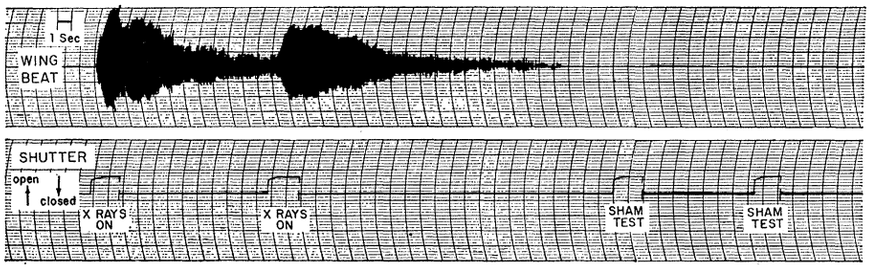


Fig. 2. Initiation and augmentation of wing beat in a moth (*Agrotis ypsilon*) on exposure to x-rays at 0.17 r/sec. For the sham exposure test the shutter was opened but power to the x-ray tube was turned off.

high-frequency sounds produced during the shutter operation and exposure. Any sounds which were within the moth's frequency and intensity range could be observed on an oscilloscope as spike potentials in the nerve preparation. (iii) This control was provided to detect possible effects of the x-ray beam on the motion transducer or the lead wires, which might in turn evoke flight activity in the moth. For this purpose, additional preparations were made with animals attached to wires which were fastened directly to the ring stand. Under dim red illumination and through a window from the control room, visual observations were made of the initiation of flight at the onset of the exposure to the x-ray beam.

Figure 2 shows the response of one moth (*Agrotis ypsilon*) to two exposures at a dose rate of 0.17 r/sec. Each exposure was 2 seconds in duration. The first stimulus was presented when the animal was inactive and the second exposure was given during evoked flight. In each moth tested, the exposure to the x-ray beam initiated wing beat or caused a change in the amplitude of the beat during flight. The dose rate required to initiate this action varied from subject to subject. Table 1 gives the minimum rate to which each of the subjects responded. The response occurred early within the first second of exposure. If the moth was inactive, sometimes a suprathreshold intensity of x-rays for that preparation did not elicit the flight pattern. The response to subsequent radiation exposures could be restored if the moth were first exposed to a brief flash of light to establish a state of heightened excitability.

The control tests indicate that the response of the moth is to the x-ray beam. When subjected to the "sham" trials the animals gave no evidence of initiation of the flight pattern. The record of spike potentials from the tympanic nerve preparation gave no indication of the presence of auditory stimuli during shutter operation and exposure. Animals not attached to the transducer were observed to exhibit vigorous flight movement at the onset of exposure, which indicates that the response could not be attributed to radiation-induced changes in the electrical detection system.

The complex motor reaction demonstrated in these moths occurs at a radiation intensity below that reported for any other organism (*1–8*). Several specimens exhibited responses to radiation intensities in the range of 0.01 to 0.12 r/sec. The latency of reaction was less than 1 second on most records. The differences in effective dose rates among specimens may reflect differences in radiosensitivity among species, the variations in physiological state among specimens, and the level of excitation upon which radiation is imposed.

It is difficult to ascribe the response to radiation action on muscle fibers in view of the low exposure dose and the short latency. It is significant that the motor response could be obtained only when the specimen had been in the darkened room for several minutes. Electroretinograms are being made of a large series of moths by using both beta radiation plaques and x-rays as the source of stimulation. The preliminary data indicate that the threshold intensity for visual activation may be comparable to that required for initiation of wing beat in the moth. It is suggested that the induction of flight activity may be the behavorial consequence of visual stimulation through low-intensity radiation (*10*).

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## Strychnine: Its Action on Spinal Motoneurons of Cats

Abstract. *In cats strychnine does not alter measurably the potential, threshold, after potentials, or refractoriness of the membrane of the spinal motoneurons. Increased reflexes probably result from increase of excitatory impingement upon motoneurons. Spikes recorded during a rapid succession of nerve impulses produced by strychnine ("strychnine burst") suggest that soma membrane resistance is appreciable during stimulation.*

Many attempts have been made to explain the mechanism by which strychnine influences spinal cord activity. A decrease in the size of inhibitory postsynaptic potentials has been shown (*1*), but strong descending inhibitory influences from vestibular stimulation occur in the cord treated with strychnine (*2*). Wall *et al.* (*3*) have shown that the drug decreases the excitability and presumably increases the level of polarization of dorsal root terminals, but does not change motoneuron excitability to extracellular stimulation. It prolongs the repolarizing phase of the antidromic spike in crayfish stretch receptors with concomitant repetitive axonal firing which presumably results from the maintained depolarization of the soma. Inhibitory influences can still be detected in this structure after strychnine poisoning (*4*).

We have studied the effects of strychnine on motoneuronal membrane in an attempt to deduce some of the characteristics of the synaptic impingement on motoneurons after administration of strychnine.

Spinal motoneurons of cats anesthetized with Nembutal and immobilized with curare were impaled with glass micropipettes filled with KCl or $K_2SO_4$, and circuits were arranged for simultaneously recording transmembrane voltages and for passing polarizing currents through the micropipettes (*6*). Strychnine was administered by rapid intravenous injection in doses up to 0.3 mg/kg. Before injection, a motoneuron was penetrated and its responses to antidromic, orthodromic, and direct stimuli were recorded. The same tests were then performed after the characteristic actions of strychnine had developed. Both the resting membrane potential and the threshold current required to elicit firing by intracellular stimulation have the same values before and after treatment. Also, the so-called "critical stimulus interval" (*5*), measured with antidromic stimuli, is unchanged. Thus, none of the properties of motoneurons which we examined was appreciably affected. It is then probable that the changes in reflex responses result from changes in the synaptic impingement upon the motoneurons. The latter could be caused by action of the drug on the postsynaptic membrane of interneurons or by action on presynaptic terminals (*3*).

In a given cell the initial phase of the response to a dorsal root shock recorded intracellularly is similar before and after administration of strychnine; a rapid depolarization develops and the first spike arises at the same membrane voltage. In normal animals, with low intensity stimulation of dorsal roots, the falling phase of the spike tends to repolarize the membrane, and only a single spike occurs. After administration of the drug, however, the slow wave is not abolished by the first spike, but further depolarization occurs which gives rise to other spikes starting at higher and higher levels of depolarization. A large hyperpolarization frequently follows the strychnine burst (Fig. 1*A*).

The magnitude of the slow wave evoked by dorsal root stimulation is markedly altered if polarizing currents are passed through the impaling micropipettes (Fig. 1, *B, C*). The height of the monosynaptic excitatory potential (lower arrows) undergoes less change

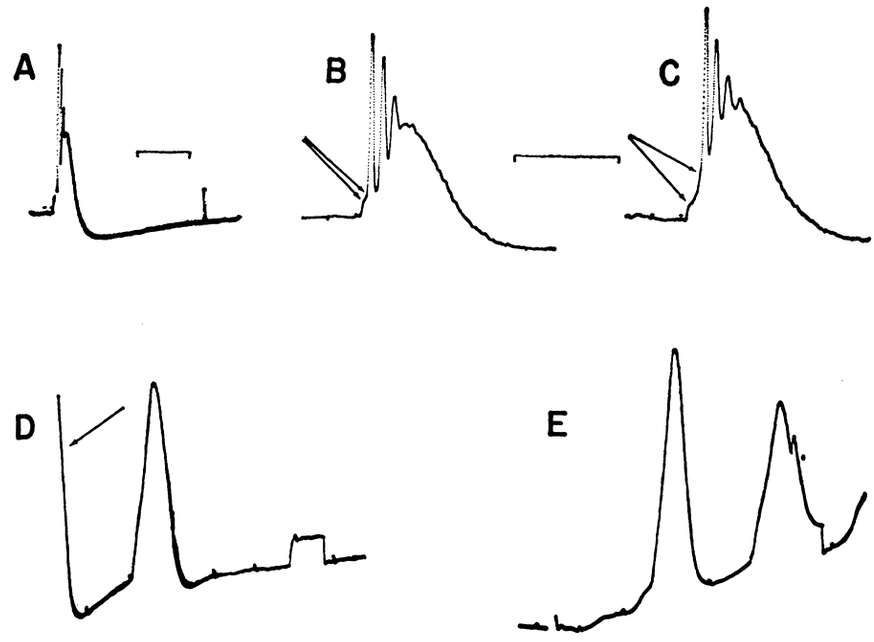


Fig. 1. *A*, Spikes and slow wave evoked after strychnine administration in a motoneuron by dorsal root stimulation; calibration 50 msec and 10 mv. *B*, Record on faster sweep obtained from same cell, with the same stimulation. *C*, Same as 1*B*, but during flow of hyperpolarizing current of $2 \times 10^{-8}$ amp through the impaling micropipette. Lower arrows in *B* and *C* indicate height of monosynaptic excitatory potential. Upper arrows tentatively indicate start of first spike. Time bar: 25 msec; voltage calibration, same as in *A*. *D*, Repetitive firing produced by direct stimulation through micropipette. First spike (arrow) is partly obscured by transient of stimulating pulse. Note greater height of second spike in this trace than in 1*E*. Time marks: 1 msec; square wave: 10 mv. *E*, Repetitive firing from same cell produced by dorsal root stimulation after strychnine administration. Calibrations as in *D*. Note that 10 mv square wave is superimposed upon falling phase of second spike.

than the late slow wave which suggests that membrane conductance is much higher during the strychnine slow wave than it is at rest or during the monosynaptic excitatory potential. It is assumed that the threshold voltage for firing (tentatively indicated by the upper arrows in Fig. 1, *B, C*) is not changed by currents (*6*).

Spikes evoked by the strychnine slow wave decrease rapidly; usually the third spike has a peak-to-peak amplitude of less than 25 mv, as illustrated in Figs. 1*A* and 1*B*. The frequency of discharge during a strychnine burst is about 200 to 300 impulses per second (Fig. 1*E*). If similar frequencies are evoked by direct stimulation with steps of current through the impaling microelectrode, there is some decrease of spike amplitude (Fig. 1*D*). However, the decrease occurring with electrical stimulation is considerably less than it is during a strychnine burst. The more marked decrease of spike amplitude during the strychnine burst may

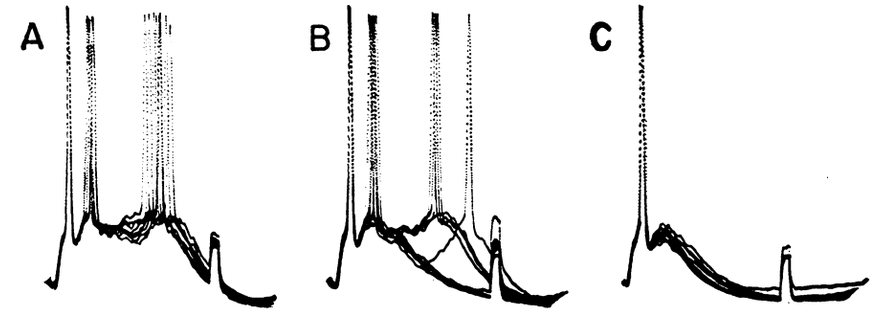


Fig. 2. Multiple traces of repetitive strychnine bursts evoked by dorsal root stimulation. *A*, 4-per-second stimulation; *B*, 7-per-second stimulation; *C*, 10-per-second stimulation. Calibration pulse is 2 msec and 10 mv in each case. Note all-or-none behavior of late wave in *B*.

be the consequence of the load imposed by the subsynaptic membrane, which is highly conducting, upon the spike generator. This conclusion implies that membrane resistance remains appreciable during spike activity, at least for the spikes in the vicinity of the peak of the strychnine wave.

The height of the strychnine slow wave increases rapidly as the strength of a stimulus to a dorsal root is increased. If supramaximal stimuli are repeated at a frequency of about 5 to 8 per second, the late component may disappear in an all-or-none manner (Fig. 2). This behavior suggests that the activity of interneurons becomes abnormally homogeneous under the influence of strychnine, as may happen with more effective coupling among the different units.

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14 March 1963

## Preference Aversion in Mice to Bitter Substance

Abstract. *Preference-aversion functions demonstrable for sweet and salty stimuli have been found for a "bitter" substance, sucrose octaacetate, at individually specific concentrations for mice. The data support Schneirla's views correlating stimulus magnitudes and approach-withdrawal. The positive reinforcing value of this bitter substance as a weak stimulus is diminished with continuous exposure and no secondary reinforcement. Avoidance is related to intensity of stimulation rather than modality or postingestion effects.*

Avoidance of bitter flavored aqueous solutions is considered to be biologically determined and of evolutionary significance, since many of the toxins found in poisonous plants are alkaloids, which are intensely bitter. Moreover, the bitter modality is the most sensitive of the tastes, responding to the lowest concentrations of naturally occurring compounds (*1*).

The typical preference-aversion curve, dependent on concentration, which has been derived for sweet and salty solutions has not been reported for bitter solutions. In studies with the two-bottle choice situation (the substance to be studied dissolved in water is offered simultaneously and continuously with unflavored water), solutions of bitter substances in low concentration may be accepted equally with water. With increasing concentrations, however, there is a marked reduction in the amount of bitter solution drunk compared with water, but no preference point has been reported.

In the course of drinking experiments five of ten NIH/N albino male mice withdrew from water flavored with $10^{-3}M$ sucrose octaacetate (SOA) after 16 hours of water deprivation when no other source of fluid was available. This synthetic substance is intensely bitter to man and is rejected by guinea pigs, rats, hamsters, and great tits at this concentration (*2*). All of the fluid-deprived mice began to drink SOA at once. Half of them stopped after their initial laps, while the other five continued to drink at the same rate as controls drinking only water. Since need should be similar in all animals, it was expected that a striking difference might exist in the sensitivity of the animals to the bitter substance. To investigate the problem the behavior toward SOA under conditions of ad libitum food and fluid was measured.

Another group of ten mice was given a two-bottle choice, $10^{-5}M$ SOA or water. The two bottles were presented for 8 days. Solutions were changed daily and sides of presentation were alternated to eliminate the effect of side preferences. The results are summarized in Fig. 1. Under these conditions, and with animals that had not previously tasted the bitter solution, half showed almost complete rejection at this weaker concentration. Of the remaining five, three drank more test substance (in excess of 55 percent) than water at least for several successive days. The two mice which showed neither preference nor rejection (they drank 55 and 46.4 percent respectively in 48 hours), drank whichever was on the preferred side. However, the animals that started out initially preferring SOA to water tended, after 5 days, to

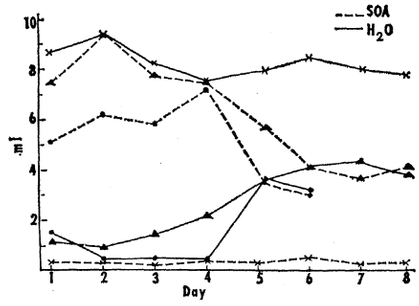


Fig. 1. Mean daily fluid intake. (Cross) Mice that rejected SOA at $10^{-5}M$. (Triangle and circle) Mice that preferred SOA at $10^{-5}M$ and $5 \times 10^{-7}M$, respectively.

a more equal daily intake regardless of prior preference for a particular side. This may be due to habituation, for there is apparently no secondary reinforcing value of the stimulus (*3*).

Since at least half of the animals did not reject the test solution at $10^{-5}M$ and some actually preferred it, a search for preference concentrations was instituted for the five animals which showed almost complete rejection. Presenting animals with rejected concentrations may affect subsequent behavior to less concentrated solutions. Nevertheless, four of the five animals drank more of the test substance at individually specific concentrations when a series of lower concentrations was presented randomly, each for 48 hours or longer.

Two of these showed a peak preference at $5 \times 10^{-7}M$ and were maintained on this concentration to determine if they also showed the apparent habituation decrease. After 4 days of high intake, these also began a daily consumption more equal to that of water (Fig. 1). The other two mice preferred SOA at $10^{-6}M$. Increasing the concentration above the preferred one resulted first in equal intake with water; only side preference was evident. With greater concentrations, the bitter substance was avoided. Two of the three mice which preferred it at $10^{-5}M$ continued their preference at $10^{-8}M$, which is near the limit of its solubility. The remaining animal drank more of it than water only when it was on the preferred side, so that a 48-hour total indicated neither preference nor rejection.

Of the two mice that originally did not prefer or reject sufficiently to overcome side preference at $10^{-5}M$, one rejected and one showed preference at $10^{-8}M$. Thus, for one animal at least, the preference point was at saturation.