Establishing an Appropriate Period of Acclimatization Following Transportation of Laboratory Animals

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Abstract

Stress associated with transportation has widespread effects on physiological systems in laboratory animals, including changes in the cardiovascular, endocrine, immune, central nervous, and reproductive systems. Although short-lived, these changes can confound research if animals are utilized before homeostasis is restored and physiological measures return to normal. Therefore, some period of acclimatization following transportation is generally suggested to restore homeostasis. The following two questions should be considered to establish an adequate period for acclimatization: (1) Will anticipated physiological changes confound the research to be conducted? (2) What is the length of time necessary for confounding physiological changes to normalize? Finding answers to those questions in the literature can be a challenge. Most literature on the physiological impact of transportation involves agricultural animals, although the limited literature in common laboratory animal species generally parallels changes documented in agricultural animals. The literature documents elevated heart rate and weight loss, as well as elevated concentrations of adrenaline, noradrenaline, glucose, cortisol, free fatty acids, and β-hydroxybutyrate. Carbohydrate, protein, and lipid metabolism (both lipolysis and lipogenesis) are altered, and plasma osmolality, albumen, protein, and pack-cell volume increase. Neutrophilia and lymphopenia are also evident. These measures generally return to baseline within 1 to 7 days of transportation, although animals that are young, severely stressed, and have stress-sensitive genotypes may show altered physiological measures for several weeks. Other measures such as circadian rhythm and reproductive performance may take several weeks to months to normalize.

Key Words: acclimatization; agricultural animals; immune system; physiological effects; rodents; stress; transportation

Introduction

Transportation unavoidably causes stress in animals. Although stress is not always an adverse experience and is a necessary and regular aspect of life, it causes changes in an animal's physiological status during transportation and for some period thereafter. Utilizing transported animals before their physiological status normalizes can have considerable and unintended effects on research results. In this article, we briefly explore some of the physiological changes that have been documented in research animals, and we consider the length of time necessary to properly acclimatize animals following transportation and before the initiation of experiments. Notwithstanding our recognition of the profound effects that transportation can have on animal behavior and subsequent behavioral studies, we focus specifically in this article on physiological changes associated with transportation and their impact on biomedical experimental data. The information herein is based partially on the recently published Institute for Laboratory Animal Research (ILAR[1]) report *Guidelines for the Humane Transportation of Research Animals* (NRC 2006).

Transportation Stress and Physiological Changes

Stress is the biological response animals exhibit in response to stimuli (stressors) that disrupt their homeostasis (Stokes 2000). Animals often adapt successfully to a stressor and return to a normal homeostatic state, resulting in positive health effects (McEwen 2002). Stressors and stress are therefore not inherently negative events; it is generally when stress is long lasting or severe that animals are unable to adapt successfully and adverse consequences occur (NRC 1992).

Mammals respond to stress by releasing a host of primary mediators such as glucocorticoids and catecholamines. These mediators have widespread effects on tissues and cells; they bind to receptors, ion channels, and intracellular proteins to cause primary effects such as activation of signaling cascades and gene expression. Cumulatively, the

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[1]Abbreviations used in this article: β-OHB, β-hydroxybutyrate; HPA, hypothalamic-pituitary-adrenocortical; ILAR, Institute for Laboratory Animal Research; NEFA, nonesterified fatty acid.

primary mediators result in secondary outcomes (McEwen and Seeman 1999). For instance, acute stress causes release of the primary mediator epinephrine, which binds to β-adrenergic receptors on the heart, increasing heart rate (Reece 2004). Secondary outcomes are possible in every physiological system including the cardiovascular system, metabolism, the central nervous system, and the immune system (McEwen and Seeman 1999). However, the magnitude and duration of secondary outcomes are greatly influenced by the intensity, duration, and type of stressor. The age, health status, genotype, and previous experiences of animals also influence secondary outcomes.

Although literature on effects of acute and chronic stressors continues to grow, it is difficult to predict physiological changes that will occur in response to transportation from available literature. Transportation involves physical (e.g., temperature changes), physiological (e.g., limited access to food), and psychological stressors (e.g., exposure to novel environments) (NRC 2006). Predicting secondary physiological outcomes resulting from transportation is a challenge because multiple stressors occur, often in combinations unique to a particular transportation scenario or species. For example, swine and cattle are often shipped in large groups on truck beds without food or water (FASS 1999). In this situation, heat/cold stress, social stress, food/water deprivation stress, noise stress, and restraint stress may all occur. Nonhuman primates, however, are often shipped by plane in environmentally controlled compartments with food and water (NRC 2006). In such cases, noise stress, vibration stress, and stress from a novel cage environment are unavoidable and should be expected.

The majority of animals utilized in research are rodents (Trull and Rich 1999) generally shipped in environmentally controlled ground vehicles by commercial vendors (NRC 2006). With the physical environment tightly controlled, the main stressors are psychological. Laboratory animals, particularly rodents, live in uniform environments. Cages, bedding, food, conspecifics, sounds, and odors animals are exposed to in laboratories and breeding facilities comprise their familiar and constant environment. During and after transportation, almost every aspect of the animal's environment changes, with no way for the animal to return to the previously familiar physical and social environments.

Due to the complexity of the stressors associated with transportation, research efforts have been, appropriately, evaluations of physiological changes resulting from actual transportation events. The majority of this literature involves agricultural animals because transport of livestock is an integral part of the production cycle in the United States, and there are social and economic pressures for animals to arrive in good condition. This literature is reviewed briefly in the following section to provide a broad overview of the major physiological changes that have been documented. For an in-depth review of transportation in agricultural animals, we advise readers to consult the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS 1999).

After review of physiological changes associated with transportation of agricultural animals, we review the limited research available on physiological changes in rodents following transportation. We refer researchers seeking additional information about the effects of transportation on research animals to the recently published ILAR report *Guidelines for the Humane Transportation of Research Animals* (NRC 2006).

Physiological Changes in Agricultural Animals Following Transportation

When an animal is transported, psychological, physiological, and physical stress result in the release of catecholamines such as adrenaline (epinephrine) (Dalin et al. 1993) and noradrenaline (norepinephrine) (Dalin et al. 1993; Parrott et al. 1998). These releases correspond to elevated heart rates (Ingram et al. 2002; Parrott et al. 1998) and blood glucose concentrations (Kannan et al. 2000; Stull and Rodiek 2000).

Transportation also activates the hypothalamic-pituitary-adrenocortical (HPA[1]) axis, which is generally considered to be a response to psychological stress (Knowles and Warriss 2000). Activation of the HPA axis leads to the release of glucocorticoids, of which cortisol is the most commonly measured (Lay et al. 1996; McGlone et al. 1993). Elevated levels of cortisol and other glucocorticoids have been shown to have a significant impact on the immune system (McEwen et al. 1997). Therefore many immunological changes are associated with transportation (see Table 1). These immune changes can lead to delayed morbidity and mortality, particularly in young animals (Swanson and Morrow-Tesch 2001). Glucocorticoids also increase blood glucose levels (Kannan et al. 2000; Kent and Ewbank 1983) through effects on carbohydrate, protein, and lipid metabolism including lipolysis, lipogenesis and gluconeogenesis (Sapolsky et al. 2000).

Many animals respond to the transportation experience by reducing their intakes of food and water (Friend et al. 1998; Knowles et al. 1999). In other cases, it may be necessary for personnel to withhold food or water during transport for safety reasons. Normally, animals lose weight during transport; however, this weight loss is greater than that lost during the same length of time without food and water (Knowles and Warriss 2000). When food intake decreases because food is withheld or due to travel-associated anorexia, the animal's body mobilizes energy stores. Triglycerides mobilized from adipose tissue and are released as nonesterified fatty acids (NEFAs[1]). NEFAs are converted, in part, by the liver to ketones, including β-hydroxybutyrate (β-OHB[1]). Both NEFA and β-OHB are utilized as energy sources in peripheral tissues (Knowles and Warriss 2000). Elevated levels of both NEFA and β-OHB have been found in the blood of transported animals (Broom 2003). A decrease in water intake also has the direct effect of decreasing

Table 1 Immunological responses to transport in agricultural animals

Species	Immunological change	Reference (see text)
Cattle	↓ Percentage of lymphocyte	Kent and Ewbank 1983
	↑ Percentage of neutrophil	Tarrant et al. 1992
	↑ Neutrophil:lymphocyte ratio	Schaefer et al. 1997
		Kegley et al. 1997
		Murata et al. 1985
		Phillips et al. 1989
Goats	↓ Percentage of lymphocyte	Kannan et al. 2000
	↑ Percentage of neutrophil	
	↑ Neutrophil:lymphocyte ratio	
Horses	↑ Neutrophil:lymphocyte ratio	Stull and Rodiek 2000
	↑ White blood cell count during transport	
Swine	↓ White blood cell and neutrophil counts	Dalin et al. 1993
		McGlone et al. 1993
	↓ Percentage of lymphocyte	
	↑ Percentage of neutrophil	
	↑ Neutrophil:lymphocyte ratio	
	↑ White blood cell and neutrophil counts (during transportation)	

intracellular and extracellular fluid volumes (Knowles and Warriss 2000). As a result, plasma osmolality, total plasma protein and albumen concentrations, and packed-cell volume increase (Broom 2003).

Physiological Changes in Rodents Resulting from Transportation

It is reasonable to suggest that rodents likely exhibit similar physiological responses during and after transport. In fact, the limited literature on transportation of rodents (Table 2) indicates that basic measures of stress and immune function are affected in a manner similar to that documented in agricultural animals. In general, it has been reported that glucocorticoid concentrations increased for 1 to 2 days, body weight decreased, and measures of the immune system indicated a suppressed immune response up to 48 hr after transport. These changes have also been documented in dogs (Bergeron et al. 2002) and rabbits (Toth and January 1990).

Acclimatization Following Transportation

Because even the overseas transport of animals takes only a few days, chronic stress resulting in long-term health effects (Irwin 1994; Kiecolt-Glaser et al. 2002) is unlikely to occur during a routine transportation event. The available, albeit limited, literature supports this supposition, documenting that the physiological changes are short lived. Nevertheless, even transient physiological changes can affect biomedical research results substantially if experiments are performed before the animal can acclimate to its new environment and before its physiological status has normalized.

Table 2 Physiological changes in rodents resulting from transportation

Strain	Age/size at transport	Length of transport	Physiological response	Recovery time	Reference (see text)
Mice					
C57BL/6NHsd	6 wk	18-42 hr	↓ Natural killer cell activity	24 hr	Aguila et al. 1988
			↑ Plasma corticosterone levels	24 hr	
Crl:COBS,CD-1(ICR)BR	30-35 g	24-48 hr	↑ Plasma corticosterone concentrations	>48 hr	Landi et al. 1982
			↓ Foot pad thickness (delayed-type immune hypersensitivity)	24 hr	
			↓ Hemagglutination titers (antibody titer)	24 hr	
			↓ Plaque-forming cell assay (antibody production)	48 hr	
Peromyscus maniculatus	2-25 mo	27-36 hr	↓ Reproductive performance (timing/frequency of reproduction delayed)	1 mo	Hayssen 1998
Tac:(SW)fBR	14.5-15.5 g	24 hr	↓ Body weight	1-4 days	Weisbroth et al. 1977
		72-88 hr	↓ Body weight	4-7 days	
CBA/FaCam, RAP, Swiss crosses	ND	28 hr	↓ Body weight	2 days	Wallace 1976
SF/Cam and SK/Cam	ND	28 hr	↓ Body weight	2 days	Wallace 1976
Rats					
Sprague-Dawley	40-55 g	1-40 hr	↓ Body weight and weight gain	12-48 hr	Dymsza et al. 1963
Sprague-Dawley	325-350 g	ND	↑ Myocardial antioxidant enzyme activity	7 days	Rowland et al. 2000
Tac:NIH(SD)fBR	115-155 g	24-88 hr	↓ Body weight	12-24 hr	Weisbroth et al. 1977
Wistar	40/41 days	15 hr	↑ Water intake	1 day	van Ruiven et al. 1998

ND, no data available.

To minimize effects of transportation-induced physiological changes on subsequent biomedical research, it is advisable to consider two factors. The first factor is whether the anticipated physiological changes could confound the research to be conducted. In general, studies suggest that the physiological responses of animals to transportation include changes in the cardiovascular, endocrine, immune, and reproductive systems as a result of stress (Knowles and Warriss 2000). Researchers should carefully consider whether the focus of their research utilizing transported animals involves physiological functions that can be affected by changes in these several systems. For example, it is reasonable to suggest that metabolic studies could be confounded by transportation-induced elevations in glucocorticoids. Researchers should also consider whether a physiological measure that is used as a humane endpoint could be altered by transportation. For example, body weight is a physiological measure commonly used as a humane endpoint in some types of research (Toth 2000). Most animals show decreases in body weight following transportation (see Tables 1 and 2) therefore use of body weight in this situation may not accurately indicate an animal's condition.

The second factor that should be considered is how long it takes for potentially confounding physiological changes to normalize. Generally, primary mediators of a stress response (i.e., catecholamines and glucocorticoids) return to normal concentrations within 24 hr of transportation. Secondary physiological outcomes may take longer to normalize. Although literature on effects of transportation on rodents is limited, it generally includes information on the length of time necessary for different physiological parameters to normalize. As shown in Table 2, most physiological changes in the immune and endocrine systems take 1 to 7 days to normalize. Similar acclimatization periods were documented in rabbits from studies in which hyperglycemia, neutrophilia, lymphopenia, and elevated plasma cortisol levels required 48 hr to normalize (Toth and January 1990). The only physiological parameters in common laboratory species that took longer than 1 wk to normalize were measures of reproductive performance, which required 1 mo for normalization (Hayssen 1998).

We advise researchers to consult the literature for guidance on effects of stress that may confound their study and the required duration for acclimatization. However, before a final determination can be made, it is necessary to consider several other factors. These factors include the intensity and duration of stress; the age, genotype, health status, and previous experience of the animal; and allometric differences. All of these factors can have an impact on the magnitude and duration of physiological changes.

Particularly lengthy or traumatic transportation events might be expected to increase the magnitude of physiological changes and the length of time for these measures to return to normal. Generally, investigators who have compared length of transport with loss of body weight have reported that the longer the animals are transported, the more body weight an animal loses and the longer it takes for animals to regain their initial condition (e.g., Brown et al. 1999). Other studies have documented that physiological measures were affected by transport duration in a linear fashion, including serum glucose, urea nitrogen, and urea nitrogen:creatinine ratio (Cole et al. 1988).

Researchers should also consider the age, genotype, health status, and previous experience of the animal being transported. Young calves, for example, do not exhibit the large changes in heart rate, cortisol, and glucose levels that mature cattle show (Knowles et al. 1997). However, after being transported, young pigs and calves have been found to have unstable metabolic rates, which require $\geq$1 wk to stabilize (del Barrio et al. 1993; Heetkamp et al. 2002; Schrama et al. 1992).

Genotype and previous experience can also increase or decrease physiological changes. For example, stress susceptibility in swine, characterized by hyperthermia and sudden death following stress, is inherited by a single recessive gene (Hal^{n}). When subjected to transport, the three Hal genotypes ($Hal^{N/N}$, $Hal^{N/n}$, $Hal^{n/n}$) had different cortisol concentrations immediately after transport as well as 2 wk after transport (Nyberg et al. 1988).

The allometric differences between small animals (e.g., rodents and young animals) and large animals (e.g., agricultural or adult animals) in energy expenditures should also be considered. Small animals require more calories per unit of body mass and become dehydrated more quickly than larger animals. For this reason, small animals may become stressed more quickly and more severely by food or water deprivation than larger animals, leading to larger and longer-lasting stress responses (NRC 2006).

Conclusion

Prudent investigators would be well advised to ensure that transported animals used in their investigations have had adequate time to acclimatize prior to the initiation of experiments because transportation causes physiological changes that can confound subsequent research. To avoid this consequence, researchers must consider both the physiological changes caused by transportation stress and the durations of these changes. Investigating pertinent literature (reviewed briefly in this article) and considering mitigating factors such as age, genotype, health, and previous experience will help guide decisions regarding the length of time necessary to properly acclimatize animals.

Researchers must be aware that the physiological changes caused by transport may not be limited to those identified in this article because comprehensive studies to identify all the physiological changes associated with transportation have not been published to date. In addition, generalities discussed in this article may not hold true for every species. For example, cortisol levels have not been found to correlate with stress in swine during transport (Brown et al. 1999; Hicks et al. 1998). Based on this and other likely differences between species, we urge researchers to thor-

oughly examine the literature for relevant information. We hope that the information in this article will guide and facilitate their search.

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