Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Classics

RAISING THE 'DEAD' – REPERFUSION FROM TORPOR

Kelly Drew discusses Paul Chatfield and Charles Lyman's classic paper 'Circulatory changes during the process of arousal in the hibernating hamster', published in the *American Journal of Physiology* in 1950.

Over 60 years ago, Paul Chatfield and Charles Lyman described fundamental physiological phenomenona in the hibernating hamster *Mesocricetus auratus* that define the transition between the torpid and euthermic states (Chatfield and Lyman, 1950). The authors reported uneven distribution of heat throughout the animal's bodies as they rewarmed and intense vasoconstriction of the hindlimb vasculature during the onset of rewarming. They also described a role of the autonomic nervous system in regulating cardiovascular responses during rewarming. The autonomic (or involuntary) nervous system controls basic physiological functions through a balanced influence of the sympathetic nervous system (SNS; known for the fight-or-flight response) and the parasympathetic nervous system (PNS; known for the rest-and-digest response). In particular, Chatfield and Lyman described a dominant role for the SNS in stimulation of heart rate (HR) during rewarming. The increase in HR was preempted by a rapid rise in blood pressure achieved by differential vasoconstriction and restriction of circulation to the posterior parts of the body. These early observations, illustrated in recent educational videos (http://www.brains-hibernation.iab.alaska.edu), provided the foundation for our current understanding of the mechanisms that coordinate the highly regulated state of hibernation and inspired researchers to ask questions about the adaptive significance of these cardiovascular changes. Chatfield and Lyman proposed that arousal from hibernation is initiated by a waking stimulus, which causes a mass discharge from the hypothalamus and leads to an increase in body temperature (T_b), HR and blood pressure. Additional methods, refined to include the use of indwelling cannulae (Toien et al., 2001) and wireless telemetry to monitor heart electrical activity (ECG) and blood pressure (Mertens et al., 2008; Swoap and Gutilla, 2009; Hampton et al., 2010), have expanded these early observations, but fundamental questions, such as the mechanisms behind interbout arousals and the adaptive significance of some of the unique circulatory changes described in Chatfield and Lyman's classic paper, remain unanswered.

Hibernation is the ultimate extreme of mammalian energy conservation that allows animals to survive without food for 5–8 months (Barnes, 1989; Sheriff et al., 2010). Energy conservation is defined by a decrease in metabolic rate and consequent T_b termed torpor. Hibernation is defined by prolonged torpor bouts during which animals may decrease metabolic rate to as low as 1–2% of basal metabolic rate (Geiser, 1988; Buck and Barnes, 2000), and core T_b decreases to within 1–2°C above ambient temperature (Buck and Barnes, 2000). Bouts of prolonged torpor are interrupted by brief (4–24 h) periods of euthermic T_b and metabolic rate known as interbout arousals. Overall, hibernation is characterized by multiple torpor bouts, with each bout consisting of an entrance phase, a maintenance phase and an arousal phase (Drew et al., 2007). Chatfield and Lyman studied the arousal phase because, as they wrote, 'The manipulations necessary to prepare the hamster for taking records invariably initiated the process of arousal from hibernation' (Chatfield and Lyman, 1950).

They made observations at an ambient temperature of 5°C by instrumenting torpid hamsters with ECG electrodes and thermocouples (one in the cheek pouch and one in the rectum), cannulating the carotid artery and vein for blood pressure monitoring and drug delivery, and exposing the vagus nerves for stimulation and recording. The authors noted a dramatic increase in HR as animals aroused from torpor and asked (1) whether the SNS stimulated HR and (2) whether the maximal HR during arousal was limited by the physical effects of the temperature of the heart, by the PNS or by intrinsic properties of the tissue. The SNS conveys a fight-or-flight response through a chemical messenger called noradrenaline (or norepinephrine), a chemical cousin of adrenaline (or epinephrine). Chatfield and Lyman determined what role the SNS played in arousal by blocking its chemical communication with a drug (veratrosine). They found that pharmacological blockade of SNS stimulation decreased HR during arousal so they knew that the SNS stimulated the heart during arousal. They next injected adrenaline to determine whether the SNS could stimulate the heart to beat even faster. They found that even after adrenaline was administered to hamsters during arousal, HR did not increase anymore than without the added drug. These results suggested that SNS stimulation of HR was maximal during arousal, but did not address what set the limit on HR. Thus came one of the first attempts to assess the competing roles of sympathetic and parasympathetic influence during hibernation and the role of temperature on cardiovascular processes in hibernating mammals.

Knowing that that thermodynamic effects of cooling on the rate of physical, chemical or biological processes are described by the Arrhenius equation, where the log of the rate of a process is a linear function of the reciprocal of the absolute temperature (i.e. temperature expressed in K), Chatfield and Lyman wondered whether an increase in temperature controlled the increase in HR. The scientists graphed the log of HR as a function of the reciprocal of the absolute cheek pouch temperature (used to approximate the temperature of the heart) and found that the Arrhenius equation was not linear. Therefore, HR was not limited by effects of temperature on physio-chemical processes.

Cardiac activity is regulated by the SNS and the PNS. Parasympathetic neurons, bundled into the vagus nerve (cranial nerve X), release acetylcholine as a neurotransmitter and decrease cardiac activity *via* activation of muscarinic acetylcholine receptors located on the heart. To determine whether PNS innervation of the heart limited HR during arousal, Chatfield and Lyman abolished PNS influence by treating the animals with atropine. Atropine is a competitive antagonist of muscarinic acetylcholine receptors and blocks the effects of acetylcholine on the heart. To ensure that the dose of atropine (1 mg kg^{-1}) was effective, the authors indicated that the dose used abolished reflex vagal slowing of the heart caused by anoxia. This same dose, however, failed to influence HR during rewarming, suggesting that PNS influence did not limit HR during arousal. Because the vagus nerve did not appear to be important in limiting HR during arousal, the authors questioned whether the vagus nerve could function at low temperatures. They found that stimulating the vagus nerve at a T_b of 10°C slowed HR and concluded that the vagus nerve could function at low temperature, but did not limit HR during arousal. Once arousal was nearly complete, however, atropine was found to reverse periods of bradycardia. By default, Chatfield and Lyman concluded that high HR during arousal was limited by intrinsic properties of cardiac tissue rather than by direct effects of temperature or by cardio-inhibitory influence of the PNS nervous system.

Reflecting on the impact that Chatfield and Lyman's classic paper has had on the study of hibernation, subsequent work has significantly refined the techniques used to monitor T_b and cardiovascular changes during hibernation. These approaches have extended our knowledge of mechanisms, primarily neural, responsible for coordinated physiological changes during the process of arousal as well as during the entrance and maintenance phases of torpor (Harris and Milsom, 1995; Milsom et al., 2001; Drew et al., 2007; Jinka et al., 2011). Observations have been made on a number of true hibernating species, including ground squirrels, which hibernate according to an endogenous circannual rhythm with little influence from environmental conditions, and hamsters, which hibernate in response to shortening of photoperiod and resource availability. Observations have also been made of fasting-induced torpor in mice. Remarkably similar qualitative changes are noted in a variety of species. For example, in all cases, torpor onset involves pronounced decreases in oxygen consumption that precedes a decline in T_b, and arousal involves uneven rewarming so that the front regions of the body rewarm before the hind regions. Moreover, a characteristic hysteresis (Milsom et al., 2001; Mertens et al., 2008; Swoap and Gutilla, 2009) in the relationship between T_b and cardiovascular changes during entrance and arousal suggests that common central nervous system (CNS) mechanisms regulate cardiovascular changes associated with hibernation and torpor in a variety of species.

More recent studies of the influence of the SNS and the PNS on cardiovascular changes during onset and maintenance of hibernation along with these earlier studies of arousal from hibernation demonstrate that the PNS dominates during onset of hibernation to slow the heart and the SNS dominates during arousal to stimulate the heart (Harris and Milsom, 1995; Milsom et al., 2001). Interestingly, while Chatfield and Lyman showed that the PNS is not essential for arousal from hibernation, the SNS is essential for onset of hibernation (Swoap and Weinshenker, 2008; Braulke and Heldmaier, 2010). The balance of SNS and PNS influence during onset, maintenance and arousal may depend on the species and ambient temperature.

Chatfield and Lyman concluded that the SNS plays an important role in arousal and rewarming, but experiments reported in this classic paper were not designed to test how the SNS was affecting rewarming. The authors suggested that pharmacological blockade of the SNS with veratrosine slowed rates of rewarming by decreasing HR and thus limiting circulation of warmed blood. More recently, Osborne et al. (Osborne et al., 2005) found that α-adrenergic receptors in the SNS mediate the hindlimb vasoconstriction noted by Chatfield and Lyman that occurs upon rewarming. Constriction of the posterior vasculature is expected to facilitate warming of the anterior portions of the body by shunting warmed blood to these regions. Blockade of this process would then be expected to slow rates of rewarming as noted in the classic paper. In addition, Swoap and Weinshenker have shown that inhibition of brown adipose tissue by the SNS is necessary for rewarming from torpor in mice (Swoap and Weinshenker, 2008). Inhibition of the SNS with veratrosine may therefore have slowed the rate of rewarming by preventing SNS stimulation of brown adipose tissue.

Many of the contributions of Chatfield and Lyman's classic paper included some of the first descriptions of circulatory changes associated with arousal from hibernation. The nature of these contributions inspired others to describe similar phenomena using more refined techniques. Measures of blood pressure during hibernation have nonetheless lagged behind other observations, in part because wireless technologies for monitoring blood pressure in small animals have become available only recently (Swoap and Gutilla, 2009). Chatfield and Lyman's measurements of blood pressure during arousal from hibernation provided the first evidence for an increase in peripheral resistance during arousal. This observation is now well documented as one of the early hallmarks of arousal (Osborne et al., 2005). Less well understood is the control of blood pressure during entrance and maintenance of torpor, which may be important in heat and energy conservation (Jinka et al., 2012a). Swoap and Guitilla showed that estimates of total peripheral resistance increase threefold during fasting-induced torpor in mice (Swoap and Gutilla, 2009), suggesting that circulatory changes may be significant throughout all phases of torpor, including entrance, maintenance and arousal.

Based on observations for the role of the autonomic nervous system in the circulatory changes observed in their classic paper, and the current understanding of the hypothalamus as a central command center to coordinate the balance between fight-or-flight and rest-and-digest responses, Chatfield and Lyman pointed to the hypothalamus as a key regulator of neural control of arousal from hibernation. While the central control of hibernation remains largely unknown (Drew et al., 2007), more progress has been made in understanding torpor onset than arousal. Torpor onset involves activation of A_1 adenosine receptors within the CNS that inhibit themogenesis to promote cooling (Tamura et al., 2005; Jinka et al., 2011; Tupone et al., 2012). A central site of action of A_1 adenosine receptor agonists also prevents cardiac arrhythmias associated with hypothermia (Miyazawa et al., 2008).

Although the hypothalamus is the most probable site of action (Shintani et al., 2005) definitive studies have not been done to test this hypothesis in spontaneously hibernating animals. Central control of arousal from hibernation is less well studied. Although autonomic nervous system control of arousal must be coordinated within the CNS, recent evidence in the arctic ground squirrel suggests that a site outside of the CNS, stimulated by removal of glutamatergic NMDA receptor stimulation, serves as a sufficient signal to induce arousal (Jinka et al., 2012b).

In summary, the classic paper of Chatfield and Lyman reviewed with other work in 1982 (Lyman, 1982) set the stage for the study of circulatory changes during hibernation. These pioneer physiologists, as well as their contemporaries John and Janet Twente (Twente and Twente, 1968; Twente and Twente, 1978), illustrated some of the remarkable physiology of hibernating mammals that has sparked continued and growing interest in this feat of mammalian energy conservation.

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