Bacterial population profiles in deep sediment layers at the four geographically distinct locations in the Pacific Ocean are remarkably similar to those in the Japan Sea (Fig. 4) and likewise decrease significantly with sediment depth ($P \ll 0.001$). Some deviations from the trend occur which can be linked to specific environmental factors. Elevated bacterial populations occur in a shallow water, high productivity Peru Margin site. Conversely, reduced populations are present in low productivity (Eastern Equatorial Pacific) and hydrothermally heated sites (Juan de Fuca Ridge). There is no indication that the rate of decrease in bacterial populations will change in deeper sediments, and hence it is likely that bacterial populations are present to much greater depths. Temperature is a limiting factor, but with a thermal gradient of 30–100 °C km^{-1} in sediments and a probable upper temperature for bacterial growth of 110–150 °C (ref. 16), it would only be limiting in very deep or hydrothermal sediments. A depth integration of bacterial numbers to only 500 mbsf (average oceanic sediment depth[1]) produces, however, a considerable total bacterial biomass of ~1.5 t ha^{-1} organic carbon (average bacterial volume = 0.21 μm^3, 310 fgC μm^{-3} (ref. 7), density = 1). Although this only constitutes a small percentage of global sedimentary organic carbon (0.004%), remarkably it is 10% of the living carbon in the surface biosphere[17].

These findings significantly extend the depth of the marine biosphere and indicate that sedimentary organic matter, including molecular fossils[18], will undergo continued bacterial modification long after burial. The deep biosphere also responds to, and modifies, geochemical fluxes (for example, CH_4; Fig. 1) analogous to, but probably more significant globally than, biological interactions at hydrothermal vents and in oil reservoirs[19]. The presence of viable bacterial populations in deep marine sediments is consistent with recent results from other deep subsurface environments such as aquifers[20]. □

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Role for supplementary motor area cells in planning several movements ahead

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To achieve a volitional goal, we need to execute multiple movements in a specific temporal order. After repetitive performance of a particular sequence of movements, we are able to memorize and execute the whole sequence without external guidance. Where and how in the brain do we store information necessary for the orderly performance of multiple movements? We have found a group of cells in the cerebral cortex of monkeys whose activity is exclusively related to a sequence of multiple movements performed in a particular order. Such cellular activity exists in the supplementary motor area[1,2], but not in the primary motor cortex[3,4]. We propose that these cells contribute a signal about the order of forthcoming multiple movements, and are useful for planning and coding of several movements ahead.

We trained two monkeys (*Macaca fuscata*) to perform three movements (push, pull or turn a manipulandum) in four different orders. Before each movement, monkeys waited for a tone signal that served as a movement trigger. After completion of an individual movement, a mechanical device returned the manipulandum to a neutral position within which the animal had to hold the manipulandum and wait for a next trigger signal. Initially, during the learning phase, green, yellow and red lights indicated that the correct movement required was to push, pull or turn the manipulandum, respectively. The animals received five learning trials and subsequently performed the sequential motor task in the absence of the visual cues. The success rate was over 95% in all motor sequences. Electromyographic analysis showed that the forelimb muscles were active for only a short period during the execution of individual movement, but not active during the period when animals were waiting for the next movement trigger signal.

The classically defined supplementary motor area (SMA) is now divided into two areas[2,5,6]: the rostrally situated presupplementary area (pre-SMA) or F6, and the caudally situated SMA proper (or F3). We have recorded from both areas delineated by the criteria reported previously[6]. This report deals with 206 cells recorded in the caudal part (for the sake of brevity, called simply the SMA). Of these, 54 cells were of interest in that they were preferentially active in relation to a particular order of forthcoming movements guided by memory. Although these cells were active during a waiting period before the first movement trigger signal, the activity increased only if the order of forthcoming three movements was a specific one. The activity of this type of SMA cell is shown in Fig. 1; for instance, it was active while the animal was waiting to perform a motor sequence of turn–pull–push (Fig. 1, bottom). It is unlikely that the activity of this cell signals a preparatory process for the initial movement in the sequence because the cell was not active when the animal was ready to perform a different sequence of turn–push–pull (Fig. 1, top). In addition, the cell was not active when the animal performed the three movements in other sequences (e.g. push–pull–turn). Furthermore, the cell was not active if the three movements were guided by a visual signal, even if the order was turn–pull–push. These cells then seem to signal a specific order of forthcoming, multiple movements to be performed on the basis of memory.

We found another group of 74 cells in the SMA that were preferentially active during the interval between two specific movements. These cells were active during a waiting period

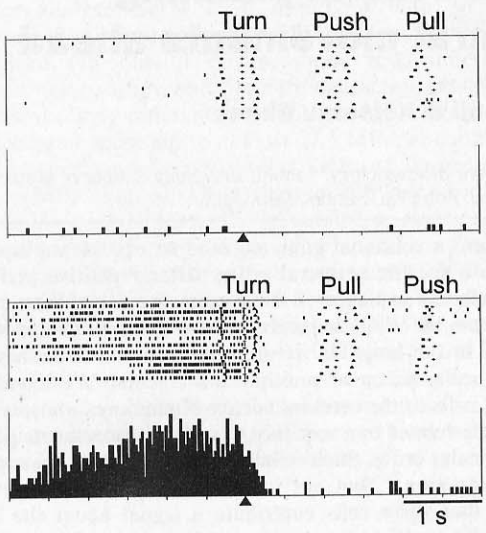

FIG. 1 Activity of a cell in the SMA exhibiting preferential relation to a specific order of three movements performed without sensory guidance. This cell is active during a waiting period, but only if the sequence of upcoming movements is in the order of turn, pull and push (bottom). In raster displays, each row represents a trial, and dots represent individual discharges of this cell. Small squares, crosses, and triangles denote the times of occurrence of the trigger signal, movement onset, and target acquisition. In histograms, discharges over 12 trials are summated. Triangles at the bottom indicate the start of the first movement. METHODS. Standard electrophysiological techniques for single-cell recording were used[27]. Monkeys, sitting in a primate chair, were required to place a manipulandum to a neutral position and wait 2.5–4.5 s for the first movement trigger signal (high-pitch tone). When the animal performed the first movement, a mechanical device returned the manipulandum to the neutral position. While keeping the manipulandum in this position, the animal had to wait about 1 s for the second and then for the third movement trigger signal. A series of three correct movements was rewarded with delivery of apple sauce, 500 ms later. The average time interval between motor sequences was 7 s. Initially the correct movement was indicated with green, yellow and red lights. During this learning period of visually guided five trials, the animal had to learn the correct sequence, after which the sequential motor task was performed on the basis of memory. After completion of six trials of the memorized sequential task, random flashing of lights (for 2 s) signalled the end of current sequence, and the beginning of new sequence.

before execution of a particular movement, but only after the performance of another particular movement. An example of this type of cell is shown in Fig. 2. This cell was active during the waiting period preceding a forthcoming pull movement and following the performance of push movement (Fig. 2, top, SEQ1 and SEQ3). The same cell was not active during the waiting period before a pull movement if the previous movement was to turn (Fig. 2, bottom; SEQ2 and SEQ4). These cells seem to signal a temporal linkage combining two specific movements.

We also analysed cellular activity from the primary motor cortex (MI, $n=106$) in the same animals, and found that properties of cellular activity were different from those in the SMA. A great majority (90%) of cells in MI was active in close temporal association with the execution of a particular movement or movements. Figure 3 shows a typical example of such movement-related cellular activity in MI. In this cell, the activity started shortly before, and lasted during execution of push movement. As demonstrated, the temporal order of the individual movements within a sequence did not influence the magnitudes of the activity changes. A smaller number of MI cells ($n=27$) exhibited activity during the waiting periods. These cells were active while the animals were waiting to perform a particular movement, regardless of the temporal sequence of the three movements.

Previous studies have reported that cells in the SMA are active in close time relation with movement execution[7–10], supporting a view that the SMA takes some part in execution of movements even when the motor task is simple[11–13]. In line with these previous reports, such movement-related activity was also found in 25 cells in the present study (an example is shown in Fig. 4). On the other hand, it has also been reported that the SMA has a more significant role when motor tasks are more demanding[14–21]. Clinical findings in human subjects with brain lesions including the SMA have found that the difficulties among patients are most pronounced in relation to sequential movements or sequential performance of multiple movements[22,23]. Monkeys with SMA lesions also showed abnormalities in sequential motor tasks[4,24]. Such motor deficits become more

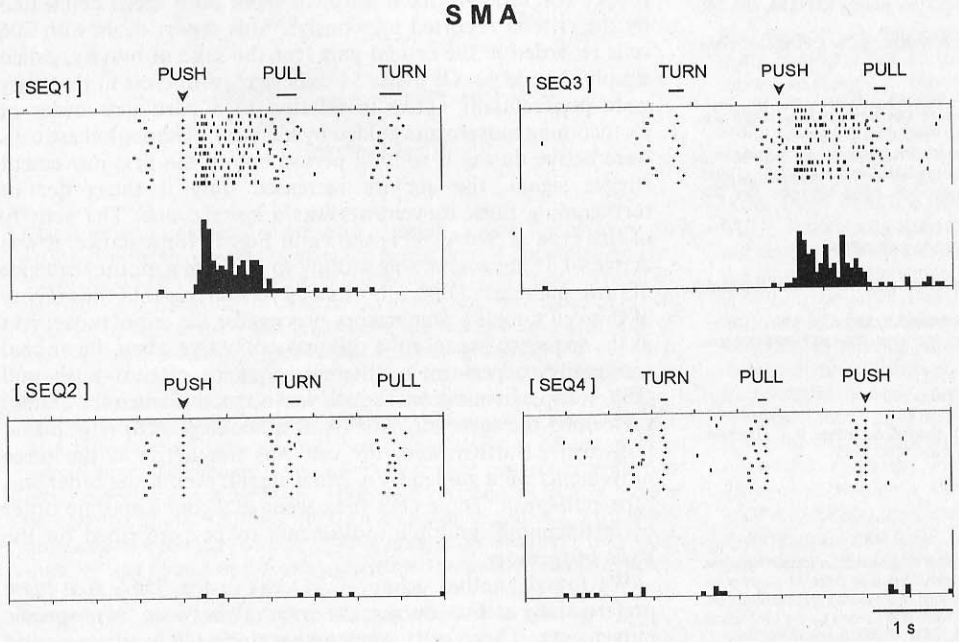

FIG. 2 Selective activity of an SMA cell during a waiting period between a single combination of two specific movements. This cell is active before the 'pull' movement if the previous movement is 'push' (top, SEQ1 and SEQ3) but not if the previous movement is 'turn' (bottom).

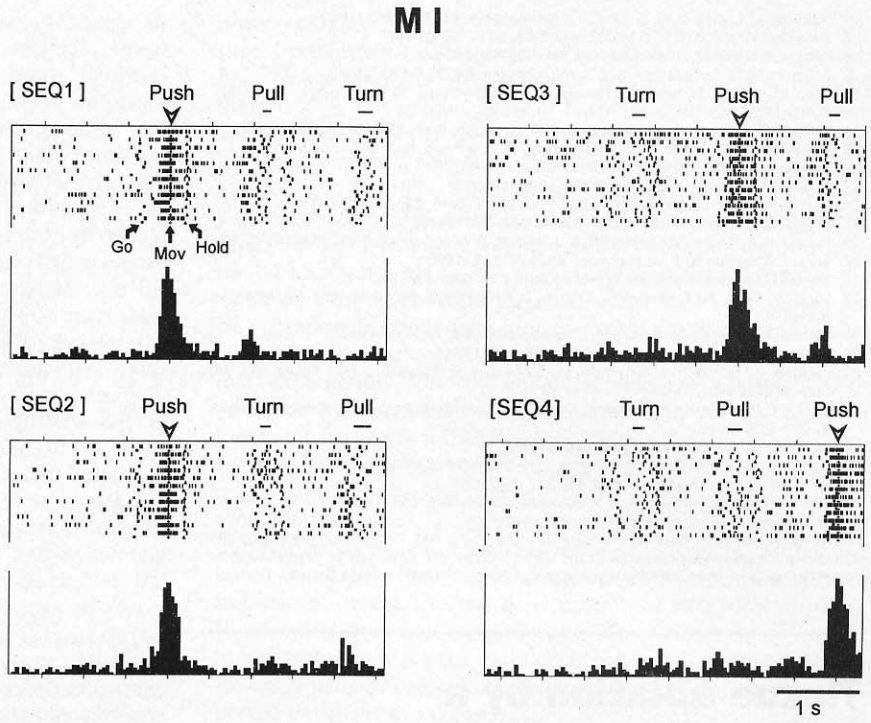

FIG. 3 Activity of a movement-related cell commonly observed in MI. This cell is active during a short period of execution of 'push' movement. The magnitude of activity is not influenced by the temporal order of the three movements.

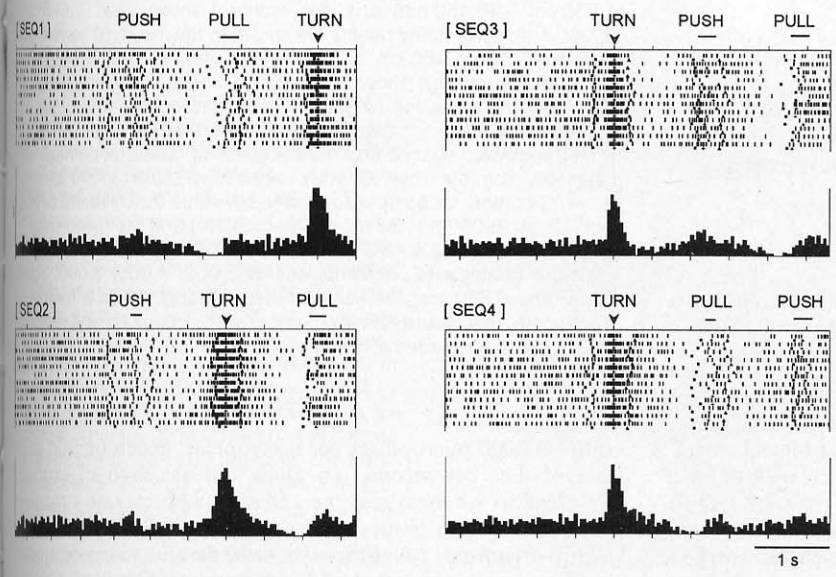

FIG. 4 Activity of an SMA cell that is categorized as movement-related. This cell is active shortly before and during 'turn' movement.

apparent when subjects had to perform the motor task on the basis of memory[25]. The abundance of the preparatory type of activity in waiting periods in this study is in accord with these reports, and indicate that cellular activity in the SMA seems different depending on the nature of the motor task required.

Our results lend support to a hypothesis that the SMA is crucial for temporal structuring of movements. The cellular activity in two different cortical areas suggest that the SMA, but not the MI, is profoundly involved in performing multiple movements in a predetermined order. The order-specific cellular activity, as shown in Fig. 1, could provide a signal about the order of forthcoming movements, useful for programming temporal structure of an intended motor act that includes multiple movements. On the other hand, the SMA cells that are specifically active during an interval between a particular combination of two movements in a specific order (as shown in Fig. 2) are useful for coding a particular time sequence of two movements. We propose that these two classes of cellular activity provide a means by which the SMA is involved in temporal sequencing of multiple movements. It is important to note that the role of the pre-SMA in motor preparation or structuring forthcoming motor action has been implicated in previous reports[6,26]. We will describe elsewhere the cellular activity in the pre-SMA during performance of the same motor task as in the present study. □

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Tissue ablation by a free-electron laser tuned to the amide II band

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EFFORTS to ablate soft tissue with conventional lasers have been limited by collateral damage and by concern over potential photochemical effects[1–5]. Motivated by the thermal-confinement model[6], past infrared investigations targeted the OH-stretch mode of water with fast pulses from lasers emitting near 3,000 nm (refs 1, 7–9). What does a free-electron laser offer for the investigation of tissue ablation? Operating at non-photochemical single-photon energies, these infrared sources can produce trains of picosecond pulses tunable to the vibrational modes of proteins, lipids and/or water. We report here that targeting free-electron laser radiation to the amide II band of proteins leads to tissue ablation characterized by minimal collateral damage while maintaining a substantial ablation rate. To account for these observations we propose a novel ablation mechanism based on compromising tissue through resonant denaturation of structural proteins.

Laser ablation aims to achieve tissue removal by 'cold' etching, that is, where the etch pattern is defined by the irradiating beam and peripheral tissue is free from thermal (collateral) damage. Free-electron lasers (FELs) are broadly tunable, pulsed sources providing both high average and high peak power[10,11] and great versatility for applications research[12–17]. The Vanderbilt FEL (ref. 18) operates in the 2,000–10,000 nm region, enabling a comparison of tissue ablation at 3,000 nm with other infrared wavelengths that were previously unavailable. It emits trains of roughly picosecond micropulses, separated by 350 ps with ~17,000 micropulses per macropulse, generating up to 3 macropulses per second. To guide our selection of exposu wavelengths we measured the absorption spectra of a range different types of tissues: sclera and especially the cornea a highly organized, collagenous tissues; dermis additionally co tains proteoglycans and elastin in a more complex fibril archite ture; brain is a non-collagenous, cellular tissue. Ocular a neural tissues have about the same water content, where dermal tissue is less hydrated. Despite these distinctions, th infrared spectrum for cornea (Fig. 1) is typical of soft tissue Figures 2–4 compare ablation of the various tissues at 6,450 an 3,000 nm. Figure 1 also summarizes the wavelength dependen for cornea, demonstrating remarkable ablative properties f wavelengths near 6,450 nm. Similar wavelength dependence were obtained in other tissues, with further reduction in colla eral damage for neural tissue at 6,450 nm (Fig. 3*a*).

FEL peak powers were in the megawatt range, raising th possibility of nonlinear or multiphoton effects. With regard t the former, the anharmonic shift (270 cm^{-1}) of the OH-stret mode of water, as measured with picosecond pulses[19], is a fra tion of spectral widths (~400 cm^{-1}) as measured here. As f the latter, the rate of tissue removal did not exhibit the powe law dependencies indicative of multiphoton processes[20].

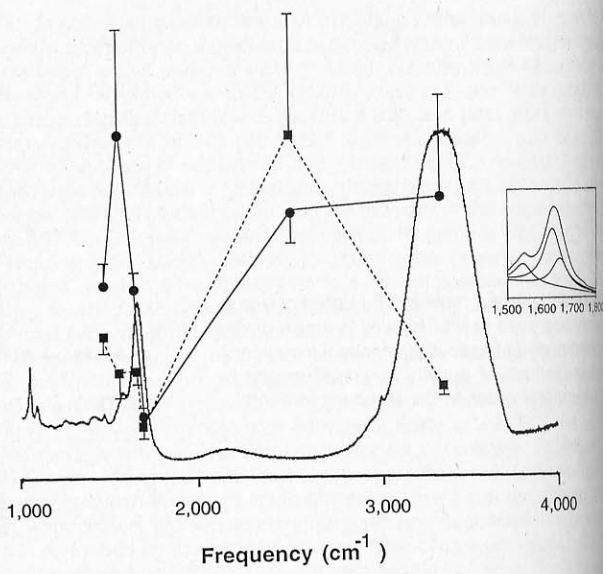

FIG. 1 Relative ablation yield (●) and collateral damage (■) plotte against the absorption spectrum for corneal stroma.
METHODS. Spectra were taken as previously described[13]. The dominan mid-infrared feature was the OH-stretch mode of water near 3,300 cm (3,000 nm) and, as such, is the most efficient wavelength for absorbi infrared (IR) radiation in the 2,000–10,000 nm range. The partial resolved feature centred near 1,640 cm^{-1} (6,100 nm) comprises th bending mode of water near 1,640 cm^{-1}, the amide II mode nea 1,550 cm^{-1} (6,450 nm) and the amide I mode near 1,665 cm (6,000 nm); curve fitting results are shown in the insert. IR wavelength between 1,500–1,700 cm^{-1} (5,900–6,600 nm) will excite both wat and proteins through these overlapping modes. At a wavelength 6,450 nm the extinction lengths of water and protein are 12 μm an 8.5 μm respectively, corresponding to more than 100 stromal layer Both the ablated volume and the thickness of collateral damage we estimated from histology. Relative values were scaled to the spectru for presentation; for comparison the mean value for collateral damag was 12 μm (6,000 nm, 13 mJ per macropulse) and the mean value f ablation yield was 2.5×10^5 μm^3 mJ^{-1} (6,450 nm). These data are f exposure to single FEL macropulses at 3,000, 4,000, 6,000, 6,10 6,450 and 6,850 nm. Ablation was also investigated as a function wavelength, macropulse energy, number of macropulses, and repetitio rate of the macropulses (not shown).