NOTE

Laboratory Routines Cause Animal Stress

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Eighty published studies were appraised to document the potential stress associated with three routine laboratory procedures commonly performed on animals: handling, blood collection, and orogastric gavage. We defined handling as any non-invasive manipulation occurring as part of routine husbandry, including lifting an animal and cleaning or moving an animal's cage. Significant changes in physiologic parameters correlated with stress (e.g., serum or plasma concentrations of corticosterone, glucose, growth hormone or prolactin, heart rate, blood pressure, and behavior) were associated with all three procedures in multiple species in the studies we examined. The results of these studies demonstrated that animals responded with rapid, pronounced, and statistically significant elevations in stress-related responses for each of the procedures, although handling elicited variable alterations in immune system responses. Changes from baseline or control measures typically ranged from 20% to 100% or more and lasted at least 30 min or longer. We interpret these findings to indicate that laboratory routines are associated with stress, and that animals do not readily habituate to them. The data suggest that significant fear, stress, and possibly distress are predictable consequences of routine laboratory procedures, and that these phenomena have substantial scientific and humane implications for the use of animals in laboratory research.

Stress induced by highly invasive procedures used in some animal studies is well recognized by regulatory authorities and Institutional Animal Care and Use Committees. Animals used in laboratory research and testing are also regularly subjected to routine maintenance or monitoring procedures, such as personnel entering the animal housing room, cage movement and cleaning, body weight collection, physical examination, injections, and collection of blood or other tissues. Because these procedures may be considered incidental in nature, their effects on laboratory animal well-being may be overlooked by ethical review committees, whose task is to try to reduce the potential for animal pain and suffering. Some studies suggest that routine procedures may cause significant stress to animals, and that even seemingly benign animal studies may raise substantial animal welfare concerns.

This paper summarizes findings from other studies, focusing on three procedures commonly performed on animals used in laboratory research: routine handling, blood collection, and orogastric gavage. We address the possibility that routine procedures are intrinsically stressful to laboratory animals regardless of the care and skill with which they are performed. Throughout this paper, we define *stress* as "the effect produced by external (i.e., physical or environmental) events or internal (i.e., physiologic or psychologic) factors, referred to as stressors, which induce an alteration in an animal's biologic equilibrium" (1). We define *distress* as "an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behaviors" (1).

Methods

Two online databases (PubMed and NLM Gateway) were used to identify studies published in English involving quantitative or behavioral measures of pain or stress in animals during routine laboratory handling, blood collection, and orogastric gavage. The following root terms were used during searches: animal, laboratory, stress, rat, mouse, primate, bird, cat, dog, handling, venipuncture, venepuncture, bleeding, gavage, force-feeding, and oral dosing. PubMed contains articles dating back to 1966, and NLM Gateway includes articles from 1953 to 1965. Other papers were found by scanning literature cited in retrieved papers. "Handling" was defined as any non-invasive manipulation that is part of routine husbandry, including lifting an animal, cage cleaning or moving an animal's cage.

For reported physiologic measurements, we calculated the difference between the mean baseline or control measure and the mean maximum measure following the intervention and converted this to a percentage change from baseline. For variables decreasing in response to stressors, mean minimum measures were used. This difference was then divided by the baseline or control measure, and the quotient multiplied by 100 to derive percentage.

Results

Handling. (i) Rats and mice. A summary of studies reporting animal responses to routine handling procedures is presented in Table 1. Heart rate (HR) and blood pressure (BP) were shown to increase in both male and female rats monitored by telemetry in response to a variety of handling procedures, including animal lifting, cage changing, simulated cage changing (rat returned to original cage), restraint with subcutaneous injection, transport to another room with subcutaneous injection, restraint with tail vein injection, being present in the room during decapitation of other rats, handling and body weight collection, and handling and vaginal lavage (2-5). These responses persisted for 45 to 90 min following the procedures. Mean HR rose significantly in adult male rats removed from their home cages once daily for 20 days and placed in an adaptation chamber for either 2-min ($n = 13$) or 1-h ($n = 7$) sessions. Although HR gradually returned to near resting levels by the end of the 1-h sessions, animals showed no signs of habituating to the handling over the course of the experiment as judged by latency of HR to return to baseline (6). A rise in core body temperature of 1°C occurred within 30 min and lasted several hours in each of five male rats after being handled three times for 30 sec for insertion of a rectal temperature probe (7).

During cage changing, physiologic perturbations were similar whether rats were picked up and placed in new cages or were returned to their original cages. This suggests that animal responses are caused by physical manipulation rather than by unfamiliar aspects of new surroundings, such as new bedding (4). Placement of soiled bedding in new cages did not reduce stress responses (5).

Routine handling has been shown to elevate serum concentrations of hormones such as corticosterone, which mediates responses to stress in addition to regulating protein and carbohydrate metabolism, and prolactin, which also mediates stress responses. Blood samples col-

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Table 1. Summary of published findings on animals' response to handling

Study	Animals	N	Procedure	Parameter measured	Calculated maximum % change[a]	P
Sharp et al. 2002b	male rats	8	cage change	heart rate	+46%	≤ 0.05
				blood pressure	+19%	≤ 0.05
Sharp et al. 2001	female rats	8	cage change	heart rate	+37%	≤ 0.05
				blood pressure	+15%	≤ 0.05
Sharp et al. 2003a	female rats	7-8	cage change	heart rate	+37%	< 0.05
				active behaviors	+190%	< 0.05
			cage change simulated	heart rate	+30%	< 0.05
				active behaviors	+223%	< 0.05
			handled and weighed	heart rate	+46%	< 0.05
				active behaviors	+441%	< 0.05
Duke et al. 2001	male rats	6-8	cage change	heart rate	+20%	< 0.05
				blood pressure	+34%	< 0.05
Gallaher et al. 1985	male rats	5	handled[b]	body temperature	+2.7%	NA
Armario et al. 1986a	male rats	6	handled	corticosterone	+300%	< 0.0001
			cage moved	glucose	+9%	< 0.05
Barrett and Stockham 1963	male rats	12	handled	corticosterone	+338%	< 0.001
Gärtner et al. 1980	male rats	8	cage moved	corticosterone	+63%	< 0.001
				prolactin	+83%	< 0.001
				TSH	+90%	< 0.001
				T3	+42%	< 0.001
				packed cell volume	+6%	< 0.05
				haemoglobin	+11%	< 0.05
				protein	+25%	< 0.01
De Boer et al. 1990	male rats	6	handled	corticosterone	+160%	< 0.05
				noradrenaline	+290%	< 0.05
				adrenaline	+220%	ns
				glucose	+195%	ns
Brown and Martin 1974	male rats	15	novel environment	corticosterone (crest)[c]	+31%	NA
				corticosterone (trough)[c]	+200%	NA
				prolactin (crest)	+140%	NA
				prolactin (trough)	+135%	NA
				growth hormone (crest)	+550%	ns
				growth hormone (trough)	+465%	ns
Zethof et al. 1993	male mice	320	handled	body temperature	+3.1%	< 0.001
Seggie and Brown 1974	male mice	355	handled	corticosterone (crest)	+190%	< 0.01
				corticosterone (trough)	+175%	< 0.01
				prolactin (crest)	+275%	< 0.001
				prolactin (trough)	+220%	< 0.001
				growth hormone	+400%	< 0.001
Tabata et al. 1998	male mice	6	cage moved	glucose	+30%	< 0.01
	female mice	6	cage moved	glucose	+24%	< 0.05
	male rats	6	cage moved	glucose	+1%	ns
	female rats	6	cage moved	glucose	+3%	ns
Clement et al. 1989	male mice	4	handled	body temperature	+4.8%	NA
Moynihan et al. 1990	male mice	36	handled	immunoglobulin (IgM)	–26%	< 0.05
				immunoglobulin (IgG)	–28%	< 0.05
				lymphocyte CD4 (L3T4)	–7.0%	ns
				lymphocyte CD8 (Lyt2)	+31%	ns
				lymphocyte Pan T (Thy 1)	+8.5%	ns
				lymphocyte IgM	+6.6%	ns
Moynihan et al. 1989	female mice	20	handled	immunoglobulin (IgG)	+37%	< 0.05
				immunoglobulin (IgM)	NA	ns
Solomon and Cohen 1968	mice	108	handled	primary antibodies	+100%	< 0.001
				secondary antibodies	+118%	< 0.02
Newton et al. 1962	rats	48	handled	post-challenge survival	+15%	< 0.01
Line et al. 1989	female rhesus macaques	6	cage change	heart rate	+46%	NA

Table 1. Summary of published findings on animals' response to handling (cont.)

Study	Animals	N	Procedure	Parameter measured	Calculated maximum % change[a]	P
Capitanio et al. 1996	male rhesus macaques	37	room entered	leukocyte (CD8) count	+50%	< 0.05
Meyer and Knobil 1967	rhesus macaques	3	various[d]	growth hormone	0 – +2,400%	NA
Matt et al. 1983	female hamsters	12	cage moved	prolactin	+125%	< 0.05
Conn et al. 1989	hamsters	12	cage change	body temperature	+3.7 – 4.6%	< 0.005
Knudtzon 1984	rabbits	5	cage moved	glucose	+21%	< 0.01
				glucagon	0%	ns
				insulin	–19%	ns
Widmaier et al. 1994	fruit bats	195	handled	cortisol	+100%	< 0.001
				corticosterone	+150%	< 0.02
Le Maho et al. 1992	male geese	7	handled	corticosterone	+160%	< 0.01
				epinephrine	+165%	< 0.01
				norepinephrine	+160%	< 0.01
Romero and Remage-Healey 2000	starlings	18	handled[c]	corticosterone	+100 – 350%	< 0.0001
Dawson and Howe 1983	starlings	57	handled	corticosterone	+400%	< 0.001
Harvey et al. 1980	ducks	90	handled	corticosterone	+385%	< 0.001
Beuving and Vonder 1978	hens	14	handled	corticosterone	+550%	< 0.05
Romero et al. 1997	sparrows	82	handled	corticosterone	+260 – 730%	< 0.0001

[a]from baseline or control.
[b]with insertion of rectal probe.
[c]of diurnal adrenal gland cycle.
[d]agitation (not defined), room entry by unfamiliar persons, telephone ringing, abdominal skin pinched forcefully.
ns, statistically non-significant.
NA, statistical significance not provided by cited authors.

lected from adult rats 15 min after a 15-sec period of handling showed significant elevations in concentrations of serum corticosterone (8, 9) and prolactin (9), which persisted for 30 to 60 min. Stress-like hormonal responses varied with the intensity of handling stressors. Cage change with transfer to a quiet room was more stressful than cage change without transfer, while cage change and transfer to a noisy room elicited the greatest hormonal elevations (9). Corticosterone and prolactin elevations following handling were pronounced at both the crest and trough of the diurnal adrenal gland cycle in rats (10, 11). Being handled for 5 sec, or being placed for 3 min in a novel environment (open-topped cardboard drum 65 cm high and wide) caused corticosterone and prolactin levels to "respond dramatically" in individually housed male rats bled by decapitation (10).

Plasma corticosterone quadrupled within 15 min, and decreased significantly 30 to 60 min later in 12 male rats in response to brief routine handling and weighing. Rats bled after being transported to a laboratory also had threefold higher plasma corticosterone levels than did rats bled in their holding room (12). Significant increases in mean plasma corticosterone and noradrenaline concentrations were reported in six individually housed, catheterized male rats in response to being lifted gently from their cage, held over another cage then returned to their home cage, or being lifted and placed for 15 min in a similar clean cage lacking bedding, food, or water (13).

Serum corticosterone levels rose significantly in eight adult male rats within 5 min of moving cages from a shelf to the floor without opening lids, peaking at 15 min and remaining above baseline up to 60 min later (14). Levels of prolactin, TSH (thyroid-stimulating hormone), and T3 (triiodothyronine) also rose significantly within 15 min and were still increasing up to 1 h later. Other blood indices, including packed cell volume, hemoglobin, and plasma protein, increased significantly within 2 min of handling, peaking at about 5 min, and reverting to baseline by about 10 min (14).

Stress stimulates the body to release glucose into the bloodstream in physiologic preparation for the "fight or flight" response. Plasma glucose concentrations increased in proportion to handling time in 40 adult male rats bled by cardiac puncture or decapitation (15), but did not rise significantly in 12 rats following handling and transport to an adjoining room (16). Growth hormone decreased markedly in male rats (n = 355) in response to being lifted and held for 5 sec (11).

Research suggests that mice are at least as sensitive as rats in their responses to potential laboratory handling stressors. Personnel entering the animal room, animal handling, and cage cleaning all caused acute increases in heart rate and body temperature in mice studied by telemetry (18). Mice habituated to personnel entering the holding room after 2 weeks, but not to handling (18). Plasma glucose concentrations also rose significantly in 12 mice following handling and transport to an adjoining room (16).

Body temperatures increased approximately 1.7°C in four male mice monitored by telemetry after being picked up and restrained for 15 sec as if for injection. In these same animals, body temperatures peaked 15 min post-handling and remained elevated for several hours (19). Animal activity also increased significantly during the first hour following handling, potentially contributing to the elevation in body temperature. Stress-induced hyperthermia, a phenomenon in which the last remaining animal from a cohort of group-housed animals removed sequentially from a home cage demonstrates the greatest elevation in body temperature, appears to be a robust and reproducible phenomenon in mice (20, 21). Stress-induced hyperthermia was observed in 12 separate groups of 10 mice, in which animals were successively handled for rectal temperature collection. Temperatures returned to baseline in 30 to 60 min (21).

Mice restrained manually for 10 or 20 sec showed no significant