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The Ontogeny of Neurochemical Systems for Control of Feeding and Drinking (41778)

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It is common knowledge that suckling gives way to feeding and drinking in the ontogeny of young mammals. The transition from nursing like an infant to eating and drinking like a miniadult is a conventional milestone in their development. But few appreciate the completeness with which ingestive behavior is changed when neonates make this transition. When suckling, the neonate is extracting a single fluid from a maternal teat positioned deep in its pharynx, to which it is attached almost continuously, and on which it sucks rhythmically. When feeding and drinking the juvenile forages for food and water as separate commodities which are ingested with behaviors (licking, biting, chewing) that are more varied than sucking and distinctly different from it. Although both result in the ingestion of food and water the behaviors of suckling, on the one hand, and of feeding and drinking, on the other, are entirely different.

Research of the past decade on the newborn rat has yielded several insights into how this transition is made. First, the newborn rat is competent at birth to identify a maternal nipple by its odor (1, 2) and to contribute to the control of its milk intake by utilizing signals from the upper GI tract (3) while it remains attached to a nipple without interruption as long as the dam is with her litter (4). The mechanisms for these behaviors are well developed at birth. Second, as it matures, behaviors are added to the suckling's repertory that make it more competent for ingestion, both for suckling and for ingestion while away from the dam. At 8 or 10 days of age the animal's nutritive state gains control of its attachment behavior (5). Now it will detach itself from the nipple when fed and will shift from nipple to nipple while suckling (32) thereby maximizing its access to milk. Later it will eat its first food, the semisolid material called "ceacotrophe" that is produced in the mother's ceacum and is excreted with her feces (7), and it will drink water (8). Then, beginning at 3 weeks of age and continuing through weaning, it adopts the mother's nocturnal mode of ingestion (9) and will prefer foods that she and the other adults in the pack are eating (10). Sometime between its fourth and fifth postnatal week it graduates from the litter and begins its career of independent feeding and drinking.

And, third, recent research has shown that the suckling rat is capable of both feeding and drinking at very early ages. When only 2 or 3 days old it will drink water when made thirsty (11, 33) and can be induced to drink excess amounts of milk by electrical activation of the medial forebrain bundle (12). Shortly thereafter it will eat and drink from puddles at its feet (13), and, if kept warm and given easy access to liquids, it can be weaned as early as at 14 to 15 days of age (14). In all of these instances the pup is performing acts of adult ingestion while away from its dam. It is, in other words, surprisingly precocious in adult-like ingestive behavior when it is not suckling.

These last facts led my thinking in an unexpected direction, unexpected, at least, to someone like myself who began his research on this problem believing that adult ingestive behavior develops in a serial fashion out of suckling in analogy to crawling and walking in the human infant. On the contrary, I now believe that suckling and adult ingestion are different behaviors and must be mediated by different neurological systems that develop in parallel. The precocity of the neonate rat for feeding and drinking while still a suckling supports this view. Here, I am adopting ideas that were first expressed by Hall (20) then elaborated upon by Blass and Cramer (15), and are discussed by Hall and Williams in a recent, comprehensive review (31).

My contributions to these insights began with a series of experiments that were done with Katherine Houpt (3) in which we showed, (i) that newborn rats respond to deprivation from mother's milk by suckling more from her, and (ii) that in doing so their suckling is controlled by upper GI fullness. We therefore demonstrated the competence of the newborn

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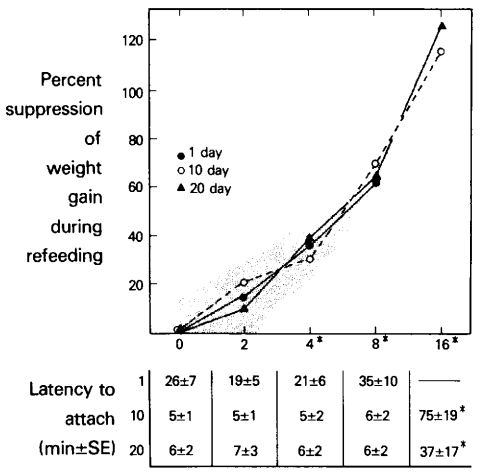
for control of its suckled milk intake. This fact has been confirmed recently and has been shown to be dependent on visceral afferents. That is, my colleagues and I (16) have repeated the Houpt experiments at ages 1 through 20 days with the same essential findings. Pups at all ages studied including the youngest age, adjusted their intakes of suckled mother's milk in precise appreciation of the contents of their upper GI tracts, as shown in Fig. 1 which is a summary of our experiments. In addition, this control by the pup of its own intake was lost by pups feeding away from their dams after their subdiaphragmatic viscera had been completely denervated. These pups were heedless of gut volume and they overate to the point of massive upper GI distension. We have therefore shown that the rat pup is equipped at birth with mechanisms for precise control of its own milk intake and that these mechanisms utilize distension signals from the upper GI tract.

Houpt and I also showed that feeding in response to glucoprivation (decreased intracellular glycolysis as produced by 2-DG (2-deoxy-D-glucose) does not control suckled intake (3). Increased intake of mother's milk is not produced by 2-DG at any time during the suckling period despite its effectiveness for elicitation of a hyperglycemic response as early as three days of age. Glucoprivation will accelerate the 21-day-old weanlings approach to food and will increase the juvenile's (40 to 45 days old) consumption when it is eating like an adult (3), that is, when it must consume solid food independently of the dam, but 2-DG does not increase the suckled intake of mother's milk. This work with 2-DG showed that the full complement of controls of feeding does not mature until the animal is on the verge of puberty and, in retrospect, that suckling and feeding are subject to different metabolic controls as would be expected for separate neurologic systems.



Latency to attach (min±SE)					
	1	26±7	19±5	21±6	35±10	—
	10	5±1	5±1	5±2	6±2	75±19*
	20	6±2	7±3	6±2	6±2	37±17*

FIG. 1. Above: Suppression of mother's milk intake (as suppression of weight gain) by pups 1, 10, and 20 days old suckling their dam immediately after upper GI preloads of milk (0, 2, 4, 8, 16% body wt, on horizontal axis). Below: latency of the same pups to reattach to their dam after the same treatments (ages 1, 10, 20 days, in horizontal rows as a function of same percentage preloads). *, $P < 0.05$. Stippling, SE of nearest mean. Note the precision with which pups suppress their intake, and the insensitivity of their latencies of attachment in response to upper GI loading. See Ref. (16).

Houpt's work therefore contained intimations of our present insights, namely: (i) competence for control of mother's milk intake by sucklings at birth, (ii) increasing complexity of ingestive behavior with advancing age, and (iii) separateness of suckling and adult ingestion. All of her work was with food intake. Jim Wirth and I (11) then studied the ontogeny of water drinking in the rat pup with results that again support our current views, especially the precocity of the pup for adult-like ingestion and the increasing complexity of its ingestive behavior with advancing age. We were able to do so because of his development of the first technique for study of ingestion by pups away from their dams. It is shown in Fig. 2 in which you see a 2-day-old rat being held at Wirth's "water-fountain" so that it can ingest water that is delivered directly into its mouth. Intake is measured by weight increases over short intervals. With this innovation we were able to study water drinking in pups of any age by subjecting them to thirst challenges just before offering them water at the fountain. Three challenges were used, all administered subcutaneously, with the results shown in Fig. 3 which are a striking illustration of the precocity of adult-like ingestive behavior by neonates that are away from their dams. Note first that all three challenges elicit water intake before the animals are 1-week old, that is, 2 weeks before they will be weaned and will be dependent on free water, and at a time when by ingesting milk their normal hydrational

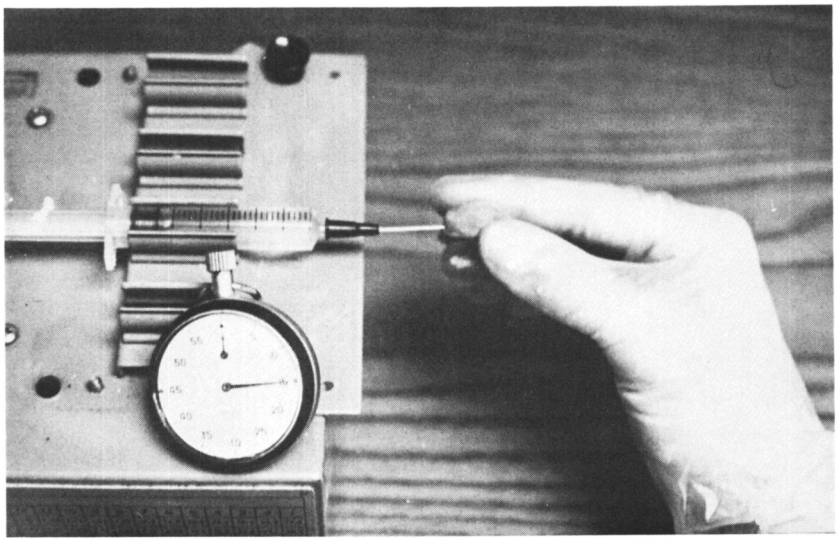


FIG. 2. Photo of Jim Wirth holding a 2-day-old pup at the "water fountain," his innovative technique for measurement of intake (milk or water) by pups of any age. See Ref. (11).

problem is one of water excess. We were struck in these early experiments with the fact that the pups drank the water rather than attempting to ingest it by sucking. That is, they licked and swallowed it as it flowed from the spout rather than closing their lips around the spout in order to suck on it. Evidence, therefore, in the behavior of the pup itself for the precocious expression of adult-like drinking behavior.

Note also in Fig. 3 that the three challenges become effective at different ages, cellular dehydration at 2 days of age, hypovolemia at 4, and release of renal renin at 6, a clear instance of increasing complexity with advancing age. That is, not only can newborn rats be made thirsty for water, but, the neurologic systems for the several kinds of thirst have different temporal programs of development.

Having discovered the precocious and sequential development of ingestive behaviors in newborn rats, and being interested in the control of adult ingestion by naturally occurring humoral agents (18), I have recently been studying the ontogeny of the neural mechanisms for the arousal of feeding and drinking by angiotensin and norepinephrine. This required intracranial injection into pups of the youngest ages. Because of the fragility of their crania and because of their rapid growth, conventional chronic implantation techniques

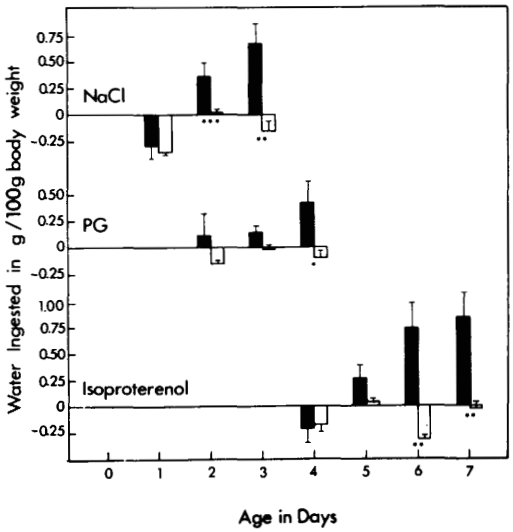


FIG. 3. Water intake from the "water fountain" of suckling rats as a function of age. Animals were given intermittent access to the fountain for 2 hr after each thirst challenge. Challenges: NaCl, 2.50 mosmole/100 g body wt; PG, 40% polyethylene glycol in isotonic saline, 1.25 ml/100 g body wt; Isoproternol, 500 μl/kg; all sc. $N = 6$ all groups. Filled bar, challenged pups; open bar, pups injected with isotonic saline. Significance begins with $P < 0.05$ (8) and graduates to $P < 0.001$ (***). Note that the pups drink to cellular dehydration (NaCl) at 2 days, to hypovolemia (PG) at 4 days, and to release of renal renin (isoproterenol) at 6 days. See Ref. (11).

were not practical. We therefore invented a technique that takes advantage of the rat pup's cartilaginous skull and of the insensitivity of the brain, and that yields an animal that is capable of normal behavior within seconds of an intracranial injection (19). The injection (1 μl) is made free-hand directly through the scalp and skull while the pup's head is held in a standard position between the experimenter's fingers. Anesthesia is unnecessary because the trauma is no greater than that which is produced by a parenteral injection in an adult rat. The animals can therefore be tested immediately after the injection is made. Half-strength india ink is the vehicle. It allows us to validate the locus of the injecta within the brain by autopsy immediately after behavioral test. This is accomplished by examination of the cut surface of a coronal section through the head, and, as is shown in Fig. 4, makes the success or failure of the injection obvious by visual inspection.

As in our earlier experiments with Wirth's water-fountain the pups are allowed to ingest water or milk while away from their dam and their individual intakes are measured by weight increases over short intervals of 1 hour or less. But we now use three improvements. One is the anterior oral catheter of Hall (20) which makes handling of individual pups unnecessary. The fluid is delivered by pulse infusions at overall rates that are greater than the pups can consume. Second, we cover the pup's perineum with a celloidin "diaper" that eliminates weight losses due to excretion of feces and urine. And third we satiate the pups for milk before testing them for the effects of our treatments.

The first results of these experiments are recalled in the Fig. 5 which shows the age of

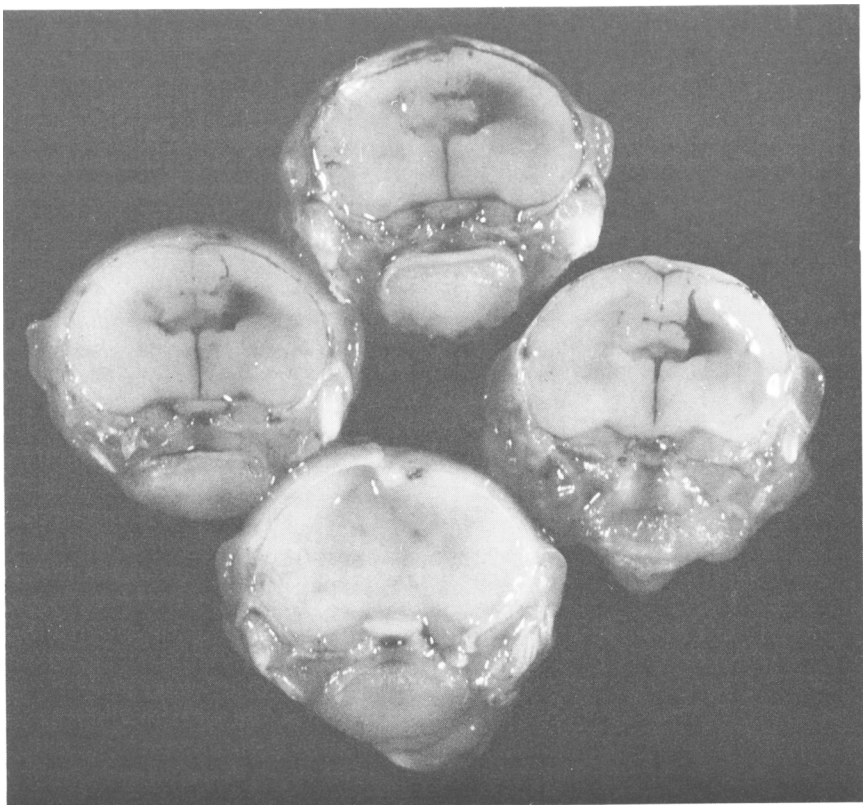


FIG. 4. Untouched photo of cut surface of pups' heads showing successful intraventricular injections (ICV) and an uninjected brain (below). Note the presence of injecta in lateral and third ventricles as revealed by india-ink vehicle. See Ref. (19).

onset of the dipsogenic effect of an injection of angiotensin II into the dorsal third ventricle. As you can see, rat pups appear to drink water in response to angiotensin at 2 days of age, then at between 4 and 5 days their response is clear and it has adult characteristics of sensitivity (they will drink to as little as 1 ng) and potency (the volumes drunk, as percentage of body weight, are equal to those drunk by adults when the same doses are used). Here, again, the precocity of the rat pup for adult-like ingestion is demonstrated.

Most recently, in work by Susan Ellis which will shortly be published in full (22), we have confirmed the early onset of two stages of angiotensin sensitivity (initial responding at 2 days, an abrupt increase to adult levels a 5 days). We suspect that these stages are an expression, in drinking behavior, of the serial maturation first, of the subfornical organ, the extra blood–brain barrier circumventricular organ that is necessary for drinking induced by blood-borne angiotensin (23) and which is structurally mature in the rat at birth (24), and then of the second angiotensin sensitive system which is in the brain parenchyma and which can also mediate the dipsogenic action of angiotension (25). Ellis and I have also found that at these early ages angiotensin-induced drinking is indiscriminate. The pups drink as much milk as water. The characteristic adult response of greater water than milk intake appears abruptly at 8 days of age.

These new techniques have allowed Ellis to discover that the orexigenic effect of norepinephrine (NE), first described in the adult rat by Grossman (26), has an abrupt onset at the transition from 9 to 10 days of age. As shown in Fig. 6, before that age NE (2 μl) injected into the ventral third ventricle has no effect on milk intake by pups fed intraorally while away from their dam. But when they are 10 days old or greater they ingest more milk after intraventricular NE despite having been satiated on it (by 40 to 60 min of intraoral infusion until they show satiation) just prior to the intracranial treatment. This interestingly is several days after adrenergic receptors are

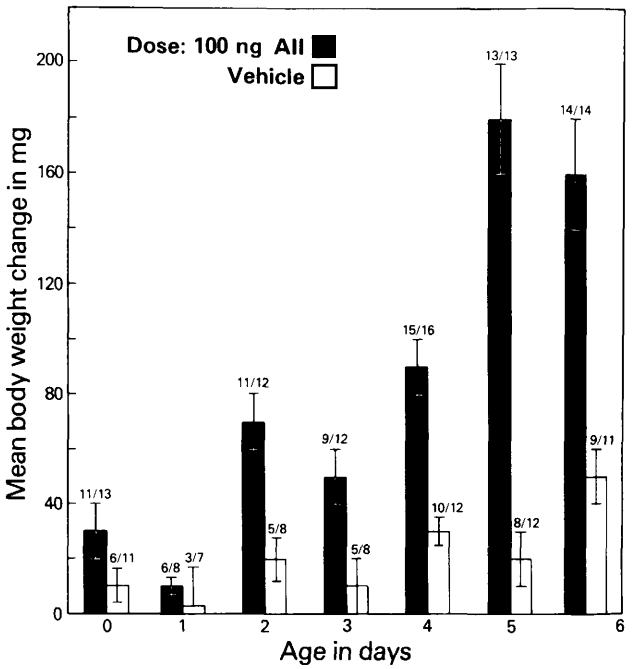


FIG. 5. The ontogeny of angiotensin-induced water drinking. The bars are water intakes (as increases in body wt = SE) produced by 100 ng of angiotensin II (filled bars) or an equal volume of isotonic saline (open bars) injected into the anterior cerebral ventricles of pups 0 through 6 days of age. Animals were offered water at the Wirth "water fountain" for 3 min after injection. Numbers above bars: denominator = number tested at each age, numerator = number that drank (gained weight). See Ref. (19).

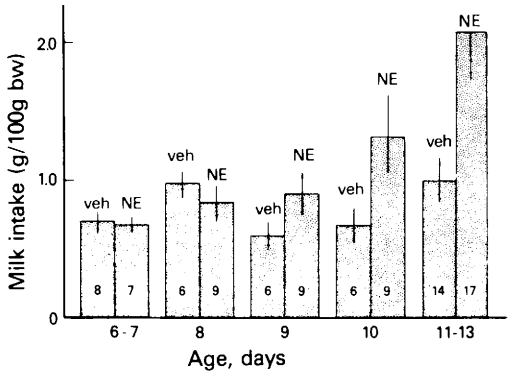


FIG. 6. Ontogeny of norepinephrine-induced eating. Bars are milk intakes of pups receiving pulsatile intraoral infusion for 1 hr immediately after ICV injection of 2 μl of norepinephrine (NE) or of isotonic saline (veh) into their third cerebral ventricles. All were satiated on milk before injection (milk preinjection). Inserted numbers are *N*'s. Note the abrupt onset of NE's orexigenic effect at 10 days. From Ellis *et al.* (21).

first found in the developing rat forebrain (27). Water intake was uneffected by intracranial NE at all ages.

And, last, this work provides our clearest evidence for the separateness of the suckling and feeding neurological systems. Recall that the orexigenic effect of intracranial NE shown above was obtained from pups that were not suckling. They had been isolated from their dams and were licking and swallowing milk infused into their mouths. When Ellis repeated the NE injections and allowed the pups to suckle, intake was not increased. That is, when removed from a litter, injected, and then returned to their dam to suckle they did not ingest more mother's milk even, as is shown in Fig. 7, when they had been prefed to satiation so that they were treated exactly like the pups that were fed by oral infusion. The orexigenic effect of intracranial NE is *not* expressed in suckling behavior. In exact analogy, amphetamine is anorexigenic in 5-day-old rats, but only when they are feeding away from their dam (28). The drug does not decrease their suckling behavior until they are 15 days old.

The work reviewed here makes some contribution to the problem of how mammals make the transition from suckers to free feeders and drinkers. It suggests that, as the result of *in utero* development, they are born with a complex and as yet incomplete neurologic system for suckling which matures postnatally and controls their ingestive behavior until weaning begins. But suckling does not appear to be the developmental prelude to adult ingestion. Instead a second neurologic system for ingestion appears to be nascent in the suckling. It seems to be developing in the neonate, without expression in behavior, in order to succeed suckling when the weanling leaves the dam. It is sufficiently advanced in development to be utilized during the suckling period for consumption of either food or water at very early ages but only when the pup is required by isolation from its dam to utilize acts of adult ingestion, and it is controlled by norepinephrine and amphetamine just as is adult feeding. Several determinants of thirst (cellular dehydration, hypovolemia, angiotensin) also control it, as shown by our earlier work, and we expect that they will be ineffective when the pup is suckling, but these experiments have not yet been completed.

We believe, therefore, that suckling is not

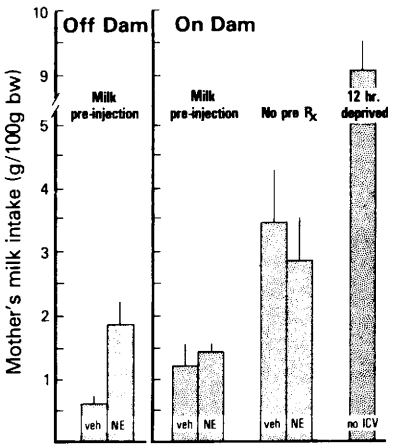


FIG. 7. On the right: Lack of effect of ICV NE on suckling. NE does not increase mother's milk intake of pups suckling from their dam—in pups that are sated on milk (Milk preinjection) and in those that are not (No pretreatment). Twelve-hour deprived pups not treated with ICV NE ingest almost 9% of their body weight in milk from the dam in the 1-hr test demonstrating that the lack of effect of NE in the other groups is not due to limited supply of mother's milk. On the left: The orexigenic effect of ICV NE in pups ingesting milk by intraoral infusion while away from the dam for comparison. From Ellis *et al.* (21).

the kindergarten for adult feeding and drinking. They do not develop from it like walking from crawling. Instead suckling appears to be the analog of neonatal walking or stepping which is done by newborn humans whose head and trunk are supported upright while the plantar surfaces of their feet touch the ground (29). Such infants make rhythmic stepping movements and extend their legs with sufficient strength to support themselves while they walk forward. But this behavior disappears from the infant's repertory at from 6 weeks to 4 months of age at which time they flex their legs when held upright with feet in contact with the ground. Infant neonatal walking is now replaced by crawling which is the true prelude to walking. It is not uncommon for vertebrates to have dual neurologic mechanisms for locomotion. Think of how many birds both walk and fly, and some of them do one (usually walk) before the other. Amphibians swim as larvae and then walk or hop as adults, and again they do so in developmental sequence (30).

We are suggesting that in mammals the neural mechanisms for ingestion are similarly organized. There are two of them. Each has its own neurology, and each has its own set of afferent and neurochemical controls. And each has its own developmental schedule such that suckling is expressed first and is then suppressed at weaning (31) as it is replaced by feeding and drinking which then emerge as the animal's exclusive ingestive behaviors.

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Elliott Blass and Ted Hall were generous in their criticism of this essay. Their colleagueship has been invaluable to me, not only in the preparation of this review, but more importantly in the progress of this research in which we have a common interest.

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