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Adaptation period of laboratory animals after transport: a review

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Introduction

Before laboratory animals are submitted to experimental procedures, they are usually transported from the breeding institute to the experimental unit. Transportation stress may disturb the physiological stable state of the animals. The extent to which the homeostasis is disturbed may depend, among other factors, on the distance, length and mode of transportation. The aim of this literature review was to determine the minimum period needed for laboratory mice, rats and rabbits, to reach a point of homeostasis again after transportation. Although the term "acclimatisation period" is used more often, "adaptation period" is considered more appropriate. "Acclimatisation period " may be interpreted as an adaptation to climate changes only, whereas "adaptation period" refers to adaptation to all environmental changes (*Köhler et al.* 1978).

Sufficient time must be given to the animals to recover from transportation stress in order to safeguard the animals' well-being and to minimize the variance of experimental results. From an economical point of view, the length of the adaptation period should be kept as short as possible. The Council of Europe (1986) provides only general guidelines on the minimum necessary length of the adaptation period after transportation: "animals may be used for procedures during the quarantine period as long as they have become acclimatised to their new environment and they present no significant risk to other animals or man". It is further stated that the quarantine period is determined by "a competent person according to the circumstances, normally the veterinarian appointed by the establishment". In the literature highly variable lengths of minimum adaptation periods after transport are indicated. Literature results describing the minimum necessary length of the adaptation period for different parameters in rats, rabbits and mice after transport, are discussed in the following.

Parameters

Body weight: A parameter which is used as a basic indicator to estimate the minimum length of the adaptation period of animals after transport, is body weight (gain) (see Table 1). After transporting rats by car (1 hour), train (26 hours), or plain (40 hours), body weight gain returned to normal in about two days, when compared to non-transported animals kept at the breeding station (*Dymsza et al.* 1963). These results may indicate that an adaptation period of 2 days will suffice for the rat, regardless of the mode or length of transport.

Wallace (1976) transported mice by car and train for 28 hours. Control animals were also transferred from group-housing in cages to individual housing in the same type of transport boxes, but remained in the animal room. In addition, the influence of water and/or food deprivation and/or supply was examined, both in transported and in control mice. Measurements during different experimental conditions were not performed simultaneously, but were said to be "at random" over a certain period of time. Recovery time was defined as the number of days needed to recover initial body weight (within 0.5 g of the initial body weight, i.e. just before transfer to the transport box). Both in control and transported mice the recovery time was on average 1.8 days. Food and water deprivation increased weight loss and delayed recovery time in both groups. In case the percentage weight loss was 17-23%, death occurred. Supply of food and water during transport is therefore recommended.

Einer-Jensen & Nielsen (1972) found no significant difference in growth rate between transported

Table 1. Overview of literature on adaptation period of laboratory animals after transport.

Species	Reference	Parameter	Minimum adaptation period*
Rat	*Bieglmayer et al.* 1980	LH	>7 days
Rat	*Weisbroth et al.* 1977	Body weight	<24 hours
			<12 hours (plain water during transport)
Rat	*Dymsza et al.* 1963	Body weight gain	2 days
Rat	*Grant et al.* 1971	Water intake	individual housing: 17 days
			group housing: 23 days
Rat	*Tobiska & Brada* 1963	Serum mucoprotein	3 weeks
Mouse	*Tuli et al.* 1995	Corticosterone	<1 day
		Behaviour	3 days
Mouse	*Landi et al.* 1982	Corticosterone	>2 days
Mouse	*Weisbroth et al.* 1977	Body weight	4 days
			0 day (plain water during transport)
Mouse	*Wallace* 1976	Body weight	2 days
Mouse	*Einer-Jensen* 1972	White blood cell count	7-8 weeks
Mouse	*Aguila et al.* 1988	Corticosterone	1 day
		Natural killer cell activity	1 day
Mouse	*Drozdowicz et al.* 1990	Corticosterone	>1 day
		White blood cell and lymfocyte count	<12 hours
Mouse	*Landi et al.* 1982	Foot pad test	1 day
		Haem aggl. test	1 day
		Plaque forming cell assay	2 days
Rabbits	*Toth & January* 1990	Glucose	4-6 days
		Corticosterone	1 day
		White blood cell diff.	<2 days

* Only significantly different results as a result of transportation stress are mentioned.

(for a period of 6-7 hours) and non-transported mice, which remained at the breeder unit.

Weisbroth et al. (1977) investigated 26 hours´ shipments of rats and mice by car and plain. The recovery period was determined as the period needed for the body weight to return to values within 1 standard deviation (SD) of the growth curve of controls that remained at the breeding establishment. The recovery period in rats provided with water (as water pouches) and pelleted food during transport, was less than 12 hours. Rats receiving food and water in the form of canned gel diet or potatoes during transport, had a recovery period of less than 24 hours. Mice provided with water pouches during transport remained within 1 SD of the control body weight curve. Mice given canned gel diet or potatoes during shipment needed 4 days to recover. The use of different food and water sources during transportation can differentially affect the length of the recovery period of the body weight.

On the basis of body weight measurements, an adaptation period of 2 days for rats and of 4 days for mice may suffice for transports up till 40 hours. The (separate) supply of food and water during transport may shorten the adaptation period and is therefore recommended.

Corticosterone: Blood corticosterone level is also used as a basic parameter to determine the mini-

mum length of the adaptation period after transportation. After transporting rabbits by car or plain for 4 hours plasma cortisol level was significantly elevated upon arrival, when compared to control values that were measured 6 or 7 days after arrival. At day 1 after transport this difference had disappeared again (*Toth & January* 1990).

Mice were transported by car (36-48 hr) or plain (24-36 hr) and obtained a moist commercial diet during transport (*Landi et al.* 1982). Corticosterone levels in blood were elevated for more than 48 hours after arrival, when compared to control mice that had been shipped to the laboratory one month before. Immune function parameters (foot pad test, hemagglutination assay and plaque-forming cell assay) had returned to normal within 48 hours after arrival, despite the increased corticosterone levels.

Blood corticosterone concentrations of mice that had been transported by car (36-42 hours) or plain (18-20 hours) were significantly increased on the day of arrival, when compared to control mice that had been shipped to the laboratory 3 weeks before (*Aguila et al.* 1988). After 1, 3 and 5 days blood corticosterone levels were similar to the controls.

Mice which were transported in a cage (*Tuli et al.* 1995) for only 12 minutes (10 min walk and 2 min lift) had significantly higher corticosterone levels immediately after this transport, when compared to baseline control values, that had been obtained from animals in an adjacent room one day earlier. Corticosterone levels returned to control levels within 24 hours.

Drozdowitcz et al. (1990) measured a significant increase in blood corticosterone concentration in mice after an in-house transport of 12 minutes, when compared to non-transported (negative) controls. Transport did not induce a maximal adrenocortical response when compared to (positive) controls which had received an injection with adrenocorticotropic hormone. Transported, positive and negative control mice were all housed in the same unit and blood collection was done simultaneously. In transported mice, the recovery time needed to regain normal circadian corticosterone periodicity, was over 24 hours.

Upon transport, animals are often being regrouped in the transport boxes. *Gärtner & Stoll* (1972) measured elevated corticosterone levels in rats for a period of at least 3 days after regrouping (without transport), as compared to controls that had been housed together for 30 days. Seven days after regrouping, similar corticosterone levels were measured in regrouped and in control rats.

In case animals are being transported from one continent to another, a light/dark-shift may occur. *Weinert & Eimert* (1994) found a disturbance of the circadian corticosterone rhythm in mice that experienced a light/dark-shift (prolongation of the dark period by 8 hr) without being transported. Resynchronization of circadian corticosterone rhythm took 1-2 weeks in juvenile (6 weeks of age) mice and more than 2 weeks in adult (18 weeks of age) mice.

Depending on the duration and the circumstances of the transport, it may take 1 to more than 14 days, before blood corticosterone levels have returned to normal values.

White blood cell parameters: Compared to blood corticosterone levels, parameters like white blood cell count and differentiation determine more directly the state of the immune system. A lowered white blood cell count and an elevated number of neutrophils combined with a lowered number of lymphocytes, generally indicates an impaired capability of the immune system to respond to antigens. This is thought to be stress-related.

Rabbits shipped by car or by car and plane for four hours (*Toth & January* 1990) had an altered white blood cell differentiation (number of neutrophils was increased and that of lymphocytes lowered) compared to values obtained from rabbits 6-7 days after transport. This change in white blood cell differentiation lasted no longer than two days. White blood cell count was not significantly influenced by transport.

Einer-Jensen & Nielsen (1972) measured – among others – white blood cell count in transported mice. The control group remained housed at the SPF-unit at the breeder. An adaptation period of 7-8 weeks was considered sufficient, however, because the mice were infected with mites at the experimental laboratory, these results cannot be interpreted reliably. *Drozdowicz et al.* (1990) found a decreased total white blood cell and lymphocyte count in mice after an in-house transport of about 12 minutes. These parameters returned to baseline values within 12 hours. Controls were housed in the same unit, and sampling was done simultaneously.

Immediately after rats had been transported by plain and car for a period of two days, the amount of segmented neutrophils and monocytes were elevated and eosinophils decreased, compared to control values from rats 12 days after transport (*Bean-Knudsen & Wagner* 1987). White blood cell counts were similar. The choice of experimental setup implies that rats were assumed to be acclimatised 12 days after arrival. As no measurements were performed at intermediate time points, one cannot determine whether a period shorter than 12 days would also suffice.

Based on white blood cell count and differentiation, no exact determination of the minimum length of the adaptation period after transport can be given. The adaptation period in rabbits should probably be two days (*Toth & January* 1990).

Glucose concentration: *Toth & January* (1990) measured a hyperglycaemia in rabbits immediately after air transportation and three days later, as compared to control values (at day 6 after transport). After rabbits had been transported by car, the blood glucose concentration followed the same pattern as observed after air transportation, but values were not significantly different from control values at day 7. A variable food intake during transport may account for different levels of blood glucose. After transport by car, food intake was significantly reduced for 24 hours, when compared to the control value of day 7. After air transportation food intake was significantly lower for a period of 5 days, when compared to day 6.

Water intake: After transporting rats by train for a period of 5 hours, an adaptation period for each individual was calculated on the basis of its increasing initial water intake. It was assumed that rats were acclimatised at the time that water intake would be "stabilized". A period of about 17 days was considered necessary for individually housed rats (*Grant et al.* 1971). Group-housed rats needed about 23 days. As the animals were quite young, the measured initial increase in water intake after transport could have been an age-dependent effect as well.

LH and FSH concentration: *Bieglmayer et al.* (1980) measured luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentration in rats that had been transported by car. The LH levels in transported rats were increased for at least 7 days as compared to the control values in rats that had remained at the breeder. LH levels measured 45 days after transport were similar to control values. FSH levels were similar in transported and non-transported rats.

Serum mucoprotein concentration: *Tobiska & Brada* (1963) found a significant increase in serum mucoprotein concentration in rats within 24 hours after they had been transported by car. Control values were obtained from animals that had been killed at the breeding station the night before transport. An elevated serum mucoprotein level was thought to be stress-related. From day 3 to 13 after transport, serum mucoprotein concentration was slightly above control. After 3 weeks the level was slightly below the control value. Thereafter, the concentration sharply increased because of a lice infestation. The authors considered an adaptation period of at least 3 weeks to be necessary, however, a significant increase was found only within the first day after transport.

Splenic Natural Killer cell activity: *Aguila et al.* (1988) only found a significant decrease in splenic natural killer cell activity within the first 24 hours after transport by car (36-42 hours) or plain (18-20 hours), when compared to controls shipped 3 weeks prior to the experiment. Controls were sampled simultaneously as the test animals at days 0, 1, 3 and 5 post-arrival.

Immune function tests: *Landi et al.* (1982) measured the disturbance of immune function in mice after transport: the foot pad test (FPT) was used as a measure for delayed type hypersensitivity, the haem agglutination test (HAT) as a quantification of the antibody level in blood and the plaque forming cell assay (PFCA) as an indication of antibody production. The FPT and HAT normalised within 24 hours and PFCA normalised within 48 hours after transport, as compared to controls that had been shipped one month prior to the experiment.

Serum AST, LDH, Potassium, Cholesterol: The levels of serum aspartate transaminase (AST), lactate dehydrogenase (LDH), and cholesterol were elevated and serum potassium was lowered in rats immediately after transport, when compared to values of controls that were sampled 12 days after shipment (*Bean-Knudsen & Wagner* 1987).

Behaviour: The behaviour (i.e. rearing, climbing, digging, grooming, eating, drinking, biting and

sexual behaviour) of both individually and group housed mice has been studied by *Tuli et al.* (1995) at one day before, and four days after an in-house transport of 12 minutes. Results showed that feeding and exploratory behaviour (rearing and climbing) increased significantly immediately after transport and became stable by the second day. Grooming was significantly decreased for one day after transport. A significant increase in sexual behaviour was found on days 1 and 3 after transport. No significant differences were found in chewing, biting (agression), digging and drinking over the 4 days of observation. Individually housed mice were less active than the group housed mice. No significant differences for grooming, chewing, biting, digging and drinking were found between individually and group housed mice. Individually housed mice ate significantly more than group housed mice until the second day, possibly due to lack of competition for food or as a substitute for lack of social interactions (i.e.boredom). Although most of the behaviours stabilized soon after transport, some other behavioral activities failed to return to baseline values even after four days (*Tuli et al.* 1995).

Discussion

Variable lengths of adaptation periods after transport have appeared in the literature. This variation may partly result from differences in experimental setup: variable duration and mode of transport, variable environmental conditions during transport, different species and different experimental parameters being studied. In most studies no proper controls were used, which complicates the interpretation of the results: controls were transported at variable time points before the experiment and/or were held at a different location and/or were not sampled at the same time point as the transported animals. Only in the study of *Drozdowitcz et al.* (1990) were control and test animals sampled at the same time and housed in the same unit: after an in-house transport of mice, blood corticosterone concentration was elevated for at least 24 hours, whereas white blood cell and lymphocyte count normalized within 12 hours. The availability and the type of food and water sources during transport, influence the length of the adaptation period (*Weisbroth et al.* 1977, *Wallace* 1976). Therefore, it should be mentioned in the materials and methods section of scientific articles, whether food and/or water have been administered during transport and in what form. The separate supply of food and water during transport is recommended (*Weisbroth et al.* 1977).

A light dark-shift in itself causes a minimum adaptation period in mice of more than 2 weeks, because of the time needed for resynchronisation of circadian corticosterone rhythm (*Weinert & Eimert* 1994). Further studies are required to investigate what the effects are of simultaneously subjecting animals to transportation and a light-dark shift.

Besides transporting animals from one institute to another, one has to consider the effects of in-house transport also. Animals that are used in experiments are often transported in-house, e.g. from the quarantine room into the animal unit, or from the animal room to the experimentation room and vice versa. These in-house transports may significantly influence experimental parameters (*Tuli et al.* 1995, *Drozdowicz et al.* 1990).

Summary

Transporting animals leads to a temporary disturbance of normal physiology (homeostasis). After an adaptation period in the new laboratory, the animals must have returned to their normal physiological state. A literature survey was performed in order to establish the minimum necessary length of the adaptation period of mice, rats and rabbits after transport. The minimum length of the period for adaptation that was considered necessary varied from none to 7-8 weeks; in most studies an adaptation period of 7 days was considered sufficient. However, in most studies no proper experimental setup had been used: either the controls were housed at a different location, or they were sampled at different time points, as compared to the transported animals. Besides transport per se, additional factors like a shift in the light-dark rythm, will also disturb the homeostasis. The separate supply of food and water sources during transport is recommended. More research into the effects of transport stress per se is needed, as well as into the interaction of transport stress with other environmental factors occurring simultaneously.

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