Effects of stimulating the cerebellar surface on the activity in penicillin foci

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The effect of stimulating the cerebellar surface on the activity of acute penicillin foci (20,000 units) was investigated in a statistically designed study. All animals were randomly assigned either to an experimental group in which the dura over the posterior lobe was opened and a bipolar surface electrode placed over the vermis of the anterior lobe, or else to a control group in which only the dura was opened. After surgery, the animals in the experimental group were randomly assigned to a stimulated or an unstimulated group. The cerebellar surface was stimulated at a frequency of 10/sec using capacitatively coupled pulses with peak current densities of 26.0 mA/sq cm and a duration of 0.1 msec. The effectiveness of the stimuli for activating neuronal elements in the cerebellum was evaluated 1) by recording the cerebellar evoked response from the sensorimotor cortex, and 2) by measuring the effect of a conditioning cerebellar stimulus on the percent change in the amplitude of the cortical response evoked by stimulating the receptive fields of the trigeminal nerve. A statistical comparison of the mean number of seizures for successive 10-minute intervals between control and experimental groups indicated that any difference in the means of these parameters occurred by chance with a high probability and did not result from any treatment. The duration of the seizures, however, was significantly affected by stimulation of the cerebellar surface. Further, the total number of seizures and the amplitude of the cerebellar evoked response in the same animal were inversely correlated. Thus, stimuli that evoked large-amplitude responses over the sensorimotor cortex may have produced a decrease in the number of seizures, whereas stimuli that evoked small-amplitude responses may have produced an increase in the total number of seizures with respect to the control group.

KEY WORDS: cerebellar stimulation, epilepsy, penicillin focus, seizures, cerebellar evoked response

RECENT clinical experiments indicating that electrical stimulation of the cerebellar surface might be therapeutically beneficial to patients with intractable, nonfocal epilepsy led to a renewed interest in determining whether the cerebellum may alter neuronal activity in experimentally induced epileptic foci. As a result, experimental studies have been performed in a variety of animal models. For example, Hutton, et al., used the penicillin focus model in acute animal preparations. More recently, the sole administration of penicillin, pentylentetrazol, or chloralose, or the combination of enflurane and a sensory stimulus in awake animals was used to create four different animal models of epilepsy that seem to bear behavioral and electroencephalographic (EEG) manifestations corresponding to those observed in some human...
CAT POOL
Female, 2-3 kg.

Randomly assigned before surgery to

Control Group
(dura open)

Experimental Group
(electrode placed)

Randomly assigned before Penicillin injection to

Unstimulated Group
Stimulated Group

FIG. 1. Diagram illustrating the assignment of the animals to the protocols. Female animals were randomly assigned before surgery to either the control group or experimental group. The latter group received twice as many animals as the control group, since the animals in this group were subsequently randomly assigned to either the unstimulated or stimulated group before the injection of the penicillin.

seizure disorders. Other models that most closely resemble psychomotor epilepsy were produced by the injection of cobalt into the hippocampus. In addition, some investigators employed electrical stimulation to evoke afterdischarges in the septum, whereas others used the augmenting response of the thalamocortical system.

In all of these studies, the effect of stimulating the cerebellar cortex on abnormal EEG activity produced by each experimental model was observed either qualitatively or quantitatively. The results from these experiments were often inconclusive (see Myers, et al., Table 1) and did not convincingly determine whether stimulation of the cerebellar surface can affect seizure activity in these animal models. One of the reasons for the differences observed between the reported effects of cerebellar stimulation might in part have depended upon the differences in the strength and characteristics of the abnormal neuronal activity of the various seizure foci, as well as on the differences in the parameters and locations used for stimulating the cerebellar surface. Equally important, however, are the differences in the degree to which these experiments were statistically designed and the extent to which the data were quantitatively evaluated.

The present experiments were undertaken to re-evaluate the effect of electrically stimulating the cerebellar surface on the abnormal neuronal activity of penicillin foci, using an experimental protocol which was designed according to strict statistical rules. The results demonstrated that the abnormal activity of these seizure foci was extremely variable between animals of the same group and, as a consequence, the statistical comparison of most of the calculated parameters between the control and treated groups indicated that many of the observed differences could have resulted by chance with high probability, rather than as a consequence of stimulating the cerebellar surface. However, the data also indicated that stimulation of the cerebellar surface increased the number of seizures of short duration with respect to the control group. Further, when stimuli were applied that evoked responses of large amplitude in the sensorimotor cortex, the total number of seizures was reduced with respect to the mean of the control group.

Materials and Methods

Experiments were performed on 68 female cats (including seven preliminary experiments) weighing between 2.0 and 3.5 kg. (Females were preferred because of their greater abundance.) All 61 animals in the experimental sequence were randomly assigned at the outset of the experiment to one of two groups (Fig. 1), with the experimental group having twice as many animals as the control group. In the experimental group, the dura over the vermis of the posterior lobe was cut, and a cerebellar stimulating electrode was placed over the vermis of the anterior lobe. In the control group, only the dura over the vermis of the posterior lobe was cut. Following surgery, but before injection of the penicillin, the animals in the experimental group were randomly assigned to either the stimulated or the unstimulated group.

All animals were initially anesthetized with halothane, intubated with a 5-mm pediatric endotracheal tube, and artificially respired following the intravenous administration of 0.5 cc gallamine triethiodide (Flaxedil). Blood pressure was measured continuously by a catheter placed in the femoral artery. End-expiratory CO2 concentration was monitored throughout the experiment and maintained between 3.5% and 4.5%. The normal body
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温度（39°C）的动物被维持在±1°C 与一个反馈设备。按照这些初步准备，
动物被放在一个立体外固定器，立即被在 C1-2 的水平切断脊髓，以防止
后期记录期间的过度动作。这种程序被爱好于麻醉的动物与 Flaxedil，
因为这种药物对中枢突触的影响和在青霉素焦点的活动是未知的。

细胞器刺激的电极*被插入一个非常小的开口在后叶的上瑞斯，然后进
一步地向前，以便它休息在前叶的后端表面，通常对侧于青霉素焦点。放
置后，电极被缝合在位，切口闭合。

之后，感觉运动皮质被暴露。两根银线电极被粘在位；一根被放在大
脑皮质的上方（参考电极）和另外一根在皮质表面的 1 到 2 mm 从注射位
点（记录电极）。皮质的 EEG 活动被放大由一个 P511 草药前置放大器†
和记录在一个 Brush 纸带记录仪。‡

青霉素钠（20,000 单位溶解在 20 μl 正常生理盐水）被通过一个破
坏玻璃微电极在大约 20 分钟（大约 1 mm 深）注射到右侧感觉运动皮
质。新鲜的稀释青霉素被混合与 Evans 蓝色染料来允许在显微镜下视
察任何可能泄漏的注射物质沿管的管。在手术的暴露和注射期间，皮
质表面被持续地湿以暖的生理盐水滴（39°C）。

在注射后，开颅术被用德士高橡胶覆盖。所有开口被随后缝合在动物
还在乙醚麻醉状态下。这时（2 到 3 小时后开始的手术），最初的麻
醉药物不再能作为由面部的运动相关于记录从青霉素焦点。动物被移
开立体外固定器和放入一个音响-和光的盒子为随后的 5 小时记录期间。

细胞器表面被刺激用双极电脉冲在 10/秒与铂板嵌入德士高（7.6 平方
毫米表面/板）的频率。信号被电容耦合和有峰值的电流密度为 26.0 mA/
平方厘米和一个持续时间 0.1 毫秒。这个刺激强度被预先展示在活化
细胞器的传入，细胞器的核和网状形成在猫。‡

*细胞器表面电极由 Medtronics, Inc., Minneapolis, Minnesota 制作。
†前置放大器由 Grass Instrument Co., 101 Old Colony Avenue, Quincy,
Massachusetts 制作。
‡纸带记录仪由 Gould, Inc., Instrument Systems Division, 3631
Perkins Avenue, Cleveland, Ohio 制作。
§正畸树脂由 L. D. Caulk Co., Milford, Delaware 制作。

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Preliminary studies indicated that halothane anesthetic can profoundly affect the activity in a penicillin focus (Fig. 2). In addition, this anesthetic can also significantly reduce the excitability of neurons in the cerebellar nuclei. Therefore, in all animals the halothane anesthetic was terminated after completion of the surgery and closure of all incisions. The 5-hour recording period was started 40 minutes after the halothane anesthetic was terminated. Preliminary indications suggested that the effects of halothane on the activity in a penicillin focus (Fig. 2) and the neurons in the cerebellar nuclei were to a large extent reversed after this time period.

Following the 5-hour recording time, all animals were perfused with formalin and the cerebellum exposed. The cerebral cortex as well as the cerebellum were removed and stored in formalin for later examination by a person not involved in other aspects of the experiment. The cerebella for all three groups were evaluated for hemorrhage, and placement and depression of the electrode.

The activity in each penicillin focus recorded over a 5-hour period was analyzed in 10-minute periods. The criterion for the occurrence of a seizure was an increase in recording amplitude above background EEG that lasted for at least 2 seconds. The paroxysmal spikes were not included but short afterdischarges which sometimes follow them were tabulated if they lasted at least 2 seconds. In each 10-minute recording period, the duration of each seizure was measured and tabulated on computer cards for later processing and calculation of the parameters on the Health Sciences CDC 3300 computer. The evaluation of the effectiveness of cerebellar-surface stimulation involved the statistical comparison between the parameters of either the unstimulated or the stimu-
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Fig. 3. Average time of seizures in successive 10-minute intervals are plotted with respect to time for the preliminary experiments (A) and for the control (B), the unstimulated (C), and stimulated (D) groups. The standard deviations are indicated by the dashed lines.

laid group with those of the control group.
Since the variability of the parameters characterizing the activity of the penicillin focus among the animals from any one group was unknown for the present experimental protocol, a preliminary study was undertaken in seven cats, all of which were surgically prepared according to the protocol established for the animals assigned to the control group. Following the tabulation of the data from these experiments, the number of seizures was determined for successive 10-minute periods for each animal. Then the average number of seizures in corresponding 10-minute periods was calculated for all seven animals. These averaged values and the corresponding standard deviations were then plotted with respect to time (Fig. 3 A). During the first 110 minutes of recording time, the variability in the number of seizures between animals was relatively small compared to the observed variability at later times. In addition, the mean values for the total number of seizures, total seizure time, and the time per seizure were evaluated and shown in Table 1.

From this preliminary study, it was concluded that if 14 experiments per group were performed in the experimental sequence, the mean value of the total seizure time and the mean number of seizures during the initial 110 recording minutes (see Table 1) would be expected to be within at least 8% of the true mean of their distribution, whereas the mean values of the total number of seizures per animal and time per seizure would only be expected to be within 23% of their respective true mean (see Table 1). Therefore, these observations indicated that, for any treatment to be effective, the mean value of the
TABLE 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total No. of Seizures</th>
<th>Total Seizure Time (sec)</th>
<th>Time/Seizure in First 110 min (sec)</th>
<th>No. Seizures Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>396</td>
<td>5930</td>
<td>16.8</td>
<td>13</td>
</tr>
<tr>
<td>standard deviation</td>
<td>173</td>
<td>827</td>
<td>5.3</td>
<td>2</td>
</tr>
<tr>
<td>95% confidence interval as % of mean</td>
<td>±23%</td>
<td>±8%</td>
<td>±17%</td>
<td>±8%</td>
</tr>
<tr>
<td>no. animals</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*The statistical tests performed on each of these parameters suggested that, if 14 experiments were performed, the mean value of the total seizure time and the number of seizures per 10-minute interval during the initial 110 minutes of recording time would be expected to be within 8% of their true mean value, whereas the mean values of the remaining parameters, total number of seizures and time per seizure would be within 23% of their respective true means.

parameters of the treated group would have to differ from the mean of the control group by at least 8% or 23%, respectively (95% confidence interval), assuming that the distributions characterizing the parameters of the control group in the experimental sequence would be similar to those obtained from the preliminary experiment. On the basis of these observations, it was decided to begin stimulation of the cerebellar surface at the onset of the 5-hour recording period and to evaluate its effectiveness in altering the activity of the penicillin focus over the entire recording period since the duration of cerebellar stimulation could also be regarded as a treatment parameter.

Results

A total of 61 experiments were performed to obtain the 14 animals required for each of the three groups (control, unstimulated, and stimulated). Nineteen experiments were excluded from the protocol exclusively for the reasons indicated in Table 2. The difference in the number of animals discarded from the protocol between each group was not statistically significant ($\chi^2$ test). For the remaining 42 experiments, the mean number of seizures during successive recording periods was calculated for each group. Figure 3 illustrates the mean values and appropriate standard deviation for the control (Fig. 3 B), the unstimulated (Fig. 3 C), and the stimulated groups (Fig. 3 D). The number of seizures in the individual foci of each group was extremely variable as indicated by the large standard deviation with respect to the mean value for each 10-minute period. As a consequence, the statistical comparison of the mean values between the control group and the experimental groups indicated that the differences observed in the mean number of seizures might have occurred by chance with high probability rather than as the result of any treatment, for instance, the placement of the electrode or electrical stimulation (two-sided t-test). In addition, the mean values for the total number of seizures in the experimen-

TABLE 2

<table>
<thead>
<tr>
<th>Reasons for Removal</th>
<th>Control Group</th>
<th>Unstimulated Group</th>
<th>Stimulated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>temporary loss of respiration (power failures)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>uncontrollable or low end-expiratory CO$_2$ (outside 3.5–4.5%)</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>low temperature (below 38°C)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>leakage of penicillin during injection (see Methods)</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>edema of injection site (postmortem)</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>surgical problems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inappropriate termination of halothane (see Methods)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total cats discarded</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
Cerebellar stimulation and penicillin seizure foci
tal groups were seemingly different from
those of the control group (Table 4), but
because of a large standard deviation within
each group, these differences could not be
ascribed to the treatments.
An attempt was made to gain an under-
standing of the large variation in the number
of seizures between animals of each group. A
comparison of the plots representing the
average number of seizures in successive 10-
minute intervals revealed that the activity in a
penicillin focus could change significantly
during the 5-hour recording period. For ex-
ample, in a single experiment illustrated in
Fig. 4, the number of seizures changed
dramatically, increasing from a value below the
mean number of seizures for the group to
values two standard deviations beyond the
mean value. The comparison of the recorded
data obtained from the 10-minute intervals
previous to the sudden change (Fig. 4 A) with
the 10-minute periods during which the
number of seizures was drastically increased
(Fig. 4 B and Fig. 5), indicated an abrupt
change in the recorded activity of the
penicillin focus. In particular, the average
duration of the seizures was much shorter
during the periods when the number of
seizures was larger. Most seizures during
these periods consisted of irregularly oc-
curring paroxysmal spikes followed by an
afterdischarge lasting for only a few seconds.
This pattern of activity was quite different
and in contrast to the activity recorded during

![Fig. 4. Comparison of the mean number of seizures in successive 10-minute recording periods (graph above) with the recorded data (A, B). Before the sudden change in the mean number of seizures (A), the pattern of activity was quite regular, that is, the seizures were of longer dura-
tion and were followed by a depression of activity and subsequent paroxysmal spikes. In Period B, the pattern of activity changed drastically and con-
sisted of irregularly occurring paroxysmal spikes followed by an afterdischarge lasting for only a few seconds (see also Fig. 5) resulting in the large number of observed seizures.](image)

![Fig. 5. Irregular paroxysmal activity recorded from a penicillin focus. This record was obtained from
the same focus illustrated in Fig. 4 at a time when the mean number of seizures was drastically increased.](image)
FIG. 6. Comparison of the mean number of seizures in successive 10-minute recording periods (graph above). The mean number of seizures for this animal in each period was approximately the same as the mean number for the particular group. Both at A and B, a consistent pattern of activity was observed (see records below). The depression of activity following each seizure was terminated by an increasing number of paroxysmal spikes that eventually lead to the occurrence of the next seizure.

earlier periods, in which the seizures were of longer duration and were followed by a depression of activity and subsequent occurrence of several paroxysmal spikes (Fig. 4 A). This latter pattern of neuronal activity was regularly observed in those experiments in which the number of seizures was approximately the same as the mean number of seizures for the particular group (Fig. 6 A and B).

These observations suggest that the effectiveness of stimulating the cerebellar surface should also be evaluated with respect to the duration rather than the number of seizures. Therefore, histograms of the durations of all seizures from each animal were constructed. Even though the configurations of these histograms were quite variable, they could be classified into four distributions (Fig. 7). The histograms of the first distribution were unimodal with a maximum number of seizures with very short durations and a rapid decline from this maximum (Fig. 7 A). The histograms characterizing the second type of configuration were also unimodal, but their mode was usually between 7 and 10 seconds and had a very small number of seizures of very short duration (Fig. 7 B). The third consisted of a multimodal distribution with a very large difference in the amplitudes between modes (Fig. 7 C). The fourth was also multimodal but with small amplitude differences between modes (Fig. 7 D). In order to determine...

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whether the treatments had any effect on the characteristics of these distributions, a person uninvolved in the experiment was asked to categorize the histograms according to the above criteria and tabulate them with respect to the three experimental groups (Table 3). Even though differences in the number of histograms in each category were observed among the three groups, the $\chi^2$ test indicated that these differences could have occurred by chance with high probability, and hence could not be ascribed to treatment effects.

This conclusion was not supported by the comparison of the histograms that combined the durations of all seizures from all animals in each group (Fig. 8). The control group and the stimulated group demonstrated a very large number of short seizures (of 2-second

<table>
<thead>
<tr>
<th>Histogram Grouping</th>
<th>Control Group</th>
<th>Unstimulated Group</th>
<th>Stimulated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>unimodal (mode at 2–3 sec)</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>unimodal (mode at 7–10 sec)</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>multi-modal (large difference)</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>multi-modal (small difference)</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

*Each histogram was constructed from the durations of all seizures recorded from an animal. No significant difference was found between groups in each category using the $\chi^2$ test. See Fig. 7.

Fig. 8. Histograms constructed from the durations of all seizures in all animals of each group. The differences between the distribution of either the unstimulated (U) or stimulated (S) groups and the control (C) group were statistically significant ($\chi^2$ test).
duration) whereas the unstimulated group did not show a significant number of short seizures with respect to larger ones. Using the $\chi^2$ test, we found that the comparison of the three distributions indicated that there was a significant difference between the histograms of the treated groups and the control group. However, the mean values and standard deviations for the total seizure time per animal in each group were not significantly different (Table 4). When the average time per seizure and animal was calculated, the treated animals seemed to have greater average seizure time, but the difference was not significant at the $p = 0.05$ level.

The question was then raised whether some of the differences among the parameters within an experimental group, especially in the unstimulated and stimulated groups, could result from either the placement of the electrode or the effectiveness of the cerebellar stimulus to activate the neural substrates. Since the histological evaluation of the cerebella indicated that the electrode impression on the cerebellar surface and the associated hematoma were different from animal to animal, a difference in the seizure activity between animals of the same group might have resulted from the placement of the electrode. This conjecture was evaluated by correlating the histological findings with the total number of seizures of the corresponding animal. However, the correlation was not significantly different from zero (coefficient = 0.36).

The very large differences in the number of seizures among animals in the stimulated group could have resulted from differences in the effectiveness of the cerebellar stimulus in altering seizure activity. In order to assess this possibility, the response evoked by the cerebellar stimulus was recorded from the surface of the sensorimotor cortex before the injection of the penicillin (Fig. 9 A). In 10 of 12 experiments in the stimulated group,* responses of different amplitudes and with averaged latency of 10.2 ms (1.63 ms SD) were evoked by the cerebellar surface stimulus. The amplitude values for each cerebellar evoked response were correlated with the total number of seizures for the appropriate animal. These calculations indicated that these two parameters were independent, since the correlation coefficient ($-0.51$) was not different from zero at the 0.05 level of significance. The primary component of the trigeminal evoked response (Fig. 9 B) was affected in nine experiments by the cerebellar-surface stimulus. The response was increased on the average by 19% in three animals and decreased in six of them (mean 41%, 13% SD). The secondary component was affected in eight experiments; in two of these experiments, the response was increased (average 51%), whereas in six of them, the response was decreased by an average of 44% (33% SD). The percent changes of amplitude of the primary and secondary components were compared to the total number of seizures from the corresponding animal. The correlation for the primary component was insubstantial.

*Note as a result of camera failure, the records of the cerebellar evoked response in two animals were not available for a quantitative evaluation.

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**TABLE 4**

**Correlation of number and duration of seizures per animal in each experimental group**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Unstimulated)</th>
<th>Group III (Stimulated)</th>
<th>Tests for Equality of Means of Distribution (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>no. seizures/animal</td>
<td>463</td>
<td>205</td>
<td>378</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>p &gt;0.2</td>
<td>p &gt;0.1</td>
<td>p &gt;0.2</td>
<td>p &gt;0.1</td>
</tr>
<tr>
<td>total seizure time/animal (sec)</td>
<td>5278</td>
<td>2299</td>
<td>5133</td>
<td>1848</td>
</tr>
<tr>
<td></td>
<td>p &gt;0.3</td>
<td>p &gt;0.2</td>
<td>p &gt;0.3</td>
<td>p &gt;0.2</td>
</tr>
</tbody>
</table>

*Even though the mean values of these parameters of the stimulated or unstimulated groups differed from those of the control group, these differences could not be ascribed to the treatments since they could have occurred by chance with high probability.
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0.37, whereas for the secondary component, it was -0.03, indicating that the effectiveness of the cerebellar-surface stimulus in altering the amplitudes of either primary or secondary components was independent of the differences observed in the total number of seizures.

Discussion

Experimental evidence that cerebellar stimulation can effectively alter the abnormal neuronal activity in experimentally induced epileptic foci in animals is important in contemplating use of this procedure for treating patients with intractable epilepsy. First, such information would provide an important neurophysiological basis for ongoing and future clinical trials. Second, if a suitable model can be established, and if cerebellar surface stimulation can be demonstrated to affect seizure activity in a well designed statistical study, the efficacy of various stimulus parameters and locations can be systematically investigated. Third, the outcome of such a study would provide appropriate models for investigating the mechanisms and pathways essential for mediating the effectiveness of cerebellar stimulation. As a consequence of the clinical importance of additional experimental evidence, several studies have recently been reported that used different animal models and experimental designs to determine successfully whether cerebellar stimulation may, in fact, reduce the severity of seizure activity. However, many of these studies were not statistically designed, and, in addition, often did not adequately quantify the data.

The present experiments were designed to circumvent some of the shortcomings of previous studies by using the following criteria: 1) the animals were assigned randomly to control, stimulated, and unstimulated groups; 2) the histological and physiological measurements, and the surgery were performed in double-blind fashion; 3) the data were analyzed quantitatively; and 4) changes in factors extraneous to those directly related to the activity in the penicillin focus and the action of the cerebellar output on the focus were minimized. The control of the fourth criterion was achieved by reducing gross movements of the animal with a cervical transection of the spinal cord rather than by using a continuous Flaxedil infusion, by

![Fig. 9. Averaged evoked responses recorded from the sensorimotor cortex. A: Averaged potential evoked by stimulating the surface (CbS) of the posterior folia of the anterior lobe vermis. B: Averaged response evoked by stimulation of the face in the distribution of the trigeminal nerve while the animal was still anesthetized (Trig) in control animals. C: Trigeminal evoked response conditioned by stimuli applied to the cerebellar surface (conditioning interval = 10 msec). D: The average cerebellar evoked response (A) was subtracted from the averaged response in C to determine the time course of the conditioned trigeminal evoked response (D). This response was then compared to the unconditioned response (B). All evoked potentials were obtained by averaging 128 consecutive responses.](image)
recording from an awake, unanesthetized animal (see Methods), and by controlling temperature and end-expiratory CO₂. The regulation of the latter two parameters was thought to be very important to this study, since previous studies as well as our own preliminary experiments indicated that the number and duration of seizures were drastically affected by any variations of both temperature and end-expiratory CO₂. The choice of the acute penicillin focus as an animal model was based on several considerations. First, the model had been previously found to be a satisfactory one for the quantitative evaluation of the effectiveness of anticonvulsant drugs. Second, the focus could be generated within minutes, and the effectiveness of the electrical stimulus on the activity could be assessed within hours. Third, the predicted number of animals needed for a statistical evaluation of the study was economically feasible. However, despite these factors, the choice of this model may still have been inappropriate for testing the therapeutic effectiveness of cerebellar stimulation, since the abnormal neuronal activity in the focus could have been too strong to allow any statistically significant modification of its characteristics by cerebellar stimulation. The strength of the focus in the present experiment (20,000 units) was chosen because the data of Stark, et al., indicated that the mean values of the parameters characterizing the activity of the foci induced by the injection of 20,000 units of penicillin were not statistically different from those representing the activity of the foci created by the injection of either 15,000 or 60,000 units. In addition, preliminary experiments suggested that the injection of 20,000 units resulted in a penicillin focus of which the activity invariably consisted of both paroxysmal spikes and ictal activity, a requirement felt to be essential for the study of the effectiveness of cerebellar-surface stimulation.

The quantitative assessment of the data from the preliminary study indicated that 14 experiments per group would be sufficient to allow a determination of the effectiveness of the cerebellar-surface stimulation in altering the number of seizures. This inference, however, assumed that the distributions of the various parameters in the preliminary experiments were the same as those for the control group. However, the activity of the penicillin foci in the experimental sequence varied considerably as compared to the values for the preliminary study, indicating that the data from preliminary experiments might not have represented an unbiased sample of the distribution of the parameters.

The variations in the number of seizures between animals of the same group in the experimental series meant that a large number of additional experiments would have to be performed in order to insure that the observed mean would be within the 95% confidence interval established for the preliminary experiment (within 15% of the mean value). As a consequence, the statistical comparison of the mean number of seizures between control and treated groups, the null-hypothesis being that they are equal, yielded no statistically significant differences, even though upon qualitative inspection, differences in the mean number of seizures seemed obvious in some cases. Hence, we may ask, how much cerebellar-surface stimulation would have to decrease the mean number of seizures in order to conclude statistically that the treatment was effective in reducing seizure activity. Given the mean number of seizures and the standard deviation of the control group, the mean number of seizures expected to be statistically different from the mean of the control group at the p = 0.05 level was calculated for each 10-minute period, assuming initially that an increase in the effectiveness of cerebellar-surface stimulation would mainly affect the number of seizures of the stimulated group but not the variability. Figure 10 illustrates the results from these calculations. The shaded area represents the region in which no significant differences in the mean number of seizures between treated and control groups would be detected. Notice that the data from the stimulated group (triangles) was entirely within the shaded area. If, in addition, the standard deviations of the treated group were reduced by one-third, then the shaded region in Fig. 10 would be narrower, but only by a small amount, since the standard deviation of the control group was large. However, this change in variability would be just large enough to place the mean number of seizures in the first and second 10-minute periods outside the shaded area. These calculations clearly demonstrate that the shaded area in Fig. 10 was too wide for small effects of cerebellar-surface stimulation to be
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FIG. 10. Illustration of the region (shaded) in which no significant differences in the mean number of seizures between the stimulated and control groups could be detected given the variability of this parameter. The mean values of the stimulated group were entirely within this area. To conclude that cerebellar-surface stimulation decreased seizure activity (that is, points for the stimulated group occurring outside the shaded area), the mean number of seizures in each 10-minute interval would have had to have been reduced from the corresponding value of the control group by at least one-third.

detectable. In order to be able to conclude that cerebellar-surface stimulation can decrease seizure activity in the penicillin focus, the mean number of seizures in each 10-minute interval would have to be reduced from the corresponding values of the control group by at least one-third so that they would lie outside the shaded region in Fig. 10. In addition, a reduction in the variability between animals in the control and the stimulated groups would also narrow the shaded region, and more subtle effects of cerebellar stimulation could then be observable.

The question arises why the activity of individual penicillin foci was more variable than the activity in similar foci reported by other investigators. For example, using the normalization procedure of Stark, et al.12 (expressing the number of seizures during the third and fourth half-hour periods as a percent of the number of seizures recorded during the first hour), the mean values and standard deviations for the control group were 139 ± 70 for the third and 196 ± 173 for the fourth period in the present study, whereas in the experiments of Stark, et al.,12 these values were 130 ± 29.7 and 113 ± 50.4, respectively, for a focus created by the injection of 30,000 units of penicillin.12 The reasons for the differences between their data and ours might have resulted from several factors. First, in our studies, the data from all animals, regardless of type of recordings, were included in the protocol. Only when complications were encountered during surgery or when the monitored physiological parameters did not fall within pre-established limits were any data discarded (Table 2). Whether similar criteria were established by Stark, et al.,12 was not evident from their description. Second, the animals in the present study were unanesthetized and were not paralyzed with Flaxedil. Instead, they were partially immobilized by sectioning the spinal cord at the C1–2 level. Since the twitching of the facial musculature during ictal events increased, the sensory inputs to the epileptic focus from the entire head area could have affected the activity in the focus. It was unknown, however, whether the elimination of sensory input with the administration of Flaxedil would have reduced the variability and whether the continuous administration of this drug12 would have affected the activity in the penicillin focus via its effect on central synaptic mechanisms.

The observation that the amplitude of the cerebellar evoked response seems to vary independently of the total number of seizures recorded from the same animal might in-
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Fig. 11. Relationship of the total number of seizures to the amplitude of the cerebellar evoked response obtained from the same animal. For the data points A and B, not only was the amplitude of the cerebellar evoked response zero, but there was, in addition, no conditioning effect by the cerebellar-surface stimuli on the trigeminal evoked response, indicating that the cerebellar electrode was not likely positioned appropriately on the cerebellar surface. Therefore, these two data points were excluded from the subsequent correlation of the total number of seizures with the amplitude of the cerebellar evoked response (solid line). The dashed line was drawn through the mean number of seizures of the control group to illustrate that stimuli applied to the cerebellar surface can increase or decrease the total number of seizures and that this effect may be related to whether stimuli of equal strength evoke a cerebellar response of small or large amplitude.

It was concluded that the neuronal substrate mediating the evoked response did not directly affect the activity in the penicillin foci. However, when the results were more closely inspected, these two parameters seemed to be inversely related. In order to reach this conclusion, the assumption was made that the electrodes could not be positioned on the cerebellar surface with the same degree of contact and at the same exact location in each animal. As a consequence, stimuli applied to the cerebellar surface could not be expected to be equally effective in activating the underlying neuronal tissue in different animals. The absence of both the cerebellar evoked response and a conditioning effect on the amplitude of the trigeminal evoked response was therefore assumed to indicate that the stimulating electrode was positioned appropriately for the stimuli to alter the cerebellar output. If this latter assumption is correct, then the data of two animals from which neither an evoked response nor a conditioning effect could be recorded can be excluded from the calculations of the correlation coefficients (see Fig. 11, data points A and B), since the stimuli in these animals likely did not activate the neuronal tissue underlying the electrode. This conclusion was supported by the histological evaluation revealing that a hemorrhagic area on the cerebellar surface was observed at the location where the stimulating electrodes were situated. A recalculation of the correlation coefficients of the data points from the remaining 10 animals (see Methods) showed a negative cor-
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relation of the total number of seizures with the amplitude of the cerebellar evoked response (correlation coefficient = -0.77), indicating that an increase in the amplitude of the cerebellar evoked response was correlated with a decrease in the total number of seizures.

The inverse relationship between the amplitude of the cerebellar evoked response and the total number of seizures is represented by the solid line in Fig. 11. The intersection of this line with the abscissa would be predicted to occur near the mean number of seizures for the unstimulated group (at 378, Table 4) if the number of seizures depended only on the amplitude of the cerebellar evoked response. But, in all animals whose total number of seizures was greater than the mean of the unstimulated group, the cerebellar-surface stimuli did alter the amplitudes of the trigeminal evoked response even though the amplitude of the cerebellar evoked response was small, if present at all. Consequently, these observations suggest that the neuronal substrate mediating the cerebellar evoked response was likely not the sole cause for the inverse relationship between this response and the total number of seizures. This conclusion gains further support from the additional observation that the pattern of cerebellar activity evoked by the surface stimulus, which resulted in the significant increase of seizures of short duration in the stimulated group as compared to the control group (Fig. 8), might not be solely related to the activation of the pathway responsible for generating the cerebellar evoked response, since the correlation of the number of short seizures with the amplitude of the cerebellar evoked response indicated that these two parameters varied independently (correlation coefficient = 0.14). In summary, then, the effectiveness of the cerebellar-surface stimulus in altering the cerebellar output might to some extent be related to changes in the activity of the penicillin focus (Fig. 8), and may be inversely related to the total number of seizures per animal (Fig. 11).

The conclusion that stimulation of the cerebellar surface may alter the activity in a penicillin focus is based solely on a statistical data analysis which indicated that the differences between histograms constructed from the durations of all the seizures in a group (Fig. 8) could not have occurred by chance with a high probability. Inspection of Fig. 8, however, suggests that the differences in seizure duration (Fig. 8) between non-stimulated and stimulated groups were small, indicating that cerebellar-surface stimulation might only weakly affect seizure activity in a penicillin focus. Even though the histograms in Fig. 8 appear to support this contention, the large variations in the values of the parameters, especially among the animals in the stimulated group, might have lessened the difference between groups. For example, if the stimulus strength were adjusted for each animal in the stimulated group such that the amplitude of the cerebellar evoked response would have been maximal and the same for all animals, then, on the basis of the inverse relationship between this amplitude and number of seizures (Fig. 11), the activity in a penicillin focus might have been affected to a greater degree and the parameters more noticeably altered. Consequently, even though the present study may indicate that the effect may only be weak, the experimental design was not appropriate to quantitate the degree of effectiveness of stimuli applied to the cerebellar surface in reducing seizure activity.

The results from our study provide some experimental support for the clinical observation that the frequency of intractable and non-focal seizures was decreased in patients by electrical stimulation of the cerebellar surface.8 This conclusion is based on the apparent inverse relationship of the total number of seizures with the amplitude of the cerebellar evoked response (Fig. 11). However, the amplitude value at which the number of seizures on the abscissa corresponds to the mean of the control group (Fig. 11, dashed line) for an animal in the stimulated group is not zero for any given experiment. On the basis of this observation, the total number of seizures can be predicted to be larger than the mean number in the control group if the cerebellar stimulus only evokes a response of small amplitude (data points to the right of dashed line). Consequently, without a determination of the degree of alteration of cerebellar and extracerebellar neuronal activity produced by the electrical stimuli, either an increase or a decrease in the number of seizures with respect to the control group might be observed in different ex-
periments even though the stimulus parameters are the same in all experiments. The lack of measuring the effectiveness of the applied stimuli in altering the excitability of the underlying neuronal tissue by many of the previous investigators may explain in part the differences in the results from their studies. However, it should be emphasized that the data from the present experiments do not conclusively demonstrate a strong inverse relationship between the amplitude of the cerebellar evoked response and the number of seizures. The analysis demonstrates only that the correlation coefficient is different from zero; however, because of the small number of data points, the 95% confidence interval is $-0.26$ to $-0.94$ (0.05 level of significance). Therefore, additional experiments are essential to establish this important relationship conclusively.

The data imply, however, that the parameter settings on a clinical stimulator might not necessarily determine the therapeutic effectiveness of the electrical stimuli. Rather, because of the expected variability in the degree of contact between the electrode and the cerebellum, an assessment of the extent to which a given set of stimulus parameters affects the activity in cerebellar output pathways appears to be essential. Without such an evaluation, the success or failure of this treatment cannot resolve satisfactorily the basic question of whether stimulation of the cerebellar surface is therapeutically effective. Therefore, standard noninvasive tests that can be administered with ease, that are quantifiable, and that are based on our present understanding of cerebellar output systems should be developed in conjunction with the evolution of this new therapeutic procedure.

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