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The identification and management of pain, suffering and distress in cephalopods, including anaesthesia, analgesia and humane killing ☆,☆☆

Paul L.R. Andrews [a,b,*], Anne-Sophie Darmaillacq [c], Ngaire Dennison [d], Ian G. Gleadall [e], Penny Hawkins [f], John B. Messenger [g], Daniel Osorio [h], Valerie J. Smith [i], Jane A. Smith [j]

[a] Division of Biomedical Sciences, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK
[b] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy
[c] Groupe Memoire et Plasticite comportamentale, University de Caen Basse-Normandie, F-14032, Caen cedex, France
[d] Home Office, Animals in Science Regulation Unit, Dundee, DD1 9WW, Scotland, UK
[e] Department of Applied Biosciences, Faculty of Agriculture, Tohoku University, Amamiya 1-1, Sendai, 981-8555, Japan
[f] RSPCA Research Animals Department, Southwater, RH13 9RS, West Sussex, UK
[g] University of Cambridge, Department of Zoology, Cambridge, CB2 1TN, UK
[h] School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK
[i] Scottish Oceans Institute, Gatty Marine Laboratory, School of Biology, University of St. Andrews, St. Andrews, Fife, KY16 8LB, Scotland, UK
[j] The Boyd Group, Hereford, UK

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ABSTRACT

From January 2013 scientific projects involving cephalopods became regulated by Directive 2010/63/EU, but at present there is little guidance specifically for cephalopods on a number of key requirements of the Directive, including: recognition of pain, suffering and distress and implementation of humane end-points; anaesthesia and analgesia, and humane killing. This paper critically reviews these key areas prior to the development of guidelines and makes recommendations, including identifying topics for further research. In particular: a) Evidence on how cephalopods might experience pain is reviewed; and a draft scheme of behavioural and physiological criteria for recognising and assessing pain, suffering and distress in cephalopods used in scientific procedures is presented and discussed. b) Agents and protocols currently used for general anaesthesia and analgesia are evaluated. Magnesium chloride, ethanol and clove oil are the most frequently used agents, but their efficacy and potential for induction of aversion need to be systematically investigated, according to the species of cephalopod and factors such as body weight, sex and water temperature. Means of sedating animals prior to anaesthesia should be investigated. Criteria for assessing depth of anaesthesia, including depression of ventilation, decrease in chromatophore tone (paling), reduced arm activity, tone and sucker adhesiveness, loss of normal posture and righting reflex, and loss of response to a noxious stimulus, are discussed. c) Analgesia should be provided for cephalopods used in scientific procedures, whenever this would be the case for vertebrates. However, research is needed to evaluate effective agents and administration routes for cephalopods. d) Techniques for local anaesthesia need to be defined and evaluated. e) Currently used methods of killing and criteria for confirmation of death in cephalopods are evaluated. Based on present knowledge, a protocol for humane killing of cephalopods is proposed. However, further evaluation is needed, along with development of humane methods of killing that will not compromise study of the brain. On humane grounds: *i.* mechanical (as opposed to chemical) methods of killing should not be used on conscious cephalopods (unless specifically authorised by the national competent authority); and *ii.* hatchlings and larvae should be killed by overdose of anaesthetic and not by immersion in tissue fixative.

Key gaps in current knowledge are also highlighted, so as to encourage research that will contribute to the evidence base needed to develop guidelines to the Directive.

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* Corresponding author at: Division of Biomedical Science, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK.
E-mail address: pandrews@sgul.ac.uk (P.L.R. Andrews).

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1. Introduction

There are strong ethical, legal and scientific obligations to avoid, recognise, and alleviate any pain, suffering and distress caused to animals used in scientific procedures.

The ethical obligation is reflected in legal requirements to minimise suffering, such as those enshrined in EU Directive 2010/63/EU (European Parliament and Council of the European Union, 2010). This new law replaces EU Directive 86/609 and, for the first time, includes "all live cephalopods" within its scope (in addition to vertebrates).

The scientific imperative to reduce animal suffering arises because adverse effects such as pain and distress are likely to confound experimental results, especially if they are unrecognised. There are many studies showing that physiological responses to discomfort, pain, stress and distress can affect the quality of data obtained in studies involving vertebrates (e.g.: Hurst and West, 2010; Karelina et al., 2009; Roedel et al., 2006) and this is highly likely to be the case in cephalopod studies too.

Similar considerations apply when animals are humanely killed to obtain tissue for *in vitro* studies where suffering should be minimised on ethical, legal and also scientific grounds, as the physiological condition of the tissue may be affected by suffering around the time of killing.

A degree of suffering may be unavoidable in the experimental protocol (e.g. surgery, administration of substances or adverse reactions in toxicology procedures), or may itself be the object of the study, for example in some aspects of psychology or pain research. Whatever the cause, suffering should be recognised and assessed in order that it can be minimised at all times and so that pre-set "humane end points" can be implemented (see below).

This paper reviews some of the evidence on whether cephalopods experience suffering, proposes an objective approach for assessing suffering in an experimental setting, and reviews approaches to anaesthesia, analgesia and humane killing.

It should be emphasised that there is still much work to be done in all of the areas discussed in this paper. We anticipate that implementation of Directive 2010/63/EU will stimulate research to address some of the questions raised in relation to pain, suffering, distress, anaesthesia and euthanasia as well as questions discussed by Smith et al. (2013–this volume).

2. Evidence of the capacity for cephalopods to experience pain

A review of the published literature for evidence of nociceptors and for the perception of pain in cephalopods can be summarised as "absence of evidence rather than evidence of absence". As pointed out by Smith et al. (2013–this volume), the time for debate about the rationale for including cephalopods within the scope of EU legislation on animal experiments has passed and the new law assumes that these animals can, and do, experience pain, suffering and distress. Nevertheless, there is a paucity of robust data in many key areas relevant to suffering in cephalopods, and so there is merit in examining the arguments put forward by the *Scientific Panel on Animal Health and Welfare (AHAW) on the Revision of the Directive* (EFSA Panel on Animal Health and Welfare, 2005), which advocated inclusion of cephalopods in the legislation, and other published literature discussing this topic in order to highlight the major 'knowledge gaps' that limit the ability to make objective assessments about some aspects of cephalopod welfare.

It must be remembered that there are many causes of suffering, including stress, distress, hunger, fear, anxiety, sensory deprivation and frustration (e.g. resulting from physical confinement), as well as pain (Andrews, 2011). However, in this section we focus on pain, for two reasons. First, the capacity of cephalopods to experience psychological distress has not been evaluated to a significant extent. Second, AHAW (EFSA Panel on Animal Health and Welfare, 2005) regarded pain as "a particularly important form of suffering", which is pivotal to many discussions of welfare, and pain caused by animal experiments using invertebrates is an area of longstanding concern and controversy (Fiorito, 1986).

In considering evidence for an animal's "capacity to experience pain", it is important to recognise that there are two components to pain responses: *i. nociception* (the physiological detection and response to painful stimuli); and *ii.* the *experience* of pain (a conscious, emotional sensation, which, in humans, is mediated by the "highest" part of the brain, the cerebrum); and that nociceptive (reflex) responses can occur without any conscious sensation of pain.

A Working Party of the Institute of Medical Ethics (Smith et al., 1991) identifies seven criteria that, taken together, might provide evidence for pain experience in animals. The seven criteria are listed below, and we discuss evidence relevant to cephalopods against each item in turn (taking criteria 6 and 7 together). In essence, the criteria cover: possession of appropriate nervous receptors and pathways for nociception; involvement of brain centres that could have the capacity to generate pain sensation, with the possibility of modulation by endogenous and/or exogenous opioids; behavioural responses to noxious stimuli that are functionally similar to those of humans; and evidence of learning in relation to painful stimuli. Note that the AHAW report (EFSA Panel on Animal Health and Welfare, 2005) utilised these criteria in a slightly modified form.

It should be noted that over the last 10 years the fish research community has dealt with a number of the issues now confronted by the cephalopod research community. For example, the sensory experience of fish exposed to a noxious stimulus is contentious (for contrasting views see: Braithwaite and Boulcott, 2007; Rose, 2007), but in contrast to cephalopods there is good neurophysiological evidence for the existence of nociceptors in fish (Mettam et al., 2012; Sneddon, 2009; Sneddon et al., 2003).

2.1. Criterion 1: Possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body and connected by nervous pathways to the lower parts of the nervous system

2.1.1. Presence in cephalopods: likely but not proven

The International Association for the Study of Pain (IASP) defines a nociceptor as "a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli" (IASP Task Force on Taxonomy, 2011). A noxious stimulus is one which is potentially or actually damaging to the organism, but note that the definition implies nothing about the conscious sensation ("what the animal may feel") resulting from activation of a nociceptor. Nociceptors are usually identified by neurophysiological studies, but indirect evidence may also come from ultrastructural studies (e.g. unspecialised afferent nerve terminals may be a characteristic of nociceptors) and behavioural studies (e.g. withdrawal of the animal in response to a noxious stimulus, possibly combined with a blunting of the response by an analgesic). A start has been made in identifying molecular signatures for nociceptors (e.g. TRPV1, ASICs; Smith and Lewin, 2009) and this may be an approach to resolving this question for cephalopods. An understanding of the properties of nociceptors (mechanical threshold, chemical sensitivity, capacity for sensitisation) in cephalopods will contribute to assessment of the severity classification of experimental procedures.

There is good neurophysiological and behavioural evidence for nociceptors sensitive to mechanical stimulation in the mollusc *Aplysia californica* (Illich and Walters, 1997). There is also evidence for nociceptors in the land snail *Cepaea nemoralis* (Kavaliers et al., 2000) and the sea-slug *Tritonia diomedia* (Getting, 1976). Although there have been no comparable studies on cephalopods, from a phylogenetic perspective this would make it likely that they possess a nociceptive system. The evidence that a receptor structure and its associated afferent axon is a "nociceptor" is essentially based on characterisation of the afferent discharge in response to graded stimuli. The EFSA (2005) report concluded that cephalopods "have nociceptors in their skin" (Section 2.4.5, p. 34 in the report) citing Wells (1978) to support this (but see below).