Are neuromuscular blocking agents being misused in laboratory pigs?

A. G. Bradbury and R. E. Clutton*

Wellcome Trust Critical Care Laboratory for Large Animals, Roslin Institute & Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin, Midlothian EH25 9RG, UK

*Corresponding author. E-mail: e.clutton@ed.ac.uk

Abstract

The literature (2012–4) describing experimental pig surgery was reviewed to estimate the extent to which neuromuscular block (NMB) is used, to examine methods for ensuring unconsciousness, and to identify the rationale for use of NMB and establish the anaesthetist’s training. In the first stage of a two-stage review, NMB use was estimated using Web of Knowledge to identify articles describing NMB during pig surgeries. In the second stage, PubMed and Google Scholar were used to increase the number of articles for determining measures taken to prevent accidental awareness during general anaesthesia (AAGA). The corresponding authors of screened articles were emailed four times to establish the reason for using NMB and the anaesthetists’ backgrounds (medical, veterinary, or technical). The first search revealed NMB use in 80 of 411 (20%) studies. Of the 153 articles analysed in the second stage, two described strategies to reduce AAGA. Some (6%) papers did not provide information on anaesthetic doses; citations supporting anaesthetic efficacy were found in only 13. Five of 69 papers using inhalation agents measured end-tidal anaesthetic concentrations based on human, not porcine, minimal alveolar concentrations. The methods in 13% of articles reporting anaesthetic depth assessment were incomplete or questionable, or both; four described using somatic motor reflexes. Corresponding authors of 121 articles reported that the principal reason for NMB was improved ‘surgical visualization’ (26%). Medical or veterinary anaesthetists supervised anaesthesia in 70% of studies; non-anaesthetists provided NMB, unsupervised, in 23. Nine respondents prioritized experimental expediency over pig welfare. In laboratory pig studies, AAGA may be prevalent; reported details of its attempted prevention are woefully inadequate.

Key words: anaesthesia; awareness; neuromuscular blockade; surgery; swine

Editor’s key points

- Neuromuscular blocking agents (NMBs) are widely used in pig surgery for research.
- Inadequate monitoring of anaesthetic depth could result in accidental awareness.
- The authors undertook a literature review and author questionnaire about studies reporting pig surgery and NMB use.
- Results suggest accidental awareness is likely to be prevalent owing to inadequate monitoring and poorly trained staff.

The use of neuromuscular blocking agents (NMBs) in conditions of inadequate anaesthesia or analgesia in humans has resulted in accidental awareness during general anaesthesia (AAGA),¹ which may have severe psychological consequences.²–⁴ The majority of affected humans report their experience of awareness verbally, but a proportion fail to report at all.⁵ Estimating the extent of AAGA in infants and children is complicated by their inability to communicate effectively regarding awareness.⁶ Difficulties are greater in animals because, unlike children, they never develop the capacity to articulate memories of AAGA that they may have experienced as infants.⁷ To date, the challenge in determining whether animals receiving NMBs may have suffered AAGA has been avoided; it being assumed, presumably, that
there is universal compliance with the unsurprising recommendation that humane NMB use is absolutely dependent on the provision of adequate anaesthesia (Flecknell; Hall and colleagues; Tranquilli and colleagues). Unfortunately, ensuring such provision is equally challenging, being based on ‘signs’ of anaesthesia that the veterinary anaesthetist assumes—without incontrovertible evidence—to be related to the cognitive, sensory, or emotional state of the animal.

Given the potential for severe effects on humans, a failure to assure unconsciousness in laboratory animals receiving NMBs constitutes a dereliction of the refinement principle (i.e. that experiments involving animals are conducted in a way that obviates their pain and suffering and optimizes their welfare). Refinement (along with replacement and reduction) forms an ethical defence for animal use in biomedical research (Burch). Legislation restricts the use of NMB in animal experiments in many countries. In the UK, NMB can only be used in procedures licensed under the Animals (Scientific Procedures) Act 1986 and the granting of specific permission. Even in licensed procedures, the individuals administering NMB must be additionally and specifically licensed to do so on the basis of ‘adequate training provided by competent research workers’. In the USA, the Institute of Laboratory Animal Resources requires that the proposed use of NMBs is carefully evaluated by the Institutional Animal Care and Use Committee (IACUC), ‘to ensure the well-being of the animal’. However, these measures per se provide no safeguards against AAGA; therefore, strategies have been proposed that attempt to reduce risk. These strategies include the following: (i) administering NMBs only once noxious stimulation demonstrably fails to elicit motor responses; (ii) (a) using an anaesthetic technique whose efficacy has been established in pilot studies on unparalysed animals or (b) using a non-NMB-based anaesthetic whose efficacy has been critically and convincingly established in previous, though not necessarily related, studies involving animals of similar breed, sex, and age, undergoing similar procedures or (c) using end-tidal anaesthetic concentration tractions shown to produce adequate anaesthesia in minimal alveolar concentration (MAC) determination studies; or (iii) peripherally allowing monitored neuromuscular block to wane to determine the presence or otherwise of spontaneous movement. With each of these strategies, the possibility that the response of an individual animal to anaesthetic agents differs from that of test animals or that the technique of delivery may change (e.g. through equipment malfunction or human error) means that methods for monitoring the depth of anaesthesia in individual animals must be available. Unfortunately, signs of anaesthetic depth in animals are poorly defined, and nociceptive responses are not always characterized by increases in arterial blood pressure and tachycardia. Training and experience of the anaesthetist may overcome these difficulties; in the 5th National Audit Project (NAPS), AAGA was less likely when senior anaesthetists were involved, and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes recommends that ‘specialist advice’ on anaesthesia should be obtained when these (NMB) agents are used.

Pigs are used extensively in biomedical research; 77 280 animals were used in the EU in 2011. A proportion of these undergo surgical procedures that are associated with postoperative pain in human patients and in which profound muscle relaxation may be beneficial. It is more likely that NMB is used to achieve this in pigs compared with other laboratory animals, because NMB is widely recommended for tracheal intubation, which is relatively difficult in this species. Two points are pertinent in considering AAGA in pigs: (i) individual pigs, like some human patients, may not show sympathetic nervous signs when paralysed and exposed to noxious stimulation; and (ii) bispectral index monitoring is unreliable in this species.

In contrast to the A(SPA) 1986, the transposition of the EU Directive 2010/63/EU into UK law makes no specific statements about the use of NMBs in laboratory animals. This, and the publication of NAPS, prompted the present literature review. The aims of the review were as follows: (i) to estimate the extent to which NMB is used in pigs undergoing experimental surgical procedures; (ii) to determine what measures were taken to ensure that pigs were unconscious; (iii) to establish the reasons why NMB was felt to be necessary; and (iv) to identify the training background of the supervising anaesthetist.

Methods

A two-stage literature review was conducted on 24 and 25 July 2014. The first stage aimed to identify the extent of NMB use and used the search engine Web of Knowledge (https://webofknowledge.com) and search criteria for articles describing pig surgery (full search queries are shown in Fig. 1) published between 2012 and 2014. Only papers available through the University of Edinburgh (25 000 journals) were used. Identified articles underwent further analysis only if they described: (i) the anaesthetic technique used; and (ii) a surgical procedure that is recognizably painful in human subjects, in other words that it has supporting citations from the medical literature or has been recognized as potentially painful in other animal species by previous reviewers or by the American College of Laboratory Animal Medicine (ACLAM) Analgesic Task Force (2007). Consequently, only articles describing skin incision, skin burning, cranio-tomies, laparotomies, laparoscopies, orthopaedics, thoracotomies, and transluminal endoscopy were examined. Studies were excluded if they: (i) described the use of fewer than three pigs; or (ii) were pharmacological assessments of NMB drugs. The materials and methods sections of the article were read (by A.G.B.) to determine whether NMB had been used or not. Articles were categorized as ‘NMB use described’ or ‘NMB use not described’. The country of origin of the article was recorded for the first-stage sample.

The second stage aimed to increase the sample size available for further analysis and used Web of Knowledge, Pubmed (http://www.ncbi.nlm.nih.gov/pubmed), and Google Scholar (http://scholar.google.co.uk). The keywords used were ‘pig’ and the names of specific NMB agents described in papers identified in the first stage. Resulting articles describing the same operations were then filtered using the same aforementioned inclusion and exclusion criteria. Articles from both stages were then combined and the methods sections examined (by A.G.B.) to identify evidence of concerns with AAGA and to record specific measures described to minimize its likelihood. Consequently, the presence (or absence) of the following were recorded: (i) the description of specific strategies (see this page, first column, paragraph 2) taken to reduce the risk of AAGA; (ii) details (drugs, doses, and dosing intervals) of the anaesthetic technique, with citations (including MAC values) supporting its efficacy; and (iii) details of the methods used to determine anaesthetic depth. Descriptions of depth of anaesthesia monitoring were recorded as being absent or present. These descriptions were examined to determine the variables used to assess depth of anaesthesia, the frequency of assessment, and the training and experience of the assessor.

The total number of pigs (mean and range) involved in articles describing NMB use was calculated.
The corresponding authors of all eligible articles were then invited by email to answer the following two questions: (i) ‘Why was neuromuscular blockade imposed in this procedure?’; and (ii) ‘What was the training background of the person responsible for the anaesthetic?’ The following five options were provided for the latter: (i) a qualified veterinary anaesthetist [i.e. a Diplomate of the European College of Veterinary Anaesthesia and Analgesia, a Diplomate of the American College of Veterinary Anaesthesiology, or a Diplomate (Veterinary Anaesthesia) of the Royal College of Veterinary Surgeons]; (ii) a qualified medical anaesthetist; (iii) a trained animal anaesthetist (veterinary nurse or animal care technician); (iv) a veterinarian or medically qualified physician without specific training in anaesthesia; or (v) ‘other’. Non-responding authors were emailed at 2 week intervals on three subsequent occasions. Corresponding authors were made aware that their anonymized answers would be used in a review of anaesthetic practices in laboratory pigs.

Justifications for the use of NMBs were categorized as ‘recognized’ or ‘novel’ on the basis of their perceived (assessed by R.E.C.) acceptance in medical or veterinary anaesthetic practice. Novel justifications were subcategorized as experiment biased, neutral, or animal biased, based on the perceived importance of...
experimental expediency over animal welfare. An attempt was made to correlate justification categories with the background of the anaesthetist.

Recorded details were entered onto a spreadsheet (Microsoft Excel; Microsoft, Redmond, WA, USA) and analysed. The results are expressed as percentages.

Results

The first literature search identified 847 potentially eligible articles. The University of Edinburgh had access to 726 of these, and a further 190 articles were excluded because they either failed to meet inclusion criteria or met the exclusion criteria (see Fig. 1 for details). Likewise, a further 125 (24%) of the remaining 536 papers were excluded because the anaesthetic technique was not described. In the 411 articles in which NMB use was detailed, 80 (20%) reported such use.

Sixty articles reporting the use of NMBs (75%) originated in Europe, with 56 (70%) from the EU. Of the remainder, 10 (13%) originated in the USA, six (8%) were from South America, and four (5%) were from North America. The second literature search identified a further 73 papers describing the use of NMBs and the anaesthetic used, giving a total of 153 of the papers available for full analysis. A total of 2661 pigs were involved in all studies describing NMB; the mean number of pigs per study was 17, and the range was 3–69. The drugs used to achieve NMB were described in 153 of the papers and were as follows: pancuronium (88), vecuronium (22), atracurium (14), rocuronium (12), cisatracurium (11), pipecuronium (3), gallamine (1), suxamethonium (1), and d-tubocurarine (1). Doses and administrative patterns were highly varied so are not detailed here.

No papers described the use of control groups of unparalysed animals before the principal study or periodically allowing monitored NMB to wane to determine the presence or otherwise of spontaneous movement.6 One article stated that NMB was administered ‘after documenting adequate depth of anaesthesia (paw [sic] pinch) to facilitate the surgical procedure (thoracotomy)’.26 A second described providing NMB for laparotomy ‘after ascertaining that anaesthesia sufficiently prevented responses (signs of awakening or withdrawal reactions) to painful stimulation between the front toes’.34 A third reported use of NMB only when withdrawal reflexes were verified as absent under anaesthesia to ensure that anaesthesia was sufficient.35

Of the 153 articles describing aspects of anaesthetic technique (jack of any detail was an exclusion criterion), dose information was recorded in 144 (94%) articles. Citations supporting the efficacy of the anaesthetic technique described were provided in 13 (9%) articles.

Of the 69 papers reporting the use of inhalation anaesthesia alone for maintenance of anaesthesia, five described the use of end-tidal concentration (FeTCO2) monitoring. Of these, two referred to desired end-tidal concentrations in terms of MAC multiples (i.e. 1.0–1.5 MAC isoflurane in an oxygen/nitrous oxide mix and ‘sevoflurane 1–1.5 MAC’), but neither indicated whether the values used were derived from human or porcine subjects. The remaining three described target end-tidal concentrations (sevoflurane 2.5%, isoflurane 0.5–1%, and isoflurane 0.8–1.2%), all of which are less than published MAC values for these agents in pigs. The term ‘awake’ was used in only one of 20 papers (5%) that described monitoring the depth of anaesthesia during NMB. Nine others used the terms ‘adequate’ or ‘satisfactory’ with respect to levels of anaesthesia or analgesia. Nine papers did not detail the variables used to indicate the depth of anaesthesia (e.g. ‘Anaesthesia was increased if any response to stimuli was observed’). Four papers used only cardiovascular indices, namely arterial blood pressure (4) heart rate (3) and the ECG (1) to monitor the depth of anaesthesia. In three articles, a combination of autonomic nervous signs, such as lacrimation, and cardiovascular signs was used in conjunction with motor responses to surgery, such as corneal reflexes or hindlimb flexion. One article reported using motor effects alone (i.e. the pedal withdrawal and palpebral reflexes, along with jaw tone) to monitor the adequacy of anaesthesia. Details were unclear or confusing in two reports: ‘during induction, the depth of anesthesia was adapted according to the heart rate and blood pressure’, and ‘adequacy of sedation and analgesia was assessed using physical examination and vital signs’. In another two articles, depth of anaesthesia was assessed using a combination of cardiovascular signs with variables that were either unrecognized or tenuously linked with anaesthetic adequacy. One explicitly referred to the use of a Datex Ohmeda S5 monitor to record MAC and the sleep threshold. In the other, ‘adequacy of anaesthesia’ was monitored by continuous ECG, pulse oximetry, and capnometry.

Human involvement in monitoring anaesthetic depth was specified in three papers, which coincidentally indicated the frequency of monitoring and the anaesthetists’ training. In the first, vital signs were taken at 30 min intervals by experienced animal care technicians. In the second, motor reflexes were evaluated using ‘standard protocol’ every 15 min. A veterinary anaesthetist subjectively assessed depth of anaesthesia throughout the procedure in the third. The continuous monitoring of signs of anaesthetic depth was also reported in a further five papers, but referred to the use of multichannel patient monitors.

Of the 20 papers providing details of the monitoring of depth of anaesthesia, three indicated the measures taken when depth of anaesthesia was judged to be inadequate. Two described fentanyl injection, one in conjunction with propofol. The third stated that, ‘When marked physical movement was observed, we additionally injected 1 ml of relaxant’.36

Monitoring of NMB using a peripheral nerve stimulator was described in one paper. Three reported the assessment of jaw tone, along with pedal withdrawal, corneal or palpebral reflexes, or both, in relation to depth of anaesthesia monitoring, not NMB. The response rate from corresponding authors was 121 of 153 (77%). These provided a total of 149 reasons for using NMB (27 respondents gave more than one justification). Of these, 113 were ‘recognized’ reasons for using NMB and are detailed in Table 1 along with supporting statements and citations. Thirty-six responses were regarded as ‘novel’. Twenty-seven of these were categorized as neutral, whereas nine were judged to be experiment biased. No respondent justified the use of NMBs in prioritizing animal benefit over experimental outcome. Novel justifications along with correspondents’ statements are detailed in Table 2.

The corresponding authors of many (47 [39%]) articles using NMB revealed that the anaesthetic had been co-supervised by personnel from more than one of the categories offered (Fig. 2). Medical and veterinary anaesthetists supervised alone in 31 and 19 instances, respectively. Veterinarians, technicians, and medical personnel without qualifications in anaesthesia supervised NMB in 16, five, and two articles, respectively. Analysis was simplified by appointing responsibility for co-supervised animals to the most qualified individual in the following rank: (i) qualified veterinary or medical anaesthetist; (ii) trained veterinary anaesthetist (nurse or animal care technician); (iii) veterinarian without specific anaesthesia training; (iv) physician without specific anaesthesia training; and (v) other. This revealed that qualified veterinary or medical anaesthetists were the predominant supervisory category, being described in 85 of 121 (70%)

Downloaded from http://bja.oxfordjournals.org/ by guest on October 6, 2016
articles, followed by veterinarians without specific anaesthesia training (28 of 121, 23%), trained veterinary anaesthetists (animal care technicians; five of 121, 4%), and physicians without specific anaesthesia training (two of 121, 2%).

Attempts to link justification categories with the anaesthetist’s background were thwarted because in most instances the corresponding authors were not responsible for the anaesthesia and so the principal motive for using NMB could not be ascertained with confidence.

**Discussion**

Although the reported use of NMB in pigs undergoing experimental surgery is not insubstantial (20%), only two of the 153 articles reviewed here reported the use of measures taken to reduce the risk of AAGA. Of 536 papers originally screened, 125 (24%) were excluded because the anaesthetic was not described. After further screening, a total of 153 papers were identified, which provided some details of anaesthesia, but 6% did not report doses, and only 13 provided citations supporting efficacy. Of 69 papers reporting the use of volatile anaesthetics alone for maintenance of anaesthesia, only five described the use of end-tidal concentration monitoring. Only 20 articles described the methods used to assess anaesthetic depth. Medical or veterinary anaesthetists were responsible for supervising NMB in 70% of instances, which was used to facilitate IPPV and improve

---

**Table 1** 'Recognized' justifications for use of neuromuscular block given by corresponding authors of articles describing pigs undergoing experimental surgery (2012–4)

<table>
<thead>
<tr>
<th>Justification</th>
<th>Number of articles using justification</th>
<th>Supporting statement from corresponding authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>To improve surgical 'visualization’</td>
<td>32</td>
<td>‘Small doses of each medication to prevent accumulation during liver failure reduce anaesthetic’</td>
</tr>
<tr>
<td>To facilitate ventilation</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>To prevent involuntary muscle contractions caused by surgery</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>To prevent shivering</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>To facilitate tracheal intubation</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>For laparoscopic surgery</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>To ‘fix’ eye position</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>To control carbon dioxide production during cardiopulmonary bypass</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>As a component of balanced anaesthesia</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** 'Novel' reasons for using neuromuscular block given by corresponding authors of articles describing pigs undergoing experimental surgery (2012–4). *Experiment-biased, animal-biased, or neutral justifications were based on the perceived prioritization of experimental expediency and animal welfare. NMBA, neuromuscular blocking agent

<table>
<thead>
<tr>
<th>Justification</th>
<th>Number of articles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>27</td>
</tr>
<tr>
<td>‘As part of standard laboratory routine’</td>
<td>16</td>
</tr>
<tr>
<td>‘To reflect medical anaesthetic practice’</td>
<td>9</td>
</tr>
<tr>
<td>‘To achieve ‘stable and easily controllable anaesthetic management’</td>
<td>1</td>
</tr>
<tr>
<td>‘Employed neuromuscular blockade for provider and animal safety’</td>
<td>1</td>
</tr>
<tr>
<td>Experiment biased*</td>
<td>9</td>
</tr>
<tr>
<td>‘To prevent interference with haemodynamic monitoring or data collection’</td>
<td>5</td>
</tr>
<tr>
<td>‘Minimize the animal interference with the experimental procedures’</td>
<td>1</td>
</tr>
<tr>
<td>‘To prevent the pig from biting the endoscope’</td>
<td>1</td>
</tr>
<tr>
<td>‘Major surgery requires NMBA’</td>
<td>5</td>
</tr>
<tr>
<td>‘Pigs suddenly (unexpectedly) recover from a depthless anaesthesia, and pain also will cause unexpected movement. Even a subtle movement will disturb delicate surgical procedures, including microsurgery’</td>
<td>1</td>
</tr>
<tr>
<td>'To control carbon dioxide production during cardiopulmonary bypass'</td>
<td>2</td>
</tr>
<tr>
<td>'As a component of balanced anaesthesia'</td>
<td>2</td>
</tr>
</tbody>
</table>

**Fig 2** The distribution of responsibility for supervision of anaesthesia among medical and veterinary anaesthetists, veterinarians, medical physicians, and technicians, in 120 articles describing experimental pig surgery. Rounded rectangles indicate the training category and number of articles ascribing sole supervisory responsibility to that category. Circles, triangles, and a non-rounded rectangle indicate the division of responsibility among two, three, and four categories, respectively.
operating conditions. Inadequately detailed descriptions of anaesthetic technique, including the assessment of depth of anaesthesia, have major and potentially stark implications for study reproducibility and animal welfare.

Most (75%) articles from the first stage originated in the EU, which may reflect the ambivalence of precautionary statements in Directive 2010/63/EU (Article 14.3). This states: ‘Member States shall ensure that animals are not given any drug to stop or restrict their showing pain without an adequate level of anaesthesia or analgesia’.23 Instructions from other authorities are more explicit. The A(SP)A 198612 amendment 2012, Section 173 stipulates that the use of NMB requires additional licensing for both the researchers and the project, and is contingent on there being ‘appropriate anaesthesia or analgesia’ in accordance with the project licence. Brazilian law 11794/2008, Article 14, §7, forbids the use of neuromuscular blocking agents or muscle-relaxing agents as substitutes for sedation, analgesia, or anaesthesia. However, ambiguity prevails in the USA. The National Institutes of Health12 guidelines stipulate that ‘if paralyzing agents are to be used, the appropriate amount of anaesthetic should first be defined on the basis of results of a similar procedure using the anaesthetic without a blocking agent’. In contrast, the Institute of Laboratory Animal Resources requires only that the proposed use of NMBs be carefully evaluated by the IACUC.13

Only three articles described measures aimed specifically at reducing the risk of AAGA, by taking the absence of pedal withdrawal reflexes in response to ‘pinching’ to signal the animals’ suitability to be paralysed and undergo thoracotomy or laparotomy. This is worrying because it indicates that the majority of articles—for whatever reason—did not describe any measures to ensure unconsciousness. Furthermore, it reveals that thoracotomy and laparotomy operations were regarded as similarly noxious to the pinching of interdigital skin. The absence of description in the remaining papers may reflect genuine oversight, a disregard of the need for detail, or may result from editorial processing. In any event, it presents a disregard of recommendations pertaining to NMB use in experimental animals (see below).

Anaesthetic techniques were described in all reviewed papers, but this was a preselected inclusion criterion and therefore does not reflect an appreciation that adequate anaesthesia is a prerequisite for NMB. However, although 144 (94%) of reviewed papers provided dose information, only 13 cited evidence for the effectiveness of the anaesthetic. This is all the more worrying given the sparse descriptions of monitoring anaesthetic depth. Regulations regarding the provision of adequate anaesthesia when NMB are used are widespread and explicit. Under the A(SP)A 1986,12 project applications must provide details of the anaesthetic and analgesic or decerebration regimens, or both, to show that they are sufficient to ensure the welfare of the animal or to justify why such methods are not required. Similar requirements are required in the Australian guidelines.16 Many authorities require evidence of this, showing that the efficacy of selected anaesthetics be: (i) first established (A(SP)A 1986),12 if necessary, in pilot studies of animals of the same species undergoing similar procedures without NMB15 and (ii) confirmed in the main study by allowing NMB to wane periodically in order to allow identification of voluntary motor responses to noxious stimulation.5 Despite the feasibility of monitoring neuromuscular transmission in pigs—a prerequisite for the second recommendation—technical descriptions of NMB monitoring were found in only one article.

Variation in reported values of MAC in pigs, which range from 1.32 to 2.04% for isoflurane and from 3.5 to 4.44% for sevoflurane, probably result from the use of a range of analysis methods.42 Nevertheless, minimal values are still marginally greater than those calculated for 30-yr-old human beings23 (i.e. 1.29 and 2.00% for isoflurane and sevoflurane, respectively). This indicates that pigs are likely to require greater end-tidal concentrations to ensure immobility than humans exposed to equally noxious stimulation. Therefore, it is worrying that the use of other anaesthetics with MAC-sparing activities was not mentioned in two papers describing the maintenance of anaesthesia with end-tidal isoflurane concentrations of 0.5–1.046 or 0.8–1.2%.47 A further two articles described the delivery of MAC multiple values (‘sevoflurane 1–1.5 MAC’ and ‘1.0–1.5 minimum alveolar concentration (MAC) of isoflurane in an oxygen/nitrous oxide mix’ but failed to disclose whether values were derived from human or porcine studies. When the former values are used then the resistance of pigs to inhalation anaesthetics increases the likelihood of AAGA. The NAP—published after the articles reviewed here—recommends that end-tidal volatile agent monitoring be used in conjunction with nerve stimulation when NMB is imposed during inhalation anaesthesia ‘to minimise the risk of any period of neuromuscular blockade without anaesthesia’. In the present review, only five of 69 papers using inhalation agents describe end-tidal anaesthetic monitoring, indicating considerable scope for improvement.

Details of the methods used to monitor anaesthetic depth are requirements for study approval under A(SP)A 198612 and the US Ag Guide.46 The finding that 133 articles in the present review did not describe such methods is less worrying than the number which did, because several of the latter provoke serious concerns regarding the competency of the anaesthetist and its implications for animal welfare. For example, four papers described the use of somatic motor activity (pedal withdrawal, palpebral and corneal reflexes, and jaw tone) to ensure adequate depth of anaesthesia during NMB. This is alarming because not only are these reflexes absent during profound block but the presence of pedal withdrawal and palpebral reflexes in the absence of NMB would indicate inadequate levels of surgical anaesthesia; the pedal withdrawal reflex should be absent in pigs undergoing major surgery.59 Three articles did not indicate whether the ‘anaesthetist’ reacted to the presence of cranial nerve or pedal withdrawal reflexes by administering more NMB agent or anaesthetic (nor was the query raised in the review’s follow-up component). However, one article stated explicitly that ‘When marked physical movement was seen, further relaxant was administered’.56 Two of the 20 papers detailing the monitoring of depth of anaesthesia described the use of fentanyl (one in conjunction with propofol) when anaesthesia was judged inadequate. Neither of these was amongst the four using motor reflexes to assess anaesthetic depth during NMB. Unfortunately, the fentanyl doses conventionally used in dogs and humans have minimal MAC-sparing effects in pigs.50

Analysis of the physiological variables used to assess anaesthetic depth in the 20 articles providing such information suggests that in general, AAGA in laboratory pigs can be identified by increased sympathetic nervous activity, a belief promoted by the A(SP)A 198612 and Australian16 guidelines, which require continuous monitoring of heart rate or blood pressure, or both, and pupil size, so as to indicate any lightening of the level of anaesthesia. However, such indicators are inconsistent in paralysed humans,19 and inadequate anaesthesia does not consistently affect these variables.5 51 52 The same appears to be true in pigs. In one study involving the electrocution of 24 pigs immobilized with succinylcholine,20 three died immediately, whereas 14 developed ventricular tachycardia. However, two animals developed sinus arrest with ventricular bradycardia, and sinus bradycardia was
seen in five pigs, two of which terminated in ventricular standstill. Although the unexpected bradycardia produced by the NMB, the possibility that the arrhythmias resulted from AAGA cannot be dismissed. Referring to human patients, NAP5 recommends that specific depth of anaesthesia monitoring be used when end-tidal volatile agent monitoring is absent or irrelevant (e.g. during total i.v. anaesthesia). Unfortunately, the bispectral index shows unconvincing ability to reflect depth of anaesthesia adequately in pigs.43-45 Raw and processed EEG indices are likewise unreliable; one study found that nociceptinsimultaneously increased blood pressure, but not spectral edge frequency 95%, median frequency, wź, θ/β or θ/β ratios, total power, or suppression ratio in isoflurane-anaesthetized pigs.52 In a subsequent study, similar stimuli caused movement in a group of pigs, even when burst suppression was present.37

The limited information available in eight papers with respect to who carried out assessment of the depth of anaesthesia and at what intervals does little to assure that the prevention of AAGA was prioritized. Only one article described the continuous monitoring of depth of anaesthesia; in two others, assessment was made at 15 and 30 min intervals. The continuous monitoring of depth of anaesthesia signs using multichannel patient monitors was reported in five papers, but only one referred (ambiguously) to the measurement of the ‘sleep threshold’. No papers reported the use of bispectral index monitoring, which may indicate either a widespread appreciation of the shortcomings of the device in pigs or a low level of concern with AAGA. The A(SPA) 198612 is unequivocal on the degree of attention required by paralysed animals; [these] ‘should be attended by a person competent to administer NMBAs and monitor anaesthesia, pain and distress, and analgesia effects’. Where surgical intervention is involved, a second person must be present at all times to ensure the maintenance of anaesthesia.

The reasons given for using NMB were largely uncontroversial, with its benefits in facilitating IPPV and tracheal intubation, improving surgical ‘visualization’, and ‘fixing’ the globe during ocular surgery being widely recognized. Justification of the use of NMB to ‘reflect medical anaesthetic practice’ is arguably defensible providing the treatment of the porcine model extends to other aspects of care, particularly the provision of analgesia. This may not be the case.58 The use of NMB ‘as standard laboratory routine’ seems less justifiable in light of the apparent indifference to the risk of AAGA revealed by the present review. The finding that NMB was used in 21 articles to prevent involuntary muscle contractions caused by the procedure or in another 13 to prevent shivering is worrying, because although these may occur as unconscious reactions to certain procedures, both may also be responses to noxious stimulation under inadequate anaesthesia.54 Equally worrying are comments indicating the prioritization of study objectives over animal welfare (e.g. ‘to prevent animal interference with experimental procedure’ or ‘to prevent pigs biting the endoscope’). The implications of one respondent’s statement, ‘pain can cause movement’, are of serious concern in terms of animal welfare and the adequacy of anaesthesia training. The ‘anaesthetic-sparing’ effect of NMB, which were used to justify its imposition in one paper, have not been demonstrated in pigs.23 The US Ag guide48 and A(SPA) 198612 require that the proposed use of NMBs be justified to the IACUC and in the animal use protocol, respectively. Marsh and Studer55 were less compromising, opining that manuscripts failing to justify NMB use convincingly in the methods section should be rejected and that the only valid indications for NMB in laboratory animals are investigations of neuromuscular blocking agents themselves or the suppression of electromyographic activity during neurophysiological recording.

Given the apparent widespread failure to prioritize the prevention of AAGA, the fact that medical or veterinary anaesthetists had shared or sole responsibility for anaesthesia in 70% of articles raises serious concerns for their clinical competency, commitment to animal welfare, or ability to report experimental methods accurately. The finding that personnel without any qualifications in anaesthesia were allowed to supervise NMB in 23 articles provokes serious doubts about the understanding of those authorizing or monitoring the conduct of animal experiments, or both, including Animal Welfare and Ethical Review Boards and IACUCs. Supervision by unqualified individuals also runs counter to recommendations (e.g. Hall and colleagues),9 which proposed that the anaesthetics used in animals that are to be paralysed should be those with which the anaesthetist is familiar without NMB and whose pharmacokinetic profile is known. Regulatory bodies make similar recommendations concerning familiarity and competence. The A(SPA) 198612 requires that ‘Applicants who wish to use NMBs in their project will have to show evidence of competence of staff or how such competence will be obtained’, whereas Canadian guidelines state, ‘It is extremely important that proper equipment and personnel with experience in the use of these agents be available’.50 Whether these criteria were met by the qualified anaesthetists (medical and veterinary) who predominated as the group responsible for NMB management in the articles surveyed or indeed, anaesthetists from other backgrounds, was not determined in the present review.

It is possible that the details analysed here differed from what genuinely took place and have spuriously created an over pessimistic view. In the follow-up phase, corresponding authors rather than those responsible for the anaesthetic were consulted, so the activities of the latter may have been misrepresented. No attempt was made to determine the involvement of corresponding authors with either the genuine or the reported details of anaesthetic management. However, in a previous study investigating analgesic use in laboratory rodents using a similar two-stage (structured review and questionnaire-based follow-up) process, differences between reported and real practice were considered to be modest,24 which confers worrying implications for the findings of the present review.

The cumbersome two-stage screening process used was necessary because the sensitivity and specificity of the available search engines differ markedly. PubMed showed low sensitivity and specificity, and the inconsistent use of MeSH terms in articles rendered its use impractical in the second stage. Its search algorithm also showed low sensitivity to articles describing surgery in pigs, making it difficult to obtain a convincingly representative sample. Google Scholar showed very low specificity even with detailed search criteria, but proved useful in identifying articles missed in previous searches. In the second stage, keywords were restricted to ‘pig’ and the name of the NMB identified in the first stage. This proved necessary because use of further terms critically weakened the specificity of the search. Use of the search engines to locate terms used once in the methods section was of limited effectiveness because of the prioritization algorithms for the results. Future studies adopting the methods used in the present review might consider a database search to facilitate this.

Using Web of Knowledge alone to estimate the extent of NMB use may have introduced selection bias, but is defended for aforementioned reasons. Although further bias13 probably arose from reviewing only articles available through the University of
Edinburgh libraries (which was necessitated by limited funding), the benefits of a broader search are questionable, in that the present review already reveals considerable cause for concern. A less biased review creating a more optimistic impression would not obviate the likelihood that some pigs undergoing experimental surgery are conscious. That this be changed by creating a pessimistic, though less accurate picture is arguably justifiable on ethical grounds.

The ARRIVE guidelines (2010)\(^{62}\) ascribe responsibility for ensuring that published articles involving animal experiments provide adequate information for the ‘research community’. This responsibility falls on subscribing journal editors and reviewers\(^{63-65}\) in addition to the authors, because editorial policy has a strong positive effect on animal care standards.\(^{62}\) Unfortunately, the ARRIVE guidelines may be at odds with editorial policies concerning article length. One corresponding author in the present review reported that the details of anaesthetic management, including NMB monitoring, that they had submitted had been dismissed by reviewers as ‘irrelevant’.

Publication of full details as supplementary data online or abolition of space restrictions on the methods section of research articles,\(^{66,67}\) as Nature has done, would enable full details of NMB to be recorded.

The publication of details pertaining to ethical review permissions is a requirement of both ARRIVE\(^{62}\) and the Committee on Publication Ethics\(^{67}\) and further indicates that Animal Welfare and Ethical Review Boards or IACUCs must assume some responsibility for the appropriate use of NMB in experimental animals.

The majority of papers describing NMB failed to provide the details necessary to determine whether the animals involved were unanesthetized, and in general, the reported use of NMB in experimental pigs creates the inescapable impression that concerns with AAGA were not prioritized. Despite the adverse consequences of AAGA being widely appreciated, 23 of the reviewed articles described the supervision of anaesthetics by unassisted non-anaesthetists. Perhaps of greater concern is the finding that trained medical or veterinary anaesthetists supervised an anaesthesia in the remainder of studies. The stark implication of the present review is that AAGA in pigs undergoing experimental surgery may be widespread. This indicates serious failures in the process that begins with a study’s ethical review and ends with its publication.

**Authors’ contributions**

Data collection and analysis; writing first draft: A.G.B. Study design and data analysis, including graphical data representation; critical revision of paper for intellectual content: R.E.C.

**Supplementary material**

Supplementary material is available at British Journal of Anaesthesia online.

**Acknowledgements**

We thank Fiona Brown of the University of Edinburgh Library Service for her assistance in the review process.

**Declaration of interest**

None declared.

**References**

13. Institute of Laboratory Animal Resources, Committee on Care and Use of Laboratory Animals, National Institutes of Health, Division of Research Resources. Guide for the Care and use of Laboratory Animals. Washington DC: National Academies, 1985
60. Olfert ED, Cross BM, McWilliam AA. Guide to the Care and use of Experimental Animals. Ottawa: Canadian Council on Animal Care, 1993
63. Festing MFW, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J 2002; 43: 244–58

Handling editor: H. F. Galley