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Modern human origins backdated

Here we report datings of a hominid cranium (specimen KNM-ER 3884) and femur (KNM-ER 999) from the Lake Turkana region, Kenya, that indicate ages of around 270,000 and 300,000 years, respectively. These hominids might represent the oldest near-modern human specimens from anywhere in the world. Our datings and other recent evidence indicate that the chronological framework of *Homo sapiens* evolution in Africa needs to be revised.

The cranium from Ileret, northeast of Lake Turkana, was first reported in 1992 (ref. 1). It was found in deposits formerly attributed to the Guomde Formation[2], most of which has since been subsumed into the Chari Member of the Koobi Fora Formation. It was derived from undifferentiated later deposits, probably representing an age of 0.5 to 0.1 Myr (ref. 2). However, the specimen came from very close to the base of the latest Pleistocene/Holocene Galana Boi Formation[1] and so the hominid could be much younger.

We have now dated two different fragments of the cranium and a part of the femur, which derived from the same deposits[3], by non-destructive γ-ray spectrometry. The technique has been used successfully to date other fossil hominids[4]. Activities of ^{234}U and ^{230}Th are determined from the γ-rays emitted at 53.3 KeV and 67.7 KeV, respectively[5], by a high-purity germanium γ-ray detector (25% relative efficiency).

The two samples of the cranium yielded concordant U–Th ages of 272,000 years (minimum age 159,000; indeterminate maximum age) and 279,000 years (minimum 162,000; maximum indeterminate). The U–Th date of 301,000 years (minimum 205,000, maximum indeterminate) for the femur supports the date of the cranium and indicates that both fossil hominid specimens came from very closely related stratigraphic levels. The U–Th dates are further supported by U–Pa dates of over 180,000 years for all three samples. Finally, an age of about 270,000 years for the cranium and 300,000 years for the femur are in agreement with the previous stratigraphic conclusions[1]. The infinite upper errors leave open the possibility that the two hominids spanned a longer timescale, but they are both probably older than 180,000 years.

The cranium belongs to an adult individual. It consists of a large posterior part of the cranial vault including most of the occipital, parietals and temporals, a nearly complete supraorbital region and a maxillary part with all teeth. Preliminary estimation of endocranial capacity points to around 1,400 cm^3. The posterior vault has thin walls (5–6 mm) and a lack of clear archaic features, and so shows close affinity to modern anatomy. In contrast, the torus-like supraorbitals are different from those seen in modern humans and closer to late archaic specimens like Florisbad and Laetoli H.18. Our observations indicate that the hominid might represent an archaic *Homo sapiens* or a transitional specimen very closely related to modern humans.

Comparative analyses showed that, in spite of its rather robust shaft, the femur has some modern features common among the earliest modern humans from Qafzeh and Skhul, Israel[6]. In view of the uranium-series dates, the femur might indicate that a very robust but basically modern morphology already existed in eastern Africa more than 200,000 years ago and probably as early as 300,000 years ago.

Such an early existence of near-modern transitional or late archaic *Homo sapiens* specimens, and the presence of early archaic *Homo sapiens* (Bodo, Ethiopia) at around 600,000 years ago[7], as well as other recent datings of African archaic and early modern fossils (Eyasi, Florisbad, Singa)[8–10] make a revision of the course of Middle Pleistocene evolution in Africa necessary (Fig. 1). Early and late archaic *Homo sapiens* and also the earliest modern humans seem to have existed considerably earlier than has been assumed[11]. This revision further supports an early evolution towards modern humans in Africa and pushes the origin of archaic *Homo sapiens* back to the earliest Middle Pleistocene. This framework strongly affects the hypotheses of the emergence of archaic *Homo sapiens* outside Africa, for example the Ante-Neanderthals of Europe and archaic *Homo sapiens* of China.

Table 1 **Results of uranium-series dating**

	KNM-ER 999 (femur)	KNM-ER 3884 (cranium sample 1)	KNM-ER 3884 (sample 2)
Mass (g)	130.8	23.3	20.1
U p.p.m.	3.95	4.03	2.28
Ratio of nuclide activities			
$^{234}U/^{238}U$	2.253±0.256	3.773±0.760	4.343±0.757
$^{230}Th/^{234}U$	1.089±0.129	1.114±0.248	1.133±0.236
$^{230}Th/^{232}Th$	46	99	>200
$^{231}Pa/^{235}U$	1.083±0.060	1.185±0.178	1.309±0.210
Age (yr)			
U-Th (s.d.)	301,000 (+∞, −96,000)	272,000 (+∞, −113,000)	279,000 (+∞, −117,000)
U-Pa	>180,000	>180,000	>180,000

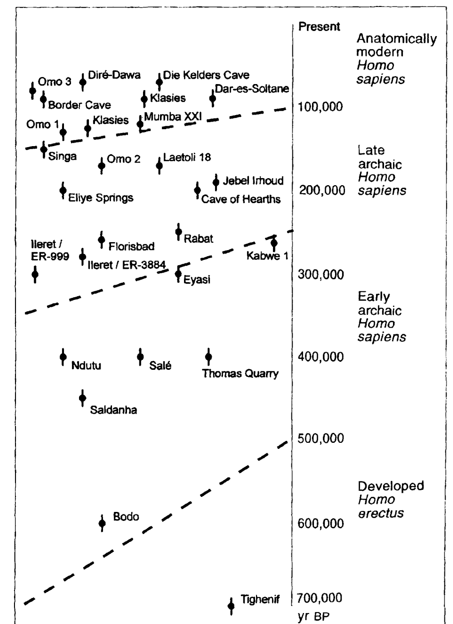

Figure 1 **Revised scheme of *Homo sapiens* evolution in Africa. The transitional *Homo erectus*/archaic *Homo sapiens* period can now be dated to at least 700,000–500,000 years ago and the transition from early to late archaic *Homo sapiens* to around 350,000–250,000 years. The origin of modern *Homo sapiens* might go back to at least 150,000 years ago. The most likely ages of the specimens are given, but they have different qualities and errors.**

Günter Bräuer
Institute of Human Biology, University of Hamburg, Allende-Platz 2, 20146 Hamburg, Germany

Yuji Yokoyama, Christophe Falguères
Laboratoire de Préhistoire du Muséum National d'Histoire Naturelle, Institut de Paléontologie Humaine, 1 Rue René Panhard, 75013 Paris, France

Emma Mbua
National Museums of Kenya, P.O. Box 40658, Nairobi, Kenya

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Endothelial nitric oxide synthase and LTP

Long-term potentiation (LTP) is often considered to be a cellular correlate of learning. During LTP induction in the CA1 region of the hippocampus, nitric oxide (NO) synthesized in the dendrites of pyramidal cells may carry retrograde signals from the postsynaptic to the presynaptic terminals[1,2]. We show that LTP is defective in hippocampal slices from mice lacking functional endothelial nitric oxide synthase (eNOS) and from wild-type mice treated with a NOS inhibitor. The endothelial isoform of NOS seems to be required for the maintenance of LTP in the hippocampus.

Extracellular application of NOS inhibitors blocks LTP in the hippocampal CA1 area[3–5], although there are reports that LTP generated by strong stimuli is not sensitive to these drugs[6–8]. Furthermore, in cultured hippocampal neurons, LTP is blocked by extracellular post- and presynaptic application of oxymyoglobin (which binds free NO), or by post-, but not presynaptic injection of a NOS inhibitor[9]. Exogenous NO paired with a weak, sub-threshold tetanic stimulus also induces LTP[9].

Mice lacking a functional copy of the neuron-specific NOS isoform (nNOS) exhibit normal LTP, but LTP in nNOS knockouts is blocked by NOS inhibitors[8]. This may be explained by the finding that eNOS, initially believed to be present only in endothelial cells, is the main isoform in CA1 pyramidal cells[10]. Consequently, eNOS, not nNOS, may be responsible for synthesizing NO postsynaptically during LTP. Indeed, injecting hippocampal slices with an adenovirus vector containing a truncated, and hence not functional, eNOS gene (a putative dominant negative) blocks LTP at synapses in the CA1 stratum radiatum[11].

We inactivated the eNOS gene by replacing exons 24 and 25 with the neomycin-resistance gene in the embryonic stem cell line E14-1. Functional inactivation of eNOS was demonstrated by the lack of endothelial NO formation in eNOS$^{-/-}$ mice, derived from two independently generated mutant clones. The hippocampi of these eNOS-deficient animals had no obvious anatomical defects and apparently normal excitatory synaptic transmission in the CA1 region. Baseline test excitatory postsynaptic potential (EPSP) amplitude (30–40% of the maximum EPSP amplitude), was not significantly different for wild-type and eNOS$^{-/-}$ mice (0.95±0.05 mV for control slices, 1.08±0.07 mV for eNOS$^{-/-}$ slices, mean±s.e.m.).

We chose a relatively weak LTP induction method (so as not to induce NO-independent LTP) that was highly sensitive to the NOS inhibitor *N*-nitro-L-arginine (NOARG). The method induced moderate potentiation in wild-type slices that lasted at least 90 min (148.4±12.8% 90 min after tetanus, $n=11$). In the presence of NOARG, EPSPs exhibited short-term potentiation (STP) but not LTP, decaying gradually to baseline in less than 90 min (94.9±4.2% 90 min after tetanus, $n=7$, Fig. 1a, b). After 90 min, the NOARG-treated group was significantly different from controls ($P<0.01$) but not significantly different from baseline. Using the same induction method, the eNOS$^{-/-}$ mice exhibited STP but no LTP (101.6±6.5% 90 min after tetanus, $n=10$, Fig. 1a, b). After 90 min, the potentiation level in the eNOS-deficient group was significantly different from wild-type slices ($P<0.01$), and not significantly different from baseline.

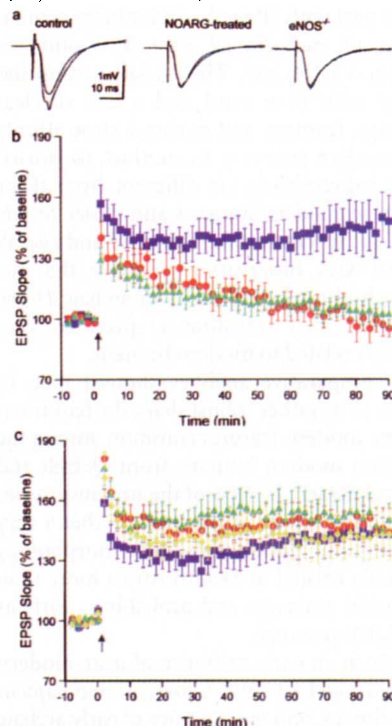

Figure 1 a, **Superimposed field EPSPs, one recorded immediately before tetanus and one 90 min after tetanus, for wild-type slices, wild-type slices treated with 200 μM NOARG, and eNOS$^{-/-}$ slices.** b, **Time course of mean normalized EPSP slopes in wild-type slices (blue squares), wild-type slices treated with 200 μM NOARG (red circles), and eNOS$^{-/-}$ slices (green triangles) subjected to weak tetanic stimulation (3 trains of 10 pulses each, 100 Hz, 20 s inter-train interval, 40 μs pulses).** c, **Time course of mean normalized EPSP slopes in wild-type slices (blue squares), wild-type slices treated with 200 μM NOARG (red circles), eNOS$^{-/-}$ slices (green triangles), and eNOS$^{-/-}$ slices treated with 200 μM NOARG (yellow diamonds) subjected to strong tetanic stimulation (80 μs pulses). Tetanic stimulation is indicated by arrows. Vertical bars show s.e.m.**

Next, we examined LTP induced by a stronger tetanic stimulation. We found that doubling the pulse duration during tetanus only (from 40 to 80 μs) induced a level of LTP that was not significantly different in wild-type slices treated with NOARG compared to wild-type slices not treated with the drug (143±11.8% 90 min after tetanus for NOARG-treated slices, $n=12$; 143±8.2% for controls not treated with NOARG, $n=8$, Fig. 1c). Using this tetanic stimulation, we observed robust LTP in eNOS$^{-/-}$ slices (150±8.6%, $n=9$) that was not significantly different at 90 min from LTP in wild-type slices or in eNOS$^{-/-}$ slices treated with NOARG (142±13.3%, $n=10$, Fig. 1c).

Our data are consistent with the hypothesis that NO synthesized by eNOS in postsynaptic CA1 pyramidal cells is a retrograde messenger required for the long-term maintenance, but not induction, of potentiation induced by weak stimuli. LTP induced by stronger stimuli does not seem to require NO, possibly because strong stimuli produce retrograde messengers in addition to NO. As LTP induced by strong stimuli in eNOS$^{-/-}$ slices is not attenuated by NOARG, it seems unlikely that the LTP we observe is due to a compensatory activity of nNOS. The resemblance between our results for eNOS$^{-/-}$ slices and for NOARG-treated wild-type slices suggests that eNOS is the main isoform participating in this process, and that any contribution from another isoform would be minimal.

Rachel I. Wilson, Jevgenij Yanovsky
Axel Gödecke, David R. Stevens
Jürgen Schrader, Helmut L. Haas
Zentrum für Physiologie und Biomedizinisches Forschungszentrum,
Heinrich-Heine-Universität,
D-40001 Düsseldorf, Germany
e-mail: haas@uni-duesseldorf.de

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