in the interpretation of the chromatograms. Human cortical bone, obtained at autopsy, was extracted according to the procedure described by M. J. Glimcher and E. P. Katz [*J. Ultrastruct. Res.* **12**, 705 (1965)]. Samples analyzed were the gelatins extracted into saturated guanidine thiocyanate, *p*H 7.4, at 4°C.
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18. Supported by PHS research grants AM-3564 and AM-4501 and by PHS training grant AM-5067. This is publication No. 432 of the Robert W. Lovett Memorial for the Study of Diseases Causing Deformities. We thank Mrs. Kathleen Grant and Mrs. Eleanor B. Pyle for technical assistance. A.J.M. is a fellow of the International Atomic Energy Agency; E.D.H. is a trainee of the National Institute of Arthritis and Metabolic Diseases.
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14 April 1967

## Inferior Olive of the Cat: Intracellular Recording

Abstract. *The action potential evoked from inferior olivary neurons of the cat by orthodromic, antidromic, or intracellular stimulation has a large prolonged depolarization, frequently with several superimposed smaller spikes. The all-or-nothing unitary response appears to be a property of the cell, and the later spikes are not secondary to synaptic excitation by recurrent axon collaterals.*

The inferior olive (IO) complex is a major source of cerebellar climbing fibers (*1*). Eccles *et al.* (*2*) demonstrated that the Purkinje cell *D*-potential, first recorded by Granit and Phillips (*3*) with cerebellar stimulation, is the climbing fiber response (Fig. 1A) and can be evoked by either direct or reflex stimulation of IO; reflex activation presumably would be conveyed through recurrent collaterals of excitatory climbing fibers. Armstrong and Harvey (*4*) recently described the extracellular responses of IO units. Approximately one-third of their units showed an initial spike followed by several smaller spikes. They concluded, largely from recorded extracellular field potentials, that the later spikes of the unitary responses probably were excited synaptically by way of postulated IO axon collaterals (*2*, *5*). However, anatomical studies have not demonstrated such collaterals. A recent report by Sedgwick and Williams (*6*) does not mention multiple spiked responses from IO. In our investigation, intracellular recording revealed an unusual all-or-nothing potential, frequently with multiple spikes, and the evidence presented here indicates that the later spikes of the unit response are not secondary to recurrent synaptic excitation.

Cats were anesthetized with pentobarbital (intraperitoneal injection of 35 mg per kilogram of body weight), and supplements were given intravenously as required. To minimize movement, a bilateral pneumothorax was performed, and the animal was paralyzed with gallamine triethiodide. Through a ventral exposure the inferior olive nucleus was explored systematically with glass microelectrodes filled with 2.7*M* KCl or 2*M* K-citrate for intracellular recording (d-c resistance, 10 to 40 megohms in brain). In some experiments KCl electrodes filled with methyl blue dye were used for intracellular marking (*7*), and 2*M* NaCl electrodes filled with fast green FCF were used for extracellular marking (*8*). Standard intracellular recording equipment included a neutralized capacitance preamplifier and a bridge circuit for passing current through the impaling micropipette. Stimulation sites included ipsilateral pericruciate cerebral cortex (by means of bipolar silver ball electrodes), just beneath the cortical surface of anterior lobe of the cerebellum (by means of an array of concentric stainless steel electrodes), and footpad of the contralateral forepaw (by means of bipolar needle electrodes). Stimulus durations were 0.1 msec or less, usually applied as single shocks, but occasionally a train of three stimuli was used at a frequency of 1000 per second.

In ten cats, 106 units were studied by either extracellular or intracellular recording. Units were located in IO histologically by marking with dye associated with micrometer measurements and physiologically by field potentials following cerebral and cerebellar stimulation (*4*). Unit potentials from olive neurons were so unusual that identification was not a problem. Forty-six units were evoked antidromically from cerebellar stimulation; these had latencies ranging between 2.5 and 4.5 msec and followed stimulation rates of 100 to 200 per second. The latency from ipsilateral cerebral cortex (38 units) was 12.8 ± 3.6 msec (mean and standard deviation); that from contralateral forepaw (87 units) was 21.7 ± 8.2 msec. Convergence of cerebral and spinal inputs was a common finding and occurred in 31 dorsal accessory olive units and two principal olive units (ventral lamella).

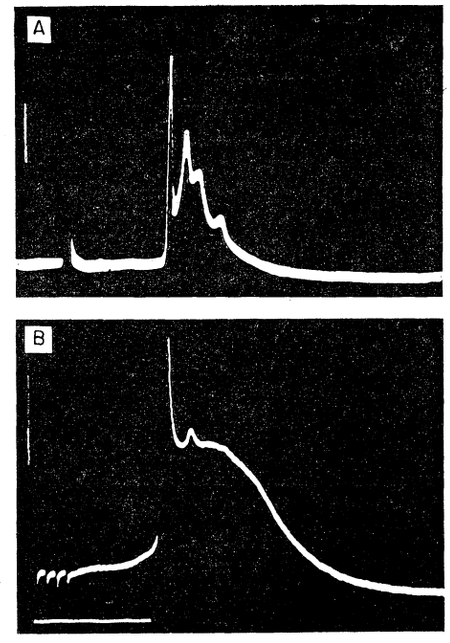


Fig. 1. (A) Response of climbing fiber recorded intracellularly from Purkinje cell. Response evoked by stimulation of inferior olive. [Reproduced with permission from Eccles *et al.* (*2*)]. (B) Intracellular action potential of an inferior olive cell evoked by stimulation of ipsilateral cerebral cortex. Top of vertical bar is zero potential and length represents 20 mv; time calibration, 10 msec.

The extracellular unitary potentials generated by the IO cells were similar to those reported by Armstrong and Harvey (*4*) and consisted of one to five initially positive diphasic or monophasic spikes (Fig. 2, I and J). The number of spikes in a unitary response was variable on successive trials, even when the strength of the stimulus was kept constant, and multiple spikes occurred more frequently with ortho-

dromically evoked responses than with the antidromically or direct intracellularly evoked ones. When the strength of orthodromic, antidromic, or intracellular stimulation was near threshold, the evoked action potential had the same variation in numbers of spikes as with a stronger stimulus, which indicates the unitary nature of the complex response pattern.

After impalement by the microelectrode, IO cells frequently lost their action potentials and had a much reduced resting potential, although synaptic potentials usually persisted. However, satisfactory intracellular spikes were recorded from 25 cells, one for more than 2 hours. The action potential heights varied from 25 to 70 mv, overshooting the zero potential in 13 of the cells. Resting potentials varied from −20 to −56 mv. The initial spike was followed by a large prolonged depolarization (PD) of variable duration; usually one to three smaller spikes were superimposed on the PD, with interspike intervals ranging between 1.2 to 2.5 msec (Figs. 1B and 2A). In cases where only a single spike was seen, the transmembrane potential of the PD plateau was readily measured and ranged from −8 to −24 mv. The rising phase of the IO action potential had an inflection, and the spike could be separated into *A* and *B* components by repetitive antidromic or intracellular stimulation (Fig. 2F). The PD, with or without the repetitive smaller spikes, was invariably associated with the *B* spike. Antidromic activation during hyperpolarization of the cell with small intracellularly applied currents blocked the *B* spike and the PD, leaving only the *A* spike (Fig. 2, G and H); further hyperpolarization then blocked the *A* spike.

A possible mechanism of the unitary multispiked complex is synaptic reactivation by IO axon collaterals. Since intracellular stimulation of a single cell gives rise to the entire complex, one must also postulate that recurrent collaterals return to the same cell. If this is the case, the synaptic drive would clearly have to be a powerful one. Moreover, the occurrence of only an *A* spike without large synaptic potentials when the hyperpolarized IO soma is antidromically invaded (Fig. 2H) implies that the PD with its multiple spikes is not evoked by collaterals distal to the *A* spike generator.

The action potential of IO cells is unlike that found in other central neurons, although it does resemble the burst firing occasionally recorded from hippocampal pyramidal cells (*9*). In contrast to the hippocampus bursts, the number of spikes in an IO response were never more than five, and prolonged depolarizing currents evoked successive bursts rather than a burst followed by solitary spikes.

The simplest intracellular response recorded from IO cells consists of a single spike and a large PD (Fig. 2, C to F). The multispiked action potential may represent temporal summation of partially inactivated spikes and their PD's. On the other hand, the small spikes on the PD could also represent the electrotonic propagation of full-sized spikes generated in the axon, their variable size reflecting an increased membrane conductance during the PD.

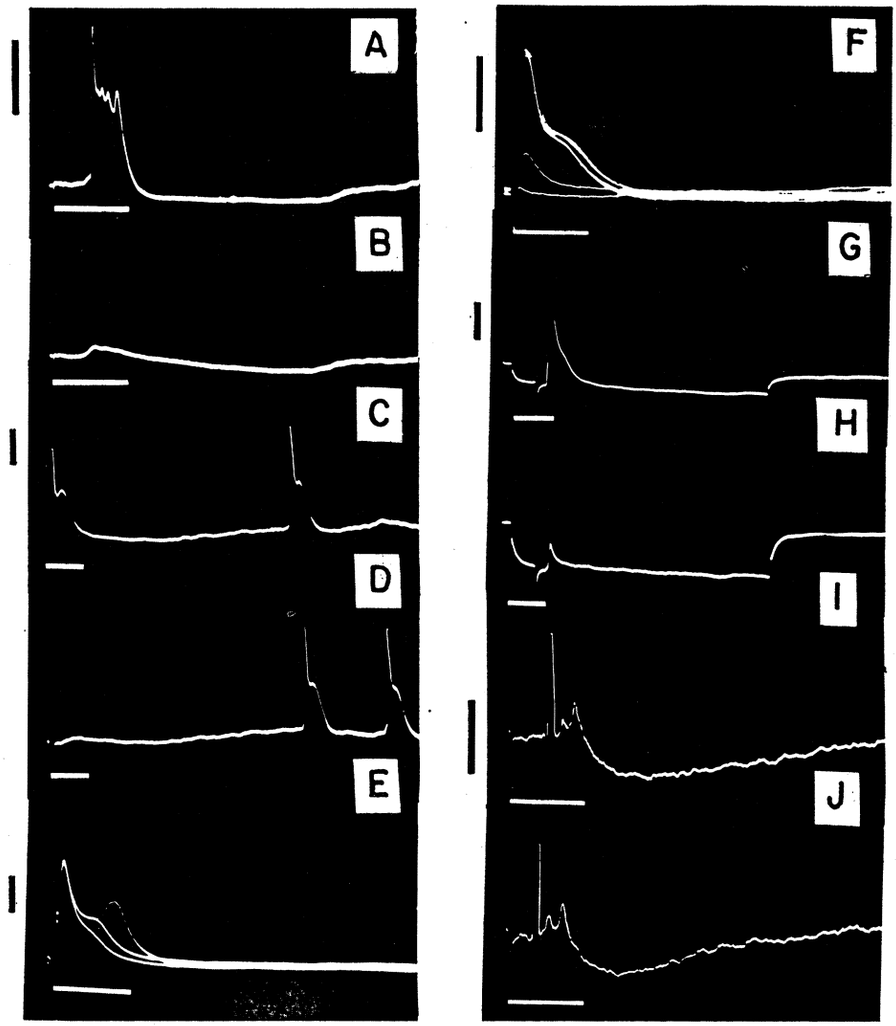


Fig. 2, A to H. Intracellular records from IO cells. (A) Action potential evoked by stimulation of ipsilateral pericruciate cerebral cortex. (B) Postsynaptic potential recorded in same cell as A to a subthreshold cerebral cortex stimulus. (C) Antidromically evoked spike from anterior lobe of cerebellum. (D) Small depolarizing-hyperpolarizing synaptic potential following cerebellar stimulation when same cell as C was not antidromically excited. Note rebound excitation in both C and D. (E and F) Superimposed traces from IO cell stimulated by a short, directly applied, intracellular current (1 per second in E and 25 per second in F). (G) Spike evoked antidromically during hyperpolarization of IO cell by intracellularly applied current. (H) Increased hyperpolarizing current blocks *B* spike and PD, leaving only *A* spike without large recurrent synaptic potential. (I) Extracellularly recorded unit transynaptically evoked from cerebellum which followed at 40 impulses per second and had a latency variation from 4.7 to 5.2 msec. (J) Antidromic excitation of the same unit as I to a stronger cerebellar stimulus (1.2 times that of I) which followed at 166 impulses per second and had a constant latency of 3.2 msec. Top of vertical bar in A to H is zero potential and length represents 20 mv. In I and J, vertical bar is 1 mv; positivity is upward. Time calibration: A to D, 20; E to F, 4; and G to J, 10 msec.

Regardless of the mechanisms responsible for the multispiked action potentials, spikes probably are conducted along the IO axon, since the interval between spikes agrees with the delay between excitatory postsynaptic potentials (2 msec) evoked by the climbing fibers in Purkinje cells (Fig. 1) (*2*).

A graded depolarizing synaptic potential followed by a graded hyperpolarizing potential occurred with orthodromic stimulation (Fig. 2B), and rebound firing was often present after the hyperpolarizing potential (Fig. 2, C and D). These synaptic potentials will be discussed in detail in a later report. It is important to note here that a similar depolarizing-hyperpolarizing potential (with mean latency of 5 msec) was frequently observed following cerebellar stimulation (Fig. 2D), in addition to the all-or-nothing spike complex. Usually the threshold for the orthodromic action potentials from cerebellum was higher than that for antidromic firing. In nine cells, however, the orthodromic discharge was evoked at a lower stimulus intensity (Fig. 2I); the mean latency for this group was 9.5 ± 4.2 msec. If the current of the stimulus was increased the variable latency of these cells to the constant value characteristic of an antidromic response (Fig. 2J) was suddenly shortened. Thus, cerebellar stimulation can evoke a transynaptic response in an IO cell by a route other than antidromic excitation of its own axon. This is supported by Eccles' data showing that the "reflex climbing fiber response" of a Purkinje cell may be evoked without exciting the climbing fibers to that cell (*2*).

This study has shown that the mechanism of the IO cell unitary repetitive discharge seen after orthodromic, antidromic, or direct intracellular stimulation does not involve reactivation by way of recurrent collaterals. It cannot yet be stated whether transynaptic excitation of IO cells after cerebellar stimulation occurs by way of recurrent IO axon collaterals, by way of axon collaterals from mossy fibers, or possibly by way of a separate orthodromic cerebello-olive pathway.

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14 June 1967

## Chemical-Cue Preferences of Inexperienced Snakes: Comparative Aspects

Abstract. *Different species of new-born, previously unfed snakes will respond with tongue flicking and prey-attack behavior to water extracts of the skin substances of various small animals. However, there are clear species differences in the type of extract responded to by previously unfed snakes, even within the same genus. These differences correspond to the normal feeding preferences shown by the various species.*

It has often been noted that animals can selectively respond to certain highly specific perceptual cues without the benefit of previous experience with those cues (*1*). The stimuli involved usually represent but a small fraction of the entire stimulus situation and are termed sign stimuli or releasers. In many instances the resulting response is also quite specific and stereotyped. For instance, newborn, previously unfed garter snakes (*Thamnophis s. sirtalis*) will respond with prey-attack behavior to extracts of the surface substances of normally eaten prey when these extracts are presented on cotton swabs (*2*). Similar specificity to chemical cues has been demonstrated in many forms of invertebrates and, to a lesser extent, in vertebrates (*1*).

Beyond the existence and analysis of such stimulus–response relations in a particular species looms the broader evolutionary implications. I here report the chemical perception aspects of feeding behavior in a number of species of neonate colubrid snakes.

I presented a variety of extracts from the surface substances of small animals to litters of individually isolated newborn snakes. The animals used in preparing extracts for the testing were: nightcrawler (*Lumbricus terrestris*), leafworm (*Lumbricus rubellus*), redworm (*Eisenia foetida*), turtle leech (*Placobdella parasitica*), slug (*Deroceras gracile*), cricket (*Acheta domestica*), minnow (*Notropis atherinoides acutus*), guppy (*Lebistes reticulatus*), goldfish (*Carassius auratus*), larval salamander (*Ambystoma jeffersonium*), metamorphosed salamander (*Ambystoma jeffersonium*), cricket frog (*Acris crepitans blanchardi*), and newborn mouse (*Mus musculus*). An extract was made by placing one or more of the intact animals in distilled water (10 ml of water per 1.5 g of body weight) at 50°C for 1 minute and stirring the water gently. The animal was then removed and the resulting liquid centrifuged and refrigerated until use. Extracts for any one experiment were always prepared on the same day.

The snakes were from litters or eggs borne by gravid females captured in the field and maintained in captivity until parturition or egg-laying. Shortly after birth or hatching the snakes were weighed, measured, and then isolated in glass tanks measuring 23 by 14 by 17 cm. Each tank was placed on white shelf paper and the four outside walls were covered with white partitions. The floor of the tank was bare except for a small plastic petri dish containing water. Except for testing periods, the aquaria were covered with glass tops. The temperature of the room in which the snakes were housed never varied more than between 22° to 26°C; during testing the temperature was maintained at 24° to 25°C.

Each member of a given litter of newborn snakes was tested only once on a series of extracts of the surface substances of potential prey. Usually twelve or thirteen different extracts were used. Distilled water was the control. Each subject received a different ordering of the test extracts, systematically balanced insofar as possible for each litter. Testing was carried out over 2 or 3 successive days beginning on the 3rd or 4th day of life and always before any previous feeding or exposure to the extracts (*3*). The testing procedure consisted of dipping a 15-cm