ulate the generation of fluorescence from optics of the fluorometer was included in the model. The result has been superimposed on the data in figure 2. The curve generated by the computer compares well on a qualitative basis with the experimental data, showing maximum absorption of both the excitation and fluorescent light halfway across the muscle. The rate of change in fluorescence as the muscle is brought in and out of the light beam differs slightly from that which the model predicts. This is believed to be a result of the physical limitations of the apparatus and reflection of fluorescent light from the inside surface of the 0.3 mm diameter aperture. Furthermore, the above equations describe the production of fluorescence in a homogeneous medium, but the actual tissue fluorescence will be influenced by many factors including the total number of fluorescent 'sites' in a given volume of tissue, quenching of fluorescence and the light scattering properties of the muscle fibers.

To estimate the thickness of muscle at which absorption should begin to predominate, a simulation was performed plotting fluorescence against the calculated light path through the muscle using a larger muscle diameter of 3.7 mm (fig. 3). The result shows that fluorescence should increase with thickness up to 0.65 mm before absorption of excitation and fluorescent light becomes too great.

To investigate the situation in which the fluorescent light is monitored from the same plane as the incident light, a second model was developed as follows

$$I_{(465)} = \alpha \int_0^t I_{0(365)}\, e^{-a_1 x} \cdot e^{-a_2 x}\, dx\,. \qquad (4)$$

The above formula describes the fluorescence output originating from points which are at a distance x from the surface of a muscle of total thickness t. All the constants have their previous meanings. Evaluating the integral for the complete thickness of the muscle produces

$$I_{(465)} = \frac{\alpha I_{0(365)}}{a_1 + a_2} \cdot \left[1 - e^{-(a_1 + a_2)t}\right]. \qquad (5)$$

Using the same values for a_1 and a_2, a simulation over 50 increments of thickness up to 3.0 mm was performed and the results shown in figure 4. At 1.00 mm thickness approximately 85% of the total fluorescence was being detected. A further doubling of the muscle thickness resulted in increasing the fluorescence signal by the remaining 15%. Thereafter, further increases in muscle thickness produced little effect on the total fluorscence output owing to almost complete absorption of the incident and fluorescent light by the first 2.0 mm of tissue.

It is apparent from both experimental and theoretical considerations that ability to detect fluorescence from deep layers of tissue is severely limited by light absorption. It is equally apparent that the method of epifluorescence as used with cardiac tissue does not exclusively give an answer as to the metabolic state of the surface region since fibers situated as deep as 0.65 mm (or 40 fiber diameters) will contribute appreciably to the recorded signal.

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Morphine selectively facilitates the inspiratory-inhibitory vagal reflex in adult rabbits

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Summary. In rabbits naive to opiates or pretreated with morphine a selective morphine-induced facilitation of the Breuer-Hering inflation reflex is described.
Key words. Breuer-Hering inflation reflex; morphine; naloxone.

Direct central action of opiates on respiration occurs at the level of chemosensitive medullary areas[7] and respiratory neurons[4]. The effects of opiates on the respiratory control system have been recently reviewed by Trippenbach[9], with some remarks on vagal modulation of respiratory timing. Champagnat et al.[3] observed that in animals with bilateral cervical vagotomy, depres-

gram of the C_5 phrenic root was continuously recorded. Series of tracheal occlusions starting randomly at different phases of inspiration were applied. Each occlusion was continued to the onset of the following inspiration. Tracheal occlusions were performed before and after the giving of i.v. doses (3–5 mg) of morphine. For comparison, pentobarbital (5 mg) and chlora-

at the closing point of each inflation as the meeasure of stimulus intensity. It was previously shown that P_{TP}, the measure of inflation depth, is also an indicator of vagal afferent input from the lungs[8].

The plot of i vs the respective closing point P_{TP} is well described by an exponential fit $i = \exp(a\ P_{TP})$, since for $P_{TP} = 0$, T_E (occl.) = T_E (ctrl.); the additive constant of the regression line is zero. In a semilogarithmic scale the regression is linear, $\ln i = a\ P_{TP}$; correlation coefficients of the least squares regression fit (r) > 0.9 in all experiments. The slope, a, is a reproducible and sensitive measure of the strength of the BH reflex. Increased slope means increased reflex strength; for a given stimulus intensity, P_{TP}, the response, i, is bigger.

A summary of the experimental data is given in the table. In all cases morphine increased the reflex strength significantly. The opiate antagonist naloxone reversed the effects of morphine, which speaks for the involvement of opiate receptors in the modulation of the reflex. After the additional dose of chloralose and urethane P_{TP} decreased, with unchanged frequency of breathing, while after pentobarbital both P_{TP} and frequency were depressed; in both cases the reflex strength remained unchanged.

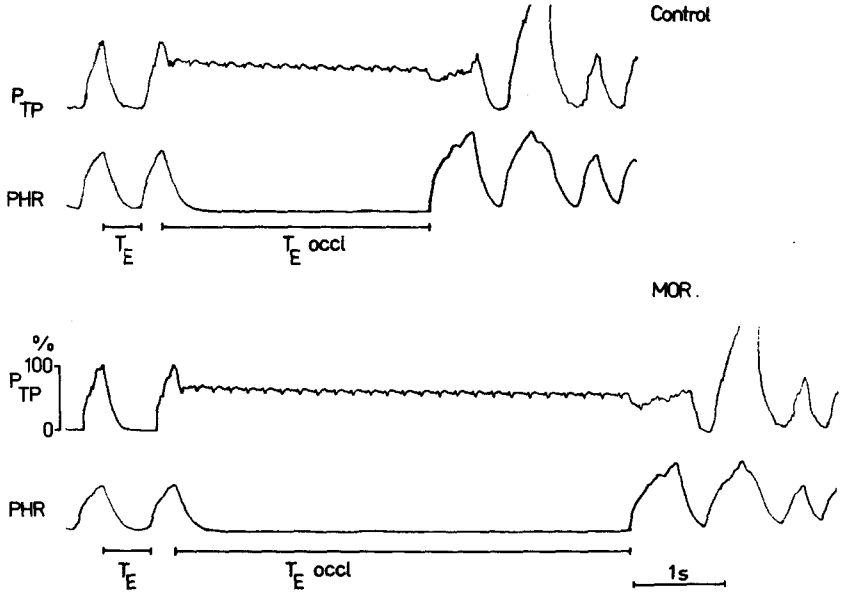
It is evident from the results obtained that the technique employed in the present study measures the strength of the BH reflex independently of the actual pattern of breathing. In the case of non-opiate anesthetics, the breathing pattern can be pharmacologically manipulated without an accompanying change in the strength of the BH reflex. Our observation that pentobarbital does not change the reflex strength differs from that of Bouverot et al.[1], but the small dose of the drug given in the present study may be responsible for this.

The present data show that following morphine the inspiratory-

Parameters of the linear regression of ln i vs P_{TP}, and breathing pattern before and after injection of the drugs

Rabbit experiment	n	Slope (10^{-3})	r	p	P_{TP}(%)	f/min
1 Control	23	10.15 ± 0.32	0.987	–	–	91.5
Morphine	27	12.04 ± 0.38	0.991	0.00045	0	62.0
2 Control	22	25.07 ± 0.80	0.992	–	–	62.0
Morphine	24	28.45 ± 0.86	0.989	0.0064	0	53.5
3 Control	23	6.84 ± 0.23	0.987	–	–	48.0
Morphine	7	24.19 ± 3.26	0.953	0.00001	−17	30.5
Naloxone	24	5.60 ± 0.27	0.978	0.00001	+2	57.5
Urethane and chloralose	19	5.79 ± 0.32	0.979	n.s.	−14	58.0
4 Control	25	10.42 ± 0.60	0.981	–	–	49.5
Morphine	16	16.84 ± 0.57	0.991	0.00001	−20	43.5
Morphine	8	19.55 ± 0.78	0.994	0.035	−16	34.0
Naloxone	19	12.41 ± 0.92	0.966	0.0016	−5	49.5
Pentobarbital	14	13.33 ± 0.40	0.994	n.s.	+2	42.0
5 Control	13	24.37 ± 1.42	0.988	–	–	47.5
Morphine	18	30.71 ± 1.86	0.969	0.012	0	36.0
Naloxone	11	22.73 ± 1.12	0.988	0.002	+5	48.0

Columns from the left: Rabbit, No. of the animal; experiment, drug tested; n, number of occlusions; slope, the slope of the least squares regression fit ln $i = a\ P_{TP} \pm$ its SD; r, correlation coefficient of the above; p, probability that the value of the slope is identical to the preceding value (by two-tail t-test); P_{TP}, percent change in transpulmonary pressure during unobstructed breathing, compared to control; f, respiratory frequency. [a]Animals pretreated with morphine. Drugs tested: control (general anesthesia); naloxone 0.04 mg; morphine 3–5 mg; pentobarbital 5 mg; urethane and chloralose, ⅓ of anesthetic dose. All drugs given i.v. Fitting of the data and statistical testing were performed according to Wine[10].



he effect of post-inspiratory tracheal occlusion. Original recordings of the transpulmonary pressure (P_{TP}) and integrated phrenic nerve activity (PHR) efore (control) and after (MOR) i.v. administration of 0.7 mg kg^{-1} of morphine. T_E = time of expiration (T_E(control)); T_E occl = time of expiration rolonged by occlusion; 100% P_{TP} = inspiratory peak of P_{TP} before occlusion.

inhibitory vagal reflex is accentuated, which is in agreement with the early observation of Ngai[6]. However, we should point out that our approach may lead to somewhat different conclusions from those of more usual experiments where the prolongation of T_E during maximal occlusion is taken as the measure of the strength of the BH reflex. An example is the result of Grunstein and Grunstein[5]. These authors concluded that enkephalin, an endogenous opioid peptide, does not change the inspiratory-inhibitory vagal reflex in the newborn. However, if the reflex strength is expressed as the ratio of T_E (occlusion) to T_E (control), their data show that the reflex strength decreases. It seems, therefore, that the ontogenesis of opiate modulation of the vagal respiratory input may require a closer investigation.

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Off-responses of newt pit organs after chemical stimulation[1]

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Summary. Distilled water rinsing after stimulation of the newt pit organ with divalent chloride salts produced neural off-responses. The off-response was selectively depressed by $HgCl_2$ treatment and was not suppressed by a water rinse containing NaCl. The results indicate that the off-response might be due to the removal of the divalent cations bound to the receptor membrane.
Key words. Newts; *Cynops pyrrhogaster;* pit organ; neural off-response; $HgCl_2$ treatment.

Some investigators have demonstrated that in aquatic vertebrates a lateral line organ, the pit organ, has a conspicuous sensitivity to various chemical stimuli[3-6]. Previously we reported that when the newt lower jaw, on which many pit organs are distributed, was rinsed with distilled water. After a chemical stimulation, a marked integrated response was obtained from a mandibular branch of the lateral line nerve supplying the pit organs[7]. There have been no studies on this kind of response in the pit organs, although a similar response, the so-called 'water response', in the gustatory organ has been studied by many investigators[8-11]. The aim of the present experiments was to elucidate the effect of a water rinse after stimulation of the newt pit organ with divalent chloride salts.

Materials and methods. Male newts *(Cynops pyrrhogaster)* anesthetized with urethan (9 mg/g b.wt, i.p.) were used in the present experiments. Impulse discharges recorded from a mandibular branch of the lateral line nerve were passed through an electronic integrator circuit (Nihon Kohden Model EI-600G) with a time constant of 0.3 s and displayed on an ink-writing recorder. Test solutions made from reagent grade chemicals dissolved in distilled water were applied to the lower jaw at the flow rate of approximate 6 ml/min, after that distilled water was delivered continuously to rinse the lower jaw at the same flow rate. The response produced by the distilled water rinse is here termed the 'off-response' against the 'on-response' produced by the test solution. The height in mm, from the level immediately before the onset of each of the integrated on- and off-responses to the peak, was used as their response measures (see inset in fig. 2). To examine the effects of pretreatment with transition metal ion on the off-response, in some cases test solution was delivered after the lower jaw was treated with $HgCl_2$ or $CuCl_2$ for 1 min. After the treatment the lower jaw was rinsed continuously until immediately before the application of test solution. The effects of chelating agents after the treatment were also examined. The room temperature was kept at 20 ± 2 °C throughout the experiment. All the solutions and distilled water used were adapted to room temperature prior to use.

Results and discussion. Figure 1 represents the relation between the off-response of the newt lateral line nerve after $CaCl_2$ stimulation and $CaCl_2$ concentration. Threshold concentration for the off-response after $CaCl_2$ was around 10^{-5} M. The magnitude of the off-response increased with increasing concentration of $CaCl_2$ at lower concentrations, but it decreased abruptly at higher concentrations above 0.3 M (fig. 1). This type of relationship between off-response and concentration was common to other divalent chloride salts, $SrCl_2$ and $MgCl_2$. The magnitude of the off-response had a tendency to depend on the duration of stimulation by $CaCl_2$; in general, it increased slightly with stimulus duration up to 1 min. However, the magnitude of the off-response was unchanged for successive $CaCl_2$ stimulations with a given stimulus duration. On the other hand, the magnitude of the on-response to $CaCl_2$ increased with the concentration from 10^{-5} M to 3×10^{-2} M, while it decreased gradually above 0.1 M, as described previously[7]. Similar concentration ranges for the production of on- and off-responses were obtained for $SrCl_2$ and $MgCl_2$.

The relationship between the magnitude of the on-response to 0.1 M $CaCl_2$, which produced the largest off-response, and that of the subsequent off-response in 21 newts is shown in figure 2. A highly significant negative correlation coefficient was noticed between the two responses ($r = -0.90$, $p < 0.001$ $n = 21$), and the algebraic sum of individual on- and off-responses showed a similar value. A similar relation between on- and off-responses was observed for 0.1 M $SrCl_2$ ($r = -0.92$, $p < 0.001$ $n = 17$) and 0.1 M $MgCl_2$ ($r = -0.92$, $p < 0.001$, $n = 17$).

The relation between the $CaCl_2$ response and the subsequent off-response in the present experiments closely resembles the results[11] obtained from the rat chorda tympani nerve, in which water applied to the tongue after stimulation with 1 M sucrose produced an off-response. There existed an inverse relation be-