SHORT COMMUNICATION

INDIVIDUAL VARIATION IN RESPONSE TO STRESSORS IN FARM ANIMALS: IMPLICATIONS FOR EXPERIMENTERS

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Abstract *Animal Welfare 1994, 3:* 213-218

Physiological and behavioural responses to stressors may affect experimental results. Since individual animals differ in their pattern of response to stressors, it is suggested that stress during experiments has the potential for increasing variability in responses to experimental treatments. Evidence supporting this is given from experiments carried out on farm animals. The main factors accounting for individual differences in response to stressors such as handling are habituation, early experiences and genetic background. Several ways of reducing stress during experiments are suggested and the need for skilful and humane handling is emphasized. It is concluded that reducing stress during experiments will have welfare benefits and may reduce the number of animals that need to be used.

Keywords: *animal welfare, farm animals, individual differences, stress, variability*

Introduction

It is widely accepted that the number of animals used in experiments should be the minimum necessary to obtain significant results (Poole 1987; Remfry 1987). Individual variation in the response to experimental treatments should therefore be minimized (Bleby 1987).

The thesis of this article is that stress during experiments may increase such variation and, consequently, the number of experimental animals needed. The rationale behind this is that 1) physiological and behavioural responses to stressors may affect the results of many experiments and 2) individual animals differ greatly in their response to stressors.

The aim of this paper is to provide evidence in support of the aforementioned thesis and discuss its implications for experimenters. The examples given will focus on farm animals and will refer only to responses to handling, for handling is the stressor most commonly encountered by experimental animals. The term 'handling' will be used in a broad sense to include varying degrees of human presence, restraint, invasive procedures and social isolation (Lawrence *et al* 1991).

Individual variation in the response of the pituitary–adrenal axis to handling

The responsiveness of the adrenal axis to stressors is so widely recognized that increases in circulating concentrations of adrenocorticotrophic hormone (ACTH) and adrenal glucocorticoids are commonly used as indicators of stress (Levine 1985; Moberg 1985). Handling has been shown to cause such a response in cattle (eg Mitchell *et al* 1988), pigs (eg Hessing *et al* 1992), sheep (eg Niezgoda *et al* 1987) and goats (eg Lyons *et al* 1988b).

An increase in the plasma concentrations of ACTH and glucocorticoids may affect the results of many experiments, including those looking at growth rate, reproduction, immune function and disease. For example, many types of stressors cause a reduction in growth rate due in part to decreased protein accretion in skeletal muscle. Glucocorticoids are thought to play an important role in such an effect (Klasing 1985). Indeed it has been shown that in pigs, aversive handling results in increased glucocorticoid levels and impaired growth (Seabrook & Bartle 1992).

Glucocorticoids can also affect the synthesis and secretion of gonadotropins and disrupt reproduction. Restraint and blood sampling have been found to alter endogenous luteinizing hormone secretion in both cows and ewes (Moberg 1991).

Finally, glucocorticoids have several effects on the immune system and on resistance to disease. Various stressors including handling are thought to play a role in the pathogenesis of some diseases in farm animals (Roth 1985).

Individual differences in the adrenal axis response to handling have been described in cattle, pigs, sheep and goats.

Cattle

Simple handling procedures involving putting the animals into a race and sampling blood have been found to cause a marked increase in both ACTH and cortisol levels. Large individual differences were found, particularly between animals habituated to handling and those that were not habituated. Mean plasma concentrations of cortisol after handling in unaccustomed animals were about seven times higher than those of accustomed animals (Mitchell *et al* 1988).

Individual differences are also affected by the animals' early experiences. For example, calves separated from their dams at 21 days of age were found to show a significantly higher adrenal response when restrained at 191 days of age than those left with their dams until weaning (Lay *et al* 1992). Housing influences on the pituitary–adrenal axis have also been reported. Dantzer *et al* (1983) showed that calves housed tied-up on slatted floors showed a higher increase in cortisol levels after venipuncture than those reared loose on straw and in groups.

Pigs

Hessing *et al* (1992) found that pigs could be classified as resistant or non-resistant according to their behavioural responses to restraint. Non-resistant pigs were subsequently found to have adrenals weighing more than those of resistant pigs, suggesting that non-resistant animals may react to environmental challenges with a more pronounced activation of the adrenal axis.

Sheep and goats

Large individual differences in cortisol levels after handling have been reported in sheep. As in cattle, differences were particularly marked between accustomed and unaccustomed animals (Siegel & Moberg 1980).

In goats, it has been shown that human presence may cause a significant increase in plasma cortisol levels. Individual kid goats greatly differed in their response and could be classified as either 'timid' or 'bold' depending on the magnitude of their response (Lyons *et al* 1988b). The mean increase in cortisol levels in timid goats was about three times higher than that of bold animals. Furthermore, activation of the adrenal axis in response to human presence was very much affected by the presence of other animals. For example, pituitary–adrenal responsiveness was always lower when kid goats were accompanied by their mother. The presence of another penmate could also reduce the increase in cortisol levels, but only if the penmate did not show fear of humans (Lyons *et al* 1988b).

Individual differences in other responses to handling

Individual differences in response to handling are not restricted to differences in pituitary–adrenal responsiveness. For example, handling has been shown to increase packed cell volume and plasma concentration of several metabolites, including glucose (Mitchell *et al* 1988). These changes may affect the results of many experiments, as packed cell volume and plasma concentration of glucose are regularly included in metabolic profile tests (Payne & Payne 1987). Large individual differences exist in these responses to handling, being particularly marked between accustomed and unaccustomed animals (Mitchell *et al* 1988).

In sheep, social isolation associated with several experimental procedures has been shown to cause several physiological and behavioural responses, including increase in heart rate and restless behaviour. According to these responses, individuals could be classified as either 'unresponsive', 'vocal' (those that responded only with the type of vocalization used to call for attention) or 'physically responsive' (those that responded with vigorous attempts to return to where the flock was held). Vocal and physically responsive individuals showed a higher heart rate when isolated than unresponsive individuals (Syme 1981; Syme & Elphick 1982). These differences may be relevant to experiments looking at metabolism or nutrition, for other studies have found that restless behaviour and increased heart rates may persist in several individuals over many days and that they may account for an increase in metabolic rate of up to 15 per cent (van Adrichem & Vogt 1993).

Implications for experimenters

From the above examples several strategies can be suggested to reduce the increase in individual variation in response to experimental treatments caused by the stress of handling.

Characteristics of the animals

Since animals reared under different conditions may show different responses to handling (eg Dantzer *et al* 1983), it would be convenient if all animals used in a given experiment had been reared under the same conditions. However, this would not completely eliminate individual differences in response to handling. For example, Kerr and Wood-Gush (1987)

found large individual differences in response to human approach in heifers reared under exactly the same conditions. Furthermore, a genetic component accounting for part of the individual variability in fear of humans between individuals reared under the same conditions has been found in several species, including cattle (Dickson *et al* 1970), pigs (Hemsworth *et al* 1990) and goats (Lyons *et al* 1988a). Therefore, using animals of a similar genetic background would also be convenient.

Habituation

Habituation seems to be one of the main factors accounting for individual differences in response to handling. Therefore, it would be convenient for experimenters to habituate the animals to the handling procedure before doing the experiments. However, habituation is not without problems. First, habituation to some handling procedures may take very long (eg Blaxter 1962) and this could be an economic problem.

Second, animals may not habituate at all to some handling procedures. In fact, repeated exposure to a stressor may sometimes lead to a gradual increase in the response rather than to a decrease (Niezgoda *et al* 1987).

Modification of the handling procedure

Any modification of the handling procedure aimed at reducing its stressful nature would be beneficial. In this regard, the necessity of handling the animals skilfully and humanely can not be overemphasized (Broom 1991).

Exposure of an animal to novelty is one of the most potent elicitors of stress (Levine 1985). Furthermore, stress increases with increases in environmental novelty (Hennessy *et al* 1979). Therefore reducing the novelty of the handling procedure (for example by handling the animal in its pen) may be beneficial (Lawrence 1991).

Finally, the results obtained by Lyons *et al* (1988b) on social modulation of the stress response in goats suggest that handling animals in the presence of habituated penmates may also be helpful.

Areas deserving further research

Work with laboratory rodents has shown that individuals reared in enriched environments show a much less pronounced response to several environmental challenges including handling than those reared in barren environments (Gray 1991). Whether this is applicable to farm animals is unknown, but the results obtained by Dantzer *et al* (1983) in calves (see before) may suggest that this topic deserves further research.

Also, handling of dogs and cats during the so-called sensitive period for socialization reduces their response to handling when adults (Karsh & Turner 1988). The possibility of farm animals having similar sensitive periods should be further explored.

Conclusions and welfare implications

Reducing stress during experiments by handling the animals as skilfully and humanely as possible has an obvious welfare benefit, for the welfare of the animals used is improved. The evidence presented in this paper indicates that it may also reduce the number of animals

needed to obtain significant results. This is important not only on welfare grounds, but also for economic reasons. It is hoped that this will help to convince sceptical scientists and animal caretakers of the need to improve welfare standards.

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