Review of Stress and the Measurement of Stress in Marine Mammals

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LONG-TERM GOALS

The goals of this review of stress are to provide for marine mammals a conceptual framework for considering stress, to use the framework to review previous studies of stress, and to suggest how to design studies that address questions about stress.

OBJECTIVES

The objectives are to comprehensively review the literature on stress in marine mammals, to present a conceptual framework for considering stress in marine mammals that can be used by marine mammal researchers to consider how animals detect and respond to stressors over short and longer time periods, and to collate and critically assess data on stress hormone measurements.

APPROACH

A comprehensive review of the published literature on stress will be performed. Definitions of stress used in different studies will be identified and related to a clear conceptual framework for the consideration of stress in marine mammals. Summary tables will be prepared to list, for each group of marine mammals, key features of all published papers. Reported values for cortisol concentrations will be summarised in tables with details of sample collection conditions in order to provide readily accessible information on mean values and ranges of measured cortisol concentrations in different situations. Dr Cockrem will write the review.

WORK COMPLETED

A database of 323 marine mammal stress references has been established, with 312 journal articles and 11 book chapters, reports and books. The database has been searched to find references with definitions of stress, to determine what factors have been proposed to be stressors for marine mammals, and to summarise variables that have been suggested to indicate stress in marine mammals. A framework based on current understanding of how mammals respond to changes in their environment has been used to develop a new conceptual framework for considering stress in marine mammals. Studies of stress in each taxa will be summarised to provide an overview for each group. Reports of
measured plasma and serum concentrations of cortisol in taxonomic groups of marine mammals have been collated and results in expressed in the three commonly used units of ng/mL, ug/dL and nM for easy comparison between studies. The conditions in which samples were collected will be examined in detail and presented so that reference values for cortisol concentrations in each taxa can be determined. Reports of hormone concentrations for other relevant hormones have also been collated. Suggestions will be presented for future research on stress in marine mammals based on criteria for asking questions that can be answered and that can inform discussions of possible effects of naval activities on marine mammals. The review will be completed early in FY2014.

RESULTS

An outline of the sections of the review is given below:
1. Introduction

2. Marine mammal classification

3. Stress, stressors and stress responses
   3.1 The literature on stress in marine mammals
   3.2 Definitions
   3.3 Potential stressors in marine mammals

4. A conceptual framework for stress in marine mammals

5. The measurement of stress

6. Stress and cortisol in marine mammals
   6.1 Cetaceans
      6.1.1 Whales
      6.1.2 Dolphins
      6.1.3 Porpoises
   6.2 Pinnipeds
      6.2.1 Elephant seals
      6.2.2 Other seals
      6.2.3 Sea lions
   6.3 Otters and polar bear
      6.3.1 Sea and marine otters
      6.3.2 Polar bear
   6.4 Sirenians

7. Other hormones in marine mammals
   7.1 ACTH
   7.2 Catecholamines
   7.3 Aldosterone
   7.4 Antidiuretic hormone (ADH; vasopressin)
   7.5 Other hormoness

8. Future research on stress in marine mammals
9. Conclusions

The current review is being conducted in the context of consideration of possible effects of naval activity on marine mammals. Questions about such effects are often posed as questions about whether particular naval activities cause stress in marine mammals. These questions can only be answered if stress is defined and measurable. However, it is clear from the literature on stress in marine mammals that different authors have different views on stress, and that the term stress is often used to refer to general animal health. This means that discussions on whether groups of animals are experiencing or have experienced stress are constrained by the absence of clear criteria for answering questions about stress.

The database for the current review contains 323 marine mammal references, 209 of which have stress, stressor or stress response in the title, abstract or keywords. 14 of these references consider oxidative stress which is intracellular, leaving 195 references (182 journal articles and 13 others) on stress in whole animals. Approximately 6% of these references have definitions of stress, stressor or stress response, so most papers that consider stress use this term without defining it. The definitions that have been given are diverse and almost invariably are couched in general terms such as as the response of the body to any threatening situation, or a state when homeostasis is threatened or disrupted. It is not possible to determine if an animal or a group of animals is experiencing stress when stress is defined in these general terms. Definitions of stress in the marine mammal literature are tabulated and discussed in the review, and a definition based on the mammalian stress physiology literature is proposed for marine mammals.

A wide variety of factors have been suggested as possible stressors for marine mammals, including anthropogenic disturbance, direct exploitation, pollution, global climate change, severe weather, food restriction, ambient noise, nonpoint source pollution, water temperature, marine biotoxins, habitat degradation, commercial and recreational fisheries, vessel disturbance, environmental contaminants, forced exercise, capture, restraint, mechanical transport, crowding, parturition, disease, changes in group dynamics, competition over resources, and unstable dominance hierarchies. It appears that any environmental factor or social stimulus or situation that could potentially have a detrimental effect on a marine mammal has been deemed to be a stressor. The absence of clear definitions of stress is also seen in the list of variables that have been proposed as measures of stress in marine mammals. These include plasma and serum concentrations of cortisol, catecholamines, ACTH, aldosterone, thyroid hormones, IGF-I, reproductive hormones, haptoglobin, haemoglobin, red blood cell count, white blood cell count, albumin, and various enzymes (AST, CK, LDH, alkaline phosphatase and alanine aminotransferase). Other variables said to indicate stress include body condition, immune measures, patterns of "stress-responsive protein" expression, and behaviour patterns. It is apparent from the marine mammal literature that the term stress is generally used interchangeably with animal health, and that discussions of stress are often based on assessments of whether animals are exposed to stimuli thought to be harmful.

Animals are continuously aware of and responding to changes in their physical and social environment. Stimuli from the internal and external environments activate neural pathways that lead to brainstem structures and to the sensory thalamus, and from these structures to the hypothalamus. The hypothalamus also receives inputs from the cerebral cortex and the limbic system, and plays a central role in initiating responses of the whole animal to internal and external stimuli. Stimuli from the external environment, and internal stimuli, can activate neural and neuroendocrine pathways that are recognised as the stress neurocircuitry (Herman et al., 2003; Boyce and Ellis, 2005; Ulrich-Lai and
Herman, 2009). The output pathways are central structures that activate the sympathetic division of the autonomic nervous system, together with corticotropin-releasing hormone (CRH) pathways at the start of the hypothalamo-pituitary-adrenal (HPA) axis. A stress response occurs when there is rapid activation of the sympathetic nervous system with release of catecholamines from the adrenal medulla, and slower activation of the HPA axis with release of glucocorticoids from the adrenal cortex. This is a stress response defined in terms of the response of an animal to changes in its environment, so the measurement of stress is the measurement of the level of activity of this biological stress response system (i.e. the measurement of catecholamine and glucocorticoid secretion). This conceptual framework, with its implications for how to measure stress, will be developed in the review.

References with stress, stressor or stress response in the title, abstract or keywords have been identified for taxonomic groups of marine mammals (see Table 1). These studies will be summarised to provide an overview of previous studies of stress in marine mammals.

**Table 1. Numbers of marine mammal references with stress in the title, abstract or keywords.**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Number of references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whales</td>
<td>33</td>
</tr>
<tr>
<td>Dolphins</td>
<td>53</td>
</tr>
<tr>
<td>Porpoises</td>
<td>5</td>
</tr>
<tr>
<td>Elephant seals</td>
<td>14</td>
</tr>
<tr>
<td>Other seals</td>
<td>51</td>
</tr>
<tr>
<td>Sea lions</td>
<td>39</td>
</tr>
<tr>
<td>Polar bears</td>
<td>18</td>
</tr>
<tr>
<td>Otters</td>
<td>10</td>
</tr>
<tr>
<td>Sirenians</td>
<td>15</td>
</tr>
</tbody>
</table>

Plasma or serum concentrations of cortisol indicate the level of activity of the HPA axis in an animal, and hence the degree of stress experienced by an animal. Cortisol is a glucocorticoid hormone, and cortisol or cortisol metabolites can also be measured in other sample matrices such as faeces and blubber. ACTH is a pituitary gland hormone that is part of the HPA axis, and ACTH concentrations can be measured in plasma. Catecholamines can also be measured; however, their concentrations change quickly and it is very difficult to collect blood samples that can be used to indicate catecholamine concentrations in animals before the start of the collection process. The marine mammal literature has been searched and papers that report plasma or serum concentrations of cortisol, ACTH, catecholamines identified for marine mammal taxa (see Table 2). Reported cortisol concentrations will be tabulated for every study and expressed in the three common units of measurement (ug/dL, ng/mL and nM) so studies can be compared using the same units. The sample collection conditions for each study will be considered in detail so the measured hormone concentrations can be compared between studies in relation to the sampling conditions. This will lead to a comprehensive presentation and summary of cortisol measurements in marine mammals. A similar approach will be taken for ACTH and catecholamines, and also for aldosterone and vasopressin.
(antidiuretic hormone, ADH) as these hormones have been considered relevant to stress in marine mammals. There are 12 papers in the literature that describe faecal glucocorticoid measurements, with 5 of these including plasma or serum measurements and 7 papers reporting faecal results alone. These results will also be analysed and summarised. This section of the review will provide, for the first time, an accessible summary of all published studies of cortisol and catecholamine stress hormones in marine mammals.

**Table 2. Numbers of papers with measurements of plasma or serum concentrations of cortisol, ACTH, catecholamines, aldosterone and vasopressin (ADH) in marine mammals.**

<table>
<thead>
<tr>
<th></th>
<th>Cortisol</th>
<th>ACTH</th>
<th>Catecholamines</th>
<th>Aldosterone</th>
<th>Vasopressin (ADH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whales</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Dolphins</td>
<td>30</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Porpoises</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elephant seals</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Other seals</td>
<td>24</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sea lions</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Polar bears</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otters</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirenians</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**IMPACT/APPLICATIONS**

There is currently no recent, comprehensive review of stress in marine mammals available to the scientific community. The review will assist researchers to readily determine what work has previously been undertaken on stress in particular species and groups of marine mammal species, to pose questions in relation to clear definitions of stress, to determine how stress can be measured in their study animals, and to interpret hormone data in relation to results from previous studies.

**RELATED PROJECTS**

This review is related to two projects which include investigations of the measurement of stress via quantification of blubber cortisol concentrations. These projects are "Variability of hormonal stress markers collected from a managed dolphin population" and "Variability of hormonal stress markers and stress responses in a large cross-sectional sample of elephant seals".

**REFERENCES**

