Comment

Does the Stress of Laboratory Life and Experimentation on Animals Adversely Affect Research Data? A Critical Review

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Summary — Recurrent acute and/or chronic stress can affect all vertebrate species, and can have serious consequences. It is increasingly and widely appreciated that laboratory animals experience significant and repeated stress, which is unavoidable and is caused by many aspects of laboratory life, such as captivity, transport, noise, handling, restraint and other procedures, as well as the experimental procedures applied to them. Such stress is difficult to mitigate, and lack of significant desensitisation/habituation can result in considerable psychological and physiological welfare problems, which are mediated by the activation of various neuroendocrine networks that have numerous and pervasive effects. Psychological damage can be reflected in stereotypical behaviours, including repetitive pacing and circling, and even self-harm. Physical consequences include adverse effects on immune function, inflammatory responses, metabolism, and disease susceptibility and progression. Further, some of these effects are epigenetic, and are therefore potentially transgenerational: the biology of animals whose parents/grandparents were wild-caught and/or have experienced chronic stress in laboratories could be altered, as compared to free-living individuals. It is argued that these effects must have consequences for the reliability of experimental data and their extrapolation to humans, and this may not be recognised sufficiently among those who use animals in experiments.

Key words: *animal welfare, cost–benefit analysis, data accuracy, glucocorticoids, psychological, stress, translational medical research.*

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Introduction

In the December 2017 issue of *ATLA*, I published an Editorial, in which I suggested that stress in animals in laboratories, resulting from their environment and experimental procedures, was not limited to welfare concerns (1). It also adversely affects, significantly and unavoidably, multiple biological systems and therefore affects the resultant experimental data. This exacerbates inter-species differences and makes extrapolation to humans even more difficult and unreliable. In an effort to encourage and promote discussion of this issue, which is underappreciated, I am expanding on that Editorial.

Stress may be thought of as "the sum of the biological reactions to any adverse stimulus, physical, mental, or emotional, internal or external, that tends to disturb the homeostasis of an organism" (2). It is increasingly acknowledged that diverse species experience pain, stress and distress (see below), and experience depression and anxiety disorders (see Ferdowsian *et al.* [3]). This includes fish — notably the ubiquitous laboratory zebrafish — which show emotional fever/stress-induced hyperthermia in response to a variety of stressors, including simple handling (4, 5). Stress in fish can result in increased aggressive behaviour (6), elevated anxiety, diminished weight-gain, and altered levels of dopamine and serotonin metabolites in the brain (7), as well as increased brain levels of extracellular adenosine, which has neuromodulatory effects (8).

While all species experience stressors and stress in their natural environments, psychological and physiological problems can arise with exposure to recurrent stressors, and/or when stress becomes chronic and too difficult to cope with. This leads to allostatic overload (excessive wear and tear on the body), which may manifest in altered physiological responses, some of which can be harmful (9) — generally thought of as a state of distress. However, the US National Research Council (NRC) accepts that there is "confusion in the scientific, regulatory, and animal welfare communities" concerning the distinction between stress and distress; and that these terms are often used interchangeably in animal wel-

fare literature and that relevant available information is "far from complete", with distress remaining "a complex and still poorly understood phenomenon" (10). Nevertheless, it is generally understood that distress manifests when an individual is unable to cope and adapt successfully to one or more stressors, resulting in compromised well-being due to the inability to return to physiological and psychological homeostasis (11). Notably, an individual can remain in distress, even when a stressor is removed.

While different species and individuals have different stressors, variable ranges of stress to which they can adapt, diverse spectra of tolerance, and dissimilar manifestations and sequelae of excessive stress, they share the same biological pathways and mechanisms that are adversely affected by stress. Briefly, stressors stimulate the hypothalamic–pituitary–adrenal (HPA) axis, the sympathetic adrenal medullary axis, and the sympathetic and parasympathetic nerve projections that directly innervate secondary lymphoid organs (12–14). Among other things, this results in the elevation of the 'stress hormones', cortisol and corticosterone (CORT). Raised glucocorticoid (GC) levels, alongside significant elevations of heart rate, blood pressure and other hormone levels, are acknowledged indicators of fear, stress and distress, and are used as biomarkers for stress in terrestrial vertebrates in laboratories (e.g. 15–17).

Direct consequences of these neuroendocrine changes include deleterious effects on innate and adaptive immunity, central nervous system pathology, and cardiovascular and reproductive perturbations (18, 19), leading to extensive and diverse adverse health effects. Psychological trauma, for instance, may result in altered health or damaging patterns of behaviour. In humans, immune perturbations manifest in poor responses to vaccines, increased susceptibility to infections, and accelerated disease progression (see Gurfein *et al.* [18]). Further, the various mechanisms underlying such effects mean that stress has ramifications beyond the individuals experiencing it. Successive generations, and individuals who have experienced prenatal and/or early-life stress, may be destined to suffer the consequences in adulthood.

Caveats and Framing the Argument

Chronic or long-term stress is not unique to animals in laboratories — it is part and parcel of life for all species in whatever environment. I do not intend to suggest otherwise, and it is accepted that animals in the wild experience acute stressors regularly (such as lack of food and shelter, predation, disease, and so on); not only is this entirely normal, but it can be beneficial in terms of building allostatic capacity. This Comment, however, argues that stresses resulting from life in the laboratory often have negative consequences — given the opportunity, the animals would avoid many laboratory stressors, and so they can hardly be considered benign. In addition, the degree, type, frequency and duration of laboratory stressors are different to those in the wild, and together could be more chronic. This may result in greater adverse consequences for animal welfare and for scientific data quality. Experiencing repeated, unnatural stressors, such as blood draws and gavage, cannot be compared to the brief elevations in CORT resulting from the natural diurnal/nocturnal adrenal cycle. For instance, the former stressors are very different from simply waking up, or being hungry. In well-designed experiments, increases in stress biomarkers are relative to baseline levels in any case (see, for example, 16, 20).

That laboratory stress can — arguably, frequently and unavoidably — have effects that compromise animal welfare and experimental results is accepted by the US NRC's Committee on Recognition and Alleviation of Distress in Laboratory Animals (10): "In the longer term… a breakdown in an animal's ability to cope with its environment is likely to lead to adverse emotional states and poor welfare. Some of these cases may be quite minor and not give rise to significant ethical concerns; but prolonged or intense circumstances would compromise the animal's welfare enough to warrant concern and also significantly affect the research results." Further: "Strong evidence in rodents has shown that mild stress of 2–3 months duration — a regimen that produces no signs of overt distress — alters the animals' performance in tests of anxiety, depression, and memory… Other findings indicate that rats' habituation to a test environment can dramatically affect their response to a toxic substance". Stress can also be beneficial: "Over a longer time frame, glucocorticoid production in response to infection helps restrict the immune system, thus preventing deleterious effects of inflammatory factors on tissues" (10). While this latter statement is true, it also supports the argument made here, that stress — even when not 'bad' — adversely affects biological systems, and consequently confounds research data.

Therefore, it is not only distress that leads to biological consequences affecting data reliability, quality and relevance. It seems clear that the consequences of stress are also an issue for welfare and data quality. To quote again the US NRC: "The impact of distress on both animal welfare and research results is likely even more pronounced than that of stress. Animals exposed to prolonged severe stress experience underlying changes in physiological functions (e.g. gastric lesions or immunosuppression) that can interfere with experimental manipulations; alter experimental variables such as behaviour, drug dosing and clearance; change the progress of a disease; and

contribute to morbidity and mortality. A variety of stressors can contribute to unintended distress, from postoperative pain or infection to barren housing conditions or the solitary confinement of an individual of a social species. Stereotypies, abnormal repetitive behaviours indicative of poor well-being that are often observed in distressed animals, are thought to reflect defective brain function and to be a result of poor animal welfare. Stereotypies are thus likely to interfere with behavioural, neuroscience, and pharmacological studies" (10); and "The impact of stress and distress on the quality of scientific research can result in the generation of compromised data, which in turn necessitates the use of more animals… Both stress and distress represent potential complications in a wide range of experiments, and should be proactively addressed by good experimental design" (10). While I (and others) disagree that the use of more animals and the careful design of experiments can have an acceptably positive impact on welfare, and on the human-relevance of the data generated, the main points in this Comment stand. Efforts to address this issue over many years are acknowledged, but I argue that, due to fundamental biology and inter-species differences, the improvement of husbandry, veterinary care, regulation, oversight, training, etc. can never be sufficient to significantly address and overcome these issues.

Stress Resulting from Laboratory Life and Research, and its Biological Impact

The issue for animals in laboratories is that life can be inherently and excessively stressful — perhaps much more than in their natural environments, from which laboratory conditions differ greatly, even when enriched and accounting for efforts to mitigate stressors. Laboratory conditions preclude or limit many natural behaviours, housing tends to be much smaller than the animals' natural ranges, and the animals are subjected to frequent manipulations and handling, as well as other alien factors that they try to resist and avoid (16, 20). Stressful procedures and environmental factors are numerous and varied. Briefly, they include, but are not limited to: general handling and manipulations, such as weighing and saline injections (15, 16, 21–23); anaesthesia (15, 24–31); restraint (21, 32–36); gavage (37–42); blood sampling (15–17, 43–50); food and water restriction (51, 52); non-natural environment (53–55) and associated factors, such as noise and light (56–62); social crowding and/or isolation (63–71); cage conditions/cleaning/changing (18, 58, 72–78); transport (19, 58, 79–83); observing procedures on, and killing of, other animals (31, 84–87); and even enrichment itself (17, 88, 89). It has been suggested that captive-bred animals may not know that these stressors are different to those in the wild, though there might be some perception that they are not similar to 'natural' stressors such as limited food, inadequate shelter, predation and so on. In any case, the point is that they are different, but more importantly, they are also frequent, regular and inescapable.

Naturally, this has animal welfare and scientific implications, acknowledged at least in some quarters. For instance, stress from handling is accepted as a source of "unexplained variation within and between animal studies", as it influences "both the behaviour and physiology of animals" (23; see also Balcombe *et al*. [16] and Meijer *et al*. [17]), and relatively poor caging conditions "may contribute to problems in translating murine research into human studies" (18, 77, 78). However, it is accepted by some that these factors are probably widely underappreciated (90). It should be noted that some consider enriched environments simply as 'less bad' rather than 'better' than those that are non-enriched. To illustrate, significant numbers of animals experiencing enrichment still go on to develop stereotypies (e.g. 91–98).

The Nature of Stress: Biological Basis/Mechanisms of Stress and its Adverse Effects

The physiological consequences of stress are varied and powerful. This, in itself, is of concern for the translation of animal data to humans, as they compound and confound existing difficulties in translation due to species differences. First, however, a consideration of the underlying mechanisms is important, to demonstrate their fundamental nature and potency.

Primary mediators of stress, such as GCs and catecholamines, are released in response to stressors, with various biological consequences. Such biological effects generally go beyond species boundaries/limits for mammalian species, though the mechanisms and specific effects differ to varying degrees. These primary mediators are extremely powerful, because they ultimately modulate the expression of many genes. Secondary outcomes have been documented "…in every physiological system, including the cardiovascular system, metabolism, the central nervous system, and the immune system", and are confounded by the characteristics of the stressor(s), as well as the attributes of the affected individual, such as age, health, status, genetic background, past experience, etc. (99).

The potency and ubiquity of the stress response has been demonstrated in a number of *in vitro* studies, revealing that it generally blocks every important cellular process, including DNA replication,

transcription, pre-mRNA processing, mRNA export, and translation, until the cells recover (100). Therefore, stress exerts its effects via varied molecular mechanisms, with far-reaching consequences. The principal ones are highlighted below.

— *Epigenetic mechanisms (histone acetylation and DNA methylation)* (101–107): Psychological stress alters gene expression via histone acetylation and DNA methylation. Much occurs in response to environmental triggers, e.g. diet, drugs, toxins, and psychological stress, e.g. fear conditioning and maternal care (103). Genes involved in HPA axis function are especially susceptible (108), e.g. in suicide victims with a history of child abuse (109), and post-traumatic stress disorder (PTSD) is strongly associated with the epigenetic modification of genes involved in immune function and inflammation (110).

— *Alternative splicing/expression of regulatory microRNAs:* Alternative splicing of gene transcripts and microRNAs (miRNAs), is a powerful means of altering gene expression that can be significantly affected by stress (111, 112). For example, acute stress in humans altered the splicing of 27 genes in peripheral leukocytes (100).

— *Oxidative damage and ageing:* Mental stress contributes to oxidative stress in the body, and therefore to oxidative damage (113). This effect has been identified in students undergoing academic examinations (114), and in the lymphocytes of psychologically stressed individuals (115). Oxidative stress is also associated with PTSD and depression (116), contributes to the ageing process (117), and is associated with neurodegenerative disease, ophthalmologic disease, cancer and cardiovascular disease (including atherosclerosis, hypertension, cardiomyopathy, chronic heart failure, myocardial ischaemia and ventricular arrhythmias; 117, 118). There may be confounding data on the effects of oxidative stress from different species/strains of mice, which further challenges the translation of data across species (119).

Direct Physiological Consequences of Stress

The physiological consequences of stress are numerous and varied. Table 1 shows how promiscuous these consequences, effects and manifestations are in many species, including humans. Given that these effects involve so many biological pathways and systems, the effects on experimental data must be significant.

Habituation/Desensitisation to Stress

Some argue that animals become habituated (120) and/or desensitised (121) to stress, so implications for welfare and experimental results may be overcome (23, 122, 123). For instance, non-human primates can be trained to approach test environments, and to present their arms for blood withdrawal and so on, seemingly voluntarily. However, it has been shown that repeat exposure to homotypic stressors of greater intensity and/or severity does not result in habituation, and could actually result in *sensitisation* of the CORT response (122). Furthermore, CORT levels might only decrease for certain types of stressor, persisting for other types (35); where desensitisation has been shown, it is only to a modest degree, in a small proportion of the animals in the studies, and in small sample sizes (70). While some studies have suggested that enrichment might decrease stress, others have shown a paradoxical increase in stress via CORT levels (18). Similarly, some studies have suggested that transferring scent-marked materials from old to new cages reduces stress-related aggression, while others found that it increases aggression (74). Furthermore, mice do not habituate to stress associated with simple handling, and indeed seem to become sensitised to it (124–126). In instances where CORT levels decrease, there is increasing evidence that this does not necessarily mean an attendant decrease in stress; other indicators, such as the neutrophil–lymphocyte ratio, might be 'better' indicators of chronic stress, which can occur in the absence of increased serum CORT (127).

Adverse Physiological Sequelae of Psychological Stress are Initiated Prenatally or in Early Life, and are Heritable

Captive-born animals can be exposed to stress prenatally via their wild-trapped mothers (128–131), and as infants in laboratory environments, often experiencing inadequate maternal contact and care (132–134; cited by Camus *et al.* [135], and Detter *et al.* [136]). If an animal's parents or grandparents lived in laboratories, and/or were born of parents that lived in laboratories and/or endured being wild-caught, then their ancestors experienced highly stressful lives and would have been affected by the adverse consequences described herein. Even if such an individual was subsequently afforded as stress-free a life as possible (difficult, if not impossible, in a laboratory), the consequences of their early lives, and the lives of their ancestors, would lead to the same adverse effects as if they had continued to experience excessive stress as adults.

That early-life experiences affect adult psychopathology is widely accepted. As Jean-Paul Sartre put it, "Childhood decides" (see Murgatroyd [102]). "A large body of data shows that stress during pregnancy causes an increase of GCs in the blood of the dams and the foetus, leading to alterations of the structure and function of the developing brain… These alterations result in the disturbance of the function of the neuroendocrine system and different kinds of behaviour throughout life" (137). Early-life/prenatal exposure to stress leads to altered adrenocorticotrophic hormone responsiveness, dysfunction of the HPA axis (138, 139), and altered autonomic modulation of immune function that may begin *in utero* (103). Social isolation in several species leads to neuroendocrine changes, increased cortisol, and ensuing behavioural problems (for references, see Champagne [101]). Maternal inflammation during pregnancy (from infection, or possibly stress) may increase the risk of neurodevelopmental disorders such as schizophrenia and cerebral palsy (140). Physiological sequelae include cardiovascular disease and metabolic disorders such as diabetes (141), compromised immune function, including poor lymphocyte proliferation upon infection and reduced placental transfer of antibodies during pregnancy (140), autoimmune disorders, chronic obstructive lung disease, asthma and obesity (see Chang [142]). Pivotal to these adverse outcomes are the aforementioned stress-related epigenetic processes and oxidative damage. Methylation of gene regulatory regions is partly involved (143), the extent of which may be set during prenatal development (141). Cord blood samples of infants of mothers with late-pregnancy depression show altered methylation of the GC receptor promoter, which also predicts elevated salivary cortisol in early life (144). Other modifications are inherited and transgenerational in nature (145); for example, poor prenatal nutrition affects GC receptor methylation, affecting the growth and metabolism of first and second-generation offspring (146), and matrilineal transmission of the effects of diethylstilbestrol (DES) occurs via hypomethylation, leading to increased cancer risk over two generations (147).

Summary

The inherent, multi-faceted stress of laboratory life — often excessive, relative to the more transient, acute and 'natural' stresses experienced in the wild — is evidenced by its often negative impact on the well-being of the animals involved, both psychologically and physiologically. Because this harm is mediated via established trans-species biological mechanisms involving the HPA axis and the sympathetic nervous system, and effected via oxidative stress and epigenetic mechanisms, which affect multiple biological pathways and systems, it can have adverse and confounding effects on experimental results. This modulation of many biochemical pathways and gene expression can result in downstream effects such as organ damage, cardiovascular diseases, attenuated immune function and autoimmune disorders, premature ageing and mortality, developmental abnormalities, elevated tumour initiation and progression, and musculoskeletal atrophy (16).

The absolute degree of translation of animal studies to humans is debatable, but undoubtedly, "Studies using animal models are more translatable to human disease when the animals' welfare is maximised" (127). Arguably, it is difficult to "maximise" welfare significantly, given the inherent, widespread, substantial and largely intractable nature of the stresses involved in animal research and laboratory life. Notably, habituation and/or desensitisation to many of the stressors is often not possible, or at least not significant, and the impact of these effects on experimental data — and their extrapolation to humans — is likely to be significant. The many sources of stress have been summarised here, along with their effects on multiple biological and physiological systems. The literature warns that: "…animals subjected to the environmental changes that occur during transportation… react with changes in their physiology, such as body weight, plasma hormonal levels, heart rate and blood pressure changes… When measurements of physiological parameters are performed using conventional measurement techniques, the results must be interpreted with caution as these conventional techniques also have effects on the animals" (148). Most importantly, "Suffering in animals can result in physiological changes which may increase the variability of experimental data" (149). Many scientists are well aware of these effects and considerations, and have cautioned against disregarding them (150–152). Yet, while accepting the negative effects of pain, stress and distress, and their influence on study outcome, such effects are often not reported or are under-reported in scientific publications (90).

The impact of stress on immunological and inflammatory responses seems particularly prevalent, and might be especially critical, seeing as much animal experimentation involves infectious agents and/or immune function (for a discussion and references, see Bailey [153] and Bailey [154]). Crucially, this impact exacerbates and compounds existing immune differences between humans and non-humans due to genetic differences. To illustrate, genomic duplications — one of the most significant causes of genetic variation among primates (155) and at the root of many aspects of intra-species and inter-species diversity — differentially affect many genes involved in immune and

Table 1: A summary of the physiological consequences and manifestations of stress, in many species, including humans

Consequences of general stress	Notes
Stereotypies (abnormal, repetitive, invariant behaviours with no obvious function; 160, 161) and self-harm (e.g. 162–165) in non-humans	These correlate with basal plasma cortisol and corticotrophin-releasing hormone (CRH) in cerebrospinal fluid (166).
Wide-ranging physiological symptoms in humans	Anger, depression, anxiety, behavioural changes, food cravings, lack of appetite, frequent crying, difficulty sleeping, tiredness, lack of concentration, chest pains, constipation, diarrhoea, cramps and muscle spasms, dizziness, fainting, nervous twitches, restlessness, sexual dysfunctions, breathlessness, and a host of diseases and illnesses with probable associated psychogenic (as well as biological) causes (167).
Long-lasting neurophysiological changes	These could "…have direct implications for electrophysiological, behavioural, and molecular studies" (see 69). — Isolated rats show "…structural and functional changes in the mesocorticolimbic dopaminergic system, exhibited hyperlocomotor activity and impaired sensorimotor gating" (168). — Isolated pigs show "…sustained changes in behavioural, neuroendocrine and immune regulation" (169). — Socially isolated humans show increased risk of death, "…genome-wide transcriptional activity of impaired GC response genes and increased activity of pro-inflammatory transcription control pathways", and higher risk of developing "…conduct disorders, personality disorders, major depression, PTSD, schizophrenia, and anxiety disorders" (see 170).
Multi-faceted modulation of the immune system	This occurs in many, if not all, mammalian species (140, 171, 172). It is especially problematic for research involving immune function, infectious diseases, etc. The expression of several hundred genes (many related to immune function) may be perturbed by simple handling of animals (173; and see 33), which may be affected by the acute or chronic nature of the stressor (see 173). Chronic stress can: — shift neutrophil–lymphocyte ratios (127, 174); — affect expression of cytokines and cytokine receptors (175, 176), such as IL-2 and IL-6 (18); — alter gene splicing in peripheral leukocytes (10); — alter global and immune gene specific methylation (177); and — differentially affect brain activity and neurotransmitter release, macrophage activity and antibody production (33). The same stressor in acute or chronic forms may have different effects: — In rodents, acute restraint increases delayed-type hypersensitivity (DTH) and leukocyte redeployment, but can increase or decrease them when chronic (33, 178). — In humans, chronic, though not acute, stress increases susceptibility to colds (179). These inconsistencies show that stress is mediated not only by GCs (180), and that accounting for the effects of stress in animal research must be difficult, if not impossible.
Observed alterations in human immune function	These include: — "attenuated responses to vaccination, poorer wound healing, exaggerated release of inflammatory mediators, & premature aging of the immune system" (181); — increased plasma and CNS cytokine levels, impaired natural killer cell activity, lower T-lymphocyte counts (in PTSD/complex PTSD patients; 182); — epigenetic changes exerting lifelong impact on immune and inflammatory function (in PTSD/complex PTSD patients; 182); and — greater inflammatory responses to vaccinations (in depressed humans; 183). Neuropeptides involved in stress responses may accentuate pathophysiological sequelae in critically ill individuals (184). In healthy humans, 49 different genetic pathways are affected by stress, including genes associated with immune function (185). Stressed students undergoing examinations have significantly increased pro-inflammatory cytokines (186).
Activation of the HPA axis	This accelerates ageing generally, with adverse effects on brain/central nervous system, immune system, skeletal muscle and bone tissue (see 187–189). HPA dysregulation may lead to excessive inflammation via increases in the levels of circulatory inflammatory cytokines, decreases in anti-inflammatory cytokines, and alterations in the expression of genes involved in immune activation of peripheral blood cells (see 177).

Table 1: continued

Effects of specific stressors	Notes
Disease susceptibility	An increase in susceptibility has been noted across several species to: — general disease and somatic disorders (see 100, 103); — various cancers (190, 191); — pancreatitis and pancreatic tumours (192, 193); — gastrointestinal disorders (194); — thyroid pathology (195); — multiple sclerosis (171); — inflammatory bowel disease (142, 196); — cardiovascular disease (197–199); — accelerated ageing and age-related disorders (187); and — musculoskeletal injury (200). Stressors can increase oxidative stress, triggering inflammatory pathways associated with type-2 diabetes, cardiovascular disease, osteoporosis, arthritis, some cancers, and susceptibility to some infections (e.g. 201–205).
Handling	Causes biological changes, affecting wellbeing and/or experimental results. Enrichment intended to mitigate stress can substantially alter brain structure, function and physiology. Light, noise, cage position/changing etc. all affect physiology, behaviour, anxiety and experimental data (206). Effects of handling stress may often be "missed" by researchers (23).
Early-life stress in various species	Results in: — abnormal brain development, leading to early-life psychopathologies and adult chronic mental illnesses (e.g. 207, 208); — epigenetic modifications associated with depression and suicidal behaviour in later life (e.g. 102, 209–211); and — elevated morbidity and mortality from chronic diseases of ageing, including vascular disease, autoimmune disorders, and premature mortality (e.g. 141, 212). Stress from maternal deprivation has negative effects on growth rates in rats, and adversely affects circadian clock and stress responses (72).
Traumatic stress in humans	Studies on PTSD patients have shown that: — acute stress affects glucose metabolism, inflammation and components of the immune system associated with type-2 diabetes (201); — serious long-term consequences include hypertension, heart attacks and stroke, as well as increased risk of obesity, Alzheimer's disease, and AIDS dementia complex (213).

inflammatory responses (156). Indels (genomic insertions and deletions) also affect major histocompatibility complex (MHC) genes, which are critical to immune responses, and are associated with differences in response to infections, as well as susceptibility to autoimmune diseases. These are further confounded by sex-related and strain-related differences. It has also been shown that the susceptibility and responsiveness of mice to stressors varies with the strain (157, 158). Stress also affects sleep, and conversely sleep perturbations exacerbate and sensitise individuals to stress, with all the attendant consequences. These consequences are also strain-dependent, and intimately linked to the CRH/HPA system (159).

Additionally, the adverse consequences of stress are multigenerational, as the associated epigenetic mechanisms affect the germline. This is likely to have significant consequences for animals, and their offspring, in laboratories: if an animal's parents or grandparents experienced a stressful laboratory life and experimental procedures, and/or if the offspring experienced significant stress in early life, then this will compound any further stress that they experience as adults, in turn compounding species differences and the translation of data to humans.

Overall, these observations of detrimental physiological effects and the general mechanisms behind them have been detailed in many species (including humans), and throughout the evolutionary scale from monkeys to rodents. The minutiae of the genes and biochemical pathways responsible, and their manifestations, may differ to some degree, but there are common mechanisms and adverse effects in all species examined to date. It must be concluded that laboratory life for animals used in experiments has serious and intractable consequences for their welfare, and for the quality and human relevance of the experimental data obtained (which, in any case, are already of debatable applicability to humans, due to species differences).

Finally, I believe that this issue should be taken much more seriously by legislators, regulators, funders, practitioners and advocates of animal experiments, and urge all involved to do so. The information presented here could, and should, be a valuable resource for project licence applicants, ethics committees and the Home Office Inspectorate, for use in experimental design and harm–benefit analyses, and to aid data interpretation. Though it argues that relatively little can be done to minimise many, if not all, stressors and stress, it could inform attempts to do so — as well as controlling variable factors and mitigating negative consequences, etc. The information could also be factored into existing guidance for the strategic planning of animal experiments, since stress impacts all areas of this planning, including study objectives, species/strain selection, experimental procedures, analgesia, training of staff, and so on.

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