Table 1. Differing LD_{50}'s (the dose which is lethal to half the flies) of the four portions of the housefly population used for comparing the effectiveness of WARF/AR plus DDT with the effectiveness of DDT alone.

Sex of flies	LD_{50} for DDT (μg/fly)	
	DDT-susceptible portion	DDT-resistant portion
Males	0.064	0.58
Females	0.12	0.85

Early emerging and late emerging flies were discarded, only those emerging during a 24-hour period being retained for testing. Flies were treated individually on the mesonotum with a 1 μl droplet of an acetone solution containing the required amount of either DDT or DDT plus WARF/AR. Control flies were treated with acetone alone. Flies were anesthetized with carbon dioxide during treatment. Groups of ten sequentially treated flies were kept at 25°C in petri dishes 4.5 cm in diameter, each containing a paper strip to which a mixture of honey, sugar, and dried milk had been applied. At each concentration of DDT, or DDT plus WARF/AR, there were from three to eight independent tests, 100 flies being used for each test. The values shown are average values of these tests.

Throughout the tests the proportion of WARF/AR to DDT was 1 to 5, as recommended by the manufacturers. It is not known whether this proportion produces the maximum effects.

In Fig. 1 (male flies) and Fig. 2 (female flies) the percentage of flies killed at each dose of either DDT or DDT plus WARF/AR is plotted against the logarithm of the weight of DDT applied to each fly (*13*).

The plateau in the dosage-mortality relationship for DDT alone, in both males and females, indicates that the test flies are not homogeneous for DDT susceptibility. One portion (about 62 percent of males and 68 percent of females) is susceptible to relatively small doses of DDT, the LD_{50}'s being shown in Table 1. These values are within the upper range of normally DDT-susceptible strains, but both are 2.5 times the 1959 LD_{50}'s of the parent ES strain (*11*), possibly indicating that in the emergence of the polymorphism for resistance, all individuals have become less susceptible. The other portions of the population (being 38 percent of males and 32 percent of females) have LD_{50}'s (Table 1) arithmetically times 9 and times 7 of the LD_{50}'s of the susceptible portions. These values are beyond the upper range for DDT-susceptible flies and therefore this portion of the population must be regarded as resistant. In this respect it resembles latter generations of the Canberra strain from which it was derived. The original Canberra line appeared homogeneous for DDT susceptibility during 1952 (*9*) but was markedly heterogenous in 1960 with 17 percent resistant males and 19 percent resistant females (*10*).

In the susceptible portion of the population there are no real differences in the percentages of flies killed with DDT or with DDT plus WARF/AR. Thus this synergist conforms with those previously investigated, most if not all (*6, 7*) of which are ineffective in increasing the kill of DDT-susceptible insects. The effects of added WARF/AR are marked in the resistant portion of the population, almost complete kill being achieved at DDT concentrations (0.291 μg males, 0.543 μg females) insufficient to kill more than a small percentage of the resistant portions of the population. The estimated LD_{50}'s of DDT plus WARF/AR to these resistant portions are, males 0.16 μg DDT/fly and females 0.30 μg DDT/fly. Thus at the LD_{50}'s the effects of added WARF/AR were to improve the effectiveness of the DDT to males by 3.6 times and to females by 2.8 times. Alternatively, it may be considered that the added WARF/AR reduced the level of resistance from times 9 for males and times 7 for females to times 2.5 for both sexes. This is a large reduction, but the failure of the synergist to convert resistant flies to fully susceptible flies conforms with the pattern of other synergists (*6*), full susceptibility never being regained (*14*).

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13. Plotting the results of the more widely used probit mortality against logarithmic dose transforms did not clarify the relationship between dose and kill. Only the plot for females treated with DDT plus WARF/AR could be regarded as a reasonable fit to a straight line; similar plots for males suggested two lines of poor fit, intersecting at about probit five, convex upward. As is to be expected from Figs. 1 and 2, the data for DDT alone plot as three intersecting lines of moderate fit. There are no indications of parallelism.
14. The sample of WARF/AR was obtained from Allied Chemical Corporation, New Jersey, through the World Health Organization, Geneva. Their assistance is gratefully acknowledged.

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Cortico-Subcortical Homeostasis in the Cat's Brain

Abstract. *Transcortical polarization of one cerebral hemisphere, while producing the well-known changes in the amplitude of the evoked potentials in the ipsilateral cortex, induced opposite behavior of these indicators in the contralateral cortex. With the corpus callosum sectioned, the reciprocal relationship was enhanced. Anesthetic doses of barbiturates not only eliminated reciprocity but made the potentials on both sides react in unison to unilateral polarization. These findings suggest the existence of a negative feedback system between the cerebral cortex and the subcortex and the existence of a "left-right equalizing" mechanism carried by pathways in the corpus callosum.*

Small amounts of Pentothal or Nembutal injected into the carotid artery of nonanesthetized, curarized cats produce a temporary reduction of the amplitude of the potentials evoked by electrical stimulation of the chiasma and recorded in the ipsilateral visual area I. In contrast, the signals recorded from the hemisphere opposite to the side of injection are enhanced after a latency of 30 to 120 seconds (Fig. 1, top). The magnitude changes last for several minutes, and both sides return to the pre-injection levels at about the same time. The depression by anesthetics in the injected side is a well-known phenomenon; however, to the best of our knowledge, potentiation of evoked responses contralaterally to the

site of administration of a depressing compound has never been described.

It occurred to us that this reciprocal behavior of the two cortices could be explained in the following way. The anesthetic-treated hemisphere is depressed, as evidenced by attenuation of the evoked potentials. This depression leads to a reduction of tonic corticofugal inhibitory discharge and thus to a release of brainstem arousal activity. Consequently, an increase is brought about in the ascending activating system which is masked in the anesthetic-treated cortex but which can exert its effect freely in the contralateral cortex, where it enhances the evoked responses (Fig. 1, bottom).

We thus suggest the existence of a negative feedback system operating between the brainstem and the cerebral cortex. A peculiar aspect of this feedback system would be that its output (that is, the ascending activating mechanism) diverges bilaterally to impinge upon two separate effector areas (that is, the cerebral cortices of both hemispheres) and that the feedbacks from these two areas converge upon the brainstem and operate through a mixer to exert their combined restraining influence (Fig. 1, bottom).

There is evidence for central nervous mechanisms with the necessary operating characteristics to function as components of such a diverging negative feedback system. Numerous investigators have confirmed and extended Moruzzi and Magoun's classical observations (*1*) on the bilaterally and diffusely projecting, ascending activating system. Evidence is also available concerning corticofugal pathways impinging on the brainstem reticular formation. Rossi and Zanchetti, in a recent review article (*2*), have summarized the anatomical data. Descending suppressor effects induced by stimulation of a number of cortical areas (*3*) strongly suggest that these cortico-reticular pathways are partly inhibitory in nature. Release phenomena appearing after decortication (*4*) indicate that the inhibitory downward discharge is tonically active. Hugelin, Dumont, and Paillas (*5*) have shown that the activating output of the reticular formation is under the restraining influence of the cerebral cortex.

To test the validity of the hypothesis of a negative feedback system more explicitly, we used a technique which would allow us to confine any excitability modulating procedure to the cortex of one hemisphere. Transcortical d-c polarization appeared to be a method which would satisfy this requirement.

Thirty-four experiments were done on cats which were given an intraperitoneal injection of Dial with urethane (*6*) (0.5 ml/kg) or were anesthetized with ether during the surgical procedures and then immobilized with Flaxedil and artificially respirated. Local anesthetics were used to infiltrate the marginal areas of the surgical openings in the curarized preparations. Both cerebral cortices were exposed over the whole convexity with a narrow bridge of bone left covering the longitudinal sinus. The skin surrounding the craniotomy was sutured to a ring to form a well. A wall of dental cement, constructed over the longitudinal bone bridge, divided the well into two pools, which were filled with tyrode solution. For d-c polarization, current from a battery supply was passed from platinum foil electrodes in contact with one tyrode pool to platinum wire depth electrodes situated in the white matter. Current flow was generally made to rise to its full value, starting from zero, in about 1 second. In the present discussion we refer to "positive polarization" as surface positive against the white matter. Direct cortical responses and evoked responses elicited by electrical stimulation of the optic nerve or the superficial radial nerve served as indicators of "cortical excitability."

Figure 2 (top left, *A*) shows an example of the results obtained in 25 nonanesthetized animals in which peripherally evoked potentials served as indicators. The typical reciprocal changes of amplitude in response to changes in polarization current are apparent. Similar results, over a smaller current range, were obtained in five animals in which direct cortical responses were used (Fig. 2, bottom).

If the preparations were given intravenous or intraperitoneal injections of barbiturates (in doses to render surgical anesthesia), unilateral polarization with currents of various strengths resulted in curves whose slopes had the same sign (Fig. 2, top left, *B*). We explain this finding by assuming that the structures required for normal functioning of the hypothetical negative

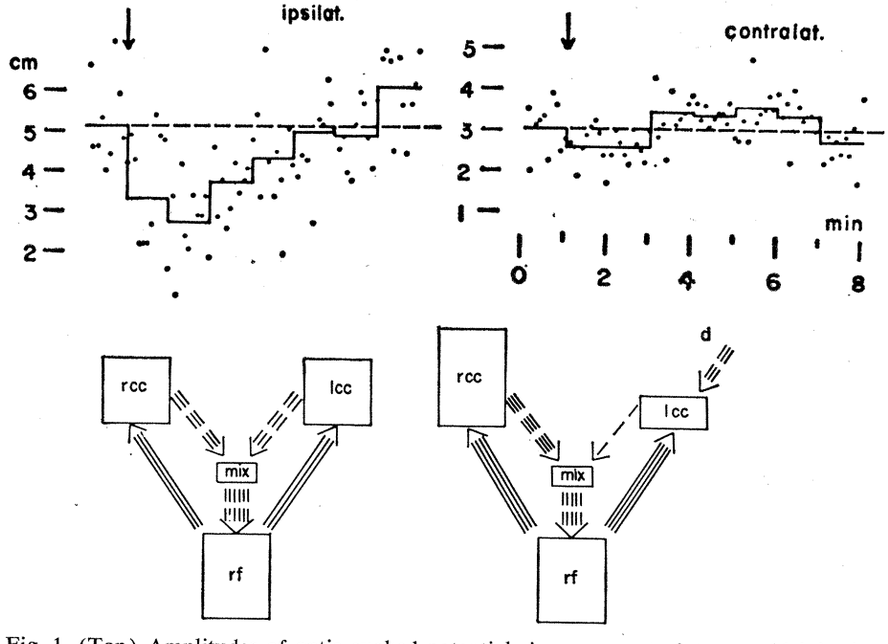

Fig. 1. (Top) Amplitudes of optic evoked potentials in response to intracarotid injection of 3 mg of Nembutal in curarized cat. *Ipsilat.*: potential amplitude (measured from peak of largest positive spike to peak of negative afterswing) on side of injection; *contralat.*: opposite side. Ordinate: arbitrary units of amplitude. Dashed horizontal line: pre-injection control level. Arrow: time of injection. (Bottom) Highly schematic representation of subcortico-cortical feedback system. Left: control level; right: after exogenous depressing factor (*d*) is introduced to left cortex (*lcc*). Note increase in excitability in right cortex (*rcc*) expressed by height of box and drop in excitability in left cortex. Excitability of reticular formation (*rf*) remains constant. Solid-line arrows: activating ascending influences; dashed-line arrows: inhibitory (corticofugal) influences acting via mixer (*mix*) represented simply as summing device.

feedback system are affected by barbiturates. This interpretation is supported by the recent observation that the extracallosal interhemispheric delayed response is eliminated by barbiturates (*7*). We also hypothesized the existence of a second system which is not susceptible to anesthetics and which serves as a tonically active "left-right equalizing" mechanism. Experiments in three cats in which the corpus callosum had been severed 2 to 15 weeks prior to test day point to this pathway as a carrier for this "equalizing" function. Within a limited range of polarizing current, reciprocity was considerably more pronounced in these callosotomized preparations than in intact animals (Fig. 2, bottom). In Dial-anesthetized and callosotomized animals, polarization had little or no effect on the contralateral cortex, whereas the ipsilateral cortex exhibited behavior similar to that in nonanesthetized callosotomized preparations (Fig. 2, bottom).

It should be noted that the relationship between current strength and amplitude of the direct cortical responses in the contralateral cortex is approximately linear for the range of polarizing current from −0.5 to +1.0 ma. Over the whole range investigated, the response amplitude is a sigmoid function of polarizing current (Fig. 2, bottom). No explanation for these aspects of the data can be offered at present.

We also studied in several cats the effect of unilateral total destruction of the cortex. The example shown in Fig. 2 (top right) demonstrates the increase of all the components of the response evoked by chiasmatic stimulation as the result of contralateral decortication.

In experiments involving the direct cortical response in animals subjected to surface-positive polarization, we observed that 3 to 8 minutes after cessation of polarization with currents as low as 1.0 ma, the potentials disappeared for as long as 2 minutes. There is some suggestion that the latency and the duration of this effect were related to the intensity and duration of polarization; however, we do not have enough data to make a definite statement. In appearance and duration, this "dip" could well be related to the phenomenon of spreading depression (*8*).

Transcortical electrical polarization of one hemisphere was found to interact with the evoked potentials not only in the polarized cortex but also in the contralateral cortex. Within a limited range, the changes observed in the contralateral hemisphere were opposite to those in the treated ipsilateral cortex.

There is evidence that the response evoked by stimulation of the optic nerve and recorded in visual area I increases while the experimental animals shift from sleep to wakefulness and as a result of stimulation of the reticular formation (*9, 10*). This observation supports our interpretation that destruction of one cortex leads to enhancement of the ascending arousal activity in the contralateral hemisphere (Fig. 2, top right).

Caspers has shown that the direct cortical response increases in amplitude with shifts toward lower levels of arousal and during surface-positive polarization, and, conversely, decreases with shifts toward higher levels of arousal and during surface-negative polarization (*11*). Similar trends are known for the responses evoked by clicks, light flashes, and electrical stimuli of peripheral nerves (*10, 12*). Our own experiments on the direct cortical response would then indicate

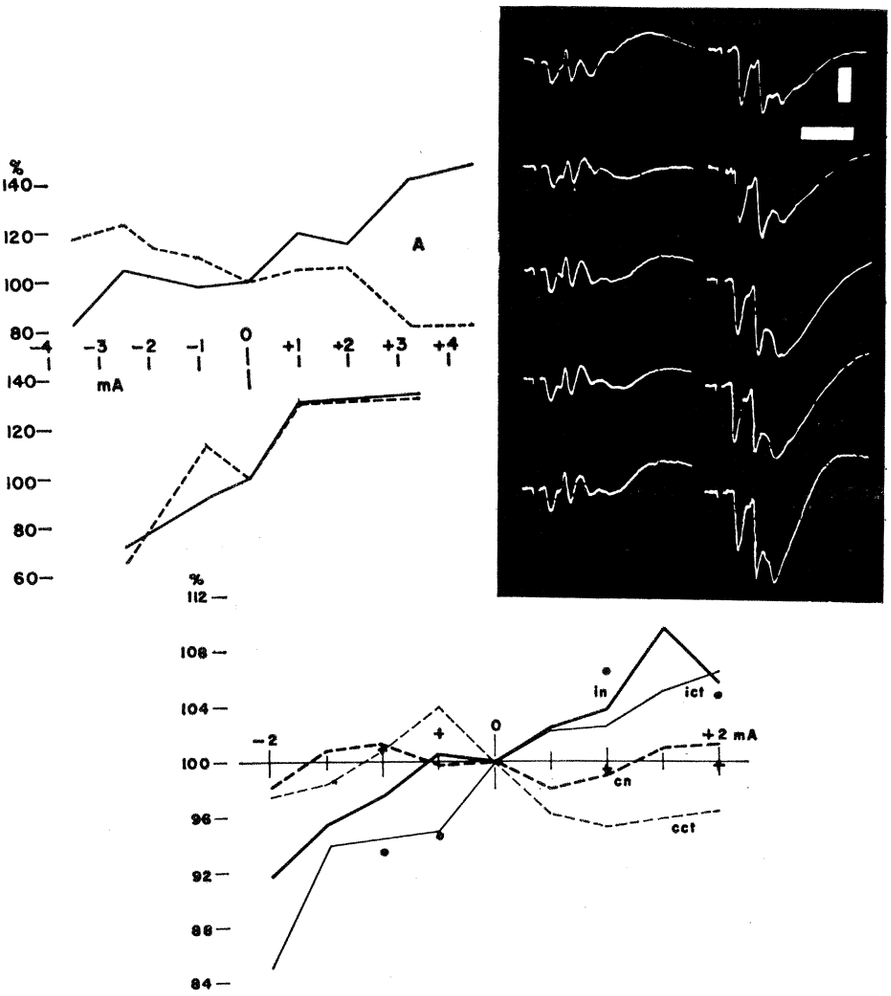

Fig. 2. (Top left) Relation between evoked potential amplitude (negative afterswing of potential, elicited by stimulation of the superficial branch of the radial nerve recorded in SI) and d-c polarization (plus and minus signs refer to surface positivity and negativity, respectively). Polarization for 2 minutes. Amplitude is in percent of control level. Solid line, polarized side; dashed line, opposite, nonpolarized hemisphere. *A*: animal administered Flaxedil; *B*: animal injected with 0.5 ml/kg of Dial with urethane. (Top right) Responses in left visual cortex evoked by single shocks to the chiasma. Left column: five consecutive responses as control; right column: five consecutive responses 2 minutes after the right cerebral cortex of the entire convexity has been sucked off. Calibration: 100 μv, 5 msec. (Bottom) Relation between potential amplitude (in percent of control) during 2-minute period of polarization, and intensity and direction of polarizing current. Solid lines: side of polarization (*i*); dashed lines: opposite, nonpolarized side (*c*); heavy lines; means of five noncallosotomized animals (*n*); thin lines: means of three chronically callosotomized animals (*ct*). Circles and crosses: amplitude of direct cortical responses (in percent of control) on ipsilateral (•) and contralateral (+) cortex of anesthetized callosotomized preparation, plotted against polarizing current (one animal). Directional signs refer to polarity at surface of cortex.

that surface-positive polarization decreases the arousal level in the ipsilateral cortex and enhances it in the contralateral cortex, whereas surface-negative polarization enhances arousal in the ipsilateral and, within a limited current range, depresses it in the contralateral cortex. A similar interpretation is justified for experiments on the peripherally evoked somatosensory response.

We also may interpret the reciprocal changes in amplitude in the two hemispheres as representing reciprocal changes in excitability. If we use identical stimuli to evoke the potentials in homologous areas of the two hemispheres, and thus activate two homologous populations of neural elements, we can expect that reciprocal changes in the overall excitability in the two populations are likely to produce reciprocal changes in the amplitude of the evoked response.

Unilateral transcortical polarization or unilateral destruction of the cortex then brings about changes which are compatible with the proposed mechanism of a diverging negative feedback system operating between the subcortex and the two cortices. Although we do not have sufficient data to quantitatively and temporally define the operational characteristics of this system, we conclude from the present results that, within a limited range, changes produced in one hemisphere are compensated for by opposite changes in the other hemisphere. This points to one important function of this hypothetical negative feedback system, namely, to keep the "mean excitability" or the "mean arousal level" of the whole cerebral cortex at a constant level, whose actual value at a given time would depend upon a "preset" homeostatic magnitude.

The homogeneous distribution of excitability level over both hemispheres would be the function of the callosal "equalizer mechanism." According to Bremer (*13*), the two-way conduction channels in the callosal system are tonically active. Bremer also claimed that these commissural fibers are predominantly excitatory in nature, whereas Chang (*14*) came to the conclusion that the callosum exerts mainly inhibitory effects. Recently it has been shown that "phenomena of both excitatory and inhibitory nature follow the arrival of a transcallosal volley" (*15*). The "equalizing function" proposed here could be subserved by excitatory and/or inhibitory elements: a facilitatory influence would be the result of increased excitatory activity and/or a decreased inhibitory activity and, conversely, depression would result from a drop in excitatory activity and/or an increase in inhibitory activity (*16*).

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Canine Antiserums Analogous to Human Allergic and "Blocking" Antiserums

Abstract. *The serums of dogs that are allergic to ragweeed can passively sensitize normal dogs. Cutaneous reactions or systemic anaphylaxis may be produced by appropriate challenge with ragweed extract. Canine antiserum produced by immunization of normal dogs with ragweed extract is shown to inhibit these reactions of the passively sensitized dogs.*

Human reagin (allergic antibody, skin-sensitizing antibody) is an antibody believed responsible for certain reactions which occur in allergic individuals after exposure to the antigen (*1*). Antiserums produced by the injection of pollen extracts in man have been shown to modify the passive cutaneous reaction, the Prausnitz-Küstner reaction (*2*), of reagin and allergen (*3*). The antibody so produced is believed responsible for this modification and has been termed "blocking" antibody. Experimental studies in clinical pollinosis demonstrating a protective effect of "blocking" antibody against the passive production of asthma and anaphylaxis in man are not feasible.

Canine hypersensitivity provides a laboratory model of allergic disease (*4*). Canine reagin (allergic antibody, skin-sensitizing antibody) in sufficient quantity transfers cutaneous, bronchial, and anaphylactic sensitivity to normal dogs (*5*). The present studies report the production of an antibody to ragweed-pollen extract in normal dogs which modifies the reaction of dogs passively sensitized with canine anti-ragweed reagin and challenged with ragweed antigen.

Normal dogs received six biweekly injections of a 15-percent pollen extract of short ragweed (*Ambrosia elatior*) in incomplete Freund's adjuvant (*6*). The dogs were bled prior to each injection. The pooled serums of dogs immunized with ragweed (IDS) were tested for antibody by hemagglutination (*7*) of tanned red blood cells coated with ragweed antigen, and by ring and gel-diffusion precipitin tests (*6*). The hemagglutination titer of the pooled serums was 1:400. Ring and gel-diffusion precipitin reactions with 5 percent ragweed antigen were negative, although four of the twelve individual serums composing the pool were weakly positive.

Two separate pools of canine reaginic serums (CRS) were collected by bimonthly bleedings for 12 months from each of two dogs proven to be spontaneously sensitive to ragweed pollen (*8*). The highest serial five-fold dilution of each CRS pool eliciting a positive passive cutaneous reaction (*5*) after an intravenous challenge injection of 1 ml of a 3 percent concentration of ragweed extract was determined in two recipient normal dogs. The two CRS pools elicited positive passive cutaneous reactions at dilutions of 1:25 and 1:125, respectively. When the same amount of ragweed antigen was incubated with 10 ml IDS for 1 hour at 37°C prior to intravenous challenge, the mixture of ragweed and IDS did not result in any positive cutaneous reactions in the recipient dogs. This experiment suggested that the ragweed antigen was neutralized by the IDS and