about $^1/_{500}$ of that of dinitrophenol, which is now classified as a classical uncoupler because of its relatively weak uncoupling power in comparison with that of a powerful uncoupler[10,11]. As described in the introductory statement the halogeno-ethanols have a significant ability to cause perturbation of the configuration of the hydrophobic groups in a protein, but 1-propanol, on the other hand, has a poor ability to cause such perturbation. The difference in their ability is probably due to the following; the nature of the hydrophobicity of the protein hydrophobic groups is different from that of the halogeno groups but identical with that of the nonpolar group of 1-propanol[3]. This leads us to a hypothesis interpreting the mode of uncoupling by these halogeno-ethanols; 2-chloroethanol, 2-bromoethanol and 2,2,2-trifluoroethanol perturb the structure of the inner membrane to cause leakage of protons from the outside to the inside. The ineffectiveness of 2-fluoroethanol in figure 1 may be due to the poor hydrophobicity of its fluoroethyl group - insufficient to cause the above uncoupling effect. It is now recognized that the enhancement of the uncoupling power the introduction of halogeno groups into a protonophore-type uncoupler results from both an increase in its hydrophobicity and a decrease in the pK_a[12-14]. This study, however, indicates that the introduction also gives the uncoupler the ability to cause an effective change in the structure of the inner membrane.

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Adenosine-3, 5-phosphate levels in brain structures of rats submitted to four different behavioral procedures

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Summary. Shuttle avoidance training decreased the cAMP content of rat brain (excluding hippocampus and caudate nucleus), amygdala and hypothalamus. Stimulation by tones alone had a similar effect, but only in the brain fraction. Pseudoconditioning or footshocks alone had no effect.

Brief aversive learning experiences in rats or mice are accompanied by increased rates of phosphorylation of brain chromosomal[2-4] and synaptosomal[5] proteins. This effect probably depends on the activation of protein kinases by adenosine-3′, 5′-phosphate (cAMP)[2,4]. Therefore, regional changes in brain cAMP levels might be expected during the initial stages of aversive conditioning. Hambley and Rose[6,7] reported an early fall, followed by a late increase, of cAMP levels in the brain of chickens submitted to another form of learning, namely, visual imprinting. The present note reports on the effect of 5- or 25-min of shuttle avoidance training, pseudoconditioning, footshocks, and tones, on the cAMP content of various brain regions of the rat.

Material and methods. Adult male Wistar rats from our own breeding stock were used (age 59–70 days; weight 135–195 g). Behavioral procedures were carried out in 50 × 25 × 25 cm noncompartmentalized shuttleboxes made of acrylic[4,8]. The floor of these boxes was a grid of 2 mm bronze bars spaced 7 mm apart. A flat, 0.5-cm-wide, piece of acrylic at the midline was the only mark between the right and left sides of the floor. A loudspeaker was attached to the rear wall 15 cm above floor level, at the midline. Animals were divided into 5 groups, each submitted to a different behavioral treatment, as follows:

1. Pseudoconditioning. 5 sec, 1 kHz, ≅ 70 db tones were presented at randomly variable intervals of 10–50 sec, at a rate of 10 tones every 5 min. Footshocks (60 Hz, 1 mA, 2 sec) were interspersed among the tones at randomly variable tone-shock intervals of 5–45 sec, as follows: 8 shocks among the first 10 tones, 7 among the following 10 tones, 5 among the next 10 tones, 3 among the next 10 tones, and 2 among the last 10 tones. This approximately matches the distribution of footshocks over time obtained in the following behavioral procedure[4,8].
2. Shuttle avoidance. Tones were presented as above, but each tone was immediately followed by a footshock (contiguity) unless the animals performed a shuttle response to the tone (avoidance contingency)[8].
3. Tones alone. Tones were presented every 10–50 sec; no shocks.
4. Footshocks alone. Footshocks delivered every 10–50 sec; no tones.
5. Intact controls. Animals taken out from their home cages and sacrificed right away.

In the 4 former groups, the behavioral treatments were carried out during either 5 or 25 min. In all cases, interstimulus intervals were programmed in such a way, that the animals submitted to 5 min sessions received 10 tones (or trials, or footshocks), and those submitted to the 25-min sessions received 50 tones (or trials, or footshocks).

Animals were sacrificed by decapitation within 30 sec from termination of each of the above described behavioral procedures. The brain was immediately taken out, put on ice and dissected into either hippocampus, caudate nucleus and rest of the brain (excluding cerebellum but including amygdala and hypothalamus), or amygdala and hypothalamus. Tissues were homogenized at less than 4 °C in 20 times their volume of 6% trichloroacetic acid, and subsequently processed for cAMP determination by radioimmuncassay[9] using the New England Nuclear Corporation kit NEX-132. Protein content of each tissue was measured in aliquots of the homogenates by the Folin phenol reagent method[10]. Statistical comparisons were by a randomized-group analysis of variance followed by a Duncan multiple range test[11].

Table 1. Performance of shuttle responses by rats submitted to pseudo-conditioning in a shuttle-box, to shuttle avoidance learning, and to tones alone

Group	Shuttle responses to the tone			Intertrial shuttlings over 25 min
	Tones 1–10	Tones 41–50	Tones 1–50	
Pseudoconditioning	1.0±0.2 (22)	0.9±0.2 (14)	5.1±0.2 (14)	2.6±1.4 (14)
Shuttle avoidance	2.4±0.3[a] (25)	4.9±0.6[a,b] (17)	18.8±0.4[a] (17)	2.2±0.8 (17)
Tones alone	0.6±0.2 (22)	0.1±0.1[a,c] (15)	1.2±0.2[a] (15)	10.1±1.3[a] (15)

Values are means±SE. Number of animals in parentheses. [a] Significant difference from the other 2 groups at 0.5% level. [b] Significant difference from performance during tones 1–10 of the same group at 0.5% level, [c] at 1% level.

Table 2. cAMP levels (pmoles/mg protein) of brain structures of rats submitted to 5 or 25 min of pseudoconditioning, shuttle avoidance, tones alone, or shocks alone in a shuttle-box (means±SE)

Group	Hippocampus	Caudate	Rest of brain	Amygdala	Hypothalamus
Intact controls	18.9±1.2 (9)	15.9±1.7 (7)	20.7±1.1 (7)	32.7±1.4 (10)	56.6±4.2 (10)
Pseudoconditioning (5 min)	16.4±0.8 (8)	15.4±1.0 (8)	17.6±1.2 (8)	–	–
Shuttle avoidance (5 min)	18.0±1.4 (8)	15.6±1.1 (8)	16.8±1.2 (8)	–	–
Tones alone (5 min)	20.7±2.2 (7)	15.6±0.8 (8)	18.5±2.0 (7)	–	–
Shocks alone (5 min)	16.3±0.7 (7)	15.1±0.7 (7)	18.0±1.6 (7)	–	–
Pseudoconditioning (25 min)	16.9±1.2 (6)	16.8±2.6 (6)	16.8±1.4 (6)	31.3±3.0 (8)	58.4±4.9 (8)
Shuttle avoidance (25 min)	18.2±1.8 (7)	15.6±2.3 (7)	15.5±1.1[a] (7)	26.9±2.0[b] (10)	41.2±4.1[b] (10)
Tones alone (25 min)	16.9±1.5 (6)	13.6±0.8 (6)	15.8±1.3[b] (6)	30.1±2.6 (9)	50.9±5.4 (9)
Shocks alone (25 min)	15.8±0.8 (6)	14.4±0.9 (6)	17.0±0.5 (6)	31.6±2.2 (8)	59.7±4.8 (8)

Number of animals in parentheses. [a] Significant difference from intact controls at 1% level, [b] at 5% level.

The main behavioral results are shown in table 1. Animals trained in the avoidance task made more shuttle responses to the tone than those submitted to pseudoconditioning, and animals trained with tones alone made less. In the avoidance group, the incidence of responses increased between the first and the last block of 10 trials (avoidance conditioning[4]. In the tones alone group, the opposite was observed (habituation[4,8,12]). The latter made more intertrial shuttlings than any of the other groups. In those tests which included footshocks, all animals responded to all shocks with shuttling (100% escape responding).

Biochemical findings are shown in table 2. No differences in cAMP levels were observed among the 5-min groups. In the 25-min groups, the cAMP content of the rest of the brain was lowered after avoidance training and after habituation. In those animals in which the amygdala and hypothalamus were studied, shuttle avoidance caused a decrease of the cAMP content of both structures.

The cAMP changes were opposite to what might have been expected from the literature of the effect of sensory stimulation or learning on brain protein phosphorylation[2–5]. In addition, they occurred much later (at 25 rather than at 5 min[2–4]), and in different brain structures (rest of the brain, amygdala and hypothalamus instead of hippocampus and caudate nucleus[4]). Hambley and Rose[6,7] also were unable to correlate their observations on the effect of imprinting on chicken brain cAMP with their own data on the effect of that variable on RNA and protein synthesis[13]. This lack of correlation is unlikely to result from the fact that in the present experiments animals were sacrificed by decapitation, instead of microwave irradiation or immersion in liquid nitrogen[6,7]. For one thing, brain cAMP levels in our animals were closely similar to those reported in the same or other species by authors using different procedures for sacrifice[7,14–16]. For another, both the effect of visual imprinting[6,7], and of norepinephrine[15], on brain cAMP, seem to be independent both of the method of sacrifice, and of the basal cAMP level.

The cAMP changes shown above occurred only in those groups in which some measurable form of learning took place: avoidance and habituation; and, in addition, they correlate at least with the initial effect of imprinting on chicken brain cAMP[6,7]. Thus, the cAMP decrease is one of the very few biochemical changes reported so far which occurs in different forms of learning, and is not seen in pseudoconditioned or footshock-stimulated groups as well[2,4,13]. The functional meaning of this phenomenon is not known. The present findings suggest that different brain areas may be involved in avoidance learning and in habituation, which is consistent with a number of pharmacological observations from this laboratory[4,8,12].

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