conditions, the results demonstrate that at least the 2 nonradioactive techniques do not allow one to calculate 'absolute' turnover rates. Both inhibitors of the catecholamine synthesis also affect central monoamines by depleting dopamine and noradrenaline (H 44/68)[5,14-16] or noradrenaline (FLA 63)[5,13-16] stores. Furthermore, inhibition by H 44/68 leads to a decrease of the physiologically elevated motor activity in rats during D[17], and also antagonizes the central stimulation induced by amphetamine, dexamphetamine, H 77/77, L-dopa and various anticholinergic drugs[18-23]. Inhibition by FLA 63 of the stimulant properties of dexamphetamine, L-dopa and H 77/77 on motility was also described[19,22,24]. In naive mice, Svensson and Waldeck[25] reported a decrease in motor activity due to FLA 63 alone, whereas in rats this was not observed[17]. However, it has been shown that basal and drug-induced increased motility[26,27], as well as a drug-induced decrease in motility, are greatly dependent on the strain of rats and mice used[27,28]. Thus, these data clearly show that both H 44/68 and FLA 63, by depleting central catecholamine stores, obviously decrease cardiac sympathetic activity. This may well explain the lower turnover rate of cardiac noradrenaline both during L and D in comparison with the data obtained with the radioactive technique. Nevertheless, the experiments with FLA 63, in accordance with those obtained after H 44/68[7] and with ^{3}H-(-)-noradrenaline[7-9], clearly demonstrate that the turnover of noradrenaline in whole rat hearts as well as in heart atria and ventricles displays a circadian-phase-dependency.

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2 Friedman, A.H., and Walker, C.A., J. Physiol., Lond. *197* (1968) 77.
3 Quay, W.B., Gen. comp. Endocr. *3* (1963) 473.
4 Wurtman, R.J., and Axelrod, J., Life Sci. *5* (1966) 665.
5 Lemmer, B., and Berger, T., Naunyn-Schmiedeberg's Arch. Pharmak. *303* (1978) 257.
6 Costa, E., Biochem. Pharmac. *2* (1970) 169.
7 Lemmer, B., and Saller, R., Naunyn-Schmiedeberg's Arch. Pharmak. *282* (1974) 75.
8 Lemmer, B., and Wenda, K., Pharmac. biochem. Behav. *10* (1979) 361.
9 Lemmer, B., and Charrier, A., Naunyn-Schmiedeberg's Arch. Pharmak. *313* (1980) 205.
10 Weimer, R., thesis. University of Frankfurt, Frankfurt 1982.
11 Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P.G., and Hefti, F., Biochem. Pharmac. *23* (1974) 2437.
12 Chang, C.C., J. Neuropharmac. *3* (1964) 643.
13 Florvall, L., and Corrodi, H., Acta pharmaceut. suec. *7* (1970) 7.
14 Corrodi, H., Fuxe, K., Hamberger, B., and Ljungdahl, Å., Eur. J. Pharmac. *12* (1970) 145.
15 Saller, R., thesis. University of Frankfurt, Frankfurt 1976.
16 Andén, N.-E., and Fuxe, K., Br. J. Pharmac. *43* (1971) 747.
17 Lemmer. B., and Berger, T., Naunyn-Schmiedeberg's Arch. Pharmak. *303* (1978) 251.
18 Weismann, E.D., and Koe, B.K., Life Sci. *4* (1965) 1037.
19 Svensson, T.H., Eur. J. Pharmac. *12* (1970) 161.
20 Thornburg, J.E., and Moore, K.E., Neuropharmacology *12* (1973) 853.
21 Thornburg, J.E., and Moore, K.E. Neuropharmacology *12* (1973) 1179.
22 Lassen, J.B., Psychopharmacologia, Berl. *37* (1974) 331.
23 Ahlenius, S., Brain Res. *69* (1974) 57.
24 Strömberg, U., and Svensson, T.H., Psychopharmacologia, Berl. *19* (1971) 53.
25 Svensson, T.H., and Waldeck, B., Eur. J. Pharmac. *7* (1969) 272.
26 Davis, W.M., Babbini, M., Pong, S.F., King, W.T., and White, C.L., Pharmac. biochem. Behav. *3* (1974) 803.
27 Lemmer, B., Caspari-Irving, G., and Weimer, R., Pharmac. biochem. Behav. *15* (1981) 173.
28 Anisman, H., and Kokkinidis, L., Psychopharmacologia, Berl. *45* (1972) 243.

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Voltage oscillations in mammalian metaphase II oocytes

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Summary. The membrane potential has been measured in ovulated mouse oocytes using conventional electrophysiological techniques. Temporal oscillations in membrane voltage have been observed in the oocytes, with periods of about 6 h. This oscillatory pattern, peculiar to oocytes in metaphase II, might explain the differences in membrane potential values reported in several studies on mammalian oocytes.

Biological oscillations span a wide range of frequencies and wave-like phenomena. Circadian rhythms in mammalian biological clocks for instance, measure periods in h, while biochemical oscillations tend to have periods of the order of min[2]. Oscillatory patterns of membrane potential with a shorter period (of the order of sec)[3-4], have been shown in vertebrate nerve cells. Wave-like oscillations of resting membrane potential (RP) with a period of about 6 h are here described in mouse oocytes in metaphase of the 2nd meiotic division.

Materials and methods. Egg collection. Unfertilized and fertilized eggs were obtained from the oviducts of unmated or mated superovulated mice (Swiss CD1). Superovulation was induced by i.p. gonadotropin injection[5]. The interval between human chorionic gonadotropin (hCG) injection and egg collection was between 12 an 24 h. The oocytes were cleared of cumulus cells by treatment with hyaluronidase, (300 IU ml^{-1}). Isolation and cell washing procedures were performed in MH, a HEPES-buffered oocyte culture medium with the following composition (mM); NaCl, 94.6; KCl, 4.8; $CaCl_2$, 1.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; Na-lactate, 23.3; Na-pyruvate, 0.33; glucose, 5.6. The medium was buffered with HEPES-NaOH, 25 mM. Following isolation, oocytes were immediately processed for electrophysiology. In some experiments, as indicated in the text, the oocytes were collected 12 h after hCG injection and cultured at 37 °C for various times before electrophysiological recordings were made.

Electrophysiology. Intracellular recordings were made using conventional electrophysiological techniques. Glass microelectrodes (20–40 MΩ resistance) were filled with 3 M KCl, and connected to a W.P. Instr. Mod. M4A electrometer. The bath was connected to ground via a standard MH-agar bridge. The membrane potential was determined by recording differentially between an intracellular microelectrode and an extracellular pipet, filled with saline-agar. The oocytes were penetrated by oscillating microelectrodes placed perpendicularly in contact with the cell surface. Microelectrodes showing a potential drift in the external solution of more than 1 mV/min were rejected. Determinations were made at room temperature (19–21 °C).

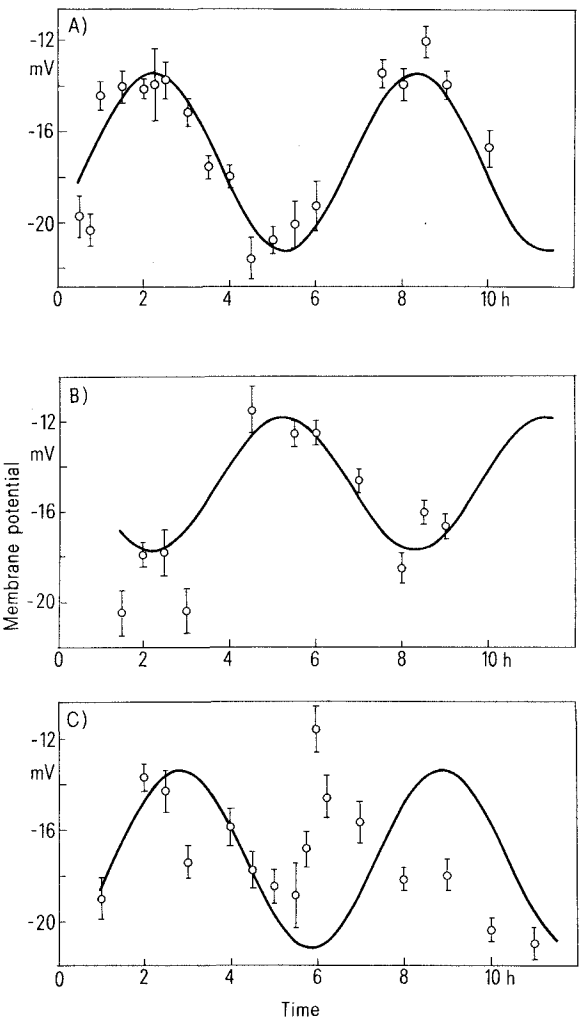
Results and discussion. Metaphase II oocytes were collected from the oviducts at various intervals following the superovulation-eliciting injection of hCG (12–24 h) and penetrated by a single electrode. The measured RP displayed an oscillatory pattern. The function best fitting the experimental data (mean values ± SEM) computed using the method of least square and reported in the figure A, was: $V = V_0 + A\sin(2\pi t/T + \phi)$ where V indicates membrane potential; V_0, mean potential around which the values oscillate; T, period; t, time; ϕ, phase angle.

Experiments were performed to investigate whether membrane voltage changes with reproducible waveform were also detectable in oocytes collected immediately after ovulation and cultured in vitro for various times. In 6 experiments, we examined oocytes ovulated before 12 h following hCG injection. Voltage temporal oscillations were still observed in cultured oocytes, and the data were best fitted by the above reported sinusoidal function, having the same period. These findings suggest that: a) rhythmic voltage activity may be expression of a sort of intrinsic biological clock triggered by ovulation; b) voltage oscillations are not correlated with the tubal environment but appear to be independent of the cell surroundings. The figure, B gives an example of this kind of experiments. The shift in time (about 2 h) in the plot of the figure, B vs A, may be related to the fact that the data of the figure, B were determined in cells ovulated 11–12 h after hCG. In the figure, A on the other hand, data represent statistical results of determinations performed in oocytes ovulated between 11.00 and 13.30 h after hCG. The smaller amplitude of the oscillations in the plot of the figure, B *vs* A (i.e. 2.92 vs 3.89 mV) was not a general result of this kind of experiments; it may be attributed to statistical fluctuations of the membrane voltage in the different cell populations examined.

Experiments of same kind as those summarized in the figure, A, have been made in fertilized eggs (fig., C), collected from the oviducts of mated mice, in order to see if the membrane voltage oscillatory pattern is peculiar to metaphase II oocytes, or is also present in fertilized eggs. Data reported in the figure, C were fitted by identical sinusoidal function, and only the phase angle ϕ, was adjusted to give the 'best' fit. As shown in the figure, C, only the data obtained at earliest postovulatory times (12–15 h after hCG), followed the same law as those obtained in oocytes collected from unmated mice. These findings indicate that fertilization, which in our system presumably occurs at 15–16 h after hCG injection[5], modifies the voltage oscillatory pattern by dumping oscillations and by shifting the membrane potential in the positive direction.

Data in the literature show that mouse metaphase II oocytes have RP-values ranging from −14 to −24 mV. MF-1 oocytes show for instance an RP of around −14 mV[6]; BDF oocytes, from −20 to −23 mV[7]; ICR and DDy oocytes, of −23.1 ± 2.9 mV[8]; CD1 oocytes, of −24 ± 1.4 mV[9]. These differences of RP-values have been usually attributed to the different strains of mice studied[7]. The present paper shows that postovulatory voltage temporal oscillations may be at least in part responsible for the reported dispersion of RP-values.

At present there is no general theory of biological oscillating phenomena which may explain the origin of the waveform voltage changes recorded in metaphase II mouse oocytes. Events which might be responsible for the membrane voltage oscillations reported here, are: a) a differential 'short-circuiting' ionic leakage around inserted electrodes during metaphse II; b) an oscillation of intracellular H^+ or Ca^{2+} concentration; c) a rhythmic transmembrane



Membrane voltage oscillations in unfertilized and fertilized eggs. *A* Oscillations in ovulated unfertilized eggs at various postovulatory times. After collection from the oviduct, the oocytes were immediately processed for electrophysiological recordings. Data represent mean ± SEM (n ranging from 30 to 50). Zero in abscissa corresponds to 12 h after hCG injection. The curve best fitting experimental data (computed using the method of least squares) was traced by a CDC 6600 computer. The data fitting equation is: $V = -17.3 + 3.89\ \sin(2\pi t/6.07 - 0.73)$. *B* Voltage temporal oscillations in cultured oocytes. Oocytes were collected from 6 oviducts, 12 h after hCG injection, and then cultured. Data (mean ± SEM; n = 10) were determined after various intervals of culture. The data fitting equation is: $V = -16.77 + 2.92\ \sin(2\pi t/6.07 + 2.73)$. Abscissa and ordinate, as in (A). *C* Non-sinusoidal oscillations of membrane potential in eggs collected from mated mice. Data (n ranging from 25 to 48) were fitted by same equation as in (A) by adjusting the phase angle, ($\phi = -1.3$). Abscissa and ordinate, as in (A) and (B).

calcium or potassium ions influx; d) oscillating transmembrane active transports; e) an oscillatory voltage affecting rearrangement of fixed charges on the membrane surface[10] during oocyte maturation. Hypothesis a) may be discarted since membrane input resistance variations (105 ± 15 MΩ; $n = 10$) are random and did not follow potential oscillations. Events b) and c) can only explain temporal non-sinusoidal oscillations of membrane potential of a shorter period (order of seconds)[11]. Hypothesis d) is unlikely since the experiments were performed at room temperature; under these conditions active transport is supposed to be reduced or abolished[12]. Consistent with hypothesis e) is a rearrangement of the cell membrane due to spontaneous release of cortical granules in ovulated oocytes[13]. Whatever the origin of this type of ascillatory pattern, it indicates a state of electrical instability of the oolemma in the ovulated oocytes that might play a role during sperm-egg interaction[14].

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2 Halberg, F., A. Rev. Physiol. *1036* (1969) 675.
3 Poulsen, J.H., and Williams, J.A., Nature *263* (1976) 156.
4 Kuba, K., J. Physiol. *298* (1980) 251.
5 Edwards, R.G., and Gates, A.H., J. Endocr. *18* (1959) 292.
6 Fulton, B.F., and Whittingham, D.G., Nature *273* (1978) 149.
7 Powers, R.D., and Tupper, J.T., Devl Biol. *38* (1974) 320.
8 Okamoto, H., Takahashi, K., and Yamashita, N., J. Physiol. *267* (1977) 165.
9 Eusebi, F., Mangia, F., and Alfei, L., Nature *277* (1979) 651.
10 Madden, K.S., and Van Der Kloot, W., J. Physiol. *276* (1978) 227.
11 Kuba, K., and Takeshita, S., J. theor. Biol. *93* (1981) 1009.
12 Thomas, R.C., Physiol. Rev. *52* (1972) 563.
13 Nicosia, S.V., Wolf, D.P., and Inoue, M., Devl Biol. *57* (1977) 56.
14 Epel, D., in: The cell surface: mediator of developmental process, p. 169. Eds S. Subtenly and N.K. Wessels. Academic Press, New York 1980.

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The chorda tympani nerve and taste in the chicken

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Summary. By recording the electrical activity in the chorda tympani nerve following chemical stimulation of the mandibular taste buds it was demonstrated that this nerve conducts gustatory information from this region of the mouth. The presence of relatively large numbers of taste buds in this region of the mouth would suggest that contrary to previous findings the chorda tympani nerve has an important role in taste perception.

The facial nerve of birds is thought not to be involved in relaying taste information[1-6] but recent work on the upper bill of the mallard has shown that taste buds are innervated by the facial nerve[7]. In the chicken, taste buds are found adjacent to the anterior mandibular salivary glands which are situated in the buccal epithelium of the lower jaw[8]. This area of the buccal epithelium is innervated by the chorda tympani branch of the facial nerve. By recording the electrical activity in the chorda tympani nerve the present experiment demonstrates that it conducts neural impulses orginating in these mandibular taste buds.

Methods. Ten 15-week-old Brown Leghorn hens were anesthetized with sodium pentobarbitone (Sagatal, May and Baker Ltd) and the forebrain removed by gentle suction. After decerebration the head was fixed on its right side by means of a metal plate screwed into the dorsal skull, and the upper and lower beaks were cemented with dental acrylic onto a fixed metal plate. The mouth was held open and the tongue was pulled to one side to expose the lower buccal epithelium. The hens were paralyzed with gallamine triethiodide (Flaxedil, May and Baker Ltd) to prevent reflex swallowing and artificially ventilated through an air tube in the trachea. A unidirectional air flow was ensured by rupturing the abdominal air sacs after laparotomy. The heart rate was continuously monitored and the air flow was adjusted to usually about 2 l/min in order to maintain a constant heart rate which was similar to that before the gallamine injection. The body temperature of the animal was maintained at 40 °C by means of a heating blanket and monitored with a rectal probe.

After removal of the overlying skin and muscle and mandible was exposed. The dorsal part of this was removed to expose the mandibular canal. The chorda tympani (CT) nerve was exposed at the point where it joins the sublingual ramus of the mandibular nerve. The CT is a small nerve (about 70 µm in diameter) and it was dissected to the jaw articulation and cut. A suitable length of nerve (1.5 mm) was desheathed and placed over 2 silver wire recording electrodes. The surrounding skin was sutured to a stainless steel loop and pulled up to make a liquid paraffin pool to prevent drying of the nerve. The electrodes were connected to a preamplifier (DAM-6A, W-P Instruments Inc), displayed on a storage oscilloscope (5103N Tektronix Inc) and recorded on a tape recorder (Store 4DS, Racal Recorders Ltd).

The anterior buccal epithelium was stimulated for approximately 10 sec with 10 ml of the required solution maintained at body temperature delivered by a syringe place close to the epithelium. The solutions used were 1 M potassium, calcium, sodium and ammonium chlorides, 1 M fructose and sucrose, 0.1 M quinine hydrochloride, 0.05 M acetic, citric and hydrochloric acids, distilled water and Tyrode ringer (without glucose). Distilled water (pH 4.5) produced a pronounced response in most but not all preparations (fig. 1,i) whereas Tyrode ringer, had little or no effect (fig. 2,a). The salts and acids had of necessity to be made up in distilled water but all the other solutions were made up in ringer.

Neural activity was recorded for 2 min after the test solution had been placed on the preparation. After this, the buccal epithelium was washed with 30 ml of distilled water and followed by 20 ml of ringer. The preparation was allowed a 3-min period before another test solution was applied.

Results and discussion. An example of the electrical activity recordings is shown in figure 1,a. The other results are