on days 14, 21, and 30, NI animals obtained higher scores (i.e., showed superior orienting ability) than did HY animals. A rearing-by-outcome interaction was obtained for **self-mouth** (F [1, 178] = 5.12, $P < 0.05$]; MR infants had minimal amounts of self-mouthing, but NR HY infants self-mouthed for longer periods than did NR NI infants (Table 4).

Statistically significant three-way interactions were detected for test items **body righting** (F [3, 440] = 15.44, $P < 0.01$), **aversion on back** (F [3, 440] = 5.69, $P < 0.01$), and **response intensity** (F [3, 440] = 3.22, $P < 0.05$). All animals manifested rapid body righting, with the exception of NR HY infants on day 7, which failed to right the body. Overall, HY infants manifested less aversion while on their back than did NI infants; however, MR HY monkeys displayed the least aversion on days 7 and 14, whereas on days 21 and 30, NR HY monkeys had the least aversion (Figure 1). Although MR monkeys had higher scores overall than did NR infants for response intensity, the lowest scores for the test item were obtained by MR HY infants on day 7, and by NR HY infants on day 30 (Figure 2).

Table 3. Placing, startle to auditory, and orientation cluster values in late-onset hydrocephalic and non-impaired infants across four test days

Test item		Test day 7	14	21	30
Placing	HY	1.75 (0.25)	0.33 (0.21)	0.25 (0.25)	0.17 (0.17)
	NI	0.36 (0.05)	0.35 (0.04)	0.34 (0.05)	0.37 (0.04)
Auditory startle	HY	1.50 (0.29)	0.75 (0.31)	0.38 (0.24)	0.33 (0.21)
	NI	0.79 (0.07)	0.71 (0.06)	0.74 (0.06)	0.64 (0.06)
Orientation cluster	HY	1.06 (0.16)	0.80 (0.19)	1.16 (0.26)	0.73 (0.23)
	NI	1.11 (0.05)	1.22 (0.05)	1.37 (0.05)	1.35 (0.04)

See Table 2 for key.

Table 4. Self-mouth in mother- and nursery-reared late-onset hydrocephalic and non-impaired infants

Hydrocephalic		Non-impaired	
MR	NR	MR	NR
0.17 (0.11)	2.0 (0)	0.33 (0.04)	0.87 (0.04)

MR = Mother-reared, NR = nursery-reared. Values represent mean, with SEM in parentheses.

Discriminant function analysis: Discriminant function analysis indicated that HY infants were clearly discriminable from NI infants matched to age and rearing condition at days 7, 14 and 21 (Table 2). Cross-validated classifications achieved 100% accuracy at days 7 and 21 and 83.3% accuracy at day 14, where one animal in each group was misclassified. In contrast, the groups were poorly discriminable at day 30; only three of six NI (50% correct) and four of six HY (67% correct) animals were correctly classified.

Discussion

In an earlier report (14), deficits in the neurodevelopmental outcome of a hydrocephalic rhesus monkey neonate during the first three weeks of life were described. That infant was more impaired in the neonatal period than were the animals of the study reported here, which appeared clinically normal on examination but were diagnosed with hydrocephalus between 5 months and 5 years of age. However, there were several similarities in the outcome of the neurodevelopmental comparison between HY and NI monkeys. Particularly noticeable in both cases were reductions in visual orienting ability, and the diminished capacity to right from a supine-to-prone position. However, it should also be noted that several characteristics of the late- onset HY animals, such as stronger placing and auditory startle reflexes, were not observed in the HY neonate.

The initial ANOVA, in which the six HY infants were compared with the general population, revealed group differences in some test items, although in many instances, the overall main effect was mitigated by test day or rearing condition interactions. The HY animals had above-average scores for passive resistance, indicating hypertonicity, when the examiner manipulated the infants' limbs. This finding may contradict previous studies in which abnormalities of muscle tone accompanied

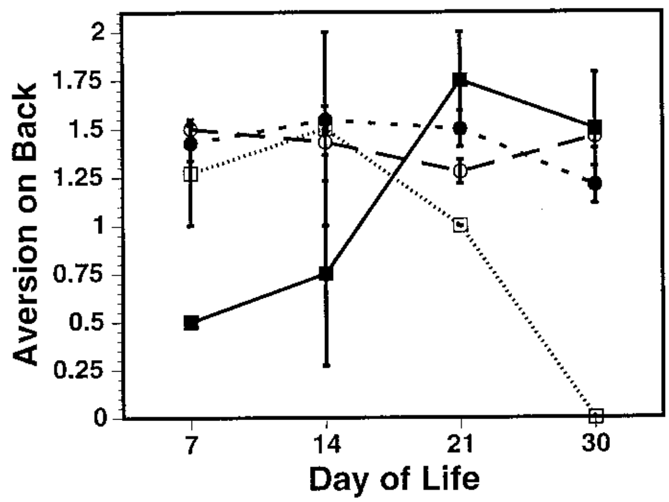

Figure 1. Aversion on back scores for rhesus infants across the first month of life (mean ± SEM). Closed squares, solid line = mother-reared late-onset hydrocephalic infants; open squares, dotted line = nursery-reared late-onset hydrocephalic infants; closed circles, short-dashed line = mother-reared non-impaired infants; and open circles, long-dashed line = nursery-reared non-impaired infants.

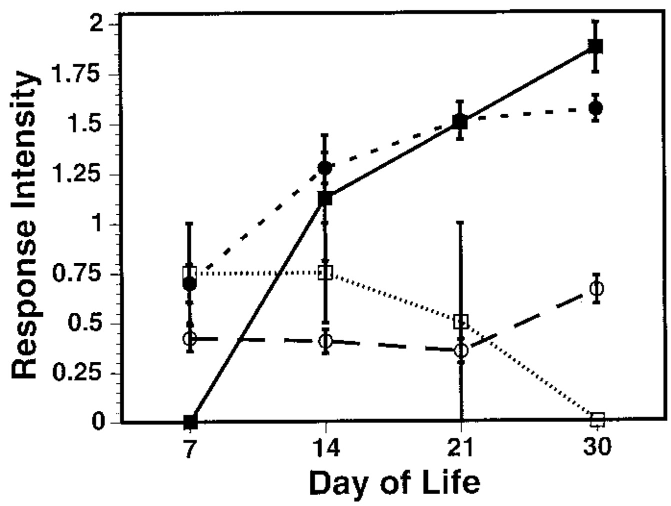

Figure 2. Response intensity scores for rhesus infants across the first month of life (mean ± SEM). *See* Figure 1 for key.

hydrocephalus in rhesus infants (experimentally induced, 23) and children (24), although it is unclear whether active or passive muscle tone was assessed in these studies. It also appears inconsistent with our finding that, in some instances, HY animals manifested inability to right themselves from the supine to prone position, as well as lack of distress when placed in the supine position. The inability to self-right indicates motor deficit and is in accord with the unusual postures, ataxia, and lethargy that have been reported in cases of hydrocephalus in humans and other species (4, 6, 10–12). The increased muscle tone in response to experimenter manipulation may be indicative of overall high reactivity to tactile stimulation (i.e., tactile defensiveness).

On most test days, HY monkeys had lower scores for the **orientation** cluster. These results are in accord with findings of deficits in visual-motor functioning in hydrocephalic humans (25, 26). There are several possible reasons for low orientation scores in HY monkeys. Low scores on this cluster can be a consequence of a total lack of visual function, as was probably the case with the hydrocephalic infant in the case study (14). Additionally, low scores can reflect a transient immaturity of the visual system, as scores on this cluster typically are low after birth but increase as the infant matures (20). Finally, temperament characteristics can influence orienting abilities; it appears that more distressed infants are less able to attend to orientation stimuli (27), and highly distractible or inattentive infants also receive lower scores on these items. Because the HY animals did not manifest high levels of emotional distress nor did they appear to be totally lacking in visual functioning, the most likely cause of their lower orientation scores was either a less mature visual system or behavioral inattention.

The HY monkeys had stronger responses to the auditory startle and placing upper extremities test items than did NI infants, but on day 7 only. The auditory startle reflex can be evoked for the first time in macaques between postnatal day 3 and 12, with an average age of emergence at 10 days (28). However, elicitation of the reflex was variable between and within animals; some infants never manifested the startle response, and others did so inconsistently. Published accounts established the earliest emergence of the placing of the upper extremities response at postnatal day 4 (29) or day 6 (30) although placing did not occur in some animals until postnatal day 14 (30). The previous studies differ in method from those of this study in two respects: they measured the presence or absence, rather than strength, of these reflexive responses and additionally, the previous studies reported emergence of reflexes as a function of age rather than group means at specific, predetermined ages. Because animals in our study were not tested daily, it is impossible to compare the HY and NI infants on the developmental progression of their reflex responding. Therefore, the stronger response of HY infants could have been due either to earlier peak in the maturation of these reflexes, or to more exaggerated response on day 7. A strong or exaggerated response to an auditory startle stimulus is considered indicative of a fearful or reactive temperament in animals and humans (31). In addition, tactile sensitivity and strong startle response are constituents of tactile defensiveness, a type of sensory integration dysfunction (32). Thus, the stronger responses of the HY infants to these reflex-evoking stimuli may reflect an underlying temperament dimension or may be associated with sensory integration dysfunction.

The study reported here must be interpreted with caution, given two limitations, the first being the small sample of late-onset hydrocephalics in the study. Hydrocephalus is a rare condition in nonhuman primates [incidence ranges from 1:25 to 1:946 in literature (1–3, 33, 34)]. Therefore, we have been limited in our ability to amass a substantive database of late-onset HY animals. The second limitation is that, with few exceptions, most of the "deficits" manifested by late-onset HY infants are within the normal range of responses for infants of that age, as befits the original intent of the neonatal assessment examination. Therefore, the response to any one item of the neonatal examination cannot be considered as a "marker" or predictor of hydrocephalus. However, by examining the responses to several test items in conjunction, our ability to construct a profile of infants at risk is enhanced. For instance, on day 7, none of the NR late-onset HY infants was able to right itself after being placed in supine position (although MR HY infants were able to perform this task). In contrast, in the control population, 139 of 143 infants were able to right themselves on day 7. In addition, a high score of 2.0 on the test item 'placing upper extremities' was obtained for three of the four HY infants on day 7 (75%); however, only 7 of 143 control infants (5%) received a 2.0 rating on that item. However, no control infants manifested the inability to right itself and high scores on placing upper extremities. These data suggest that an infant with high placing scores and unable to right itself on day 7 of life could be at risk for developing hydrocephalus later in life. Our discriminant function analysis was able to correctly assign animals to their respective conditions on most test days, indicating that group differences emerge when several variables are considered simultaneously.

It must be emphasized that the six animals that sustained a diagnosis of hydrocephalus appeared clinically normal in the neonatal period; in other words, the behavioral differences between these animals and the control population predated the clinical signs and diagnosis of hydrocephalus. This raises the issue of whether these six animals were actually hydrocephalic during the first month of life. In the absence of confirmatory information (e.g., magnetic resonance imaging scans), we are unable to satisfactorily reach a conclusion on this issue. If the animals were hydrocephalic as neonates, then in our neonatal examination, we were observing early indications of a progressively developing hydrocephalus in our study, prior to the onset of more overt clinical signs. If, on the other hand, the six infants were not hydrocephalic as neonates, the question becomes whether we were witnessing markers predictive of susceptibility to hydrocephalus later in life, and whether subtle structural abnormalities in the neonatal period would distinguish those infants that would later become hydrocephalic. Whether or not the six late-onset HY animals were actually hydrocephalic during the period of testing, the fact remains that they differed from NI animals for several significant dimensions. Thus, a major question raised by this study is what relationship, if any, exists between the structural changes caused by hydrocephalus and the behavioral propensities manifested by normal-appearing and normal-acting neonates. Of interest would be whether subtle structural abnormalities in the neonatal period distinguish infants that will later become hydrocephalic.

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References

1. **vanWagenen, G.** 1972. Vital statistics from a breeding colony. J. Med. Primatol. **1:**3–28.
2. **Korte, R., F. Vogel, I. Osterburg, *et al.*** 1988. Prenatal waste and spontaneous malformation in macaques, p. 141–150. *In* D. Neubert, H.-J. Merker, and A.G. Hendrickx (eds.), Non-human primates: Developmental biology and toxicology. Überreuter-Wissenschafts Verlag, Berlin.
3. **King, N. W., Jr., and L. V. Chalifoux.** 1986. Prenatal and neonatal pathology of captive nonhuman primates, p. 763–770. *In* K. Benirschke (ed.), Primates: The road to self-sustaining populations. Springer-Verlag, New York.
4. **Hoerlein, B. F., and E. D. Gage.** 1978. Hydrocephalus, p. 733–760. *In* B. F. Hoerlein (ed.), Canine neurology. W. B. Saunders Company, Philadelphia.
5. **Berthe, J., G. Barneon, G. Richer, *et al.*** 1980. A medulloblastoma in a baboon (*Papio papio*). Lab. Anim. Sci. **30:**703–705.
6. **Malaga, C. A., R. E. Weller, G. E. Dagle, *et al.*** 1991. Spontaneous hydrocephalus in an adult owl monkey (*Aotus nancymae*). Lab. Anim. Sci. **41:**182–184.
7. **Banish, L. D., R. Sims, D. Sack, *et al.*** 1993. Prevalence of shigellosis and other enteric pathogens in a zoologic collection of primates. J. Am. Vet. Med. Assoc. **203:**126–132.
8. **Chrisman, C. L.** 1991. Behavior and personality disorders, p. 133–175. *In* C. L. Chrisman, Problems in small animal neurology. Lea & Febiger, Philadelphia.
9. **Jones, T. C., and R. D. Hunt.** 1983. The nervous system, p. 1637–1688. *In* T. C. Jones, and R. D. Hunt, Veterinary pathology. Lea & Febiger, Philadelphia.
10. **Brown, R. J., J. M. Hardman, and M. Kessler.** 1981. Hydrocephalus in a Guyanan squirrel monkey, *Saimiri sciureus*. J. Med. Primatol. **10:**187–188.
11. **de Lahunta, A.** 1977. Cerebrospinal fluid and hydrocephalus, p. 33–56. *In* A. de Lahunta, (ed.) Veterinary neuroanatomy and clinical neurology. W. B. Saunders Company, Philadelphia.
12. **Munson, L., and R. J. Montali.** 1990. Pathology and diseases of great apes at the National Zoological Park. Zoo Biol. **9:**99–105.
13. **Neville, B. G. R.** 1993.Clinical features of hydrocephalus in childhood and infancy, p. 100–118. *In* P. H. Schurr, and C. E. Polkey, (eds.), Hydrocephalus. Oxford University Press, Oxford.
14. **Champoux, M., M. F. Kriete, M. A. Eckhaus, *et al.*** 1997. Behavioral and physical concomitants of congenital hydrocephalus in a rhesus macaque (*Macaca mulatta*) neonate. Contemp. Top. Lab. Anim. Sci. **36:**56–61.
15. **Cioni, G., H. F. R. Prechtl, F. Ferrari, *et al.*** 1997. Which better predicts later outcome in fullterm infants: quality of general movements or neurological examination? Early Hum. Dev. **50:**71–85.
16. **Ferrari, F., H. F. R. Prechtl, G. Cioni, *et al.*** 1997. Posture, spontaneous movements, and behavioural state organisation in infants affected by brain malformations. Early Hum. Dev. **50:**87–113.
17. **Prechtl, H. F. R.** 1997. State of the art of a new functional assessment of the young nervous system. An early predictor of cerebral palsy. Early Hum. Dev. **50:**1–11.
18. **Ruppenthal, G. C.** 1979. Survey of protocols for nursery-rearing infant rhesus macaques, p. 165–185. *In* G. C. Ruppenthal (ed.), Nursery care of nonhuman primates. Plenum Press, New York.
19. **Brazelton, T. B.** 1973. Neonatal behavioral assessment scale (Clinics in developmental medicine, No. 50). William Heinemann Medical Books, London.
20. **Schneider, M. L., and S. J. Suomi.** 1992. Neurobehavioral assessment in rhesus monkey neonates (*Macaca mulatta*): Developmental changes, behavioral stability, and early experience. Infant Behav. Dev. **15:**155–177.
21. **Gunnar, M. R., C. A. Gonzalez, B. L. Goodlin, *et al.*** 1981. Behavioral and pituitary- adrenal responses during a prolonged separation period in infant rhesus macaques. Psychoneuroendocrinology **6:**65–75.
22. **Klecka, W. R.** 1980. Discriminant analysis, vol. 19. *In* M. S. Lewis-Beck (ed.), Quantitative applications in the social sciences. Sage Publications, Newbury Park, CA.
23. **Michejda, M., K. Bayne, M. Schneider, *et al.*** 1991. Functional and structural recovery of the brain in in utero treated hydrocephalic monkeys: Follow-up of neurobehavioral development, p. 157–176. *In* S. Mancuso (ed.), Achievements in gynecology 1989–90. Karger, Basel.
24. **Roth, S. C., J. Baudin, D. C. McCormick, *et al.*** 1993. Relation between ultrasound appearance of the brain of very preterm infants and neurodevelopmental impairment at eight years. Dev. Med. Child Neurol. **35:**755–768.
25. **Tirosh, E., and M. Jaffe.** 1995. Apparent life-threatening event: A neurologic persepective. J. Child. Neurol. **10:**216–218.

26. **Fletcher, J. M., S. R. McCauley, M. E. Brandt, *et al.*** 1996. Regional brain tissue composition in children with hydrocephalus. Relationships with cognitive development. Arch. Neurol. **53:**549–557.
27. **Champoux, M., S. J. Suomi, and M. L. Schneider.** 1994. Temperament differences between captive Indian and Chinese-Indian hybrid rhesus macaque neonates. Lab. Anim. Sci. **44:**351–357.
28. **Mowbray, J. B., and T. E. Cadell.** 1962. Early behavior patterns in rhesus monkeys. J. Comp. Physiol. Psychol. **55:**350–357.
29. **Taylor, E. M., D. Sutton, and R. C. Lindeman.** 1980. Somatic reflex development in infant macaques. J. Med. Primatol. **9:**205–210.
30. **Hines, M.** 1942. The development and regression of reflexes, postures, and progression in the young macaque. Contrib. Embryol. **30:**155–209.
31. **Schmidt, L. A., and N. A. Fox.** 1998. Fear-potentiated startle responses in temperamentally different human infants. Dev. Psychobiol. **32:**113–120.
32. **Ayres, A .J.** 1972. Sensory integration and learning disorders. Western Psychological Services, Los Angeles.
33. **Centers for Disease Control.** 1971. Primate zoonoses surveillance. Report #6. Centers for Disease Control, Atlanta, Georgia.
34. **Hendrickx, A. G., and S. Prahalada.** 1986. Teratology and embryogenesis, p. 333–362. *In* W. R. Dukelow, and J. Erwin (eds.), Comparative primate biology, vol. 3: Reproduction and development. Liss, New York.