Effects of intraventricular administration of insulin on immunoreactive TRH contents in the hypothalamus, plasma immunoreactive TRH, TSH, T_4, T_3 and blood glucose levels

After drug administration	0	5	10	30	60	90 min
Saline-treated						
TRH contents in the hypothalamus (ng)	4.1 ± 0.3	4.1 ± 0.2	3.9 ± 0.2	4.1 ± 0.3	4.0 ± 0.3	4.2 ± 0.3
TRH in plasma (pg/ml)	6.4 ± 2.0	6.3 ± 1.9	5.2 ± 2.0	6.1 ± 2.1	5.6 ± 1.8	6.4 ± 2.0
TSH in plasma (ng/ml)	270 ± 24	264 ± 24	266 ± 25	270 ± 26	264 ± 24	266 ± 25
T_4 in plasma (µg/dl)	4.9 ± 0.3	4.9 ± 0.4	5.0 ± 0.3	5.1 ± 0.4	5.2 ± 0.3	4.9 ± 0.2
T_3 in plasma (ng/dl)	51 ± 3.6	52 ± 3.8	49 ± 3.4	47 ± 3.4	48 ± 4.0	47 ± 3.2
Blood glucose (mg/dl)	88 ± 4.5	85 ± 4.6	80 ± 4.0	80 ± 3.8	78 ± 3.9	77 ± 3.7
Insulin-treated						
TRH contents in the hypothalamus (ng)		3.6 ± 0.2	3.0 ± 0.3[c]	3.4 ± 0.3	3.8 ± 0.3	4.2 ± 0.3
TRH in plasma (pg/ml)		10 ± 3.4	30 ± 6.4[b]	16 ± 3.6[c]	11 ± 3.4	7.0 ± 2.6
TSH in plasma (ng/ml)		290 ± 25	340 ± 27	480 ± 29[a]	360 ± 29[c]	260 ± 23
T_4 in plasma (µg/dl)		5.2 ± 0.3	5.1 ± 0.3	5.2 ± 0.4	5.4 ± 0.4	5.6 ± 0.4
T_3 in plasma (ng/dl)		50 ± 3.6	55 ± 4.0	58 ± 4.2	72 ± 4.6[b]	86 ± 4.2[a]
Blood glucose (mg/dl)		86 ± 4.2	82 ± 3.8	78 ± 4.1	77 ± 3.9	75 ± 3.6

Values are expressed as the mean ± SE in each group of 5 rats. Differences from the saline-treated group are shown by [a]$p < 0.001$, [b]$p < 0.005$ and [c]$p < 0.05$.

ficantly, but that of T_4 did not. The blood glucose levels did not change significantly.

Discussion. It has been reported that the peripheral administration of insulin stimulates TSH release in rats[6,7], and the authors found that intraventricular injection of insulin increased plasma ir-TRH and TSH levels. This suggests that insulin may act on the hypothalamus. The hypothalamic ir-TRH content and its plasma concentration may expressed a balance between TRH release, synthesis and degradation, so the inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro was investigated. The inactivation of TRH immunoreactivity by plasma or hypothalamus after insulin injection did not differ from that of the control[13]. These data suggest that insulin may affect TRH release or synthesis. The question can be raised as to whether the effect of insulin on TSH release might be a direct action of insulin on the hypothalamus or mediated via a fall in blood glucose levels. The present data indicate that the effects of insulin on TSH release are not mediated via a fall in blood glucose levels. Plasma T_3 levels increased significantly after insulin injection, confirming the results obtained by peripheral administration of insulin[6,7]. The present study revealed that plasma ir-TRH levels increased followed by increases in plasma TSH and T_3. The time course of these changes is similar to that with exogenous TRH administration[14]. These data suggest that insulin acts directly at the hypothalamus level to stimulate TRH and TSH release.

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2 Ovine insulin was kindly supplied from Shimizu Pharm. Co., Ltd. (Japan).

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Can postictal suppression of the perforant path – fascia dentata responses account for the ECS-induced anterograde amnesia in rats?

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Summary. Electroconvulsive shock (ECS) decreases fascia dentata responses to entorhinal stimulation by 50% in unanesthetized rats. Synaptic potentials and population spikes return to pre-ECS level during 1 h and 3 h, respectively. This recovery rate is compared with the dynamics of ECS-induced anterograde amnesia.

The assumption that the hippocampus plays an important role in memory processes[3] is based not only on results of lesion studies but also on conditioning-elicited changes of hippocampal evoked potentials[4,5] and on anterograde and retrograde amnesia caused by epileptic activity of this structure[6,7]. Hesse and Teyler[8] demonstrated in anesthetized rats that hippocampal afterdischarge suppresses the long-term potentiation elicited in CA1 stratum radiatum by low frequency tetanization of Schaeffer's collaterals. The authors suggested that this effect can account for retrograde amnesia observed after similar intervention in behavioral experiments. This plausible hypothesis cannot be easily tested, however, since the time courses of the 2 phenomena are different. Whereas the seizure interfered with the long-term potentiation regardless of the number of tetani applied and time after initial potentiation (up to 1 h), the memory-disrupting effect of epileptic seizure has a characteristic temporal gradient and rapidly decreases with increasing acquisition-seizure delay. It seems, therefore, that long-term potentiation in the hippocampus is not a necessary prerequisite for preservation of memory traces, the consolidation of which has already progressed beyond a certain level. Since a more straightforward correlation of the behavioral and electrophysiological after-effects of hippocampal seizure activity can be expected in the case of anterograde amnesia, we have attempted to establish the time course of the postictal changes of the fascia dentata responses and to compare them with the available evidence on postictal impairment of acquisition or retrieval.

The experiments were performed in 6 adult male hooded rats of the Druckray strain weighing approximately 250 g. Under pentobarbital anesthesia, a pair of stimulating electrodes (twisted stainless steel wires 0.1 mm in diameter, interelectrode distance 1.0 mm) were inserted into the entorhinal cortex (stereotaxic coordinates AP 6.0, L 4.0, V 3.0 according to Fifkova and Marsala[9]), while a similar recording electrode was inserted into the dorsal hippocampus (AP 2.5, L 2, V 2.5). 2 silver screws (2 mm in diameter) in the frontal bone 7 mm rostral to bregma, served as reference and ground electrodes, respectively. Single pulses (0.1 msec, 10–20 V, 0.2 Hz) were applied to the stimulating electrodes while hippocampal activity was amplified with a wide band amplifier (1–5000 Hz) and displayed on a CRO. Hippocampal EEG was simultaneously recorded with a polygraph. Using 2 independent stereotaxic carriers both stimulating and recording electrodes were moved until optimum fascia dentata response was obtained according to the criteria of Douglas and Goddard[10]. The electrodes together with the 5-pin female socket were then fixed to the skull with anchoring bolts and a layer of acrylate.

Several days after surgery the animal was placed in a tall glass cylinder. The electrodes were connected with a flexible counterbalanced cable to the stimulating and recording equipment and the fascia dentata responses were recorded during 3.5 h. The intensity of the stimulation pulses (0.1 msec, 0.1 Hz) was adjusted so as to elicit the population spike attaining about 50% of maximal amplitude. The responses were averaged in groups of 16 with a laboratory computer (LINC 8). The stimulation was either continuous throughout the 3.5-h experiment or was applied in isolated trains of 16 pulses spaced at 30-min intervals.

In control experiments the animals received sham ECS treatment after 30 min of recording. The animals were restrained by hand while light alligator clips smeared with conductive jelly were connected to their pinnae. The recording was resumed 2 min later. No change of the fascia dentata responses was caused by this treatment and the averaged evoked potentials remained stable throughout the 3.5 h of the experiment.

Application of ECS (80 mA, 1.5 sec) elicited full tonic extension seizure[11] accompanied by afterdischarge in the hippocampus, followed by depression and secondary afterdischarge 30–40 sec later. Fascia dentata responses recorded in the first 3 min after cessation of the seizure activity were significantly reduced and only gradually returned to the pre-ECS level. The regular arrangement of dentate neurons makes it possible to recognize in the gross evoked potential 2 distinct components corresponding to the extracellularly recorded population EPSP (excitatory postsynaptic potential) and population spike of granular cells (fig. 1). Since results obtained with continuous stimulation and discrete test trains were essentially similar, they were joined in the average curve shown in figure 2.

The population EPSP and population spike decreased in the first post-ECS average by about 35% and 50%, respectively. Whereas the population EPSP almost reached the control level 30 min later, the population spike required more than 3 h for full recovery (fig. 2). The difference between the recovery of the population EPSP and the population spike was maximal 30 min after ECS. No significant changes of response latencies accompanied the changes of the evoked potential amplitude.

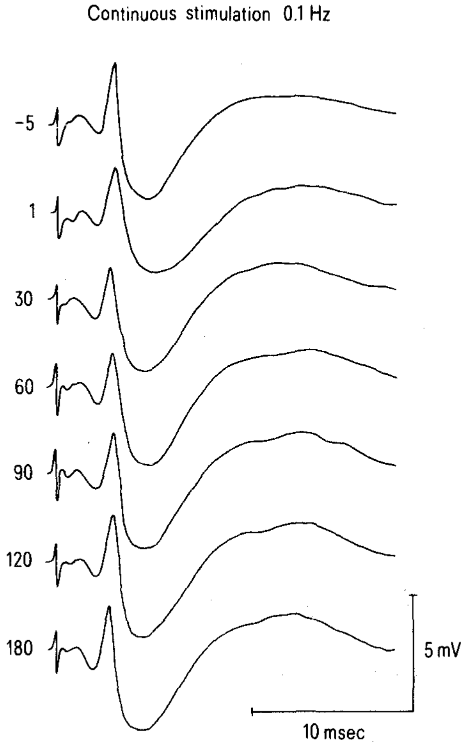

Figure 1. Averaged evoked response (n = 10) elicited in fascia dentata by entorhinal stimulation before and at various intervals after ECS. The numbers indicate time before (−5) or after ECS in minutes.

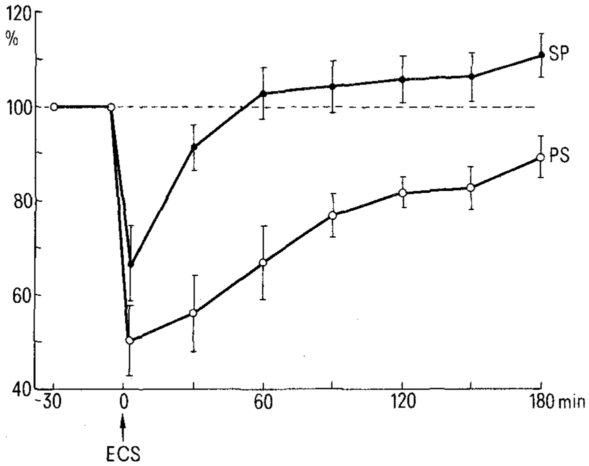

Figure 2. Mean changes of population EPSP (SP) and population spike (PS) amplitudes ater ECS treatment. Abscissa: Time before and after ECS Ordinate: Amplitude in percentage of the pre-ECS value. The vertical bars indicate SEM.

The change of the population EPSP is consonant with the report by Hesse and Teyler[8] who found complete postictal depression lasting up to 10 min followed by gradual recovery lasting 15–25 min. No population spike was recorded in the latter study, however, which examined a different part of hippocampus (CA1) in deeply anesthetized rats. The different recovery rates of the population spike and population EPSP indicate that ECS interferes not only with synaptic transmission but that it elicits an even more prolonged decrease of postsynaptic excitability.

Comparison of the above electrophysiological findings with the behaviorally established anterograde effects of ECS brought controversial results. Electrically elicited hippocampal after-discharge caused only a short-lasting (10 min or less) anterograde impairment of spatial working memory of rats[12–14]. Since these tasks are all critically dependent on hippocampal integrity[15], it is obvious that the 30–40% reduction of the fascia dentata response does not impair this memory function.

On the other hand the recovery of the population spike amplitude has a similar time course to the ECS-induced anterograde impairment of the acquisition of passive avoidance[16] or of conditioned taste aversion[17] and of the retrieval of conditioned emotional reactions[18, 19]. The significance of these positive correlation is diminished, however, by the fact that neither of the above conditioned reactions is impaired by hippocampectomy[3].

The above evidence indicates that the postictal behavioral deficit cannot be simply accounted for by interference with the transhippocampal information flow.

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Habitat marking: males attracted to residual odors of two *Drosophila* species

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Summary. We have found that males of the sibling species *D. melanogaster* and *D. simulans* are preferentially attracted to sites marked by the residual odors of conspecific females, especially if non-virgin. In natural populations, this could enhance sexual selection among males, and cause some isolation at the microhabitat level.

The potential importance of habitat selection is being increasingly debated[1]. Many studies are based upon gene (in particular electrophoretic) and chromosomal polymorphisms. Since these genotypic assessments are not directly relatable to the field situation interpretations are often difficult, and stress the need to study ecobehavioral traits important in determining the distribution and abundance of organisms in habitats. In insects, such traits can be related to abiotic and biotic (especially resources) features of the environment[2]. In *Drosophila*, there are recent indications of the feasibility of this approach in habitat selection studies for phototaxis[3].

Cosmopolitan *Drosophila* species typically utilize resources and court in the same habitats. The attraction of flies to resources is therefore an important ecobehavioral component of habitat selection. While there is good evidence that certain chemicals act as attractants[1, 4], it has not been explored whether flies themselves mark habitats. Here we present experimental evidence at the interspecific level for 2 cosmopolitan sibling species, *D. melanogaster* and *D. simulans*, which predominantly involves 'marking' of habitats by females.

Adults aged 2–3 days were placed into 470-ml glass cylinders covered with gauze at both ends; 600 virgin females, 600 non-virgin females or 700 males of each species were separately used as 'marker' populations. The higher number of males compensated by volume for the size difference between sexes. After 8 h, the flies were discarded, and the cylinders placed in a wind tunnel (figure). Light was provided by a single fluorescent tube (40 W) placed vertically behind the tunnel. After starving for 8 h exposed to water-saturated vapour at 20°C, 300 males and females of each species were then simultaneously released into the observation chamber (B). Each species was marked with a different fluorescent dust and colors were alternated between experiments. Odors were drawn from the marked glass cylinders (C) into the observation chamber