Inferior Olive: Its Role in Motor Learning

Abstract. *Specific chemical lesion of the rat inferior olive by intraperitoneal administration of 3-acetylpyridine prevents recuperation from motor abnormalities generated by unilateral labyrinthine lesion. Moreover, in animals that have recuperated from the labyrinthine lesion, 3-acetylpyridine produces a reversal of the symptoms within 2 hours of administration. These results indicate that the integrity of the olivo-cerebellar system is necessary for the acquisition and retention of this form of motor learning, but that the cerebellum itself is not the seat of such learning.*

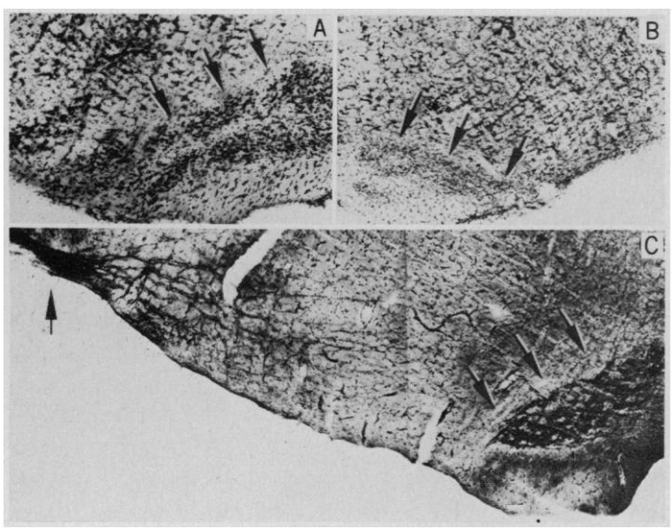
Bechterew (*1*) discovered in 1883 that the characteristic posture and motor abnormalities which follow unilateral vestibular nerve transection normally disappear a few days after the lesion. A subsequent set of experiments by Magnus (*2*) further demonstrated that this compensation, a type of motor learning, was largely impaired by removal of the cerebellum. Recently, equivalent experiments, based on an experimental paradigm of Gonshor and Melvill-Jones (*3*), have been performed by Robinson (*4*) in the visual system. He has demonstrated in cats that after cerebellectomy the reorganization of the vestibulo-ocular reflex, which follows the use of inverting prisms, does not occur. Robinson proposed as an explanation for his results that the cerebellum is probably the seat of motor learning and that the presence of changeable synapses in this cortex could be the underlying mechanism, as suggested by Marr (*5*). Marr's hypothesis proposed that the sole function of the climbing fiber synapses on Purkinje cells is to modify the properties of the parallel fiber–Purkinje cell synapse and that this modification is the basis for motor learning in the cerebellum.

To test this hypothesis, we designed a simple paradigm. After selective destruction of the inferior olive (IO) by intraperitoneal administration of 3-acetylpyridine (3AP) (*6*), a unilateral lesion of the membranous labyrinth was made. If Marr's hypothesis is correct, no motor learning should occur if the climbing fiber system is disrupted. In contrast, once a motor task is learned, disruption of the climbing fiber system will not destroy the engram, since it would have been "transferred" to the parallel fiber–Purkinje cell system.

In agreement with the experimental evidence of Desclin and Escubi (*7*), we have found that a complete lesion of the IO may be obtained with 3AP. Furthermore, we have developed a variation of the 3AP technique that restricts the central lesion to the IO. We give an intraperitoneal injection of 75 mg of 3AP per kilogram of body weight, and a subsequent injection of harmaline (15 mg/kg) 3 hours later. The 3AP apparently damages IO cells by interfering with cellular metabolism, with a resultant increase in abnormal nucleotides (*8*); harmaline produces repetitive synchronous activity of the IO (*9*), which apparently accelerates the metabolic changes in this nucleus. This 3AP dose would ordinarily be lethal to most animals. We found, however, that injection of niacinamide (*10*) (300 mg/kg) 4.5 hours after the 3AP injection prevented death and protected the rest of the nervous system from otherwise extensive lesions, especially in the brainstem nuclei (*7*). In its absence, animals show a large weight loss and complex respiratory problems even with smaller 3AP doses (*7*).

Histological study of the IO with Nissl stain shows complete chromatolysis of IO neurons 3 days after treatment with the three drugs (Fig. 1, A to C). In the absence of harmaline, however, the lesion was often incomplete; the medial accessory olive generally remained. Both light (*11*) and electron microscopy (*12*) revealed that 3AP disrupts the climbing fiber system without affecting the mossy fiber–granule cell–Purkinje cell pathway.

In the normal rat, electrical stimulation of the underlying cerebellar white matter evokes molecular layer negativity that is specifically related to the climbing fiber system (Fig. 2, A and B). This negative field potential is absent after 3AP (Fig. 2, C and D). The early negative field potential evoked in the molecular layer by the mossy fiber afferent system [the so-called N_3 wave (*13*)] is seen in both instances (Fig. 2, A and C). In both cases this field potential is blocked by Golgi cell inhibition (*13*) after local stimulation of the surface of the cerebellar cortex (Fig. 2, B and D). These data corroborate the morphological finding of an exclusive climbing fiber lesion resulting from the 3AP injection, since the mossy fiber–granule cell–Purkinje cell system is intact, as are the Golgi and basket cell inhibitory systems. Furthermore, in the presence of harmaline, 3AP totally blocks the rhythmic firing of the climbing



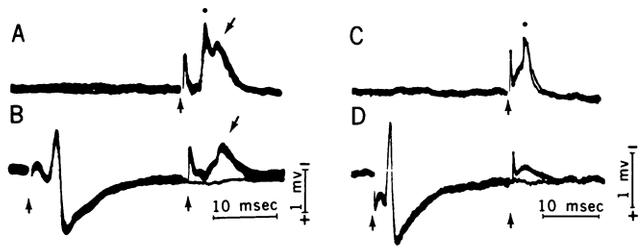


Fig. 1 (left). Anatomical results demonstrating damage to the olivocerebellar system after 3AP injection. (A) Nissl stain of the normal brainstem showing the inferior olive. (B) A similar brainstem section 3 days after 3AP administration. Staining of the IO cells is completely replaced by that of glial elements. (C) Ebbesson-Heimer stain of the brainstem showing degeneration of the inferior olive and the olivo-cerebellar pathway (arrows). Fig. 2 (right). Electrophysiological results demonstrating damage to the olivo-cerebellar system after 3AP injection. (A and B) Field potential recorded from the molecular layer of the cerebellar cortex of normal rats after electrical stimulation of the cerebellum. In A, a brief pulse to the underlying white matter evoked, (i) immediately after the artifact (upward arrow), a short latency negativity (produced by the antidromic invasion of Purkinje cells), and (ii) a later negativity consisting of two peaks produced by the mossy (dot) and climbing fiber (arrow) afferents. When this stimulus is preceded by surface activation of the cortex in B (first arrow), both the antidromic and the mossy fiber negativity are depressed. The second, or climbing fiber, negativity (arrow) remains. (C and D) Similar recordings made after 3AP injection in a rat. In C only the first two negativities are observed; no climbing fiber field potential is seen. In D a preceding local stimulation markedly reduces the amplitude of these potentials. Time, voltage, and polarity are indicated by calibration bars.

fiber afferents within 2 hours of its administration, without producing any abnormality in the firing properties of the Purkinje cells.

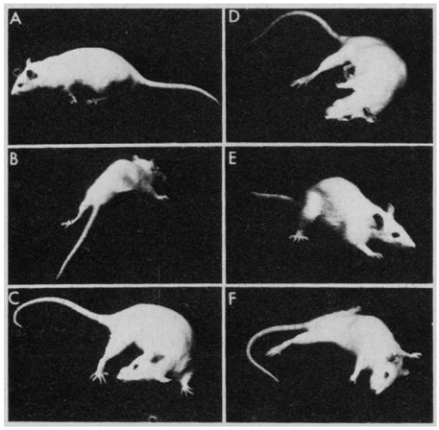
After the IO lesion, the animals became grossly ataxic but recovered some of their motor performance after this acute phase of general motor disruption. However, their movements remained sluggish and a distinctive gait which we called "mud-walking" developed (Fig. 3, A and B). This gait is characterized by exaggerated flexion of limbs and an abnormal shift of body weight from one side to the other. For the most part, however, the animals showed no obvious noncerebellar abnormalities. Unilateral vestibular damage produced in these animals a vigorous rolling toward the side of the lesion (Fig. 3C). No improvement in the motor disability was seen in any of the eight animals tested even several months after vestibular injury. In contrast, normal rats (*14*) were found to recuperate almost totally within 24 hours (Fig. 3, D and E). Since 3AP treatment produces selective damage of the olivo-cerebellar pathway, we propose that the lack of motor learning following vestibular lesion results from the absence of this system, even in the presence of an otherwise intact cerebellum. At this stage two alternative possibilities were tested. (i) The IO is necessary for the "teaching" of new tasks to Purkinje cells through the climbing fiber system, by modifying the parallel fiber–Purkinje cell synapses by the climbing fiber, as proposed by Marr (*5*). (ii) The modifications do not occur in the cerebellar cortex but either at or prior to the olive level, the IO system being a link in the pathway necessary for compensation.

In order to differentiate between these possibilities, we gave 3AP to rats that had recuperated for as long as 6 months from a previous vestibular lesion. Within 2 to 3 hours after injection, all rats tested returned to the abnormal motor behavior previously seen after vestibular lesion was made (Fig. 3F). This was long before any ataxia could be observed (Fig. 4). Histological studies made after 3AP treatment showed the distinct and isolated disruption of the IO and, on some occasions, destruction of hypoglossal neurons. This experiment was performed on 12 rats; none recovered from this abnormal motor behavior, even after a period of 6 months.

We propose, therefore, that the underlying mechanism for motor correction involves the olivo-cerebellar system, but that the climbing fibers cannot transfer this correction to the Purkinje cells. Rather, the olivo-cerebellar system is probably part of a motor system that compensates for motor abnormalities and possibly for other types of so-called motor learning. This suggestion is corroborated by our finding that, after cerebellar decortication (sparing the cerebellar nuclei and their connections to the brainstem), acquisition and retention of vestibular compensation is present. This finding suggests that the most important variable in this form of motor learning is the integrity of the olivo-cerebellar nuclear and olivo-vestibular pathways, which are generally destroyed with cerebellectomy. These results point out that some of the conclusions drawn from cerebellar lesion may actually result from the interruption of pathways rather than from indigenous function of this center.

The exact manner in which this system modifies motor patterns is obscure. It is evident, however, that this system plays a key role in the function of the cerebellum, since animals without olivo-cerebellar input show a distinct ataxia in the absence of lesions to other circuits in the cerebellar cortex or in the brainstem. This finding suggests that the IO plays an important role in the normal organization of movement, particularly that of locomotion. We were surprised, however, to find that many of the purely instinctive movements, such as grooming, were not impaired even in animals that had difficulty walking and keeping a proper stance.

In summary, we propose that the IO plays a central role in the compensation for this type of motor abnormality. We further propose that the neuronal modifications that accompany this behavioral change, although requiring the olivo-cerebellar system, do not occur at cerebellar level but are rather of extracerebellar origin. The circuits that may be modified are probably those that project through the olivo-cerebellar nuclear system, although other systems may be modified as well.



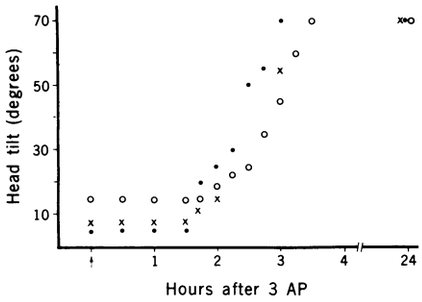


Fig. 3 (left). Motor abnormalities of rat after 3AP administration. (A) Normal locomotion in the rat. (B) Example of mud-walking after 3AP administration. Note the excess amplitude of flexion movement during locomotion. (C) Abnormal posture after vestibular damage in a 3AP animal. (D) Normal animal with labyrinthine lesion. (E) Same animal 24 hours after the lesion, showing complete recuperation of posture and locomotion. (F) Complete reversion to precompensated state 3 hours after 3AP administration. Fig. 4 (right). Time course of reversion to abnormal head position after 3AP injection in animals that have compensated for vestibular lesion. The angle of head-tilt is plotted against time after 3AP injection. The maximum tilt was 70°; at this angle the animal's head was against the floor. The 3AP was given on day 3 (filled circle), day 8 (x), or day 19 (open circle) after lesion.

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14. In this report, a normal rat is one not injected with 3AP.

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