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A RELATIONSHIP BETWEEN ENDOGENOUS BRAIN TYROSINE HYDROXYLASE AND SOCIAL DOMINANCE BEHAVIOR OF RATS

by

SHERRY L. SALMAN

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York.

1983

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August 8 1983
date

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RELATIONSHIP BETWEEN ENDOGENOUS BRAIN TYROSINE HYDROXYLASE AND SOCIAL DOMINANCE BEHAVIOR OF RATS

by

Sherry L. Salman

Adviser: Professor Jay Weiss

A significant body of experimental work has focused on the neurochemical correlates of experimenter-induced stressful conditions, such as electric shock. However, relatively little research has explored the neurochemical correlates of behavior in the natural, social environment of a species. Therefore, the present study asked the question: are there differences in endogenous catecholamine activity among rats in a population which are related to social dominance behavior?

Male hooded rats were left undisturbed in a large colony enclosure while they established a dominance hierarchy. Behavioral observations were scored on various measures of agonistic behavior and dominance ranks computed for each animal. Tissue samples were then taken of locus coeruleus (LC), hypothalamus, substantia nigta, olfactory tubercles, amygdala, caudate, and frontal cortex. These regions were then assayed for tyrosine hydroxylase (TH) activity. Correlations were determined between brain TH and dominance rank for four colonies of rats. The position of an animal in the dominance hierarchy consistently correlated highly with TH activity in the LC. No significant relationship was found between TH and dominance in the dopaminergic brain regions. In addition, only winning at 'broadsiding', a threat behavior, was signif-

icantly correlated with TH, again in the LC, across colonies. These data suggest that brain noradrenergic function is related to social dominance in rats.

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INTRODUCTION

The present study asked the question: is there a relationship between endogenous brain tyrosine hydroxylase (TH) activity and dominance behavior among rats in a social colony? Animals were placed in a colony setting and left to establish a dominance hierarchy. Dominance behavior was then measured by the observation and statistical evaluation of naturally-occuring units of rodent social behavior. The behavioral observations and dominance ranks of each of the colony members were correlated with their TH activity in locus coeruleus (LC), hypothalamus, substantia nigra (SN), caudate nucleus, amygdala, olfactory tubercles, and frontal cortex. This study investigated, in particular, the relationship between animals who were socially dominant and endogenous levels of TH (the rate-limiting enzyme in catecholamine (CA) synthesis) in the locus coeruleus (a nucleus that contains 80% of noradrenergic cell bodies, Swanson, 1976). A variety of evidence indicates that: 1) CA metabolism is related to agonistic behavior, and there are heritable differences in the agonistic behavior of rats which are related to endogenous CA metabolism; 2) Studies using social colonies of rats (Ellison, 1976; Ellison, 1977) have suggested that the noradrenergic (NE), dopaminergic (DA), and serotonergic (5-HT) systems contribute differentially to social behavior, and in particular, animals with lesions placed in the LC were dominated by

others in the social environment (Eison, Stark, &Ellison, 1977); 3) CA metabolism, and particularly increases in TH activity, have been related to situations in which animals are exposed to long-term stress; 4) NE depletion in the LC is correlated with stress-induced behavioral depression, and this depression can be overcome by pharmacological stimulation of alpha 2 receptors in the LC (see review: Weiss, Bailey, Goodman, Hoffman, Ambrose, Salman, & Charry, 1982). The time course of behavioral recovery suggests that induction of TH in the LC is among the active processes which promote recovery from the behavioral deficits exhibited by rats after exposure to stress.

Thus, agonistic behavior, social behavior, and stress-induced behavioral depression are all related to TH metabolism and/or to the LC. 'Dominance' is assumed to be a complex behavior, involving at least in part, both agonistic behavior and an adaptive response to long-term stress, both of which are related to TH metabolism. The present study, then, asked the question: what is the relationship between endogenous TH activity and dominance behavior among rats in a social colony?

Heritable variations in agonistic behavior & CA metabolism

Evidence for the heritability of various aspects of agonistic behavior comes from numerous studies. For example, some rats and mice are natural fighters while others are not (Moyer, 1976; Silverman, 1978; Barr,

Sharpless, & Gibbons, 1979). Offspring of dominant rats have been found to be more dominant than offspring of subordinates, even after crossfostering (Uyeno, 1960). And furthermore, rats and mice can be selectively bred for reactivity and aggressiveness (Sudak, & Maas, 1964; Barr, Gibbons, & Bridger, 1976; Moyer, 1976; Slater, Blizard, & Pohorecky, 1977; Lagerspetz, Tirri, & Lagerspetz, 1968).

Some aspects of these heritable differences in agonistic behavior have neuropharmacological correlates: specifically, NE and DA play a role in several types of aggression, particularly intraspecific or 'affective' aggression, while 5-HT is related to predatory attack (Reis, 1972; Reis, 1974; Avis, 1974; Kaada, 1972; Bernard, 1975). Drugs such as amphetamine, which cause release of DA and to a lesser extent NE, cause increased intraspecific aggression in rodents but diminish predatory attack (Panksepp, 1971a). An increased utilization of DA (turnover) has been found in aggressive strains of mice, as compared to non-aggressive strains (Bernard, 1975). In general, intraspecific agonistic behavior can be characterized as having autonomic activation components, being enhanced by drugs which stimulate CA activity and inhibited by drugs which stimulate cholinergic or 5-HT activity, and as being androgen dependent -- while the characteristics of predatory aggression are the reverse (Reis, 1972; Reis, 1974; Stolk, Conner, Levine, & Barchas, 1974; McLain, Cole, Schreiber, & Powell, 1974; Eichelman, & Thoa, 1973).

Regarding TH and intraspecific agonistic behavior, Axelrod and co-workers (Ciaranello, Lipsky, & Axelrod, 1974) have shown that not only did related sublines of mice show marked differences in their spontaneous fighting behavior, but the strain exhibiting most fighting behavior also had elevated levels of TH, relative to non-fighters. This elevation of TH was not attributable to the brief fighting period. Analysis of the hybrid progeny of these mice suggested that the difference in enzyme activity had a heritable basis as did the difference in fighting behavior. Administration of AMPT, which blocks TH synthesis, has been found to decrease intraspecific aggression in male mice (Matte, & Tornow, 1979).

Especially relevant is a study by Barr et al. (1979) which found that spontaneously aggressive male rats differed from non-aggressive rats with regard to increased endogenous levels of DA in hypothalamus. In addition, a positive correlation was found between the number of offensive sideways movements ('broadsiding') and concentration of DA in the aggressive animals. Broadsiding is a major component of the intraspecific agonistic behavior pattern in rodents (Blanchard, & Blanchard, 1977; Grant, 1963), and is considered to be a 'threat' behavior. It is important to note that dominance, which is established primarily through overt agonistic behavior, may be maintained by threat behavior and other responses which do not involve overt fighting. Thus, threat behavior such as broadsiding,

because it was previously paired with overt agonistic behavior, may come to elicit a CER from other animals.

Although there is evidence for the heritability of agonistic behavior and its neuropharmacological correlates, caution is needed in generalizing from rodents to other species. For example, in monkeys the best predictor of dominance status is the rank of an animal's mother, but due to the fact that mothers defend juveniles by threatening other juveniles and their protective mothers (Koford, 1963; Marsden, 1968). Thus, although agonistic behavior undoubtedly has an heritable component which may be reflected in brain biochemistry, the influence of the social and ecological environment cannot be underestimated, even in rodent populations.

Colony studies

A few studies have examined CA metabolism in relation to the social environment. Studies with hooded rats have shown that only certain strains are disposed to establish definite social dominance hierarchies (Friedman, & Iwai, 1976; Lockwood, & Turney, 1981), and that strains showing the least amount of social interaction had lower levels of NE in hypothalamus than more active strains (File, & Vellucci, 1979). This suggests that animals who are active socially might be functioning in a high CA metabolic state: support for this hypothesis comes from the fact that animals exposed to a colony environment show elevated blood

pressure, adrenal weights, and levels of adrenal TH, as compared with controls (Henry, Stephens, Axelrod, & Mueller, 1971). Studies using colonies of rhesus monkeys with lesions placed in the amygdaloid nuclei (Kling, Lancaster, & Benitone, 1970; Kling, & Dunne, 1976) found that lesioned animals engaged in less social interaction and agonistic behavior than operated controls, and fell in dominance rank. A methodological problem with these studies is that it is unknown as to whether the placement of a lesion in the amygdala caused the effects, or whether just being impaired per se was responsible.

In distinction to this type of study, work by Ellison and colleagues on the "low NE" and "low 5-HT" rat, does suggest a relationship between NE and dominance: animals were placed in a colony and allowed to grow, then captured and injected with the neurotoxins 6-hydroxydopamine (6-OHDA, 25 ug), 5,6-dihydroxytryptamine (5,6-DHT, 10 ug), or saline on three successive days (Ellison, 1976). Animals were all returned to the colony and their behavior during the next 50 days was observed. The animals depleted of NE (6-OHDA lesions) and 5-HT (5,6-DHT lesions) developed particular syndromes over time: the "low NE" rat behaved like an animal in a state of high 5-HT activity, being lethargic and subordinate in the colony but confident in a novel environment (Ellison, 1977; Ellison, 1975). The "low 5-HT" rat behaved like an animal in a high NE state: anxious, aggressive, socially dominant, but fearful in a novel

environment. The opposed behavioral syndromes fit with evidence which indicates that the NE and 5-HT systems are anatomically interrelated, and exert opposed and balanced actions on diverse functions such as temperature regulation, arousal, and pain threshold (Feldberg, & Myers, 1964; Geyer, Segal, & Mandell, 1976; Jouvet, 1977; Ellison, 1975; Eison et al., 1977; Cedarbaum, & Aghajanian, 1978; Bodnar, Ackerman, Kelly, & Glusman, 1978; Lewis, Renaud, Buda, & Pujol, 1976).

Group differences in dominance appeared 25 days after lesioning: the "low 5-HT" rats (high NE state) were observed to be the winners of spontaneous wrestling bouts and were always first to gain entry to the feeding area of the colony (Ellison, 1976). As is usual in a colony, an 'alpha' rat emerged: this animal was aggressive, not fearful in the colony, and socially dominant. The alpha was a "low 5-HT" rat (high NE state) in both colonies sampled, had outbursts of violent aggressive activity, and terrorized the colony. It was particularly the behavior of this alpha rat in Ellison's studies which suggested the hypothesis of the present study: that there might be endogenous differences in NE metabolism related to dominance. A subsequent study (Eison et al., 1977) found that animals with lesions placed in the LC were dominated in the colony by animals with lesions placed in substantia nigra. This again suggested that elevated CA activity, particularly in LC might be related to social dominance behavior.

One additional note of interest is the work by Redmond and colleagues on the behavior of rhesus monkeys living in free-ranging colonies. An inverse correlation was found between stable social affiliative (such as grooming and play) and agonistic behaviors of individual animals and platelet monoamine oxidase (MAO) activity. Specifically, socially active monkeys had low MAO activity. The differences between individuals were quite large, while within animal variation was small, suggesting that the normal range of enzyme activity is adaptive, and supports stable social patterns (Redmond, & Murphy, 1975; Redmond, Murphy, & Baulu, 1979). While not directly related to brain CA metabolism, this study indicates that there are stable endogenous physiological parameters which correlate with the agonistic and social behavior of particular individual animals.

Stress, behavioral depression, and TH metabolism

It is well established that animals exposed to short and long-term stressors show increased CA synthesis, usually by induction of new TH molecules (Zigmond, Schon, & Iversen, 1974; Reis, Joh, & Ross, 1975; Glazer, Weiss, Pohorecky, & Miller, 1975), and that LC and its projection systems play a basic role in the central response to stress. Cold-stress, electric shock, and reserpine administration all increase TH activity in LC. This increase is evident to a lesser extent in LC terminal areas, as well as noradrenergic nuclei A1 and

A2, but not in the dopaminergic areas of substantia nigra and caudate nucleus (Thoenen, 1970; Joh, Geohman, & Reis, 1973; Reis, Joh, Ross, & Pickel, 1974; Weiss, Glazer, Pohorecky, Brick, & Miller, 1975; Renaud, Joh, Snyder, & Reis, 1979b) thus indicating that stress-induced induction of TH is confined to the NE system.

Behaviorally, animals exposed to stressors show increases in aggressive behavior which remain, along with elevated TH activity, after the removal of the stressor (Welch, & Welch, 1969; Lamprecht, Eichelman, Thoa, Williams, & Kopin, 1972). It has also been found that the inability to avoid or escape a stressful situation (i.e. to "cope" successfully) will result in decreased NE concentration (Weiss, Stone, & Harrell, 1970; Anisman, 1975; Weiss, Glazer, & Pohorecky, 1976). Animals who have been defeated in aggressive encounters also show a decrease in NE levels (Eleftheriou, & Church, 1968).

Especially pertinent to the present study is work by Raab and Oswald (1980) which indicates that different conditions of social conflict have opposed effects on TH activity: when a defeated tree shrew had to live in the visual presence of a victorious dominant animal, the defeated animal showed decreased TH in septum (compared with isolated controls), and died within a week even though it was physically separated from the winner. Conversely, other subordinates who coexisted with dominants but could withdraw from their visual presence showed increased TH in septum and

hypothalamus (compared with isolated controls) and lived for many months.

A series of studies by Weiss and colleagues are particularly suggestive as to how TH, stress, and behavioral depression may be related to social dominance (for review, see Weiss et al., 1982). Weiss' animal model of depression states that sufficient exposure to a severe uncontrollable stressor disrupts normal CA transmission in brain (causing a depletion of NE), and produces behavioral depression, measured primarily as poor avoidance-escape behavior in active tasks (Weiss et al., 1975; Glazer et al., 1975; Weiss et al., 1976). Observational data suggest that the stressor (shock) results in a loss of normal aggressiveness coupled with a state of high arousal, in addition to the behavioral depression, so that stress-induced depression appears to model agitated depression in humans. However, repeated exposures to shock-stress protects NE from depletion and prevents behavioral depression. This apparently occurs because the activity of TH is induced after repeated exposure to shock, thus preventing NE depletion (Thoenen, 1970; Musacchio, Julou, Kety, & Glowinski, 1969; Reis et al., 1974). In Weiss' studies, after repeated exposure to shock, animals were protected from behavioral depression and TH activity was indeed increased. In addition, the behavioral depression and NE depletion could be averted by pretreating animals with tetrabenazine (a drug which depletes NE, causing a subsequent increase of TH) before

exposing them to shock-stress (Glazer et al., 1975).

This stress-induced behavioral depression has a particular time-course; it is present up to 48 hours post stress, then decreases steadily and disappears after 72-96 hours. Animals showing behavioral depression also show decreased levels of NE in LC 48 hours post stress, an effect which also disappears after 72-96 hours. There are no shock-induced changes of DA levels in striatum or olfactory tubercles (Weiss, Bailey, Pohorecky, Korzeniowki, & Grillone, 1980) suggesting that depletion of brain DA is not responsible for behavioral depression. (However, other investigators (Anisman, Irwin, & Sklar, 1979a) have found that both NE depletion as well as DA receptor blockade successfully disrupt escape performance, so the relative contributions of NE and DA have not been fully ascertained.) Nevertheless, both the behavioral depression and the NE depletion begin recovery after 48 hours. At first it was presumed that shock-induced changes in CA's simply dissipated over time (Weiss, Krieckhaus, & Conte, 1968) so that both CA function and active behavior thereby return to normal. However, recent data suggest that conditioned emotional responses (CER) are learned during behavioral depression; these responses can increase NE turnover and induce NE depletion long after the acute effects of shock have disappeared (Cassens, Kuruc, Roffman, Orsulak, & Schildkraut, 1981; Anisman, & Sklar, 1979c; Sherman, Allers, Petty, & Henn, 1979). This suggests that recovery from

behavioral depression is not due simply to dissipation of acute shock-induced NE depletion, but requires an active process which interferes with the effect of CER's. The time course of behavioral recovery suggests that the active process may be the induction of TH molecules. Induction of TH, specifically in the LC appears between 48-72 hours after NE depletion (Reis et al., 1974; Zigmond, 1979), the same time period during which recovery from behavioral depression occurs. The importance of TH is underlined by Anisman and colleagues (1979 a, b) who found that escape deficits could be produced in rodents by injection of alpha-methyl-para-tyrosine (AMPT, which blocks TH synthesis), without ever exposing them to shock-stress. It should also be noted that depletion of NE by DBH inhibitors, blockade of DA receptors, and increasing Ach concentration by treatment with anticholinesterase all induce deficits which mimic those elicited by inescapable shock (Anisman, Ritch, & Sklar, 1981).

The establishment and maintainence of a position of dominance within a colony involves numerous agonistic encounters, many severe enough to be considered stressors (Miczek, Thompson, & Shuster, 1982). If induction of TH is among the active processes promoting recovery from stress-induced behavioral depression, this stress-initiated mechanism could protect an animal from the effects of severe stress. The present study suggests that dominant animals come to occupy their position in the hierarchy because high

TH activity protects them from behavioral depression during confrontations with other colony members. It has been suggested above, that TH in the LC is particularly important, and recent experiments have shown that infusion of clonidine (a drug which stimulates alpha 2 receptors) into the LC will promote recovery from behavioral depression (Goodman, Weiss, Hoffman, Ambrose, Bailey, & Charry, 1982).

Locus Coeruleus

Appendix A contains a detailed description of the anatomy, distribution, and functional significance of the LC (also see reviews by Amaral, & Sinnamon, 1977; McNaughton, & Mason, 1980; van Dongen, 1981). Here, discussion is confined to evidence which supports the LC as being a structure related to dominance behavior. Briefly, this small nucleus has been implicated in functions as diverse as respiration (Johson, & Russell, 1952; Ward, & Gonn, 1976), regulation of cerebral blood flow (Scriabine, Clineschmidt, & Sweet, 1976), cardiovascular regulation (the LC mediates the fall in blood pressure observed after shock-induced fighting, Snyder, & Reis, 1975; Williams, Richardson, & Eichelman, 1978), micturition (Kuru, 1965; Lally, Degroat, & McLain, 1972), various spinal reflexes (Ader, Postema, & Korf, 1979), appetitive approach toward food (Osumi, Oishi, Fujiwara, & Takaori, 1975; Redmond, Huang, Snyder, Maas, & Baulu, 1977), sleep stages (Jouvet, 1977; Bubenick, & Monnier, 1972), anterior pituitary activity (Ogren, & Fuxe,

1974), sensory transmission (Sasa, Igarashi, & Takaori, 1977; Geyer, 1976), self-stimulation (ICSS, Wise, 1978), learning (review: Mason, 1981), memory (Zornetzer, Wickliffe, & Appleton, 1978), extinction (Mason, 1979), pain (Korf, Bunney, & Aghajanian, 1974; Bodnar et al., 1978), and general arousal (Korf, 1976; Jones, Bobillier, Pin, & Jouvet, 1973).

The role of the LC in the stress response and in behavioral depression has been discussed previously. The LC has also been implicated in the mediation of noxious stimuli: Neurons in LC will respond with an initial increase of activity to noxious stimulation, and elevations in nocioceptive thresholds have been demonstrated following LC lesions (Bodnar et al., 1978). The sensitivity of LC to pain and/or stress is supported by the fact that morphine decreases the spontaneous firing of the LC and reduces the increase in firing brought on by toe pinch (Korf et al., 1974).

Mason (Mason, & Fibiger, 1979) has suggested an 'attentional' function for LC whereby incoming stimuli are screened out if not relevant, providing a system for mediating information by 'stimulus sampling'. This idea is indirectly supported by data from Redmond and colleagues, who suggest that LC plays a role in fear and anxiety. Lesions placed in LC of monkeys resulted in a placid animal which did not show appropriate species-typical signs of fear, i.e. withdrawal from a rubber snake (Redmond, & Huang,

1979). However, lesions placed in ascending fibers from LC in rats failed to affect a social interaction test of anxiety, and resulted in increased boxing and wrestling behavior (Crow, Deakin, File, Longden, & Wendlandt, 1978). Lesions placed in the LC have also resulted in increased shock-induced fighting in rats, (intraspecific aggression), while mouse-killing (predatory attack) was not affected (Kostowski, 1978). Thus, lesions placed in the LC may not result in fear or anxiety per se, but may impair an animal's ability to make appropriate emotional responses. After LC lesions, rats are impaired in their ability to extinguish responding after reward has ceased (Mason, 1981). Animals with lesions placed in LC were dominated by animals with lesions placed in SN in a colony environment (Eison et al., 1977).

Thus, the LC is involved with the stress-response, behavioral depression and its recovery, pain, 'screening' of sensory information (Foote, Friedman, and Oliver, 1975; Davis, Cedarbaum, Aghajanian, & Gendelman, 1977; Segal, & Bloom, 1976), some aspects of intraspecific agonistic behavior, and possibly appropriate response to emotional and other stimuli. All of these variables can be assumed to be related to dominance in a colony situation, in which an animal needs to respond efficiently and appropriately to social stimuli, as well as be protected from the stress of agonistic encounters.

Summary

Given the foregoing findings it seemed reasonable to suggest that animals who are socially dominant in a colony environment may have higher levels of TH in LC than subordinate animals. The hypothalamus, amygdala, and olfactory tubercles were examined because of their involvement in intraspecific agonistic behavior (Avis, 1974; Kaada, 1972; Flynn, Vanegas, Foote, & Edwards, 1970; Panksepp, 1971b; Clemente, & Chase, 1973; Egger, & Flynn, 1967). The frontal cortex was selected to represent an NE terminal field. The SN and caudate represent a DA nucleus and terminal field as controls for the activity of dopaminergic TH (Appendix B contains the anatomical distribution, functional mechanics, and pharmacology of TH). No other NE nuclei were examined.

The present study is different from those involving neurochemical correlates of experimenter-induced behaviors: phenomena such as shock-induced depression (Weiss, & Glazer, 1975; Anisman, 1975; Overmier, & Seligman, 1967) and shock-elicited aggression (Bernard, 1975; Bernard, & Pavlino, 1975; Ulrich, Wolff, & Azrin, 1964; Moyer, 1968; Stolk et al., 1974) have been studied extensively, but usually as isolated behavioral responses to experimental manipulation, dissociated from the social and ecological matrix of the species and divorced from the functional significance of all behavior, which exists in relation to

the species as a whole. Whether constructing animal models of pathological or normal behavior, it seems highly desirable to develop models in which behavior can be identified and studied in a social context which approximates the naturally occuring social environment, since abnormal and normal behavior in humans are typically identified within such a social context.

METHODS

Subjects and Colony

The experiment was run in four sequential replications (ABCD; N=9,8,9,7). In each, male hooded rats (Blue Spruce breeders) weighing 140-150 g. (100 days old) were introduced into a large (8' x 6' x 4') colony enclosure and left for eight weeks to grow and establish stable dominance hierarchies. The animals were not handled again until sacrifice.

The colony design was adapted from Ellison (1976) and is shown in Fig. 1. It was built of wood and wire mesh, and consisted of two levels: a main floor, and a smaller feeding platform connected to the main floor by a plastic tube. The floors were covered with sawdust which was changed every two weeks. Water was available ad libitum, and the rats were fed (Purina Lab Chow) on a schedule, described below. A large running wheel as well as several wooden play objects, were located on the main floor.

The colony was built in a room isolated from the rest of the laboratory. It had its own ventilation system, and was kept at a constant temperature (21-22°C) year-round. One or two experimenters could sit quietly in the room and observe the animals. The colony could also be seen through a peephole window outside the room. The entire colony room was kept on a reverse day-night lighting cycle (overhead white light from 10:00 PM - 10:00 AM, and dim overhead red lighting from 10:00 AM - 10:00 PM.)

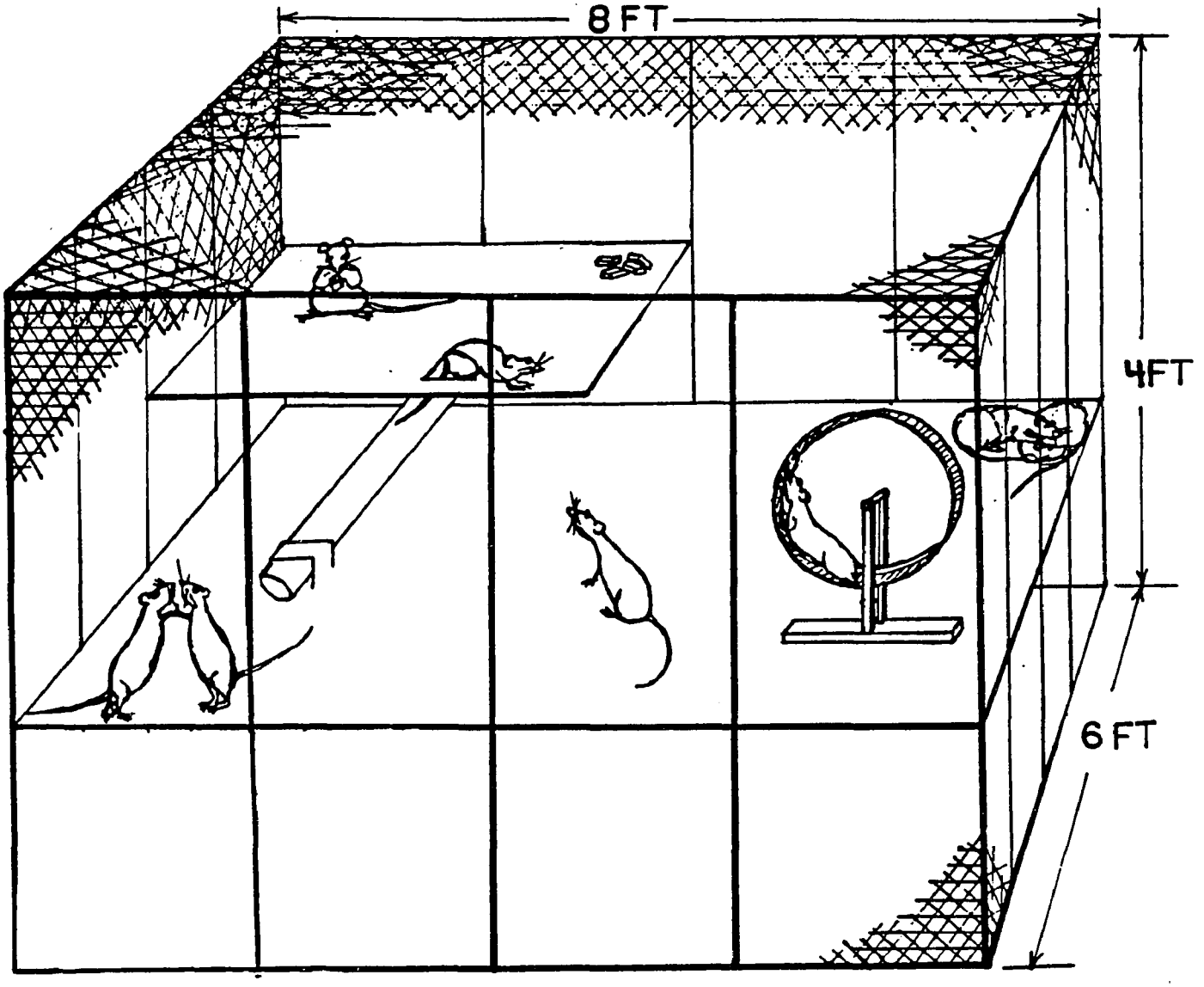

Fig. 1. Representational drawing of the rat colony in which 7-10 rats were allowed to live undisturbed for approximately four months. On the basis of social interactions, a dominance hierarchy emerges that can be discerned by observing the encounters between animals.

Behavioral observation and scoring

Individual animals (readily distinguishable by their natural patterned markings) were randomly assigned identification numbers. After dominance hierarchies were established (8 weeks, Grant, & Chance, 1958; Baenninger, 1966; Blanchard, & Blanchard, 1977), the animals were observed over a period of two weeks, 4-6 hrs. per day, for a total of 25-30 hrs. Observations were made during four standard periods: from 10-12 AM, 2-4 PM, 4-5 PM, and 5:30-7:30 PM. This covered the periods of maximal activity in the colony. The hour from 4-5 PM was feeding time. A few minutes prior to 4 PM food was placed on the feeding platform, a whistle was blown, and rats would compete for access to the tube connecting the main floor with the feeding platform. At 4 PM the tube was opened, and animals went up to the platform, one by one. The tube was closed at 5 PM, by which time animals had left the platform, having also hoarded food pellets. (During the first 3 days of this procedure any animals remaining on the platform were chased off before the tube was closed. By day 4, no animals remained on the platform, and hoarding became common.)

The experimenter would quietly enter the colony room prior to an observation period, and then record all occurrences of the social interactions described below, noting the identification numbers of participating animals as well as the 'winner' and 'loser', if relevant. A sample data sheet is shown in Fig. 2.

DATE: June 6, 1980
GROUP: A

	BOX	BRDS	WRES	FIGHT	CHASE	WHEEL	MOUNT	COMMENTS
Time 2		8w3L 8w3L 8w10L		8w5L		10 8 8		
2:30								
	8-3	8w2L	2w8L 8w2L 9w7L	8w10L 8w10L 8w4L 8w2L	8w9L 3w7L 3w1L	8 2		*8 showing piloerection of fur.
3								
	8-3 9-5 5-10	8w3L 8w4L 9w10L 5w10L	8w9L	8w2L	8w10L 3w5L 8w7L	5 3 1 8 1		All scatter when *8 approaches.
3:30								
		8w3L 3w10L 8w3L 8w3L	8w3L 8w3L	8w10L 8w1L 8w7L	9w10L	10 4 2 2 2		
4								

Fig. 2. Data sheet from group A, showing observation periods and behavioral categories. Abbreviations are Box (boxing), Brds (broadsiding), Wres (wrestling), Fight (fighting), Chase (chasing), Wheel (wheel running), and Mount (mounting). Numbers indicate identity of animal participating and 'w' and 'l', winner or loser. For example, between 2-2:30 PM, rat #8 won a fight with #5.

The rat displays a limited repertoire of acts and postures during social interactions (Lehman, & Adams, 1977; Blanchard, Blanchard, Takahaski, & Kelley, 1977) which can be reliably identified by a trained observer, and which resemble behavior seen among wild rats (Baenninger, 1966; Barnett, 1963). The particular behaviors chosen as measures have been described and standardized in detail as 'elements' of rodent social behavior (Grant, 1963; Grant, & Mackintosh, 1963) which are seen during the establishing and maintainance of dominance orders (Grant, & Chance, 1958; Blanchard, & Blanchard, 1977).

Boxing. (Fig. 3a) Two or more rats stand upright and paw at one another. Each animal participating was recorded; no winner or loser.

Broadsiding. (Fig. 3b) One rat approaches another, repeatedly pushing him sideways with the hip area. The identity of both winner (the pusher) and loser were recorded.

Wrestling. (Fig. 3c) Two animals grapple together on the floor, clutching and striking at each other, until one successfully 'pins' the other beneath him. Winner (the top animal) and loser of each bout were recorded.

Fighting. (Fig. 3d) Violent encounters between two rats, always accompanied by squeals and biting episodes. The animal bitten would usually attempt to break away and flee. Winner (the biter) and loser (the fleeing rat) were recorded.

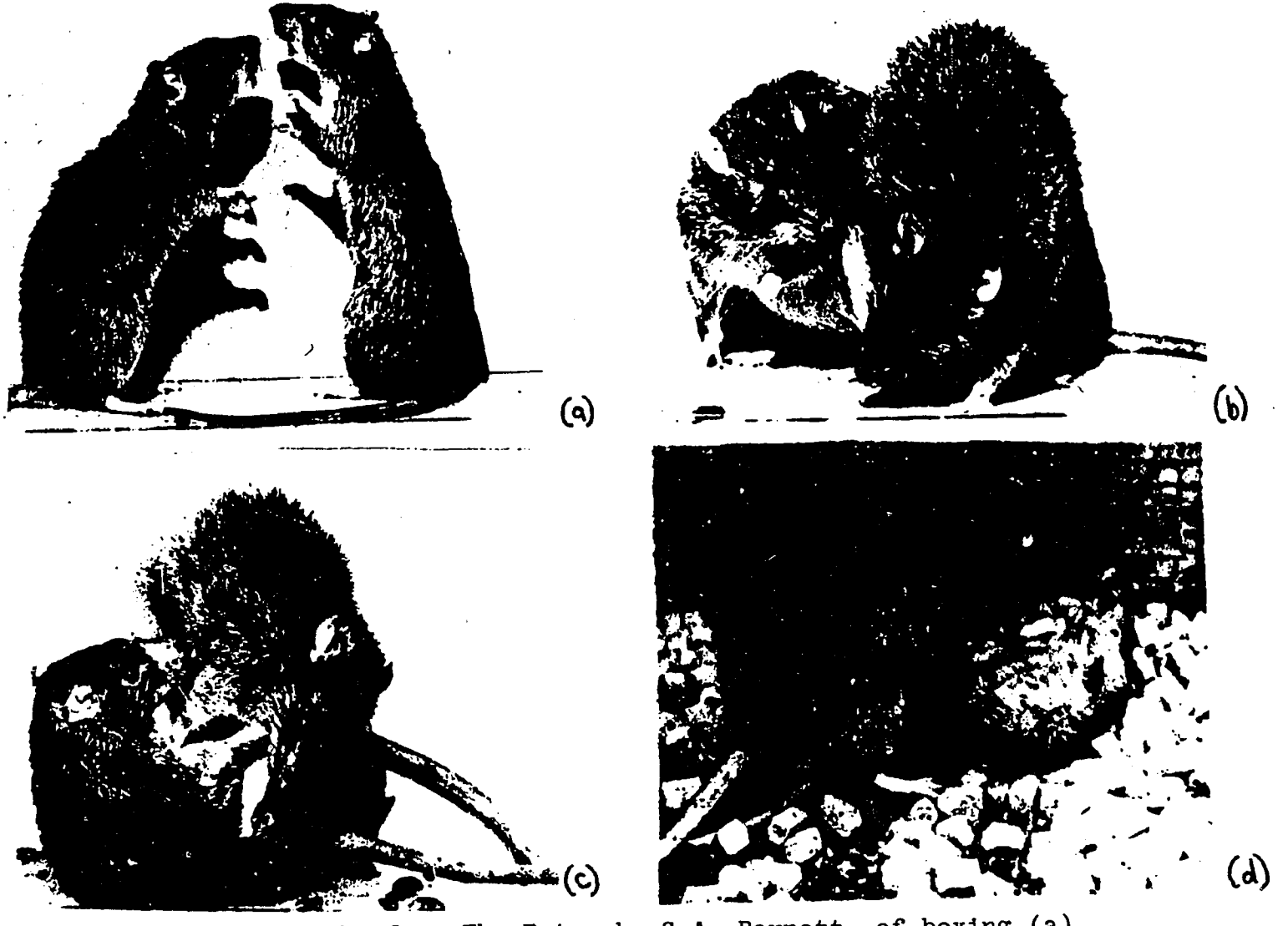

Fig. 3a-d. Photographs from The Rat , by S.A. Barnett, of boxing (a), broadsiding (b), wrestling (c), and fighting (d).

Chasing. One rat chases another around the floor for more than 3 secs. There is no fighting or other behavior. Winner (the chaser) and loser are recorded.

Mounting. Occassionally, one rat would mount another. This behavior seemed idiosynchratic to particular individuals and was only observed in one colony. Mounter and 'mountee' were noted.

Wheel running. An individual runs in the wheel for more than 30 secs. The individual is recorded.

Order of entry. The order in which animals entered the tube to the feeding platform at feeding hour was recorded (adapted from Ellison, 1976).

Dominance Ranking

Using these observations, animals were ranked according to dominance in the following manner: ratio of wins/losses was computed for each behavior, and these were summed across behaviors for each animal. These scores were then ranked, and used as a 'baseline' dominance hierarchy. However, a linear rank-order is an abstraction, which is used to simplify a complex set of relationships. Thus, ranking in the present study utilized other variables such as order of entry up the feeding tube -- the animal going up first received the highest score, the next animal received the next score, i.e. 10,9,8, etc.. These scores were added to the overall ratio-score. In addition, preliminary observation indicated that particular behaviors were favored

by particular animals. For example, dominant animals engaged in broadsiding with others close in rank, while low-ranking animals tended not to broadside at all. All animals wrestled. So, one additional point was given for every broadsiding win. In the case of final scores which were close together, the observer considered additional factors such as the selectivity animals exhibit for interacting with particular others, i.e. dominants usually did not interact with middle-range animals, but rather with betas and low-ranking animals. The hierarchy thus constructed typically consisted of an alpha, the dominant rat, one or two betas (animals who challenged and interacted with alpha, but did not win), a few animals occupying middle positions who did little interacting at all, and one or two animals at the bottom of the hierarchy who were chased and harrassed by others. An example of dominance ranking is presented in Results.

Interrater reliability was obtained for the behavioral categories (Pearson's r= .93, based on 78 observations, in which two experimenters recorded the behaviors which occurred and category totals were compared) and dominance hierarchies (Kendall's tau = .88 Colony B, .90 Colony C). The second experimenter knew the purpose of the experiment, but had no prior knowledge of dominance rank.

Computer Program

In an effort to further objectify dominance ranking, a computer program was created which calculated efficiency scores from raw behavioral data, for each animal in the colony. The program was designed to duplicate the evaluations and decisions made by an observer regarding behavioral observations. All programming was done on a Polymorphic Systems computer #8813, and appears in Appendix C. The formula from which the program was derived, as well as discussion, validity data, and computer generated efficiency scores (CES) are also found in Appendix C.

Sacrifice

At the end of the observation period animals were captured at random, removed from the colony, weighed, and decapitated. Brains were immediately dissected on ice into hypothalamus, locus coeruleus, olfactory tubercles, substantia nigra, caudate, amygdala, and frontal cortex. Tissues were wrapped in aluminum foil and stored at -70°C (Puymirat, Jaway-Agid, Gaspar, Ploska, Prochiantz, & Agid, 1979) until assay for TH activity. The sacrifice and dissection procedure took 4 hrs. to complete, and was done during the dark cycle between 11 AM - 3 PM.

Dissection

Brain regions were dissected in accordance with cytoarchitectural landmarks and boundaries as indicated in a

standard atlas of the rat brain (Konig, & Klippel, 1970). Particular characteristics of our dissection procedure were as follows: First, the hypothalamus was removed. The cerebellum was then discarded, exposing the top of the brainstem, and the locus coeruleus removed. The LC sample extended approximately 3mm in the rostrocaudal direction and 1mm in the dorsoventral direction, as seen in Fig. 4. The densest area of cells extends approximately 1.2mm from the level of the genu of the facial nerve to the midportion of the dorsal tegmental nucleus (Grzanna, & Molliver, 1980). Our LC sample included the traditional A4 and A6 groups of Dahlstrom and Fuxe (1964). Turning the brain ventral side up, the brain stem was discarded by cutting along the vascular boundary line from the forebrain. Looking down on the brain, the substantia nigra sample was removed by cutting just inside the cortex and taking the tissue which remained at the hypothalamic level and slightly below. The olfactory tubercles were removed by cutting along the vascular boundaries. The thalamus, hippocampus, internal capsule and septum were discarded, and the remaining cortex rolled out smoothly. Cutting along the sulcus from the dorsal side a sample was removed containing some entorhinal cortex and the amygdala. Orienting the brain ventral side up, the globus pallidus was discarded,and the caudate removed. The sample of frontal cortex was determined by laying the cortex flat, dorsal side up, dissecting away any remaining structures lateral to the rhinal sulcus, and

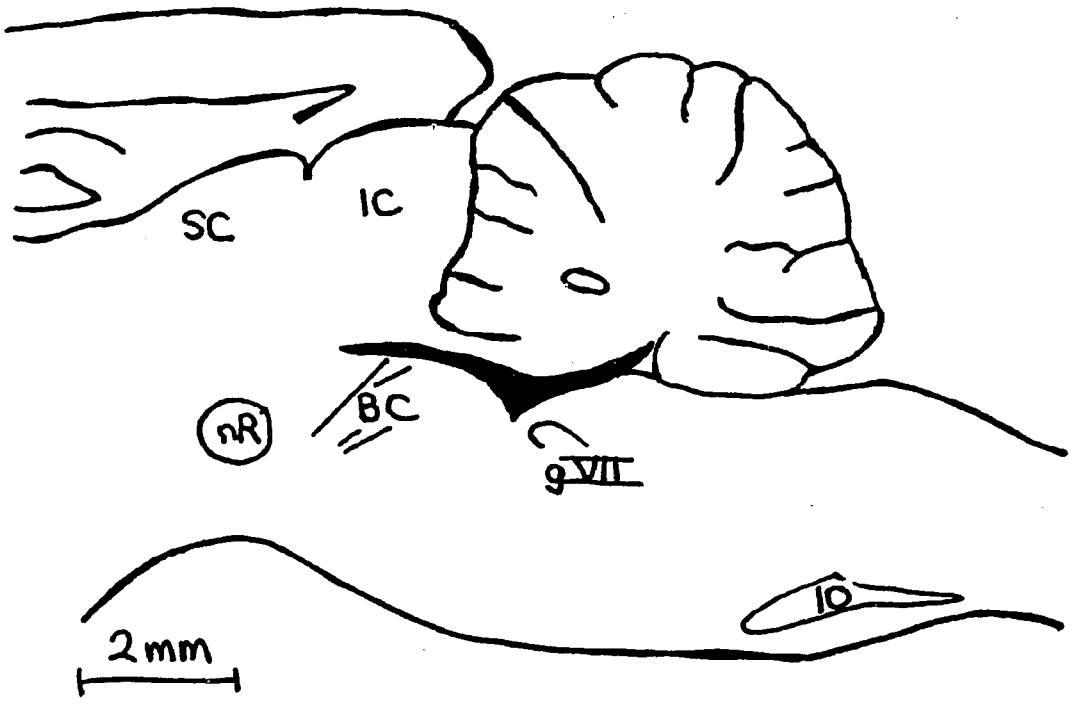

Fig. 4. Drawing adapted from Grzanna and Molliver (1980) of a parasagittal section of rat brain. The shaded area indicates the location of the LC. Abbreviations: BC, brachium conjunctivum; gVII, genu of the 7th nerve; IC, inferior colliculus; SC, superior colliculus; nR, red nucleus; IO, inferior olive.

removing approximately 350 mg. of tissue with a horizontal cut across the midline determining the posterior boundary of the sample.

The approximate weights of tissue samples obtained was as follows (for bilateral structures, total weight is given): hypothalamus, 50 mg; locus coeruleus, 20 mg; substantia nigra, 25 mg; olfactory tubercles, 50 mg; amygdala, 115 mg; caudate, 30 mg; cortex, 330 mg.

Assay

Prior to assay, samples were recoded to eliminate experimenter bias. Tissues were assayed according to the method of Tong Joh (Reis et al., 1975) adapted from Coyle (1972), whereby TH activity is measured by isolating the L-(^{14}C(U)) DOPA formed after conversion from L-(^{14}C(U)) tyrosine. Briefly, tissues were homogenized in 5mM K-phosphate buffer (pH 6.5-7.0) containing .1% Triton X-100 (Sigma). Small tissues or those with low TH activity were homogenized in a standard aliquot of buffer: locus coeruleus in 300 ul, amygdala in 500 ul, and cortex in 2 mls. Other tissues were homogenized by a standard dilution factor: hypothalamus, 1 mg. tissue in 15 ul buffer; olfactory tubercles, 1 mg. in 70 ul; substantia nigra, 1 mg. in 50 ul; and caudate, 1 mg. in 50 ul.

Tissues were centrifuged at 4°C for 10 minutes at 6000 x g., and 50 ul aliquots of supernatant taken as samples. Phosphate buffer blanks and samples were assayed in

duplicate. At this point, any remaining homogenate was frozen for future analysis, if necessary. The reaction cocktail was then added to the samples: 5.0 ul ^{14}C tyrosine in ethanol (New England Nuclear), 5.0 ul tyrosine (non-radioactive), 7.5 ul 1.0M NaAc (pH 5.8), 5.0 ul fresh 19mM DL-6 Methyl-5,6,7,8 tetrahydropterine hydrochloride ($6MPH_4$, Calbiochem-Behring Corp.) in 420mM Mercaptoethanol, 1.0 ul Catalase (Boehringer-Mannheim), and 1.5 ul H_2O. Samples were incubated in a gently shaking waterbath at 30°C for 20 minutes, then placed on ice and the reaction stopped by addition of 1.0 ml .4N $HClO_4$ containing 10 ug L-DOPA (Sigma).

Samples were centrifuged again and equilibrated with 5 vol. 2% disodium ethylenediamine tetraacetic acid (EDTA), plus 1.5 vol. 0.35M KH_2PO_4 (monobasic), and adjusted to pH 8.6 with NaOH. Alumina columns (400 mg, pH 3.4, Woelm Pharma, West Germany) were washed with 20 mls H_2O, and the ^{14}C- DOPA eluted over columns with 4 mls .5N acetic acid. Liquiscent was added to the samples which were collected in minivials and analyzed by liquid scintillation spectrometry (Packard Tri-Card Liquid Scintillation Spectrometer).

The following modifications were introduced to increase the efficiency and sensitivity of the assay: for tissues with low TH activity only, i.e. cortex, ^{14}C tyrosine was used in the reaction cocktail. For a normal assay the final concentration of tyrosine was $2 \times 10^{-4}M$. For samples

with low activity final concentration could be as low as 1 x 10^{-5}M. The assay could be interrupted, and samples stored overnight in a cold-room, after incubation and addition of the $HClO_4$ containing L-DOPA.

Previous to actual data analysis it was determined that recovery of ^{14}C DOPA adsorbed to the alumina and subsequently eluted was 82%, which is standard for this procedure (Acheson, Kapatos, & Zigmond, 1981). The assay was found to be linear over incubation times of 10-30 minutes, and over tissue concentrations of 25-75 uls.

TH activity of control tissues compared with those obtained in other laboratories yielded comparable values. TH activity was calculated as pico- or nanomoles L-DOPA/per hour/per locus coeruleus (Note*) or per milligram of tissue (other brain areas). Sample results from our laboratory expressed as means ±S.E.M. were: LC 171 ± 12 pmol/LC/hr, hypothalamus 212 ± 14 nmol/g/hr, and substantia nigra 870 ± 85 nmol/g/hr. Corresponding values from other studies are LC 187.1 ± 12.6 pmol/LC/hr, hypothalamus 142 ± 8 nmol/g/hr, and substantia nigra 560 ± 42 nmol/g/hr (Renaud et al., 1979; Reis et al., 1975). The slightly higher values we obtained are due to improved assay conditions (Tong Joh, personal communication).

Note - LC activity was expressed this way since it is the most accurate and reliable method for assessing TH activity in this area (Lewander, Joh, & Reis, 1977). Immunocytochemistry has shown that within the LC the TH is contained within the cell bodies and proximal processes of intrinsic neurons (Pickel, Joh, & Reis, 1975). The tissue without TH is a great source of variability in the dissection, so expression of activity per mg of tissue gives greater variation in results than expressing activity per LC as an entire tissue.

Data Analysis

Behavior and TH

Spearman Rank-Order (rho) and Pearson (r) correlations were calculated comparing body weight, TH activity, and 24 various aspects of behavior, for the animals in each colony. The matrices included these variables:

	TH activity in:	Behavior:
Body Weight	Locus coeruleus	Dominance hierarchy
	Hypothalamus	CES (Computer ranks)
	Olfactory tubercles	Overall wins
	Amygdala	Overall losses
	Caudate n.	Overall activity
	Substantia nigra	Overall $\frac{\text{Wins}}{\text{Losses}}$ x Activity (W/L x A)
	Frontal cortex	Broadsiding wins
		Broadsiding losses
		Broadsiding activity
		Broadsiding W/L x A
		Fighting wins
		Fighting losses
		Fighting activity
		Fighting W/L x A
		Chase wins
		Chase losses
		Chase activity
		Chase W/L x A
		Wrestling wins
		Wrestling losses
		Wrestling activity
		Wrestling W/L x A
		Boxing activity
		Wheel running

Tetrabenazine Experiments

In two further studies, an attempt was made to test the hypothesis that high TH results in dominance-related behavior. Individual animals under varying colony conditions were treated with tetrabenazine (TBZ), a drug which results in a depletion of CA's and a subsequent induction of TH (Chan, & Webster, 1971). Social behavior in the colony was then observed. Unless otherwise indicated, conditions are the same as those described for the colony experiment..

In the first study (TBZ Colonies), four small groups (N's: A=5, B=4, C=5, D=5) of hooded rats were left undistrubed for six weeks to grow and form a social hierarchy. The colony enclosure was 4'x2'x2', constructed of wood and wire mesh. After this period, behavior was rated for two hours on three successive days (total six hrs.), and a dominance hierarchy compiled. Animals were then removed and injected: The beta rat (2nd highest in rank) received 5mg/kg of TBZ (Hoffman-LaRoche), while all others received saline. Animals were returned to cages, left undisturbed for 72 hrs. after which time behavior was again rated and a 'post injection' dominance hierarchy compiled. The pre- and post injection social hierarchies were compared using Spearman Rank-Order correlations.

Prior to actual data analysis it was determined that 5mg/kg TBZ reduced catecholamine concentration: NE (ng/gm) was reduced by 10% in hypothalamus, 17% in frontal cortex,

and 30% in locus coeruleus (as compared with controls); DA (ng/gm) was reduced 44% in hypothalamus, 35% in cortex, and 11% in locus coeruleus. A TH induction study indicated that three days after injection with 5mg/kg TBZ, TH activity was 40% higher than controls in hypothalamus, and almost twice control levels in locus coeruleus.

In the second study (TBZ trios) four groups of six animals were run. Upon arrival at the laboratory, three animals received TBZ and three saline, all six were then placed in a colony cage and left undisturbed for two weeks, after which they were re-injected. After ten days the animals were injected for a third time. Two days after the last injection animals were tested for competitive dominance (Zook & Adams, 1975) over four, ten minute test periods, during which the experimenter placed a piece of apple in the colony and recorded the time (mins./secs.) that the apple was controlled by the drug animals, and by the saline animals (the 'apple test'). The total test time for each colony was 40 mins. Results were evaluated by Student's t-tests for differences between related means.

RESULTS

Behavior

A dominance hierarchy was readily constructed in each colony in accord with the procedures described in the Methods section. Table 1 shows data used to compile a dominance hierarchy. Ranking was done in three successive stages:

Stage I -- Total scores. Each animal's scores were added across rows, total scores (TS) computed and preliminary ranks assigned. Particular characteristics of scoring were as follows: animals received only 1/4 point for boxing, as this behavior was judged relatively unimportant in evaluating dominance (Baenninger, 1966). As discussed in Methods, each 'broadsiding win (bw)' received an extra point, as this behavior was judged to be very important in dominance behavior (Blanchard et al., 1977). In addition, the tube entry scores were divided by 10 in order to reduce the weight of this behavior in the TS.

Stage II -- Differentiating between similar scores. If TS were similar, one animal was ranked higher than another on the basis of a larger 'bw' score. For example, in Table 1, rat F has a TS of 7.5, but a 'bw' score of 0.0, while rat E had a TS of 7.4, but a 'bw' score of 1.0. Therefore, rat E was ranked higher than rat F.

Stage III -- Further differentiation between low-ranking animals. Further discrimination between low-ranking animals was done on the basis of whether animals lost many behavioral encounters, or did little interacting. For example, although rats H and I both have a TS of 5.0, rat I is ranked lower than H because raw observational data revealed that it was persistently chased and threatened by other animals, while rat H did little interacting at all.

After all stages of ranking were completed, final dominance ranks were assigned to each animal. These appear in "Ranks" column in Table 1.

TABLE 1
For colony A, raw scores used in calculation of dominance hierarchy.

Rat	Ratio W/L(a)				Box(b)	Tube(c) Entry	Brdsg Wins	Total	Ranks
	Brdsg	Wrestle	Chase	Fight					
A	39.0	4.0	16.0	25.0	3.0	9	39	135.0	1 Alpha
B	.5	.7	11.0	1.3	3.5	8	10	35.0	2 Beta
C	2.5	0.0	4.0	0.0	.5	5	5	17.0	3
D	.4	1.0	2.0	3.0	2.0	4	4	16.4	4
E	.2	0.0	.5	.2	1.5	4	1	7.4	5
F	0.0	1.0	.2	.3	1.0	5	0	7.5	6
G	0.0	0.0	.1	0.0	0.0	6	0	6.1	7
H	0.0	0.0	0.0	0.0	2.0	3	0	5.0	8
I	0.0	1.0	0.0	0.0	2.0	2	0	5.0	9 Omega

(a) total wins divided by losses, over the 2 week observation period
(b) each participant in boxing bout received 1/4 point
(c) total tube entry score divided by 10

The behavior of the alpha, or most dominant, animal was characterized by winning (as defined in Methods) virtually every encounter in which it engaged. The alpha's behavior was selective towards other individuals depending on their rank as seen in Table 2: it engaged mainly in broadsiding with beta animals (score of 20), but did not fight with betas (score of 3). It seldom interacted with middle-range animals (6 and 2), but chased and fought with the lowest-ranking rat (10) but did not broadside with the omega (1). Alphas were also observed to be extremely emotional when handled during the sacrifice procedure, squealing and struggling vigorously, while other individuals were relatively passive. Beta rats won encounters with other animals except the alpha, with whom betas engaged (but lost) in broadsiding. The behavior of middle-range animals was characterized by grooming and wrestling with each other, winning bouts with the omega

TABLE 2
For colony A, distribution of wins and losses in broadsiding and fighting between alpha, beta, middle, and lowest-rank (omega) animal.

	BROADSIDING				FIGHTING			
Loser	Alpha	Beta	Middle	Omega	Alpha	Beta	Middle	Omega
Winner								
Alpha		20	6	1		3	2	10
Beta	0		4	2	0		4	3
Middle	0	0		2	0	0		4
Omega	0	0	0		0	0	0	

rat (2 and 4), but seldom interacting with alpha. The one or two lowest-ranking animals lost behavioral encounters (scores of zero), and were chased and sometimes bitten by the others (loser scores of 10, 3, and 4).

When the feeding tube was opened, the alpha went up first, pulling others out of the tube entrance if they attempted to go up first. The alpha remained on the feeding platform for the entire hour, often chasing other animals off after 30 mins. Lowest ranking animals went up the tube last, and would make several trips up and down hoarding pellets. Occasionally all animals fed undisturbed on the platform for 20-30 mins. until behavioral encounters began, usually between alpha and beta(s).

Stylistic differences were observed among the colonies regarding behaviors used to maintain the dominance hierarchy (see Table 3): in colonies A and D, broadsiding and fighting occurred more frequently than other behaviors, while in colony B wrestling occurred most frequently and was engaged in by the alpha (who did not wrestle in A and D). Colony C was also characterized by broadsiding, as well as chasing. Chi-square analysis ($p < .01$) indicated that particular alpha animals and specific behaviors are significantly related.

TABLE 3
Total number of occurrences of broadsiding, wrestling, chasing, and fighting in the alpha rat per colony.

Behavior	Alpha			
	Colony A	B	C	D
Broadsiding	39	23	32	36
Wrestling	5	33	5	6
Chasing	16	27	61	8
Fighting	25	12	21	24

Chi-square = 99.38, $p < .01$, C = .459

Relationship between TH activity and dominance rank

As can be seen in Table 4, the position of an animal in the dominance hierarchy consistently correlated highly with the TH activity in one region of the brain -- the LC (See p. 41). Significant correlations between TH and dominance rank were also obtained in hypothalamus, but only in two of the four colonies sampled. No significant correlation was found between TH and dominance rank in any of the other five brain regions.

Figure 5 shows the TH activity in the LC and SN of individual animals from a representative colony. There was a strong relationship (pearson product moment correlation, $r = .88$) between an animal's dominance rank and the TH activity in LC, while the relationship between rank and TH in other brain regions (the SN for example, $r = .10$) appears to be random.

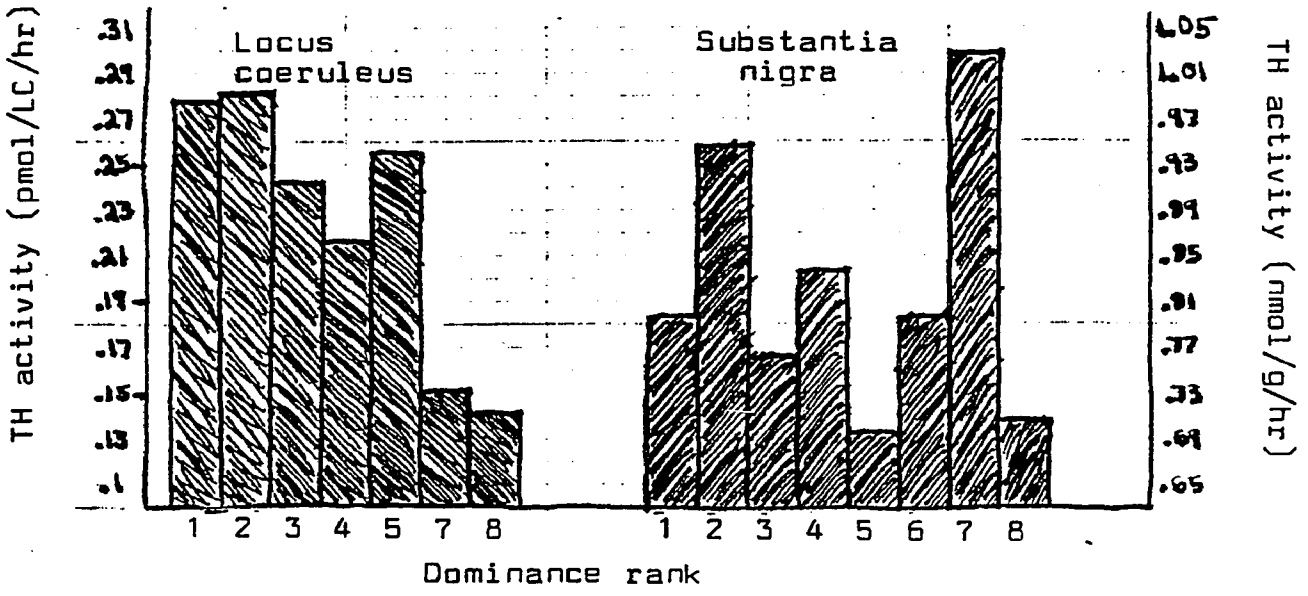

Fig. 5. TH activity in LC and SN from colony B. Values from individual rats are shown in descending order of dominance (left to right, #1 to #8). The LC of #6 was lost.

It is interesting to note that the brain regions sampled appear independent of each other regarding TH activity, with a possible exception of a relationship existing between TH activity in LC and cortex. Very few significant correlations were found between TH in various brain regions, and those that existed were not consistent (i.e. in some colonies the correlation was positive, while in others it was negative). For example, the correlations between TH in LC and hypothalamus were r = .68, (.57), (.75) in colonies A, B, and D; between LC and caudate, r = (.86), (.70), and .31 in colonies A, B, and D; between cortex and hypothalamus, r = .68 and (.48) in colonies C and D. The TH in LC and cortex was correlated at r = .71 in the one colony (D) sampled. No

other relationships were found between TH activity in other brain regions.

TABLE 4
Correlations of TH activity and dominance rank.

Brain region	Correlation Colony A (N=9)	B (N=8)	C (N=9)	D (N=7)
Locus coeruleus	.84*	.88*	+	.76*
Hypothalamus	.84*	.45	.68*	(.60)
Amygdala	++	++	.11	.27
Frontal cortex	++	++	.08	.20
Substantia nigra	.55	.10	.29	.44
Caudate	(.40)	.11	.08	.19
Olfactory tubercle	.63	.56	.51	+

* p <.05
+ samples lost
++ brain region not analyzed
Negative correlations are indicated in parentheses ( ).

Relationship between TH Activity and Specific Behaviors

Correlations between TH activity and specific behaviors are seen in Table 5. Of the 24 original behavioral categories scored, only 9 showed significant correlations with TH, and these are included in Table 5. Significant correlations between behaviors and TH were found only in the LC, hypothalamus, and SN.

Of the individual behavioral categories, the dominance hierarchy and all the 'wins' categories were most highly related to TH, particularly in the LC. Dominance hierarchy and broadsiding wins were found to correlate with TH activity in the LC across colonies.

TABLE 5

Correlations of TH activity and specific behavioral variables.

Behavior	Correlation											
	Locus Coeruleus				Hypothalamus				Substantia nigra			
	Colony A	B	C	D	Colony A	B	C	D	Colony A	B	C	D
Hierarchy	.84*	.88*	++	.76*	.84*	.45	.68*	(.60)	.55	.10	.29	.44
Overall Wins	.69*	.74*	++	.46	.84*	(.30)	.56	(.25)	.71*	.40	.21	.62
Broadsiding Wins	.84*	.74*	++	.74*	.79*	(.17)	.54	.07	.59	.36	.26	.58
Broadsiding Encounters	.54	.57	++	.57	.69*	(.37)	.25	(.30)	.84*	.40	.16	.71*
Fight Wins	.92*	.56	++	.32	.71*	.01	.58	(.04)	.67	.38	.31	.41
Chase Wins	.85*	.56	++	.31	.80*	.01	.58	.06	.62	.38	.17	.41
Wrestle Wins	.56	.81*	++	.28	.63	(.36)	.57	(.14)	.90*	.50	.21	.61
Wrestle Encounters	.89*	.63	++	.14	.62	(.43)	.83*	(.32)	.34	.20	.09	.43
Boxing	.43	.32	++	.44	.32	(.24)	.41	(.19)	.70*	.47	.23	.69*

* $p < .05$

++ samples lost

Negative correlations are indicated in parentheses

The remaining data indicate differences between colonies as to which behaviors were favored by the animals: in colony A, chase wins and fight wins also correlated with TH in LC, while in colony B wrestling wins was related to TH in LC. The specific behaviors in each colony found to be related to TH activity, as seen in Table 5, reflect the stylistic differences in maintaining dominance observed in each colony, noted in the Behavior section, and seen in Table 3. In the hypothalamus, the TH activity followed the LC in colony A, but showed an opposed trend in colonies B and D.

Broadsiding encounters and boxing were the behaviors most highly related to TH activity in the SN, while neither of these behaviors correlated with TH in LC (see Table 5). This suggests that TH in the SN is related to a variable other than dominance.

Of the 9 specific behaviors related to TH activity, 7 were found to be correlated very highly with each other: dominance hierarchy, overall wins, broadsiding wins, broadsiding encounters, fight wins, chase wins, and wrestle wins were intercorrelated from .70 to .96. (Therefore linear regression analysis was not done.) These behaviors all correlated with TH in the LC, and it seems reasonable to assume they were measuring the same factor or variable, which appears to be dominance. Conversely, boxing and broadsiding encounters correlated with TH activity in the SN. Boxing did not correlate significantly with the 7 'dominance behaviors'

(except broadsiding encounters, .69) nor with TH in the LC. Therefore, it is likely that the correlation between TH in the SN and boxing reflects some variable other than dominance, perhaps activity.

There was no correlation between body weight and TH in any of the seven brain regions sampled. Body weight correlated significantly with dominance rank in only one colony (C), and was not consistently related to any other specific behaviors.

Tetrabenazine Experiments

TBZ Colonies

In this study, the beta animals were injected with TBZ and returned to the colony. However, these drug-treated animals did not rise in social rank, and the 'pre-treatment' alpha animals were not displaced. Pre- and post-treatment hierarchies remained stable, and were correlated from .8 to 1.00.

TBZ Trios

In this study, drug-treated and saline animals vie for possession of food (competitive dominance). However, there was no significant difference (t-test) in the time that the food was in possession of either group: TBZ animals -- 77 mins., saline animals -- 83 mins.

It would appear that neither of the TBZ experiments was successful in producing dominant animals. Unfortunately, it was not possible to adequately evaluate these results,

because the motor behavior of the TBZ animals was impaired: they moved about the colony in jerky, hopping motions. Thus, the question as to whether inducing TH will result in dominance-related behavior remains unanswered by the present pilot studies; this issue is adressed more fully in the Discussion.

DISCUSSION

The results of this study suggest that the social behavior of individual animals is related to TH activity in the brain. All of the brain regions investigated contained substantial amounts of TH, as expected. However, only TH in the LC and hypothalamus showed significant correlations with dominance-related behavior. TH in the LC correlated significantly in all colonies in which it was measured, while TH in the hypothalamus correlated significantly in only half the cases. Thus, only TH in the LC was consistently correlated with dominance-related behavior.

Regarding the relationship between TH and specific behaviors, winning in broadsiding was consistently related to dominance rank, suggesting the possibility that dominance may be maintained in the colony largely via such threat behavior. Thus, a distinction between dominance behavior and aggressive behavior, i.e., fighting, is important, since dominance-related behavior may actually serve to minimize overt aggressive behavior. Numerous studies have linked DA with aggressive behavior (Reis, 1972, 1974; Avis, 1974; Panksepp, 1971; Bernard, 1975) but have not distinguished overt aggressive behavior from dominance behavior. The present findings reveal that TH activity in the predominantly dopaminergic brain regions -- olfactory tubercles, caudate, and SN -- did not correlate with dominance. Therefore, the present data suggest that

noradrenergic, rather than dopaminergic systems are implicated in dominance-related behavior. Moreover, TH in the SN correlated highly with boxing and broadsiding encounters (which were not dominance-related behaviors), suggesting that this dopaminergic region is related to a variable other than dominance per se, perhaps motor activity. Ellison and co-workers found different behavioral syndromes in colony animals following lesions placed in LC or SN (Eison et al., 1977): animals with lesions placed in the LC were dominated by those with lesions placed in the SN, who were hyperaggressive. Thus, NE and DA systems may exert different effects on the CNS, and on behavior (Antelman, & Caggiula, 1977). However, Crow et al. (1978) found <u>increased</u> boxing and wrestling after placing lesions in the LC. These authors interpreted their findings as indicating that lesioned animals engaged in more 'aggressive' behavior than controls. However, the present study suggests that boxing, and also wrestling behavior, are not strongly related to dominance behavior, but rather to another variable such as motor activity. Thus, the data from Crow et al. is not inconsistent with the conclusions of the present study, that the LC is implicated in dominance behavior.

It is not clear from the present findings if the NE and DA systems exert different and <u>opposed</u> effects on dominance behavior, or if there is an NE/DA interaction with respect to dominance. Turning to the findings of the present study,

boxing and broadsiding encounters, which correlated significantly with TH in the SN, did not correlate highly with dominance-related behaviors or with TH in the LC. However, the lowest ranking animals rarely engaged in boxing bouts at all, suggesting that this activity is only associated with middle to upper-range dominance status. Thus, there may be a complex interaction between the DA and NE systems with respect to dominance behavior, as has been suggested in the analysis of uncontrollable stress effects (Anisman et al., 1981).

Returning to the main findings, the studies by Weiss and co-workers which found that shock-induced behavioral depression is related to decreased NE in the LC (see Introduction) suggest intriguing possibilities regarding what may be involved in the relationship between dominance and TH in the LC. The alpha rats in the present study were active, efficient in behavioral encounters, and aggressive -- these observations fit well with the findings of Weiss' shock studies in which high TH in the LC protects against behavioral depression. It is possible that the alpha rat comes to occupy the dominant position because high TH in the LC protects it from the adverse behavioral effects that arise from confrontation with other animals in the colony during active competition for dominance. Conversely, low-ranking animals with low TH might have a susceptibility to stress-induced depression. Do these differences reflect a predisposition to aggress? It is possible that the

behavior of the animals in the present study results, in part, from differences in underlying 'emotional' traits, which in turn are related to specific genetic factors such as brain enzyme activity. These factors may predispose individuals to respond differently to the same conditions, such as the stress of agonistic encounters. This hypothesis is indirectly supported by Anisman et al. (1979b) who found large within-species variations in response to inescapable shock and treatment with AMPT (which interferes with TH synthesis): sublines of mice whose escape performance was minimally affected by shock pretreatment, were the same sublines least affected by drug pre-treatment. Presumably in these particular sublines, synthesis of TH keeps pace with increased demand for utilization, and prevents CA depletion. These authors have also suggested that the neurochemical change induced by stressors such a shock, are subject to conditioning and/or sensitization; namely, once an animal is exposed to a stressor resulting in NE depletion, subsequent limited exposure to this stressor (or associated stimuli) results in a 'conditioned' NE depletion (1979c). Thus in the present study, the dominant animals may be protected by high TH and not affected neurochemically by the stress of agonistic encounters, while the low-ranking animals, who are affected by this stress, may also be subject to repeated conditioned NE depletions.

On the other hand, it is also possible that aggressive, emotional encounters in the colony <u>per se</u> simply induce high

TH in the alpha rats, as it is well documented that stressful events induce TH (see Appendix B for review). However, if this factor alone were operating, it would also be reasonable to expect the beta rats and the low-ranking 'scapegoats', who are both involved in stressful encounters, to show evidence of high TH induction, and this is not the case. Perhaps then, the alpha animals are distinguished by the fact that they cope successfully with the stressors of the colony situation, i.e. become 'dominant', and this dominance is reflected in high TH .

Thus, the question remains: does high TH in the LC result in dominance, does dominance result in high TH, or do these variables interact in a long feedback loop and remain intertwined? The tetrabenazine studies attempted to separate these variables by inducing TH and observing the effects of this treatment on dominance behavior. The results indicated that the induction of TH did not change previous dominance status, or produce dominant individuals. However, the existence of confounding problems due to the drug treatment, plus data from other studies indicating the difficulty of pharmacologically inducing changes in a social hierarchy (Silverman,1978; Poshivalov, 1980), leave this question unanswered. An attractive theoretical possibility is that dominance and high TH interact, such that each successively reinforces the other: high TH in the LC enables an animal to cope with the stress of agonistic encounters, while this successful coping behavior, i.e. dominance,

results in a further induction of TH in the LC.

In any event, what might be the functional effects of high TH in the LC which contribute to dominance behavior? Exposure to the stressors inherent in the colony situation may cause release of NE (see Weiss et al., 1982 for review). If this happens within the LC the result is an increase in the activity of LC neurons, due to understimulation of the inhibitory presynaptic receptors (alpha$_2$) on LC neurons (Aghajanian, Cedarbaum, & Wang, 1977; Cedarbaum, & Aghajanian, 1978). However, in a converse situation, high TH might enlarge the amount of NE available, making an animal more resistent to NE depletion and preserving the stimulation of alpha$_2$ receptors in the LC. Recent data (Weiss, personal communication) indicate that understimulation (by piperoxane, which blocks receptors) of alpha$_2$ receptors in the LC mimics behavioral depression produced by shock, while stimulation (by clonidine) of these receptors in the LC produces the opposite effect, behavioral activation. The dominant animals in the present study, because of high TH in the LC, may be functioning in an analogous state of behavioral activation.

Several morphological characteristics of the LC lend support to this hypothesis: the efferents from LC project virtually everywhere in the CNS, including other NE nuclei (see Appendix A), but seem poorly designed to carry discrete information because of the profuse branching of axons and the extensive terminal areas. However, LC fibers would be

very well-designed to activate the brain in some general way, perhaps mediating a state of emotional tone and/or arousal (Amaral, & Sinnamon, 1977). If the dominant animals in the present study have decreased activity in LC neurons, due to stimulation of presynaptic receptors, the end result will be less inhibition of other brain areas, therefore behavioral activation. It is of interest in this regard, that Redmond et al. (1979; 1981) associate an increase in activity in LC with an increase in anxiety; the dominant animals in the present study, who are presumably in the opposite physiological state, namely decreased activity in LC, also appear to be behaviorally quite different from Redmond's animals, and do not show the signs of anxiety found by Redmond and co-workers.

There are several questions that remain unanswered by the present study. The first is whether the high level of TH in the LC of dominant animals represents activation of, or induction of this enzyme? In delayed activation, which is confined to NE systems, the increase in enzyme activity is due to enhanced catalytic activity of enzyme molecules without change in number (see Appendix B) and appears to be initiated by stimulation of central cholinergic receptors. In induction, which is also confined to NE systems, the increase in enzyme is due to actual synthesis of new enzyme molecules, which can be shown immunologically in the CNS with antiserum prepared against purified TH. Induction has been shown to be initiated by drug treatments which deplete

NE, and by stressors, such as shock and cold exposure. Although both delayed activation and induction have been observed in the LC, and both function as long-term regulatory mechanisms for changes in tyrosine hydroxylation which promote availability of transmitter, induction is the mechanism initiated by stressful conditions, and thus is most likely to be responsible for the high TH in the LC of dominant animals. Confirmation of this hypothesis awaits immunological analysis.

Another issue is the interpretation of the hypothalamic TH, which was found to correlate with dominance rank positively, negatively, and not at all. One possible explanation for this discrepancy is that although the hypothalamus is involved with both agonistic behavior and the stress response (see Introduction) these are only partial aspects of dominance behavior, and may have been more, or less, present in different colonies. For example, in colony A where there was a good deal of fighting, the correlation between hypothalamic TH and rank was $r = .84$, whereas in colony D where dominance was maintained by broadsiding alone, the correlation between hypothalamic TH and rank was $r = (.60)$.

It is also important to note that the LC was the only NE nucleus investigated, and thus the conclusion that dominance is related to high TH in the LC is restricted to this nucleus alone, and cannot be extended to the NE system as a whole. However, it can be pointed out that the

regulation of TH may be qualitatively comparable in all central NE systems: nuclei A1 and A2 have been induced to synthesize TH, like the LC, in response to reserpine treatment (Renaud et al., 1979b).

Finally, the procedure of the present study has the inherent limitation of being able to demonstrate only a correlation between dominance and TH in the LC. In addition, it is unlikely that high TH in the LC is the sole mechanism mediating dominance behavior. Moreover, assuming high TH in the LC is a major component of dominance-related behavior, LC transmission directly affects other transmitter systems such as DA (Antelman, & Caggiula, 1977; Anden, & Grabowski, 1976), cholinergic (Mason, & Fibiger, 1979; Visi, 1980), 5-HT (Lewis et al., 1976) and GABAminergic systems (Moroni, Bianchi, Moneti, Tanganelli, Guandalini, & Beani, 1982), and these undoubtedly contribute to dominance behavior.

Concluding comments

The findings of the present study, that high TH in the LC is correlated with social dominance in rats, are compatible with the results of numerous studies on the neuropharmacology of depression and arousal. The CA hypothesis (Schildkraut, & Kety, 1967) initially identified disturbances in NE function as a basis of depression -- drugs which increased the availability of NE at receptors are clinical antidepressants and produce behavioral arousal

in animals, while drugs which decrease NE levels cause depression in humans, and produce behavioral depression in animals. Recent data indicate that electroconvulsive shock therapy, an effective treatment for 'endogenous' depression, produces increased TH activity in the LC of rats, as well as in LC terminal fields, while no change in TH was found in DAminergic brain areas (Masserano, Takimoto, & Weiner, 1981). Thus high TH in LC is associated with recovery from depression. The naturalistic 'rat colony' which contains animals with both high and low TH in LC, plus concomitant behavioral characteristics, provides an unique setting in which to investigate the neuropharmacology of behavioral depression and arousal.

Appendix A -- Locus Coeruleus

The locus coeruleus is a small noradrenergic nucleus located in the dorsolateral pontine tegmentum, bilaterally lateral to the IV ventricle, and bounded by n. mesencephalic V nerve laterally, and the superior cerebellar peduncles dorsolaterally (for detailed reviews of LC anatomy see Grzanna, & Molliver, 1980; van Dongen, 1981; Cedarbaum, & Aghajanian, 1978; Moore, & Bloom, 1979; Cuello, 1978; Swanson, 1976; Lindvall, & Bjorklund, 1978). This nucleus has an analogue or homologue in all mammalian, avian, and reptilian classes. The LC is the largest NE cell group, with 1400-1600 cells, and reaches the most extended target region. Neurons of LC contain TH and DBH (as well as some AChE activity), have an unusual dark pigmentation, and are surrounded by a dense glial and vascular network. There are receptors in LC for numerous neuroactive substances, including NE, 5-HT, Ach, opiate substances, neurotensin, glutamate, and substance P (Usdin, Weiner, & Youdim, 1977; van Dongen, 1981).

LC axons are beaded fibers consisting of 1-2 um intensely fluorescent varicosities separated by thin intervaricose segments. Each axon branches profusely, sending terminals over a wide area. In addition to synaptic contacts, there are large numbers of terminals without synaptic specializations in LC target areas. The whole nucleus extends about 3.5 mm rostocaudally, and there is

morphological evidence from immunocytochemical mapping that cells of the LC can be divided into contiguous but cytologically distinct subgroups (Swanson, 1976; Amaral, & Sinnamon, 1977; Grzanna, & Molliver, 1980: The LC 'proper' (the A6 or "dorsal" area), has tightly packed medium-size cells, and long, thin axons oriented in a rostrocaudal direction. Another group (A4) extends into cerebellum and the roof of the IV ventricle, has spindle-shaped cells and longitudinally oriented dendrites. A ventral group, at the caudal end of the LC proper has long, thin dendrites which branch and radiate far outside the nucleus, as do most of the LC's spiny dendrites. And lastly, there is a rostral group which has large multi-polar cells, and few dendrites.

Does each subgroup have different afferent and efferent connections? The differences in axonal and dendritic morphology suggest possible different patterns of convergence and integration of input as well as different patterns of outgoing information. Also, there are recurrent collateral axons in the LC which can modulate adjacent cells, providing intra- as well as intergroup communication.

The initial delineation of the LC and its projections was due to the work of Dahlstrom and Fuxe (1964) and Ungerstedt (1971). At this time it was thought that because of its profusely branching axons, every LC cell innervated all terminal regions, so that the same neuron innervated cortex, hippocampus, amygdala, cerebellum, spinal cord, and a host of other areas. Recently, more sensitive techniques

(see Lindvall, & Bjorklund, 1978; Cedarbaum, & Aghajanian, 1978; Clark, 1979) have revealed a more complex innervation and specific pathways. For example, only cells in the ventral LC project to spinal cord, while all of LC except the ventral tip innervates the hippocampus. Hypothalamus, septum, amygdala, thalamus, and cortex each form a different pattern of labelling. This certainly suggests that all LC cells do not project to all terminal areas, but rather that some cells project to many regions while others project to limited areas. There also appears to be a general scheme of topographic innervation, i.e., caudal cells project to brain stem, those cells in the middle of LC project to the raphe, and those in the dorsal area to hippocampus, cortex, etc.

In general, LC cells send highly branched axons rostrally to innervate wide portions of the higher brain, and caudally down into spinal cord and brain stem, and also into cerebellum. Efferent projections may be summarized as follows: descending pathways to spinal cord, central gray, the raphe system, solitary nucleus, dorsal motor n. vagus, and other brainstem nuclei, as well as other NE cells groups A1 and 2, A5, and A7. There is a separate tract to cerebellum where fibers terminate on the Purkinje cells. Ascending efferent terminals include the anterior, ventral, and lateral thalamic nuclei, the geniculate bodies and colliculi, hypothalamus (although this area receives 75% of its innervation from ventral NE cell groups), median eminence, area postrema, and contralateral LC cells as well

as ipsilateral LC. Terminals are also found on small blood vessels, neurosecretory fibers, and ependymal cells, and some NE released in this area may reach the IV ventricle. There is extensive innervation to hippocampus, dentate gyrus and cingulate cortex, plus an extensive but more diffuse projection to anterior and frontal cortex, with terminals found mostly in the molecular layer. The LC also sends efferent projections to primarily dopaminergic limbic areas: septum, amygdala, piriform cortex, striatum, and olfactory tubercles.

The afferent projections to LC have been less well delineated but include; spinal cord, A1 through A5 NE cell groups, solitary nucleus, brainstem reticular nuclei, central gray and pontine nuclei, the ipsi- and contralateral LC, and the raphe nuclei. There is an afferent projection from cerebellar nuclei. Afferents have also been found from amygdala, stria terminalis, hypothalamic nuclei, thalamic nuclei, and substantia nigra.

Several things may be noted about these projections to and from the LC: there is a great deal of reciprocal innervation within the LC itself, with other NE cells groups and brainstem nuclei, and with the raphe system. Four days after lesioning in the dorsal and central raphe, TH activity in LC was elevated 30% and 82%, respectively (Lewis et al., 1976) providing neurochemical evidence that LC has a direct relationship with the serotonergic neurons of the raphe system. The afferents to the LC seem to bring sensory

information as well as 'feedback' information from other brain areas. The efferents from LC project virtually everywhere but seem poorly designed to carry very discrete information because of their profuse branching and extensive projections, but would be very well-designed to activate the brain in some general way, perhaps mediating a state of emotional tone and/or arousal, providing the animal with a flexible and appropriate system for assessing and responding to the environment.

The question of the LC's function is complex, but it is probably not specific to a particular behavior. A review of the literature suggests that no consistent pattern of change results from lesions in the LC, and no predictable syndrome appears after LC destruction (Clark, 1979; Amaral, & Sinnamon, 1977) in contrast to, for example, the hyperphagia resulting from lesions in the area of the ventromedial hypothalamus.

A review of the learning literature indicates that the LC is not necessary for the acquisition of simple learning and does not mediate a general positive reinforcement system as was previously suggested (Mason, 1979; Mason, 1981; Mason, & Fibiger, 1979). Stimulation of LC elicits ICSS but is not the sole system operating, nor is the integrity of the LC necessary for ICSS to appear. In addition, animals with LC lesions have been found to be impaired, improved, and normal in the acquisition of learned behavior (Amaral, & Sinnamon, 1977). However, lesions made <u>after</u> learning

extend the time-course of labile memory, suggesting that the LC normally delimits the labile stage of memory (Zornetzer et al., 1978). Also, LC lesioned animals do have impaired ability to extinguish a response after reward has ceased (McNaughton, & Mason, 1980), and they fail to ignore irrelevant stimuli (Davis et al., 1977).

Instead of being involved in discrete, specific behaviors, the LC may have a modulatory role with wide-ranging influence, whose loss might only become apparent under extreme conditions like stress. Since the LC is activated during stress, and inhibits its terminal areas, such as hypothalamus and cortex which are also activated during stress, the LC might function to dampen an organisms response to stress. How might this work hypothetically? In cortical areas, NE from diffuse, unspecialized LC axons would lower the ongoing 'noise', allowing relevant afferent information to be processed. In brain areas which respond quickly to stress, the LC could dampen activity quickly, through conventional synapses, thus preventing a hasty behavioral response. At the same time the LC projections to brainstem nuclei might decrease the transmission of somatosensory information, while the projection to lateral geniculate could allow an increased transmission of visual information. This filtering and gating process would allow the animal to adapt the 'signal-to-noise' ratio to an appropriate balance, depending on various conditions.

There is experimental evidence supporting this

hypothesis: the ongoing activity in monkey auditory and somatosensory cortex is suppressed by NE from the LC, while the responses to species-specific vocalization and to tactile stimuli remained intact or were enhanced (Foote et al., 1975). This suggests that during an LC-induced reduction in ongoing activity the response of terminal areas to other stimuli remain intact, resulting in an increased signal-to-noise ratio. In hippocampus, spontaneous firing which was inhibited by LC stimulation or a loud tone, changed to an excititory response when the tone signalled food (Segal, & Bloom, 1976), and lesions placed in LC produce a decrease in magnitude of the acoustic startle response in rats (Davis et al., 1977) -- all suggesting a change in the signal-to-noise ratio.

Appendix B -- Tyrosine Hydroxylase

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, was first described in 1964 (Nagatsu, Levitt, & Udenfriend), and purified for antisera preparation in 1973 (Joh et al.). The enzyme is an oxidase which converts tyrosine to L-dihydroxyphenylalanine (L-DOPA) by addition of a hydroxyl group. TH oxidizes only the naturally occuring amino acid L-tyrosine, shows a high degree of substrate specificity, and requires molecular oxygen plus a reduced pteridine cofactor. The activity (Vmax) of TH is much lower than that of DOPA decarboxylase or dopamine-B-hydroxylase (DBH), the next two enzymes in the biosynthetic pathway.

Various techniques have been developed to identify and localize neuroactive substances. Particularly relevant to the study of TH have been fluorescence histochemistry and immunocytochemistry, whereby a virtually complete picture of the cellular distribution of the monoamine-containing neurons in the CNS is now available (Falck, Hillarp, Thieme, & Torp, 1962; Dahlstrom, & Fuxe, 1964; Hokfelt, Johansson, Fuxe, Goldstein, & Park, 1976, 1977; Lindvall, & Bjorklund, 1978; Cuello, 1978). Immunohistochemical and immunoenzymatic techniques in which specific antibodies to various substances bind chemically or immunologically to these substances, are much more precise than the classical histofluorescence techniques and allow a very sensitive

detection of TH in CA cells (Pickel et al., 1975; Hokfelt et al., 1976,1977). These experimental approaches combined with electron autoradiography, studies using neurotoxins such as 6-OHDA (Acheson, Zigmond, & Stricker, 1980) synthesis inhibitors such as AMPT (Jouvet, 1977), as well as radioenzymatic assays (Reis et al., 1975; also see Method section) have yielded a detailed account of the localization, distribution, and activity of TH.

This discussion will be limited to TH in CNS, although it is also found in adrenal medulla and all sympathetic NS tissue. In brain, the enzyme is associated with the synaptosomal area, and has been studied in its soluble form, as the membrane-bound form represents only a small fraction of overall TH activity (Acheson et al., 1981).

As the rate-limiting enzyme in CA biosynthesis, TH is found in all dopaminergic (DA) and noradrenergic (NE) neurons. In general, TH is localized in neuronal processes, particularly in structures resembling neurotubules, which probably provide a mechanism for transport of TH out of the cell body where it is synthesized (Pickel et al., 1975). In NE neurons such as those in the locus coeruleus (LC), TH is localized in the proximal portion of axons, and in dendrites, while in DA neurons fluorescence is very intense and distributed throughout the axons and up to synaptic terminals.

TH is present in neuronal systems with the distribution of known DA and NE fiber systems and cell bodies (see

Lindvall, & Bjorklund, 1978; Dahlstrom, & Fuxe, 1964). Thus there is a widespread occurrence of TH-positive neuron systems in spinal cord, brain stem nuclei, mesencephalon, basal ganglia, substantia nigra, hypothalamus, thalamus -- in the diencephalon, with a dense innervation in caudate, n. accumbens, olfactory tubercles, septum and hippocampus -- and in telencephalon, mainly confined to subcortical and limbic cortical structures such as amygdala, entorhinal cortex, cingulate cortex, and prefrontal cortex. TH-positive cells are found in all CA groups, A1 through A14, with particularly dense innervation in A6 and A4, the LC (see Hokfelt 1976 and 1977 for review). Although the LC represents only 3% of the weight of the entire brain stem, it contains 23% of the TH activity there, twice as much as in hypothalamus, and 20 times more than in hippocampus (Zigmond et al., 1974.)

What mechanisms regulate the amount and activity of TH? For schematic purposes, TH can be said to controlled by three mechanisms: 1) <u>availability</u> of substrate and cofactors, 2) inhibition or stimulation of enzyme <u>activity</u> through changes in surrounding neuronal activity, and 3) <u>induction,</u> or increased synthesis of new enzyme molecules resulting from long-term or severe changes in neuronal activity.

Under normal physiological conditions, TH is saturated with tyrosine, but under unusual conditions such as hypo- and hypertension addition of tyrosine to the diet will

increase or decrease blood pressure, respectively (Conlay, Maher, & Wurtman, 1981), presumably by affecting the saturation of TH. Addition of cofactor will increase TH activity, and increasing cyclic AMP-dependent protein phosphorylation of TH increases the enzyme's affinity for both substrate and cofactors (Schwartz, 1981).

Early studies demonstrated that TH activity can be inhibited by feedback or end-product inhibition by NE and DA, which probably act by competing with binding of the oxidized cofactor (Udenfriend, 1962). In addition, stimulation of CA fiber systems was found to result in increased NE synthesis due to enhanced activity of TH (Morgenroth, Boadle-Biber, & Roth, 1974). A model emerged which suggested that stimulation of NE systems results in NE being released and metabolized, thereby depleting a functional pool of NE and thus removing end-product inhibition on TH, which would allow for increased activation of the enzyme. This short-term activation could compensate for any rapid changes in transmitter concentration. Correspondingly, during quiescent periods when NE accumulates and end-product inhibition is functioning, TH activity is decreased.

These short-term mechanisms which control for moderate variation in functional activity involve the activation of pre-existing TH molecules:-stimulation of CA systems results in an activation of TH which is maximal after 10 mins. of stimulation, and persists after the stimulation has ended

(see Cooper, Bloom, & Roth, 1978). Kinetic studies indicate that this activation is mediated by an increased affinity of TH for tyrosine and cofactor, and a decreased affinity for end-product inhibitors. The molecular mechanism responsible for the changes in TH affinity is cAMP phosphorylation, which is increased during neuronal activity. The short-term mechanisms regulating TH activity are rapidly reversible and occur within minutes.

However, most recent studies of activation and induction of TH refer to long-term mechanisms for control of synthesis which come into play under stressful conditions which result in intense sympathetic activity, or during prolonged increases in neuronal activity. These processes have been studied extensively by Joh and co-workers, as well as others (Lewis et al., 1976; Reis et al., 1975; Lewander et al., 1977; Renaud et al, 1979a and b), and may be separated into those employing 'delayed activation' of TH molecules, and those resulting in induction of new enzyme molecules.

Delayed activation occurs after 24-48 hours, may persist for several weeks, and appears to be initiated by stimulation of central cholinergic receptors (Renaud 1979b; Lewander et al., 1977). The increase in enzyme activity is due to enhanced catalytic activity of enzyme molecules without change in number. Delayed activation has been observed in LC, A1 and A2 cell groups, n. tractus solitarius (NTS), and is presumed to exist in all noradrenergic

systems. In brain, delayed activation is confined to NE systems, and is also specific to TH, as DBH is not similarly affected. The molecular mechanisms responsible are unclear, but the cholinergic stimulation may initiate removal of a noncompetitive inhibitor, or accumulation of a different molecular species of TH with greater affinity properties. The latency and protracted course of delayed activation suggest that it functions as a long-term regulatory mechanism for changes in tyrosine hydroxylation which promote availability of transmitter.

The other long-term mechanism regulating TH is induction: the synthesis of new enzyme. The existence of new enzyme is shown immunologically in the CNS with antiserum prepared against purified TH. Results of various studies have shown that treatment with reserpine, electric shock, and cold stress, increase TH in the cell bodies of LC, A1 and A2, with the response in LC being 4-5 times greater than in the other NE cell groups (Zigmond et al., 1974; Renaud et al., 1979b; Weiss et al., 1982). Induction is also observed in NE terminal areas such as NTS, hypothalamus, hippocampus, frontal cortex and cerebellum. However, there is a delayed increase in TH in terminal fields due to the rate of axoplasmic flow: induction will be evident after hours in LC (maximal response by 48-72 hours and recovered by 3 weeks), but may take many days until seen in nerve terminals (Reis et al., 1975). There is also a smaller magnitude of response in terminals as compared to

cell bodies. The reason for this is unclear -- possibly the quantity of TH in one LC neuron is diluted by the wide extent of it's terminal fields, or new enzyme is suppressed by regulatory mechanisms at terminal sites such as end-product inhibition and availability of cofactors, or the TH degrades over time (Reis et al., 1975; Boarder, & Fillenz, 1979).

Other proteins like MAO and decarboxylases are not subject to induction, which like delayed activation is specific to NE neurons. Unlike with activation however, DBH in the LC is induced by reserpine, with the response being small and more variable than that of TH (Reis et al., 1975). The nature of the molecular mechanisms initiating induction of TH and DBH is unknown. Possibly the reduction of intracellular levels of NE in terminals signals the LC cell body to synthesize new enzyme. Studies of the time course for depletion of NE and elevation of TH show them to be virtually identical (Reis et al., 1975).

There is no evidence that delayed activation or induction occur in DA neurons; these capabilities appear restricted to NE systems. This emphasizes the fact that the TH in NE and DA cells is different. This difference includes molecular weights and responses to immunochemical staining; TH in DA neurons may be maximal under normal conditions, may be more labile, or may simply be regulated differently (Joh, & Reis, 1975). Although DA neurons respond to short-term stimulation with an activation of TH

and removal of end-product inhibition as do NE neurons, their response to cessation of stimulation is different. During quiescent periods, DA neurons will rapidly increase synthesis despite expanding supply of transmitter (Roth, Salzman, & Nowycky, 1978). Apparently, the TH is not sensitive to inhibition in these neurons until a particular level of DA is reached which reinstates end-product inhibition. These data again suggest a difference in the physical properties of dopaminergic and noradrenergic TH.

This brief review suggests several characteristics of TH which make this enzyme suitable as a mediator of dominance-related behavior. For the NE system, long-term mechanisms like delayed activation and induction suggest means by which long-range adaptation to increased transmitter demand would be possible. The short-term activation of TH would allow an animal to compensate quickly for episodes of acute stress. Another form of plasticity is suggested by recent studies in which intraventricular injections of 6-OHDA resulted in a delayed increase in TH activity in cell bodies of LC, followed by an increase in TH activity and NE synthesis in terminal fields of hippocampus, which was still present after 11 weeks (Acheson et al., 1980). This appears to be a relatively permanent adaptation of an NE system in response to injury. By contrast, the cholinergic system shows no changes in amount or activity of enzyme in response to utilization of the pathways, and synthesis is dependent largely on availability of substrate alone.

Appendix C

Computer-generated efficiency scores (CES)

Fig. 6 shows the formula for calculation of an efficiency score for behavior. The computer program (Appendix C) calculates efficiency scores from this formula for each animal in broadsiding, wrestling, chasing, and fighting behavior, using raw observational data. An average efficiency score (CES) is calculated by pooling behavioral categories*. These CES's are then ranked, yielding a dominance hierarchy.

$$\frac{\#\text{ of Total Wins} \times \text{Activity Rank} \times (2^{\text{Wins Rank}})}{(2^{\text{Activity Rank}}) \times \#\text{ of Total Activity} \times \text{Wins Rank}}$$

Fig. 6. Formula for calculation of efficiency scores, where
# of Total Wins = the total number of an animal's wins in a category i.e., fighting.
# of Total Activity = the total number of an animal's encounters in a category.
Wins Rank = an animal's rank regarding total wins in a category.
Activity Rank = an animal's rank regarding total encounters in a category.

*Boxing behavior was included in the CES, since subordinates do not box, by giving 1 activity point and 1/2 a win point for each boxing bout to each participant. In addition, every animal received 1/4 'free win' point in order to keep all individuals in the calculations.

The formula incorporates the following assumptions and was tailored to 'best fit' the four sets of colony observations: efficiency of behavior is characteristic of dominance, so animals must be ranked according to both activity (Activity rank) and wins (Wins rank) and these compared. For example, rat 'A' may have a total activity score of 18 of which only 5 are wins, while rat 'B' has a total activity score of 10 of which 5 are wins. A comparison yields 5/18 = .26 for 'A' and 5/10 = .50 for 'B', with rat 'B' having the higher score, signifying efficiency. These scores are then ranked, but ranking includes an exponential component because a dominance hierarchy is not linear. An exponential value allows a meaningful comparison between animals since there is an important separation between the alpha and beta animals in a hierarchy, which does not exist between two middle range animals. A win for the alpha may be 'equivalent' to many wins for a middle animal in terms of the effect this win produces on the subsequent behavior of other animals in the colony. The exponential function puts numerical weight on activity at the top of the hierarchy, which accurately reflects the reality of rodent social interaction.

The CES's shown in TABLE 6 indicate the non-linear nature of a dominance hierarchy, with the alpha rat (#1) having a much larger score than the beta (#2), who in turn is far ahead of the middle range animals, who do not differ too much from each other. The correlation coefficient (rho)

comparing the computer ranks with the dominance hierarchy is .89 ($p < .01$).

The computer-ranking program was validated against observer-ranking of four independent colonies: correlations (rho) were 1.00, .80, .90, and .90 ($p < .01$).

TABLE 6

Dominance rank and CES rank for colony A

Observer Dominance Ranks	CES Score	Computer Ranks
1 (Alpha)	1395	1
2	216	2
3.5	67	3
3.5	44	4
5	13	6
6	1	8.5
7	14	6
8	14	6
9	1	8.5

Detailed description and categorization of rodent social behavior has developed from the initial classifications by Grant and co-workers (Grant, & Chance, 1958; Grant 1963; Grant, & Mackintosh, 1963) to videotaped

and computer-assisted analysis of social interaction (Crawley, Pryor, Creveling, & Bernard, 1982). This program differs from previous analyses of dominance ranking in rodents (Baenninger, 1966; Grant, & Chance, 1958; Lehman, & Adams, 1977). These studies, concerned with the stability of dominance orders have yielded conflicting results, due in part, to the different aspects of behavior observed: spontaneous social behavior (Grant, 1963), competitive fighting for possession of food (Zook, & Adams, 1975), or territorial aggression (Blanchard et al., 1977). Another source of error has been the methods by which dominance is determined. Grant (1958; 1963; 1963) used the relative magnitude of an animal's ratio of wins to losses (wins/losses) to determine position in the hierarchy. Other investigators have used similar formulas such as wins/losses x encounters (Baenninger, 1966; Blanchard, & Blanchard, 1977), or Chi-square paradigms that determine which interactions between animals are not occurring at chance level of frequency (Lehman, & Adams, 1977). During observation it is apparent that the alpha rat does not fight equally with all animals, but that his attacks are directed towards betas and toward the lowest animal. Thus his behavior is generally selective and efficient, i.e. at the top of the hierarchy one encounter goes a long way. The present program incorporates this selectivity and efficiency dimension of rodent dominance behavior by analyzing the ratio of wins to activity in a complex manner using an

exponential function, which recognizes the fact that dominance ranks are not linear.

A formula for calculating dominance from raw observational data has advantages of standardization and elimination of experimenter bias. It is particularly useful in controlling for the fact that prior knowledge of early dominance may influence an observer. However, inspection of the correlation matrix in Appendix D indicates that the CES hierarchy did not correlate as highly with TH as did the observer-ranked hierarchy. Possible reasons for this discrepancy are: 1) the CES formula was not able to identify the lowest ranking animal, whose identity is evident from observation; 2) There are factors which the CES does not take into account, such as a particular behavior which is favored by a particular animal. For example, observation suggests that fighting is always a dominance-related activity, while wrestling is not. It appears that although such subjective factors influence the observer's ranking, they do not reverse the CES ranking: the CES correlations with TH activity are lower than, not opposite from, the observer-ranked correlations with TH.

APPENDIX C

```
100 REM INPUT INFO INTO DATA-RATS
120 REM COPYRIGHT SAM MAGEE 1981 ALL RIGHTS RESERVED
130 PRINTCHR$(12)
140 DIM A$(7:30),R(10,10,7,2),A(7,2),C1$(1:4),C2$(1:4)
150 DATA "Broadsiding",1,1,"Boxing",1,0,"Wrestling",1,1,"Chase",1
160 DATA "Wheel Running",0,0,"Approaching Observer",0,0
170 FOR X=1 TO 7\READ A$(X),A(X,1),A(X,2)\NEXT X
175 FILE:4,OPEN,"DATA-RATS",INOUT
176 PRINT"Reading File...",
177 MAT INPUT:4,R
178 F1=0\REM FILE CHANGED FLAG
180 ON ESCAPE EXIT 190
190 ON ERROR EXIT 190
200 PRINTCHR$(12)\PRINT"ENTER or CHANGE BASIC DATA"
210 PRINT
212 PRINT"ACTIVITIES..."
220 PRINT\PRINTTAB(10)," 0. Exit"
230 FOR X=1 TO 7
240 PRINTTAB(10),%2I,X,". ",A$(X)
250 NEXT X
260 PRINT
270 INPUT"Your Selection : ",B
275 PRINTCHR$(12)
280 IF B=0 THEN 600
290 IF B<1 OR B>7 THEN 190
300 REM B=ACTIVITY #
301 ON ERROR GOTO 301
302 PRINTCHR$(12)
303 INPUT"Start at RAT # (1-10) : ",X5
304 IF X5<1 OR X5>10 THEN 301
305 PRINTCHR$(12)
308 A1=A(B,1)\A2=A(B,2)\REM ATTRIBUTES OF ACTIVITY
310 ON ERROR GOTO 310
312 FOR X9=X5 TO 10\REM FIRST RAT LOOP
315 IF A1<>0 THEN 380
320 PRINTCHR$(12)\PRINT"Enter Data for '",A$(B),"'"
330 PRINT
340 PRINT"RAT # : ",%2I,X9\PRINT
350 C2,C1=R(X9,1,B,1)\IF C1=0 THEN C1$="" ELSE C1$=STR$(C1,%#4I)
364 PRINT"Number of observations : ",
365 Z=CALL("FUN$",6,MEM(C1$),4,0)\PRINT
366 IF C1$="" THEN C1$="0"
367 C1=VAL(C1$)
368 IF C1<0 THEN C1$=""\GOTO 364
369 IF C1<>C2 THEN F1=1
370 R(X9,1,B,1)=C1\GOTO 510
380 REM SECOND RAT LOOP
390 FOR X8=1 TO 10
395 IF X8=X9 THEN 500
400 PRINTCHR$(12)\PRINT"Enter Data for '",A$(B),"'"
403 PRINT\PRINT"RAT # ",%2I,X9," VERSUS RAT # ",%2I,X8
405 C1,C2=R(X9,X8,B,1)
410 IF A2<>0 THEN 430
412 IF C1=0 THEN C1$="" ELSE C1$=STR$(C1,%#4I)
415 PRINT\PRINT"Number of Observations : ",
420 Z=CALL("FUN$",6,MEM(C1$),4,0)\PRINT
422 IF C1$="" THEN C1$="0"
425 C1=VAL(C1$)
427 IF C1<0 THEN C1$=""\GOTO 415
428 IF C1<>C2 THEN F1=1
429 R(X9,X8,B,1)=C1\GOTO 500
```

```
440 C1,C2=R(X9,X8,B,1)
450 IF C1=0 THEN C1$="" ELSE C1$=STR$(C1,%#4I)
455 PRINT\PRINT"No. of WINS for RAT # ",%2I,X9," : ",
460 Z=CALL("FUN$",6,MEM(C1$),4,0)\PRINT
465 IF C1$="" THEN C1$="0"
470 C1=VAL(C1$)
471 IF C1<0 THEN C1$=""\GOTO 455
472 IF C1<>C2 THEN F1=1
473 R(X9,X8,B,1)=C1
475 C1,C2=R(X9,X8,B,2)\IF C1=0 THEN C1$="" ELSE C1$=STR$(C1,%#4I)
480 PRINT\PRINT"No. of LOSSES for RAT # ",%2I,X9," : ",
482 Z=CALL("FUN$",6,MEM(C1$),4,0)\PRINT
485 IF C1$="" THEN C1$="0"
487 C1=VAL(C1$)
488 IF C1<>C2 THEN F1=1
489 IF C1<0 THEN C1$=""\GOTO 480
490 R(X9,X8,B,2)=C1
500 NEXT X8
510 NEXT X9
550 GOTO 190
600 PRINTCHR$(12)
605 IF F1=0 THEN 650
610 PRINT"Writing to file...",
620 FILE:4,REW
625 PRINT %#4I
630 MAT PRINT:4,R
640 FILE:4,CLOSE
650 LINK"PROG<MENU"
```

```
100 REM 12 PLOT FROM DATA-RATS
120 REM
140 REM COPYRIGHT SAM MAGEE 1981 ALL RIGHTS RESERVED
160 PRINTCHR$(12)
180 DIM R(10,10,7,2),D4(10),D5(10),R2(10),R3(10),D8(10),W4(10)
200 DIM A$(7:30),A(7,2),C1$(1:4),C2$(1:1),C$(1:1),S2(10),S3(10)
210 DIM R4(10),R5(10),W5(10)
220 DATA "Broadsiding",1,1,"Boxing",1,0,"Wrestling",1,1,"Chase",1
240 DATA "Wheel Running",0,0,"Approaching Observer",0,0
260 FOR X=1 TO 7\READ A$(X),A(X,1),A(X,2)\NEXT X
270 INPUT"WHICH DATA SET (1-4) : ",J7
280 FILE:4,OPEN,"RATS"+STR$(J7,%#1I),INPUT
300 INPUT"DO YOU WANT FULL GRAPHS ? ",C$
320 PRINTCHR$(12)\G5=10
340 FILE:2,LIST
360 PRINT"Reading Data...",
380 MAT INPUT:4,R
400 PRINT\PRINT"Counting...",
420 GOSUB 1980\REM FILL D4 AND W4
440 GOSUB 1600\REM GET RANKS INTO R2(n)&R3 FROM D4(n)
450 GOSUB 2300\REM GET RANKS INTO R4(n)&R5 FROM W4(n)
460 REM   OUTSIDE LOOP FOR VALUE CRUNCHING
480 FOR E1=1 TO 1
500 REM PRINT:2\PRINT:2,"VAR=",%6F3,E1\PRINT:2
520 PRINT\PRINT"Printing...",
540 FOR X9=1 TO 10\C8,C3=0
560 IF C$<>"Y" THEN 680
580 PRINT:2,CHR$(12)
600 PRINT:2,"Efficiency Rating...  TOTAL ACTIVITY : ",%4I,D4(X9),'
620 PRINT:2,"GRAPH # AAA"
640 PRINT:2
660 PRINT:2,"RAT # ",%2I,X9\PRINT:2
680 C7=0\FOR B=1 TO 7\REM ACTIVITY LOOP
700 IF A(B,2)=0 AND A(B,1)=0 THEN 1120
720 REM CALC TOTAL OCCURENCES
740 C6=0
760 FOR X8=1 TO 10\REM SECOND RAT LOOP
780 IF X8=X9 THEN 820
800 C6=C6+R(X9,X8,B,1)\REM WINS
820 NEXT X8
830 IF R5(X9)=0 THEN R5(X9)=1
840 IF B=2 THEN C6=INT(C6/2)\REM 1/2 POINT FOR BOXING
860 IF C6<2 THEN C6=.2\REM SMOOTH OUT ONE-TIMERS
880 IF D4(X9)=0 THEN C4=0\GOTO 940
900 K6=2^R3(X9)/(D4(X9)*R3(X9))\REM ****
920 C4=(C6/(K6+1)*100)/R5(X9)\REM CONSTANTS FOR LOWSIDE SMOOTHING
930 C4=INT(C4)
940 IF C$<>"Y" THEN 1000
960 FOR D2=1 TO 80\PRINT:2,"-",\NEXT D2
980 PRINT:2\PRINT:2,A$(B)," (",%6I,C4,")",TAB(30),
1000 C8=C8+C4\C7=C7+C6
1020 IF C$<>"Y" THEN 1120
1040 FOR D1=1 TO INT(C4/8)
1060 PRINT:2,"*",
1080 NEXT D1
1100 PRINT:2
1120 NEXT B
1140 S2(X9)=INT(C8/5)\REM SORT LIST
1160 IF C$<>"Y" THEN PRINT:2,%2I,X9,%6I,INT(C7),%6I,INT(C8/5),%4I
1180 FOR D2=1 TO 80\PRINT:2,"-",\NEXT D2\PRINT:2
1200 PRINT:2,TAB(30),"0",TAB(76),"400"
```

```
1240 C8=0
1260 NEXT X9
1280 MAT S3=0
1300 FOR T2=1 TO 10\K9,K0=0
1320 FOR T3=1 TO 10
1340 IF S2(T3)>K9 THEN K9=S2(T3)\K8=T3\K0=1
1360 NEXT T3
1380 IF K0=1 THEN S3(T2)=K8\S2(K8)=0
1400 NEXT T2
1420 MAT S2=0
1440 PRINT#2,%#4I\MAT PRINT#2,S3
1460 PRINT#2
1480 NEXT E1\REM CRUNCH LOOP
1500 FILE#4,CLOSE
1520 PRINTCHR$(12)
1540 LINK"PROG<MENU"
1560 REM SORT ACTIVITY RANKING
1580 REM LOW TO HIGH
1600 PRINT\PRINT"Sorting...",\I1=1\MAT D5=D4\MAT R2=0
1620 REM REMOVE DEAD RATS
1640 FOR L0=1 TO 10
1660 IF D5(L0)=0 THEN D5(L0)=9999
1680 NEXT L0
1700 K1=9999\REM GET LOWEST NUMBER POSITION (1-10)
1720 FOR L2=1 TO 10
1740 IF D5(L2)<K1 THEN K1=D5(L2)\K2=L2
1760 NEXT L2
1780 R2(I1)=K2\D5(K2)=9999
1800 I1=I1+1
1820 IF I1<11 THEN 1700
1840 REM SECOND SORT
1860 FOR L3=1 TO 10
1880 FOR L4=1 TO 10
1900 IF R2(L4)=L3 THEN R3(L3)=L4\L4=11
1920 NEXT L4\NEXT L3
1940 RETURN
1960 REM CALCULATE TOTAL ACT. NUMBERS IN D4(n) WINNERS IN W4(n)
1980 MAT D4,W4=0
2000 FOR J=1 TO 10
2020 FOR K=1 TO 10\IF K=J THEN 2100
2040 FOR L=1 TO 7
2042 IF (A(L,1)=0 AND A(L,2)=0) THEN 2060
2043 IF L=2 THEN U2=.5 ELSE U2=1\REM 1/2 BOXING
2045 W4(J)=W4(J)+R(J,K,L,1)*U2\REM WINNERS
2060 D4(J)=D4(J)+R(J,K,L,1)+R(J,K,L,2)\REM TOTALS
2080 NEXT L
2100 NEXT K
2120 NEXT J
2140 RETURN
2300 REM LOW TO HIGH
2310 PRINT\PRINT"Sorting...",\I2=1\MAT W5=W4\MAT R4=0
2360 K1=0\REM GET HIGHEST NUMBER POSITION (1-10) INTO R4
2370 FOR L2=1 TO 10
2380 IF W5(L2)>K1 THEN K1=W5(L2)\K2=L2
2390 NEXT L2
2400 R4(I2)=K2\W5(K2)=0
2420 I2=I2+1
2440 IF I2<11 THEN 2360
2460 REM SECOND SORT
2480 FOR L3=1 TO 10
2500 FOR L4=1 TO 10
2520 IF R4(L4)=L3 THEN R5(L3)=L4\L4=11
2540 NEXT L4\NEXT L3
2560 RETURN
```

```
100 REM 12 PLOT FROM DATA-RATS
120 REM
140 REM COPYRIGHT SAM MAGEE 1981 ALL RIGHTS RESERVED
160 PRINTCHR$(12)
180 DIM R(10,10,7,2),D4(10),D5(10),R2(10),R3(10),D8(10),W4(10)
200 DIM A$(7:30),A(7,2),C1$(1:4),C2$(1:1),C$(1:1),S2(10),S3(10)
210 DIM R4(10),R5(10),W5(10)
220 DATA "Broadsiding",1,1,"Boxing",1,0,"Wrestling",1,1,"Chase",1
240 DATA "Wheel Running",0,0,"Approaching Observer",0,0
260 FOR X=1 TO 7\READ A$(X),A(X,1),A(X,2)\NEXT X
270 INPUT"WHICH DATA SET (1-4) : ",J7
280 FILE:4,OPEN,"RATS"+STR$(J7,%#1I),INPUT
300 INPUT"DO YOU WANT FULL GRAPHS ? ",C$
320 PRINTCHR$(12)\G5=10
340 FILE:2,LIST
360 PRINT"Reading Data...",
380 MAT INPUT:4,R
400 PRINT\PRINT"Counting...",
420 GOSUB 1980\REM FILL D4 AND W4
440 GOSUB 1600\REM GET RANKS INTO R2(n)&R3 FROM D4(n)
450 GOSUB 2300\REM GET RANKS INTO R4(n)&R5 FROM W4(n)
460 REM   OUTSIDE LOOP FOR VALUE CRUNCHING
480 FOR E1=1 TO 1
500 REM PRINT:2\PRINT:2,"VAR=",%6F3,E1\PRINT:2
520 PRINT\PRINT"Printing...",
540 FOR X9=1 TO 10\C8,C3=0
560 IF C$<>"Y" THEN 680
580 PRINT:2,CHR$(12)
600 PRINT:2,"Efficiency Rating...  TOTAL ACTIVITY : ",%4I,D4(X9),'
620 PRINT:2,"GRAPH # AAA"
640 PRINT:2
660 PRINT:2,"RAT # ",%2I,X9\PRINT:2
680 C7=0\FOR B=1 TO 7\REM ACTIVITY LOOP
700 IF A(B,2)=0 AND A(B,1)=0 THEN 1120
720 REM CALC TOTAL OCCURENCES
740 C6=0
760 FOR X8=1 TO 10\REM SECOND RAT LOOP
780 IF X8=X9 THEN 820
800 C6=C6+R(X9,X8,B,1)\REM WINS
820 NEXT X8
830 IF R5(X9)=0 THEN R5(X9)=1
840 IF B=2 THEN C6=INT(C6/2)\REM 1/2 POINT FOR BOXING
860 IF C6<2 THEN C6=.2\REM SMOOTH OUT ONE-TIMERS
880 IF D4(X9)=0 THEN C4=0\GOTO 940
900 K6=2^R3(X9)/(D4(X9)*R3(X9))\REM ****
920 C4=(C6/(K6+1)*100)/R5(X9)\REM CONSTANTS FOR LOWSIDE SMOOTHING
930 C4=INT(C4)
940 IF C$<>"Y" THEN 1000
960 FOR D2=1 TO 80\PRINT:2,"-",\NEXT D2
980 PRINT:2\PRINT:2,A$(B)," (",%6I,C4,")",TAB(30),
1000 C8=C8+C4\C7=C7+C6
1020 IF C$<>"Y" THEN 1120
1040 FOR D1=1 TO INT(C4/8)
1060 PRINT:2,"*",
1080 NEXT D1
1100 PRINT:2
1120 NEXT B
1140 S2(X9)=INT(C8/5)\REM SORT LIST
1160 IF C$<>"Y" THEN PRINT:2,%2I,X9,%6I,INT(C7),%6I,INT(C8/5),%4I
1180 FOR D2=1 TO 80\PRINT:2,"-",\NEXT D2\PRINT:2
1200 PRINT:2,TAB(30),"0",TAB(76),"400"
```

REFERENCES

Acheson AL, Zigmond MJ, & Stricker EM. 1980. Compensatory increase in tyrosine hydroxylase activity in rat brain after intraventricular injections of 6-hydroxydopamine. Science 207: 537-539.

Acheson AL, Kapatos G, & Zigmond MJ. 1981. The effects of phosphorylating conditions on tyrosine hydroxylase activity are influenced by assay conditions and brain region. Life Sciences 28: 1407-1420.

Ader JP, Postema F, & Korf J. 1979. Contribution of the locus coeruleus to the adrenergic innervation of the rat spinal cord: a biochemical study. Journal Neural Transmission 44: 159-173.

Aghajanian GK, Cedarbaum JM, & Wang RY. 1977. Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. Brain Research 136: 570-577.

Amaral DG, & Sinnamon HM. 1977. The locus coeruleus: neurobiology of a central noradrenergic nucleus. Progress in Neurobiology 9: 147-196.

Anden NE, & Grabowski M. 1976. Pharmacological evidence for stimulation of dopamine neurons by noradrenaline neurons in the brain. European Journal Pharmacology 39: 275-282.

Anisman H. 1975. Time-dependent variations in aversively motivated behaviors: Nonassociative effects of cholinergic and catecholaminergic activity. Psychological Review 82: 359-385.

Anisman H, Irwin J, & Sklar LS. 1979a. Deficits of escape performance following catecholamine depletion. Psychopharmacology 64: 163-170.

Anisman H, Grimmer L, Irwin J, Remington G, & Sklar LS. 1979b. Escape performance after inescapable shock in selectively bred lines of mice: response maintenance and catecholamine activity. Journal of Comparative and Physiological Psychology 93: 229-241.

Anisman H, & Sklar LS. 1979c. Catecholamine depletion in mice upon reexposure to stress: mediation of the escape deficits produced by inescapable shock. Journal of Comparative and Physiological Psychology 93: 610-625.

Anisman H, Pizzino A, & Sklar LS. 1980. Coping with stress, norepinephrine depletion and escape performance. Brain Research 191: 583-588.

Anisman H, Ritch M, & Sklar LS. 1981. Noradrenergic and dopaminergic interactions in escape behavior: analysis of uncontrollable stress effects. Psychopharmacology 74: 263-268.

Antelman SM, & Caggiula AR. 1977. Norepinephrine-dopamine: interactions and behavior. Science 195: 646-653.

Avis HH. 1974. The neuropharmacology of aggression: a critical review. Psychological Bulletin 81: 47-63.

Bacopoulos NG, & Bhatangar RK. 1977. Correlation between tyrosine hydroxylase activity and catecholamine concentration on turnover in brain regions. Journal of Neurochemistry 29: 639-643.

Baenninger LP. 1966. The reliability of dominance orders in rats. Animal Behavior 14: 367-371.

Barnett SA. 1963. A study in Behavior. The Rat. London, Methuen.

Barr GA, Gibbons JL, & Bridger WH. 1976. Neuropharmacological regulation of mouse killing by rats. Behavioral Biology 17: 143-159.

Barr GA, Sharpless NS, & Gibbons JL. 1979. Differences in the level of dopamine in the hypothalamus of aggressive and non-aggressive rats. Brain Research 166: 211-216.

Bernard BK. 1975. Aggression and the brain monoamines: what are the answers, but of more importance, what are the questions? In Aminergic Hypotheses of Behavior: Reality or Cliche? Natl. Instit. of Drug Abuse, Research Monograph #3, Rockville, Maryland.

Bernard BK, & Paolino RM. 1975. Temporal effects of castration on emotionality and shock-induced aggression in adult male rats. Physiology and Behavior 14: 201-206.

Blanchard RJ, & Blanchard DC. 1977. Aggressive behavior in the rat. Behavioral Biology 21: 197-224.

Blanchard RJ, Blanchard DC, Takahaski T, & Kelley MJ. 1977. Attack and defensive behavior in the albino rat. Animal Behavior 25: 622-634.

Boarder MR, & Fillenz M. 1979. Absence of increased tyrosine hydroxylation after induction of brain tyrosine hydroxylase following reserpine administration. Biochemical Pharmacology 28: 1675-1677.

Bodnar RJ, Ackermann RF, Kelly DD, & Glusman M. 1978. Elevations in nociceptive thresholds following LC lesions. Brain Research Bulletin 3: 125-130.

Bruning JL, & Kintz BL. 1977. Computational Handbook of Statistics, 2nd edition. Scott, Foresman & Co., Glenview, IL.

Bubenick G, & Monnier M. 1972. Nuclear size variations in cells of locus coeruleus during sleep, arousal, and stress. Experimental Neurology 35: 1-12.

Cassens G, Kuruc A, Roffman M, Orsulak PJ, & Schildkraut JJ. 1981. Alterations in brain norepinephrine metabolism and behavior induced by environmental stimuli previously paired with inescapable shock. Behavioral Brain Research 2: 387-407.

Cedarbaum JM, & Aghajanian GK. 1978. Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. Journal of Comparative Neurology 178: 1-16.

Chan OL, & Webster RA. 1971. Effect of tetrabenazine and alpha-methyl-m-tyrosine on exploratory activity and brain catecholamines in rats. British Journal of Pharmacology 41: 691-699.

Ciaranello RD, Lipsky A, & Axelrod J. 1974. Association between fighting behavior and catecholamine biosynthetic enzyme activity in two inbred mouse sublines. Proceedings of the National Academy of Sciences USA 71: 8, 3006-3008.

Clark TK. 1979. The locus coeruleus in behavior regulation: evidence for behavior-specific versus general involvement. Behavioral Neurology & Biology 25: 271-300.

Clemente CD, & Chase MH. 1973. Neurological substrates of aggressive behavior. Annual Review of Physiology 35: 329-356.

Conlay LA, Maher TJ, & Wurtman RJ. 1981. Tyrosine increases blood pressure in hypotensive rats. Science 212: 559-560.

Cooper JR, Bloom FE, & Roth RH. The Biochemical Basis of Neuropharmacology 3rd edition. 1978. Oxford University Press, NY.

Coyle JT. 1972. Tyrosine hydroxylase in rat brain -- cofactor requirements, regional and subcellular distribution. Biochemical Pharmacology 21: 1935-1944.

Crawley JN, Szara S, Pryor GT, Creveling CR, & Bernard BK. 1982. Development and evaluation of a computer-automated color TV tracking system for automatic recording of the social and exploratory behavior of small animals. Journal of Neuroscience Methods 5: 235-247.

Crow TJ, Deakin JFW, File SE, Longden A, & Wendlandt S. 1978. The locus coeruleus noradrenergic system -- evidence against a role in attention, habituation, anxiety and motor activity. Brain Research 155: 249-261.

Cuello, AC. 1978. Immunocytochemical studies of the distribution of neurotransmitters and related substances in CNS. In Handbook of Psychopharmacology, Vol. 9. eds. LL Iversen, DS Iversen, SH Snyder. Plenum Press, NY.

Dahlstrom A, & Fuxe K. 1964. Evidence for the existence of monoamine containing neurons in the central nervous system. Acta. Physiology Scandinavia 62: Suppl. 232: 1-55.

Davis M, Cedarbaum JM, Aghajanian GK, & Gendelman DS. 1977. Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate and locus coeruleus lesioned rats. Psychopharmacology 51: 243-253.

Diagnostic and Statistical Manual of Mental Disorders Third Edition, 1980, American Psychiatric Assoc., Washington, DC.

Egger MD, & Flynn JP. 1967. Further studies on the effects of amygdaloid stimulation and ablation on hypothalamically elicited attack behavior in cats. In Progress in Brain Research, Vol. 27. eds. WR Adey, Amsterdam, Elsevier.

Eichelman BS Jr., & Thoa NB. 1973. The aggressive monoamines. Biological Psychiatry 6: 143-164.

Eison MS, Stark AD, & Ellison G. 1977. Opposed effects of locuc coeruleus and substantia nigra lesions on social behavior in rat colonies. Pharmacology Biochemistry & Behavior 7: 87-90.

Eleftheriou BE, & Church RL. 1968. Brain levels of serotonin and norepinephrine in mice after exposure to aggression and defeat. Physiology & Behavior 3-977-980.

Ellison G. 1975. Behavior and the balance between norepinephrine and serotonin. Acta. Neurobiology Experimentia 35: 499-515.

Ellison G. 1976. Monoamine neurotoxins: selective and delayed effects on behavior in colonies of laboratory rats. Brain Research 103: 81-92.

Ellison G. 1977. Animal models of psychopathology; the low-norepinephrine and low-serotonin rat. American Psychologist Dec: 1036-1045.

Falck B, Hillarp N A, Thieme G, & Torp A. 1962. Fluorescence of catecholamines and related compounds condensed with formaldehyde. Journal of Histochemical Cytochemistry 10: 348-354.

Feldberg W, & Myers R. 1964. Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. Journal of Physiology (London) 173: 226-237.

File SE, & Vellucci SV. 1979. Behavioral and biochemical measures of stress in hooded rats from different sources. Physiology & Behavior 22: 31-35.

Flynn J, Vanegas H, Foote W, & Edwards S. 1970. Neural mechanisms involved in a cat's attack on a rat. In The Neural Control of Behavior ed. RF Whalen, Academic Press, NY.

Foote SL, Freedman R, & Oliver AP. 1975. Effects of putative neurotransmitters on neuronal activity in monkey auditory cortex. Brain Research 86: 229-242.

Friedman R, & Iwai J. 1976. Genetic predisposition and "stress-induced" hypertension. Science 193: 161-162.

Fuxe K, Hokfelt T, & Ungerstedt U. 1971. Morphological and functional aspects of central monoamine neurons. International Review Neurobiology 13: 93-126.

Geyer MA, Segal DS, & Mandell AJ. 1976. Opposite behavioral effects produced by locus coeruleus and median raphe lesions. Neuroscience Abstracts 2: 488.

Glazer HI, Weiss JM, Pohorecky LA, & Miller NE. 1975. Monoamines as mediators of avoidance-escape behavior. Psychosomatic Medicine 37: 535-543.

Glick SD, & Stanley ME. 1975. Neurochemical correlate of body weight in rats. Brain Research 96: 153-155.

Glowinski J. 1975. Some characteristics of the 'functional" and 'main storage' compartments in central catecholamine neurons. Brain Research 62: 489-493.

Goodman PA, Weiss JM, Hoffman LJ, Ambrose MJ, Bailey WH, and Charry JM. 1982. Reversal of behavioral depression by infusion of an alpha 2 adrenergic agonist into the locus coeruleus. Neuroscience Abstracts 8: 362.

Grant EC, & Chance MRA. 1958. Rank order in caged rats. Animal Behavior 6: 183-194.

Grant EC. 1963. An analysis of the social behavior of the male laboratory rat. Behavior 21: 260-281.

Grant EC, & Mackintosh JH. 1963. A comparison of the social postures of some common laboratory rodents. Behavior 21: 246-259.

Gray JA. 1977. Drug effects of fear and frustration. In Handbook of Psychopharmacology. Vol. 8, (Ed. Iversen), Plenum Press, NY.

Groves PM, & Wilson CJ. 1980. Fine structure of rat locus coeruleus. Journal Comparative Neurology 193: 841-852.

Grzanna R, & Molliver, ME. 1980. The locus coeruleus in the rat: an immunohistochemical delineation. Neuroscience 5: 21-40.

Henry JP, Stephens PM, Axelrod J, & Mueller RA. 1971. Effect of psychosocial stimulation on the enzymes involved in the biosynthesis and metabolism of noradrenaline and adrenaline. Psychosomatic Medicine 33: 3, 227-237.

Hokfelt T, Johansson O, Fuxe K, Goldstein M, & Park D. 1976. Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain. I. Tyrosine hydroxylase in the mes- and diencephalon. Medical Biology 54: 427-453.

Hokfelt T, Johansson O, Fuxe K, Goldstein M, & Park D. 1977. Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain. II. Tyrosine hydroxylase in the telencephalon. Medical Biology 55: 21-40.

Joh TH, Geohman C, & Reis DJ. 1973. Immunochemical demonstration of increased accumulation of tyrosine hydroxylase protein in sympathetic ganglia and adrenal medulla elicited by reserpine. Proceedings National Academy of Sciences USA 70: 2767-2771.

Joh TH, & Reis DJ. 1975. Different forms of tyrosine hydroxylase in central dopaminergic and noradrenergic neurons, sympathetic ganglia and adrenal medulla. Brain Research 85: 146-151.

Johnson FH, & Russell GV. 1952. The locus coeruleus as a pneumotoxic center. Abstract, Anatomical Records 112: 348.

Jones BE, Bobillier P, Pin C, & Jouvet M. 1973. The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking of the cat. Brain Research 58: 157-177.

Jouvet, M. 1977. Neuropharmacology of the sleep-waking cycle. In Handbook of Psychopharmacology, Vol. 8. eds. Iversen LL, Iversen DS & Snyder SH. Plenum Press, NY.

Kaada, BR. 1972. Stimulation and regional ablation of the amygdaloid complex with reference to functional representations. In BE Eleftherious (Ed.), The Neurobiology of the Amygdala, Plenum, NY.

Kling A, Lancaster J, & Benitone J. 1970. Amygdalectomy in the free-ranging vervet. Journal of Psychiatric Research 7: 191-199.

Kling A, & Dunne K. 1976. Social-environmental factors affecting behavior and plasma testosterone in normal and amygdala lesioned M. Speciosa. Primates 17: 23-42.

Koford CB. 1963. Rank of mothers and sons in bands of rhesus monkeys. Science 141: 356-357.

Konig JFR, & Klippel RA. 1970. The Rat Brain: A Stereotoxic Atlas , Krieger, Huntington, NY.

Korf J, Bunney BS, & Aghajanian GK. 1974. Noradrenergic neurons: morphine inhibition of spontaneous activity. European Journal of Pharmacology 25: 165-169.

Korf J. 1976 Locus coeruleus, noradrenaline metabolism and stress. In Catecholamines and Stress (ed. E. Usdine), Pergamon, NY.

Kostowski W, Czlonkowski A, Jerlicz M. Bidzinski A, & Hauptmann N. 1978. Effects of lesions of the locus coeruleus on aggressive behavior in rats. Physiology and Behavior 21: 695-699.

Kuru M. 1965. Nervous control of micturition. Physiology Review 45: 425-494.

Lagerspetz KYH, Tirri R, & Lagerspetz XMJ. 1968. Neurochemical and endocrinological studies of mice selectively bred for aggressiveness. Scandinavian Journal of Psychology 9: 157-160.

Lalley P, Degroat W, & McLain P. 1972. Activation of the sacral parasympathetic pathway to the urinary bladder by brain stem stimulation. Federation Proceedings 31: 386.

Lamprecht F, Eichelman B, Thoa NB, Williams RB,& Kopin IJ. 1972. Rat fighting behavior: serum dopamine-B-hydroxylase and hypothalamic tyrosine hydroxylase. Science 177: 1214-1215.

Leckman J, Gershon E, & Nichols A. 1977. Reduced platelet MAO activity in the first degree relatives of patients with bipolar affective disorders. Archives of General Psychiatry 34: 601-606.

Lehman MN, & Adams DB. 1977. A statistical and motivational analysis of the social behaviors of the male laboratory rat. Behavior LXI 3-4.

Lewander T, Joh TH, & Reis DJ. 1977. TH:delayed activation in central noradrenergic neurons and induction in adrenal medulla elicited by stimulation of central cholinergic receptors. Journal of Pharmacology and Experimental Therapeutics 200: 523-534.

Lewis BD, Renaud B, Buda M, & Pujol JF. 1976. Time-course variations in tyrosine hydroxylase activity in the rat locus coeruleus after electrlytic destruction of the nuclei raphe dorsalis or raphe centralis. Brain Research 108: 339-349.

Lindvall O, & Bjorklund A. 1978. Organization of catecholamine neurons in the rat central nervous system. In Handbook of Psychopharmacology Vol. 9 Plenum Press, NY.

Lockwood JA, & Turney TH. 1981. Social dominance and stress-induced hypertension: strain differences in inbred mice. Physiology & Behavior 26: 547-549.

Marsden H. 1968. Agonistic behavior of young rhesus monkeys after changes induced in social rank of their mothers. Animal Behavior 16: 38-44.

Mason ST. 1979. Noradrenaline: reward or extinction? Neuroscience Biobehavioral Review 3: 1-10.

Mason ST, & Fibiger HC. 1979. Current Concepts I. Anxiety: the locus coeruleus disconnection. Life Sciences 25: 2141-2147.

Mason ST. 1981. Noradrenaline in the brain: progress in theories of behavioral function. Progress in Neurobiology 16: 263-303.

Masserano JM, Takimoto GS, & Weiner N. 1981. Electroconvulsive shock increases tyrosine hydroxylase activity in brain and adrenal gland of the rat. Science 214: 662-664.

Matte AC, & Tornow H. 1979. Dissociated effects of inhibition of catecholamine synthesis on motor activity, "emotionality", and aggression in mice. Journal of Neuroscience Research 4: 239-245.

McLain WC, Cole BT, Schreiber R, & Powell DA. 1974. Central catechol- and indolamine systems and aggression. Pharmacology Beiochemistry & Behavior 2: 123-126.

McNaughton N, & Mason ST. 1980. The neuropsychology and neuropharmacology of the dorsal ascending noradrenergic bundle -- a review. Progress in Neurobiology 14: 157-219.

Miczek K, Thompson ML, & Shuster L. 1982. Opoid-like analgesia in defeated mice. Science 215: 1520-1522.

Moore RY, & Bloom FE. 1979. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annual Review of Neuroscience 2: 113-148.

Morgenroth VH III, Boadle-Biber MC, & Roth RH. 1974. Tyrosine hydroxylase: Activation by nerve stimulation. Proceedings of the National Academy of Sciences USA. 71: 4283.

Moroni F, Bianchi C, Moneti G, Tanganelli S, Guandalini P, & Beani L. 1982. Release of GABA from the guinea pig neocortex induced by electrical stimulation of the locus coeruleus or by nor-epinephrine. Brain Research 232: 216-221.

Moyer KE. 1968. Kinds of aggression and their physiological basis. Behavioral Biology 2: 65-87.

Moyer KE. 1976. The Psychobiology of Aggression NY: Harper & Row.

Musacchio JM, Julou L, Kety S, & Glowinski J. 1969. Increase in rat brain tyrosine hydroxylase activity produced by electroconvulsive shock. Proceedings of the National Academy of Sciences USA 63: 1117-1119.

Nagatsu J, Levitt M, & Udenfriend S. 1964. Tyrosine hydroxylase, the initial step in norephinephrine biosynthesis. Journal of Biology & Chemistry 239: 2910-2917.

Nies A, Robinson DS, & Harris LS. 1974. Comparison of monoamine oxidase, substrate activities in twins, schizophrenics, depressives and controls. Advances in Biochemistry & Psychopharmacology 12: 59-70.

Ogren SO, & Fuxe K. 1974. Learning, brain noradrenaline, and the pituatary-adrenal axis. Medical Biology 52: 399-405.

Osumi Y, Oishi R, Fujiwara H, & Takaori S. 1975. Hyperdipsia induced by bilateral destruction of the locus coeruleus in rats. Brain Research 86: 419-427.

Overmier JB, & Seligman MEP. 1967. Effects of inescapable shock upon subsequent escape and avoidance learning. Journal of Comparative and Physiological Psychology 63: 23-33.

Panksepp J. 1971a. Drugs and stimulus-bound attack. Physiology & Behavior 6: 317-320.

Pickel VM, Joh TH, & Reis DJ. 1975. Immunohistochemical localization of TH in brain by light and electron microscopy. Brain Research 85: 295-300.

Poshivalov V. 1980. The integrity of the social hierarchy in mice following administration of psychotropic drugs. British Journal of Pharmacology 70: 367-373.

Puymirat J, Javoy-Agid F, Gaspar P, Ploska A, Prochiantz A, & Agid Y. 1979. Post mortem stability and storage in the cold of brain enzymes. Journal of Neurochemistry 32: 449-454.

Raab A, & Oswald R. 1980. Coping with social conflict: impact on the activity of tyrosine hydroxylase in the limbic system and in the adrenals. Physiology & Behavior 24: 387-394.

Redmond DE Jr, & Murphy DL. 1975. Behavioral correlates of platelet monoamine oxidase in the rhesus monkey. Psychosomatic Medicine 37:80.

Redmond DE Jr, Huang YH, Snyder DR, Maas JW, & Baulu J. 1977. Hyperphagia and hyperdipsia after locus coeruleus lesions in the stumptailed monkey. Life Sciences 20: 1619-1628.

Redmond DE Jr, Murphy DL, & Baulu J. 1979. Platelet monoamine oxidase activity correlates with social affiliative and agonistic behaviors in normal rhesus monkeys. Psychosomatic Medicine 41: 2, 87-100.

Redmond DE Jr, & Huang YH. 1979. Current Concepts II. New evidence for a locus coeruleus-norepinephrine connection with anxiety. Life Sciences 25: 2149-2162.

Redmond DE Jr. 1981. Clonidine and primate locus coeruleus: evidence suggesting anxiolytic and antiwithdrawl effects. In H. Lal & S. Fielding (Eds.) Psychopharmacology of Clonidine Alan R. Liss, NY.

Reis DJ. 1972. The relationship between brain norepinephrine and aggressive behavior. Research Publication Association for Research of Nervous & Mental Disorders 50: 266-297.

Reis DJ. 1974. Central neurotransmitters in aggression. Research Publication Association for Research of Nervous & Mental Disorders 52: 119-146.

Reis DJ, Joh TH, Ross RA, & Pickel VM. 1974. Reserpine selectively increases tyrosine hydroxylase and dopamine-B-hydroxylase enzyme protein in central noradrenergic neurons. Brain Research 81: 380-386.

Reis DJ, Joh TH, & Ross RA. 1975. Effects of reserpine on activities and amounts of tyrosine hydroxylase and dopamine-B-hydroxylase in catecholamine neuronal systems in rat brain. Journal of Pharmacology & Experimental Therapeutics 193: 775-784.

Renaud B, Joh TH, & Reis DJ. 1979. An abnormal regulation of tyrosine hydroxylase restricted to one catecholamine nucleus in the brain stem of spontaneously hypertensive rats. Brain Research 173: 164-167.

Renaud B, Joh TH, Snyder DW, & Reis DJ. 1979b. Induction and delayed activation of tyrosine hydroxylase in noradrenergic neurons of A1 and A2 groups of medulla oblongata of rat. Brain Research 176: 169-174.

Roth RH, Salzman PM, & Nowycky MC. 1978 Impulse flow and short-term regulation of transmitter biosynthesis in central catecholaminergic neurons. in Psychopharmacology - A Generation of Progress. (Ed. ME Lipton). Raven Press, NY.

Sachar EJ. 1981. Psychiobiology of affective disorders. In Principles of Neural Science Eds. Eric Kandel and James Schwartz, NY, Elsevier North Holland, Inc.

Sasa M, Igarashi S, & Takaori S. 1977. Influence of the locus coeruleus on interneurons in the spinal trigeminal nucleus. Brain Research 125: 369-375.

Schildkraut JJ, & Kety SS. 1967. Biogenic amines and emotion. Science. 156: 21-30.

Schwartz, JH. 1981. Biochemical Control Mechanisms in Synaptic Transmission. In Principles of Neural Science Eds. Eric Kandel and James Schwartz, NY Elsevier North Holland, Inc.

Scriabine A, Clineschmidt BV, & Sweet CS. 1976. Central noradrenergic control of blood pressure. In Annual Review of Pharmacology and Toxicology. Vol. 16. (ed. HW Elliott). Palo Alto, CA.

Segal M, & Bloom FE. 1976. The action of norepinephrine in the rat hippocampus. IV. The effects of locus coeruleus stimulation on evoked hippocampal unit activity. Brain Research 107: 513-525.

Sheard MH. 1969. The effect of p-chlorophenylalanine on behavior in rats: relation to 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Brain Research 15: 524-528.

Sherman AD, Allers GL, Petty F, & Henn FA. 1979. A neuropharmacologically-relevant animal model of depression. Neuropharmacology 18: 891-893.

Silverman, P. 1978. Animal Behavior in the Laboratory Pica Press, NY. Chs. 20-22.

Slater J, Blizard DA, & Pohorecky LA. 1977. Central and peripheral norepinephrine metabolism in rat strains selectively bred for differences in response to stress. Pharmacology Biochemistry & Behavior 6: 511-520.

Snyder DW, & Reis DJ. 1975. Sudden death following bilateral lesions of nucleus locus coeruleus. Neuroscience Abstracts 1: 425.

Stolk JM, Conner RL, Levine S, & Barchas JD. 1974. Brain norepinephrine metabolism and shock-induced fighting behavior in rats: differential effects of shock and fighting on the neurochemical response to a common foot shock stimulus. Journal of Pharmacology & Experimental Therapeutics 190: 193-209.

Stone EA. 1975. Stress and catecholamines. In Catecholamines and Behavior, Vol. 2, Neuropsychopharmacology (Ed. AJ Friedhoff) Plenum Press, NY.

Sudak HW, & Mass JW. 1964. Behavioral-neurochemical correlation in reactive and non-reactive strains of rats. Science 146: 418-420.

Sullivan J, Stanfield CN, & Dackis D. 1977. Platelet MAO activity in schizophrenia and other psychiatric illnesses. American Journal of Psychiatry 134: 1098-1104.

Swanson LW. 1976. The locus coeruleus: a cytoarchitectonic, Golgi and immunohistochemical study in the albino rat. Brain Research 110: 39-56.

Thoenen H. 1970. Induction of tyrosine hydroxylase in peripheral and central adrenergic neurones by cold exposure of rats. Nature 228: 861-862.

Udenfriend S. 1962. Fluorescence Assay in Biology and Medicine. Academic Press, NY.

Ulrich RE, Wolff PC, & Azrin NH. 1964. Shock as an elicitor of intra- and inter-species fighting behavior. Animal Behavior 12: 14-15.

Ungerstedt U. 1971. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiology Scandinavia Supplement 367: 1-49.

Usdin E, Weiner N, & Youdim MBH. 1977. Structure and Function of Monoamine Enzymes. Marcel Dekker, Inc. NY.

Uyeno ET. 1960. Hereditary and environmental aspects of dominant behavior in the albino rat. Journal of Comparative and Physiological Psychology 53: 138-141.

Valeri P, Angelucci L, & Palmery M. 1978. Specific (3H) corticosterone uptake in the hippocampus and septum varies with social settings in mice: hormone-receptor autoregulation may be involved. Neuroscience Letters 9: 249-254.

Valzelli L. 1974. 5-Hydroxytryptamine in aggressiveness. Advances in Biochemical Psychopharmacology 11: 255-263.

Van Dongen PAM. 1981. The central noradrenergic transmission and the locus coeruleus: a review of the data, and their implications for neurotransmission and neuromodulation. Progress in Neurobiology 16: 117-143.

Ward DG, & Gunn CG. 1976. Locus coeruleus complex: elicitation of a pressor response and a brain stem region necessary for its occurrence. Brain Research 107: 401-406.

Weiss JM, Krieckhaus EE, & Conte R. 1968. Effects of fear conditioning on subsequent avoidance behavior. Journal of Comparative and Physiological Psychology 65: 413-421.

Weiss JM, Stone EA, & Harrell N. 1970. Coping behavior and brain norepinephrine level in rats. Journal of Comparative and Physiological Psychology 72: 153-160.

Weiss JM, Glazer HI, Pohorecky LA, Brick J, & Miller NE. 1975. Effects of chronic exposure to stressors on avoidance-escape behavior and on brain norepinephrine. Psychosomatic Medicine 37: 522-534.

Weiss JM, & Glazer HI. 1975. Effects of acute exposure to stressors on subsequent avoidance escape behavior. Psychosomatic Medicine 37: 499-521.

Weiss JM, Glazer HI, & Pohorecky LA. 1976. Coping behavior and neurochemical changes: an alternative explanation for the original "learned helplessness" experiments. In G. Serban and A. King (Eds.) Animal Models in Human Psychobiology. Plenum Press, NY.

Weiss JM, Bailey WH, Pohorecky LA, Korzeniowksi D, & Grillone G. 1980. Stress-induced depression of motor activity correlates with regional changes in brain norepinephrine but not in dopamine. Neurochemistry Research 5: 9-22.

Weiss JM, Goodman PG, Losito BG, Corrigan S, Charry JM, & Bailey WH. 1981. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of the rat. Brain Research Review 3: 167-205.

Weiss JM, Bailey WH, Goodman PA, Hoffman LJ, Ambrose MJ, Salman SL, & Charry JM. 1982. A model for neurochemical study of depression. In Behavioral Models and the Analysis of Drug Action. Elsevier, Amsterdam, NY.

Welch BL, & Welch AS. 1969. Sustained effects of brief daily stress (fighting) upon brain and adrenal catecholamines and adrenal, spleen, and heart weights of mice. Physiology 64: 100-107.

Williams RB, Richardson JS, & Eichelman BS. 1978. Location of CNS neurons mediating the blood pressure fall after shock-induced fighting in the rat. Journal of Behavioral Medicine 1: 177-185.

Wise RA. 1978. Catecholamine theories of reward: a critical review. Brain Research 152: 215-247.

Zigmond M, & Harvey J. 1970. Resistance to central norepinephrine depletion and decreased mortality in rats chronically exposed to electric foot shock. Journal of Neural & Visceral Research 31: 373-381.

Zigmond RE, Schon F, & Iverson LL. 1974. Increased TH activity in the LC of rat brain stem after reserpine treatment and cold stress. Brain Research 70: 547-552.

Zigmond RE. 1979. Tyrosine hydroxylase activity in noradrenergic neurons of the locus coeruleus after reserpine administration: sequential increase in cell bodies and nerve terminals. Journal of Neurochemistry 32: 23-29.

Zook JM, & Adams DB. 1975. Competitive fighting in the rat. Journal of Comparative and Physiological Psychology 88: 1, 418-423.

Zornetzer SF, Wickliffe CA, & Appleton R. 1978. Locus coeruleus and labile memory. Pharmacology Biochemistry & Behavior 9: 227-234.