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The Effects of Different Levels of L-Dopa on the Aggressive Biting Behavior of Rats

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THE EFFECTS OF DIFFERENT LEVELS OF L-DOPA ON THE AGGRESSIVE BITING BEHAVIOR OF RATS

by

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INTRODUCTION

Parkinson's Disease is frequently controlled through the use of L-Dopa (L-3,4 dihydroxyphenylalanine). This drug seems to have some undesirable side effects, particularly in the area of aggressive behavior, suggesting that further analyses of its effects are necessary. The biochemical symptoms of Parkinson's Disease consist of a decrease of melanin, a dark colored pigment, in the corpus striatum and substantia nigra. This disease manifests itself behaviorally with tremor, rigidity, loss of associated movements, muscular weakness and loss of facial expression (Cotzias, VanWoert and Schiffer, 1967). L-Dopa is the synthetic form of dopa, a naturally occurring substance in the brain and the immediate precursor of dopamine (Esplin, 1970). L-Dopa is preferred to dopamine in clinical treatments because it readily crosses the blood brain barrier which dopamine does not do. It is rapidly metabolized into dopamine: thus, placing L-Dopa into the bloodstream is eventually the same as placing dopamine directly into the brain (Fisher and Christie, 1973). Dopamine is concentrated mainly in the substantia nigra of the extrapyramidal system with terminals in the neostriatum (Barbeau, 1972). On its release it becomes the immediate precursor of adrenaline and noradrenaline

in the metabolic pathway by means of which these neurotransmitters are synthesized (Fisher and Christie, 1973). Noradrenaline and adrenaline activate cells in the sympathetic nervous system leading to the effector organs thus creating a state of general arousal (Butter, 1968).

Studies relating L-Dopa to behavior have produced conflicting results. For example, increased locomotor activity, biting, striking and various other signs of extreme arousal in cats, rats and mice have been reported in several studies (Everett and Borcherding, 1970; Benkert, Gluba and Matussek, 1973; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973). When a change in behavior is correlated with the L-Dopa it is frequently characterized as increasing alertness, irritability and rage behavior (VandeWende and Spoerlein, 1962; Everett and Borcherding, 1970; Reis, Moorhead and Merlino, 1970). Reis, Moorhead and Merlino (1970) and Strömberg (1970) both found that high doses are correlated with highly aggressive behavior while low doses do not have the same effect in cats and mice. In contrast, other studies using rats and mice have found either no change in behavior as compared to controls or that the subjects appeared to be somewhat sedated (Rossum and Hurkmans, 1964; Eichelman and Thoa, 1973). However, several of these studies used only one level of L-Dopa and then studied the effect at that level in combination with other drugs

(Blaschko and Chrusciel, 1960; Rossum and Hurkmans, 1964; Thoa, Eichelman and Ng, 1972; Benkert, Gluba and Matussek, 1973). It is not clear whether the effects found in these studies are due to the L-Dopa, the other drugs or the combination. Secondly, these studies used different routes of administration, such as intravenous injection (VandeWende and Spoerlein, 1964; Geyer and Segal, 1974), injection into the brain via cannulas (Yen, Stanger and Millman, 1959; Ernst and Smelik, 1966; Rolinski, 1973), and intraperitoneal injection (Rossum and Hurkmans, 1964; Benkert, Gluba and Matussek, 1973; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973). This could affect the results in that metabolic rates could vary with differing routes of administration and the quantity necessary to produce the same behavioral effects could vary with the route of administration. Finally, the measures of aggression were not consistent in these studies. For example, some used open field activity (McKenzie, 1971; Benkert, Gluba and Matussek, 1973; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973), shock elicited aggression (Thoa, Eichelman and Ng, 1972; Geyer and Segal, 1974) or presentation of novel objects (Yen, Stanger and Millman, 1959; VandeWende and Spoerlein, 1962; Rossum and Hurkmans, 1964; Rolinski, 1973). These different designs make it difficult to compare results across studies. It would seem then that one could clarify these

conflicting results by carefully studying the relationships between L-Dopa and aggressive behaviors while attempting to eliminate the problems associated with the previous studies.

Thompson and Schuster (1968) have suggested that shock elicited aggression would provide a good baseline for pharmacological research. Early studies of shock elicited aggression employed two rats that were placed in a small enclosure with shock being delivered through a grid floor. Such shocks typically produced a stereotyped posture, striking and biting behavior (O'Kelly and Steckle, 1939; Ulrich and Azrin, 1962). This was an attractive procedure in that the shock elicited behavior highly resembled that of rats engaging in aggressive behavior in their environment (Eibl-Eibesfeldt, 1961; Lorenz, 1966). However, this procedure has two major problems. First, the observation and quantification of shock elicited behavior between two rats require a subjective determination which makes it difficult to obtain consistent data. That is, two observers are required to judge what behavior has occurred, its intensity, and its duration. If the observers do not agree on what occurred the data are not considered reliable (Ulrich and Azrin, 1962). Secondly, it is difficult to control the parameters of the shock, such as shock density or shock intensity, in that contact with the grids is not continu-

ous. Recent experiments have shown that monkeys, rats and other organisms, in addition to attacking members of their own species, will also attack inanimate objects (Azrin, Hutchinson and Sallery, 1964). One might argue, however, that aggression against an inanimate object is not equivalent to aggression against a member of the same species. Such an argument is not supported by Hutchinson (1972) who demonstrated that using the same experimental conditions, a subject attacked either a member of its own species or an inanimate object equally, depending on which was present at the time of shock. Azrin, Rubin and Hutchinson (1968) later developed an apparatus designed to measure the biting attacks of a restrained rat. Using this apparatus it has been shown that duration and frequency of biting can be recorded in an objective manner (Azrin, Rubin and Hutchinson, 1968; Hutzell and Knutson, 1972; Wetzel, 1972). Thus, based on the above information, it appears that the restrained rat design eliminates many of the problems of the earlier studies suggesting that this design would be most appropriate for the present study.

The purpose of this study, therefore, is to stabilize the behavioral data between L-Dopa and aggressive behavior employing the restrained rat design.

METHOD

Subjects

Ss were 24 naive Sprague Dawley male albino rats approximately 100-120 days old. They were obtained from The Upjohn Company and housed in separate cages with ad lib food and water.

Apparatus

Each rat was restrained in a plexiglass chamber 202 mm long, 64mm high and 83mm wide mounted on a plywood base. An insert consisting of a 13mm thick plywood floor had one 13mm thick plywood wall attached to the floor so that the wall was 3mm from the right edge of the floor at the back and 9mm from the edge at the front. The insert was placed inside the plexiglass chamber to prevent Ss from twisting around. Attached to the insert was a tail rest 191mm long and 13mm wide. Directly in front of the plexiglass chamber, a MICRO BZ-2RW80 microswitch was attached to the base. An alligator clip was attached to the microswitch, holding an Archer 2781632 4" wire tie that was level with and 13mm from the S's mouth. The chamber was covered with black felt to decrease distraction. Unscrambled shock was produced by a Grason Stadler Shock Generator E60708 in conjunction with a Gerbrands Model PR 1A tape programmer. Number of bites and

number of shocks were recorded on two frequency counters on a Lehigh Valley Electronics four counter panel and on a Gerbrands Model C-3, 24V-D.C. cumulative recorder.

Procedure

Measurement of Aggression: Ss were placed in the chamber with their tails attached to the tail rest with adhesive tape. Two NUWAY snap leads were used as electrodes and attached approximately 1" apart using Grass electrode paste and adhesive tape. Biting was recorded during the first twenty minutes of the session (pre-shock condition). A bite consisted of a pull on the wire tie sufficient to close the microswitch, a distance of 5mm. During the second twenty minutes shocks were delivered every 15 seconds for .5 seconds with an intensity of 8ma (after Azrin, Rubin and Hutchinson, 1968). Biting was again recorded during the final twenty minutes with no shock present (post-shock condition).

Drug Injection: L-Dopa was suspended in .08 mg/ml of .5% methylcellulose and injected intraperitoneally 30 minutes prior to each S being placed in the chamber. Four groups of six each received the following doses of L-Dopa: Group I received 0 mg/kg, Group II received 250 mg/kg, Group III received 500 mg/kg and Group IV received 750 mg/kg.

RESULTS

The data shown in Table 1 indicate that the frequency of biting of Group IV was clearly higher than for the other three groups. It can also be seen in Table 1 that all groups had a higher frequency of biting during the shock condition than during either the pre- or post-shock conditions. Table 1 shows that all Ss in Group IV

Table 1

Frequency of Biting for Each Rat in Each Condition

	Dosage											
	0 mg/kg			250 mg/kg			500 mg/kg			750 mg/kg		
S	Pre	S	Post	Pre	S	Post	Pre	S	Post	Pre	S	Post
1	0	2	0	4	30	0	0	13	0	6	162	0
2	0	26	0	0	0	0	0	1	0	4	112	0
3	0	0	0	0	0	0	1	20	0	11	21	1
4	0	2	0	0	4	0	0	16	0	14	187	3
5	0	0	0	0	28	3	0	14	2	70	215	6
6	0	2	0	0	0	0	0	15	0	8	136	0
Mean	0.0	5.3	0.0	0.7	10.3	0.5	0.2	13.2	0.3	18.8	138.8	1.7

had a greater tendency to bite under all conditions of the shock condition. A treatment x treatment x subjects ANOVA showed the dose effect to be significant at the

less than one percent level ($F=16.910$, $d.f.=3,6$). The repeated measure of pre-shock, shock, post-shock was also found to be significant at the less than one percent level ($F=32.712$, $d.f.=3,6$). The interaction of the dose level and pre-shock, shock, post-shock conditions was also significant (Figure 1, pg. 10) at the less than one percent level ($F=19.147$, $d.f.=6,40$). This interaction indicated that a statistical subtest might clarify the results further. A test of covariance showed that the frequency of biting of Group IV increased significantly from the pre-shock condition to the shock condition indicating that the main effect was primarily due to this group ($F_{.01}=943.74$, $d.f.=3,6$). Biting then decreased significantly from the shock condition to the post-shock condition for Group IV ($F_{.01}=96.75$, $d.f.=3,6$).

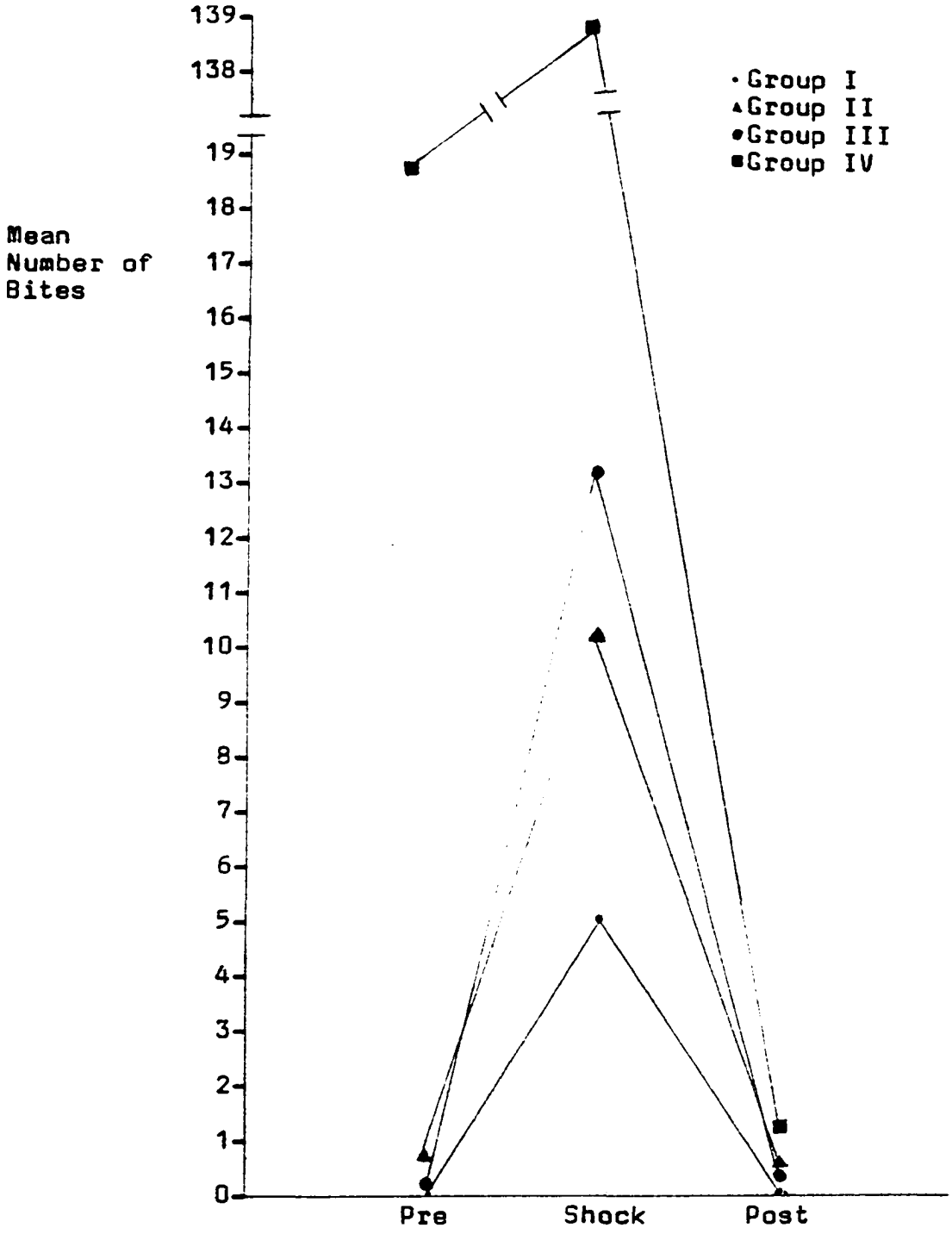

Figure 1. The interaction between the dose level and the shock conditions.

DISCUSSION

In this study the dose effect was found to be significant as were the differences between the pre-shock, shock and post-shock conditions. That is, Group IV (750 mg/kg of L-Dopa) had a higher frequency of biting than any of the other three groups in the three shock conditions. Also there was a higher frequency of biting for all groups in the shock condition as compared to the pre- and post-shock conditions. The interaction was also found to be significant. These findings are in agreement with several studies which have found that low doses of L-Dopa produced little or no change in behavior while higher doses produced rage, aggressive postures and attack responses (Blaschko and Chrusciel, 1960; Everett and Borcherding, 1970; Reis, Moorhead and Merlino, 1970; Strömberg, 1970; VandeWende and Spoerlein, 1972; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973).

However, this is contradicted by Geyer and Segal (1974) who found that low doses increased shock elicited fighting while high doses suppressed it. Smith and Dews (1962) injected rats with doses from 10 mg/kg to 1000 mg/kg with the same result. Smith and Dews suggest that higher doses suppress general motor activity. Other studies have found aggression with doses as low as 200 mg/kg

(Henning and Rubenson, 1970; Benkert, Gluba and Matussek, 1973). There are several possible explanations for this wide variety of results. First, while most of these studies used intraperitoneal injections for the L-Dopa (Rossum and Hurkmans, 1964; Benkert, Gluba and Matussek, 1973; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973), several used other routes of administration, such as injection into the brain via cannulas (Yen, Stanger and Millman, 1959; Ernst and Smelik, 1966; Rolinski, 1973) or intravenous injection (VandeWende and Spoerlein, 1964; Geyer and Segal, 1974). As a result the L-Dopa may have been metabolized at different rates. Also the amount needed to produce the same results may vary with different procedures. For example, when the drug is placed directly into the brain via cannulation, unlike the intraperitoneal injection, it is not necessary to allow for loss through the metabolic process. Secondly, the design of several of these studies was such that only one level of L-Dopa was used, alone and in combination with other drugs (Rossum and Hurkmans, 1964; Thoa, Eichelman and Ng, 1972). This confounds the studies in that it is not clear what the effect is due to: the L-Dopa, the combination of L-Dopa with the other drugs or the other drugs. The study of this thesis has been designed to determine only the effect of different levels of L-Dopa without confounding by the addition of other drugs. It seems as

though this sort of study would be essential before examining other effects.

Finally, some of these studies had at least one of two procedural problems. First, the parameters were varied in that those studies which employed shock did not use the same combination of intensity, duration and current (Henning and Rubenson, 1970; Geyer and Segal, 1974). Secondly, different measures of aggression were used to determine the effect of L-Dopa, such as shock elicited fighting (Thoa, Eichelman and Ng, 1972; Geyer and Segal, 1974), open field activity (McKenzie, 1971; Benkert, Gluba and Matussek, 1973; Narotzky, Griffith, Stahl, Bondareff and Zeller, 1973) or presentation of novel objects Yen, Stanger and Millman, 1959; VandeWende and Spoerlein, 1962; Rossum and Hurkmans, 1964; Rolinski, 1973). It would seem that in certain situations the effect of the drug would be more clearcut than others, such as a situation designed to elicit an aggressive response rather than an open field where there are no objects present toward which an aggressive behavior could be directed.

In summary, the results of this study indicate a positively accelerated monotonic dose response relationship between L-Dopa and aggressive behavior. One might have more confidence in these results, which are in contrast with some of the other studies, because the present results were obtained in a somewhat more carefully

controlled manner. First, the study was designed so that there was a clear baseline of nonshock in the first twenty minutes with which to compare the effect of the shock condition. This was followed by a return to the nonshock baseline demonstrating the effect of the combination of shock and L-Dopa more clearly. Secondly, the shock was more consistent than in other studies in that the rat was in constant contact with the electrodes rather than being able to move around on grids. A third reason for these results is that in having the subject attack an inanimate object, it was possible to narrow the study down to one specific aggressive response and observe the effects on that response. Also, the L-Dopa was not studied in combination with other drugs but was used alone in all Ss. It would appear that these results would be cause for concern in the clinic for patients receiving large doses of L-Dopa. It is suggested that patients receiving comparable doses be observed for changes in behavior.

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