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A novel method for in situ fixation of whale brains

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Abstract

A new method of in situ formalin fixation was used on 38 brains from minke whales (*Balaenoptera acutorostrata*). The method was developed because traditional ways of fixing brains are poorly suited to the collection of whale brains. The whole brain was preserved uncut in its meninges and then excised undamaged from the skull at a later opportunity. There was no handling of the brain in the fresh state. Fixation was started within a couple of hours post mortem. All brains were subjected to gross and light microscopy examination. The results showed that both the gross and microscopic architecture of the brains were adequately preserved, with no massive gross or histological changes due to insufficient fixation apparent. The occurrence of fixation artifacts was low. Microscopic examination showed well-preserved cells and myelin in all parts of the brain. We report the mean fixed weight of the minke whale brain as 2741 g, which is the lowest among the baleen whales. The cerebellum constituted 22% of the total brain weight, which conforms to findings in other baleen whales. This in situ method can probably be used without any particular modifications in other whale species and also in large terrestrial mammals.

Keywords: Central nervous system; Minke whale; *Balaenoptera acutorostrata*; *Mysticeti*; *Cetacea*

1. Introduction

Most cetaceans are inaccessible creatures and whale brains available for examination have always been difficult to obtain. Consequently, there are many gaps in our knowledge of their central nervous system, mainly because of the difficulty in obtaining specimens in a suitable state for histological examination. The literature on the cetacean brain is based largely on observations from a single or very few and often poorly fixed specimens, predominately from toothed whales (suborder *Odontoceti*) and dolphins in particular (Oelschläger and Oelschläger, 2001). Over the years, some sound data have accumulated on gross, histological and ultrastructural features of the brain of a few dolphin species and perfusion fixation of brains has been performed both on stranded animals and in experimental studies (Morgane and Jacobs, 1972; Glezer and Morgane, 1990; Glezer et al., 1990). When considering the larger whales and baleen whales (suborder *Mysticeti*) in particular, the significant logistical and technical problems in obtaining adequately fixed brains have definitely limited the progress in our understanding of their central nervous system. Some scattered data do exist and sampling has almost exclusively been carried out in connection with hunting operations (Ries and Langworthy, 1937; Jansen, 1950; Breathnach, 1955; Jacobs and Jensen, 1964; Pilleri, 1966a,b,c; Pfeiffer and Kinkead, 1990). In addition to the scarcity of available material, the constant recurring problem has been preserving the brains of large baleen whales in such a state that detailed examination could be performed. When brains were fixed shipboard during hunting operations, the time span from death to immersion fixation could on many occasions exceed 20 h (Jacobs and Jensen, 1964), easily rendering the brain in a poor condition prior to immersion. Jansen (1953) tried to fix four brains from fin whales (*Balaenoptera physalus*) by immersing the brains in formalin whilst enclosed in the immediately surrounding parts of the skull. To facilitate the penetration of fixative, he drilled some holes in the bones over the convexity of the hemispheres. The brains were adequate for gross morphological studies, but were apparently not satisfactorily fixed for histological ex-

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amination. This technique was also used on brains from fin and blue whale (*B. musculus*) fetuses (Jansen and Korneliussen, 1977).

In the traditional Norwegian hunt of minke whales (*B. acutorostrata*) ≈500 animals are annually harvested from the Northeast Atlantic stock that was estimated at ≈112,000 individuals by the Scientific Committee of the International Whaling Commission (IWC, 1996). The minke whale belongs to the baleen whales and is the smallest of the rorquals. In the North Atlantic, it may reach a length of 10 m and a weight of ≈8500 kg (Jonsgård, 1992). The head constitutes about a quarter of the body length. In an average sized minke whale (7 m), the brain is ≈20 cm long, 20 cm wide and 15 cm in height (Knudsen et al., 1999). The hunt is carried out from small specially licensed fishing boats, which range from 15 to 40 m in length (average 20.3 m) and are rigged and equipped for minke whaling in the hunting season. The boats carry harpoon guns and use grenades with an explosive charge of penthrite to kill the whales (Øen, 1995a). According to Øen (2001), 78% loose consciousness or die instantaneously and most of the remaining animals are dead within a few minutes. The dead whale is hauled transversely across the deck with the tail out along a pole with a pulley block and tackle and with the head usually hanging out over the other side. The whale is bled and butchered (flensed) and the meat and blubber are stored in ice in the hold before being brought back to port for further processing for the market.

During a scientific program on marine mammals in Norway in 1991–1993, Øen conducted a study (Øen, 1993, 1994, 1995b) to evaluate the effectiveness of the killing methods for minke whales. He carried out several post mortem examinations and also tried to excise fresh brains for further studies. The limited space on board did not allow any excision to take place before the flensing was finished, the head separated from the body and moved to a place away from other activities. The time from death to necropsy could take >24 h and the risk of damage and incipient decomposition of the brain were considerable. The excision itself was also a very time consuming operation, even with appropriate tools. The soft brain had to be dissected out with great care, otherwise the brain ruptured under its own weight. This agrees with the experience of others who have tried to excise fresh brains from baleen whales (Guldberg, 1885; Jansen, 1952). The use of a circular saw and chemicals under additional adverse conditions, such as time constraints, on a small platform and with the boat rocking in the swells, compounded the difficulties of the procedure.

In situ preservation would improve and simplify the sampling and allow excision and collection of the brains at a convenient opportunity, independent of weather conditions and other factors. Øen (1995b) performed a pilot study on one brain during scientific whaling in 1992 and on three brains during scientific whaling in 1993. A triangular opening was made with a circular saw in the skulls and formalin was poured into the brain compartments. Fixative was frequently refilled and after 36 h (one brain), 48 h (two brains) and 60 h (one brain), the brains were removed as a whole and transferred to containers with 4% formalin solution. The brain with the shortest in situ fixation period partly fell apart due to poor hardening and those fixed in situ for 48 h were still quite soft and had to be dissected out with great care. The final brain, which had stayed in the skull for 60 h, was firm and was removed without being damaged. All brains were described as excellently fixed during histological examination some months later (Øen and Mørk, 1999). As these results were very promising, it was decided to continue developing and evaluating the method during a larger and more extensive study on blast induced injuries caused by the detonation of the penthrite grenade used in the Norwegian minke whale hunt. This article describes and analyses this in situ fixation method used for sampling minke whale brains on two boats during the traditional hunt in 1998 and 1999. The weights of the fixed brains are reported and we present, to our knowledge, the first histological preparations from the central nervous system of minke whales.

2. Material and methods

In this study, brains were sampled from 38 minke whales of both genders. Ten brains were sampled during the traditional hunt in 1998 and 28 in 1999. The fixative comprised of 8% neutral formalin solution made from 2 l of 36% (w/w) formaldehyde in 9 l of water, with 80 g NaCl and 480 g hexamethylenetetramine. Due to limited space and supplies of fresh water on board, the fixative usually had to be mixed for each whale using seawater instead of fresh water. When seawater was used, NaCl was not added to the solution.

To prepare and open the skull, sharp painter scrapes, a circular bone saw with an adjustable blade, chisels and hammers were needed. The use of chemicals and saw on a very unstable working platform and the risk of spatters of bone splints, necessitated that the operator wore protective glasses, gasmask, chemical resistant industrial gloves, heavy oilskins and strong boots.

During regular hunting, flensing of a minke whale normally takes 1–2 h and in the current study the fixation of the brains started, in most cases, some 1–3 h post mortem. After the flensing, the head was parted from the body at the atlantooccipital joint. The mandible was cut free and the skull moved to the quarterdeck and placed resting on the baleens (Fig. 1A). Most of the blubber and meat were removed from the skull roof at

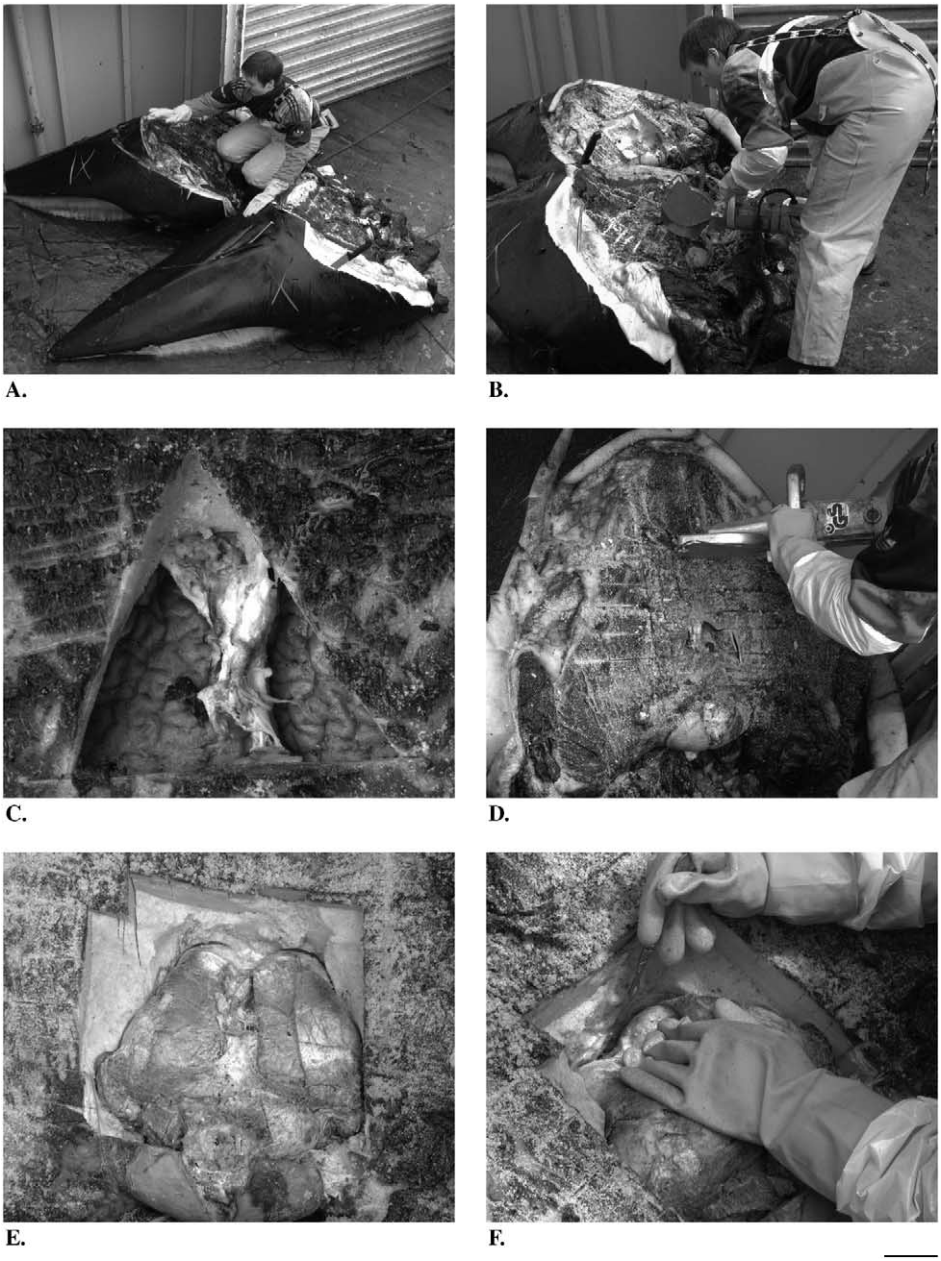

Fig. 1. In situ fixation of minke whale brains. Scale bar for A ≅ 25 cm; B ≅ 21 cm; C ≅ 2.4 cm; D ≅ 10 cm; E ≅ 5.1 cm; F ≅ 4.7 cm.

flensing, but the remains had to be scraped off to expose the bones. With the saw, three 10–15 cm long incisions were made in the skull bones over the convexity of the cerebral hemispheres ≈15 cm above the atlantooccipital joint (Fig. 1B). The cuts formed a triangle with its base close to the foramen magnum and two cuts running rostromedially with the apex in the midline (Fig. 1C). Based on experience from the pilot study (Øen, 1995b), the cutting depth of the blade of the circular saw was adjusted according to the thickness of the skull bones to avoid damaging the underlying brain tissue. The triangular bone piece was removed with the chisel and dura was opened with a knife to reveal parts of the cerebral hemispheres (Fig. 1C). Fixative was carefully poured into the epidural and subdural space until it leaked out through foramen magnum. The bone piece was replaced to protect the brain and reduce the loss of fixative, whilst the foramen magnum was plugged with a piece of soft paper or cotton rag. The fixative level was checked and, if necessary, refilled every 4 h. The position of the triangular hole and the length of the incisions affected the amount of leakage. If the hole was too caudal or rostral, too large, or if one of the cuts was too long, leakage of fixative increased considerably due to a more vertical position of the hole or due to loss at the cross-points of the incisions.

The pilot study (Øen, 1995b) had indicated that the brains had to be fixed in situ for at least 60 h to prevent

damage. In the present study, the in situ fixation period was set to 72 h to ensure that even the largest brains were sufficiently fixed prior to excision. To fully open the skull, several longitudinal and transversal cuts were made with the saw over the whole dorsal skull surface (Fig. 1D), forming almost a squared pattern of bone pieces of $\approx 4 \times 4$ cm. The bone pieces were carefully worked loose one by one with the chisel before the brain could be excised from its compartment (Fig. 1E). At excision, the dura of the cerebral frontal lobes was detached from the periost using the chisel, the optic nerves were cut with a scalpel in front of the chiasma and the cerebellum was loosened in its dura by cutting through the underlying epidural rete mirabile close to the periost (Fig. 1F). The lateral and ventral part of cerebrum enclosed in dura was carefully loosened from the periost using the fingers. The cranial nerves were cut with a scalpel before the whole brain and first segment of the spinal cord, with meninges, was lifted out, visually inspected and immersed in 8% formalin solution in large (30 l) containers.

The brains were stored in formalin for at least 2 months. Prior to further examination, the brains were washed in running fresh water overnight to reduce the formalin vapour. All brains were weighed and length and width measurements were collected from the last ten brains that were analysed. After gross external examination, the cerebrum was cut into 1 cm thick coronal slices, while the brainstem and first portion of the cervical spinal cord were sliced into 5 mm thick disks. In total, ≈ 40 specimens for histological examination were collected from the same anatomical sites in all brains: two tissue blocks from dura; 14 from cerebrum; three from cerebellum; and the whole brainstem and first portion of cervical spinal cord (20–25 tissue blocks). The blocks were embedded in paraffin wax, sectioned at 5 μm with a microtome and stained with hematoxylin/eosin (H&E) using standard protocols (Culling et al., 1985). Preparations from the brainstem and spinal cord from the ten brains sampled in 1998 were also stained with luxol fast blue-Cresyl violet (LFB-CV) (Culling et al., 1985). The quality of the fixation was evaluated by examining the gross appearance of the tissue (texture, colour, occurrence of artifacts) and by light microscopy to assess the quality and intensity of staining with both H&E and LFB-CV. Photomicrographs of the micro slides were taken using a Zeiss Axioscop (Carl Zeiss Vision GmbH, Hallbergmoos, Germany), a Sony Power HAD Camera Adapter (Sony Corporation, Tokyo, Japan) and Matrox Imaging Intellicam (Matrox Electronic System Ltd., Dorval, Canada). Adobe Photoshop 6.0 (Adobe System Inc., San Jose) and Imaging for Windows® (Eastman Software Inc./Kodak, Billerica) were used for digital processing of all illustrations (adjusting of contrast, brightness and sharpness).

3. Results

During gross examination, the brain surface was generally found to be a homogenous light grey/brown colour. In some brains though, hypostasis was noted in the vessels ventrally, which gave the surface a darker appearance. In the cut-up brain and brainstem, the texture was generally firm and the slice surfaces were dry. There were no cases where the brain tissue was liquefied or smelled sour due to post mortem bacterial growth and the occurrence of artifacts and autolytic changes due to incomplete fixation was generally low. The overall results showed that the gross and microscopic architecture in all brains was adequately preserved and no massive gross or histological changes in the tissue due to insufficient fixation were observed. Table 1 gives an overview of the fixation quality in different brain areas after gross and microscopic examination. 'Excellent' refers to brain areas or specimens without autolytic changes. 'Good' fixation refers to the occurrence of mild autolytic changes in the actual brain area, but to such a low degree that it would not affect on any anatomical or pathological evaluation of the material. 'Acceptable' refers to specimens where the fixation was not optimal, but the tissue could still be analysed using traditional methods. In Table 1, histological evaluation of the cerebellum, cerebrum, brainstem and spinal cord have been summarized into 'CBL Micro', 'CBR Micro' and 'BS/SC Micro', respectively.

A distinct pink coloration appeared on the slice surface at gross examination in 30 of the brains. It was observed both in white and grey matter, most distinct in the tissue adjacent to the ventricular system, but it disappeared after a few minutes of air exposure. In all these cases, the smell of formalin vapour was very strong. It was noted that the pink coloration was much weaker when the brains had been washed for more than 1 day in fresh water prior to examination. Also a dark, bluish discoloration on the cerebellar surface was observed in some cases at gross examination. However,

Table 1
Classification and evaluation of the quality of fixation of tissue after gross and microscopic (micro) examination of minke whale brains fixed in situ

Material	Fixation			Total
	Excellent (%)	Good (%)	Acceptable (%)	
Gross BS/SC	31 (81.6)	6 (15.8)	1 (2.6)	38
Micro BS/SC	24 (63.2)	14 (36.8)	0	38
Gross CBL	27 (71.1)	10 (26.3)	1 (2.6)	38
Micro CBL	30 (78.6)	7 (18.4)	1 (2.6)	38
Gross CBR	20 (52.6)	16 (42.1)	2 (5.3)	38
Micro CBR	17 (44.7)	20 (52.6)	1 (2.7)	38

Abbreviations: BS, brain stem; SC, spinal cord; CBL, cerebellum; CBR, cerebrum.

the histological examination of the cerebellar cortex did not reveal any dissolution of the granular cell layer. Microscopic examination showed generally well-preserved tissue (perikarya, glia fibretracts, ependyma, endothelia) in all parts of the brain (Fig. 2A–F) and the quality and intensity of staining with both H&E and LFB-CV were entirely satisfactory. The larger neurons were recognizable by their prominent nucleoli and spots of Nissl substance in the cytoplasm (Fig. 2E,F), indicating well-preserved and well-stained material.

So-called 'Swiss cheese' artifacts were observed at gross examination in restricted areas in 20 brains. In all cases, these changes were very mild, the vacuoles were few (one to five) and small (1–3 mm). They were located

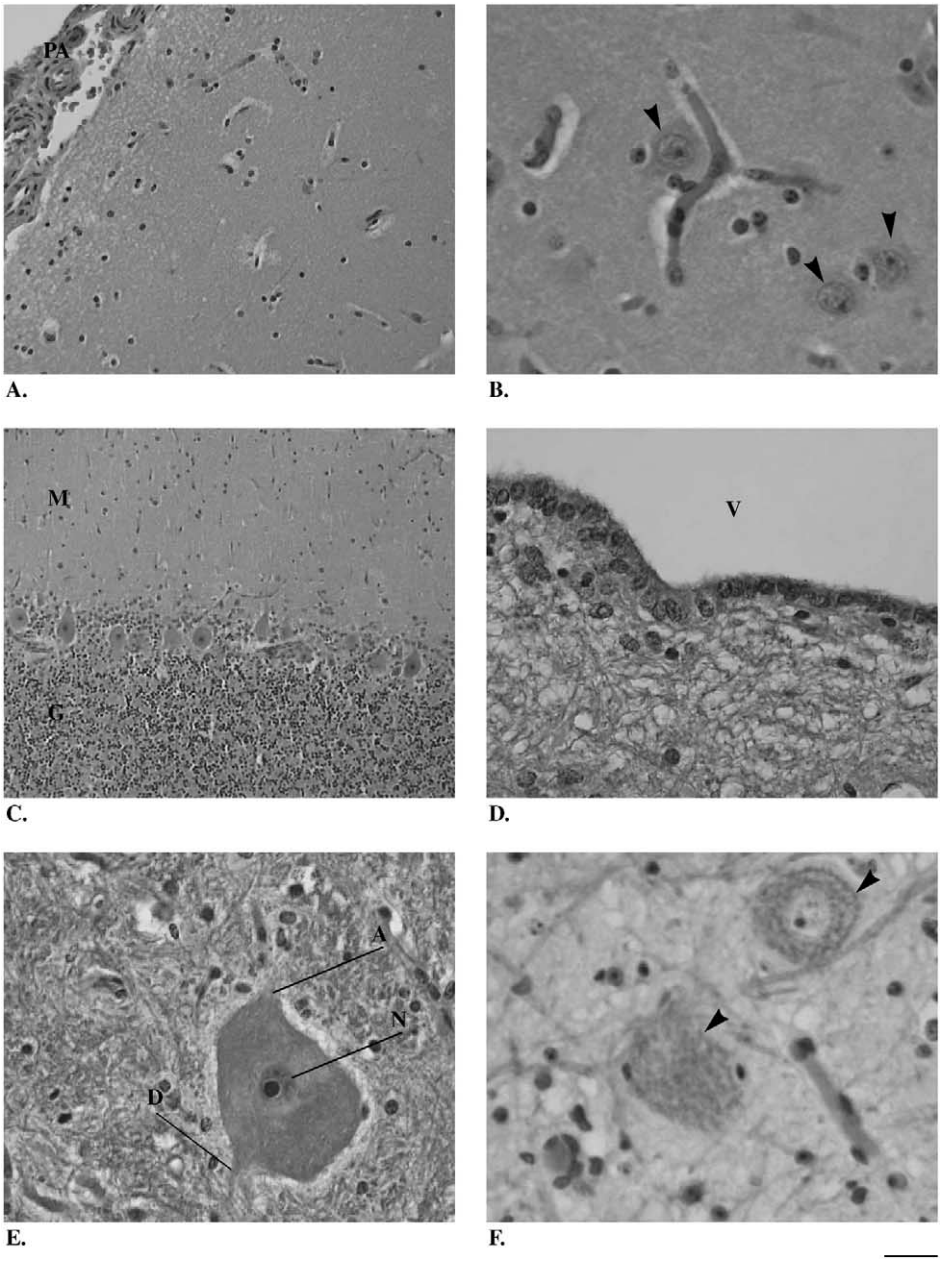

Fig. 2. Montage of microphotographs taken of different brain areas in the minke whale brain visualised using H&E or LFB-CV staining techniques. (A) The outer cerebral cortex (layer I) contains few cell bodies. PA, Pia-Arachnoids (frontal lobe, H&E). (B) Capillaries, neurons (arrowheads) and glial cells in the frontal cerebral cortex (H&E). (C) The cerebellar cortex has all the characteristic mammalian features consisting of the molecular layer (M), the granular layer (G) and the large Purkinje neurons in between (H&E). (D) Ependyma of the fourth ventricle (V) forms a ciliated one-cell thick lining of the ventricular system (upper medulla oblongata, H&E). (E) A giant pyramidal cell in the medulla oblongata. A, axon hillock; D, dendrite; N, nucleus (H&E). (F) Nissl granules are evident in motor neuron perikarya (arrowheads). Notice the clear visualization of myelinated axons shown by the myelin stain (Medulla oblongata, LFB-CV). Scale bar for A = 135 μm; B = 50 μm; C = 86 μm; D = 71 μm; E = 286 μm; F = 200 μm.

in the most central parts of the brain, especially in the thalamus, upper brain stem or the cerebellar vermis. In some of these brains, the affected tissue was slightly softer than normal. During microscopic examination, 'Swiss cheese' artifacts were discovered in two more brains (Fig. 3A) and white matter vacuolation was revealed in the most central parts of some brains, especially in the cerebellar vermis and thalamus (Fig. 3B). These vacuoles, small blood vessels and the adjacent tissue occasionally contained bacteria.

In 23 of the brains, minor lesions (wounds) caused by the saw were apparent on the top of both cerebral hemispheres. In almost all of these cases, the cuts formed a triangle. In nine brains, very shallow (<5 mm) and small (1–3 cm) cuts were noted on one of the hemispheres. Fourteen brains showed signs of compression on the part that had been resting on the bottom of the container. Although no histological changes that could be attributed to the compression were seen, gross examination was made somewhat more difficult.

During the collection of the brains, we noted that the dorsal part of the skull bones in the minke whales vary considerably in thickness and structure. Caudally, the skull consists of relatively thin solid bone (usually <1 cm), while rostrally, it is 5–15 cm thick with a fatty and porous character. Further, we noted that the dura mater is thick (2–3 mm) and runs deep into the longitudinal fissure (the falx cerebri) separating the two hemispheres (Fig. 1C). The general appearance of the brain of the minke whale is shown in Fig. 4. The mean weights of the fixed brains are presented in Table 2. The mean anterior–posterior diameter from the cranial pole of the cerebral hemispheres to the caudal pole of the cerebellum was 20.9 cm (S.D.: 2.1; range: 19–25 cm; $n=10$) and the mean transverse diameter of the cerebral hemispheres was 23.5 cm (S.D.: 1.6; range: 22–27 cm; $n=10$). The mean body length of the animals was 732 cm (95% CI: 679–785 cm; range: 420–870 cm).

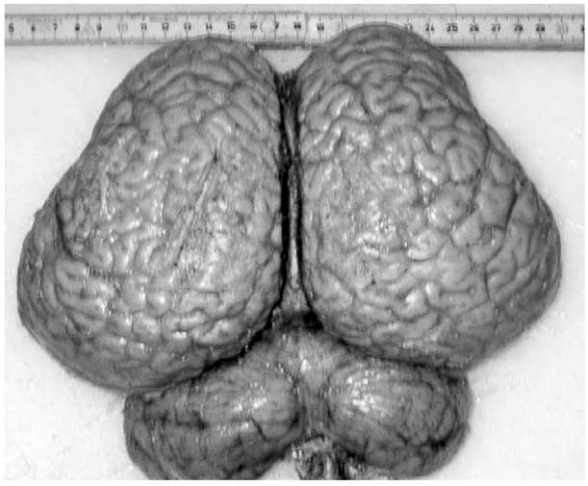

Fig. 4. Dorsal view of the minke whale brain with intact pia mater. Scale bar = 4.7 cm.

4. Discussion

The brains of cetaceans have intrigued scientists for more than a century. Having adapted to an aquatic way of life, whales are quite valuable for comparative anatomical studies. Due to inaccessibility, there are very few studies on the central nervous system of the baleen whale and traditional methods used for excision and fixation have proven to be poorly suited to the collection of brains from these very large species. The attempt to excise whole fresh brains from minke whales by Øen (1995b) was unsuccessful, which is concurrent with the experience of Jansen (1952), who attempted the same procedure on fin whales. A method of preservation prior to excision of the brains therefore had to be developed if more advanced studies of the central nervous system of the larger whale species were to be carried out. The results from this study using in situ

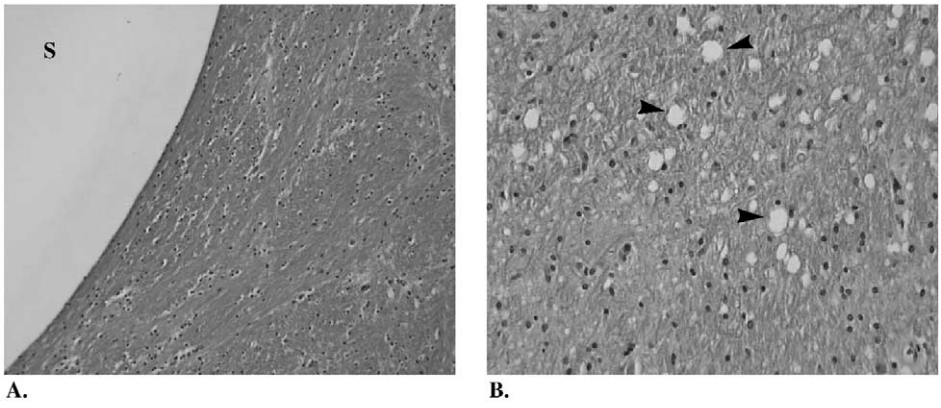

Fig. 3. (A) Part of a typical sized 'Swiss cheese' artifact (S) adjacent to quite unaffected tissue (to the right) in the thalamus (H&E). (B) White matter autolytic vacuoles (arrowheads) in the cerebellar vermis (H&E). Scale bar for A = 18.5 mm, B = 75 μm.

Table 2
Weights (in grams) of fixed minke whale brains

	Whole brain, with rete and dura, ($n = 35$)[b]	Whole brain, without dura, ($n = 35$)[b]	Cerebrum, ($n = 31$)[b]	Cerebellum, ($n = 29$)[b]
Mean (S.D.)	3463 (423.6)	2741 (296.3)	2159 (238.6)	481 (77.7)
95% CI[a]	3325–3801	2501–2641	2075–2243	453–509
Range	2350–4389	1950–3322	1600–2683	350–710

[a] 95% CI = 95% Confidence Interval.
[b] Due to technical failure and human error during gross examination, data on some brains are missing.

fixation show that brains from large whales may be successfully sampled and analysed.

The overall results showed that the described in situ fixation technique rendered the brains suitable for excision. The brains fixed in situ were far better protected from being damaged or degraded during the process of excision than fresh brains. The subsequent histological examination showed that these brains were, in many ways, better preserved than the routine autopsy brains of human and veterinary medicine. We regard the time span from death to start of fixation as the most decisive or crucial factor for this successful result. In ≈ 75% of the cases, fixation started within 2 h post mortem. For logistical reasons there were instances when fixation started later, but never exceeded 6 h, which is earlier than most routine necropsies. Some variations in the fixation quality were however observed (Table 1), as by the occurrence of mild autolytic changes in some brains. These changes may be explained by variations in the collecting procedure and the external circumstances during sampling. The in situ fixation period was set to 72 h and the results showed that this was apparently sufficient. However, due to *force majeur* (bad weather, bad weather forecast, excessive hunting etc.) some brains had to be excised earlier (48 h) or later (up to 5 days). The exact time of in situ fixation was unfortunately not recorded for every brain, but no brains were excised earlier than 48 h after the start of fixation. Brains that were excised this early had a softer consistency, making the excision much more difficult. This concurs with the observations made by Øen (1995b). There were considerable variations in air temperature (−5 to +25 °C) during the sampling period as the boats operated both in Arctic waters and in southern waters off the Norwegian coast. Although the exposure of each individual brain to high or low temperatures during the whole fixation period was not recorded systematically, none of the brains froze.

The absorption of fixing fluid into normal brain tissue is rapid, but rarely is fixed brain tissue totally devoid of fixation or preparation artifacts, which have to be considered during histological evaluation of the specimens (Esiri, 1996). An advantage with in situ fixation is the lack of handling of the brain in the fresh state. Even careful handling is known to produce artifacts (Cammermayer, 1962). 'Swiss cheese' artifacts are caused by the invasion of gas forming anaerobic bacteria (Esiri, 1996) and are not an unusual feature in fixed brains. In the present study, this occurred in restricted areas in ≈ 53% of the brains at gross examination, though always in mild forms. Microscopic examination revealed white matter vacuolation in inner parts of some brains (Fig. 3B). Such vacuolation might occur during the processing of the lipid rich tissue due to the organic solvent used for paraffin embedding, especially if the tissue undergoes some autolytic change before fixation (Summers et al., 1994). From a pathological point of view, both 'Swiss cheese' artifact and white matter vacuolisation may influence evaluation of non-inflammatory white matter degeneration, although such artifacts are of less importance for anatomical studies of the brain.

A common finding at gross examination was a pink coloration on the slice surface, which occurred in 79% of the brains. In all cases where this coloration occurred, the fixation was very good. The significance of this coloration is uncertain. It might simply be an indication of good diffusion of the fixative and of much formalin in the tissue. However, this pink coloration should not be confused with so-called 'pink haloes' or 'Pink spots of Hedley-Whyte' (Hedley-Whyte, 1985; Mitchell et al., 1990), which are artifacts caused by bacteria that have left the bloodstream and proliferated in the tissue between death and perfusion with fixative (Esiri, 1996). 'Pink halo' artifacts were not observed in any of the studied brains.

Storage compression damage was noted on 37% of the brains. There are several recommended procedures to avoid such damage, including suspension of the brain in the container using stitches through the dura (Esiri, 1996). Such methods are difficult, if not impossible, to apply to the relatively heavy brains of minke whales under field conditions. In this case, an increased risk of damage and tearing of the tissue during storage, transport and unloading would have been likely.

The general form and proportion of the various parts of the brain in the minke whale conform to the typical cetacean pattern (Fig. 4). Compared to non-aquatic mammals, the most conspicuous features are the marked brachycephaly of the cerebrum (telescoping), the great transverse diameter of the cerebral hemispheres and the large cerebellum (Table 2). As in other baleen whales, the telescoping of the cerebral hemispheres in the minke

whale is not as prominent as in most toothed whales. In minke whales, the cerebellum is more rounded and hourglass shaped in the dorsal aspect, whereas the odontocete cerebellum is more flattened dorsoventrally as a result of the greater telescoping of the brain. In terrestrial mammals, the weight of cerebellum amounts to $\approx$10–12% of the total brain weight. It has been reported by several authors that the cerebellum in cetaceans is large and may account for up to 15% of the total brain weight in odontocetes and 20% in baleen whales (Breathnach, 1959; Jansen and Jansen, 1969; Morgane and Jacobs, 1972; Bullock and Gurevich, 1979; Ridgway and Wood, 1988; Marino et al., 2000). However, the larger percentage of cerebellum in mysticetes is apparently due to a lesser forebrain, since relative to body mass, the cerebellum is not as voluminous as in larger toothed whales (Ridgway and Tarpley, 1996). The present study reveals that the cerebellum in minke whales constitute 22% of the total brain weight, which is concurrent with findings in other baleen whales. For comparison, Table 3 gives a summary of brain weights of different species of baleen whales previously reported in the literature. This summary shows that the mean fixed weight of 2741 g (Table 2) in the minke whale is the lowest brain weight reported in baleen whales, which is not surprising as the minke whale is the smallest of the baleen whales listed. Table 2 shows that the weight of the minke whale brain varied considerably, in the present material from $<$2000 g to $>$3300 g. In humans, the average brain weight is $\approx$1350 g, with normal variation between 1100 and 1800 g (Skullerud, 1985). Such variations have also been reported in whales, even from animals with approximately the same body length (Jansen, 1952). Brain weight is correlated to body size (Skullerud, 1985; Creutzfeld, 1995). The mean body length of the minke whales in our study was 732 cm, with a range from 420 to 870 cm. It was beyond the scope of this article to perform statistical correlation analyses between body length and brain weight, but taking into consideration the wide range in body length and the normal variations in brain weights, our findings are not surprising.

5. Conclusions

This study has demonstrated an in situ formalin fixation method for minke whale brains, which has several advantages compared to more traditional methods. It proved to be suitable for preserving these large brains, where excision of undamaged fresh brains is almost impossible. The whole brain is preserved uncut, resting in its meninges and the skull, and can be removed almost undamaged at a more convenient time. Handling of the brain in the fresh state is avoided and the fixation can commence very shortly post mortem. The in situ method provides acceptable, generally excellent, fixation of the brain tissue and can probably be used without any particular modifications for other whale species and also for large terrestrial mammals. In minke whales, the in situ fixation period should be at least 3 days to prevent damage at excision. However, for use in animals with smaller brains than the minke whale, the in situ fixation period may be shortened.

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Table 3
Summary of data on brain weights of baleen whales previously reported in the literature

Species	No. of specimens	Individual weight or mean weight (min–max) in grams	Reference
Blue whale	1	6700	(Guldberg, 1885)
	1	5678	(Wilson, 1933)
	1	6500	(Pilleri, 1966b)
Fin whale	1	5950	(Ries and Langworthy, 1937)
	7	6930 (5970–7875)	(Jansen, 1952)
	2	5100 (5000–5200)	(Jacobs and Jensen, 1964)
Sei whale	1	4900	(Jacobs and Jensen, 1964)
	12	4636 (3000–5200)	(Pilleri, 1965, 1966a)
Bowhead whale	Not stated	2845	(Ridgway and Tarpley, 1996)
Humpback whale	1	4030	(Breathnach, 1955)
	2	6100 (4700–7500)	(Jacobs and Jensen, 1964)
	8	4675 (4320–5200)	(Pilleri, 1966c)
Minke whale	1	2650	(Jansen and Jansen, 1969)
	7	2201 (1825–3000)	(Øen, 1995b)

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