Severe conditioned taste aversion elicited by venom of Russell's viper[1]

S. Islam

Department of Zoology, Government College Haripur, Hazara (Pakistan), 18 December 1978

Summary. Conditioned taste aversion (CTA) established in rats by associating saccharin drinking with subsequent poisoning by Russell's viper venom. Retention test revealed 76 and 56% reduction of saccharin intake in venom and antivenom-venom groups. No CTA was observed in antivenom and control groups.

Efficient control of feeding depends not only on innate reflexes and instincts, but also on acquired habits based on the experience of the animal with various diets. Conditioned taste aversion (CTA)[2] is an example of such learning which has attracted increasing attention of neurophysiologists. The bulk of CTA studies[3] stressed the importance of nausea, malaise and gastrointestinal distress for establishing the taste aversion. Nonvisceral noxious stimuli like footshock do not lead to CTA formation[4,5]. Ionescu and Burešová failed to induce CTA by drugs causing muscular paralysis. Many experimenters concluded that taste cues must be associated with gastrointestinal symptoms of sickness. This conclusion, though quite appealing, does not explain the lack of direct relationship between effectiveness of CTA treatment and intensity of sickness[7]. CTA can be induced by psychoactive drugs[8], which cause no obvious sickness. Nachman and Hartley[9] compared various rodenticides for their effectiveness in eliciting CTA to sucrose offered 2–4 min before administration of poisons. An additional approach to assessing the relative importance of nausea and malaise in production of CTA is to test the aversion-producing properties of natural poisons which are not ingested but parenterally applied. In the present work venom of Russell's viper was used as US (unconditioned stimulus) to elicit CTA and its effects were blocked by the use of antivenom.

Method. The subjects were 90 Sprague-Dawley rats 90–110 days old and weighing 170–250 g. Both males and females were used to demonstrate that results are not sex linked. They were housed in group cages (10 animals per cage) and had free access to food and water. The 1st experimental session was preceded by 24 h water deprivation. The rats were placed in a drinking box of $30 \times 22 \times 17$ cm, in the front wall of which was a glass drinking spout (8 cm above the floor) connected with a callibrated 50 ml pipette containing tap water. The animal was allowed to drink for 15 min and the amount of water consumed was measured with 0.1 ml accuracy. The procedure was repeated on day 2. During acquisition (day 3) the drinking burette was filled with 0.1% sodium saccharin, the flavour of which served as the CS (conditioned stimulus). Then the animals were divided into 4 groups. Group 1 received s.c. injection of 320 µg of Russell's viper venom 30 min after saccharin intake. Group 3 was s.c. injected with 0.1 ml of antivenom (polyantivenin prepared by National Health Laboratories Islamabad), 30 min after saccharin. Group 2 was injected 15 min after saccharin intake with 0.13 ml of antivenom and 15 min later with 320 µg of venom. The control group C received 30 min after saccharin intake i.p. injection of physiological saline (4% b.wt).

On days 4, 5 and 6 all animals had free access to water in their home cages. Water was removed on day 7. Consumption of water was tested in the drinking box on days 8 and 9, saccharin intake was measured on day 10.

The appropriate dose of the venom was established in preliminary experiments. It must not be fatal to a significant number of animals, yet should produce clearly observable signs of sickness, such as inactivity, muscular paralysis or haemorrhage. The concentration of the venom was gradually increased from 50 µg/ml to 400 µg/ml. Additional 18 rats were used to determine LD_{50}. Injection of 320 µg resulted into 100% survival, at 400 µg there was 33% death, the LD_{50} was 440 µg. All the rats not surviving died within 48 h. As 1 ml of antivenom can neutralize 2.5–3 mg of venom, rats were injected with 0.13 ml of antivenom providing complete protection against 320 µg of venom.

The venom of *Vipera russelli* was extracted with a syringe from an anaesthetized animal and centrifuged at 3000 rpm, incubated for 24 h at 27 °C and dried in a desiccator at 4 °C.

Results. Average fluid consumption in various phases of the experiment is shown in the table. All the groups consumed more water on day 2 than on day 1. Water consumption on days 8 and 9 was similar to days 1 and 2. Saccharin intake during retention test on day 10 dropped to 24% of the saccharin consumption on the acquisition day 3 in the venom group ($df = 25$, $t = 11.63$, $p < 0.001$) and to 43% in the antivenom venom group ($df = 17$, $t = 8.22$, $p < 0.001$). Comparison of saccharin intake in various groups on day 10 showed significant difference of venom versus control ($df = 44$, $t = 10.84$, $p < 0.001$) and antivenom venom versus control ($df = 36$, $t = 7.84$, $p < 0.001$), whereas other differences were not significant.

Discussion. To explain the differential effectiveness of various poisons in eliciting CTA, Nachman and Hartley[9] considered 3 factors: a) delay of onset of poisoning, b) duration of symptoms produced and c) physiological system involved in the toxic effects.

It has been argued that learned aversion is inversely related to the delay between flavour ingestion and toxic effects[10]. Little aversion is learnt with delays exceeding 24 h. However, delayed onset of poisoning is not the only explanation of CTA failure since there are extremely fast acting agents like sodium cyanide which do not elicit CTA. The duration of illness also does not appear to account for the differential effectiveness of poisons. Lithium chloride is more effective in producing CTA than red squill, but the toxic effects of LiCl are of much shorter duration than those of red squill. Garcia and Ervin[11] explained absence or presence of CTA by assuming that poisons which do not elicit learned aversion, do not stimulate the physiological system respon-

Table

Group		n	1 (W*)	2 (W*)	3 (S**)	8 (W*)	9 (W*)	10 (S**)	D10–D3
1	Venom	26	7.20±0.40	10.06±0.43	10.69±0.47	8.24±0.42	10.24±0.34	2.61±0.51	−8.03±0.69
2	Antivenom venom	18	6.64±0.44	8.78±0.46	9.12±0.53	7.23±0.34	9.74±0.49	4.02±0.45	−5.1 ±0.62
3	Antivenom	20	6.68±0.43	9.0 ±0.41	9.18±0.37	7.25±0.32	7.89±0.36	8.51±0.36	−0.67±0.50
4	Saline	20	6.78±0.37	9.43±0.31	9.65±0.29	7.04±0.35	8.32±0.29	9.76±0.37	+0.11±0.28

* Water, ** Saccharin.

sible for CTA acquisition. On the other hand, CTA producing poisons stimulate neural circuits which can be associated with taste inputs.

The venom of vipers is predominantly haemotoxic and cytolytic. Forbes et al.[12], and Omori et al.[13] reported that haemorrhage was the outstanding symptom of viper poisoning due to damage of vascular endothelium by the venom constituent haemorrhagin. Formation of clots was more common. Dubois and Geiling[14] argued that haemotoxic effects of viper venom are due to enzymatic destruction of cell membranes and tissues. The cytolytic venoms cause extensive haemorrhages at the site of injection and the resulting haemorrhage produce functional impairment throughout the body. Muscular paralysis has also been observed after viper's bite. CTA observed in the present experiment indicates that the venom elicits internal malaise. CTA seems to be due to the toxoid substances circulating in the blood rather than to haemorrhagia and muscular paralysis.

This conclusion is supported by the failure of the antivenom, which blocks the overt symptoms of poisoning, to counteract the CTA-eliciting properties of the venom. Further research is needed to find out whether higher antivenom dosages are more efficient. The possibility of using the CTA paradigm for detecting sublethal effects of the venom extends the toxicological characteristics of natural poisons and can serve for assessment of various therapeutic interventions.

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Decrease in motor activity – an early symptom in the course of experimental allergic encephalomyelitis (EAE)[1]

D. Englert and K. Hempel[2]

Institut für Medizinische Strahlenkunde der Universität, Versbacher Str. 5, D-8700 Würzburg (Federal Republic of Germany), 15 November 1978

Summary. Motor activity has been followed in rats during an experimental allergic encephalomyelitis (EAE). The disease was produced by transfer of lymph node cells from sensitized syngenic donors. Small and large movements were permanently registered by an electric activity meter. It could be demonstrated that a decrease of the motility is an early symptome of the disease. Therefore the measurement of the motoric activity might be a useful parameter in the classification of EAE.

When experimental allergic encephalomyelitis (EAE) is produced by passive transfer with immunized cells, clinical signs, such as paralysis of tail and hindlimbs, are often faint. Therefore we looked for an additional objective indicator which can help us define the course of the disease. With help of the Animax activity meter (ABFARAD) we could follow changes in motor activity. The sensing unit of the instrument consists of an oscillator operating at a frequency of 1.2 MHz and a resonance circuit tuned to this frequency. The weight, and characteristics, of the body of the subject determine the change in the resonance circuit, that is, they determine the voltage across the capacitor in the tuning circuit. Advantages of this system are that there is no detectable interference with the animal and that the instrument is independent of light, so that the nocturnal behaviour can be studied. Although we observed in our tests that not all movements were registrated by the activity meter, we suggest that in the statistical average always the same percentage of large and small movements will be recorded.

Figures 1 and 2 show the motor activity of a full-grown female Lewis rat which was injected with 5×10^8 lymphocytes from immunized syngenic donors. The animal housed

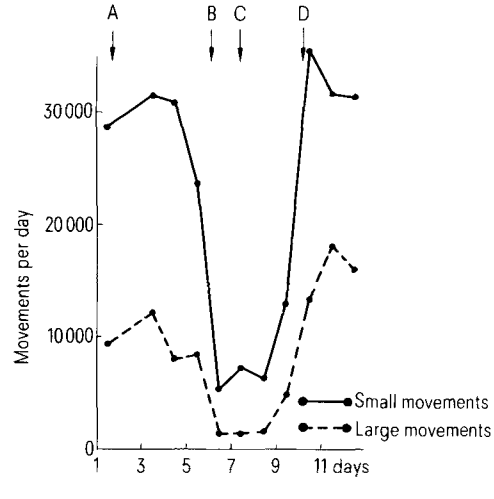

Fig. 1. Changes in activity before and after transfer of EAE by lymphocytes (Lewis rat ♀). A, Transfer of lymphocytes; B, flaccid tail; C, hindlimb paralysis; D, recovery.