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Preserved brain of the Woolly mammoth (*Mammuthus primigenius* (Blumenbach 1799)) from the Yakutian permafrost

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ABSTRACT

This paper describes the treatment and results of the fixation procedure, extraction, dissection, and data collection of the preserved Late Pleistocene brain of the mummified specimen from permafrost. The partial carcass of the Woolly mammoth (*Mammuthus primigenius* (Blumenbach 1799)) nicknamed "Yuka" was found in 2010 on the Dmitrii Laptev Sea coast (Northern Yakutia, Russia). It dates to 39,440–38,850 cal. BP. This fossil presents the unique opportunity to study the preserved brain from permafrost, with well-defined major gross anatomy features of the cerebrum and cerebellum and internal structures revealed by Computed tomography (CT) and Magnetic resonance imaging (MRI). Anatomical description, CT data including volumetry, and results of the histological and electron microscopy study of the specimen are provided.

1. Introduction

The vertebrate brain is an evolutionarily stable structure and its morphological features contain indications of the ancient evolutionarily events. Even the cerebrum, the most variable structure of the mammalian brain, has gyrification patterns, which contain family and species specificity (Saveliev, 2001).

The Woolly mammoth (*Mammuthus primigenius* (Blumenbach, 1799)) is the latest representative of an evolution lineage of mammoths, which members belong to the family *Elephantidae* (Gray, 1821). This family also includes genus of modern elephants *Elephas* (Linnaeus, 1758) and *Loxodonta* (Anonymous, 1827) (Maglio, 1973; Shoshani and Tassy, 2005). Modern elephants stand out from the other mammals in their longevity (Shoshani and Eisenberg, 1982), social structure with complicated group hierarchy, and communication (Langbauer, 2000; Schulte, 2000, 2006). They have ability to use tools and demonstrate a range of complex learning skills and behavior (Chevalier-Skolnikoff and Liska, 1993; Hart et al., 2001). Modern elephants have a large amount of folded cerebral cortex (with disproportionally large temporal lobe) with complex gyral pattern and well-developed olfactory system. Brains of the modern elephants exhibit well-developed cerebral frontal, parietal, temporal, limbic, and insular lobes and poorly-developed occipital lobes, and relatively large and also highly convoluted cerebellum (Shoshani et al., 2006).

Previous findings of the well-preserved Woolly mammoth specimens from permafrost demonstrated only "… a uniform structureless brown mass …" as the brain's vestige (Kreps et al., 1981). Our knowledge on the mammoth brain is based only on the data from endocranial casts. Until now, there was no opportunity to examine the whole brain of a Woolly mammoth, which might have had complicated behavior, similar to modern elephants. With this opportunity, proper preservation of the specimen became our primary goal. The main question was, would the state of preservation of this material allow us to get data suitable for detailed comparative anatomy?

Mummification processes of the soft tissues in the Siberia permafrost have been poorly examined. The conditions of long-term mummification in permafrost do not preclude multiple freezing-thawing. Participation of the flood bog biotopes in the initial preservation of the mummified specimens from Siberian permafrost is suggested (Popov, 1959). The naturally mummified soft tissues are widely examined by anthropologists, including use

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of the histological technics and light and electron microscopy (Hess et al., 1998; Shin et al., 2003; Eklektos et al., 2006; Prats-Muñoz et al., 2012). The most common findings in anthropology are human mummies desiccated in arid conditions. There are also bog bodies, and several unique freeze-dried mummies are known (Aufderheide, 2003; Lynnerup, 2007). Preserved brain structures are very rare even among numerous findings of these naturally mummified human remains (Tkocz et al., 1979; Radanov et al., 1992; Gerszten and Martinez, 1995; Previgliano et al., 2003; Eklektos et al., 2006; Kim et al., 2008; Maixner et al., 2013). All this findings are much younger than the current specimen (39,440–38,850 cal BP).

There are few published studies dealing with histology of mammoth tissues from permafrost. Tissues of the Woolly mammoth from the Berosovka River (Zalenskii, 1903), the baby mammoth nicknamed "Dima" (Barnhart et al., 1980; Shoshani et al., 1981) and the late Pleistocene mammals, including Woolly mammoth, from Alaska (Zimmerman and Tedford, 1976) were investigated with histological methods. The samples from the second specimen were secondarily frozen after its extraction, transported to the laboratory, and then were thawed and air-dried for electronic microscopy and immunological studies. Freezing is applicable for small objects during some histological procedures, but it is poorly adapted for handling during extraction and sustained anatomical study. The fluid fixation method (formaldehyde, alcohol-formaldehyde (Schaffer, Bouin) solutions) is widely used for fixation of rehydrated soft tissues of human mummies (Aufderheide, 2003; Mekota and Vermehren, 2005). The nervous tissue is one of the fastest decomposing tissues, and thus the fixation and preservation of the brains of large modern mammals presents some difficulties (Shoshani et al., 1982; Manger et al., 2009; Knudsen et al., 2002). The method of the perfusion-fixation with 4% formaldehyde solution is generally used to obtain high quality material of mammals with large brains, including elephants (Manger et al., 2009). There was no possibility of applying the perfusion-fixation due to decomposition of the main blood vessels of the neck of the specimen and the unknown condition of its cranial vasculature.

2. Material and methods

2.1. General information of the specimen

A mummified carcass of the Woolly mammoth nicknamed "Yuka" was found in August 2010 on the Laptev Sea coast near Yukagir (for details see Maschenko et al., 2012). It had been transported from the coast over 4 days at 5–7 °C and put into ice storage (below -5–9 °C) about 150 km from the site. At the end of 2011 it was given to the Sakha (Yakutia) Academy of Sciences for study, and transported to Yakutsk by the end of the year (below −18 C°). Since 2012 it was stored at constant temperature below freezing. The Yuka mammoth frozen carcass became available for study in February 2012. It had been examined over 3 days at room temperature and then was repeatedly frozen.

The radiocarbon date for the sampled mammoth bone is 39,440–38,850 cal BP (GrA-53289) (Boeskorov et al., 2013). The individual age of Yuka is 6–9 years old based on the state of the cranial sutures, teeth generation and wear (DP4/M1) observed in Asian elephants (*Elephas maximus*, Linnaeus 1753) (Roth and Shoshani, 1988). No frozen remains of a Woolly mammoth of such age had been found previously. Since then there were different opinions on the Yuka's age, it was decided to perform X-

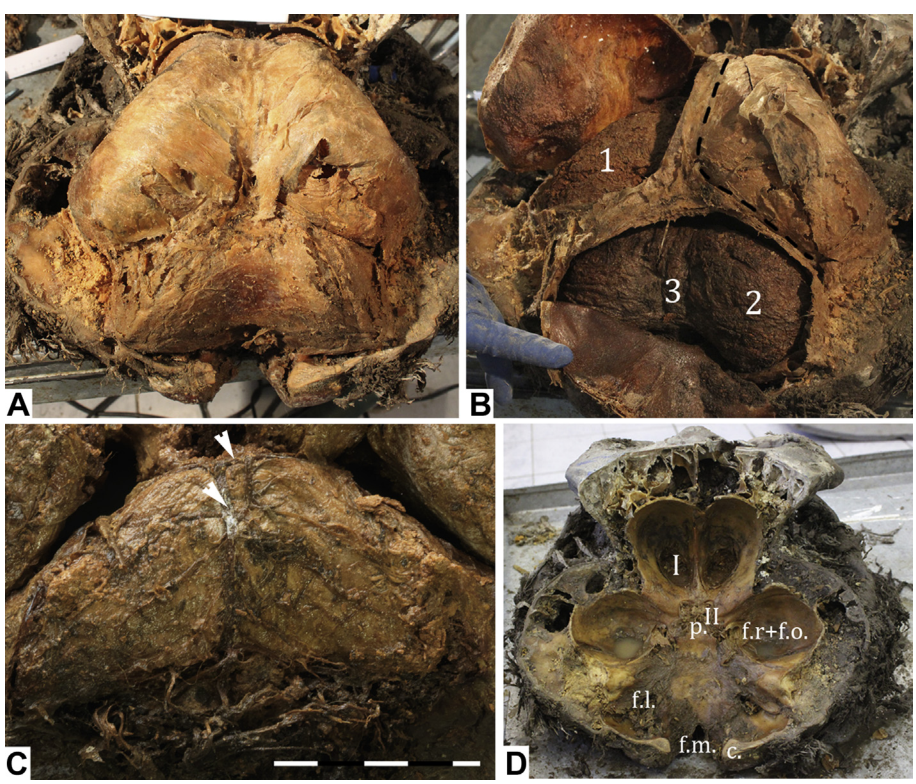

Fig. 1. Intact Yuka's brain with meninges from dorsal view after the fornix of the cranium was removed (A). Yuka's brain under the dissected *dura mater*: third dissection line is dash-marked (B): 1 – *hemisphaerium cerebri*, 2 – *cerebellum*, 3 – *vermis cerebelli*. The pontine area of the Yuka's brain from the ventral view: basilar arteria (arrow) with transverse pontine branches, bar = 5 cm (C). The basal neurocranium of the removed brain: neurocranium foramens with remnants of the nerves and vessels (D): I – the cavity and remnants of *bulbus olfactorius* above the *lamina cribrosa*, II – *foramen opticum*, p. – pituitary fossa, f.l. – *foramen lacerum*, f.r. – *foramen rotundum*, f.o. – *foramen ovale*, c. – *condylus occipitalis*, f.m.– *foramen magnum*.