Similar experiments were made with p(Tyr,Glu)- -Lys. Four rabbits were injected with 50 mg of antigen at birth, then at the 68th day with a further dose of 30 mg of antigen. At the age of 3, 4, and 5 months these four rabbits and four control littermates received three injections of 15 mg of antigen in adjuvant.

All four test rabbits remained unresponsive, whereas three out of four control animals showed a typical precipitin reaction (at least 200 μg of antibody per milliliter of serum). The unresponsive animals were then injected twice intravenously with 1.8 mg of antigen. The negative response persisted. The specificity of the unresponsive state was tested by injecting 25 mg of ovalbumin in alum; all four experimental animals and the four control rabbits gave a similar positive reaction to this antigen (600 to 2000 μg of antibody per milliliter of serum).

Tolerance to p(Tyr, Glu)- -pLys in adult rabbits treated with 6-mercaptopurine was attempted in four animals injected with 75 mg of antigen. All of the animals were unresponsive toward subsequent immunization with the antigen, whereas three out of four control animals showed a typical positive reaction.

Thus tolerance to synthetic polyamino acids can be conferred either by exposing newborn rabbits to the antigen or by treatment of adult rabbits with 6-mercaptopurine and antigen (*9*).

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14 November 1962

Midbrain Reticular Influences upon Single Neurons in Lateral Geniculate Nucleus

Abstract. *The effect of electrical stimulation of the midbrain reticular formation upon patterns of discharge of single lateral geniculate neurons was studied. Data were processed by means of a 256-channel scaler analyzer. The rate of spontaneous discharge of geniculate neurons was raised by electrical stimulation of the reticular formation and their ability to respond to intermittent light was enhanced.*

Visual messages on their way from the retina to the cerebral cortex are known to be modified at the level of the lateral geniculate nucleus (LGN) by electrical stimulation of the brainstem reticular formation (*1*, *2*). This report, which is concerned with the influences of the midbrain reticular formation on single geniculate neurons, extends the earlier studies (*1*, *2*).

Cats were lightly anesthetized and paralyzed by a continuous intravenous infusion of a mixture of sodium pentobarbital and gallamine triethiodide in normal saline. Photic stimuli were delivered by means of a multibeam ophthalmoscope (*3*). Single unit spikes were picked up with tungsten microelectrodes and recorded on one track of a twin-track magnetic tape recorder. Recycle pulses, synchronous with the end of the light flash or with the stimulus applied to the midbrain, were recorded on the other track. Brief shocks (0.1 msec in duration, 3 to 5 volts) were applied by means of bipolar silver electrodes (*2*) to the midbrain reticular formation centered on Horsley-Clarke coordinates, $A = 2.0$, $LL = 3.0$ and $H = -1.0$.

The data recorded on tape were analyzed by means of a 256-channel scaler analyzer and displayed as poststimulus time histograms (*4*). These were constructed as follows. Spikes were sorted out by the scaler analyzer and stored in channels according to their time of occurrence after the stimulus as indicated by the recycle pulse. A relatively large number of stimulus cycles were used in order to obtain a reliable mean response pattern.

In these experiments units were held for at least 3 hours, which was the minimum time necessary for an adequate analysis of the pattern of the response.

Stimulation of the mesencephalic reticular formation by means of a train of four pulses (300 per second) delivered every 3.5 seconds increased the rate of the spontaneous discharge of a single geniculate neuron when the retina was adapted to low-level photopia.

The data for each histogram (Fig. 1, *A*, *B*, and *C*) required 20 minutes of recording time. Histogram *B* followed *A* immediately. Recording for *C* commenced 1 minute after intravenous injection of 32 mg of sodium pentobarbital (Sagatal).

The poststimulus time histogram constructed for 300 successive cycles of mesencephalic stimulation with 16 msec per channel in the analyzer (Fig. 1*B*) is to be compared with the spontaneous background discharge of the same unit without change in conditions except for the absence of mesencephalic stimulation (Fig. 1*A*). The excitatory action of the midbrain stimulation is evident, consisting of an early, sharp increase in firing, with a peak at about 135 msec after the shock, followed by a fairly well-maintained increase throughout the

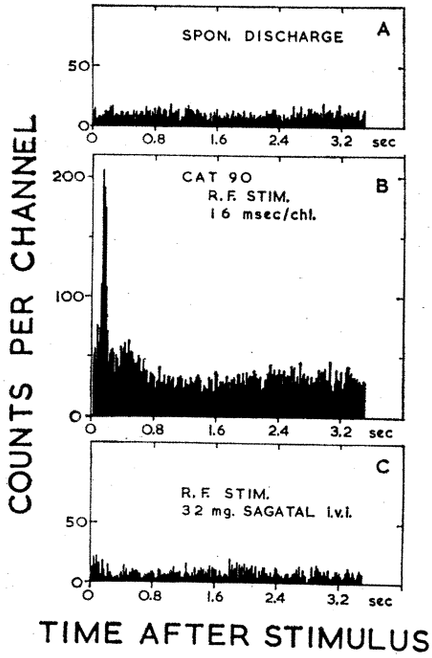

Fig. 1. Effect of electrical stimulation of midbrain reticular formation (R.F.) upon spontaneous activity of single lateral geniculate neuron. By means of a scaler-analyzer channel width of 16 msec, a total of 300 stimulus cycles were analyzed, each having a duration of 3.5 seconds. In each poststimulus time histogram, ordinates represent counts per channel and abscissae time after stimulus (recycle pulse). *A*, Spontaneous discharge under dim-light-adapted state without reticular stimulation. *B*, Midbrain reticular formation stimulated by a train of four pulses (300 per second, 0.1 msec, 3 volts). *C*, Thirty-two mg of sodium pentobarbital (Sagatal) injected intravenously under the same conditions as in *B*.

remainder of the interval before the next shock. This excitatory action was readily abolished by intravenous injection of 32 mg of sodium pentobarbital (Fig. 1*C*).

In addition to this relatively direct action on the LGN, reticular stimulation improved the temporal resolution of a geniculate unit in response to intermittent light (Fig. 2). The data for each histogram in Fig. 2 required 4 minutes of recording time, the recordings following each other immediately in the order *A*, *C*, *B*, and *D*, respectively. The dark-adapted cat's eye was stimulated by intermittent flashes of fixed intensity. A geniculate neuron was able to discharge spikes in response to each flash for frequencies up to 24 cy/sec. At 32 cy/sec (Fig. 2*A*) the unit could no longer follow each flash, frequently failing to discharge. This can be readily appreciated from the fact that the total number of spikes discharged over the 2.5 minutes of stimulation was 1294, in contrast to the total number of flashes, that is, 32 cy/sec × 150 sec = 4800. The presence of a peak at approximately 25 msec after the end of the flash indicates the correlation between the light stimulus and the response. When the stimulating shocks were applied to the midbrain reticular formation in synchrony with the end of each flash, the ability of the unit to respond to the high frequencies of flash was greatly improved (Fig. 2*B*). Not only is there a great increase in the total number of spikes but also spikes are more sharply restricted to a particular phase of the flicker cycle.

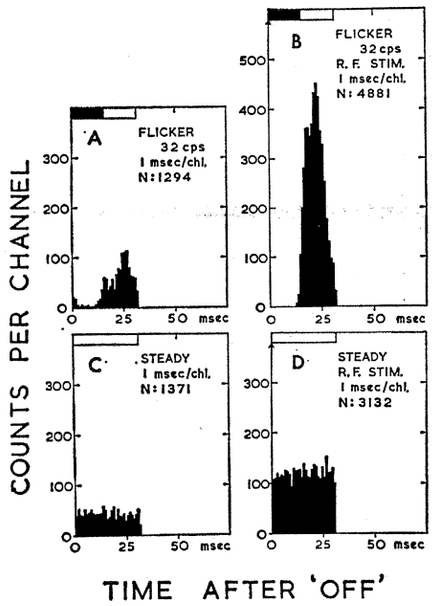

Fig. 2. Effect of reticular stimulation upon responsiveness of single geniculate neuron to intermittent light stimulation. Horizontal black and white bars stand for light off and light on, respectively. By means of a scaler-analyzer channel width of 1 msec, a total of 4800 stimulus cycles were analyzed, each having a duration of 1/32 second. Ordinates represent counts per channel and abscissae time after end of flash (recycle pulse). *N* means total number of spikes analyzed during 2.5 minutes. Arrows indicate stimulation of midbrain reticular formation. *A*, Poststimulus time histogram with only flicker stimulation. *B*, Poststimulus time histogram with flicker stimulation and single-shock stimulation of midbrain reticular formation. *C*, Unitary activity to steady illumination without reticular stimulation. *D*, Unitary activity to steady illumination with reticular stimulation.

Under steady illumination (half intensity of flickering light) the effect of midbrain stimulation was to raise the mean rate of discharge of the geniculate neuron without any special correlation between the stimulating shock and the time of occurrence of spikes (Fig. 2*D*), as compared with Fig. 2*C* in which no reticular stimulation was used.

In evaluating these results two obvious sources of error need to be excluded. First, the effect of the direct spread of stimulating current from the midbrain to the LGN is unlikely to be significant, since the effect long outlasts the duration of the shock. Furthermore, as shown in Fig. 2*D*, there is no correlation between the time of occurrence of the shock and the discharge of spikes. Second, there is also the possibility that midbrain stimulation antidromically excites optic tract axons, thus affecting the geniculate neurons after the manner of an axon reflex. This possibility is excluded because the direct recording from single optic tract axons has failed to reveal any evidence that they are activated by stimulation of the midbrain reticular formation, at least from the stimulating site used in these experiments. This finding also excludes the possibility that centrifugal influences from the midbrain to the retina (*5*) are indirectly affecting the LGN by a subsequent centripetal discharge from the retina.

While the effects of midbrain stimulation reported here have all been excitatory as far as geniculate neurons are concerned, the possibility that inhibitory influences also occur is not excluded, since the number of units so far tested is only small (*6*).

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30 October 1962

Citrus Flavonoid Complex: Chemical Fractionation and Biological Activity

Abstract. *A study was made of the anti-inflammatory activity of the fractionated components of a citrus flavonoid complex. Several highly active components, distinct from hesperidin and naringin, were isolated and tested for anti-inflammatory activity. A method for evaluation of this biological activity is recommended.*

Following Szent-Gyorgyi's original observations of the effects of his vitamin P or citrin preparations (*1*), investigations by the many workers produced an experimental and clinical literature of contradictory thought and observations that culminated in the recommendation that the designation vitamin P be abandoned (*2*). Nevertheless, work in this field has continued and the term "bioflavonoids" was coined to denote those flavonoid compounds having biological activity.

In view of the increasing number of reports of the therapeutic utility of a water-soluble citrus flavonoid complex (*3*) prepared from citrus (mixed orange and grapefruit) peel and pulp (*4*) which have appeared continuously since 1948, we undertook to determine

Table 1. Anti-inflammatory (A.I.) activity of components of a bioflavonoid complex. Test substances were administered orally 2 hours prior to challenge.

Test substance	ED_{25} (mg/kg)	Potency (A.I. units/g)
Hesperidin*	Inactive	0
Naringin*	Inactive	0
Nobiletin	20	50
Pentamethoxyflavone	20	50
RS–1	3	333
Hydrocortisone phosphate (reference)	13.5	74

* Tested at doses between 200 and 400 mg/kg.