solid except for a small volume which was melted by copper block just before dipping the specimen. The frozen ganglion was placed in a specially made ganglion holder and dried by vacuum in a deep freezing box (below −35 °C) without passing through a liquid phase. The completely dried ganglion was dissected with fine forceps under a binocular microscope and the identifiable neurons were selected according to their localization and characteristic orange color. The isolated dried single neuron was placed on a hand-made carbon disk, fitted in the EPXMA holder and coated with carbon in a vacuum chamber. The JEOL 50A X-ray microanalyser was used. The analysis of calcium and magnesium was performed by the spot analysis method using 2 separate X-ray detectors for calcium and for magnesium simultaneously on the same spot. The analysis of whole cell was performed with a spot size of about 50 µm which is smaller than the neuron diameter, and the different type of cells were analysed successively with the same analyser parameters. To obtain the same analysing conditions on a single carbon disk, in the case of analysis of different spots on a single neuron, we used several isolated neurons and analysed them at the four spots mentioned below on a single neuron successively with the same analyser parameters. The analysis was performed with an accelerating voltage of 15 kV and an absorbed current of 10^{-8} A.

Figure A is the secondary electron image of an example of the RC-cluster of *Euhadra*[3]. Figure C shows the calcium Kα and magnesium Kα X-ray counts of each identifiable neuron with the same spot size and the analyser parameters. This clearly demonstrates that the calcium and magnesium content of the neuron is different depending on the cell type.

The D cells are located in the lower part of the cluster and can easily be picked up by fine forceps. Figure B is the secondary electron image of an example of an isolated D neuron in which the cell body and its axonhillock can be recognized; a, b, c and d indicate the localization of the analysis spots. Figure C shows the results of a spot analysis of Ca Kα and Mg Kα by EPXMA. The calcium distribution in the axonhillock region is about twice that in the cell body. Magnesium shows exactly the reverse relationship of calcium distribution, i.e. rich in the cell body and poor in the axonhillock.

The analysing depth of EPXMA was found to be about 5.0–7.5 µm in an examination using copper Kα detection of a copper mesh with ultrathin sectioned epon embedding specimens of various thicknesses. The unequal distribution pattern of divalent cations described above shows changes in the cell membrane and the cytoplasm near the cell membrane. The glial cells which are usually scattered surrounding the neurons were not observed when an isolated single freeze-dried cell was embedded in epoxy resin and examined by transmission electron microscopy. This distribution pattern showed few changes when the ganglion was incubated in a calcium-free medium for 10 min. This means that the above finding, uneven distribution of divalent cations, was not due to the results of surface-attached free calcium in the incubated solution but originated from the cell itself.

In the neurons of *Aplysia* and *Helix pomatia*, synapses are not found on the surface of the cell body but are situated exclusively in the region of the initial part of the axon. The fact that the region of the initial part of the axon is rich in calcium suggests a close relationship between the synaptic region and the calcium-bound cell structure. Hydén demonstrated that the S-100 protein is normally bound to calcium in the synaptic region and conformational changes were proposed with synaptic transmission[4]. Calissano demonstrated 8 calcium binding sites with S-100 brain specific protein[5]. Magnesium is well known as an ion which has antagonistic action on synaptic transmission. In the postsynaptic region, however, the intracellular quantities of calcium and magnesium bound to the membrane structure, or subcellular structure such as the endoplasmic reticulum or mitochondria, are probably always higher and lower respectively than in the other regions.

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Folic acid levels in blood and seminal plasma of normo- and oligospermic patients prior and following folic acid treatment

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Summary. Folic acid was estimated in blood and seminal plasma of normo- and oligospermic men. Following folic acid administration (10 mg TID for 30 days), the levels in blood and semen increased. However, sperm counts, motility and DNA content of spermatozoa were not affected.

Folinic acid, the biologically active form of folic acid, is known to participate in the synthesis of thymine and purine, leading to the formation of DNA.

Since the reports on DNA content in spermatozoa in relation to sperm counts and fertility disorders are inconclusive and often contradictory[1-3], we postulated that in some cases of oligospermia, decreased DNA values[2] may result from folic acid deficiency. With this possibility in mind, we examined the basal levels of folic acid in both the blood and seminal plasma of 40 normo- and oligospermic patients, and their sperm concentrations, sperm motility and DNA contents of spermatozoa. Subsequently, these patients were treated with folic acid, 10 mg TID for 30 days. The above parameters were reexamined on the 14th and 30th days of the treatment and also a month after its completion.

The spermatozoa of the examined semens were counted and the percentage of motile cells was estimated by routine

laboratory techniques. Seminal plasma was separated from semen, using centrifugation at 15,000 rpm for 30 min. Folic acid was determined by the method of Cooperman[4], and estimations of DNA were based on a modification of the diphenylamine reaction[5] and calculated per 10^6 sperms.

Prior to treatment, the mean value of folic acid in the blood was found to be 138.9 ± 11.5 IU/ml (mean + SE), similar to values reported in a healthy population[6-7], and the mean value in the seminal plasma was 44.5 ± 7.1 IU/ml. From this observation, it is evident that the basic levels of folic acid in seminal plasma (to the best of our knowledge, not previously estimated) are about 30% of the mean level in the blood. No correlation was found between the levels of folic acid either in the blood or seminal plasma and the sperm counts.

Following treatment with folic acid, the level in the blood increased about 5fold, whereas in the seminal plasma it increased, on average, only 3fold and additional treatment did not change these results. No significant changes in sperm count, percentage of motile cells and DNA content of spermatozoa were observed.

Since the number of sperms per ml semen and the degree of their motility are not affected by an increase of folic acid level, in both blood and seminal plasma, we think that oligospermia in man is not correlated with folic acid deficiency.

The elevation of folic acid in seminal plasma following treatment would suggest that either the seminiferous tubules are permeable to molecules of folic acid, or that the high dose which was administrated overcame the testis blood barrier.

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Motilities of isolated and aggregated mice; A difference in ultradian rhythmicity[1]

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Summary. Isolated mice display higher locomotor activity and greater sensitivity to d-amphetamine than aggregated mice. Ultradian motility rhythms can be shown to differ both quantitatively and qualitatively between isolated and aggregated mice.

Many differences in cerebral chemistry and morphology and in emotional reactivity characterize 'long-term' differentially-housed male mice[2-7]. 2 such differences are : a) higher locomotor activity of 'isolated' mice[5,6]; b) enhanced behavioral and toxicological effects of injected sympathomimetic amines (e.g. d-amphetamine) in 'isolated' mice[2-7]. It should be noted that the latter difference refers to the effect of these drugs on long-term differentially-housed mice. When mice are housed in colonies and then either grouped or isolated just after injection of sympathomimetic amines, these drugs produce quite opposite results; i.e., 'acutely-grouped' mice are more sensitive to the injected drugs than 'acutely-isolated' mice[8-12]. In the present study, the evolution and ultradian periodicity of the difference in motility were examined in control and d-amphetamine-treated long-term differentially-housed mice.

Materials and methods. Male, randomly-bred Swiss mice (15-20 g), weaned at about 20-22 days of age, were housed either singly (isolated) in opaque cages ($15 \times 20 \times 25$ cm) or in groups of 24 (aggregated) in wire-mesh cages ($15 \times 25 \times 45$ cm) for 16-47 days before experiments. All experiments were performed between September and November. Motilities of the mice were measured with 2 balanced electromagnetic platforms (Panlab). All animals were tested individually (1 mouse per recording cage) to avoid fighting among the isolated mice. Biological rhythm processors[13] were used to accumulate output pulses over 5-min intervals, and total counts were printed on a thermal paper (6-digit format), local time being internally generated. Animals were subjected to 12-h light-dark cycles, the dark phase being set at 19.00 h-07.00 h. Results are presented as cpm and as power spectra.

Animals were injected i.p. with either d-amphetamine-SO_4 (2 mg/kg; K&K Laboratories, Plainview, N.Y.), or with an equivalent volume of vehicle (0.9% NaCl), and then placed into recording cages 1 min after injection. Motor activity was recorded for either 90 min or 23 h after injections. Except for 90-min studies (figure 2, A), animals were injected and placed onto platforms at the same time of day. Animals had food and water ad libitum while in their home

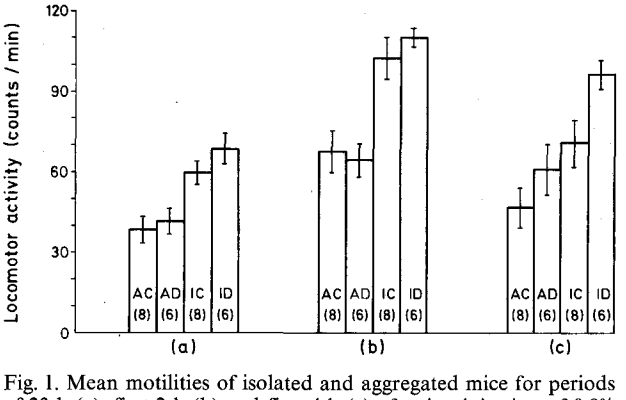

Fig. 1. Mean motilities of isolated and aggregated mice for periods of 23 h (a), first 2 h (b) and first 4 h (c) after i.p. injection of 0.9% NaCl or d-amphetamine-SO_4 (2 mg/kg). AC, aggregated control; AD, aggregated with drug; IC, isolated control; ID, isolated with drug. Vertical lines indicate SEM; numbers of animals in parentheses; all values for isolated mice are significantly greater than corresponding values for aggregated mice ($p < 0.02$; Wilcoxon's sum of rank test). The effect of d-amphetamine on isolated mice was significant only when measurements taken over the first 4-h period were combined (c); the drug produced no significant effect on the locomotor activity of aggregated mice. Animals were differentially housed for 25-47 days before testing, and were tested individually to avoid fighting activity among isolated mice.