Assessment of Stress During Handling and Transport

Journal of Animal Science (1997) volume 75: 249-257

T. Grandin
Department of Animal Sciences
Colorado State University
Fort Collins, Colorado 80523

ABSTRACT: Fear is a very strong stressor, and the highly variable results of handling and transportation studies are likely to be due to different levels of psychological stress. Psychological stress is fear stress. Some examples are restraint, contact wi th people, or exposure to novelty. In many different animals, stimulation of the amygdala with an implanted electrode triggers a complex pattern of behavior and autonomic responses that resemble fear in humans. Both previous experience and genetic factors affecting temperament will interact in complex ways to determine how fearful an animal may become when it is handled or transported. Cattle trained and habituated to a squeeze chute may have baseline cortisol levels and be behaviorally calm, whereas extensively reared animals may have elevated cortisol levels in the same squeeze chute. The squeeze chute is perce ived as neutral and non-threatening to one animal; to another animal, the novelty of it may trigger intense fear. Novelty is a strong stressor when an animal is suddenly confronted with it. To accurately assess an animal's reaction, a combination of behavioral and physiological measurements will provide the best overall measurement of animal discomfort.

Introduction

Studies to determine the amount of stress on farm animals during routine handling and transport often have highly variable results and are difficult to interpret from an animal welfare standpoint. This paper will cover some of the factors that influence how an animal may react during handling. Much of the variability between handling studies is likely to be due to different levels of psychological stress. Animals can be stressed by either psychological stress:

- restraint
- handling
- or novelty

or physical stresses:

- hunger
- thirst
- fatigue
- injury
- or thermal extremes

Procedures such as restraint in a squeeze chute do not usually cause significant pain, but fear may be a major psychological stressor in extensively raised cattle. Many apparently conflicting results of different studies may be explained if the varying amounts of psychological stress and physical stress within each study are considered. Fear responses in a particular situation are difficult to predict because they depend on how the animal perceives the handling or transport experience. The animal's reactions will be governed by a complex interaction of genetic factors and previous experiences. For example, animals with previous experiences with rough handling will remember it and may become more stressed when handled in the future than animals that have had previous experiences with gentle handling. Previous handling experiences may interact with genetic factors. Rough handling may be more detrimental and stressful to animals with an excitable temperament compared to animals with a more placid temperament. For example, Brahman cross cattle had higher cortisol levels when restrained in a squeeze chute than English crosses (Zavy et al., 1992). An animal's social rank within the group can also affect stress levels. McGlone et al. (1993) found that subordinate submissive pigs were more stressed by 4 hours of transport than dominant pigs. This paper will only address short-term stressors such as handling and transport. The measurement of chronic stress imposed by the environment or different housing systems is much more complex.

Importance of Fear and Effects of Novelty

Fear is a universal emotion in the animal kingdom and motivates animals to avoid predators. All vertebrates can be fear-conditioned (LeDoux, 1994). The amygdala in the brain is probably the central fear system that is involved in both fear behavior and the acquisition of conditioned fear (Davis, 1992). Davis (1992) cited over 20 animal studies from many different laboratories that showed that electrical stimulation of the amygdala with an implanted electrode triggers a complex pattern of behaviors and changes in autonomic responses that resembles fear in hum ans. In humans, electrical stimulation of the amygdala elicits feelings of fear (Gloor et al., 1981). Studies have also shown that electrical stimulation of the amygdala will increase plasma corticosterone in cats (Setekleiv et al., 1961; Matheson et al., 1971) and in rats (Redgate and Fahringer, 1973). Lesioning of the amygdala will block both unconditioned and conditioned fear responses (Davis, 1992). Large lesions in the amygdala will reduce emotionality in wild rats as measured by flight distance (Kemble et al., 1984). Kemble et al. (1984) also noted that lesioning of the amygdala had a taming effect on wild rats. LeDoux (1994) explains that fear conditioning takes place in a subcortical pathway and that extinguishing a conditioned fear response is difficult because it requires the animal to suppress the fear memory via an active learning process. A single, very aversive event can produce a strong conditioned fear response, but extinguishing this fear response is much more difficult.

Observations by the author on cattle ranches have shown that to prevent cattle and sheep from becoming averse and fearful of a new squeeze chute or corral system, painful or highly aversive procedures should be avoided the first time the animals enter the facility. The same principle also applies to rats. Rats that receive a strong electrical shock the first time they enter a novel alley will refuse to enter it again (Miller, 1960). However, if the rat is subjected to a series of shocks of gradually increasing intensity, it will continue to enter the alley to get a food reward. Therefore, Hutson (1993) recommends that stress in sheep during routine handling could be reduced if the animals were conditioned gradually to handling procedures. Less severe procedures should be done first (Stephens and Toner, 1975; Dantzer and Mormede, 1983).

Novelty is a very strong stressor (Stephens and Toner, 1975; Moberg and Wood, 1982; Dantzer and Mormede, 1983). This is especially true when an animal is suddenly confronted with it. In the wild, novelty and strange sights or sounds are often a sign of danger (Grandin, 1993a). Cattle will balk at shadows or differences in flooring during movement through handling facilities (Grandin, 1980). Pigs that have been trained to laboratory procedures will respond to deviations in their daily routine with a rise in blood pressure (Miller and Twohill, 1983). Reid and Mills (1962) have suggested that livestock can be trained to accept changes in management routines that would cause a significant increase in physiological measurements in animals that had not been trained. Gradual exposure of animals to novel experiences enables them to become accustomed to nonpainful stimuli that had previously evoked a flight reaction. Grandin et al. (1995b) reported that training nyala antelope to cooperate during blood sampling had to be done very slowly to avoid triggering a massive flight reaction. The animals are very vigilant and will react to any unfamiliar sights and sounds.

There are some situations in which novelty is attractive to animals. Cattle and pigs often approach and manipulate a piece of paper dropped on the ground. The author has observed that the same piece of paper will cause animals to balk and jump away if they are being forced to walk toward it. Therefore, the paper may be perceived as threatening in one situation and non-threatening in another. The author has observed that cattle in the Philippines seldom react to cars, trucks, and other distractions when they graze on the highway median strip. Cars and trucks are no longer novel because they have seen them since birth. In the nyala antelope, animals born after the adults had been trained to blood sampling procedures learned to cooperate more quickly (Grand in et al., 1995b).

Cattle can become accustomed to repeated nonaversive procedures such as weighing or drawing blood through an indwelling catheter (Peischel et al., 1980; Alam and Dobson, 1986). Sheep, pigs, and giraffes have been trained to voluntarily enter a restraint device (Panepinto, 1983; Wienker, 1986; Grandin, 1989).

However, animals do not habituate to procedures that are very aversive (Hargreaves and Hutson, 1990a). A procedure can be highly aversive without being painful. Full inversion to an upside-down position is extremely aversive to sheep. The time required to drive sheep down a race into a restraint device that inverted them increased the following year (Hutson, 1985). Cortisol levels did not decrease with experience when cattle were subjected to repeated truck trips during which they fell down (Fell and Shutt, 1986). Hargreaves and Hutson (199Oa) found that repeated trials of a sham shearing procedure failed to reduce the stress response. Sheep also did not habituate to 6 hours of restraint with their legs tied (Coppinger et al., 1991). Apple et al. (1995) found that in sheep, 6 hours of restraint stress caused dark cutting meat and very high (>110 ng/mL) levels of cortisol. Epidural blockage with lidocaine, which prevents the animals from contracting their muscles and straining against the restraint, failed to inhibit glycogen metabolism. This experiment indicates that psychological stress was probably a significant factor.

Cattle are very sensitive to the relative aversiveness of different parts of handling procedures. When they were handled every 30 days in a squeeze chute and a single animal scale, balking at the scale decreased with successive experience and balking at the squeeze chute increased slightly (Grandin, 1992). The animals learned that the scale never caused discomfort. Cattle that had been mishandled in a squeeze chute and struck hard on the head by the headgate were more likely to resist entry into the chute in the future (Grandin et al., 1994) compared with cattle that had never been hit with the headgate.

Effects of Adaptation to Handling on Stress

Tame animals that are accustomed to frequent handling and close contact with people are usually less stressed by restraint and handling than animals that seldom see people. Binstead (1977), Fordyce et al. (1985), and Fordyce (1987) report that training weanling heifer calves produced calmer adult animals that were easier to handle. Training these extensively raised calves involved walking quietly among them, teaching them to follow a lead horseman and quiet walking through chutes. How an animal is handled early in life will have an effect on its physiological response to stressors later in life. Calves on a university experiment station that had become accustomed to petting by visitors had lower cortisol levels after restraint than calves that had less frequent contact with people (Boandle et al., 1989). Lay et al. (1992a) found that restraint in a squeeze chute was almost as stressful as hot iron branding for extensively reared beef cattle. In hand-reared dairy cows, branding was much more stressful than restraint (Lay et al., 1992b).

Taming may reduce the physiological reactivity of the nervous system. Hastings et al. (1992) found that hand-reared deer had lower cortisol levels after restraint compared with free-ranging deer. Even though the physiological response to restraint was lower in the tame animals, hand-reared deer struggled just as violently as free-range deer (Hastings et al., 1992). Associations that animals make seem to be highly specific. Mateo et al. (1991) found that tame sheep approached a person more quickly, but behavioral measurements of struggling indicated that taming did not generalize to other procedures. Similar findings by Hargreaves and Hutson (199Oa,b) showed that gentling and reduction of the sheep's flight zone failed to reduce aversion to shearing. Tame animals can sometimes have an extreme flight reaction when suddenly confronted with novelty that is perceived as a threat. Reports from ranchers and horse trainers indicate that horses and cattle that are calm and easy to handle at their home farm sometimes become extremely agitated when confronted with the novelty of a livestock show or auction. The animal's behavioral reaction seems to be less likely to generalize to other procedures than its physiological reaction. Moberg and Wood (1982) found that experiences during rearing greatly affected behavior in an open field test but had little effect on adrenocortical response of lambs. Exposing piglets to novel noises for 20 min. increases both heart rate and motor activity. Heart rate habituated to a recording of abattoir sounds more quickly than motor activity (Spensley et al., 1995).

The effects of previous experience on an animal's fear response may provide one explanation for the often variable results in handling and transport studies. For example, extensively raised animals may have more psychological or fear stress during loading and unloading for transport compared to more intensively reared animals. British researchers have found that loading and unloading of sheep and calves was the most stressful part of the journey (Trunkfield and Broom, 1990; Knowles, 1995). Kenney and Tarrant (1987) reported that for Irish cattle, the actual journey was more stressful than loading and unloading. The physical stresses of the trip, such as jiggling, were more stressful than the psychological stresses of loading or unloading. A possible explanation for this discrepancy between these two studies may be the amount of contact the animals had with people. There may be a big difference in the degree of fear stress between U.S. cattle reared on range land where they seldom see people and European pasture-reared cattle. Differences in the degree of psychological stress may explain why too many rest stops during long-distance transport is detrimental to the health of weaner calves raised under U.S. conditions. Cattle feeders have learned from practical experience that 200 to 300-kg calves shipped from the southeast to Texas will have fewer health problems if they are transported non-stop for the entire 32-hour trip. For these extensively reared calves, rest stops may possibly turn into stress stops. Research is needed to conclusively determine what factors cause the rest stops to be stressful. Legislating too many rest stops may be detrimental to welfare. One possibility is fear stress during loading and unloading at rest stops and the second possibility is that the calves become infected with diseases at the rest stop. Many of the calves shipped on these trips are not properly vaccinated. There may be an interaction between rest stops and disease. Frequent rest stops may be more beneficial to fully vaccinated calves.

Genetics

Genetic factors such as temperament interact in complex ways with an animal's previous handling experiences and learning to determine how it will react during a particular handling procedure. Wild species are usually more reactive to novel stimuli than domesticated animals. Price (1984) maintains that the domestic phenotype have reduced responses to changes in the environment. Domesticated animals are more stress-resistant because they have been selected for a calm attitude toward people (Parsons, 1988). When deer or antelope are tamed, the flighty temperament is masked until they are confronted with a novel stimulus that is perceived as threatening. A tame deer or antelope can have an explosive reaction to a novel event. A wild species has a more intense flight response because this enables it to flee from predators.

Temperament in cattle is a heritable trait that may affect the animal's reaction to handling (Le Neindre et al., 1995). There are differences in temperament both between and within cattle breeds. Within the Brahman breed, temperament is heritable (Hearns haw et al., 1979; Fordyce et al., 1988). Temperament differences between breeds have also been reported by Stricklin et al. (1980) and Tulloh (1961). Genetics also affects an animal's response to stress. Brahman cross cattle had higher cortisol levels while restrained in a squeeze chute compared to English crosses (Zavy et al., 1992). Recent research by Grandin et al. (1995a) and replicated by H. Randle (1995, personal communication, University of Plymouth, U.K.) indicated that the spiral hair whorl on a bovine's forehead is an indicator of temperament. Cattle with spiral hair whorls above the eyes became more agitated while restrained than animals with hair whorls below the eyes.

Temperament may be under genetic control in many different animals. Research with rats has shown that they can be selected for either high or low emotionality (Fujita et al., 1994) or for reduced fear induced aggressiveness toward humans (Popova et al., 1993). Phenotypic characteristics are also related to temperament. Interestingly, it seems that different genetic factors control fearinduced aggression and inter-male aggression. Selection for reduced fear induced aggression had no effect on aggressive behavior toward other male rats.

Temperament is a trait that seems to be stable over time. In European Continental-cross cattle, certain individuals became extremely agitated every time they were handled in a squeeze chute and others were always calm (Grandin, 1992). The agitated animals failed to adapt to being held in the squeeze chute during four handling sessions spaced 30 days apart. Cattle with a very excitable temperament may have greater difficulty adapting to repeated non painful handling procedures than cattle with a calmer temperament. The two types of animals may have differing physiological and behavioral reactions to the same procedure. Animals with a calm temperament may adapt more easily and become less stressed with repeated handling treatments and animals with a very excitable temperament may become increasingly stressed with each repeated handling treatment. Lanier et al. (1995) found that some pigs habituated to a swimming task and maintained near baseline levels of epinephrine and norepinephrine and other animals failed to habituate and never adapted.

At five slaughter plants in the United States, Holland, and Ireland, the author has observed increasing problems with very excitable pigs and cattle from certain genetic lines that become highly agitated. It is almost impossible to drive them quietly through a high-speed slaughter line. These animals seem to have a much stronger startle reaction to novelty, are more likely to balk at small distractions such as shadows or reflections in the race, and are more likely to bunch together. Observations at slaughter plants and reports from ranchers also indicate that excitable cattle are more likely to injure themselves when they are confronted with the novel, unfamiliar surroundings of an auction market or slaughter plant. The appearance of greater numbers of more excitable pigs and cattle may possibly be related to the increasing emphasis of the livestock industry on lean beef and pork. In both cattle and pigs, the author has observed that excessive excitability occurs most often in animals bred for leanness that have a slender body shape and fine bones. Cattle and pigs bred for large, bulging lean muscles usually have a calmer temperament. This is an area that needs to be researched. Practical experience indicates that the excitable animal problem needs to be corrected because excessive excitability creates serious animal welfare problems during handling at auction markets and slaughter plants.

Cattle and pig producers need to select animals with a calm temperament, but care must be taken not to over-select for any one particular trait. A good example of over selection for a single trait is the halothane gene in pigs. Pigs with this gene have increased meat production, but the price for this increased production is poor meat quality (Pommier and Houde, 1993). Over-selection for calm temperament may possibly have detrimental effects on economically important traits, such as maternal ability. Researchers in Russia found that selecting foxes for calmness over 80 years produced animals that lost their seasonal breeding pattern and had strange piebald black and white colored coats (Belyaev, 1979; Belynev and Borodin, 1982). The foxes turned into animals that acted and looked like Border collies.

Fear Pheromones

Another factor that could confound handling stress studies is fear pheromones. Vieville-Thomas and Signoret (1992) found that urine from a stressed gilt caused other gilts to avoid a feed dispenser and urine from an unstressed animal had no effect. Both the results of this experiment and observations by the author indicate that it takes 10 to 15 min. for the fear pheromone to be secreted. Observations by the author indicate that cattle will voluntarily walk into a restraining chute that is covered with blood, but if an animal becomes extremely agitated for several minutes, the other animals refused to enter (Grandin, 1993b). In a laboratory setting pigs witnessing slaughter had no increases in either beta endorphins or cortisol. These were calm animals fitted with jugular catheters (Anil et al., 1995). Eibl-Eibesfeldt (1970) observed that if a rat is instantly killed by a trap, the trap will remain effective and can be used again. Rats will avoid a trap that failed to instantly kill. Research with rats indicates that blood may contain a fear pheromone (Stevens and Gerzog-Thomas, 1977). Stevens and Saplikoski (1973) found that blood and muscle tissue from stressed rats was avoided in a choice test, whereas brain tissue and water had no effect. Blood from guinea pigs and people also had little effect (Hornbuckle and Beall, 1974).

Short-Term Stress Measurements

This discussion will be limited to measuring shortterm stress induced by handling procedures such as being held in a squeeze chute. Assessment of stress and discomfort should contain both behavioral and physiological measures. Behavioral indicators of discomfort are attempting to escape, vocalization, kicking, or struggling. Other behavioral measures of how an animal perceives a handling procedure are choice tests and aversion tests. Common physiological measures of stress are cortisol, beta endorphin, and heart rate. Cortisol is a useful indicator of short-term stresses from handling or husbandry procedures such as castration. Researchers must remember that cortisol is a time-dependent measure that takes 10 to 20 min to reach peak values (Lay et al., 1992a).

A review of many studies indicates that cortisol levels in cattle fall into three categories:

1. baseline
2. levels that occur during restraint in a headgate, and
3. extreme stress (Table 1)

Cortisol levels are highly variable and absolute comparisons should not be made between studies, but the figures on Tables 1 and 2 would make it possible to determine whether a handling or slaughter procedure was either very low stress or very high stress. One could tentatively conclude that a mean value of >70 ng/mL in either steers or cows would possibly be an indicator of either rough handling or poor equipment, and low values close to the baseline values would indicate that a procedure was either low stress or was very quick. Quick procedures would be completed before cortisol levels could rise. Restraint in a headgate for blood sampling and slaughter produced similar values (Tables 1 and 2). Mature bulls have much lower cortisol levels than steers, cows, or heifers (Tennessee et al., 1984). In one study, there was an extreme mean of 93 ng/mL for inverting cattle on their backs for 103 seconds (Dunn, 1990). This very high figure is not due to differences in assay methods because this same researcher obtained more reasonable values of 45 ng/mL for upright restraint. Properly performed cattle slaughter seems to be no more stressful than farm restraint (Tables 1 and 2).

Table 1: Mean cortisol values in cattle during handling

Cortisol level, ng/mL	Breed	Gender	Study
Baseline			
.5 to 2	Friesian	Bulls	Tennessen et al., 1984
2	Friesian	Cows	Alam and Dobson, 1986
3	Angus cross	Bull calves	Henricks et al., 1984
6	Angus cross	Heifer calves	Henricks et al., 1984
9	Friesland and Nuguni	Cows	Mitchell et al., 1988
Restraint in headgate			
13	Holstein cows	Hand-reared	Lay et al., 1992a
24 (weaned 2 wk before test)	Unknown British or European	Weanlings, mixed genders	Crookshank et al., 1979
27	Brahman cross	Steers	Ray et al., 1972
28	Angus X Hereford	Steers	Zavy et al., 1992
30	Simmental X Hereford X Brahman	83% Steers	Lay et al., 1992b
36	Angus X Brahman	Steers	Zavy et al., 1992
46 (weaned day of test)	Unknown British or European	Mixed genders	Crookshank et al., 1979
63	Brahman X Hereford X Afrikander	Steers and heifers	Mitchell et al., 1988
Extreme Value			
93	Unknown British or European	Mixed	Dunn, 1990

Table 2: Mean cortisol values during slaughter

Cortisol level, ng/mL	Handling Methods	Study
Baseline quiet research abattoir		
15	Held in head restraint, shot immediately with captive bolt	Tume and Shaw, 1992
Commercial slaughter plant		
24	Handled quietly in conventional stunning box	Ewbank et al., 1992[a]
32	Unknown	Mitchell et al., 1988
44	Conventional stunning box	Tume and Shaw, 1992
45	Conventional stunning box	Dunn, 1990[a]
51	Poorly designed head restraint only 14% of cattle voluntarily entered it	Ewbank et al., 1992[a]
63 (median)	Electric prod all cattle, 38% animals slipped, conventional stun box	Cockram and Corley, 1991[a]
Extreme stress		
93	Inverted on back for 103 s	Dunn, 1990[a]

[a] Conducted in either England or Ireland with Bos taurus cattle.

Less clear cut ranges have been obtained in sheep. Pearson et al. (1977) found that slaughter in a quiet research abattoir produced lower cortisol levels than slaughter in a noisy commercial plant. The values were 40 vs 61 ng/mL. Values for shearing and other on-farm handling procedures were 73 ng/mL (Hargreaves and Hutson, 1990c,d) and 72 ng/mL (Kilgour and de Langen, 1970). Prolonged restraint and isolation for 2 hours increased cortisol levels up to 100 ng/mL (Apple et al., 1993).

Creatine phosphokinase (CPK) and lactate seem to be useful measures for assessing handling stresses in pigs (Warris et al., 1994). Warris et al. (1994) found that the sound level of squealing pigs in a commercial abattoir was highly correlated with CPK measurements. White et al. (1995) also reported that vocalizations in pigs were indicative of stress and were correlated with other measures of acute stress, such as heart rate. Cattle that become behaviorally agitated have higher cortisol levels (Stahringer et al., 1989). Heart rate in cattle during restraint in a squeeze chute was highly correlated with cortisol levels (Lay et al., 1992a,b). Stermer et al. (1981) found that rough handling in poorly designed facilities resulted in greater heart rates than quiet handling in well designed facilities.

Isolation is also a factor in handling stress. During restraint for routine husbandry procedures, animals are often separated from their conspecifics. Stookey et al. (1994) found that cattle became less behaviorally agitated during weighing on a single animal scale if they could see another animal in the chute less than 1 meter away in front of the scale. Agitation was measured electronically by measuring movement and jiggling via the scale load cell system. Numerous studies have shown that isolation from conspecifics will raise cortisol and other physiological measures (Kilgour and deLangen, 1970; Whittlestone et al., 1970; Arave et al., 1974).

Aversion Tests

Aversion to a handling procedure can be measured by either choice testing or measuring aversion. One measure of aversion is the time required to induce an animal to re-enter a chute where it was previously handled (Rusher, 1986a,b 1995). In a choice test, the animals are allowed to choose between two different chutes that lead to different procedures (Grandin et al., 1986; Rushen and Congdon, 1986a,b). Another useful measure is the degree of force required to induce an animal to move through a race. In some cases, measuring the degree of force provides a more accurate assessment of aversion than time. Examples of force are the number of pats on the rump or number of electrical prods. Experience and genetic factors can confound aversion tests. Rushen (1996) warns that to accurately measure aversion in a race, the animal must experience the aversive procedure more than once. Observations by the author indicate that excitable cattle sometimes run through a single file chute quickly in an attempt to escape. Research (in progress by Bridgette Voisinet and the author) reveals that bulls trained to move through a race to a squeeze chute exhibit no aversion in the race after a single noxious treatment. After one aversive treatment, they continued to voluntarily walk through the race into the squeeze chute, but balking and turning back in the crowd pen at the entrance to the race greatly increased. At this point, the animals may perceive that they may be able to avoid re-entering the race. In aversion studies, balking and other behaviors indicative of aversion must be measured in both the single file race and in the pens and alleys that lead up to the entrance of the single file race. This is especially important if the aversive procedure is performed only once. After the animal is forced to enter the chute that leads to the squeeze, it may perceive that it may be able to escape by running quickly through it toward the squeeze chute.

Under certain conditions, choice tests may be unreliable for measuring choices between mildly aversive procedures. Research conducted by Grandin et al. (1994) showed that cattle are reluctant to change a previously learned choice if the two choices in a choice test are only mildly aversive. Other research showed that sheep immediately switched sides to avoid highly aversive electro-immobilization (Grandin et al., 1986).

Implications

Both researchers and people making decisions about animal welfare must understand that fear during non-painful routine handling and transport can vary greatly. Fear is a very strong stressor. Cattle that have been trained and habituated to a handling procedure may be completely calm and have baseline cortisol and heart rate measurements during handling and restraint. Extensively reared cattle with an excitable disposition may have very high cortisol levels and show extreme behavioral agitation during the same procedure. For one animal, a squeeze chute may be perceived as neutral and non-threatening, but to another it may trigger an extreme fear response. The animal's response will be determined by a complex interaction of genetics and previous experience. Studies to assess animal welfare during handling and transport should contain both behavioral and physiological measurements.

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