Anticipatory intervals (average values with standard deviation)

Tones in pattern	Before 1st tone	Before 2nd tone	Before 3rd tone	Before 4th tone
Sequence-condition 1 (500 ms intertone interval)	– 32.6 (± 36.7)	– 31.8 (± 38.4)	– 30.4 (± 35.2)	– 32.3 (± 34.8)
Sequence-condition 2 (700 ms intertone interval)	– 28.6 (± 37.3)	– 30.4 (± 39.4)	– 36.9 (± 42.6)	– 34.1 (± 38.4)

Minus sign means tapping onset precedes stimulus onset. For details see text.

The acoustic system is capable of differentiating much shorter time intervals between tones than 30 ms[14]. It is therefore obvious that the anticipatory error in following rhythmic stimuli is not caused by functional limitations of hearing. Instead, it is apparently related to temporal programming of a highly automatized, stereotypically repeated sequence of simple and probably ballistic movements. Because the response is anticipatory it is obviously not related to the stimulus proper but to the preceding stimulus, 500 or 700 ms earlier. As there is no difference in anticipation for the two interstimulus conditions (see table), the discrete temporal advance by approximately 30 ms is embedded in a higher order temporal control mechanism. Thus, not only temporal control in the perceptual domain, but also in the sensorimotor domain appears to be organized in a hierarchical way[15].

1 This work was supported by DFG, Po 121/13.
2 Fraisse, P., L'année psychol. *66* (1966) 15.
3 Fraisse, P., in: Psychology of Music, p. 119. Ed. D. Deutsch. Academic Press, New York 1982.
4 Clynes, M., and Walker, J., in: Music, Mind and Brain, p. 171. Ed. M. Clynes. Plenum Press, New York 1981.
5 Franek, M., Radil, T., and Indra, M., Activitas nerv. sup. *27* (1985) 222.
6 Franek, M., Radil, T., Indra, M., and Lánsky, P., Int. J. Psycho-physiol. *5* (1987) 187.
7 Pöppel, E., Psychol. Forsch. *43* (1970) 1.
8 Pöppel, E., in: Handbook of Sensory Physiology, Vol. VIII, Perception, p. 713 Eds R. Held, H. Leibowitz and H.-L. Teuber. Springer, Berlin 1978.
9 Galambos, R., Makeig, S., and Talmachoff, P. J., Proc. natl Acad. Sci. USA *78* (1981) 2643.
10 Ilmberger, J., Naturwissenschaften *73* (1986) 743.
11 Pöppel, E., and Logothetis, N., Naturwissenschaften *73* (1986) 267.
12 Madler, Ch., and Pöppel, E., Naturwissenschaften *74* (1987) 42.
13 Gray, C. M., König, P., Engel, A. K., and Singer, W., Nature *338* (1989) 334.
14 Hudspeth, A. J., Nature *341* (1989) 397.
15 Pöppel, E., Mindworks. Time and Conscious Experience. Harcourt Brace Jovanovich, Boston 1988.

0014-4754/90/070762-02$1.50 + 0.20/0

Gustatory sensitivity of an anuran to cantharidin[1]

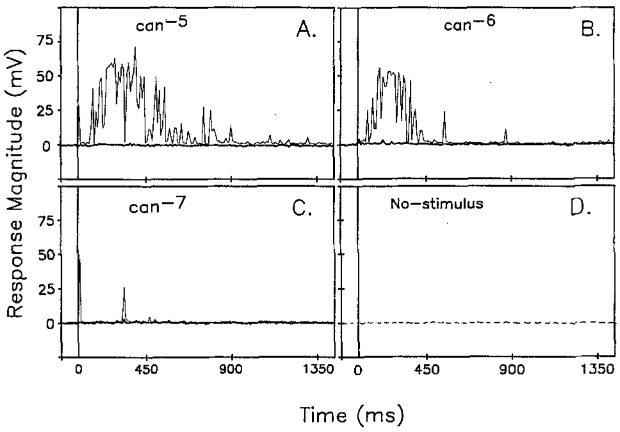
S. T. Kelling, B. P. Halpern and T. Eisner

Department of Psychology and Section of Neurobiology and Behavior, Cornell University, Ithaca (New York 14853, USA)
Received 11 December 1989; accepted 22 February 1990

Summary. Glossopharyngeal nerve stimulation of the bullfrog, *Rana catesbiana,* revealed responsiveness to low levels of cantharidin (1.3×10^{-6} M), providing a first demonstration of neural gustatory sensitivity of an animal to this defensive chemical from blister beetles (Meloidae).
Key words. Cantharidin; taste; glossopharyngeal nerve; Amphibia; Meloidae.

Cantharidin, the active principle of 'Spanish Fly', is one of the oldest-known poisons of insect origin[2]. Present as a defensive agent in the blood of blister beetles (family Meloidae) at concentrations in the order of 10^{-1} to 10^{-3} M, it is a strong feeding deterrent to ants and carabid beetles[3], and toxic to many vertebrates[4]. Nonetheless, several invertebrates[3,5], as well as vertebrates such as Japanese quail[6], armadillos[7], and a number of Amphibia[8,9], are able to eat blister beetles with apparent impunity. We here present evidence that in one known predator of blister beetles, the bullfrog *Rana catesbiana*[9], the ability to consume these insects is not attributable to an inability to taste cantharidin. We have shown this frog to have a high neural gustatory sensitivity to this compound, a capacity not previously demonstrated for an animal.

The experiments were carried out on 5 wild-caught bullfrogs (Ithaca, New York; body mass 48 ± 14 g). Each was anesthetized (10% aqueous urethane, intraperitoneal, 20 mg/kg b.wt), placed ventral side-up with the



Whole glossopharyngeal nerve activity *(Rana catesbiana)* during the 100 ms preceding and 1500 ms following stimulus onset. Vertical line at 0 ms denotes stimulus onset. *A–C*; median response to cantharidin (fine line), and to the paired Ringers control (heavy line). *D*; non-stimulus condition. The three cantharidin concentrations tested (can^{-5}, can^{-6}, can^{-7}) correspond respectively to 1.3×10^{-5}, 1.3×10^{-6}, and 1.3×10^{-7} M cantharidin in Ringers solution.

tongue pinned outstretched to a holder, and surgically prepared by standard procedure for exposure of the lingual branch of the glossopharyngeal nerve[10–12]. Stimulation (room temp. 23 ± 2 °C) was with Ringers solution[13], and with cantharidin (Sigma Chemicals, St. Louis, MO) in Ringers at three concentrations: 1.3×10^{-5}, 1.3×10^{-6}, and 1.3×10^{-7} M (henceforth given as can^{-5}, can^{-6}, and can^{-7}).

Stimulus presentation was through two automated repetitive micropipets[10], one for delivery of cantharidin solutions, the other for delivery of Ringers. Pipet tips were kept positioned for the duration of experimentation with each frog, at 0.6 mm above the frog tongue, and 0.4 mm from one another. Each stimulus (10 ± 0.003 µl) flowed for 70 ± 7 ms at 150 ± 10 µl/s and was allowed to remain on the tongue for 10 s. Prior to administration of a stimulus, the tongue was always flooded with 10 ml Ringers and left thus wetted for 2 min. As determined through preliminary testing, this sufficed to induce neural adaptation to Ringers. Cantharidin presentations were each followed by a control stimulation with Ringers, and were repeated 10 times per frog for each concentration. The 10 presentations per concentration were administered in two groups of five, in randomized sequence with the groupings for the other concentrations.

Recordings from the glossopharyngeal nerve were in accord with the convention[11, 14]. Amplified neural activity was processed with a digitally controlled summator, which integrated the input over time[14–16]. Integration intervals (10 ms) were digitized at 500 Hz for 1600 ms.

The results, summed for the five frogs (fig.), clearly show a concentration-dependent glossopharyngeal sensitivity to cantharidin. While with can^{-5} and can^{-6} the response relative to Ringers and the non-stimulus control was significantly positive ($p \leqq 0.001$; Friedman's non-parametric analysis of variance), with can^{-7} it was not. The bullfrog is evidently gustatorily sensitive to cantharidin at concentrations 3 or more orders of magnitude below those prevailing in blister beetle blood. Responses to the highest concentration tested (can^{-5}) were at a par with those we recorded for 10^{-3} M $CaCl_2$[10], a solution typically used as a frame of reference in frog gustatory studies[17]. It is clear that *R. catesbiana* must be able to taste blister beetles when it flips these into its mouth with the tongue. Blister beetles externalize their cantharidin by 'reflex bleeding' when disturbed[3]. Even the slightest emission of blood on their part should be tasteable to a bullfrog.

One wonders why an animal that is seemingly tolerant of a poison should have the gustatory capacity to detect it. Might cantharidin be of long-term toxicity to amphibians, and the bullfrog eventually, on the basis of experience with blister beetles, able to develop a gustatory aversion to the compound? Or is the sensitivity to cantharidin in no way an adaptive concomitant of co-evolution with blister beetles? Might the receptors responsible for cantharidin sensitivity in the bullfrog be attuned to other molecules as well, molecules more fundamental to the life of this animal?

1 Acknowledgments. Study supported in part by grant AI 02908 from NIH.
2 Stork, G. E., van Tamelen, E. E., Friedman, L. J., and Burgstahler, A. W., J. Am. chem. Soc. *75* (1953) 384.
3 Carrel, J. C., and Eisner, T., Science *183* (1974) 755.
4 Graziano, M. J., Waterhouse, A. L., and Casida, J. E., Biochem. biophys. Res. Commun. *149* (1987) 79.
5 Selander, R. B., and Mathieu, J. M., Univ. Ill. Biol. Monogr. *41* (1969) 1.
6 Carrel, J. C., PhD. Thesis, Cornell University, Ithaca, New York 1971.
7 Wirtz, W. O., Austin, D. H., and Dekle, G. W., Food habits of the common long-nosed armadillo *Dasypus novemcinctus* in Florida, 1960–1961, in: Evolution and Ecology of Armadillos, Sloths, and Vermilinguas, pp. 439–451. Ed. G. G. Montgomery. Smithsonian Institution Press, Washington 1985.
8 Smith, C. C., and Bragg, A. N., Ecology *30* (1949) 339.
9 Korschgen, L. J., and Moyle, D. L., Am. Midl. Nat. *54* (1955) 332.
10 Kelling, S. T., Masters Thesis, Cornell University, Ithaca, New York 1989.
11 Akaike, N., and Sato, M., Comp. Biochem. Physiol. *54* (1976) 149.
12 Dean, J., J. comp. Physiol. *135* (1980) 51.
13 Oakley, B., and Schafer, R., Experimental Neurobiology: A Laboratory Manual, p. 355. The University of Michigan Press, Ann Arbor, Michigan 1978.
14 Kelling, S. T., and Halpern, B. P., Chemical Senses *14* (1989) 716.
15 Walsh, L. F., and Halpern, B. P., J. of appl. Physiol. *37* (1974) 748.
16 Marowitz, L. A., and Halpern, B. P., Chemical Senses *2* (1977) 457.
17 Nomura, H., and Sakada, S., Jap. J. Physiol. *15* (1965) 433.

0014-4754/90/070763-02\$1.50 + 0.20/0