5

The Developmental Context of Thermal Homeostasis

MARK S. BLUMBERG

INTRODUCTION

Developmentalists are concerned with origins. For some, the search for origins in developing animals provides little more than an opportunity to clarify the factors that contribute to adult behavior and physiology. Although this is an understandable justification for developmental research, it is also important to understand that developing organisms are not simply small adults or adults-in-waiting. On the contrary, infant animals face many problems that are unique to their physical, physiological, social, and ecological circumstances (Alberts & Cramer, 1988; Hall & Oppenheim, 1987; West, King, & Arberg, 1988). These problems cannot be put off; rather, to survive, infants must solve each problem as it is encountered during ontogeny. Therefore, the "dual infant" must meet the needs of the moment as well as prepare for later life, a vital combination of adaptation and anticipation (Alberts & Cramer, 1988).

Because some of the problems faced by infants are transient, some of the solutions are transient as well, and these solutions are referred to as ontogenetic adaptations (Alberts, 1987; Hall & Oppenheim, 1987). Although a central theme of developmental psychobiology, the concept of an ontogenetic adaptation can be easily misused, as can any adaptationist concept (Williams, 1966). Nonetheless, there is no other concept that so powerfully captures the temporary utility of the umbilical cord or of suckling behavior. There is also no other concept that so simply reminds us of the dangers of judging the physiological and behavioral capabilities of infants against an adult standard. When holding infants to adult standards, we lose sight of

MARK S. BLUMBERG Department of Psychology, University of Iowa, Iowa City, Iowa 52242.

Developmental Psychobiology, Volume 13 of *Handbook of Behavioral Neurobiology*, edited by Elliott Blass, Kluwer Academic / Plenum Publishers, New York, 2001.

the context of development and the necessity of using appropriate experimental tools and methods to reveal the organizational complexity of our infant subjects.

This chapter examines a recent transformation in our understanding of the homeostatic control of body temperature in developing infants and the physiological and behavioral implications of endothermy. This chapter especially focuses on a specialized thermogenic organ called brown adipose tissue, the source of endothermy in the infants of many mammalian species. Contrary to a long-standing perspective, it will become clear that thermogenesis by brown adipose tissue during cold exposure protects and modulates diverse aspects of the infant rat's physiology and behavior. The picture of infant thermoregulation that is now emerging reveals previously unappreciated competence and complexity and points the way to a richer understanding of the developmental and evolutionary significance of endothermy. Despite these broad implications, the focus of this chapter is necessarily limited. Therefore, for broader perspectives on physiological and behavioral thermoregulation in infants and the influence of temperature on morphology and function during development, the reviews of Leon (1986), Brück and Hinckel (1996), Satinoff (1991, 1996), and Blumberg and Sokoloff (1998) are of interest.

Homeostasis, Homeothermy, and Development

Early Efforts

Thermoregulation in adult mammals is a classic example of a homeostatic process and was addressed at length in W. B. Cannon's (1932) treatise, *The Wisdom of the Body*. Cannon's ideas emerged from the foundation provided by Claude Bernard, who first described the compensatory physiological processes that establish constancy in the internal environment. In coining the term *homeostasis*, Cannon aimed to call attention to those "coordinated physiological processes which maintain most of the steady states in the organism [and which] are so complex and so peculiar to living beings" (p. 24). Furthermore, although he titled his chapter on thermoregulation "The constancy of body temperature," he was careful to stress that homeostasis denotes a condition of *relative constancy*, not stagnation, and that this relative constancy arises from "the various physiological arrangements which serve to restore the normal state when it has been disturbed" (p. 25). The early focus on physiological mechanisms was offset somewhat when, beginning with the work of Curt Richter, the contributions of behavior to homeostasis also came to be appreciated (Richter, 1943; see also Bartholomew, 1964, for a forceful endorsement of an integrative approach to homeostasis).

The publication of Wiener's (1961) book on cybernetics and the explanatory power of control systems theory (Houk, 1988) consolidated a connection between homeostasis and a few specific mechanisms derived from control theory. W. B. Cannon (1932) himself contributed to this connection when, in his chapter on body temperature, he stated that the "delicate control of body temperature indicates that somewhere in the organism a *sensitive thermostat* exists" and that this mechanism "is located in the base of the brain, in the diencephalon" (p. 199, italics added). The promise of control systems theory seemed to be realized when investigators began providing explicit mathematical descriptions of the set point mechanism (e.g., Hammel, Jackson, Stolwijk, Hardy, & Strömme, 1963). Over the years, however, numerous authors have cautioned against the reflexive invocation and reification of

such control-theoretic concepts as set point and negative feedback, and have emphasized instead the hierarchical, dynamic control of interacting homeostatic mechanisms (e.g., Booth, 1980; Hogan, 1980; Mrosovsky, 1990; Satinoff, 1978). Thus, given that stability in some physiological systems can be achieved without comparators or feedback loops, it is argued that the concept of homeostasis provides a functional description of a regulatory system but does not proscribe the mechanisms involved (Hogan, 1980).

Developmental studies of homeostasis have been relatively rare. Nonetheless, in the field of thermoregulation, over 50 years of research has contributed to a perspective according to which infant mammals, especially those of altricial species such as the Norway rat, are homeostatically immature. This perspective was the logical outcome of the use of a particular methodology that, in turn, derived from a theoretical stance that was fundamentally nondevelopmental. That methodology entailed the isolation of an individual infant rat in a cold environment (e.g., 15°C: Brody, 1943; 10°C: Hahn, 1956) and the measurement of rectal temperature throughout the period of isolation. Pups of different ages were tested using identical procedures and in this way the researcher tracked the "development of homeothermy," that is, the development of a high and stable body temperature. For example, using this experimental approach, Hahn (1956) concluded that "physical thermoregulatory mechanisms develop between the 14th and 18th day" postpartum (p. 430). In a similar vein, Brody (1943) stated that it "is generally known that human infants, and young mammals of many species, do not possess the ability to maintain a constant body temperature; their homeothermic mechanisms are not well developed" (p. 230).

With the benefit of hindsight, we can identify the fundamental flaws in these early studies. First, the exposure of pups of different ages to the same *arbitrary* air temperature structured the outcome of the experiments. For example, if Hahn (1956) had exposed all pups to 0°C rather than 10°C, homeothermy would have appeared to develop at a later age; conversely, if he had exposed all pups to 30°C, homeothermy would have appeared to develop at an earlier age. Although this criticism may now seem obvious, it was not obvious to earlier researchers because their interest was not in the regulatory capabilities of infant rats but in the attainment of a particular *adult characteristic.* The notion that infant capabilities reflect "a balance between meeting the needs of the moment and preparing for later life" (Hall & Oppenheim, 1987, p. 95) was not yet appreciated.

A related, and perhaps more fundamental flaw in the approach of Brody (1943) and Hahn (1956) is the confusion of process (i.e., homeostatic regulation) and product (i.e., homeothermy). Exposing pups of different ages to the same cold environment provides a measure of the development of insulation (i.e., fur and subcutaneous fat) or, in other words, the developing control of heat loss. The rate of heat loss, however, is largely (but not entirely) a passive feature of an organism; for example, a dead elephant may have more thermal inertia than a dead mouse and thus may cool more slowly, but we do not conclude that the dead elephant, even in the short term, is exhibiting homeostasis. On the contrary, we reserve the concept of homeostasis for the correction of a system variable in response to a disturbance and, in the case of isolated infant rats, such mechanisms primarily involve the activation and deactivation of heat production, not the regulation of heat loss. Therefore, early investigations of the development of homeothermy provided little information about the development or control of homeostatic mechanisms.

The confusion between homeostasis and homeothermy was exacerbated fur-

ther by the method of exposing pups to, what is for younger infants, an extremely cold environment. As was eventually learned, exposure to extreme cold and the rapid heat loss that results overwhelms the infant's ability to produce heat endogenously. It was only as investigators began testing pups in moderately cold environments that it was found that a newborn rat can increase metabolic heat production (Taylor, 1960). This point was brought home most clearly by Conklin and Heggeness (1971) when they stated that their infant subjects were tested at air temperatures "that would evoke, but not overwhelm, regulatory functions" (p. 333).

Brown Adipose Tissue

Although Taylor (1960) demonstrated that newborn rats can increase metabolic heat production in the cold, the source of this heat production was still not known. At that time, physiologists recognized two forms of heat production—shivering and nonshivering thermogenesis. Although a chemical, or nonshivering, form of thermogenesis was well established by the 1950s (Hsieh, Carlson, & Gray, 1957), the source of this heat production remained a mystery until Smith's (1961) suggestion that it was brown adipose tissue (BAT). In short order, investigators were using a variety of pharmacologic and physiological techniques to demonstrate that, during cold exposure, BAT is an important and sometimes exclusive source of heat production in the newborns of a number of species including rabbits, guinea pigs, and humans (e.g., Brück & Wünnenberg, 1970; Dawkins & Scopes, 1965; Hull & Segall, 1965). Over the next three decades, the "hibernating gland" first described by Gesner in the 16th century (Smith & Horwitz, 1969) was to become a focus of considerable scientific interest.

Distribution and Anatomy.

Brown fat has been reported in a wide variety of mammalian species but not in birds (Johnston, 1971; Smith & Horwitz, 1969). Among mammals, BAT is found most notably in newborns, hibernators, and cold-acclimated adults. In rodents, BAT is distributed throughout the body but is primarily concentrated in large lobes surrounding the thoracic cavity (Smith & Horwitz, 1969). The largest deposits are the interscapular pad (iBAT), which is easily identified in newborn rats as a butterfly-shaped mass lying just under the skin in the interscapular region, and the superior cervical pad, which overlies the cervical spinal cord and is separated from the interscapular pad by layers of muscle.

It has been noted that the various deposits of BAT appear ideally suited for the warming of venous blood returning from the periphery (Smith, 1964). Furthermore, iBAT appears to exhibit a unique vascular anatomy; specifically, the bilateral arterial vessels supplying iBAT lie next to corresponding veins, thus allowing countercurrent heat exchange between the arteries and veins (for a lucid introduction to countercurrent exchange mechanisms, see Schmidt-Nielsen, 1981). This countercurrent system can be bypassed by diverting venous blood flowing from BAT to the Sulzer vein, a large, unpaired vessel that returns warmed blood directly to the heart (Smith & Roberts, 1964). There is little information that bears directly on the neural control of venous outflow from iBAT.

The location of iBAT in the week-old rat and the thermal gradient established by BAT thermogenesis are shown in Figure 1. This figure presents an infrared thermograph of the dorsal surface of a week-old pup that has been isolated at room temperature (~22°C). The white zone at the center of the thermograph overlies

iBAT and indicates the region of highest temperature. Furthermore, the thermal gradient from the interscapular region to the base of the tail is substantial, approximately 2.5°C over a distance of approximately 3.5 cm. Therefore, this thermograph makes clear that heat production, at least as viewed from the skin surface, is localized to the thoracic region.

BIOCHEMISTRY AND PHARMACOLOGY. Brown fat gets its name from its high concentration of mitochondria filled with cytochrome C. When a lipid-filled brown adipocyte is stimulated by norepinephrine, a chain of events ensues in which free fatty acids are used by the mitochondria as substrate for heat production. Moreover, the free fatty acids may also participate in activating or modulating the activity of thermogenin, the uncoupling protein that is necessary for mitochondrial heat production (B. Cannon, Jacobsson, Rehnmark, & Nedergaard, 1996). Thermogenin is believed to be the rate-limiting factor for BAT thermogenesis (Nedergaard, Connolly, & Cannon, 1986).

Although it has been known for many years that norepinephrine stimulates BAT thermogenesis, the receptor subtype responsible for this effect was unknown until recently. It now appears that activation of the β_3 adrenoceptor, a subtype found both on white and brown adipocytes, triggers the liberation of free fatty acids in the brown adipocyte, which in turn initiates the cascade of events culminating in heat production (B. Cannon *et al.*, 1996; Zhao, Unelius, Bengtsson, Cannon, & Nedergaard, 1994). The contributions of other adrenergic receptor subtypes to BAT thermogenesis are still less well understood, although it appears that α_1 and β_1 receptors participate in brown fat cell proliferation (B. Cannon *et al.*, 1996). In addition, blood flow to iBAT, which increases appreciably during heat production to sustain the 10- to 40-fold increase in cellular respiration, may be modulated via α-adrenergic receptors on the vascular smooth muscle of BAT (Girardier & Seydoux, 1986).

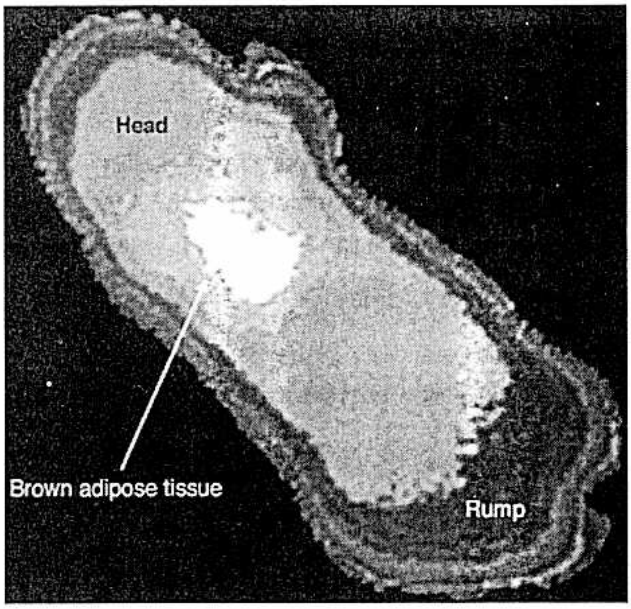

Figure 1. Infrared thermograph of the dorsal skin surface of a week-old rat during cold exposure. The region of highest temperature is coded in white, with decreasing skin temperatures coded by successively darker shades. The white region overlies the interscapular deposit of brown adipose tissue. A thermal gradient of approximately 2.5°C extends from the interscapular region to the base of the tail.

NEURAL CONTROL. It has been long recognized that BAT thermogenesis is under sympathetic nervous system control, although only the interscapular pad has been studied extensively (Girardier & Seydoux, 1986). In iBAT, each brown adipocyte is surrounded by fine fibers whose varicosities are filled with norepinephrine. These fibers arise from five pairs of nerves that enter the tissue after emerging from the intercostal muscles; these nerves originate in the first five thoracic ganglia. A final pair of perivascular nerves terminate along the arterial supply to iBAT. As would be expected from physiological studies of BAT thermogenesis, functional innervation of iBAT is present in newborn rats (Schneider-Picard & Girardier, 1982).

The central nervous system circuitry governing iBAT activation is largely unknown. Interest in brown fat as a regulator of energy balance has led some to investigate hypothalamic structures implicated in feeding. Specifically, it has been shown that electrical (Freeman & Wellman, 1987) or pharmacologic (Amir, 1990) stimulation of the periventricular nucleus in adult rats induces thermogenesis by iBAT. Investigations of the neural control of BAT at other levels of the neuraxis, however, suggest hierarchical control with multiple excitatory and inhibitory outputs. For example, decerebration experiments in anesthetized adult rats indicate a locus of inhibitory control in the region of the lower midbrain and the upper pons (Rothwell, Stock, & Thexton, 1983; Shibata, Benzi, Seydoux, & Girardier, 1987). This inhibitory influence is already present in infant rats (Bignall, Heggeness, & Palmer, 1975; Blumberg, Schalk, & Sokoloff, 1995). In addition, even in 1-day-old rats, a second locus of inhibitory control appears to exist in the basal forebrain (Blumberg *et al.*, 1995). Unfortunately, beyond this relatively crude outline, we have little understanding of the input/output relations of this hierarchically organized system, and we also know little about the neural control of the multitude of other BAT deposits throughout the body.

BROWN FAT THERMOGENESIS AND HOMEOTHERMY

An explosion of interest in brown adipose tissue over the last decade derives from its hypothesized role in the regulation of energy balance and feeding behavior and the onset of obesity (Lowell *et al.*, 1993). Our main concern here, however, is with the behavioral and physiological significance of BAT thermogenesis for infant animals; ironically, our increasing understanding of BAT as a thermogenic organ over the last 30 years has not substantially modified the prevailing view that cold-exposed altricial infants exhibit many of the characteristics of poikilothermy (e.g., Johanson, 1979; Satinoff, 1996).

The perspective that altricial infant mammals are poikilothermic reflects a number of common assumptions, some implicit and some explicit. First, Alberts' (1978) demonstration that rat pups within a litter actively huddle and, by doing so, reap substantial metabolic savings during cold exposure placed a new emphasis on the thermoregulatory capacities of the group while seemingly clarifying and justifying the deficiencies of the individual. Second, the conclusion that BAT thermogenesis is nearly useless for the individual is based upon the cold-exposed pup's inability to maintain *rectal* temperature at *adult* levels. Implicit in this conclusion is the notion that heat, whatever its source of production, is dispersed uniformly throughout the body core. Finally, when the concepts of homeothermy and homeostasis are commingled, it is not difficult to understand how a falling rectal temperature in the cold can be interpreted as evidence that homeostatic mechanisms are also absent.

Some investigators have departed somewhat from the above perspective. Brück and Hinckel (1996), for example, are particularly clear:

> Temperature regulation is frequently referred to as immature at the time of birth. However, one should be very cautious in asserting immaturity of the thermoregulatory mechanisms, even though the neonate shows more fluctuations in body temperature than does the adult. Greater fluctuations of body temperature are to be expected in smaller organisms because of their large surface area/volume ratio, the relatively small insulating body shell, and the smaller body mass that acts as a heat buffer in large organisms. Because of these peculiarities in body size and shape, a *reduced range of regulation* may be expected in the neonate. (p. 602, italics added)

It appears, however, that Brück and Hinckel (1996) are specifically directing the above comments toward the precocial infant mammals (e.g., guinea pig, lamb) that are typically larger in size when born, with fur, open eyes, significant behavioral mobility, and the capability of producing heat using shivering and nonshivering thermogenesis. In contrast, when discussing altricial species (e.g., rat, rabbit) they state that "either the capacity of the effector systems or the threshold temperatures or both are not sufficiently adjusted to the smaller body size. *Body temperature drops on exposure to environmental temperatures slightly less than thermal neutrality*" (p. 603, italics added). Although this statement is not inaccurate, we will see that the physiological and behavioral responses of even altricial infants within their "reduced range of regulation" are more interesting and complex than was previously thought.

A New View of Thermoregulatory Competence in Infant Rats

Thermoregulatory Effectors

Temperature is a fundamental physical property that modulates nearly all chemical and biological activity. Perhaps the most obvious expression of temperature's central biological importance is the lawful, exponential relationship between temperature and cellular metabolism. This relationship generally expresses itself as a doubling or tripling of metabolic rate with each 10°C increase in ambient temperature (Schmidt-Nielsen, 1991). Although the vast majority of the earth's biomass is constrained by this lawful relationship, some animals have responded to this constraint by evolving diverse physiological and behavioral mechanisms for controlling heat exchange between the internal and external environments.

Because of their relatively large surface-to-volume ratio, infant mammals are primarily concerned with problems of heat loss. In the case of newborn rats, lack of fur and subcutaneous fat add to the problem of small size to pose a continual threat of heat loss and hypothermia. This problem is countered in rats and other altricial species through the production of large litters, which provides each individual pup with huddling littermates (Alberts, 1978). Because the surface area of a huddle of pups is less than the cumulative surface areas of the individual pups, huddling provides a behavioral means of reducing the individual's radiative heat loss at any given air temperature. Heat loss is further reduced by the construction and maintenance of an insulating nest by the mother (Jans & Leon, 1983; Kinder, 1927).

In addition to behavior, infant mammals possess a variety of physiological mechanisms that contribute to survival in the cold. Chief among these mechanisms is nonshivering thermogenesis by BAT, which we discussed at length above. In addition to nonshivering thermogenesis, precocial infants and older altricial infants also exhibit shivering thermogenesis, a prominent form of heat production in adult

mammals (Brück & Wünnenberg, 1970; Taylor, 1960). For reasons that are not clear, however, nonshivering thermogenesis appears to be the favored form of heat production in young mammals. For example, in newborn guinea pigs, heat production by BAT suppresses the expression of shivering by inhibiting neural elements in the cervical spinal cord (Brück & Wünnenberg, 1970).

While most thermoregulatory effectors "evolved out of systems that were originally used for other purposes" (Satinoff, 1978, p. 21), BAT thermogenesis may be the rare exception. Thus, while shivering entails a unique, desynchronized form of thermogenic muscle activity and peripheral vasomotor activity entails the use of the circulatory system for modulating heat flow to the external environment, BAT likely evolved primarily as a thermogenic organ. In infant rats, at least until the age of 10 days, BAT is the sole source of heat production (Taylor, 1960) and is therefore the primary focus of the research described below.

Responses of Isolated Infants to Thermal Challenge

Conceptual and Methodological Considerations.

Adult mammals use a diversity of heat gain and heat loss mechanisms, both physiological and behavioral, to maintain a stable core temperature under a wide range of environmental conditions. This range is restricted, however, in infant mammals, especially those of altricial species, by small body size, poor insulation, limited locomotor abilities, and inadequate physiological mechanisms for heat loss and gain. With age, and as body size, insulation, and thermal stability increase, the range of air temperatures tolerated by the infant expands. These thermal factors contribute to the process by which pups engage in repeated egressions from the nest and eventually are weaned (Gerrish & Alberts, 1996).

Although much has been made of the thermoregulatory limitations of infant rats, these deficits should be interpreted in context. That context includes the habitat in which pups are reared, consisting of external sources of heat (i.e., littermates and mother) and thermal buffering from the ambient environment (i.e., huddle, nest, and burrow). When those external sources of heat and insulation are removed, the physiological capabilities of the infant can be probed; in doing so, however, we cannot use the same thermal challenges that we would use to probe the thermoregulatory capabilities of a huddle. Rather, we must scale the thermal challenges to the size and insulation of the individual. Again, as Conklin and Heggeness (1971) stressed, if our aim is to understand regulation, then an animal should be tested in conditions that evoke regulatory functions without overwhelming them. Thus, is it possible that the common conclusion that infant rats "quickly become hypothermic at ambient temperatures below their thermoneutral zone" (Satinoff, 1991, p. 171) is derived from testing conditions that overwhelm their regulatory capabilities?

The answer to this last question is "no": even when infant rats are gradually exposed to cold environments and heat production is observed to increase gradually, rectal temperature nonetheless decreases (e.g., Conklin & Heggeness, 1971). Thus, although it may be somewhat of an exaggeration to say that an "individual newborn rat's rectal temperature closely approximates the surrounding temperature" (Satinoff, 1991, p. 172), it is clearly the case that even moderate decreases in air temperature elicit what is, by common definition, a state of hypothermia.

Common definitions and concepts, however, may not be adequate here. Specifically, it is not necessarily the case that rectal temperature is the variable of interest for

assessing the thermoregulatory success of infant rats. First, even though rectal temperature can sometimes provide valuable information about the balancing of heat gain and heat loss, its value is limited when trying to assess the dynamics of the processes involved (similarly, it would be difficult to understand the operating principles of Hoover Dam by simply monitoring the water level downstream). Second, even in adults, rectal temperature is not always the most relevant measure for assessing thermoregulatory mechanisms (Lovegrove, Heldmaier, & Ruf, 1991). For example, the singular importance of rectal temperature is contradicted by the fact that many avian and mammalian species are capable of selectively regulating brain temperature (Baker, 1979; Caputa, 1984). In other words, there is no single body temperature that is likely to provide a comprehensive description of an animal's thermal state.

In our attempt to reassess the thermoregulatory capabilities of infant rats, we evaluated the impact of a series of air temperatures on individual pups. Our approach identifies three ranges of air temperature, the exact specifications of which depend on the species, strain, age, and body size of the infant, in addition to other factors. The *thermoneutral zone,* or the zone of least thermoregulatory effort, includes those air temperatures at which an animal does not exhibit increased metabolic heat production (Satinoff, 1996); typically, oxygen consumption is minimal within this zone. As air temperature decreases, a point is reached where heat production and oxygen consumption begin to increase; this point is called the lower critical temperature and, for a week-old rat, is approximately 34°C (Spiers & Adair, 1986). As air temperature decreases further, oxygen consumption increases progressively until an air temperature is reached where it no longer increases; for a week-old rat, this occurs at an air temperature of approximately 25°C. The range of air temperatures defined from the lower critical temperature to the point of maximal oxygen consumption has been designated as *moderate.* Finally, air temperatures below the moderate zone have been designated as *extreme.*

In addition to clearly operationalizing the definitions of moderate and extreme cold exposure, it was also important to reevaluate the logic of designating rectal temperature as the variable by which to judge thermoregulatory success or failure. Given that compartmentalization of heat is now known to be a common thermoregulatory strategy, and keeping in mind that infant rats generate heat primarily in the BAT deposits in the interscapular–cervical region, it could be argued that measuring temperature in that region of the body, at the source of heat production, might provide greater insight into the regulatory dynamics of the system. Furthermore, just as moths selectively warm their thorax before taking flight (Heinrich, 1993), perhaps infant rats selectively warm their thorax during cold exposure. If so, then rectal temperature might prove to be an inadequate measure of the thermoregulatory concerns of infant rats and we would once again see that concepts derived from research on adults are sometimes inappropriately applied to infants. Of course, the flip side of this latter notion is also important: that the new concepts derived from developmental research can in turn inform our understanding of the more complex and interwoven systems of adults.

Physiological and Behavioral Responses to Cold Exposure. A guiding theme of the research described below is that BAT thermogenesis modulates the physiological and behavioral responses of infant rats during cold exposure. In developing this theme, it has been useful to perform experiments on infants that lack the ability to produce heat endothermically. One obvious and effective strategy,

discussed below, is to block BAT thermogenesis pharmacologically in infant rats; specifically, we have used chlorisondamine, a ganglionic blocker, to prevent neural activation of BAT during cold exposure (e.g., Sokoloff & Blumberg, 1998). Of course, pharmacological interventions are never as clean as one would like; specificity is always an issue and a specific antagonist for the β_3 adrenoceptor is not yet available. In addition, techniques for denervating BAT, either chemically or surgically, suffer from limitations as well (Girardier & Seydoux, 1986).

Pharmacologic or surgical interventions are not the only experimental options available for assessing the importance of a given structure or system for a physiological or behavioral process. The comparative method also provides a useful framework for such investigations by specifying a procedure for comparing different species that differ along a dimension of interest to the investigator. Syrian golden hamsters (*Mesocricetus auratus*) were chosen by us as a comparison species because they do not develop the capacity for BAT thermogenesis until they are 2 weeks of age (Hissa, 1968; Sundin, Herron, & Cannon, 1981). In other words, unmanipulated infant hamsters exhibit a natural "blockade" of BAT thermogenesis, thus providing a second avenue for assessing the contributions of BAT thermogenesis to the infant's physiological and behavioral responses to cold.

Of course, infant golden hamsters differ from rats on dimensions other than the ability to activate BAT thermogenesis. Their gestation length of just 16 days is 6 days shorter than that of rats; nonetheless, they are relatively precocial with respect to rats in that they locomote more effectively at birth and become independent feeders, develop fur, and open their eyes at an earlier postnatal age (Daly, 1976; Schoenfeld & Leonard, 1985). Their designation by some as an "immature" species (e.g., Nedergaard *et al.*, 1986) reflects the fact that they are not easily designated either as a precocial or altricial species. Thus, one challenge for our understanding of species differences in infant thermoregulation is to assess which developmental features are related to the presence or absence of endothermy.

Brown Fat Thermogenesis and the Significance of Interscapular Temperature. In rats, BAT depots are located in a number of places throughout the body, but the largest are the cervical and interscapular BAT pads (Smith & Horwitz, 1969). Under most circumstances, activation of heat production by BAT can be inferred from (1) a significant difference between skin temperatures measured in the interscapular and lumbar regions and (2) increased oxygen consumption (Blumberg & Stolba, 1996; Heim & Hull, 1966). The usefulness of these measures is illustrated in Figure 2, which presents the real-time thermoregulatory responses of two individual week-old rats acclimated at a thermoneutral air temperature (i.e., 35°C) and then challenged at either a moderate (30°C) or extreme (21°C) air temperature. These plots demonstrate that thermal challenges evoke pronounced increases in BAT thermogenesis, as indicated by the increasing difference between interscapular temperature (T_{is}) and a neutral skin temperature measured in the lumbar region (T_{back}), coupled with an increase in oxygen consumption. It can also be seen that while moderate cold exposure results in a fall in interscapular temperature, this fall is less than that which occurs during extreme cold exposure. Moreover, the moderately cooled pup stabilizes its interscapular temperature without having to maximize heat production.

We can compress much of the information contained in Figure 2 into a two-dimensional state space in which oxygen consumption is plotted against interscapular temperature. Figure 3 presents five such plots for individual week-old rats that were exposed to varying degrees of cold challenge. Relative to Figure 2, these state

space plots do not convey some relevant information (e.g., time, air temperature); they do, however, readily capture the orderly relationships between the metabolic and thermal dimensions of BAT thermogenesis. Most importantly for this discussion, these plots reveal at a glance the fundamental distinction between moderate and extreme cold exposure. In addition, these state space plots help us to focus upon and appreciate the regulatory processes that underlie BAT thermogenesis.

The two upper plots in Figure 3 present the data from two week-old rats. In both cases, the pup was acclimated at an air temperature of 35°C; as indicated on the state space, each pup settled at a high interscapular temperature (~38°C) and a low rate of oxygen consumption (~4 ml O_2/100 g/min). After the acclimation period, air temperature was decreased to 30°C or 27°C and the two pups exhibit similar trajectories; in both cases, interscapular temperature falls passively before oxygen consumption increases (the horizontal portion of the plots). In contrast, when oxygen consumption begins to increase at an interscapular temperature of approximately 36.5°C, the trajectories now follow vertical paths. The verticality of the trajectories indicates that the pups are modulating heat production such that the temperature of iBAT remains relatively constant.

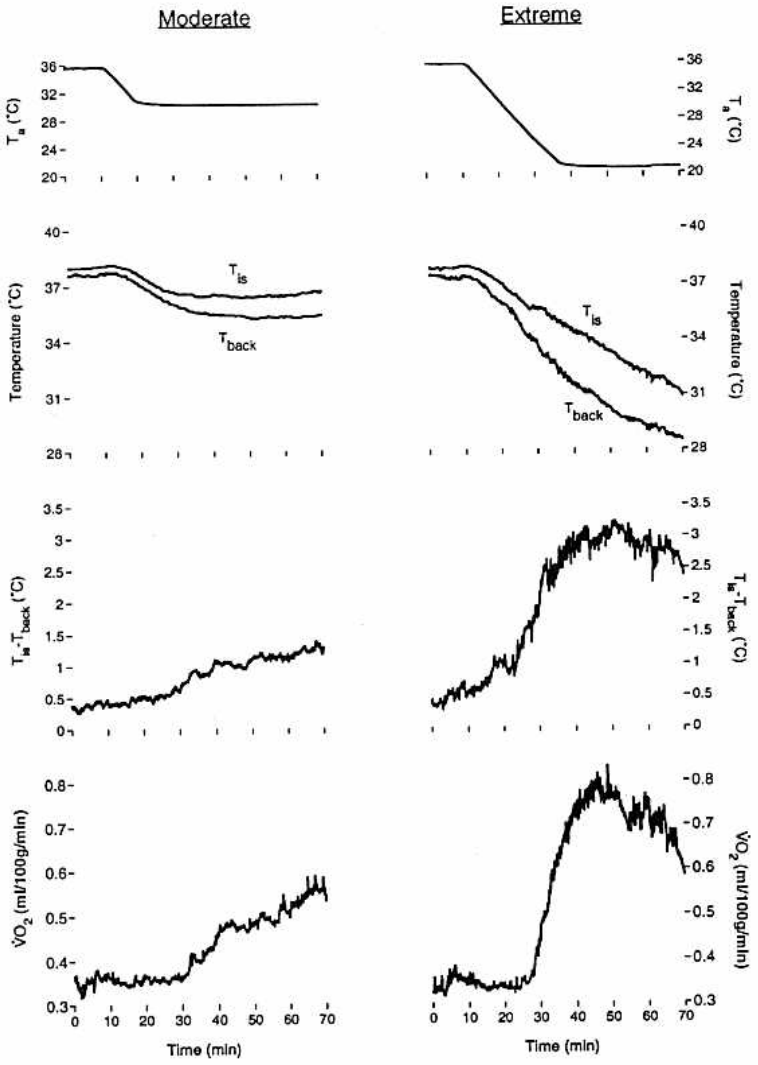

Figure 2. Air temperature (T_a), interscapular temperature (T_{is}), lower back temperature (T_{back}), $T_{is} - T_{back}$, and oxygen consumption ($\dot{V}O_2$) for two individual week-old rats challenged in a moderate (30°C) or extreme (21°C) environment. Data are from Blumberg and Stolba (1996).

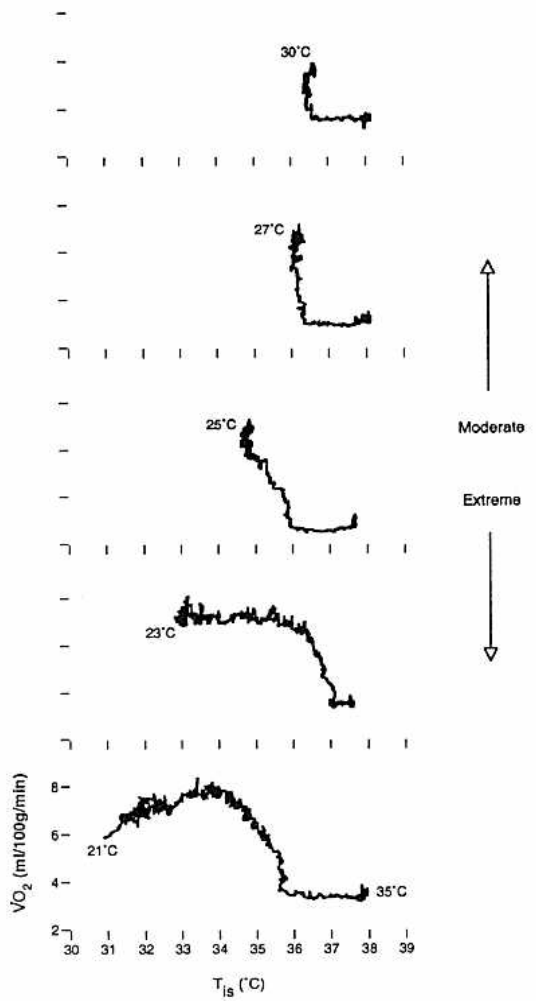

Figure 3. Space-state diagrams for individual week-old rats in which oxygen consumption ($\dot{V}O_2$) is plotted against interscapular temperature (T_{is}). Air temperature at the beginning of each test was 35°C. Temperatures at the left of each plot indicate the final air temperature for that test. Data from the top and bottom plots correspond to the T_{is} and $\dot{V}O_2$ values in Figure 2. (Reprinted from Blumberg & Sokoloff, 1998, with permission. Copyright 1998 John Wiley & Sons, Inc.)

The lower three plots in Figure 3 present the trajectories of three week-old rats cooled to 25°C, 23°C, and 21°C. We see that the vertical portions of the trajectories become progressively skewed toward lower interscapular temperatures as BAT thermogenesis is maximized. Furthermore, as cooling becomes even more extreme in the lowest plot, oxygen consumption for this pup decreases due to the suppression of cellular metabolism by cold.

As Figure 3 illustrates, pups arrive at settling points regardless of whether they are exposed to moderate or extreme air temperatures. To determine whether pups adjust metabolic heat production to defend these settling points, and to determine whether these adjustments are different at moderate and extreme air temperatures, perturbation experiments were performed (Blumberg & Sokoloff, 1997). Pups were cooled either to a moderate air temperature (30.5°C) or to an extreme air temperature (23°C). After they had settled at one of these two air temperatures, pups were exposed to a brief 3.7°C air temperature perturbation in the positive or negative direction. It was found that the pups in the moderate condition were better able to defend interscapular temperature against the perturbation than were the pups in the extreme condition. Moreover, the moderately cooled pups rapidly and effec-

tively defended interscapular temperature to negative and positive perturbations by increasing or decreasing BAT thermogenesis, respectively. In other words, these pups responded to air temperature perturbations in either direction by exhibiting symmetric homeostatic responses that are similar to those that would be expected from any successful thermoregulator.

Respiratory Adjustments. BAT thermogenesis is an aerobic process and thus one would expect to see respiratory adjustments to cold challenge that mirror changes in heat production. In one study, strain gauges were used to measure respiratory rate during moderate and extreme cold exposure (Sokoloff & Blumberg, 1997). It was found that week-old rats increase respiratory rate approximately 80% over baseline values during cold exposure (i.e., from ~2.5 Hz at thermoneutral to ~4.5 Hz at the boundary between moderate and extreme air temperatures). It was also found that at extreme air temperatures and as oxygen consumption decreases, respiratory rate decreases. The correspondence between the responses of oxygen consumption and respiratory rate during cold exposure reflects the supportive role of respiration in providing oxygen to BAT. Furthermore, it is likely that maximum levels of BAT thermogenesis are determined in part by limitations in the delivery of oxygen to BAT (Dotta & Mortola, 1992), which in turn are partly due to biomechanical and energetic constraints on the respiratory system of the infant (Mortola, 1983, 1987).

Cardiovascular Adjustments: Cardiac Rate. Smith and Roberts (1964), in noting the venous outflow from BAT, suggested that the "direct venous connection between interscapular brown fat and the azygous vein could be readily adduced as indicative of a direct convective heat transfer to the heart" (p. 146). This observation, in conjunction with studies demonstrating the direct effects of cooling on heart rate *in vitro* (Lyman & Blinks, 1959; see also Fairfield, 1948; Tazawa & Nakagawa, 1985), suggested a possible role for BAT thermogenesis in the maintenance of cardiac function during cold challenge in infants.

Given that the largest deposits of BAT are located in the thoracic region, and in light of Smith and Roberts' (1964) hypothesis that BAT thermogenesis is primarily directed toward maintenance of cardiac function, it is reasonable to suspect that heat produced by BAT is compartmentalized in the thoracic cavity (Blumberg & Sokoloff, 1998; Blumberg, Sokoloff, & Kirby, 1997). If so, then it is certainly justified to question the customary reliance on rectal temperature as an indicator of thermoregulatory success in infant rats. Instead, interscapular temperature may provide more direct and useful information.

As suggested above, there is now substantial evidence that compartmentalization is a general theme in thermoregulatory biology. Just as oxygenated blood is directed toward metabolically active organs, so, too, may thermal resources be directed toward selected bodily compartments during cold exposure. In addition, numerous thermoregulatory mechanisms have been studied that protect particular organ systems from excessive heating or cooling. These processes and mechanisms are ubiquitous, and are found in a wide variety of insects (Heinrich, 1993), fish (Block, 1986), reptiles (Crawford, 1972), birds (Caputa, 1984), and mammals (Baker, 1979). Perhaps most striking is the finding that some moths, weighing less than 250 mg, can exhibit thermal gradients between thorax and abdomen of as much as 25°C (Heinrich, 1987); keeping the thorax warm is important because the temperature-sensitive flight muscles are located in that region. Similarly, in adult golden hamsters, the thoracic cavity is substantially warmer than the abdominal cavity during

cold exposure as well as during rewarming from hibernation (Lyman & Chatfield, 1950; Pohl, 1965). Therefore, in assessing the thermoregulatory capabilities of any animal at any age, it is essential that the thermoregulatory needs of the animal be considered. As we will now see, for infant rats those needs may center on the cardiovascular system.

To assess the contribution of BAT thermogenesis to cardiac rate regulation, the electrocardiogram of week-old rats was monitored as pups were exposed to a series of moderate and extreme air temperatures (Blumberg *et al.*, 1997). When pups were cooled from thermoneutral to moderate air temperatures, cardiac rate did not change; in contrast, when pups were cooled further to extreme air temperatures, and when interscapular temperature fell, cardiac rate fell significantly (in fact, over the small air temperature drop from 26°C to 21°C, cardiac rate fell from 388 to 227 beats/min, a decrease of over 40%). In a separate experiment, when pups were pretreated with chlorisondamine to block control of the heart as well as BAT, heart rate fell in lock step with air temperature and interscapular temperature. Overall, in both untreated and ganglionically blocked pups, over 95% of the variance in cardiac rate could be accounted for by interscapular temperature.

In order to explore further the relations between cardiac rate and BAT thermogenesis, a β_3 agonist was used to selectively stimulate BAT thermogenesis in pups acclimated in a thermoneutral environment (Sokoloff, Kirby, & Blumberg, 1998). As expected, it was found that cardiac rate increased when BAT thermogenesis was stimulated and as interscapular temperature increased; this tachycardia occurred even when pups were pretreated with the ganglionic blocker, thus providing further support for the notion that cardiac rate was affected directly by warming of the heart. Finally, using a water-cooled thermode to manipulate interscapular temperature, we cooled the interscapular region of pups that had been pretreated with the β_3 agonist and found that the increase in cardiac rate could be reversed. Therefore, the results of these studies provide the strongest support for Smith and Roberts' (1964) original hypothesis that BAT thermogenesis is ideally suited to the maintenance of cardiac function during cold exposure. It is not known, however, whether rat pups enhance the effectiveness of BAT thermogenesis by selectively distributing blood flow to the thoracic cavity, as has been shown to occur in adult hamsters rewarming from hibernation (Lyman & Chatfield, 1950).

Based on the above findings relating BAT thermogenesis and cardiac rate in infant rats, we hypothesized that hamsters can only maintain cardiac rate in the cold when thermogenic mechanisms begin maturing at the end of the second week postpartum (Hissa, 1968; Sundin *et al.*, 1981). When this hypothesis was tested, it was found that cardiac rate fell in lock step with interscapular temperature even during mild cooling in hamsters 12 days of age and younger (Blumberg, 1997). In contrast, by 13 days of age when pups began exhibiting BAT thermogenesis, interscapular temperature and cardiac rate were maintained. Therefore, as in infant rats, young hamsters exhibit a striking relation between endogenous heat production and cardiac rate regulation.

It should be noted that the absence of metabolic heat production in infant hamsters precludes the use of the term "moderate" to describe the air temperatures to which they are exposed. That term is defined above on the basis of thermogenic responses to cold exposure. Therefore, given the absence of thermogenic responding by infant hamsters younger than 13 days of age, any air temperature below thermoneutral constitutes an extreme challenge for these animals.

Cardiovascular Adjustments: Arterial Pressure. Cardiac rate is just one of two variables that determine cardiac output (i.e., the amount of blood pumped by the heart per unit time). The second component of cardiac output is stroke volume, that is, the volume of blood forced out of the left ventricle during each individual contraction. In those infant mammals studied thus far, cardiac output is primarily determined by cardiac rate due to apparent limitations in the infant's ability to increase stroke volume above resting levels (e.g., Shaddy, Tyndall, Teitel, Li, & Rudolph, 1988; Teitel *et al.*, 1985). Therefore, the falling cardiac rate of infant rats during extreme cooling suggested that cardiac output was falling as well, which in turn suggested that such pups were facing related hemodynamic difficulties such as decreased arterial pressure.

To address this issue, the blood pressure responses of unanesthetized week-old rats were monitored during moderate and extreme cooling (Kirby & Blumberg, 1998). To do this, it was necessary to fashion a hair-thin catheter, insert this catheter into the femoral artery, and guide the tip to the junction of the descending abdominal aorta. When this catheter was used to monitor blood pressure during cooling to air temperatures as low as 17°C, we were surprised to find that pups were able to maintain blood pressure even in the face of substantial decreases in cardiac rate. Therefore, blood pressure is the one variable measured thus far that does not exhibit a change across the transition from moderate to extreme cold exposure. Pups maintain arterial pressure by increasing peripheral resistance (Blumberg, Knoot, & Kirby, submitted). In addition, as is discussed below, pups may engage an additional mechanism to help them maintain cardiovascular function during extreme cold exposure.

Ultrasonic Vocalizations. When isolated from the mother and littermates, infant rats emit a high-frequency, 40-kHz vocalization that is perceived by the mother and that can elicit retrieval behaviors (Allin & Banks, 1972). For many years, it has been recognized that an important stimulus for ultrasound production is the decrease in ambient temperature that normally accompanies isolation from the nest (Allin & Banks, 1971; Blumberg, Efimova, & Alberts, 1992a, b; Okon, 1971). Although the importance of cold exposure as a stimulus for ultrasound production is acknowledged, it appears that olfactory and tactile stimuli can have modulatory effects on ultrasound production (e.g., Hofer, Brunelli, & Shair, 1994).

While some investigators value the vocalizing rat pup as a potential model for the study of distress or anxiety (e.g., Miczek, Weerts, Vivian, & Barros, 1995; Winslow & Insel, 1991), we have chosen instead to focus on the environmental stimuli that elicit ultrasound production and the physiological changes that accompany it (Blumberg & Sokoloff, 2001). In one study, Blumberg and Alberts (1990) showed that rat pups emit ultrasonic vocalizations as heat production commences during cold exposure; at that time, however, we were not distinguishing between moderate and extreme air temperatures. Subsequently, when we examined ultrasound production in response to moderate and extreme cold exposure (Blumberg & Stolba, 1996), we found to our surprise that moderately cooled rat pups remain quiet even as BAT thermogenesis and oxygen consumption increase. In contrast, exposure to an extreme air temperature evoked high rates of ultrasound production. Furthermore, emission of the vocalization was found to be sensitive to air temperature differences of only 2°C across the bondary from moderate to extreme exposure (Sokoloff & Blumberg, 1997).

Although ultrasound production is described here as a behavioral response to cold, the underlying mechanisms that give rise to this behavior may actually fall within the purview of traditional physiology (for our purposes here we will leave aside the issue as to whether such distinctions between behavior and physiology are useful). Based on our earlier work on this vocalization and the physiological responses that accompany it, we hypothesized that ultrasound production is an acoustic by-product of laryngeal braking, a respiratory maneuver that is used, for example, by premature human infants as a mechanism for improving gas exchange in the lungs (Blumberg & Alberts, 1990); significantly, human infants emit an audible grunt during laryngeal braking. As we refined our procedures and measured more variables, however, an additional explanation for the rat pup's vocalization presented itself.

As described above, our investigation of arterial pressure in week-old rats indicated maintenance of pressure even during extreme cold exposure when cardiac rate (and presumably cardiac output) fell substantially (Kirby & Blumberg, 1998). It was surmised that maintenance of arterial pressure under these conditions required increased peripheral resistance, which turned out to be the case (Blumberg *et al.*, submitted). In addition, the cooccurrence of decreased cardiac rate and increased ultrasound production raised the possibility of a causal connection between them. Specifically, a decreasing cardiac output creates a backward force that impedes venous return (Guyton & Hall, 1996), and one little-known maneuver for increasing venous return is the abdominal compression reaction (ACR). The ACR entails contraction of the abdominal muscles during or after expiration, thus increasing intraabdominal pressure and thereby propelling blood back toward the heart (Youmans *et al.*, 1963; Youmans, Tjioe, & Tong, 1974). We hypothesized that pups employ the ACR during extreme cold exposure and that the ultrasonic vocalization is the acoustic by-product of the forceful expulsion of air during the ACR. We examined this possibility by monitoring intraabdominal pressure during cold exposure (Kirby & Blumberg, 1998). As expected, we detected sizable increases in intraabdominal pressure during each ultrasonic pulse, suggesting that ultrasound production is attended by forceful abdominal compressions that could, in theory, propel blood back to the heart during periods of decreased cardiac output. In addition, detection of arterial pressure pulses during ultrasound production provided additional support for this hypothesis.

Although it is clear that ultrasound production in infant rats during extreme cold exposure is associated with pulsatile increases in intraabdominal and arterial pressure, direct measurement of venous pressure during ultrasound production would provide even stronger evidence that venous return is increasing. Therefore, we conducted a critical test of the ACR hypothesis by measuring venous pressure in 15-day-old rats after injection with the α_2-adrenoceptor agonist clonidine (Blumberg, Sokoloff, Kirby, & Kent, 2000). Clonidine, like extreme cold exposure, evokes high rates of ultrasound production and simultaneously produces substantial bradycardia (Blumberg, Sokoloff, & Kent, 2000). We chose 15-day-olds for this study because they exhibit maximal ultrasonic responses to clonidine administration (Hård, Engel, & Lindh, 1988; Kehoe & Harris, 1989) and because their body size is sufficiently large to permit catheterization for measurement of venous pressure. As required by the ACR hypothesis, we found that ultrasonic vocalizations are associated with substantial pulsatile increases in venous pressure, indicative of increased venous return. Thus, these results suggested a functional linkage between the physiological conditions that evoke ultrasound production (i.e., decreased venous

return) and the physiological consequences of the maneuver that produces ultrasound (i.e., increased venous return).

One possible depiction of the physiological and behavioral consequences of extreme cold exposure is presented in Figure 4. The cascade of events begins with the direct effects of cooling on the functional properties of heart muscle and blood. First, cardiac rate falls and results in decreases in cardiac output and venous return; second, the infant rat's blood becomes more viscous as it cools (Blumberg, Sokoloff, & Kent, 1999), thus resulting in an additional impediment to venous return (Goslinga, 1984). Recruitment of the ACR improves venous return and, in conjunction with increased peripheral resistance, contributes to the maintenance of arterial pressure. Initiation of the ACR also results in the production of ultrasound as a by-product. If, as a consequence of ultrasound production, the mother retrieves the pup to the nest, the pup's exposure to extreme cold terminates and the stresses on the cardiovascular system are alleviated.

Expression of Active Sleep Behaviors during Cold Exposure and the Role of BAT Thermogenesis. In addition to physiological mechanisms of heat production, animals use behavior to manipulate their thermal environment and to locomote to a more hospitable thermal environment (Satinoff, 1996). In addition to its role in thermoregulation, behavior can also provide important information to the investigator regarding thermoregulatory success. Specifically, as is shown below, changes in behavioral state during cold exposure provide an index by which to judge an infant rat's thermoregulatory capabilities.

For our studies of behavioral state changes during cold exposure, we focused on

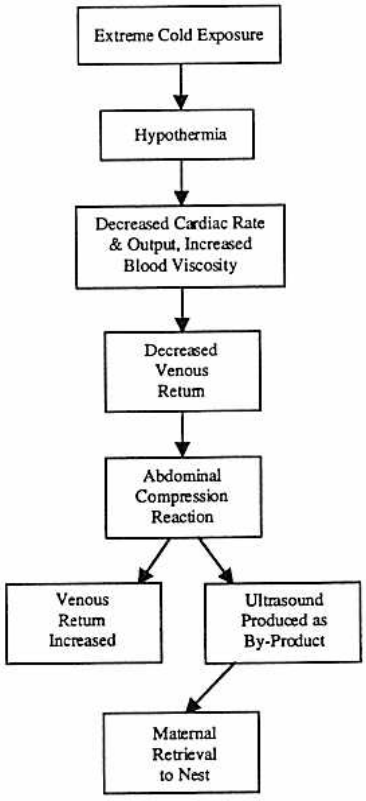

Figure 4. Cascade of events hypothesized to result from extreme cold exposure in infant rats.

myoclonic twitching, a behavior closely associated with active sleep in infants (Jouvet-Mounier, Astic, & Lacote, 1970). Myoclonic twitching consists of rapid, independent, and phasic movements of the distal limbs and tail of infant rats (Gramsbergen, Schwartze, & Prechtl, 1970). Moreover, epochs of twitching occupy as much as 70–80% of a rat pup's daily activity (Jouvet-Mounier *et al.*, 1970). Thus, although active (or rapid eye movement, REM) sleep in adults is defined on the basis of many components including a desynchronized electroencephalogram, rapid eye movements, and muscle atonia, the situation is more complicated in infants because these components are not expressed or are difficult to detect (Blumberg & Lucas, 1996). In the work described below, we have avoided the difficulties inherent in defining active sleep and other behavioral states in infants by focusing primarily on the presence or absence of myoclonic twitching.

In our first study of sleep behavior during cold exposure, isolated week-old rats initially were acclimated to a metabolic chamber at a thermoneutral air temperature (35.5°C). After acclimation, the test itself consisted of a 10-min baseline period followed by a 60-min period during which air temperature was decreased to either a moderate (30°C) or extreme (21°C) level (Blumberg & Stolba, 1996). The observer scored the incidence of myoclonic twitching continuously throughout the 70-min test. As expected, extreme cooling substantially reduced myoclonic twitching over the course of the test, but moderate cooling did not. Thus, during moderate cold exposure, it appeared that BAT thermogenesis maintains a permissive thermal environment for active sleep maintenance, as indexed by the expression of myoclonic twitching.

This hypothesis that BAT thermogenesis "protects" the expression of sleep during moderate cold exposure was tested further in a series of experiments (Sokoloff & Blumberg, 1998). The results of these experiments are presented in Figure 5. First, the findings of Blumberg and Stolba (1996) were replicated and extended in week-old rats by testing them at two moderate and two extreme air temperatures. As expected, twitching rates were high at the moderate, but not extreme air temperatures. Furthermore, rates of twitching were more closely associated with changes in interscapular temperature than oxygen consumption (Figure 5). Awake behaviors (e.g., kicking, stretching, locomotion) were also monitored in this experiment and it was found that, in general, the incidence of awake behaviors increased as rates of twitching decreased.

In the second experiment in this series (Sokoloff & Blumberg, 1998), pups were pretreated with a ganglionic blocker (i.e., chlorisondamine) and exposed to cold; now, with BAT thermogenesis inhibited, rates of twitching decreased in lock step with decreases in air temperature and interscapular temperature. Then, in the final experiment, week-old golden hamsters were tested as well and it was found that their twitching profiles during cold exposure were nearly identical to the ganglionically blocked rats. Overall, approximately 75% of the variance in levels of twitching was accounted for by interscapular temperature for the three experimental groups.

The contribution of endothermy to the maintenance of sleep behaviors in infant rats was explored further in two additional experiments (Sokoloff & Blumberg, 1998). For one experiment, a small thermode was constructed through which temperature-controlled water could flow. With this thermode attached to the interscapular region, and with a pup exposed to an extreme air temperature, supplemental heat could be added to that provided by iBAT. When this experiment was performed, pups given supplemental heat exhibited higher levels of myoclonic twitching than control animals. In a second experiment, pups were pretreated with

the ganglionic blocker and were then exposed to an air temperature of 30°C (as shown in Figure 5, for ganglionically blocked pups, an air temperature of 30°C was sufficient to suppress twitching). Then, when the pups were injected with a β_3 agonist to stimulate BAT thermogenesis, both interscapular temperature and levels of myoclonic twitching increased significantly.

In their totality, these experiments on infants rats and hamsters provide strong evidence that myoclonic twitching is sensitive to the prevailing air temperature and the activation of BAT thermogenesis. Furthermore, the studies of myoclonic twitching provide additional behavioral evidence of thermoregulatory competence in untreated infant rats in that these sleep behaviors are maintained at baseline levels during moderate cold exposure but decrease significantly during extreme cold exposure.

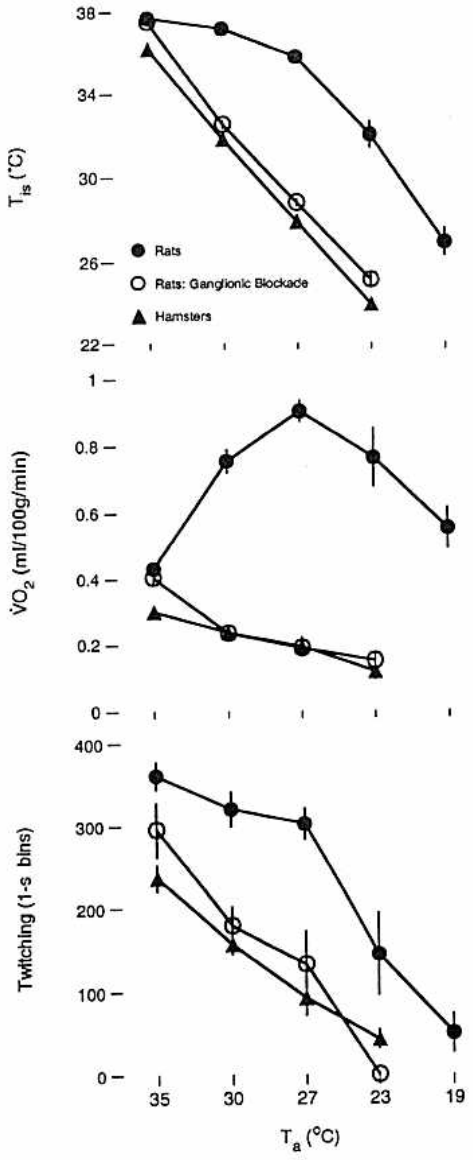

Figure 5. Interscapular temperature (T_{is}), oxygen consumption ($\dot{V}O_2$), and myoclonic twitching (per 15-min observation period) for week-old rats and hamsters during cold exposure. Rat pups were either untreated or pretreated with the ganglionic blocker chlorisondamine. The data demonstrate a strong relationship between BAT thermogenesis in the cold and the maintenance of myoclonic twitching, an index of active sleep. (Adapted from Sokoloff & Blumberg, 1998.)

Individual Differences and Developmental Changes. The research described above has concentrated on week-old rats in which the moderate air temperature range extends from approximately 25°C to 34°C. The lower boundary of the range (i.e., 25°C) is an approximation because, as stated above, the exact value can differ depending on the strain, age, size, insulation, and physiological condition of the pup, among other factors. Indeed, any experiential factor (e.g., rearing temperature, litter size) or experimental manipulation (e.g., food deprivation, surgery) that influences a pup's ability to produce heat can expand or contract the size of the moderate "zone." From a developmental standpoint, it is particularly important to recognize that the range of moderate air temperatures expands rapidly during the first 2 weeks postpartum, in part due to increases in body size (and therefore decreases in relative surface area). For example, some of the studies described above were also conducted on 2-day-old rats whose range of moderate air temperatures extends from thermoneutrality down to only 30–31°C. Nonetheless, within this narrow moderate range, 2-day-olds maintain cardiac rate (Blumberg *et al.*, 1997) and remain asleep (Blumberg & Stolba, 1996). Therefore, the concepts of moderate and extreme cold exposure are defined in relation to the individual pup being studied and its prevailing physiological state.

Summary. Figure 6 schematically summarizes the findings described above, based primarily on week-old rats. The figure should be scanned from left to right as if a pup were being exposed sequentially to decreasing air temperatures, from thermoneutral to moderate to extreme. For each variable, thicker lines represent higher values. Thus, we see that air temperature, the independent variable, decreases steadily throughout the test while the other variables exhibit distinct patterns during cooling. First, interscapular temperature, cardiac rate, and myoclonic twitching all retain high levels throughout moderate cooling and only decrease during extreme

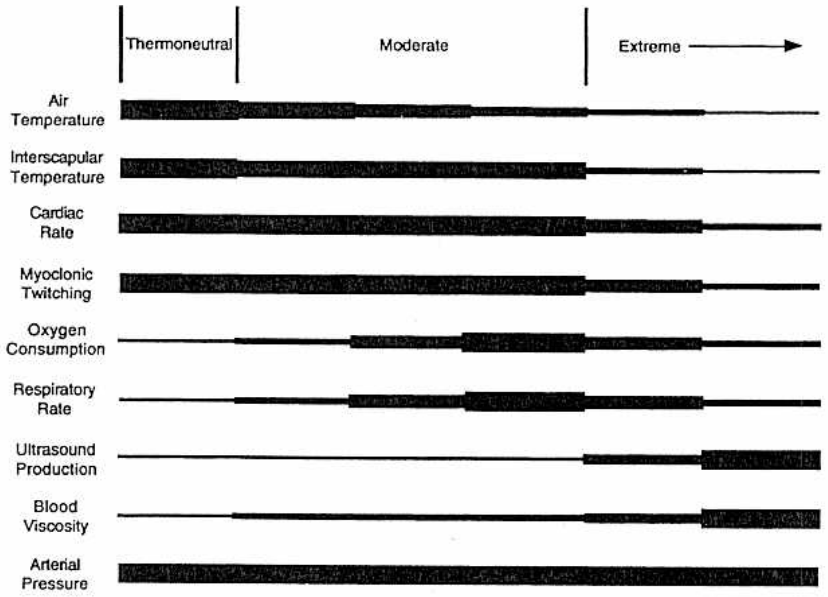

Figure 6. Schematic diagram of physiological and behavioral responses of infant rats to moderate and extreme cold exposure. Lines of greater thickness denote higher values for the given variable. The diagram is based on data collected from week-old rats, for whom the moderate range of air temperature is from approximately 25°C to 34°C. Extreme air temperatures below 17°C have not been systematically investigated.

cooling. Second, oxygen consumption and respiratory rate are similar in that they increase progressively during moderate cooling and then decrease during extreme cooling. Third, ultrasound production is rarely detected during moderate cooling but increases substantially during extreme cooling, as does blood viscosity. Finally, arterial pressure is maintained throughout the range of air temperatures tested.

BEHAVIORAL THERMOREGULATION AND GROUP REGULATORY PROCESSES IN INFANTS. With a new-found appreciation for the thermoregulatory capabilities of infant rats, we must now reconsider the individual in its natural environment, that is, as a member of a huddle of littermates. Huddling is a ubiquitous feature in mammals that give birth to multiple young. Its thermoregulatory properties were brought into sharp focus by Alberts' (1978) classic examination of huddling in rats. Huddling has also been investigated in other species including rabbits (Hull & Hull, 1982), Syrian golden hamsters (Leonard, 1982), Djungarian hamsters (Newkirk, Silverman, & Wynne-Edwards, 1995), and mice (Ogilvie & Stinson, 1966). Huddles are not static entities: individual pups within a huddle actively regulate their own temperature by diving into the middle of the huddle when cold and emerging when warm (Alberts, 1978). Therefore, individual behavioral thermoregulation is an essential component of group regulatory behavior. What has been less clear over the last two decades is how endogenous heat production contributes to the efficacy of the huddle. Stated simply, do rat pups use BAT thermogenesis while huddling, or is BAT thermogenesis activated only as an "emergency mechanism" when huddling littermates are not available?

Alberts (1978) showed that each individual pup, by huddling with its littermates, saves metabolic energy at any given air temperature. Looked at another way, the huddle can be seen as a means of expanding the range of air temperatures within which the littermates can thermoregulate. Thus, one should be able to define moderate and extreme air temperatures in the context of a huddle in the same way that this has been done for the individual. We have conducted such an experiment using infant rats and hamsters in huddles composed of two, four, or six pups (Sokoloff, Blumberg, & Adams, 2000). Not surprisingly, we have found that, for infant rats, the opportunity to huddle expands the range of air temperatures that can be defined as moderate. In contrast to rats, however, infant hamsters gain little advantage from huddling; even in huddles of six pups, heat loss cannot be significantly retarded. In other words, it appears that huddling benefits the individual only if endogenous heat production is also available. Thus, these data suggest that BAT thermogenesis is not an emergency mechanism in huddling infant rats but is an integral contributor to the huddle's effectiveness during cold exposure.

The ineffectiveness of hamster huddling does not imply that they are poor behavioral thermoregulators. On the contrary, newborn hamsters are generally considered superior to rats in their ability to move rapidly toward warmth (rats: Johanson, 1979; Kleitman & Satinoff, 1982; hamsters: Leonard, 1974, 1982). Although infants of these two species need to be tested using identical methodologies before a firm conclusion can be drawn, it is tempting to speculate with Leonard (1982) that the infant rat's larger size and endothermic capability retards cooling during cold exposure and thus suppresses its behavioral response relative to the hamster. Furthermore, it is possible that the infant hamster's lack of endothermy and the apparent ineffectiveness of huddling has made necessary the mother's nearly continual presence in the nest as a source of heat during the first week postpartum (Leonard, 1974). Thus, as we move toward a more complete under-

standing of the thermal context of infancy among different species, it is important that we consider the behavioral and physiological capabilities of individual infants within a huddle as well as the thermal contributions of the mother (Leon, 1986).

Homeostasis, Pathology, and the Reality of Separate Systems

We saw above that when early investigators attempted to document the "development of homeothermy" (e.g., Brody, 1943), there was an implicit assumption that adult standards of thermoregulatory success could be safely applied to infants. This assumption, however, is mistaken. A better appreciation of thermoregulatory capacities is obtained when thermal challenges are scaled appropriately to the physical properties of the animal, when heat loss and heat gain mechanisms are considered separately, and when thermoregulatory success is evaluated using physiological and behavioral measures. Thus, while BAT may once have been considered a generic source of heat that contributes to the maintenance of body temperature, it is becoming apparent that in some species BAT may play a transitional thermoregulatory role during development, providing heat to the vital thoracic organs during cold exposure. In other words, BAT may be most appropriately conceptualized as an ontogenetic adaptation (Hall & Oppenheim, 1987) that is suited to the needs of developing animals; its early peak activity and involution prior to weaning (unless pups are reared in a cold environment; Nedergaard *et al.*, 1986) attests to a specific role during early development.

The thermoregulatory system is composed of multiple heat loss and heat gain mechanisms that are controlled at many levels of the neuraxis (Blumberg *et al.*, 1995; Satinoff, 1978). Peripheral feedback is evident; for example, as described earlier, young guinea pigs possess neural elements in the cervical spinal cord that are responsible for the initiation of shivering and that are suppressed by heat produced by overlying BAT (Brück & Wünnenberg, 1970). This "meshed" organization of effector mechanisms, in which feedback from one heat gain mechanism suppresses the initiation of a second heat gain mechanism, may be a general feature of homeostatic systems. The challenge for the developmental study of thermal homeostasis is to understand how these multiple mechanisms develop individually and how they become integrated such that, under normal conditions, they exhibit coordinated activation. Such a level of understanding, however, will only emerge from focused developmental studies of the responses of specific effector systems to well-defined environmental challenges. Measurement of a single outcome variable (e.g., rectal temperature) is no longer sufficient.

In this chapter and elsewhere, it is commonplace to read of a "thermoregulatory system" or a "cardiovascular system." Although these systems are discussed separately for ease of communication, one has to wonder whether we can advance our understanding of homeostasis while retaining this convenient fiction. In this regard, Satinoff's (1978) observation that most thermoregulatory mechanisms originally evolved as components of other regulatory processes is particularly significant. For example, temperature regulation and cardiovascular regulation rely on the use of circulatory adjustments to control heat loss through the skin and blood flow to metabolically active organs, respectively. Thus, to what "system" does vasomotor activity belong?

In addressing this question, consider Bartholomew's (1964) observation that "biologists, because of our limitations, divide ourselves into categories of specializa-

tion and then pretend that these categories exist in the biological world. As everyone knows, organisms are functionally indivisible and cannot be split into the conventional compartments of morphology, physiology, behaviour, and genetics" (p. 8). It should give us pause that over three decades later, Blessing (1997) was moved to make a similar point, encouraging us to critically reconsider the concept that the nervous system and its functions can be divided into independent parts: autonomic versus somatic, central versus peripheral, physiological versus behavioral. Therefore, using similar logic, we must wonder whether the tendency to focus our attention on one homeostatic system at a time will continue to be an effective approach to the study of homeostasis. That approximately 50% of the thermosensitive preoptic hypothalamic neurons examined by Boulant and Silva (1987) were also sensitive to other stimuli (e.g., glucose, osmotic pressure) provides a rational justification for investigations that are explicitly interactionist. Fortunately, such investigations are becoming more common (e.g., Pleschka & Gerstberger, 1994).

The benefits of an interactionist perspective are especially apparent from studies implicating developmental thermoregulatory deficits in the origins of such pathological conditions as obesity and hypertension. For example, researchers have developed a variety of mouse and rat strains whose offspring become obese (e.g., Krief & Bazin, 1991; Lowell *et al.*, 1993; Pelleymounter *et al.*, 1995). Investigations of these strains have helped lead to the identification of a fat cell-produced protein called leptin that provides feedback information to the hypothalamus and, by doing so, promotes either fat storage (when leptin levels are low) or fat wastage (when leptin levels are high). Wastage of fat is accomplished through heat production by brown adipose tissue. These and other findings are providing important new insights into the functional interplay between metabolism and feeding.

The various obese strains differ as to which part of the leptin–hypothalamic–BAT system are disrupted (Seeley & Schwartz, 1997). For example, *ob/ob* mice do not produce leptin, and *db/db* mice do not express the leptin receptor. Regardless of the actual mechanism that triggers obesity, however, it is clear that the precursors to obesity are present in the newborn and infant. For example, although onset of obesity in the Zucker *fa/fa* rat (a strain that, like the *db/db* mouse, expresses a defective leptin receptor; Chua *et al.*, 1996) becomes visually apparent when young are 4–5 weeks of age, thermal and metabolic disturbances can be detected as early as the first week postpartum (Krief & Bazin, 1991). Specifically, BAT thermogenesis is attenuated in *fa/fa* rats as early as 2 days of age (Planche, Joliff, & Bazin, 1988), and reduced body temperatures are found even among huddling pups (Schmidt, Kaul, & Carlisle, 1984). Significantly, daily pharmacological activation of BAT thermogenesis from 8 to 16 days of age is sufficient to prevent the onset of obesity in *fa/fa* rats (Charon, Dupuy, Marie, & Bazin, 1995). Therefore, it appears that thermoregulatory responses in the cold during infancy affect the future development of such related responses as energy storage and food intake.

Although BAT thermogenesis factors into the infant's overall energy balance, it does not follow that BAT thermogenesis exists to modulate energy balance. Nonetheless, as discussed above, in the context of leptin's modulatory effects on energy balance in adults, BAT thermogenesis is interpreted as a mechanism for "wasting" or "burning off" excess fat. For the infant, however, whose main concern is rapid growth and development, there is no such thing as "excess energy." Therefore, we wondered whether leptin plays a role in modulating BAT thermogenesis in non-obese rat strains under conditions of thermal challenge when energy use and conservation are in conflict. To do this, we revisited the phenomenon of starvation-

induced suppression of thermogenesis during cold exposure, first investigated more than two decades ago by Bignall and his colleagues (e.g., Bignall *et al.*, 1975). When 4- to 5-day-old rats were isolated in an incubator away from maternal care for 18 hr, during which time leptin levels likely declined (Dessolin *et al.*, 1997), administration of leptin was sufficient to disinhibit BAT thermogenesis during cold exposure (Blumberg, Deaver, & Kirby, 1999). Therefore, the modulation of BAT thermogenesis by leptin in adults may emerge from a thermoregulatory role during infancy.

Like the Zucker rat for the study of obesity, the spontaneously hypertensive rat (SHR) has become the most widely used rat model of hypertension. This strain is characterized by high adult blood pressures due in large part to neurogenically induced vasoconstriction that, by 3 months of age, is irreversible (Yamori, 1984). Because, as noted above, vasoconstriction of peripheral resistance vessels is a mechanism that is important for maintaining blood pressure, we wondered whether infant SHRs exhibit deficient thermoregulatory responses during cold exposure in relation to pups of the normotensive control strain and, if so, whether they also exhibited deficiencies in their ability to maintain cardiac rate. As expected, infant SHRs were indeed less able to maintain interscapular temperature and oxygen consumption during cold exposure and, most importantly, also exhibited significant decreases in cardiac rate in comparison with their control strain (Kirby, Sokoloff, Perdomo, & Blumberg, 1999). In addition, the SHRs were less sensitive than the normotensive control strain to pharmacologic activation of BAT using a selective β_3-adrenoceptor agonist, suggesting that the SHR's thermoregulatory deficiencies were due in part to a diminished capacity to stimulate BAT. These findings raise the possibility that the SHR has been selected in part for developmental deficits in thermoregulation and that extreme demands placed on vasomotion in the service of heat retention in the infant reduce the range of vasomotion available to the adult for regulation of blood pressure (see Fregly, 1994, for a discussion of the induction of hypertension in adult rats after chronic cold exposure).

It is intriguing and instructive that our understanding of the most important animal models of obesity and hypertension has benefited substantially by considering the developmental mechanisms that contribute to these pathologies. It is particularly interesting that, for both of these animal models, thermoregulatory deficits have been detected during the first week postpartum. Such early deficits may initiate a cascade of events, for example, competing demands on multifunction effector mechanisms, that eventually lead to the adult pathology of interest. Clearly, more research is necessary to explore the possibility that the adult regulatory range of individual effector mechanisms is calibrated during development within a context that reflects the immediate functional needs of the young animal.

Conclusions and Future Directions

After a half-century of investigations of the thermoregulatory competence of altricial infants, it had become commonplace to refer to them as poikilotherms (Blumberg & Sokoloff, 1998; Nedergaard *et al.*, 1986). The use of this term reflected a common belief that individual newborn rats lack the size and/or insulation necessary to retain the heat produced by shivering or nonshivering mechanisms (Satinoff, 1996). However, as we have seen, recent work demonstrates that isolated infant rats during moderate cold challenges exhibit responses that are consistent with thermoregulatory success, not failure.

With this new appreciation of the contributions of endothermy to the adaptation of infant rats to cold challenge, we are now in a position to reassess the contributions of endothermy to the group regulatory processes of the huddle. Although this work is only beginning, it appears that BAT thermogenesis plays a central role in making the rat huddle work as a thermoregulatory unit. In contrast, huddling infant golden hamsters are more behaviorally active than huddling rats, a characteristic that may be due to their lack of BAT thermogenesis. Additional comparative studies will be needed to determine those features of early development that are most directly influenced by the presence or absence of endothermy.

Although this chapter has focused largely on the contributions of endothermy to infant thermoregulation, it must be stressed that infant animals exhibit simple orienting responses toward warmth that are essential for the maintenance of the huddle. Indeed, the ability to thermoregulate behaviorally is fundamental and ubiquitous, being found in diverse animals including lice (Fraenkel & Gunn, 1961), worms (Mori & Ohshima, 1995), and reptiles and mammals (Hammel, Caldwell, & Abrams, 1967; Satinoff, 1996). In animals that cannot produce heat endogenously, behavioral thermoregulation is the only means by which the internal thermal environment can be regulated. In a recent exciting development, researchers working with a nematode worm showed that selective manipulation of a thermosensory neuron or of the interneurons that mediate the worm's behavioral attraction to warmth and cold resulted in predictable disruption of thermotaxic behavior (Mori & Ohshima, 1995). Most interesting was their finding that two identifiable interneurons appear to mediate the worm's thermoregulatory behavior, and that thermotaxis results from a balance between these "cryophilic" and "thermophilic" interneurons. This work is significant for its focus on an aspect of behavior that is so essential for survival but is, as is readily acknowledged, so poorly understood (Thomas, 1995).

Our fundamental lack of knowledge concerning the rules and mechanisms that guide behavioral thermoregulation in newborn mammals is especially apparent. Although excellent descriptive studies of behavioral thermoregulation exist (e.g., Hull & Hull, 1982; Kleitman & Satinoff, 1982; Leonard, 1982), relatively few studies have examined the behavioral rules and neural mechanisms involved in thermal orientation behaviors. Such studies are needed, and they will benefit from considering the possibility that BAT thermogenesis, perhaps by influencing the rate of heat loss (Leonard, 1982), contributes to species differences in the rapidity and accuracy of behavioral responding to cold exposure. Thus, broad experimental approaches that incorporate the methods and perspectives of multiple disciplines will bring us closer to a comprehensive understanding of the myriad mechanisms and responses by which infant mammals adjust successfully to thermal challenges.

Acknowledgment

Preparation of this chapter was supported by National Institute of Mental Health Grant MH50701.

References

Alberts, J. R. (1978). Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology, 92,* 231–245.

Alberts, J. R. (1987). Early learning as ontogenetic adaptation. In N. A. Krasnegor, E. M. Blass, M. A. Hofer,

& W. P. Smotherman (Eds.), *Perinatal development: A psychobiological perspective* (pp. 11–37). New York: Academic Press.

Alberts, J. R., & Cramer, C. P. (1988). Ecology and experience: Sources of means and meaning of developmental change. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology*, Vol. 8, pp. 1–39. New York: Plenum Press.

Allin, J. T., & Banks, E. M. (1971). Effects of temperature on ultrasound production by infant albino rats. *Developmental Psychobiology, 4*, 149–156.

Allin, J. T., & Banks, E. M. (1972). Functional aspects of ultrasound production by infant albino rats (*Rattus norvegicus*). *Animal Behaviour, 20*, 175–185.

Amir, S. (1990). Stimulation of the paraventricular nucleus with glutamate activates interscapular brown adipose tissue thermogenesis in rats. *Brain Research, 508*, 152–155.

Baker, M. A. (1979). A brain-cooling system in mammals. *Scientific American, 240*, 130–139.

Bartholomew, G. A. (1964). The roles of physiology and behaviour in the maintenance of homeostasis in the desert environment. *Symposia of the Society for Experimental Biology, 18*, 7–29.

Bignall, K. E., Heggeness, F. W., & Palmer, J. E. (1975). Effect of neonatal decerebration on thermogenesis during starvation and cold exposure in the rat. *Experimental Neurology, 49*, 174–188.

Blessing, W. W. (1997). Inadequate framework for understanding bodily homeostasis. *Trends in Neuroscience, 20*, 235–239.

Block, B. A. (1986). Structure of the brain and eye heater tissue in marlins, sailfish, and spearfishes. *Journal of Morphology, 190*, 169–189.

Blumberg, M. S. (1997). Ontogeny of cardiac rate regulation and brown fat thermogenesis in golden hamsters (*Mesocricetus auratus*). *Journal of Comparative Physiology B, 167*, 552–557.

Blumberg, M. S., & Alberts, J. R. (1990). Ultrasonic vocalizations by rat pups in the cold: An acoustic by-product of laryngeal braking? *Behavioral Neuroscience, 104*, 808–817.

Blumberg, M. S., & Lucas, D. E. (1996). A developmental and component analysis of active sleep. *Developmental Psychobiology, 29*, 1–22.

Blumberg, M. S., & Sokoloff, G. (1997). Dynamics of brown fat thermogenesis in week-old rats: Evidence of relative stability during moderate cold exposure. *Physiological Zoology, 70*, 324–330.

Blumberg, M. S., & Sokoloff, G. (1998). Thermoregulatory competence and behavioral expression in the young of altricial species—Revisited. *Developmental Psychobiology, 33*, 107–123.

Blumberg, M. S., & Sokoloff, G. (2001). Do infant rats cry? *Psychological Review, 108*, 83–95.

Blumberg, M. S., & Stolba, M. A. (1996). Thermogenesis, myoclonic twitching, and ultrasonic vocalization in neonatal rats during moderate and extreme cold exposure. *Behavioral Neuroscience, 110*, 305–314.

Blumberg, M. S., Efimova, I. V., & Alberts, J. R. (1992a). Ultrasonic vocalizations by rat pups: The primary importance of ambient temperature and the thermal significance of contact comfort. *Developmental Psychobiology, 25*, 229–250.

Blumberg, M. S., Efimova, I. V., & Alberts, J. R. (1992b). Thermogenesis during ultrasonic vocalization by rat pups isolated in a warm environment: A thermographic analysis. *Developmental Psychobiology, 25*, 497–510.

Blumberg, M. S., Knoot, T. G., & Kirby, R. F. (Submitted). Neural and hormonal control of arterial pressure during cold exposure in unanesthetized week-old rats.

Blumberg, M. S., Schalk, S. L., & Sokoloff, G. (1995). Pontine and basal forebrain transections disinhibit brown fat thermogenesis in neonatal rats. *Brain Research, 699*, 214–220.

Blumberg, M. S., Sokoloff, G., & Kirby, R. F. (1997). Brown fat thermogenesis and cardiac rate regulation during cold challenge in infant rats. *American Journal of Physiology, 272*, R1308–R1313.

Blumberg, M. S., Deaver, K., & Kirby, R. F. (1999). Leptin disinhibits BAT thermogenesis in infants after maternal separation. *American Journal of Physiology, 276*, R606–R610.

Blumberg, M. S., Sokoloff, G., & Kent, K. J. (1999). Cardiovascular concomitants of ultrasound production during cold exposure in infant rats. *Behavioral Neuroscience, 113*, 1274–1282.

Blumberg, M. S., Sokoloff, G., & Kent, K. J. (2000). A developmental analysis of clonidine's effects on cardiac rate and ultrasound production in infant rats. *Developmental Psychobiology, 36*, 186–193.

Blumberg, M. S., Sokoloff, G., Kirby, R. F., & Kent, K. J. (2000). Distress vocalizations in infant rats: What's all the fuss about? *Psychological Science, 11*, 78–81.

Booth, D. A. (1980). Conditioned reactions in motivation. In F. M. Toates & T. R. Halliday (Eds.), *Analysis of motivational processes* (pp. 3–21). New York: Academic Press.

Boulant, J. A., & Silva, N. L. (1987). Interactions of reproductive steroids, osmotic pressure, and glucose on thermosensitive neurons in preoptic tissue slices. *Canadian Journal of Physiology and Pharmacology, 65*, 1267–1273.

Brody, E. B. (1943). Development of homeothermy in suckling rats. *American Journal of Physiology, 139*, 230–232.

Brück, K., & Hinckel, P. (1996). Ontogenetic and adaptive adjustments in the thermoregulatory system. In M. J. Fregly & C. M. Blatteis (Eds.), *Handbook of physiology* (pp. 597–611). Oxford: Oxford University Press.

Brück, K., & Wünnenberg, B. (1970). "Meshed" control of two effector systems: nonshivering and shivering thermogenesis. In J. D. Hardy, A. P. Gagge, & J. A. J. Stolwijk (Eds.), *Physiological and behavioral temperature regulation* (pp. 562–580). Springfield, IL: Thomas.

Cannon, B., Jacobsson, A., Rehnmark, S., & Nedergaard, J. (1996). Signal transduction in brown adipose tissue recruitment: Noradrenaline and beyond. *International Journal of Obesity, 20* (Supplement 3), S36–S42.

Cannon, W. B. (1932). *The wisdom of the body.* New York: W. W. Norton.

Caputa, M. (1984). Some differences in mammalian versus avian temperature regulation: Putative thermal adjustments to flight in birds. In J. R. S. Hales (Ed.), *Thermal physiology* (pp. 413–417). New York: Raven Press.

Charon, C., Dupuy, F., Marie, V., & Bazin, R. (1995). Effect of the β-adrenoceptor agonist BRL-35135 on development of obesity in suckling Zucker (*fa/fa*) rats. *American Journal of Physiology, 268,* E1039–E1045.

Chua, S. C., Jr., Chung, W. K., Wu-Peng, S., Zhang, Y., Liu, S.-M., Tartaglia, L., & Leibel, R. L. (1996). Phenotypes of mouse diabetes and rat fatty due to mutations in the *ob* (leptin) receptor. *Science, 271,* 994–996.

Conklin, P., & Heggeness, F. W. (1971). Maturation of temperature homeostasis in the rat. *American Journal of Physiology, 220,* 333–336.

Crawford, E. C., Jr. (1972). Brain and body temperatures in a panting lizard. *Science, 177,* 431–433.

Daly, M. (1976). Behavioral development in three hamster species. *Developmental Psychobiology, 9,* 315–323.

Dawkins, M. J. R., & Scopes, J. W. (1965). Nonshivering thermogenesis and brown adipose tissue in the human new-born infant. *Nature, 206,* 201–202.

Dessolin, S., Schalling, M., Champigny, O., Lönnqvist, F., Ailhaud, G., Dani, C., & Ricquier, D. (1997). Leptin gene is expressed in rat brown adipose tissue at birth. *FASEB Journal, 11,* 382–387.

Dotta, A., & Mortola, J. P. (1992). Effects of hyperoxia on the metabolic response to cold of the newborn rat. *Journal of Developmental Physiology, 17,* 247–250.

Fairfield, J. (1948). Effects of cold on infant rats: Body temperatures, oxygen consumption, electrocardiograms. *American Journal of Physiology, 155,* 355–365.

Fraenkel, G. S., & Gunn, D. L. (1961). *The orientation of animals.* New York: Dover.

Freeman, P. H., & Wellman, P. J. (1987). Brown adipose tissue thermogenesis induced by low level electrical stimulation of hypothalamus in rats. *Brain Research Bulletin, 18,* 7–11.

Fregly, M. J. (1994). Induction of hypertension in rats exposed chronically to cold. In K. Pleschka & R. Gerstberger (Eds.), *Integrative and cellular aspects of autonomic functions: Temperature and osmoregulation* (pp. 201–208). Paris: John Libby Eurotext.

Gerrish, C. J., & Alberts, J. R. (1996). Environmental temperature modulates onset of independent feeding: Warmer is sooner. *Developmental Psychobiology, 29,* 483–495.

Girardier, L., & Seydoux, J. (1986). Neural control of brown adipose tissue. In P. Trayhurn & D. G. Nicholls (Eds.), *Brown adipose tissue* (pp. 122–151). London: Edward Arnold.

Goslinga, H. (1984). *Blood viscosity and shock: The role of hemodiluation, hemoconcentration and defibrination.* New York: Springer-Verlag.

Gramsbergen, A., Schwartze, P., & Prechtl, H. F. R. (1970). The postnatal development of behavioral states in the rat. *Developmental Psychobiology, 3,* 267–280.

Guyton, A. C., & Hall, J. E. (1996). *Medical physiology.* Philadelphia: Saunders.

Hahn, P. (1956). The development of thermoregulation. III. The significance of fur in the development of thermoregulation in rats. *Physiologia Bohemoslovenica, 5,* 428–431.

Hall, W. G., & Oppenheim, R. W. (1987). Developmental psychobiology: Prenatal, perinatal, and early postnatal aspects of behavioral development. *Annual Review of Psychology, 38,* 91–128.

Hammel, H. T., Jackson, C. D., Stolwijk, J. A. J., Hardy, J. D., & Strømme, S. B. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set point. *Journal of Applied Physiology, 18,* 1146–1154.

Hammel, H. T., Caldwell, F. T., Jr., & Abrams, R. M. (1967). Regulation of body temperature in the blue-tongued lizard. *Science, 156,* 1260–1262.

Hård, E., Engel, J., & Lindh, A. (1988). Effect of clonidine on ultrasonic vocalization in preweaning rats. *Journal of Neural Transmission, 73,* 217–237.

Heim, T., & Hull, D. (1966). The blood flow and oxygen consumption of brown adipose tissue in the new-born rabbit. *Journal of Physiology, 186,* 42–55.

Heinrich, B. (1987). Thermoregulation by winter-flying endothermic moths. *Journal of Experimental Biology, 127,* 313–332.

Heinrich, B. (1993). *The hot-blooded insects: Strategies and mechanisms of thermoregulation.* Cambridge, MA: Harvard University Press.

Hissa, R. (1968). Postnatal development of thermoregulation in the Norwegian lemming and the golden hamster. *Annales Zoologici Fennici, 5,* 345–383.

Hofer, M. A., Brunelli, S. A., & Shair, H. (1994). Potentiation of isolation-induced vocalization by brief exposure of rat pups to maternal cues. *Developmental Psychobiology, 26,* 81–95.

Hogan, J. A. (1980). Homeostasis and behavior. In F. M. Toates & T. R. Halliday (Eds.), *Analysis of motivational processes* (pp. 3–21). New York: Academic Press.

Houk, J. C. (1988). Control strategies in physiological systems. *FASEB Journal, 2,* 97–107.

Hsieh, A. C. L., Carlson, L. D., & Gray, G. (1957). Role of the sympathetic nervous system in the control of chemical regulation of heat production. *American Journal of Physiology, 190,* 247–251.

Hull, J., & Hull, D. (1982). Behavioral thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology, 96,* 143–147.

Hull, D., & Segall, M. M. (1965). The contribution of brown adipose tissue to heat production in the new-born rabbit. *Journal of Physiology, 181,* 449–457.

Jans, J., & Leon, M. (1983). Determinants of mother–young contact in Norway rats. *Physiology and Behavior, 30,* 919–935.

Johanson, I. B. (1979). Thermotaxis in neonatal rat pups. *Physiology and Behavior, 23,* 871–874.

Johnston, D. W. (1971). The absence of brown adipose tissue in birds. *Comparative Biochemistry and Physiology, 40A,* 1108–1108.

Jouvet-Mounier, D., Astic, L., & Lacote, D. (1970). Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. *Developmental Psychobiology, 2,* 216–239.

Kehoe, P., & Harris, J. C. (1989). Ontogeny of noradrenergic effects on ultrasonic vocalizations in rat pups. *Behavioral Neuroscience, 103,* 1099–1107.

Kinder, E. F. (1927). A study of nest-building activity of the albino rat. *Journal of Experimental Zoology, 147,* 117–161.

Kirby, R. F., & Blumberg, M. S. (1998). Maintenance of arterial pressure in infant rats during moderate and extreme thermal challenge. *Developmental Psychobiology, 32,* 169–181.

Kirby, R. F., Sokoloff, G., Perdomo, E., & Blumberg, M. S. (1999). Thermoregulatory and cardiovascular responses of infant SHR and WKY rats to cold exposure. *Hypertension, 33,* 1465–1469.

Kleitman, N., & Satinoff, E. (1982). Thermoregulatory behavior in rat pups from birth to weaning. *Physiology and Behavior, 29,* 537–541.

Krief, S., & Bazin, R. (1991). Genetic obesity: Is the defect in the sympathetic nervous system? A review through developmental studies in the preobese Zucker rat. *Proceedings of the Society for Experimental Biology and Medicine, 198,* 528–538.

Leon, M. (1986). Development of thermoregulation. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology* (Vol. 8, pp. 297–322). New York: Plenum Press.

Leonard, C. M. (1974). Thermotaxis in golden hamster pups. *Journal of Comparative and Physiological Psychology, 86,* 458–469.

Leonard, C. M. (1982). Shifting strategies for behavioral thermoregulation in developing golden hamsters. *Journal of Comparative and Physiological Psychology, 96,* 234–243.

Lovegrove, B. G., Heldmaier, G., & Ruf, T. (1991). Perspectives of endothermy revisited: The endothermic temperature range. *Journal of Thermal Biology, 16,* 185–197.

Lowell, B. B., SSusulic, V., Hamann, A., Lawitts, J. A., Himms-Hagen, J., Boyer, B. B., Kozak, L. P., & Flier, J. S. (1993). Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature, 366,* 740–742.

Lyman, C. P., & Blinks, D. C. (1959). The effect of temperature on the isolated hearts of closely related hibernators and non-hibernators. *Journal of Cellular and Comparative Physiology, 54,* 53–63.

Lyman, C. P., & Chatfield, P. O. (1950). Mechanism of arousal in the hibernating hamster. *Journal of Experimental Zoology, 114,* 491–516.

Miczek, K. A., Weerts, E. M., Vivian, J. A., & Barros, H. M. (1995). Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. *Psychopharmacology, 121,* 38–56.

Mori, I., & Ohshima, Y. (1995). Neural regulation of thermotaxis in *Caenorhabditis elegans. Nature, 376,* 344–348.

Mortola, J. P. (1983). Comparative aspects of the dynamics of breathing in newborn mammals. *Journal of Applied Physiology, 54,* 1229–1235.

Mortola, J. P. (1987). Dynamics of breathing in newborn mammals. *Physiological Reviews, 67,* 187–243.

Mrosovsky, N. (1990). *Rheostasis: The physiology of change.* Oxford: Oxford University Press.

Nedergaard, J., Connolly, E., & Cannon, B. (1986). Brown adipose tissue in the mammalian neonate. In P. Trayhurn & D. G. Nicholls (Eds.), *Brown adipose tissue* (pp. 152–213). London: Edward Arnold.

Newkirk, K. D., Silverman, D. A., & Wynne-Edwards, K. E. (1995). Ontogeny of thermoregulation in the Djungarian hamster (*Phodopus campbelli*). *Physiology and Behavior, 57,* 117–124.

Ogilvie, D. M., & Stinson, R. H. (1966). The effect of age on temperature selection by laboratory mice (*Mus musculus*). *Canadian Journal of Zoology, 44,* 511–517.

Okon, E. E. (1971). The temperature relations of vocalization in infant Golden hamsters and Wistar rats. *Journal of Zoology* (London), *164,* 227–237.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., & Collins, F. (1995). Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. *Science, 269,* 540–543.

Planche, E., Joliff, M., & Bazin, R. (1988). Energy expenditure and adipose tissue development in 2- to 8-day-old Zucker rats. *International Journal of Obesity, 12,* 352–360.

Pleschka, K., & Gerstberger, R. (1994). *Integrative and cellular aspects of autonomic functions: Temperature and osmoregulation.* Paris: John Libbey Eurotext.

Pohl, H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology, 20,* 405–410.

Richter, C. P. (1943). Total self regulatory functions in animals and human beings. *Harvey Lectures, 38,* 63–103.

Rothwell, N. J., Stock, M. J., & Thexton, A. J. (1983). Decerebration activates thermogenesis in the rat. *Journal of Physiology, 342,* 15–22.

Satinoff, E. (1978). Neural organization and evolution of thermal regulation in mammals. *Science, 201,* 16–22.

Satinoff, E. (1991). Developmental aspects of behavioral and reflexive thermoregulation. In H. N. Shair, G. A. Barr, & M. A. Hofer (Eds.), *Developmental psychobiology: New methods and changing concepts* (pp. 169–188). Oxford: Oxford University Press.

Satinoff, E. (1996). Behavioral thermoregulation in the cold. In M. J. Fregly & C. M. Blatteis (Eds.), *Handbook of Physiology* (pp. 481–505). Oxford: Oxford University Press.

Schmidt, I., Kaul, R., & Carlisle, H. J. (1984). Body temperature of huddling newborn Zucker rats. *Pflüger's Archive, 401,* 418–420.

Schmidt-Nielsen, K. (1981). Countercurrent systems in animals. *Scientific American, 244,* 118–128.

Schmidt-Nielsen, K. (1991). *Animal physiology: Adaptation and environment.* Cambridge: Cambridge University Press.

Schneider-Picard, G., & Girarder, L. (1982). Postnatal development of sympathetic innervation of rat brown adipose tissue reevaluated with a method allowing for monitoring flavoprotein redox state. *Journal of Physiology* (Paris), *78,* 151–157.

Schoenfeld, T. A., & Leonard, C. M. (1985). Behavioral development in the Syrian golden hamster. In H. I. Siegel (Ed.), *The hamster: Reproduction and behavior* (pp. 289–321). New York: Plenum Press.

Seeley, R. J., & Schwartz, M. W. (1997). The regulation of energy balance: Peripheral hormonal signals and hypothalamic neuropeptides. *Current Directions in Psychological Science, 6,* 39–44.

Shaddy, R. E., Tyndall, M. R., Teitel, D. F., Li, C., & Rudolph, A. M. (1988). Regulation of cardiac output with controlled heart rate in newborn lambs. *Pediatric Research, 24,* 577–582.

Shibata, M., Benzi, R. H., Seydoux, J., & Girardier, L. (1987). Hyperthermia induced by prepontine knife-cut: Evidence for a tonic inhibition of non-shivering thermogenesis in anesthetized rat. *Brain Research, 436,* 273–282.

Smith, R. E. (1961). Thermogenic activity of the hibernating gland in the cold-acclimated rat. *Physiologist, 4,* 113.

Smith, R. E. (1964). Thermoregulatory and adaptive behavior of brown adipose tissue. *Science, 146,* 1686–1689.

Smith, R. E., & Horwitz, B. A. (1969). Brown fat and thermogenesis. *Physiological Reviews, 49,* 330–425.

Smith, R. E., & Roberts, J. C. (1964). Thermogenesis of brown adipose tissue in cold-acclimated rats. *American Journal of Physiology, 206,* 143–148.

Sokoloff, G., & Blumberg, M. S. (1997). Thermogenic, respiratory, and ultrasonic responses of week-old rats across the transition from moderate to extreme cold exposure. *Developmental Psychobiology, 30,* 181–194.

Sokoloff, G., & Blumberg, M. S. (1998). Active sleep in cold-exposed infant Norway rats and Syrian golden hamsters: The role of brown adipose tissue thermogenesis. *Behavioral Neuroscience, 112,* 695–706.

Sokoloff, G., Blumberg, M. S., & Adams, M. M. (2000). A comparative analysis of huddling in infant Norway rats and Syrian golden hamsters: Does endothermy modulate behavior? *Behavioral Neuroscience, 114,* 585–593.

Sokoloff, G., Kirby, R. F., & Blumberg, M. S. (1998). Further evidence that BAT thermogenesis modulates cardiac rate in infant rats. *American Journal of Physiology, 274*, R1712–R1717.

Spiers, D. E., & Adair, E. R. (1986). Ontogeny of homeothermy in the immature rat: Metabolic and thermal responses. *Journal of Applied Physiology, 60*, 1190–1197.

Sundin, U., Herron, D., & Cannon, B. (1981). Brown fat thermoregulation in developing hamsters (*Mesocricetus auratus*): A GDP-binding study. *Biology of the Neonate, 39*, 141–149.

Taylor, P. M. (1960). Oxygen consumption in new-born rats. *Journal of Physiology, 154*, 153–168.

Tazawa, H., & Nakagawa, S. (1985). Response of egg temperature, heart rate and blood pressure in the chick embryo to hypothermal stress. *Journal of Comparative Physiology B, 155*, 195–200.

Teitel, D. F., Sidi, D., Chin, T., Brett, C., Heymann, M. A., & Rudolph, A. M. (1985). Developmental changes in myocardial contractile reserve in the lamb. *Pediatric Research, 19*, 948–955.

Thomas, J. H. (1995). Some like it hot. *Current Biology, 5*, 1222–1224.

West, M. J., King, A. P., & Arberg, A. A. (1988). The inheritance of niches: The role of ecological legacies in ontogeny. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology* (Vol. 9, pp. 41–62). New York: Plenum Press.

Wiener, N. (1961). *Cybernetics or control and communication in the animal and the machine.* Cambridge, MA: MIT Press.

Williams, G. C. (1966). *Adaptation and natural selection.* Princeton, NJ: Princeton University Press.

Winslow, J. T., & Insel, T. R. (1991). The infant rat separation paradigm: A novel test for novel anxiolytics. *Trends in Pharmacological Sciences, 12*, 402–404.

Yamori, Y. (1984). Development of the spontaneously hypertensive rat (SHR) and of various spontaneous rat models, and their implications. In W. de Jong (Ed.), *Handbook of hypertension, Volume 4: Experimental and genetic models of hypertension* (pp. 224–239). New York: Elsevier.

Youmans, W. B., Murphy, Q. R., Turner, J. K., Davis, L. D., Briggs, D. I., & Hoye, A. S. (1963). Activity of abdominal muscles elicited from the circulatory system. *American Journal of Physical Medicine, 42*, 1–70.

Youmans, W. B., Tjioe, D. T., & Tong, E. Y. (1974). Control of involuntary activity of abdominal muscles. *American Journal of Physical Medicine, 53*, 57–74.

Zhao, J., Unelius, L., Bengtsson, T., Cannon, B., & Nedergaard, J. (1994). Coexisting b-adrenoceptor subtypes: Significance for thermogenic process in brown fat cells. *American Journal of Physiology, 267*, C969–C979.