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**Classical conditioning in planarians and an attempt
to transfer by cannibalism**

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Biologia. — *Classical conditioning in planarians and an attempt to transfer by cannibalism* (*). Nota di PATRIZIA MESSERI e FRANCESCO DESSÌ FULGHERI, presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — Nella planaria *Dugesia dorotocephala* è stato instaurato un condizionamento classico in una situazione luce-shock vibratorio. Successivamente individui condizionati sono stati dati in pasto ad individui non trattati: questi ultimi, sottoposti allo stesso procedimento di condizionamento delle loro vittime, hanno mostrato una facilitazione in confronto ai controlli.

Under an evolutive point of view planarians show traits of great interest. Actually encephalization, unipolar synapses and bilateral symmetry are found for the first time in Turbellarians.

Thompson and McConnell conditioned planarians in a Pavlovian way since 1955, associating light with electric shock [1]. Halas *et al.* repeated this experiment in 1962 with similar results [2]. In 1959 McConnell *et al.* evidenced a retention of memory in planarians regenerated from heads and tails of conditioned subjects [3], and in 1962 a transfer of memory in planarians was obtained through cannibalism by McConnell [4]. Since then many experiments were designed to test the hypothesis of the transfer of memory by cannibalism [5, 6, 7]. Presently there still are two controversial points which have delayed full acceptance of these findings [8, 9]: the first concerning the conditioning of planarians, because of the lack of adequate control groups of sensitization as defined by Beecroft [10] and pseudo-conditioning as defined by Lockhart and Grings [11]; the second concerning the transfer of memory by cannibalism, because of non homogeneous evidences in this respect.

The present work was carried out in an attempt to reach a well-controlled classical conditioning, and subsequently to add new evidences about transfer of memory by cannibalism.

EXPERIMENT I

Forty fissiparous specimens of the planarian *Dugesia dorotocephala* (Woodworth), all at the same stage of regeneration and feeding, experimentally naïve, were used. Between experimental sessions the subjects were

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maintained, each group in its trough, in dimmed light provided by the window. During the six days of the experiment they were never fed.

An experimental device for classical conditioning was used. An automatic Crouzet programmer controlled the succession of stimuli. A 60 Watt lamp, 70 cm from the experimental troughs, provided the conditioned stimulus

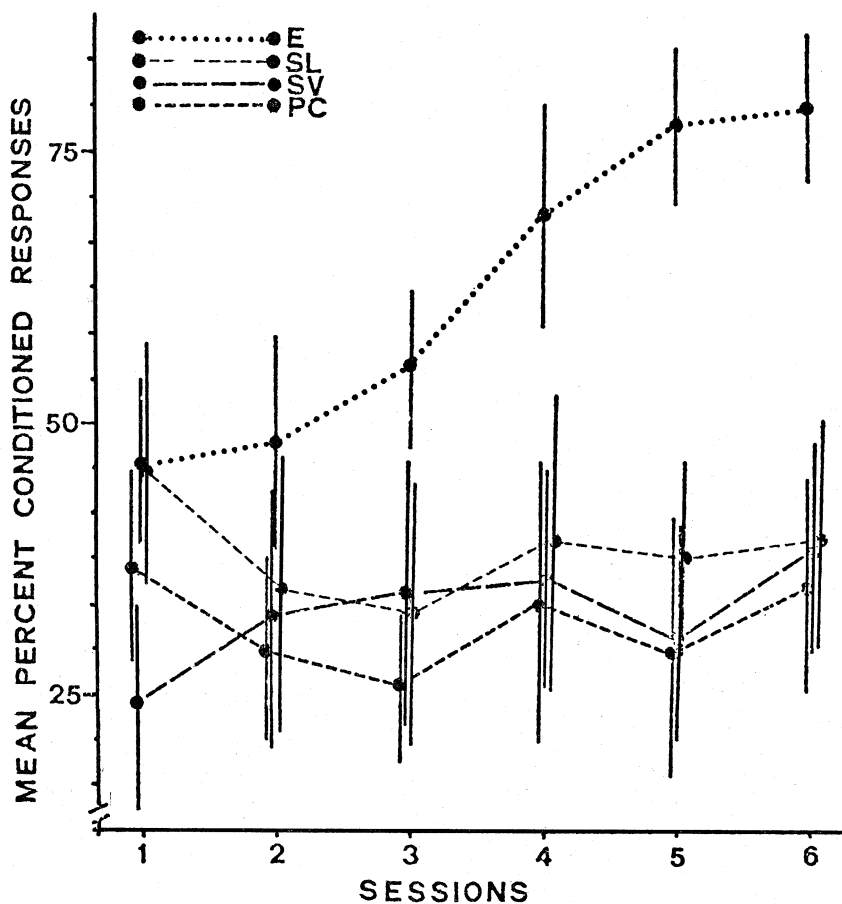


Fig. 1. - Mean percent conditioned responses $\pm f.l.0.05$ during six days of training in four groups of ten planarians. Group E: experimental; Group SL: control of sensitization to light; Group SV: control of sensitization to vibration; Group PC: control of pseudoconditioning.

(a 6 sec light of 150 lux on 1.5 environmental lux). During the last 3 sec the unconditioned stimulus (a vibration produced by a buzzer) was added. The vibration was conveyed to a vibrating plane which supported planarian troughs. The troughs were hemispherical bowls (common coffee saucers), in order to avoid vertical planes which might affect the response of planarians to light. The intertrial interval lasted 60 sec.

The experiment was performed in a thermostatic room, in which the temperature was kept within 21–23°C range. Trials began 5 min after the subjects had been gently transferred with a pipette from the housing to the experimental trough. Before every session water was changed, but slime never removed. Daily sessions were conducted at the same time during 6 days. Each session was limited to 48 training trials and 12 testing trials, administered in 1:4 ratio. Subjects were randomly assigned to four groups (10 subjects per group). In the training trial, Group E (experimental) received light paired to vibration, Group SV (control of sensitization to vibration) received vibration only, Group SL (control of sensitization to light) received light only, Group PC (control of pseudoconditioning) received a stimulation every 30 sec randomly represented either by light or by vibration. In the testing trials light only was administered. Two blocks of replication were carried out, but this was discounted in the analysis since no significant difference emerged.

As a rule the unconditioned response to vibration in planarians is represented by a contraction of the body. Since light evokes no contractions, a response to light was scored as conditioned when subjects exhibited a slight contraction followed by: *a*) a vigorous contraction, *b*) stopping, *c*) head retraction, *d*) rapid head turn, *e*) head rise from the substratum. Actually all these behavioral patterns are immediately subsequent to the contraction produced by vibration.

Fig. 1 shows the mean percentage of conditioned responses made by each of the four groups on each day of acquisition. The figure clearly shows that the experimental group made increasingly more responses than the three control groups.

Analysis of variance of the acquisition data revealed that the differences among groups were significant ($F = 39.45$; d.f. = 3/216; $P < 0.001$), the overall increase in responding over days was significant ($F = 4.65$; d.f. = 5/216; $P < 0.001$) and their interaction was significant as well ($F = 2.55$; d.f. = 15/216; $P < 0.001$), showing differences in the slope of performance curves among groups. The analysis of the differences among groups reveals that the three control groups do not differ significantly within themselves ($P > 0.05$) while their overall mean differs significantly from that of group E ($P < 0.001$). As a consequence of such data neither sensitisation nor pseudo-conditioning may be responsible for the performance of group E. Therefore we deemed that a reliable and lasting conditioning had been achieved and undertook the second part of the experiment.

EXPERIMENT II

Each group of experiment I was cannibalized by naïve subjects in order to obtain the following groups: CaE, CaSL, CaSV, CaPC. In addition a fifth group, CaN, was formed with subjects fed with naïve planarians. Before

cannibalism victims were marked during 12 hs in a I_{131}^- solution ($15 \mu\text{C/cc}$ activity), then washed in flowing pond water during 1 h, and counted in a SELO 102 A scintillation-counter to control the homogeneity of marking, discarding subjects out of $\pm 20\%$ range. Later they were cut into pieces with a blade. From this moment on, 70 cannibals (14 per group) were allowed 20 hs to cannibalize, then they too were counted to control the homogeneity of cannibalism, and 10 subjects per each group were selected. All the groups thus formed received the same training as group E. In this second experiment we have taken into account the number of sessions necessary to reach the criterion of 10 conditioned responses on 12 trials, as shown in Table I.

TABLE I.

Individual trials to criterion (10 conditioned responses on 12 trials), means and standard errors in five groups of ten cannibal planarians.

GROUP	Individual trials to criterion										$\bar{x} \pm \text{s.e.}$
CaE	2	2	2	3	3	4	4	5	5	5	3.5 ± 0.401
CaSV . . .	3	3	3	3	6	7	7	8	9	9	5.8 ± 0.813
CaN	4	4	4	5	6	6	7	8	10	10	6.4 ± 0.733
CaPC . . .	4	4	4	5	7	7	7	8	10	10	6.7 ± 0.789
CaSL . . .	4	5	5	7	7	8	8	9	10	10	7.3 ± 0.667

Group CaN: cannibal of naïves; Group CaE: cannibal of experimentals; Group CaSL: cannibal of controls of sensitization to light; Group CaSV: cannibal of controls of sensitization to vibration; Group CaPC: cannibal of controls of pseudoconditioning.

Analysis of variance gave a significant difference among groups ($F = 4.430$; d.f. = 4/45; $P < 0.01$). The analysis of this difference reveals that group CaE was significantly faster than the four control groups ($F = 15.312$; d.f. = 1/45; $P < 0.001$), which did not differ within themselves ($F = 0.802$; d.f. = 3/45; $P > 0.2$). From these comparisons a specific facilitation emerges in Group CaE.

These findings agree with results of previous works [4, 6, 7]. Some contentions, however, are still alive about this argument: actually Hartry *et al.* [5] failed to achieve a transfer of memory through cannibalism testing cannibals 12 hs after cannibalism limitedly to one session. In a temporal

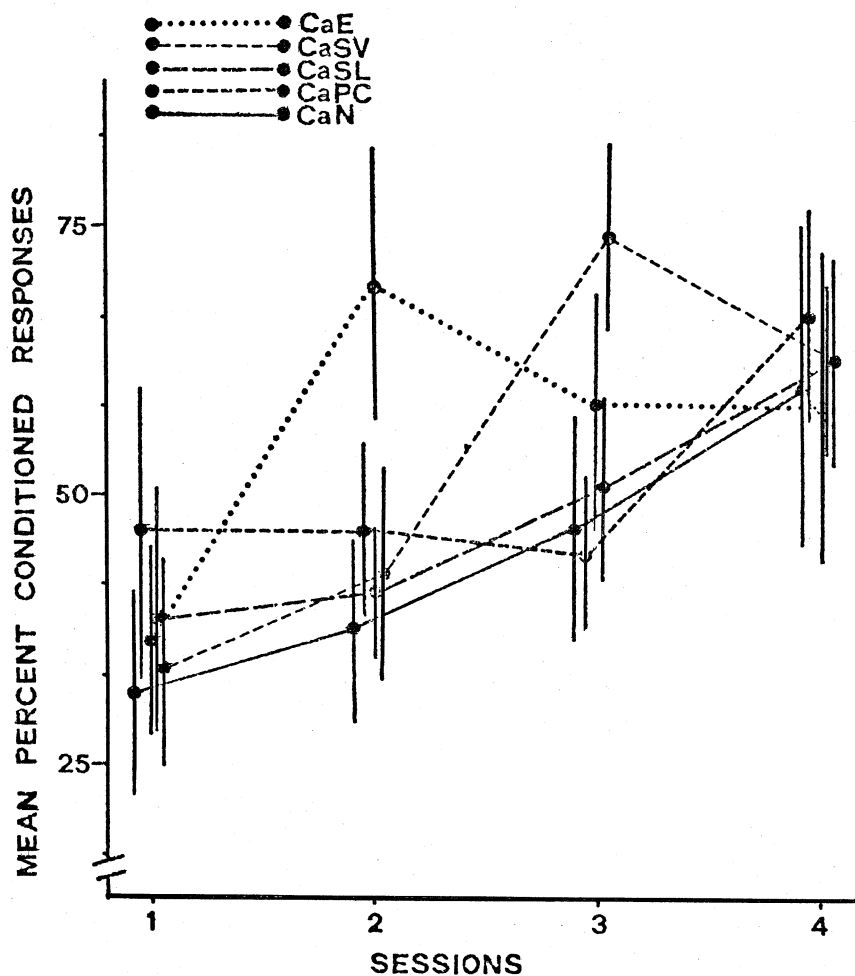


Fig. 2. - Mean percent conditioned responses $\pm$ f.l.0.05 during the first four days of training in five groups of ten cannibal planarians. Group CaN: cannibal of naïves; Group CaE: cannibal of experimentals; Group CaSL: cannibal of controls of sensitization to light; Group CaSV: cannibal of controls of sensitization to vibration; Group CaPC: cannibal of controls of pseudoconditioning.

representation, as shown in fig. 2, facilitation of Group CaE is evident during the second session, and absent in the first one. As a consequence we suggest that time has to be taken into account as a considerable factor in evaluation of data.

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