THE RELATIONSHIP OF RESISTANCE AND CURRENT TO VARIOUS VOLTAGES APPLIED TO CHRONIC ELECTRODE IMPLANTS AROUND THE TIBIAL NERVES OF ADULT MALE ALBINO RATS

Thesis for the Degree of M. A.

MICHIGAN STATE UNIVERSITY

David W. Beamer

1966

THE RELATIONSHIP OF RESISTANCE AND CURRENT

TO VARIOUS VOLTAGES APPLIED TO CHRONIC

ELECTRODE IMPLANTS AROUND THE TIBIAL

NERVES OF ADULT MALE ALBINO RATS

By

David W. Beamer

AN ABSTRACT OF

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Health, Physical Education and Recreation

1966

Approved: W W Heusner

ABSTRACT

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The purpose of this study was to determine the relationship of resistance and current to various values of voltage applied to chronic electrode implants around the tibial nerves of adult male albino rats.

Thirty-five animals were arbitrarily selected for the study. A total of twelve either died or were unfit for data collection, reducing the size of the final sample to twenty-three. This number was considered adequate for the purpose of this investigation.

Stimulating electrodes of Silastic-insulated multifilament surgical steel wire were permanently implanted around the tibial branch of the sciatic nerve of each animal. Implants were made so that a clean planter flexion would result upon stimulation. The surgical technique was routine and involved minimal trauma.

All animals were given a recovery period after implantation, during which daily activity records were kept. When activity reached a constant minimum level, recovery was considered complete.

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Each animal was then anesthetized singly in an ether chamber, and one leg was arbitrarily chosen to be placed in series with a Grass electrical stimulator and an ammeter. Electrical stimulation ranging from .2 -150 volts, at specified intervals, was applied to the animal, with the resulting current flow being recorded from the ammeter. Resistance values for each applied voltage were calculated. Polynomial regression equations for the curves of best fit to the observed currents and resistances were then determined. Corrected multiple correlation coefficients and standard errors of estimate were calculated. The voltage producing the best maximal contraction was determined, as were post-stimulation chronaxie and rheobase values.

Analysis of the data has led to the following conclusions:

1. Investigations involving permanently-implanted electrodes are feasible.

2. The relationship of both current and resistance to applied voltage follows a definite curvilinear pattern, and can be used as a basis for prediction.

3. The practical limits of applied voltage ranges from 1 - 100 volts when electrodes are chronically implanted as in this investigation.

4. An application of 35 volts appears to produce the best maximal contraction under the circumstances of this study.

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CHAPTER I

THE PROBLEM

Introduction

The advanced methods of instrumentation and measurement presently available to the researcher have enabled a few physiology of exercise studies to be conducted using electrical stimulation, via chronically implanted electrodes, to induce activity in animals. Such studies not only allow maximum control of variables, but also allow precise and accurate measurements to be made. They may, therefore, allow more accurate information to be gained concerning the internal effects of physical activity.

At the present time, electrical stimulation studies must, of necessity, use animals as subjects. For such studies to have much value, it is first necessary to know the effects and limitations of electrical stimulation itself upon the animals used as subjects. In fact, it may be necessary to know these characteristics before such a study can be effectively carried out.

Statement of the Problem

The purpose of this study was to determine the relationship of resistance and current to various values of voltage applied to chronic electrode implants around the tibial nerves of adult male albino rats. It was hypothesized that electrode-implanted nerves of rats show a definite pattern of electrical characteristics, and that this pattern could be used as a basis for prediction. Such information would be invaluable in further studies using electrical stimulation.

Limitations of the Study

1. Although the results of this small animal study are useful, they can be applied only to rats under the conditions of this study, not to other animals or to man.

2. The lack of similar studies prevents any comparison or verification of results.

3. In some cases, the internal electrical contacts were damaged by infection in the area of the implant or were useless by a short circuit within the animal.

4. The electrical equipment used did not permit precise readings and was impossible to calibrate accurately at two points in the current scale.

CHAPTER II

REVIEW OF RELATED LITERATURE

In attempting to determine the internal electrical characteristics of resistance and current in chronically electrode-implanted rats, it was necessary to learn the results of similar previous studies. Pertinent related literature was reviewed in order to gain information concerning: (a) electrodes themselves and their effect on animals, and (b) the characteristics of resistance and current in relation to various applied voltages.

Electrodes

As early as 1933, Cannon (2) implanted rubber-insulated wire electrodes in cats for the purpose of studying the effects of stimulating efferent nerves in unanesthetized animals. His electrodes proved to be readily applicable to nerve trunks in the cat, and were left in place for weeks without disturbance of the animal.

Straw and Mitchell (11) implanted rats with steel wire electrodes coated first with Teflon and then insulated with Silastic. Upon examining the tissues, they found that little or no pathological alterations had occurred, and that there was no tissue reaction to the Silastic. Infection, however, caused the failure of two out of sixteen animals.

Straw's results were verified by McCarty (9), who found no evidence of tissue reaction when rats were implanted with Silastic-insulated metal electrodes and left for a period of time.

Mauro (8) described an insulated metal electrode which could be left implanted for prolonged periods of time without danger of contaminating surrounding tissues with electrode products.

Cohen (3) designed electrodes consisting of two tin foil strips backed by a piece of Parafilm. The electrodes were implanted by bending the foil strips around the nerve with the exposed foil in contact with the nerve, sealing the electrode around the nerve with melted paraffin, and attaching insulated conducting wires to the foil strips. The wires protruded from a closed incision, permitting freedom of movement for the animal. The electrodes described by Cohen (3) were used satisfactorily in a peripheral nerve stimulation study by Coutts (4).

Van Linge (12) implanted two stimulating electrodes along the sciatic nerve of three anesthetized rats. Ten weeks later, he conducted a one-week training program using electrical stimulation through the electrodes to induce maximal contractions of the plantaris muscle.

In an attempt to devise a method of detecting nystagmus, Cutt _et al_. (5) implanted stainless steel wire electrodes, fitted into nylon bolts, into the skulls of squirrel monkeys. No evidence of infection was found. He concluded that implanted electrodes are useful for long-term experimentation and that they are well-tolerated by the animal and convenient for the investigator.

Braley (1) feels that a material which is to be implanted within a living body must not be hard and must produce no undesirable body responses. In his opinion, silicone products are ideal because of : (a) heat stability, (b) the absence of deterioration with time, (c) non-adherance to other substances, and (d) the lack of tissue reaction.

In order to determine the tissue reaction to silicone itself, Speirs and Blocksma (10) implanted subcutaneously four types of silicone sponges in 38 rats. After periods of time ranging from three days to eight months, animals were sacrificed and tissues studied. The entire process of tissue reaction was interpreted as being a normal healing response to a sterile wound. In addition, all implants were retained and no evidence of infection occurred.

Electrical Characteristics

No previous literature concerning the relationship of resistance and current to various values of applied voltages could be found.

CHAPTER III

EXPERIMENTAL DESIGN

Sample

Thirty-five adult male albino rats were selected for the study. This number was arbitrarily chosen as being adequate for the purposes of the study, while allowing for the possible loss of several animals.

In fact, 12 animals could not be used for data collection. A few died from prolonged exposure to anesthesia, several died as a result of the implantation and others, due to infection or internal short-circuit, were rendered useless. This reduced the size of the final sample to 23 animals.

Electrode Design and Implantation

The electrode design and the operative techniques of the implantation were exactly as described by Heusner *et al.* (6).

Recovery Period

After implantation, all animals were given a recovery period. During this time they were housed in standard 10 x 8 x 7-inch small animal cages with attached spontaneous activity drums, 5 inches wide and 14 inches in diameter, in which the animals could run at will. Counters attached to the drums recorded revolutions as the animals exercised. When post-operative daily activity reached a constant level, recovery was considered complete.

Stimulation Procedures

The 23 animals considered suitable for data collection were anesthetized singly in an ether chamber and placed in series with a Grass electrical stimulator and an ammeter by attaching alligator clips to the exposed electrode ends. One leg only of each animal was arbitrarily chosen to be stimulated. Each animal was then subjected to electrical stimulation ranging from .2 volts up to a maximum of 150 volts, at specified intervals. (Stimulator voltage settings were found to slightly inaccurate; they were calibrated with a voltmeter and corrected. The stimulator readings and actual values can be found in Appendices A-D). Stimulation was continued until 150 volts was reached or until the animal could not tolerate further stimulation. The frequency and duration of all stimuli were held constant at .2 stimuli per second (1 contraction each 5 seconds) and 1000 milliseconds, respectively. The long duration was required to allow the ammeter to reach peak values.

Evaluative Techniques

The current through each animal, for each voltage used, was recorded from the ammeter. Values which were not in the expected direction within a single animal were smoothed by interpolation, and the mean and standard deviation of the 23 current values obtained for each voltage were calculated. Smoothing deviant values was felt to be justified since, in every case, such deviations occurred at one of the two points on the ammeter scale which could not be precisely calibrated. This smoothed data is shown in Appendix A.

Since reliable values could not be obtained on all animals, data for voltages less than 1.0 volt and greater than 102.5 volts were dropped from further consideration at this point. Very few animals could tolerate applied voltages much greater than 102.5 volts.

With each voltage and current known, each corresponding resistance was calculated by the formula: R (ohms) = E (volts) / I (amps). The smoothed values for the currents were used for these calculations. The mean and standard deviation of the resistances were also calculated for each given voltage. This data is shown in Appendix B.

Polynomial regression equations for various powers of voltage for the curves of best fit through the observed currents and resistances were determined, and corrected multiple correlation coefficients and standard errors of estimate were calculated. For the purpose of calculating the polynomial regression equations only, data curves for both the resistance and current were begun at 6.9 volts and hypothetically extended to 218.1 volts by mirroring the data obtained between 6.9 and 102.5 volts onto the interval of 122.5 to 218.1 volts.

The voltage producing the best maximal contraction--the most powerful contraction involving only the gastrocnemius--was subjectively determined. For this, the duration of each stimulus was held at 100 milliseconds and the frequency at .5 stimuli per second (1 contraction each 2 seconds).

Post-stimulation chronaxie and rheobase values were determined on 11 animals to insure an adequate level of stimulation. Post-stimulation values were selected since these values were found to increase during the stimulation period, and values were desired which would insure adequate stimulation over an extended period of time.

CHAPTER IV

RESULTS AND DISCUSSION

Results

Data was analyzed in order to determine: (a) the resistance versus voltage curve (b) the current versus voltage curve (c) the voltage producing the best maximal contraction and (d) chronxie and rheobase.

Resistance. Figure 1 compares the mean observed resistance values with the predicted values obtained from the regression equation for each of the voltages used from 6.9 through 102.5 volts. It also shows the observed standard deviation at each of these voltages. The observed (and mirrored observed) and predicted mean resistances for each voltage from 6.9 through 218.1 volts are shown in Appendix C.

The polynomial regression equation for the curve of best fit for voltages from 6.9 - 218.1 volts was determined to be:

$$R = -0.0010442\ E^3 + 0.3524138\ E^2 - 33.7542264\ E + 1638.8591649$$

Obviously, this equation cannot be used to predict resistance values in the biological situation outside the range of applied voltages of 6.9 - 102.5 volts since all predicted values for voltages greater than 102.5 volts were based on mirrored data which was hypothetically extended, not actually observed.

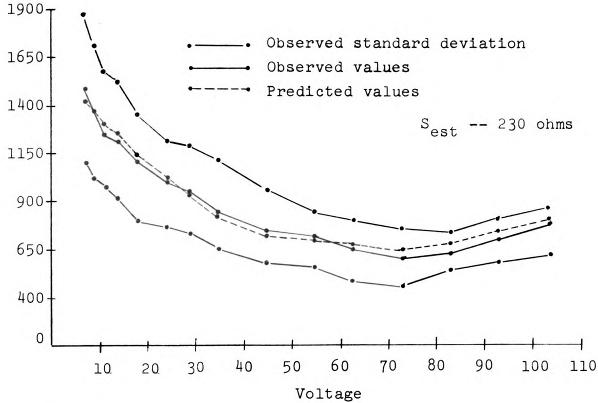

Figure 1.--Mean resistance vs. voltage.

The observed (and mirrored observed) resistance values and the resistance values predicted from the regression equation were found to have a corrected multiple correlation coefficient of .80.

The standard error of estimate of the predicted resistance values was calculated to be 230 ohms.

Current. A comparison of the smoothed observed current values with the current values predicted from the regression equation is shown in Figure 2. The observed standard deviation at each applied voltage from 6.9 through 102.5 volts is also shown. The observed (and mirrored observed) and predicted mean current values for each voltage from 6.9 through 218.1 volts are shown in Appendix D.

For the curve of best fit comparing currents and voltages from 6.9 - 218.1 volts, the following polynomial regression equation was calculated:

$$I = +0.0000009\ E^4 - 0.0003966\ E^3 + 0.0442160\ E^2 + 0.0903081\ E + 2.7692278$$

Again, this equation cannot be used to predict current values in the biological situation outside the range of applied voltages of 6.9 - 102.5 volts since 102.5 volts was the highest voltage actually applied, and no current values were observed for greater voltages. Predicted current values for voltages larger than 102.5 volts were based on mirrored data which was hypothetically extended; predictions from such data cannot be justified.

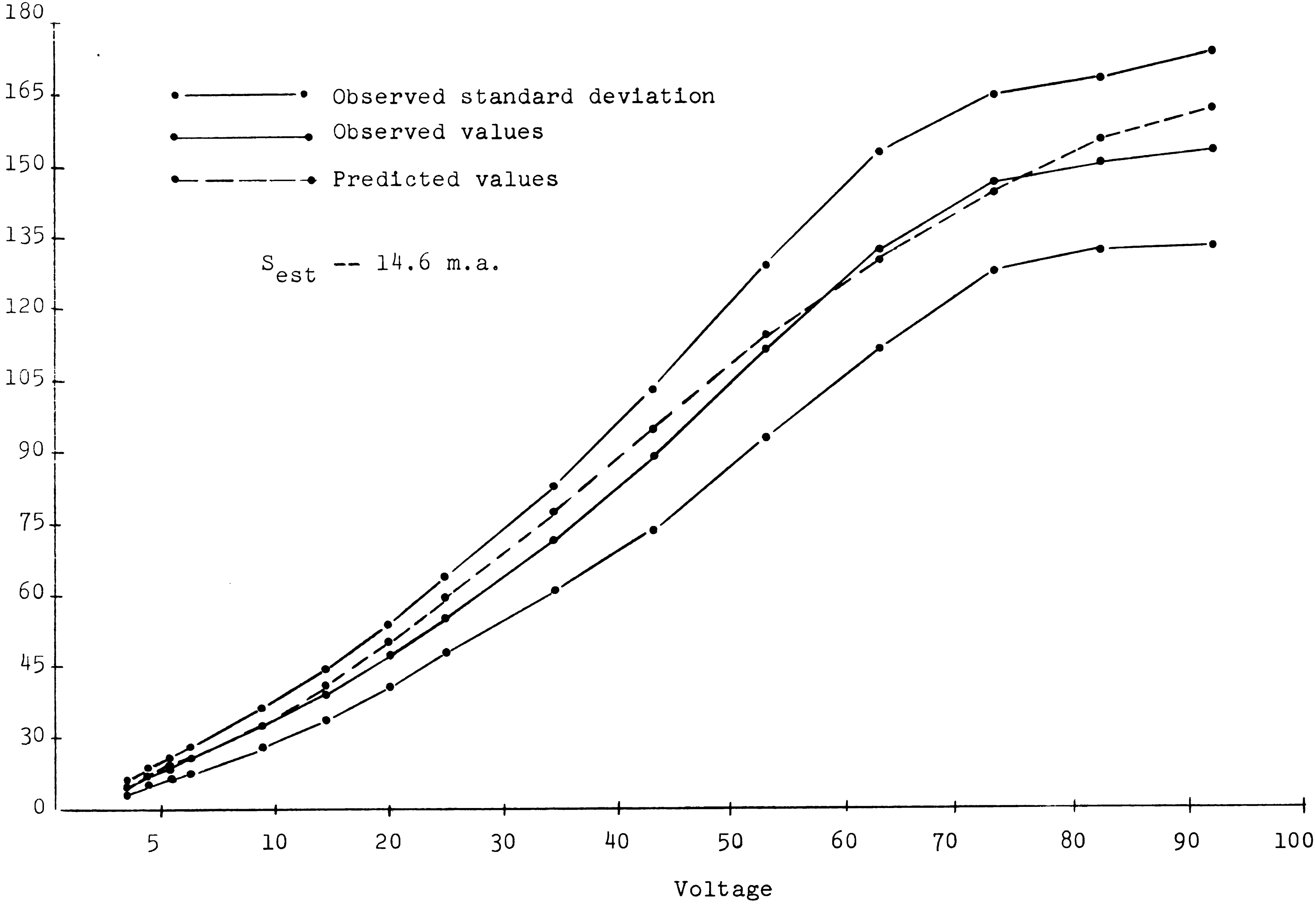

Figure 2.--Mean current vs. voltage.

The corrected multiple correlation coefficient for the observed current values and the predicted current values was .96.

The standard error of estimate of the current values predicted from the polynominal regression equation was determined to be 14.6 milliamperes.

Best Maximal Contraction. When each animal was stimulated at a frequency of .5 stimuli per second with a duration of 100 milliseconds, the most powerful contraction involving only the gastrocnemius was subjectively determined to occur, in a large majority of cases, at either 30 or 40 volts. 35 volts was accepted as being the single value most acceptable for use on all animals.

Chronaxie and Rheobase. Chronaxie values taken on 11 animals after considerable previous stimulation ranged from .10 - .75 milliseconds, with a mean value of .31 milliseconds.

Rheobase values ranged from .10 -.75 milliamperes, with the mean being .35 milliamperes. Complete chronaxie and rheobase data is shown in Appendix E.

Discussion

Resistance. Figure 1 shows that the relationship between resistance and voltage is definitely curvilinear; not linear, as might be suspected. In addition, the curve itself is not constant; it decreases rapidly when voltages are small, tends to level off, reaches a minimum point, and finally increases as maximum voltages are approached.

Resistance values would presumably continue to increase up to some maximum point if higher voltages could be tolerated by the animals.

Lloyd (7) considers a nerve fiber membrane to be a core conductor, implying a cylindrical cable-like tube having a conducting inside separated from a conducting outside by a resistive and capacitive membrane. Before a current can be induced, both the resistance and the capacitance must be overcome. The infinite number of capacitors do not break down simultaneously, however, but progressively. As each capacitor breaks down, a small parallel resistance is added to the circuit, lowering the total resistance. This total resistance is lowered exponentially, however, with each new capacitance which is broken down lowering the total resistance less than the one preceding it. Thus, when a certain number of capacitors are overcome, any additional ones breaking down have a negligible effect upon further lowering of the resistance. This produces a leveling effect, and might explain the curvilinear relationship between resistance and voltage.

After completion of data collection, a check of the stimulator indicated that, at higher voltage values, there was a leveling out of current supply. In fact, no such leveling should have occurred. It is hypothesized that this explains the final upswing of the resistance curve. It is recommended that this part of the study be repeated.

It should also be noted that the standard deviation decreases regularly until the general area of the minimum resistance is reached; it then begins to increase with increased voltage. This trend would be expected to continue if voltage could be increased still further.

The polynomial regression equation would indicate that, if voltage were dropped to zero, the predicted resistance would be 1639 ohms. It is exremely doubtful that this is correct. However, the relatively high correlation (.80) between the observed and predicted values would seem to indicate that the resistance do follow a fairly definite pattern of behavior, making it possible to predict values from the equation with a reasonable degree of confidence.

All calculations were significant at the .01 level of confidence.

Current. Like the resistance data, the current values follow a curvilinear pattern. Figure 2 also indicates that the standard deviation tends to increase as the voltage increases.

The polynomial regression equation indicates that, with no voltage, the current flow would be 2.8 amps. Obviously, this is impossible, since without voltage there can be no resulting current. Again, however, the high correlation (.96) enables predictions to be made with reasonable confidence.

The same factors accounting for the curvilinear relationship between resistance and voltage are also assumed to account for the curvilinear relationship between current and voltage.

Best Maximal Contraction. No explanation can presently be advanced for the best maximal contraction occurring almost exclusively at either 30 or 40 volts.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the relationship of resistance and current to various values of voltage applied to chronic electrode implants around the tibial nerves of adult male albino rats.

Thirty-five animals were arbitrarily selected for the study. A total of twelve either died or were unfit for data collection, reducing the size of the final sample to twenty-three. This number was considered adequate for the purpose of this investigation.

Stimulating electrodes of Silastic-insulated multifilament surgical steel wire were permanently implanted around the tibial branch of the sciatic nerve of each animal. Implants were made so that a clean plantar flexion would result upon stimulation. The surgical technique was routine and involved minimal trauma.

All animals were given a recovery period after implantation, during which voluntary daily activity records were kept. When activity reached a constant minimum level, recovery was considered complete.

Each animal was then anesthetized singly in an ether chamber, and one leg was arbitrarily chosen to be placed in series with a Grass electrical stimulator and an ammeter. Electrical stimulation ranging from .2 - 150 volts, at specified intervals, was applied to the animal, with the resulting current flow being recorded from the ammeter. Resistance values for each applied voltage were calculated. Polynomial regression equations for the curves of best fit to the observed currents and resistances were then determined. Corrected multiple correlation coefficients and standard errors of estimate were calculated. The voltage producing the best maximal contraction was determined, as were post-stimulation chronaxie and rheobase values.

Analysis of data showed that both current and resistance have a definite, predictable, curvilinear relationship to applied voltage. Values of applied voltages below 1.0 volts and above 102.5 volts, however, did not produce desirable results. The best maximal contraction was observed to occur almost exclusively at either 30 or 40 volts. Chronaxie and rheobase values both ranged from .10 - .75 milliseconds, respectively, with respective mean values of .31 milliseconds and .35 milliamperes.

Conclusions

Analysis of the results of this investigation has led to the following conclusions:

1. Investigations involving permanently-implanted electrodes are feasible.

2. The relationship of both current and resistance to applied voltage follows a definite curvilinear pattern, and can be used as a basis for prediction.

3. The practical limits of applied voltage ranges from 1-100 volts when electrodes are chronically implanted as in this investigation.

4. An application of 35 volts appears to produce the best maximal contraction under the circumstances of this study.

Recommendations

1. Due to the absence of similar studies, this investigation should be repeated to provide comparative results.

2. In future studies of this nature, electrode implants should be performed with greater care and under more antiseptic conditions to reduce loss of animals.

3. Electrical equipment should permit precise calibration of all scales used. Perhaps an oscilloscope should be used to record actual voltage.

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APPENDICES

APPENDIX A

MICHIGAN STATE UNIVERSITY
DEPARTMENT OF HEALTH, PHYSICAL EDUCATION, AND RECREATION

GRADUATE STUDIES AND RESEARCH

APPENDIX A

TABULATION SHEET

DATE OF TABULATION ____________

TOPIC ____________

TABULATED BY ____________

Raw Resistance Data

Stimulator Voltage	1.0	3.0	5.0	7.0	9.0	11.0	13.0	15.0	20.0	25.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0		
Actual Voltage	1.0	3.2	5.0	6.9	8.8	10.8	12.7	18.5	24.5	29.8	34.8	44.3	53.0	63.0	73.0	83.0	92.5	102.5		
1 R	12,500	2462	1389	1113	1000	900	876	861*	845	826*	809	726	707	525	480	512	561	603		1
2 L	16,667	5517	2778	2226	2000	1964	1868	1370	1289	1268	1141	1106*	1074*	1037*	1000	754	771	854		2
3 R	8333	2540	1344	1062	978	915	876	872*	867*	863*	859*	852	768	700	570	615	651	692		3
4 R	8333	3478	1852	1408	1313	1187	1104	1057	961	871	757	715	663	630	629	588	656	727		4
5 F	10,000	3855	2101	1663	1544	1367	1280	1039	946	898	782	777	768	741	730	674	701	743		5
6 L	11,111	3441	1492	1150	1035	991	927	912*	897*	883*	870	829*	791	761*	730	680	712	765		6
7 R	14,286	4156	2212	1683	1571	1500	1366	1142	1124	1098*	1069*	1014*	964	887	793	754	826	884		7
8 L	10,000	3368	1916	1438	1419*	1399*	1380	1370	1354	1346*	1338	886	757	700	664	692	752	820		8
9 R	11,111	4444	2174	1663	1660	1543	1497*	1331	1150	955	851	738	688	600	579	634	665	732		9
10 R	8333	4102	1976	1497	1419	1312*	1210	1028	939	874	825	777	746	630	608	629	680	732		10
11 R	6667	4102	2083	1675	1600	1565	1549	1542	1512	1490	1265	1051*	855	829	768	680	712	765		11
12 R	8333	2424	1323	1113	989	923	876	665	636	608	591*	559*	530	485	490	535	574	621		12
13 L	10,000	3556	1445	1045	871	800	751	661	590	580*	570	547	411	386	429	488	544	603		13
14 L	11,111	5161	2427	1797	1600	1500	1411	1217	1029	931	859	764	688	630	521	572	625	679		14
15 R	10,000	3855	1908	1411	1313	1200	1143*	968	875	801	753	726	589	630	730	830	925	1025		15
16 L	10,000	3200	1908	1533	1443	1385	1337	1225	1114	1072	969	836	736	624	598	610	638	707		16
17 L	12,500	5818	3311	2464	2056	1960*	1868	1667	1485	1284	1105	955	869	750	709	783	856	932		17
18 R	8333	4638	2230	1663	1517	1367	1270	1000	817	698	669	624	589	573	529	576	621	674		18
19 R	8333	4706	1916	1568	1535*	1500	1443	1412	1324	1263	1141	1108	1039	875	768	741	804	891		19
20 L	7692	4571	2000	1494	1375	1256	1154	979	907	803	733	651	589	516	529	589	656	727		20
21 L	9091	3902	1613	1278	1100	1019	969	841	738	664	653*	633	582	538	503	546	601	641		21
22 L	10,000	3636	1639	1190	1073	982	927	784	710	677	657	615	552	525	521	572	638	707		22
23 R	6667	4102	1667	1278	1073	1000	969	911	845	754	669	591	473	450	451	497	544	586		23
24																				24
25 Mean	9974	3958	1944	1496	1369	1284	1219	1081	998	935	867	786	714	653	623	633	683	744		25
26 S. D.	2375	889	470	380	339	322	308	279	262	243	217	186	171	155	129	108	107	123		26
27																				27
28																				28
29 *	resistance values calculated from smoothed current values										All resistance values are in ohms									29
30																				30
31																				31
32																				32
33																				33

Animal Number and leg

APPENDIX B

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DEPARTMENT OF HEALTH, PHYSICAL EDUCATION, AND RECREATION
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Actual Voltage	1.0	3.2	5.0	6.9	8.8	10.8	12.7	18.5	24.5	29.8	34.8	44.3	53.0	63.0	73.0	83.0	92.5	102.5		
1 R	0.1	1.3	3.6	6.2	8.8	12.0	14.5	21.5*	29.0	36.1*	43.0	61.0	75.0	120.0	152.0	162.0	165.0	170.0		1
2 L	0.1	0.6	1.8	3.1	4.4	5.5	6.8	13.5	19.0	2335	30.5	40.0*	49.3*	60.8*	73.0	110.0	120.0	120.0		2
3 R	0.1	1.3	3.7	6.5	9.0	11.8	14.5	21.2*	28.2*	34.5*	40.5*	52.0	69.0	90.0	128.0	135.0	142.0	148.0		3
4 R	0.1	0.9	2.7	4.9	6.7	9.1	11.5	17.5	25.5	34.2	46.0	62.0	80.0	100.0	116.0	141.0	141.0	141.0		4
5 R	0.1	0.8	2.4	4.2	5.7	7.9	10.0	17.8	25.9	33.2	44.5	57.0	69.0	85.0	100.0	123.0	132.0	138.0		5
6 L	0.1	0.9	3.4	6.0	8.5	10.9	13.7	20.3*	27.3*	33.7*	40.0	53.4*	67.0	82.8	100.0	122.0	130.0	134.0		6
7 R	0.1	0.8	2.3	4.1	5.6	7.2	9.3	16.2	21.8	27.1*	32.6*	43.7*	55.0	71.0	92.0	110.0	112.0	116.0		7
8 L	0.1	1.0	2.6	4.8	6.2*	7.7*	9.2	13.5	18.1	22.1*	26.0	50.0	70.0	90.0	119.0	120.0	123.0	125.0		8
9 R	0.1	0.7	2.3	4.2	5.3	7.0	8.5*	13.9	21.3	31.2	40.9	60.0	77.0	105.0	126.0	131.0	139.0	140.0		9
10 R	0.1	0.8	2.5	4.6	6.2	8.2*	10.5	18.0	26.1	34.1	42.2	57.0	71.0	100.0	120.0	132.0	137.0	140.0		10
11 R	0.2	0.8	2.4	4.1	5.5	6.9	8.2	12.0	16.2	20.0	27.5	42.2*	62.0	76.0	95.0	122.0	130.0	134.0		11
12 R	0.1	1.3	3.8	6.2	8.9	11.7	14.5	27.8	38.5	49.0	58.9*	79.2*	100.0	130.0	149.0	155.0	161.0	165.0		12
13 L	0.1	0.9	3.5	6.6	10.1	13.5	16.9	28.0	41.5	51.4*	61.0	81.0	129.0	163.0	170.0	170.0	170.0	170.0		13
14 L	0.1	0.6	2.1	3.8	5.5	7.2	9.0	15.2	23.8	32.0	40.5	58.0	77.0	100.0	140.0	145.0	148.0	151.0		14
15 R	0.1	0.8	2.6	4.9	6.7	9.0	11.1	19.1	28.0	37.2	46.2	61.0	90.0	100.0	97.0	100.0	100.0	100.0		15
16 L	0.1	1.0	2.6	4.5	6.1	7.8	9.5	15.1	22.0	27.8	35.9	53.0	72.0	101.0	122.0	136.0	145.0	145.0		16
17 L	0.1	0.6	1.5	2.8	4.3	5.5*	6.8	11.1	16.5	23.2	31.5	46.4	61.0	84.0	103.0	106.0	108.0	110.0		17
18 R	0.1	0.7	2.2	4.2	5.8	7.9	10.0	18.5	30.0	42.7	52.0	71.0	90.0	110.0	138.0	144.0	149.0	152.0		18
19 R	0.1	0.7	2.6	4.4	5.7*	7.2	8.8	13.1	18.5	23.6	30.5	40.0	51.0	72.0	95.0	112.0	115.0	115.0		19
20 L	0.1	0.7	2.5	4.6	6.4	8.6	11.0	18.9	27.0	37.1	47.5	68.0	90.0	12220	138.0	141.0	141.0	141.0		20
21 L	0.1	0.8	3.1	5.4	8.0	10.6	13.1	22.0	33.2	44.9	53.3*	70.0	91.0	117.0	145.0	152.0	154.0	160.0		21
22 L	0.1	0.9	3.0	5.8	8.2	11.0	13.7	23.6	34.5	44.0	53.0	72.0	96.0	120.0	140.0	145.0	145.0	145.0		22
23 R	0.2	0.8	3.0	5.4	8.2	10.8	13.1	20.3	29.0	39.5	52.0	75.0	112.0	140.0	162.0	167.0	170.0	175.0		23
24																				24
25 Mean	0.1	0.8	2.6	4.6	6.4	8.4	10.4	17.1	24.5	31.9	40.1	56.4	74.2	96.5	117.2	131.1	135.4	137.8		25
26 S. D.	0.02	0.1	0.5	0.9	1.2	1.7	2.1	3.5	5.1	6.6	8.0	10.8	14.3	18.5	20.1	19.1	18.3	19.6		26
27																				27
28																				28
29 *	current values smoothed by interpolation										All current values are in milliamperes									29
30																				30
31																				31
32																				32
33																				33

Animal Number and leg

APPENDIX C

Observed and Predicted Mean Resistance Values

Stimulator Voltage	Actual Voltage	Observed $\overline{R}$ (ohms)	Predicted $\overline{R}$ (ohms)
7.0	6.9	1496	1422
9.0	8.8	1369	1368
11.0	10.8	1284	1314
13.0	12.7	1219	1265
15.0	18.5	1081	1128
20.0	24.5	998	1008
25.0	29.8	935	918
30.0	34.8	867	847
40.0	44.3	786	744
50.0	53.0	714	684
60.0	63.0	653	650
70.0	73.0	623	647
80.0	83.0	633	668
90.0	92.5	683	706
100.0	102.5	744	757

	Hypothetically Extended Actual Voltage	Mirrored Observed R (ohms)	Predicted R (ohms) Based on Mirrored Data
	122.5	886	873
	132.5	947	924
	142.0	997	962
	152.0	1007	983
	162.0	977	980
	172.0	916	946
	180.7	844	886
	190.2	763	783
	195.2	695	712
	200.5	632	622
	206.5	549	502
	212.3	411	365
	214.2	346	316
	216.2	261	262
	218.1	134	208

APPENDIX D

Observed and Predicted Mean Current Values

Stimulator Voltage	Actual Voltage	Observed $\bar{I}$ (m.a.)	Predicted $\bar{I}$ (m.a.)
7.0	6.9	4.6	5.4
9.0	8.8	6.4	6.7
11.0	10.8	8.4	8.4
13.0	12.7	10.4	10.2
15.0	18.5	17.1	17.2
20.0	24.5	24.5	26.0
25.0	29.8	31.9	34.9
30.0	34.8	40.1	44.0
40.0	44.3	56.4	62.4
50.0	53.0	74.2	79.7
60.0	63.0	96.5	98.7
70.0	73.0	117.2	115.7
80.0	83.0	131.1	129.9
90.0	92.5	135.4	140.1
100.0	102.5	137.8	146.8

	Hypothetically Extended Actual Voltage	Mirrored Observed I (m.a.)	Predicted I (m.a.) Based on Mirrored Data
	122.5	137.8	146.8
	132.5	135.4	140.1
	142.0	131.1	129.9
	152.0	117.2	115.7
	162.0	96.5	98.7
	172.0	74.2	79.7
	180.7	56.4	62.4
	190.2	40.1	44.0
	195.2	31.9	34.9
	200.5	24.5	26.0
	206.5	17.1	17.2
	212.3	10.4	10.2
	214.2	8.4	8.4
	216.2	6.4	6.7
	218.1	4.6	5.4

APPENDIX E

Chronaxie and Rheobase

Animal Number	Chronaxie (milliseconds)	Rhoebase Voltage	Rheobase (milliamps)	2 x Rheobase (milliamps)
10	.35	1.1	.20	.40
11	.12	0.6	.20	.40
13	.34	1.6	.28	.56
14	.29	1.6	.40	.80
15	.34	1.5	.50	1.00
17	.14	1.5	.30	.60
19	.34	2.0	.40	.80
20	.36	0.9	.10	.20
21	.10	1.3	.40	.80
22	.23	1.3	.30	.60
23	.75	2.2	.75	1.50
Mean	.31	1.4	.35	.70