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PITUITARY-ADRENAL HORMONES, BRAIN NORADRENALINE AND
ACQUISITION OF ACTIVE AVOIDANCE BEHAVIOUR IN RATS

by:

Robert J. Bialik

Thesis presented to the Faculty of Graduate Studies of
Carleton University in partial fulfillment of the
requirements for the degree of Master of Arts.

Ottawa, January 1982

The undersigned recommend to the Faculty
of Graduate Studies and Research acceptance of the thesis
"Pituitary-adrenal hormones, brain noradrenaline and acquisition
of active avoidance behaviour in rats"

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Abstract

The ability of rats to acquire a shuttle-box avoidance response following depletion of brain norepinephrine (NE) by lesion of the dorsal tegmental noradrenergic bundle combined with depletion of plasma corticosterone by either adrenalectomy or administration of the corticosterone synthesis inhibitor, Metopirone, was examined. It was found that the NE depleted rats tended to acquire the avoidance response more quickly than sham-lesioned rats, while depletion of plasma corticosterone by either method retarded acquisition of the avoidance response. A possible mechanism of action for this effect of corticosterone depletion was suggested. There was no evidence that the depletion of brain NE in corticosterone depleted rats caused an impairment in avoidance behaviour greater than seen following corticosterone depletion alone. These latter results were inconsistent with earlier studies reporting that the combination of brain NE depletion and adrenalectomy severely retarded acquisition of an active avoidance response. It was suggested that there might be an essential parameter(s) that differed between the earlier studies and the present experiment which could explain this discrepancy in results. This presumed dependence of the avoidance deficit on parameters of the learning situation was interpreted to indicate that possibly the previously observed deficit in avoidance performance caused by the combination of brain NE depletion and adrenalectomy was of a non-associative nature rather than reflective of a strict learning or memory impairment.

Metopirone (50 mg/kg ip) effectively blocked corticosterone

synthesis and resulted in more than 50% depletion of plasma corticosterone levels two hours after administration. As well as showing a severe acquisition impairment in the active avoidance task, rats showed a decreased responsiveness to pain in both the tail-flick and hot-plate tests (unlike adrenalectomized rats) two hours following Metopirone treatment. These results indicate that this drug may prove to be a useful pharmacological tool in the study of the relationship between the pituitary-adrenal axis and behaviour. The behavioural effects of Metopirone did not parallel those of adrenalectomy in all tests, which might be explained by the acute vs chronic nature of the depletion of corticosterone produced by the two treatments or possibly by an additional direct effect of Metopirone on the brain. These possibilities will be addressed in future experiments.

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ABREVIATIONS USED IN TEXT

ACTH	adrenocorticotrophic hormone
ADX	adrenalectomy
a-MSH	alpha-melanocyte stimulating hormone
B-END	B-endorphin
CA	catecholamine
CAR	conditioned avoidance response
CRF	corticotropin releasing factor
CS	conditioned stimulus
DA	dopamine
DBHI	dopamine-beta-hydroxylase inhibitor
DDC	diethyldithiocarbamate
DNB	dorsal tegmental noradrenergic bundle
OSP-4	N-2-chloroethyl-N-ethyl-2-bromobenzyl-amine
hr	hour
ip	intraperitoneal
ITC	intertrial crossing
ITI	intertrial interval
MET	Metopirone
mg/kg	milligram per kilogram of body weight
ml	millilitre
NE	norepinephrine
US	unconditioned stimulus
ug/100 ml	microgram per 100 millilitre
ul	microlitre

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particular event or act may function to modulate storage so that, "...even a hint of the experience will in the future evoke the affective state and increase the probability of the response." (p.322). This postulated role of neurohumoral secretions at or shortly after training is not unlike the view held by proponents of "state-dependent" memory processing (e.g. Overton, 1978). That is, the chemical state of the organism at the time of training may be stored with the training experience and act as a "chemical combination" which must be reconstructed before the memory can be unlocked from storage (ie. retrieved).

One other alternative to these views is that the chemical secretions elicited by a training experience may modulate processes involved in memory storage (consolidation). Still further, it has been postulated that the neurochemical state of an organism prior to an experience will determine how the organism selects and associates stimuli in constructing an "internal representation" or package to submit to memory processing (eg. Oades, 1979).

These hypotheses that have been outlined in the previous paragraphs may be related and may all be relevant to learning and performance. The present paper examines a crucial issue addressed by all of these hypotheses, namely how the rat's neurohumoral state prior to and during avoidance training may affect its ability to acquire the avoidance response. More specifically, it examines the relevance of an intact pituitary-adrenal axis and the combined relevance of this axis and the brain's dorsal tegmental noradrenergic system for such

acquisition.

An animal's ability to attend to relevant stimuli and make appropriate associations among these stimuli influences the rate at which a conditioned response will be acquired. Although there has been little theorizing as to the nature of the cognitive process(es) involved in task acquisition that might be altered by neurohumoral substances, there is evidence indicating that natural or experimental changes in the neurohumoral state of an organism prior to conditioning can facilitate or retard acquisition performance in many different tasks. Early studies had pointed to the level of arousal at the time of training as an important determinant of task performance. The Yerkes-Dodson Law refers to the fact that learning occurs best at an optimal level of arousal, lower or higher levels of arousal result in poorer learning capacity. Presently, there is great interest in the effects on mental performance of the pituitary hormones normally associated with states of arousal. For example, specific fragments of adrenocorticotrophic hormone or ACTH (which also occur in other pituitary peptides) have been found to improve attentional and retrieval processes in normal human adults (van Riezin, Rigter & de Wied, 1977; Ward, Sandman, George & Shulman, 1979) as well as interpersonal and environmental awareness in mentally retarded adults (Sandman, Walker & Lawton, 1980).

Synthetic analogues of endogenous vasopressin (antidiuretic hormone) improve cognitive performance in elderly men (Legros, Gilot, Seron, Claessens, Adam, Moeglen, Audibert & Berchier,

1978) and in depressed patients without altering mood (Weingartner, Gold, Ballenger, Smallberg, Summers, Rubinow & Post, 1981). Vasopressin can also significantly reverse electroconvulsive shock-induced retrograde amnesia in depressed patients (Weingartner et al, 1981). Not all clinical reports concerning vasopressin treatment have shown therapeutic effects, however. Neither intranasal nor intramuscular administration of several different vasopressin analogues affected post-traumatic amnesia in the few cases studied (Jenkins, Mather, Coughlan & Jenkins, 1981; Koch-Henriksen & Nielson, 1981). A further interesting finding is that the structurally (eg. Acher, 1981) and anatomically (eg. Buijs, 1980) related peptides, vasopressin and oxytocin, have been reported to have opposite effects on memory. While vasopressin improved recall in healthy volunteers oxytocin impaired recall (Ferrier, Kennett & Devlin, 1980).

These recent studies conducted on humans were prompted by evidence in the animal literature indicating a facilitatory role of pituitary hormones on the acquisition and retention of conditioned responses. Animals depleted of pituitary hormones as a result of hypophysectomy, do not acquire a shuttlebox avoidance response (de Wied, 1964) and this impairment can be alleviated by administration of steroids, ACTH, alpha-melanocyte stimulating hormone (α -MSH) or fragments of ACTH devoid of steroidogenic properties (de Wied, 1964; 1969; Bohus, Gispen & de Wied, 1973). The natural fluctuation in hormone secretions is also correlated with acquisition of a learned response. Animals will learn a shuttlebox avoidance task quicker when tested during the peak

rather than the trough of the diurnal ACTH-corticosterone cycle (Pagano & Lovely, 1972). Exogenous ACTH can facilitate acquisition of several different tasks in intact animals (Flood, Jarvik, Bennet & Orme, 1976; de Wied & Gispen, 1977; Gold & McGaugh, 1977) and α -MSH can facilitate reversal learning (Kastin, Sandman, Stratton, Schally & Miller, 1975; Beckwith & Sandman, 1978), while Methionine-enkephalin (which is present in the pituitary as part of B-endorphin and in the brain as a free molecule) impairs acquisition of an active avoidance response (Rigter, Jensen, Martinez, Messing, Vasquez, Liang & McGaugh, 1980). A molecule identical to the fourth to tenth amino acids of ACTH [ACTH(4-10)] and vasopressin analogues reverse experimentally-induced amnesia for a number of different responses (Rigter & van Riezen, 1975). Further, it has been demonstrated that chronic treatment with ACTH(4-9) or bilateral adrenalectomy (which causes a 10 fold increase in plasma ACTH) slow the neuromorphological and behavioural deterioration normally seen in aged rats (Landfield, Baskin & Pitler, 1981).

These and other peptides that traditionally have been considered to act only as releasing factors, pituitary hormones or gut peptides, have been found to exist in brain tissue outside the hypothalamus and are thought to function as neurotransmitters or neuromodulators (eg. Renaud, 1978). Fibers containing vasopressin or oxytocin have been found to project from hypothalamic nuclei to many limbic structures (Buijs, 1978; 1980; Buijs & Swaab, 1979; Poulan, Ellendorff & Vincent, 1980; Sofroniew, 1980), to the brainstem and spinal cord (Buijs, 1978;

Sofroniew, 1980) and to the pineal gland (Buijs & Pevet, 1980) in rats and to the brainstem, spinal cord (Sofroniew, 1980), locus coeruleus, periaquiductal grey and substantia nigra (Rossor, Iversen, Hawthorn, Ang & Jenkins, 1981) in the human brain. An ACTH-like substance has been identified in the rat in some hypothalamic nuclei which are not directly, functionally coupled with the anterior pituitary (Toubeau, Desclin, Parmentier & Pasteels, 1979; Leranth, Williams, Chretien & Palkovits, 1980) and also in a number of fiber bundles with extensive projections from the hypothalamus to both cortical and subcortical areas (Joseph, 1980). It is also seen in the human brain in areas of the hypothalamus unrelated to the anterior pituitary, in the periaquiductal gray area and to a lesser extent in the forebrain (Kleber, Gramsch, Holtt, Mehraein, Pasi & Herz, 1980). Many other peptides have also been found to exist in the brain, and some have even been identified within neurons which are thought to release monoamines as their transmitter. Serotonin and Substance P, serotonin and thyrotropin releasing hormone, dopamine and cholecystokinin (Hokfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980), vasopressin and Angiotensin II (Kilcoyne, Hoffman & Zimmerman, 1980), B-endorphin and α -MSH (Watson & Akil, 1981) and possibly NE and ACTH (Schwartzberg & Nakane, 1981; Schwartzberg, D.J., 1981, personal communication to B.A. Pappas) are diads which have been identified in the same neurons in the rat brain.

Specific receptors have been hypothesized for the behaviourally active peptides, vasopressin (Castel, 1978; Buijs,

1980), ACTH (Wiegant, Dunn, Schotman & Gispen, 1979; Dunn, Childers, Kramarcy & Villiger, 1981) and B-endorphin (For a review see Olson, Olson, Kastin & Coy, 1980). Further, antagonistic effects have been observed between ACTH and B-endorphin on receptor mechanisms and on behaviour (Terenius, Gispen & de Wied, 1975; Gispen, Buitelaar, Wiegant, Terenius & de Wied, 1976; Jacquet, 1979; Akil, Hewlett, Barchas & Li, 1980; Riley, Zellner & Duncan, 1980; Smock & Fields, 1981; Botticelli & Wurtman, 1981).

Although these findings strongly suggest a role of certain neuropeptides as neuromodulators or neurotransmitters in the central nervous system, such systems do not necessarily represent the physiological basis for the effects of these neuropeptides on brain chemistry or on behaviour. For example, following hypophysectomy animals are unable to learn an active avoidance response although α -MSH, ACTH, B-END and their common precursor molecule, pro-opiocortin (See Figure 1), are still found in hypothalamic and extra-hypothalamic areas of the brain (eg. Krieger, Liotta & Brownstein, 1977; Larsson, 1977; Oliver & Porter, 1978). It seems that the continued presence of these peptides in the brain is not sufficient to prevent acquisition deficits following hypophysectomy. What is not clear however, is the role if any, the brain peptide systems play in mediating the behavioural effects of exogenously administered peptides.

One possibility is suggested by the finding that in monkeys in the superior central and dorsal nuclei of the raphe complex and in the locus coeruleus (and to a lesser extent in other brain

Figure 1. In the pituitary gland, and probably in brain, a single large precursor molecule (pro-opiocortin) contains within its amino acid sequence the entire ACTH and B-lipotropin (B-LPH) molecules. In turn, the ACTH sequence contains α -MSH, and the B-LPH sequence contains B-MSH and B-endorphin. Furthermore, the sequence of amino acids 4-10 in ACTH is repeated within the B-LPH sequence. Although the amino acid sequence of met-enkephalin is contained in the first five residues of B-endorphin, free met-enkephalin has not been detected in significant quantities in the pituitary gland. (From Iversen & Iversen, 1981).

rat removed its tail from the water was recorded and taken as an indicator of sensitivity to pain. Several minutes later rats again had their pain threshold estimated, this time by the hot-plate procedure. Rats were placed on the 27.5 cm diameter stainless steel plate (55 ± 0.5 degrees C) and the latency to the first paw-lick or until they escaped over a 4 cm high barrier at the perimeter of the plate was recorded and taken as an indicator of sensitivity to pain.

Plasma Corticosterone:

Immediately following each avoidance session rats had their tail snipped at the tip and milked for approximately 1.5 ml of blood. The blood was frozen and stored until assayed for total (i.e. bound plus free) plasma corticosterone levels as described in Experiment one. At the time of decapitation a sample of trunk blood was collected and used as an indicator of baseline corticosterone level.

Two-way Active Avoidance Training:

Rats were trained in one of two identical shuttleboxes for the acquisition of a conditioned avoidance response (CAR). The shuttleboxes were enclosed in separate sound attenuating wooden chambers. Air circulation was provided by exhaust fans which also emitted 65 db background noise.

The shuttleboxes were made of black plexiglass (measuring 82

feedback control by adrenal corticosteroids (mainly corticosterone in rodents) (Simantov, 1979; Imura and Nakai, 1981; Andersson, Fuxe, Eneroth, Blake, Agnati & Gustafsson, 1981; Silverman, Gadde & Zimmerman, 1980; Silverman, Hoffman, Gadde, Krey & Zimmerman, 1981; Roßsier et al, 1979).

It has been known for several years now that ACTH and B-endorphin are derived from a common precursor, pro-opiocortin (Mains, Eipper & Ling, 1977; Nakanishi, Inoue, Kita, Nakamura, Chang, Cohen & Numa, 1979; Seidah, Gnanoulakis, Crine, Lis, Benjannet, Routhier & Chretien, 1978) (See Figure 1). Further, corticosteroids have been found to act directly on corticotropic cells of the anterior pituitary to affect the activity of messenger RNA specific for pro-opiocortin, indicating that the negative feedback action of corticosteroids is, at least in part, at the level of transcription (c.f. Imura & Nakai, 1981). Thus, circulating corticosteroids play a role in the synthesis and secretion of ACTH and B-endorphin from the anterior pituitary. It has yet to be determined if corticosteroids alter the cleavage of ACTH, B-endorphin, or some smaller fragment such as α -MSH or ACTH(4-10), from pro-opiocortin and in this way control the relative proportions of these neuroactive agents that are released under normal or stressful conditions.

For some time, it has generally been believed that NE exerted a tonic inhibitory effect on ACTH secretion (Van Loon, Scapagnini, Cohen & Ganong, 1971; Smelik, 1967; Car & Moore, 1968) and that alpha-NE receptors mediated this effect (Scapagnini, 1974; Lancranjan, Ohnhaus, & Girard, 1979; Rudolph,

Kaplan & Ganong, 1980). However, there are a number of studies which have reported a facilitatory effect of NE on ACTH release (Noumenko, 1968; Krieger & Krieger, 1970; Kawa, Taniguchi, Miziguchi, Ryu, Ariyama, Kamisaki, Koreeda & Kanehisa, 1978). Recently, Andersson et al (1981) have identified a site at the level of the median eminence where NE inhibits the secretion of a corticotropin releasing factor (CRF) and another site in the hypothalamus where NE facilitates the release of vasopressin, and thus increases ACTH secretion. Further, these authors reported that corticosteroids enhance the inhibitory effect of NE on CRF neurons while suppressing the stimulatory effect of NE on vasopressin secretion.

It is especially significant that destruction of various limbic structures abolish the effects of some neuropeptides on certain behaviours. Behavioural effects of ACTH or vasopressin are not evident in animals with lesions to the antero-dorsal hippocampus (van Wimersma Greidanus, 1977), parafascicular area (van Wimersma Greidanus, Bohus & de Wied, 1975) or amygdala (van Wimersma Greidanus, Croiset, Bakker & Bouman, 1979). All of these areas receive a noradrenergic input from the ascending NE system and both ACTH (See Dunn & Gispen, 1977 for a review) and vasopressin (Versteeg, de Kloet, van Wimersma Greidanus & de Wied, 1979; Tanaka, de Kloet, de Wied & Versteeg, 1977) influence activity of NE containing neurons in these limbic brain regions. Further, it has been shown that treatment with the catecholamine (CA) synthesis inhibitor alpha-methylparatyrosine (α -MPT) (Kovacs, Vecsei, Szabo & Telegdy, 1977) or destruction of the

major ascending NE System (the dorsal tegmental noradrenergic bundle or DNB which rises from the locus coeruleus) (Kovacs, Bohus & Versteeg, 1979) abolish the behavioural effects of vasopressin. Thus, central NE is implicated as the mediator of the effects of these neuropeptides on the acquisition of conditioned responses.

Norepinephrine containing neurons originating in the nucleus locus coeruleus of the brainstem, and ascending in the DNB, have diffuse projections to forebrain areas (Fuxe, Hamburger & Hokfelt, 1968). This NE system was originally implicated in learning and memory by Kety (1970) and Crow (1968; 1973), who independently postulated that its widespread distribution in the forebrain made it ideally suited to promote consolidation of appropriate responses at individual synapses. However, until recently there has been little support for the role of NE in the acquisition of learned responses.

Specific forebrain NE depletion caused by six-hydroxydopamine (6-OHDA) injection into the DNB consistently failed to produce acquisition deficits in one-way or two-way active avoidance tasks (eg. Fibiger & Mason, 1978; Mason & Fibiger, 1979; Ogren & Fuxe, 1977) or in a variety of food or water motivated tasks (eg. Amaral & Foss, 1975; Mason & Iversen, 1975; 1979; Roberts, Price & Fibiger, 1976). However, recently reported data indicates that animals receiving lesions of the DNB attend to more stimulus dimensions, and thus sample more irrelevant stimuli from the environment, than do intact animals (See Mason & Iversen, 1979 and McNaughton & Mason, 1980, for reviews of this data). This

alteration in stimulus sampling caused by NE depletion can disrupt or facilitate performance in a behavioural task depending on whether stimulus changes relevant to solving the task are normally attended to by intact animals (disruption) or not attended to by intact animals (facilitation) (Mason & Lin, 1980).

This alteration in the information processing capacity of DNB lesioned animals is rather subtle and it is possible that more profound deficits associated with NE depletion are masked by a recovery of function which may have developed as a result of compensatory physiological responses. It is known that following 6-OHDA induced lesions of the DNB there is an increase in the number of postsynaptic alpha- and beta-noradrenergic receptors in the hippocampus and forebrain (U'Prichard, Reisine, Mason, Fibiger, & Yamamura, 1980) and an increase in the level of NE in the brainstem (Pappas, Saari, Peters, Roberts & Fibiger, 1978). Indirect support for a recovery of function hypothesis comes from studies using acute depletion of NE or pharmacological blockade of NE transmission, procedures which do not allow compensatory mechanisms to develop.

Hraschek and Endroczi (1979) and Hraschek, Pavlick and Endroczi (in press) have shown that high brain NE turnover is correlated with good active avoidance acquisition and that systemic or intracerebral (locus coeruleus) administration of beta-noradrenergic receptor blockers retard the acquisition of active avoidance behaviour. As well, a low dose of clonidine (100-400 ug/kg), which inhibits transmission in the ascending NE system by stimulating presynaptic autoreceptors (eg. Langer,

1980; Starke, 1980), disrupts active avoidance performance and this effect of clonidine is potentiated by the postsynaptic beta-noradrenergic blocker, propranolol (Hawkins & Monti, 1979). Depletion of NE by injection of the dopamine-beta-hydroxylase inhibitor (DBHI), diethyldithiocarbamic acid (DDC), also retards acquisition of both active (Osborne & Kerkut, 1973) and passive avoidance behaviour (Randt, Quartermain, Goldstein & Anagnoste, 1971). Thus, there is a discrepancy between the effects of chronic NE depletion (DNB lesions) and acute inhibition of NE transmission (short-acting pharmacological agents) on acquisition of a conditioned response.

While the development of compensatory mechanisms following the lesion may explain the discrepancy, Ogren, Archer and Ross (1980) have made another suggestion. These investigators found that the newly discovered NE neurotoxin, N-2-chloroethyl-N-ethyl-2-bromobenzyl-amine (DSP4), profoundly impaired acquisition of both one-way and two-way active avoidance behaviour. This neurotoxin is injected systemically and selectively destroys NE neurons in a pattern similar to that caused by injection of 6-OHDA into the DNB, except that spinal cord and brainstem NE are depleted by DSP4 while spinal NE is spared and brainstem NE is elevated following 6-OHDA lesions of the DNB (Pappas et al, 1978). Ogren et al suggest that DSP4 produces a deficit because it causes more extensive damage to locus coeruleus projections than DNB lesions, thus, a more widespread depletion of NE results. This argument can also be applied to the other studies finding deficits in acquisition

performance since when using a DBHI to deplete NE or a receptor blocker to inhibit NE transmission, all NE systems would be affected.

In conclusion, the studies reviewed in this section report data indicating that an alteration in the neurohumoral balance of the rodent prior to training can either impair or facilitate the acquisition of a conditioned response. Furthermore, it has been shown that most of the neurohumoral manipulations affecting acquisition performance involve brain NE systems either directly or indirectly.

A different line of research has implicated an interaction between corticosterone and forebrain NE in the performance of a learned response. In their original investigation, Ogren and Fuxe (1974) discovered that neither 6-OHDA induced lesion of the DNB alone or bilateral adrenalectomy (ADX) alone altered retention of a two-way active avoidance response but that the combination of DNB lesion plus ADX caused a profound retention deficit and further, these animals were unable to relearn this avoidance response. Subsequently, it was reported that this combination of DNB lesion plus ADX caused a retention deficit for a passive avoidance response in rats (Roberts & Fibiger, 1977; Mason, Roberts & Fibiger, 1979; Wendlend & File, 1979) and mice (Wenzel, Prado de Carvalho, Zornetzer & Dunn, unpublished observations, c.f. Dunn, 1980, p. 379).

Following their initial discovery that the combination of DNB plus ADX caused a performance deficit for a two-way active

avoidance response, Ogren and Fuxe (1977) showed that this same treatment impaired acquisition of a one-way active avoidance response and that the deficit was prevented by twice daily injections of corticosterone. Recently however, their experiments have been criticized on the grounds that they tested their animals too soon after the operations, at a time when the impaired animals sensitivity to pain was elevated (Wendlendt & File, 1979) and when these animals may have not yet recovered from the stress of the combined operations. Therefore, Wendlendt and File (1979) performed a similar experiment to that of Ogren and Fuxe (1977), except that they tested animals three weeks following surgery (when the DNB plus ADX animals no longer had elevated pain thresholds) and used a two-way active avoidance task with different stimulus parameters than the original Ogren and Fuxe (1974) study. Wendlendt and File failed to find a deficit in DNB plus ADX animals compared to controls and concluded that the acquisition deficit reported by Ogren and Fuxe (1977) was the result of the behavioural test being carried out too soon after the operations, when animals were generally debilitated and/or had elevated pain thresholds, both of which could have interfered with the acquisition of the avoidance task.

An alternate explanation for the lack of an effect in the Wendlendt and File DNB plus ADX group is that compensatory physiological mechanisms had developed by three weeks post-lesion and these may have mediated recovery of function. In addition to the compensatory responses in the NE system described previously (page 12), it is known that there is an increase in the uptake of

(page 12), it is known that there is an increase in the uptake of corticosterone in the limbic system following ADX (McEwen, Wallach & Magnus, 1974).

Further, several compensatory physiological responses appear to be the result of an interaction between brain NE and corticosterone systems. Mobley and Sulser (1980a,b) have reported an increase in the NE-induced accumulation of cyclic-AMP in the frontal cortex and limbic forebrain of rats following ADX, but not after adrenal demedullation or ADX plus corticosterone replacement. It has also been demonstrated (Roberts, Shoemaker, Back & Bloom, 1981; Roberts & Bloom, 1980) that there is a greater increase in the number of beta-noradrenergic receptors in the hippocampus following the combination of DNB plus ADX than there is following DNB alone or DNB plus ADX with corticosterone replacement. It is important to realize that serum corticosterone values of ADX rats remained at 20-30% of control levels in the behavioural studies (Ogren & Fuxe, 1974; 1977; Wendlendt & File, 1979). This residual amount of serum corticosterone combined with increased uptake of this steroid in the brain may have played a part in the recovery of function in the DNB plus ADX animals three weeks after the operation. An additional very important finding is that the active avoidance deficit induced by DSP4 (and presumed to be a result of NE depletion) could be reversed by daily administration of corticosterone (Ogren, Archer, Fuxe & Eneroth, 1981). Thus, it may be that while these or other compensatory physiological responses could not mediate recovery of function at one week

(Ogren & Fuxe, 1977), they could mediate the recovery of function manifested at three weeks following the combination of DNB plus ADX (ie. Wendlend & File, 1979).

In the present study I attempted to eliminate the contribution that the 20 to 30% residual serum corticosterone and the increased brain uptake of this steroid might play in the acquisition of a shuttle-box avoidance response in DNB plus ADX rats three weeks following surgery. The logic is that if serum corticosterone is depleted to very low levels, the increased capacity of the brain to bind corticosterone should have no functional significance to the behaviour of the rat. Low serum corticosterone levels were achieved by administering the corticosterone synthesis inhibitor, Metopirone, to adrenalectomized rats. Metopirone inhibits the enzyme, 11-B-hydroxylase, which is responsible for converting 11-deoxycortisol to cortisol and deoxycorticosterone to corticosterone (Dominguez & Samuels, 1963; Gower, 1974) and has been reported to cause substantial depletion of corticosterone in intact rats (Parvez & Parvez, 1973). Severe depletion of corticosterone alone is of interest since no deficit in avoidance behaviour has been reported in ADX animals (eg. Ogren & Fuxe, 1974; 1977; Roberts & Fibiger, 1977; Weiss, McEwen, Silva & Kalkut, 1969) but such a deficit might be seen if the effect of corticosterone on the brain is more completely eliminated.

The first two experiments determined the appropriate temporal pattern and dosage of Metopirone to be used in the behavioural

experiment. The third experiment replicated the groups used by Ogren and Fuxe (1974; 1977) and Wendlendt and File (1979). The stimulus parameters used were those found to produce optimum learning in this laboratory. Since Wendlendt and File tested their animals three weeks following surgery and found no deficits, additional groups receiving MET to further deplete corticosterone were included. As explained above, this treatment controlled for compensatory responses in the limbic corticosterone system, which may have mediated recovery of the behavioural response in Wendlendt and File's study.

Experiment One

Subjects were 48 male, Wistar rats weighing 200-250 grams upon delivery (Woodlyn Farms, Guelph, Ontario). All rats were housed singly in wire-mesh hanging cages and maintained on a reversed 12 hr light-dark cycle (lights on at 2000 hr). Water and Purina Lab Chow were provided ad libitum throughout the experiment. Adrenalectomized animals were given ad libitum 0.9% saline instead of water.

After at least one week of acclimatization rats were randomly assigned to one of six treatment groups. Groups were designated according to treatment as sham adrenalectomy plus vehicle (VEH), sham plus 50 mg/kg Metopirone (MET50), sham plus 100 mg/kg Metopirone (MET100), adrenalectomy plus vehicle (ADX), adrenalectomy plus 50 mg/kg Metopirone (ADX+MET50), and

adrenalectomy plus 100 mg/kg Metopirone (ADX+MET100). The Metopirone was generously donated by CIBA pharmaceuticals.

Surgical Procedure:

Bilateral adrenalectomies were performed using the dorsal approach. Briefly, an incision was made in the skin on the dorsal surface, the abdominal wall was cut inferior to the lowest rib, the adrenal capsule was clamped and the adrenal gland and some surrounding tissue were removed. An antiseptic powder was applied to the wound, the abdominal wall and fascia were sutured and the skin incision closed with wound clips. All adrenalectomized rats received a single intramuscular injection of the mineralocorticoid, deoxycorticosterone (2.5 mg/kg), immediately prior to the operation in order to prevent surgical shock (Miller & Ogawa, 1962). Adrenalectomized rats were maintained on 0.9% saline (prepared from distilled water) in the home cage. Rats were allowed 21 ± 1 days to recover before the experiment began. All sham operated rats survived surgery while 24 of 34 adrenalectomized rats survived and were included in the experiment.

Drug Injections:

One third of the rats (i.e. 8 ADX and 8 sham) received 100 mg/kg (ip) Metopirone dissolved in 40% propylene glycol, another third received 50 mg/kg (ip) Metopirone dissolved in 40% propylene

glycol, and the final third received an injection (ip) of only the 40% propylene glycol vehicle. The size of the injection was 1 ml/kg body weight.

Serum Extraction:

One rat at a time was allowed to burrow into a rolled towel where it remained without struggling until approximately 2 ml of blood was milked from its tail into an ice-cooled, heparin rinsed, glass centrifuge tube. The blood was immediately centrifuged at 3000 RPM in a table-top centrifuge (International Equipment, Model CL). This procedure yielded approximately 1.0 ml of serum (or plasma) which was then pipetted into 1.5 ml plastic tubes with caps. The samples were immediately placed into a freezer until assayed for total (i.e. bound plus free) plasma corticosterone concentration by the method modified from Givner and Rochefort (1965). Briefly, the iso-octane step was eliminated and 0.2 ml plasma was analyzed in duplicate for each sample of plasma.

Serum Corticosterone:

Blood samples were collected from the tail of the rats 6 and 24 hr following a single injection of 00, 50 or 100 mg/kg Metopirone. The 6 hr samples were collected between 1400 and 1700 hr on the day of injection, and the 24 hr samples were collected between 800 and 1100 hr the following day.

Results and Discussion

Analysis of Variance performed on these data revealed significant main effects due to surgery [$F(1,42)=72.04$, $p < .001$] and time after injection [$F(1,42)=34.42$, $p < .001$], as well as, a surgery by time after injection interaction [$F(1,42)=12.45$, $p < .001$] (See Table 1a). Adrenalectomized rats had a 75% depletion of plasma corticosterone levels ($x = 4.90 \pm 0.59$ ug/100 ml plasma) compared to sham operated rats ($x = 20.19 \pm 1.74$ ug/100 ml plasma). Corticosterone levels were lower at 6 hr ($x = 8.54 \pm 1.02$ ug/100 ml plasma) than at 24 hr ($x = 16.56 \pm 2.03$ ug/100 ml plasma) following Metopirone or vehicle injection.

Since it appeared that Metopirone had no significant effect on mean corticosterone levels in the present experiment, the difference between levels at 6 hr (i.e. 1400-1700 hr) and 24 hr (i.e. 800-1100 hr) after injections was most likely due to the natural circadian fluctuation in corticosterone levels in the blood. In light of this suggestion, the surgery by time interaction was interpreted as a surgery by time of day effect. Analysis of this interaction by the Newman-Keuls procedure of post-hoc comparisons revealed that corticosterone levels were significantly higher at 800-1100 hr than at 1400-1700 hr for the sham operated rats ($x = 26.6 \pm 2.7$ vs 13.8 ± 1.3 ug/100 ml plasma) but not for ADX rats despite a 97% increase in plasma corticosterone levels ($x = 6.5 \pm 1.0$ vs 3.3 ± 0.5 ug/100 ml plasma) in these ADX rats (See Table 1-1). It is not clear whether the natural fluctuation is not present in ADX rats or

whether the fluorometric assay procedure was not sensitive enough to determine plasma corticosterone levels accurately below concentrations of 3 ug/100 ml plasma in the blood. If a floor sensitivity effect had occurred, then for blood samples taken at 1400-1700 hr from adrenalectomized rats plasma corticosterone concentrations at the lower portion of the distribution would be more closely clustered about the mean than concentrations taken at 800-1100 hr. The Chi Square procedure for the analysis of differences between sample variances (Mendenhall & Ramey, 1973) was used to test this hypothesis. It was found that the variability in plasma corticosterone levels collected at 1400-1700 hr was significantly less than that for plasma corticosterone levels collected at 800-1100 hr [$\chi^2(7) = 2.08$, $p < .05$].

The lack of an effect of Metopirone on corticosterone levels in the present experiment was a surprise. Even if Metopirone was ineffective in intact rats, it was expected that adrenalectomized rats would have shown a reduction in corticosterone levels. They possess many fewer corticosterone synthesizing cells and therefore, much less of the 11-B-hydroxylase enzyme that Metopirone would have to act as a substrate for in its action of inhibiting corticosterone synthesis. Although not significantly lower in the Analysis of Variance, the ADX+MET100 group had a mean plasma corticosterone level 52% ($x = 2.38 \pm 0.38$ ug/100 ml plasma) and the ADX+MET50 group 65% ($x = 2.98 \pm 0.43$ ug/100 ml plasma) of group ADX ($x = 4.55 \pm 1.31$ ug/100 ml plasma) at 6 hr following Metopirone administration. When the Chi Square

procedure for the analysis of differences between sample variances was used it showed that Metopirone significantly reduced the variability of plasma corticosterone concentrations in both the ADX+MET100 [$X(7)=0.59$, $p < .005$] and ADX+MET50 [$X(7)=0.73$, $p < .005$] groups compared to the ADX group. As with the corticosterone rhythm in adrenalectomized rats, the inability to observe a significant Metopirone-induced reduction of mean corticosterone levels in adrenalectomized rats may have been due the lack of sensitivity of the assay at low plasma corticosterone concentrations. Clearly, however, Metopirone did cause a dose dependent alteration of plasma corticosterone concentrations in adrenalectomized rats without affecting corticosterone levels of intact rats.

Since Metopirone had no observable effect on plasma corticosterone levels of intact rats at 6 hr following injection, a 2 hr interval was used in the next experiment. As well, in order to determine whether blood corticosterone increased during the time it took to get blood samples from the tail (3 to 6 minutes), blood was collected from the tail (as in Experiment One) and also from the trunk following decapitation.

Experiment Two

Subjects were 32 male Wistar rats weighing 200-250 grams upon arrival from Woodlyn Farms, Guelph, Ontario. All rats were housed singly in wire-mesh hanging cages and maintained on a reversed 12 hr light-dark cycle (lights on at 2000 hr). Water

and Purina Lab Chow were provided ad libitum throughout the experiment. Animals were allowed one week to become acclimated to their new environment before the start of the experiment.

Drug Injection:

Half of the rats were injected with 50 mg/kg (ip) Metopirone dissolved in a 40% propylene glycol vehicle, while the other half of the rats received a vehicle injection.

Plasma Corticosterone:

Blood samples were collected 2 hr following injection of Metopirone or vehicle. Blood was obtained from the tail for half of the Metopirone and half of the vehicle treated rats, and blood was collected from the trunk following decapitation for the other half of the Metopirone and vehicle rats. Plasma corticosterone was determined as in Experiment One.

Results and Discussion

Analysis of Variance performed on these data revealed a significant main effect due to Metopirone administration [$F(1,28)=41.9$, $p < .001$] (Table 2a). Two hours following administration of Metopirone plasma corticosterone was reduced to 36.5% of control values ($x = 13.3 \pm 1.0$ vs 36.6 ± 3.7 ug/100 ml). Plasma corticosterone levels were independent of the method used

to collect blood from the rats [$F(1,28)=2.85$, $p > .10$].

In conclusion, 50 mg/kg Metopirone was a sufficient dose to substantially reduce corticosterone levels in the blood of intact animals 2 hr following injection. Therefore, this dose and time interval was used in the next, behavioural experiment. This experiment examined the ability of adrenalectomized rats with DNB lesions to acquire an active avoidance response. One half of the rats in this group were also injected with Metopirone 2 hr prior to training to determine whether circulating corticosterone levels are sufficient to mediate behavioural recovery in these rats through a mechanism involving supersensitivity of the brain corticosterone receptor system, as outlined earlier. In addition, half of the sham, adrenalectomized and DNB-lesioned groups received this Metopirone treatment.

Experiment Three

Subjects were 96 male, Wistar rats weighing 200-250 grams upon delivery (Woodlyn Farms, Guelph, Ontario). All animals were housed singly in wire-mesh hanging cages and maintained on a reversed 12 hr light-dark cycle (lights on at 2000 hr). Water and Purina Lab Chow were provided ad libitum throughout the experiment. Adrenalectomized rats were given 0.9% saline in the home cage. The experiment was run in three replications.

After at least one week of acclimatization rats were randomly assigned to one of eight treatment groups. Groups will be

designated according to treatment as sham operated controls (S), bilateral adrenalectomy (ADX), ADX plus Metopirone (ADX+MET), Metopirone only (MET), bilateral 6-OHDA induced lesion of the dorsal noradrenergic bundle (DNB), DNB+MET, DNB+ADX and DNB+ADX+MET.

Surgical Procedures:

Lesions of the DNB were performed using a Kopf stereotaxic apparatus while rats were anaesthetized with Equi-Thesin (4.2 cc/kg). The head of the rat was held in the plane of König and Klippel (1963) and two burr holes drilled in the skull at the following coordinates; Anterior-Posterior + .26 cm from interaural zero, Midline $\pm$.11 cm from midline suture (determined at bregma). A dual cannula system (prepared from 30 gauge stainless steel tubing) was lowered stereotaxically to a level .37 cm above the interaural line and infusions of 2 μ l, containing 4 μ g of 6-OHDA (prepared as free base, Regis Chemical Co.) dissolved in 0.9% saline with 0.2 mg/ml ascorbic acid, were performed at a rate of 0.4 μ l/min. and the cannulae left in for an additional 30 seconds to permit diffusion of the drug. Thus, rats simultaneously received 4 μ g of 6-OHDA into each DNB. An antiseptic powder was applied to the wound and the incision closed with wound clips.

Rats in the ADX+DNB group were adrenalectomized bilaterally immediately following the DNB lesion while the DNB group received a sham adrenalectomy operation (ie. both adrenals were located

Results

The Analysis of Variance summary tables for each amine in each brain part assayed are presented in the Appendix, Tables 3a to 3k. The means ($\pm$ S.E.M.) for each of the treatment groups are presented in Table 3-1. Infusion of 6-OHDA into the DNB resulted in NE depletion in the cortex to 5% of the level of vehicle infused rats, to 2% in hippocampus and to 28% in the hypothalamus. Brainstem NE was significantly elevated to 122% of vehicle infused controls. The DNB lesioned rats also had significant depletions of DA and serotonin in the neostriatum. DA was 68% and serotonin was 86% of control levels in the neostriatum.

There was no alteration of serotonin in the cortex, hippocampus, hypothalamus or brainstem and no alteration of DA in the hypothalamus due to the 6-OHDA infusions into the DNB. Adrenalectomy caused a slight but significant increase in serotonin levels in the hippocampus to 107% of sham operated rats. Further, Metopirone treated rats showed significantly increased serotonin levels in the hypothalamus to 108% of vehicle injected rats. Even though the lesion by drug interaction was not significant [$F(1,48)=3.63$, $p < .065$] analysis of this interaction effect using the Newman-Keuls procedure for post-hoc comparisons revealed that hypothalamic serotonin concentrations were higher (by 13%) in DNB+MET rats compared to DNB rats but not in group MET compared to group S (2% increase) (See Table 3-2).

Analysis of the lesion by drug interaction for NE in the

rat removed its tail from the water was recorded and taken as an indicator of sensitivity to pain. Several minutes later rats again had their pain threshold estimated, this time by the hot-plate procedure. Rats were placed on the 27.5 cm diameter stainless steel plate (55 ± 0.5 degrees C) and the latency to the first paw-lick or until they escaped over a 4 cm high barrier at the perimeter of the plate was recorded and taken as an indicator of sensitivity to pain.

Plasma Corticosterone:

Immediately following each avoidance session rats had their tail snipped at the tip and milked for approximately 1.5 ml of blood. The blood was frozen and stored until assayed for total (i.e. bound plus free) plasma corticosterone levels as described in Experiment one. At the time of decapitation a sample of trunk blood was collected and used as an indicator of baseline corticosterone level.

Two-way Active Avoidance Training:

Rats were trained in one of two identical shuttleboxes for the acquisition of a conditioned avoidance response (CAR). The shuttleboxes were enclosed in separate sound attenuating wooden chambers. Air circulation was provided by exhaust fans which also emitted 65 db background noise.

The shuttleboxes were made of black plexiglass (measuring 82

x 35 x 15 cm) with a grid floor made of steel bars (2 mm in diameter). The conditioned stimulus (CS) was a buzzer (71 db) and a light (15w), and the unconditioned stimulus (US) was an A.C. electrical current (0.5 mA) delivered through the grid floor. The grids were connected by neon bulbs to permit scrambling of the shock. Each trial was initiated by the onset of the CS and terminated when a shuttle-crossing was made to the safe side (detected by photo-cell beam disruption), or when 30 seconds had elapsed. The US (shock) followed the CS by 7 seconds. There was a 30 second interval between trials (ITI). A microprocessor, fabricated by Carleton University Science Workshop, both controlled the shuttle-box sequence and collected performance data which was printed on a paper tape.

On both days of training rats were first habituated to the darkened shuttlebox for 10 minutes prior to receiving 100 trials (which took approximately 60 minutes) of two-way active avoidance training. Each shuttle-crossing made by the rat was recorded on a paper tape by the printer module. Shuttles during the ITI (intertrial crossings; ITC) were indicated on the printout tape as zeroes, while the responses during a trial (escapes or CAR) were indicated by a latency time (from 00.01 to 30.00). The unconditioned response was a shuttle to the safe side after the onset of the shock (latency $\geq$ 7.00 seconds; escape response) while the conditioned response was a shuttle in response to the CS (latency $\leq$ 06.99 seconds; avoidance response or CAR). Rats received the second 100 trial session one week following the first session. All shuttle-box testing was carried out between

1000 and 1700 hr. The following dependent measures were obtained from the computer print-outs and subjected to the appropriate Analysis of Variance procedure. The number of avoidance responses for each block of 20 trials, the mean response latency for each block of 20 trials, the number of ITCs for each block of 50 trials, and the mean escape latency for the first and last 10 escape responses in the first avoidance session and for the first 10 escape responses in the second avoidance session were calculated for each rat.

Dissection and Assay Procedure:

The brains of seven rats from each of the eight groups were assayed for NE, DA and serotonin content between one and two weeks following the completion of testing. Rats were decapitated, brains removed and dissected on saline-rinsed, ice-cooled plates following the procedure previously used in this laboratory (Saari & Pappas, 1978). The brainstem, hypothalamus, neostriatum, cortex and hippocampus were retained separately. Immediately after dissection, tissues were frozen in liquid nitrogen and then transferred to a freezer (-70 degrees F) until assayed for NE, DA and serotonin by a modification of the fluorescence method of Jacobowitz and Richardson (1978) as described in Ings (1980). Each brain part sampled was used as its own blank in the fluorescence calculations.

Results

The Analysis of Variance summary tables for each amine in each brain part assayed are presented in the Appendix, Tables 3a to 3k. The means ($\pm$ S.E.M.) for each of the treatment groups are presented in Table 3-1. Infusion of 6-OHDA into the DNB resulted in NE depletion in the cortex to 5% of the level of vehicle infused rats, to 2% in hippocampus and to 28% in the hypothalamus. Brainstem NE was significantly elevated to 122% of vehicle infused controls. The DNB lesioned rats also had significant depletions of DA and serotonin in the neostriatum. DA was 68% and serotonin was 86% of control levels in the neostriatum.

There was no alteration of serotonin in the cortex, hippocampus, hypothalamus or brainstem and no alteration of DA in the hypothalamus due to the 6-OHDA infusions into the DNB.

Adrenalectomy caused a slight but significant increase in serotonin levels in the hippocampus to 107% of sham operated rats. Further, Metopirone treated rats showed significantly increased serotonin levels in the hypothalamus to 108% of vehicle injected rats. Even though the lesion by drug interaction was not significant [$F(1,48)=3.63$, $p < .065$] analysis of this interaction effect using the Newman-Keuls procedure for post-hoc comparisons revealed that hypothalamic serotonin concentrations were higher (by 13%) in DNB+MET rats compared to DNB rats but not in group MET compared to group S (2% increase) (See Table 3-2).

Analysis of the lesion by drug interaction for NE in the

brainstem using the Newman-Keuls procedure for post-hoc comparisons revealed that while adrenalectomy had no effect in sham-lesioned rats the DNB-lesioned rats that were also adrenalectomized had lower NE levels than DNB-lesioned rats receiving sham-adrenalectomies (Table 3-3).

These results of 6-OHDA infusion into the DNB are generally consistent with previous reports from this laboratory (Pappas et al, 1978) and from other laboratories (Ogren & Fuxe, 1974; 1977; Roberts & Fibiger, 1977; Mason et al, 1979; Wendlend & File, 1979). The somewhat greater depletion of NE in the hypothalamus and of DA in the neostriatum observed in the present experiment is an indication that the 6-OHDA infusions were placed slightly more ventral than in previous studies. Thus, the 6-OHDA infusions probably damaged more fibers from the ventral noradrenergic bundle (leading to lower hypothalamic NE) and more fibers from the nigrostriatal pathway (leading to lower DA levels in the neostriatum).

Plasma Corticosterone:

Analysis of Variance performed on the baseline corticosterone data revealed a main effect due to surgery [$F(1,88)=14.11$, $p < .001$] (See Table 4a). Baseline corticosterone levels (taken at the time of decapitation approximately 6 weeks following the operation) were 43% lower in adrenalectomized rats ($x = 9.16 \pm 0.96$ ug/100 ml plasma) than in sham rats ($x = 15.96 \pm 1.53$ ug/100 ml plasma). The mean corticosterone level in adrenalectomized

rats in the present experiment (57% of sham levels) was higher than in Experiment one (25% of shams). The major difference between the two experiments was that blood samples were taken 6 weeks following adrenalectomy in the present experiment while samples were taken 3 weeks following adrenalectomy in Experiment one. Regeneration of any residual adrenal tissue or compensatory development of the extra-adrenal source of corticosterone (as yet unidentified) may explain these findings.

Analysis of Variance performed on the corticosterone values determined from plasma collected immediately following the avoidance sessions revealed significant main effects due to surgery [$F(1,88)=508.51$, $p < .001$], drug administration [$F(1,88)=141.24$, $p < .001$] and day of training [$F(1,88)=3.98$, $p < .05$] and a significant surgery by drug interaction [$F(1,88)=113.82$, $p < .001$] (Table 4b). Adrenalectomized rats had plasma corticosterone levels which were only 18% of the level for sham operated rats ($x = 6.52 \pm 0.37$ vs 35.43 ± 1.79 ug/100 ml plasma) and Metopirone treated rats had plasma corticosterone levels which were 47% of those for vehicle treated rats ($x = 13.36 \pm 0.89$ vs 28.59 ± 2.39 ug/100 ml plasma). Although the Analysis of Variance indicated a significant day of training effect, inspection of the mean plasma corticosterone levels for day one and day two of training revealed differences of no real importance ($x = 20.15 \pm 1.87$ vs 21.79 ± 2.06 ug/100 ml plasma respectively).

Analysis of the surgery by drug interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that

sham operated, Metopirone treated rats had plasma corticosterone levels significantly lower (42%) than sham operated, vehicle treated rats but that adrenalectomized, Metopirone treated rats had corticosterone levels not significantly different (79%) than adrenalectomized, vehicle treated rats (See Table 4-1). However, as in Experiment one Metopirone reduced the variability in plasma corticosterone concentrations in adrenalectomized rats [$X(47)=17.00$, $p < .005$]. This reduction in variance may indicate that a floor effect, caused by the corticosterone assay procedure not being able to read values accurately below about 3 ug/100 ml plasma, existed which artificially elevated very low corticosterone readings. The majority of the very low corticosterone readings most likely were from adrenalectomized rats treated with Metopirone.

Pain Sensitivity:

Analysis of Variance performed on the data from the tail-flick procedure revealed only a significant main effect due to drug administration [$F(1,88)=10.12$, $p < .005$] (Table 5a). The latency until the first flick of the tail was elevated in Metopirone treated rats compared to vehicle injected rats ($x = 2.63 \pm 0.10$ vs 2.12 ± 0.09 seconds).

Analysis of Variance performed on the data from the hot-plate revealed significant main effects due to surgery [$F(1,88)=3.97$, $p < .05$] and drug administration [$F(1,88) = 7.37$, $p < .01$], as well as a lesion by surgery interaction [$F(1,88)=4.88$, $p < .05$] and a

lesion by drug by day interaction [$F(1,88)=4.24$, $p < .05$] (Table 5b). Adrenalectomized rats had a longer latency to respond to the heated plate than intact rats ($x = 3.44 \pm 0.29$ vs 2.74 ± 0.14 seconds). Analysis of the lesion by surgery interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that rats in the DNB+ADX groups had an elevated latency to respond compared to the DNB, ADX and Sham groups, which did not differ (See Table 5-1).

Metopirone treated rats had increased latencies compared to vehicle injected rats ($x = 3.56 \pm 0.27$ vs 2.61 ± 0.16 seconds). Analysis of the lesion by drug by day interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that the DNB+MET rats had elevated response latencies on the second test day compared to DNB, ADX and Sham rats but that the response latency for DNB+MET rats did not differ from the other groups on the first test day (See Table 5-2).

Intertrial Activity:

Analysis of Variance performed on the intertrial crossing (ITC) data revealed significant main effects due to surgery [$F(1,88)=11.93$, $p < .005$], drug administration [$F(1,88)=11.93$, $p < .001$], day of testing [$F(1,88)=11.73$, $p < .001$], block of trials [$F(1,88)=20.39$, $p < .001$], as well as significant interactions for surgery by day of testing [$F(1,88)=6.54$, $p < .05$] and day by block of trials [$F(1,88)=4.46$, $p < .05$] (Table 6a). Adrenalectomized rats made fewer ITCs than did intact rats

Figure 2. The mean ($\pm$ S.E.M.) number of intertrial crossings per block of 50 trials is presented for all eight treatment groups as a function of block of trials and day of test.

INTERTRIAL CROSSINGS
(PER BLOCK OF 50 TRIALS)

($x = 4.99 \pm 0.51$ vs 8.89 ± 0.72 crossings) and Metopirone treated rats made fewer ITCs than vehicle injected rats ($x = 4.85 \pm 0.52$ vs 9.03 ± 0.71 crossings). Overall, rats made more ITCs on the second compared to the first day of testing ($x = 5.38 \pm 0.56$ vs 8.5 ± 0.70 crossings) and on both days rats made more ITCs during the first 50 trials than during the last 50 trials of the avoidance session ($x = 5.83 \pm 0.52$ vs 8.05 ± 0.73).

Analysis of the surgery by day of testing interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that intact rats made more ITCs than adrenalectomized rats on the second test day but not on the first day of testing (See Table 6-1).

Analysis of the test day by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that rats made more ITCs during the last 50 trials than during the first 50 trials of the avoidance session on the second day of testing but not on the first day of testing (See Table 6-2).

Escape Latency:

Analysis of Variance performed on the escape response latency data revealed significant main effects due to surgery [$F(1,88)=8.73$, $p < .005$] and block of trials [$F(2,176)=10.61$, $p < .001$] (Table 7a). The latency to perform the unconditioned escape response was higher for adrenalectomized rats compared to intact rats ($x = 9.61 \pm 0.27$ vs 8.40 ± 0.19 seconds). Analysis of the block of trials main effect using the Newman-Keuls procedure

for post-hoc comparisons revealed that overall, the mean escape response latency was higher for the first 10 escape responses on the first test day ($x = 9.81 \pm 0.31$ seconds) than for both the last 10 escape responses of the first test day and the first 10 escape responses on the second test day (See Table 7-1).

Avoidance Performance:

The mean response latency (i.e. this includes both the unconditioned escape responses and conditioned avoidance responses) for each block of 20 trials on both the first and second test days for all eight treatment groups is presented in Figure 2. Analysis of Variance performed on the response latency data revealed main effects due to surgery [$F(1,88)=13.95$, $p < .001$], drug administration [$F(1,88)=7.91$, $p < .01$], day of test [$F(1,88)=6.72$, $p < .05$] and block of trials [$F(4,352)=78.59$, $p < .001$] as well as significant interactions for surgery by block of trials [$F(4,352)=4.99$, $p < .001$], drug by block of trials [$F(4,352)=3.81$, $p < .01$] and test day by block of trials [$F(4,352)=6.75$, $p < .001$] (Table 8a). It should also be pointed out that the main effect due to lesion was very close to the 5% significance level [$F(1,88)=3.29$, $p < .075$], indicating that there was a tendency for DNB-lesioned rats to respond more quickly in the shuttle-box than sham-lesioned rats ($x = 5.34 \pm 0.17$ vs 6.18 ± 0.16 seconds).

Adrenalectomized rats had longer response latencies than intact rats ($x = 6.62 \pm 0.20$ vs 4.90 ± 0.11 seconds) and

Figure 3. The mean ($\pm$ S.E.M.) response latency (in seconds) per block of 20 trials for all eight groups is presented as a function of block of trials and day of test.

Metopirone treated rats took longer to respond than vehicle injected rats ($x = 6.41 \pm 0.18$ vs 5.11 ± 0.15 seconds). In general, rats had longer response latencies on the first test day than on the second test day ($x = 6.23 \pm 0.16$ vs 5.29 ± 0.17 seconds). Analysis of the block of trials main effect using the Newman-Keuls procedure for post-hoc comparisons revealed that response latencies got progressively shorter across blocks of trials. Response latencies were longer on the first block of trials than on blocks 2, 3, 4 & 5, longer on the second block of trials than on blocks 3, 4 & 5, and longer on the third block of trials than on blocks 4 & 5, whereas there was no difference between response latencies on the fourth and fifth blocks of trials (See Table 8-1).

Analysis of the surgery by block interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that adrenalectomized rats showed a gradual decrease in response latency across blocks of trials while intact rats did not show this gradual decrease across trials. In adrenalectomized rats, the response latency for block 1 was longer than for each of blocks 2, 3, 4 & 5, the response latency for block 2 was longer than for each of blocks 3, 4 & 5, the response latency for block 3 was longer than for block 4 & 5, and there was no difference between blocks 4 & 5 (See Table 8-2). For sham operated rats, there was no difference in response latency among blocks 3, 4 & 5, the response latency for block 2 was longer than blocks 3 & 4 but not different from block 5, whereas the response latency for block 1 was longer than for each of blocks 2, 3, 4 & 5 (See Table

8-2).

Analysis of the drug ~~by~~ block of trials interaction using the Newman-Keuls procedure for post-hoc analysis revealed that, like the adrenalectomized rats, the Metopirone treated rats showed a gradual decrease in response latency across blocks of trials but the vehicle treated rats did not show this gradual progression (See Table 8-3). In Metopirone treated rats, the response latency was longer for the first block of trials than for each of blocks 2, 3, 4 & 5, for the second block of trials than for each of blocks 3, 4 & 5, and for the third block of trials than for each of blocks 4 & 5, while the response latencies for the fourth and fifth blocks did not differ (See Table 8-3). In vehicle treated rats, the response latency for the first block of trials was longer than for each of blocks 2, 3, 4 & 5 and there were no other differences among blocks of trials (See Table 8-3).

Analysis of the day of test by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that rats had longer response latencies on the first compared to the second test day during blocks 1 and 2 but not during blocks 3, 4 & 5 (See Table 8-4).

The mean number of avoidances for each block of 20 trials on both the first and second test days for all eight groups is presented in Figure 3. Analysis of Variance performed on the avoidance response data revealed main effects due to surgery [$F(1,88)=4.52$, $p < .05$], drug administration [$F(1,88)=15.37$, $p < .001$], day of test [$F(1,88)=94.82$, $p < .001$] and block of trials

Figure 4. The mean ($\pm$ S.E.M.) number of avoidance responses per block of 20 trials for all eight groups is presented as a function of blocks of trials and day of test.

SHAM SHAM VEH SHAM SHAM VEH DNB SHAM VEH
 SHAM SHAM MET SHAM SHAM MET DNB SHAM MET
 SHAM ADX VEH SHAM ADX VEH DNB ADX VEH
 SHAM ADX MET SHAM ADX MET DNB ADX MET

NUMBER OF AVOIDANCES

DAY 2

DAY 1

BLOCKS OF 20 TRIALS

[$F(4,352)=201.55$, $p < .001$] as well as significant interactions for drug treatment by day of test [$F(1,88)=4.52$, $p < .05$], drug treatment by block of trials [$F(4,352)=3.86$, $p < .005$], day of test by block of trials [$F(4,352)=8.12$, $p < .001$], surgery by day of test by block of trials [$F(4,352)=4.17$, $p < .005$] and drug by test by block of trials [$F(4,352)=2.42$, $p < .05$] (Table 9a).

It should also be pointed out that the main effect due to lesion was very close to the 5% significance level [$F(1,88)=3.83$, $p < .055$] suggesting that there was a tendency for DNB-lesioned rats to make more conditioned avoidance responses (CARs) than sham-lesioned rats ($x = 12.60 \pm 0.33$ vs 10.60 ± 0.35 CARs). Adrenalectomized rats made significantly fewer CARs than intact rats ($x = 10.29 \pm 0.35$ vs 12.90 ± 0.33 CARs), Metopirone treated rats made significantly fewer CARs than vehicle treated rats ($x = 9.60 \pm 0.36$ vs 13.60 ± 0.31 CARs) and overall, all rats made fewer CARs during the first as compared to the second avoidance session ($x = 9.63 \pm 0.35$ vs 13.57 ± 0.32 CARs). Analysis of the blocks of trials main effect using the Newman-Keuls procedure for post-hoc comparisons revealed that the rats made fewer CARs during the first block of trials than during each of blocks 2, 3, 4 & 5, fewer CARs during the second block of trials than during each of blocks 3, 4 & 5, fewer CARs during the third block of trials than during each of blocks 4 & 5, while there was no difference in the number of CARs during the fourth and fifth blocks of trials (See Table 9-1).

Analysis of the drug treatment by day of test interaction using the Newman-Keuls procedure for post-hoc comparisons

revealed no information that was not already indicated by the main effects of drug treatment and day of test (See Table 9-2), which was not surprising since this interaction effect was only marginally significant at the 5% level (See Table 9a).

Analysis of the drug treatment by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that the main effect of a progressive increase in the number of CARs across blocks of trials was observed in Metopirone treated rats but vehicle treated rats showed increases on the first two blocks of trials only (See Table 9-3). Metopirone treated rats made fewer CARs during the first block of trials than during each of blocks 2, 3, 4 & 5, fewer CARs during the second block of trials than during each of blocks 3, 4 & 5 and fewer CARs during the third block of trials than during blocks 4 & 5, while the number of CARs for the fourth and fifth blocks of trials did not differ (See Table 9-3). Vehicle treated rats made fewer CARs during the first block of trials than during each of blocks 2, 3, 4 & 5 and they made fewer CARs during the second block of trials than during each of blocks 3, 4 & 5, while the number of CARs during the third, fourth and fifth blocks of trials did not differ (See Table 9-3).

Analysis of the day of test by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that for the first test day rats made fewer CARs on block 1 than on blocks 2, 3, 4 & 5, on block 2 than on blocks 3, 4 & 5, on block 3 than on blocks 4 & 5, and blocks 4 & 5 did not differ, whereas on the second test day rats showed the majority

68

of their increase across trials from the first to the second block (See Table 9-4).

Analysis of the surgery by day of test by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that the day of test by block of trials interaction held for sham-operated rats but not for adrenalectomized rats. Inspection of mean numbers of CARs presented in Table 9-5 revealed that sham rats showed an increase in the number of CARs across blocks of trials which was more noticeable for the first compared to the second test day, whereas adrenalectomized rats showed increases on the first three blocks of trials on both test days.

Analysis of the drug treatment by day of test by block of trials interaction using the Newman-Keuls procedure for post-hoc comparisons revealed that the day of test by block of trials interaction held only for Metopirone treated rats and not for vehicle injected rats. Inspection of the mean numbers of CARs presented in Table 9-6 indicates that the vehicle treated rats showed the majority of their increase across blocks of trials between the first and second blocks for both test days, whereas Metopirone treated rats showed gradual increase in the number of CARs across blocks of trials on the first test day only and made the majority of their increase in the number of CARs across blocks of trials between blocks one and two on the second test day.

Discussion

Lesion of the DNB by intracerebral infusion of 6-OHDA caused virtually total depletion of NE in the forebrain of rats and a lesser depletion of NE in the hypothalamus. An increase in NE concentration in the brainstem was also observed following these lesions, presumably reflecting compensatory mechanisms which are discussed elsewhere by Pappas et al (1978). Moderate depletion of DA to 68% of control levels occurred in the neostriatum of DNB-lesioned rats. This amount of DA depletion should not have affected avoidance performance in the present experiment since previous studies have shown that depletions of DA to as much as 30 or 40% of control levels (induced by intraventricular infusion of 6-OHDA) did not affect acquisition of a CAR (Cooper, Breese, Howard & Grant, 1972; Cooper, Breese, Grant & Howard, 1973). These authors also reported that depletions of DA to 76% of control levels combined with depletion of NE to 39% of control levels facilitated acquisition of shuttle-box avoidance behaviour, a behavioural effect which has been reported following NE depletion alone (Mason & Fibiger, 1979). Consistent with these reports, DNB-lesioned rats in the present experiment tended to acquire a CAR more quickly than sham-lesioned rats (See Figures 1 and 2; DNB rats are represented by closed symbols in both figures).

Removal of both adrenal glands was a more effective method than inhibition of 11-B-hydroxylase activity (by administration of Metopirone) in reducing plasma corticosterone levels both in

unstressed rats (Experiments one and two) and in rats exposed to avoidance training (Experiment three), although the major behavioural effects of these two treatments were similar. Further, there was some evidence that Metopirone reduced plasma corticosterone concentrations in adrenalectomized rats in a dose-dependent manner, although this must be varified with a more sensitive assay procedure than was used in the present experiment. Both adrenalectomy and Metopirone treatment reduced the mean number of ITCs made per block of 50 trials in the shuttle-box. Both treatments also impaired acquisition of the CAR in the shuttle-box, as was indicated by both a higher mean response latency and a lower mean number of CARs per block of 20 trials. Since both treatments reduced intertrial activity in the shuttle-box and retarded acquisition of the CAR, it might be suggested that the avoidance deficits were a result of the general reduction in activity seen in these rats. Other studies have also reported decreased intertrial activity in rats showing impaired CAR acquisition (eg. Cooper et al, 1972; 1973) and no difference in intertrial activity when there were no differences in CAR performance (eg. Wendlendt & File, 1979).

The acquisition impairment observed three weeks following adrenalectomy in the present experiment is consistent with a recent study by Ogren et al (1981). These authors reported that a deficit in CAR acquisition was not observed until at least 14 days following adrenalectomy, and that at 7 or 10 days after the operation adrenalectomized rats acquired the CAR more quickly than intact rats did. The Ogren et al study and the present

experiment are the only two reports of an active avoidance deficit in adrenalectomized rats. Previous studies showed either no effect or facilitated acquisition (Weiss et al, 1969; Ogren & Fuxe, 1974; 1977) when rats were tested 7 to 10 days following adrenalectomy, the latter finding of course consistent with Ogren et al's results which showed increased acquisition at this time. Reduction of plasma corticosterone by administration of a single dose of Metopirone two hours before testing also impaired acquisition of a CAR in the present experiment. This is, to my knowledge, the first report of the effect of Metopirone on the acquisition of a conditioned response. Earlier, Halmy, Bohus, Frey & Endroczi (1970) had reported that Metopirone caused an impairment in the acquisition of an one-trial passive avoidance task. It was difficult to determine the importance of this finding since there was no experimental protocol or data presented concerning this effect.

The effects of adrenalectomy and Metopirone treatment were not, however, similar in all tests that were carried out. Only the Metopirone treated rats showed reduced responsiveness to pain as indicated by both the tail-flick and hot-plate procedures, whereas, only adrenalectomized rats had an escape deficit in the shuttle-box (shown by a significantly elevated escape response latency). The reduced responsiveness to pain in Metopirone treated rats may be due to the increase in B-endorphin release which occurs within several hours following administration of Metopirone (eg. Nakao et al, 1978; Hary et al, 1981). When B-endorphin levels are maintained chronically at high levels, as

after chronic Metopirone treatment for eight weeks or after 3 weeks following adrenalectomy, hyperalgesia may even be observed (Mousa, Miller & Couri, 1981). This is consistent with the present report that an analgesic effect is only observed following acute Metopirone treatment and not three weeks following adrenalectomy.

It might be suggested that the avoidance deficit in Metopirone treated rats was the result of their reduced responsiveness to painful or noxious stimuli. However, doses of morphine that produce reliable analgesia (2-4 mg/kg) actually enhance the acquisition of a shuttle-box CAR (eg. Ageel, Chin, Trafton, Jones & Piochioni, 1976).

The escape deficit observed in adrenalectomized rats might indicate that these rats had an increased tendency to freeze in response to the shock, which is consistent with previous studies indicating that adrenalectomized rats were more fearful than intact rats (Weiss et al, 1969).

A possible explanation for the avoidance acquisition deficit in adrenalectomized rats has been put forward recently. Ogren et al offer the suggestion that a secondary increase in circulating ACTH levels, which reaches its peak two to three weeks following adrenalectomy (Cox, Hodges & Vernikos, 1958) is responsible for the impairment in CAR acquisition in adrenalectomized rats. Ogren et al made this claim based on their finding that daily injection of dexamethasone but not corticosterone, at doses at which dexamethasone was more potent at inhibiting ACTH release than corticosterone was, prevented this behavioural deficit. It

is also possible that the Metopirone-induced avoidance deficit was the result of an increase in ACTH secretion. The decrease in circulating corticosterone levels following administration of Metopirone causes an increase in the release of ACTH by way of eliminating the normal inhibitory effect of this steroid on the synthesis and release of ACTH (Rossier et al, 1979; Andersson et al, 1981; Hary et al, 1981). Further work is required, however, to rule out the possibility that this avoidance deficit is due to other secondary changes which are known to occur following Metopirone administration.

Following the Metopirone-induced suppression of 11-B-hydroxylase activity and the subsequent decrease in circulating corticosterone levels an increase of the precursors, 11-deoxycorticosterone and 11-deoxycortisol occurs (Jenkins, Meakin, Nelson & Thorn, 1958; Liddle, Lowe & Harris, 1958; Bongiovanni & Root, 1963). These authors were unable to detect 11-deoxycortisol in untreated animals. The increased production of these and other steroids following the experimental blockade of a normal synthetic pathway (See Samuels & Nelson, 1975) may lead to abnormal physiological and/or behavioural responses in the animal, and might have mediated the avoidance deficit seen in the present experiment. Finally, it is not possible to rule out a direct effect of Metopirone on the brain, as has been suggested by one group of investigators (Halmy et al, 1970).

In the present experiment there was no evidence that corticosterone depletion combined with depletion of brain NE

impaired the acquisition of a conditioned active avoidance response more than either depletion did alone. In fact, when the DNB lesion was combined with adrenalectomy, Metopirone, or adrenalectomy plus Metopirone there was a tendency for performance to be facilitated. This is clearly seen in Figures 1 and 2, where most of the DNB groups (represented by filled symbols in both figures) are shown to have better performance than their appropriate control groups which received a sham lesion. These results support those of Wendlendt and File (1979) who found that there was no difference between DNB+ADX rats and Sham rats three weeks following the operations.

There appears to be little doubt that Ogren and coworkers have identified an interaction between ~~corticosterone~~ and brain NE controlling some aspect of avoidance behaviour, since their effect has been replicated a number of times (Ogren & Fuxe, 1974; 1977; Ogren et al, 1981; Roberts, D.C.S., 1980, personal communication). Furthermore, that DNB+ADX animals show a passive avoidance deficit has also ^{been} reproduced in several laboratories (Roberts & Fibiger, 1977; Mason, et al, 1979; Wendlendt & File, 1979; Wenzel et al, 1980). However, the inability of at least two laboratories to reproduce the original shuttle-avoidance effect, including the attempt to further deplete corticosterone in adrenalectomized rats in the present experiment, indicates that this effect may be dependent on some essential parameter(s) of the conditioning situation that differ among the reports. For example, both Wendlendt & File and the present author used massed presentation of trials while Ogren and coworkers used 20 trials

per day during acquisition training. Ogrén's group also used a relatively high shock intensity compared to that used by Wendlent and File and the present author. This dependence of the effect on parameters of the learning situation leads one to believe that the alteration in avoidance performance caused by the combination of DNB and adrenalectomy is of a non-associative nature rather than the reflection of a strict learning or memory deficit. Future experiments should determine which parameters are essential to the appearance of the avoidance deficit.

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APPENDIX A

Table 1a

Analysis of Variance for Plasma Corticosterone
Concentrations as a Function of Surgery and
Drug Treatment and Time of Blood Collection

Source	df	SS	MS	F	SIG
Surgery	1	5609.00	5609.00	72.04	p<.001
Drug Treatment	2	83.78	41.89	0.54	.588
Surgery x Drug Treatment	2	154.91	76.96	0.99	.381
Error	42	3270.20	77.86		
Time of Blood Collection	1	1542.40	1542.40	34.42	p<.001
Surgery x Time of Blood Collection	1	557.77	557.77	12.45	.001
Drug Treatment x Time of Blood Collection	2	68.43	34.21	.76	.472
Surgery x Drug Treatment x Time of Blood Collection	2	18.63	9.32	.21	.813
Error	42	1882.20	44.82		
Total	95	13186.00			

Table 2a

Analysis of Variance Summary Table for Plasma Corticosterone
Concentrations as a Function of Drug Treatment
and Method of Blood Collection

Source	df	SS	MS	F	SIG
Drug treatment	1	4310.60	4310.60	41.90	p<.001
Method of blood collection	1	292.82	292.82	2.85	.103
Drug treatment x Method of blood collection	1	295.25	295.25	2.87	.101
Within cells	28	2880.40	102.87		
Total	31	7779.00			

Table 3a

Analysis of Variance for Norepinephrine Concentration
in the Cortex as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.46057	.46057	525.09	p<.001
Surgery	1	.18931E-02	.18931E-02	2.16	.148
Lesion x Surgery	1	.22530E-02	.22530E-02	2.57	.116
Drug Treatment	1	.31209E-03	.31209E-03	0.36	.554
Lesion x Drug Treatment	1	.31778E-03	.31778E-03	0.36	.550
Surgery x Drug Treatment	1	.35000E-05	.35000E-05	0.00	.950
Lesion x Surgery x Drug Treatment	1	.46068E-04	.46068E-04	0.05	.820
Within cells	48	.42102E-01	.87713E-03		
Total	55	.50750			

Table 3b

Analysis of Variance for Norepinephrine Concentration in the
Hippocampus as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.65810	.65810	672.67	p<.001
Surgery	1	.34850E-03	.34850E-03	0.36	.553
Lesion x Surgery	1	.24070E-03	.24070E-03	0.25	.622
Drug Treatment	1	.67693E-03	.67693E-03	0.69	.410
Lesion x Drug Treatment	1	.38660E-03	.38660E-03	0.40	.533
Surgery x Drug Treatment	1	.10175E-02	.10175E-02	1.04	.313
Lesion x Surgery x Drug Treatment	1	.93058E-03	.93058E-03	0.95	.334
Within cells	48	.46960E-01	.97833E-03		
Total	55	.70866			

Table 3c

Analysis of Variance for Norepinephrine Concentrations in
the Hypothalamus as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	3.9134	3.9134	154.04	<.001
Surgery	1	.19751E-01	.19751E-01	0.78	.382
Lesion x Surgery	1	.33277E-01	.33277E-01	1.31	.258
Drug Treatment	1	.15795E-01	.15795E-01	0.62	.434
Lesion x Drug Treatment	1	.45198E-02	.45198E-02	0.18	.675
Surgery x Drug Treatment	1	.20725E-01	.20725E-01	0.82	.371
Lesion x Surgery x Drug Treatment	1	.64697E-02	.64697E-02	0.25	.616
Within cells	48	1.2194	.25404E-01		
Total	55	5.2333			

Table 3d

Analysis of Variance for Norepinephrine Concentrations in
the Brainstem as a Function of Treatment Groups

Source	df	SS	MS	F	SIG.
Lesion	1	.10401	.10401	25.23	<.001
Surgery	1	.20232E-02	.20232E-02	0.49	.487
Lesion x Surgery	1	.27893E-01	.27893E-01	6.77	.012
Drug Treatment	1	.73878E-03	.73878E-03	0.18	.674
Lesion x Drug Treatment	1	.11479E-03	.11479E-03	0.03	.868
Surgery x Drug Treatment	1	.44607E-02	.44607E-02	1.08	.303
Lesion x Surgery x Drug Treatment	1	.21354E-02	.21354E-02	0.52	.475
Within cells	48	.19790	.41230E-02		
Total	55	.33928			

Table 3e

Analysis of Variance for Dopamine Concentrations in the
Neostriatum as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	38.293	38.293	41.92	<.001
Surgery	1	.19130	.19130	0.21	.649
Lesion x Surgery	1	.17440E-01	.17440E-01	0.02	.891
Drug Treatment	1	.59209	.59209	0.65	.425
Lesion x Drug Treatment	1	.17741E-05	.17741E-05	0.00	.999
Surgery x Drug Treatment	1	.12025E-01	.12025E-01	0.01	.909
Lesion x Surgery x Drug Treatment	1	.17770	.17770	0.19	.661
Within cells	48	43.844	.91342		
Total	55	83.128			

Table 3f

Analysis of Variance for Serotonin Concentrations in the
Neostriatum as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.16799	.16799	8.56	.005
Surgery	1	.21639E-01	.21639E-01	1.10	.299
Lesion x Surgery	1	.23477E-01	.23477E-01	1.20	.280
Drug Treatment	1	.64286E-02	.64286E-02	0.33	.570
Lesion x Drug Treatment	1	.31171E-02	.31171E-02	0.16	.692
Surgery x Drug Treatment	1	.10441E-02	.10441E-02	0.05	.819
Lesion x Surgery x Drug Treatment	1	.38913E-03	.38913E-03	0.02	.889
Within cells	46	.94214	.19628E-01		
Total	55	1.1662			

Table 3g

Analysis of Variance for Serotonin Concentrations in the
Cortex as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.48156E-02	.48156E-02	1.02	.318
Surgery	1	.68798E-02	.68798E-02	1.46	.233
Lesion x Surgery	1	.15445E-05	.15445E-05	0.00	.986
Drug Treatment	1	.45342E-02	.45342E-02	0.96	.332
Lesion x Drug Treatment	1	.42788E-02	.42788E-02	0.91	.346
Surgery x Drug Treatment	1	.18366E-02	.1836E-02	0.39	.536
Lesion x Surgery x Drug Treatment	1	.16906E-03	.16906E-03	0.04	.851
Within cells	48	.22669	.47327E-02		
Total	55	.24921			

Table 3h

Analysis of Variance for Serotonin Concentrations in the
Hippocampus as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.23660E-04	.23660E-04	0.01	.918
Surgery	1	.17332E-01	.17332E-01	7.75	.008
Lesion x Surgery	1	.56964E-02	.56964E-02	2.55	.117
Drug Treatment	1	.50236E-02	.50236E-02	2.26	.140
Lesion x Drug Treatment	1	.16070E-04	.16070E-04	0.01	.933
Surgery x Drug Treatment	1	.15779E-03	.15779E-03	0.07	.792
Lesion x Surgery x Drug Treatment	1	.15815E-02	.15815E-02	0.71	.404
Within cells	48	.10730	.22354E-02		
Total	55	.13713			

Table 3i

Analysis of Variance for Serotonin Concentrations in the
Hypothalamus as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.45198E-02	.45198E-02	0.05	.817
Surgery	1	.11891	.11891	1.43	.238
Lesion x Surgery	1	.34353E-03	.34353E-03	0.00	.949
Drug Treatment	1	.59203	.59203	7.12	.010
Lesion x Drug Treatment	1	.30154	.30154	3.63	.063
Surgery x Drug Treatment	1	.11622	.11622	1.40	.243
Lesion x Surgery x Drug Treatment	1	.30266E-02	.30266E-02	0.04	.850
Within cells	48	3.9915	.83156E-01		
Total	55	5.1281			

Table 3j

Analysis of Variance for Serotonin Concentrations in the
Brainstem as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.23040E-02	.23040E-02	0.56	.457
Surgery	1	.38379E-02	.38379E-02	0.94	.338
Lesion x Surgery	1	.63431E-02	.63431E-02	1.55	.219
Drug Treatment	1	.82717E-02	.82717E-02	2.02	.162
Lesion x Drug Treatment	1	.14964E-01	.14964E-01	3.65	.062
Surgery x Drug Treatment	1	.43402E-02	.43402E-02	1.06	.309
Lesion x Surgery x Drug Treatment	1	.26606E-04	.26606E-04	0.01	.936
Within cells	48	.19668	.40974E-02		
Total	55	.23676			

Table 3k

Analysis of Variance for Dopamine Concentrations in the
Hypothalamus as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	.95004E-02	.95004E-02	1.08	.303
Surgery	1	.11250E-02	.11250E-02	0.13	.722
Lesion x Surgery	1	.48844E-02	.48844E-02	0.56	.459
Drug Treatment	1	.90018E-04	.90018E-04	0.01	.920
Lesion x Drug Treatment	1	.55402E-02	.55402E-02	0.63	.431
Surgery x Drug Treatment	1	.56682E-02	.56682E-02	0.65	.425
Lesion x Surgery x Drug Treatment	1	.15846E-03	.15846E-03	0.02	.894
Within cells	48	.42073	.87652E-02		
Total	55	.44770			

Table 4a

Analysis of Variance Summary Table for Baseline

Plasma Corticosterone Concentrations

as a Function of Treatment Groups

Source	df	SS	MS	F	SIG
Lesion	1	21.57	21.57	0.27	.602
Surgery	1	1109.10	1109.10	14.11	$p < .001$
Lesion x Surgery	1	5.95	5.95	0.08	.784
Drug Treatment	1	24.50	24.05	0.31	.578
Lesion x Drug Treatment	1	295.75	295.75	3.76	.056
Surgery x Drug Treatment	1	39.40	39.40	0.50	.481
Lesion x Surgery x Drug Treatment	1	68.85	68.85	0.88	.352
Within cells	88	6916.40	78.60		
Total	95	8481.50			

Table 4b

Analysis of Variance for Plasma Corticosterone
Concentration as a Function of Treatment

Groups and Day of Test

Source	df	SS	MS	F	SIG
Lesion	1	3.41	3.41	0.04	.836
Surgery	1	40113.00	40113.00	508.51	$p < .001$
Lesion x Surgery	1	15.18	15.18	0.19	.662
Drug Treatment	1	11142.00	11142.00	141.24	$p < .001$
Lesion x Drug Treatment	1	78.28	78.28	0.99	.322
Surgery x Drug Treatment	1	8979.00	8979.00	113.82	$p < .001$
Lesion x Surgery x Drug Treatment	1	56.54	56.41	0.72	.400
Error	88	6941.80	78.89		
Day of Test	1	129.36	129.36	3.98	.049
Lesion x Day of Test	1	58.52	58.52	1.80	.183
Surgery x Day of Test	1	112.84	112.84	3.47	.066
Lesion x Surgery x Day of Test	1	30.43	30.43	0.94	.336
Drug Treatment x Day of Test	1	79.31	79.31	2.44	.122
Lesion x Drug Treatment x Day of Test	1	19.38	19.38	0.60	.442

Table 4b cont'd

Source	df	SS	MS	F	SIG
Surgery					
x Drug Treatment	1	16.23	16.23	0.50	.482
x Day of Test	1	16.23	16.23	0.50	.482
Lesion					
x Surgery	1	0.15	0.15	0.00	.946
x Drug Treatment	1	0.15	0.15	0.00	.946
Error	88	2862.10	32.52		
Total	191	70637.00			

Table 5a

Analysis of Variance for the Response Latency
in the Tail-Flip Procedure as a Function
of Treatment Groups and Day of Test

Source	df	SS *	MS	F	SIG
Lesion	1	3.05	3.05	2.43	.123
Surgery	1	0.35	0.35	0.03	.867
Lesion x Surgery	1	1.30	1.30	1.03	.312
Drug Treatment	1	12.71	12.71	10.12	.002
Lesion x Drug Treatment	1	0.15	0.15	0.12	.729
Surgery x Drug Treatment	1	0.92E-01	0.92E-01	0.07	.787
Lesion x Surgery x Drug Treatment	1	1.37	1.37	1.09	.300
Error	1	110.57	1.26		
Day of Test	1	0.19	0.19	0.42	.521
Lesion x Day of Test	1	0.75	0.75	1.66	.201
Surgery x Day of Test	1	1.33	1.33	2.95	.087
Lesion x Surgery x Day of Test	1	0.56	0.56	1.25	.267
Drug Treatment x Day of Test	1	0.91	0.91	2.01	.160
Lesion x Drug Treatment x Day of Test	1	1.47	1.47	3.25	.075

Table 5a cont'd

Source	df	SS	MS	F	Sig
Surgery					
x Drug Treatment					
x Day of Test	1	0.24	0.24	0.53	.467
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test	1	0.75E-01	0.75E-01	0.02	.898
Error	88	39.75	0.45		
Total	191	174.49			

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Table 5b

Analysis of Variance for the Response Latency in
the Hot-plate procedure as a Function of
Treatment Groups and Day of Test

Source	df	SS	MS	F	SIG
Lesion	1	13.60	13.60	2.32	.131
Surgery	1	23.31	23.31	3.97	.049
Lesion x Surgery	1	28.60	28.60	4.88	.030
Drug Treatment	1	43.23	43.23	7.37	.008
Lesion x Drug Treatment	1	3.60	3.60	0.61	.435
Surgery x Drug Treatment	1	2.73	2.73	0.47	.497
Lesion x Surgery x Drug Treatment	1	15.24	15.24	2.60	.111
Error	88	516.14	5.87		
Day of Test	1	0.28E-01	0.28E-01	0.01	.923
Lesion x Day of Test	1	2.32E-01	2.32E-01	0.01	.923
Surgery x Day of Test	1	3.33	3.33	1.13	.291
Lesion x Surgery x Day of Test	1	0.12E-01	0.12E-01	0.00	.950
Drug Treatment x Day of Test	1	4.53	4.53	1.53	.219
Lesion x Drug Treatment x Day of Test	1	12.56	12.56	4.24	.042

Table 5b

Source	df	SS	MS	F	SIG
Surgery					
x Drug Treatment					
x Day of Test	1	2.02	2.02	0.68	.411
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test	1	9.06	9.06	3.06	.084
Error	88	260.47	2.96		
Total	191	940.78			

Table 6a

Analysis of Variance for the Total Number of Intertrial
Crossings per Block of 50 Trials in the Shuttle-box as
a Function of Treatment Groups and Blocks of Trials

Source	df	SS	MS	F	SIG
Lesion	1	77.04	77.04	0.55	.461
Surgery	1	1457.00	1457.00	10.38	.002
Lesion x Surgery	1	433.50	433.50	3.09	.082
Drug Treatment	1	1675.00	1675.00	11.93	p<.001
Lesion x Drug Treatment	1	17.51	17.51	0.12	.725
Surgery x Drug Treatment	1	21.09	21.09	0.15	.699
Lesion x Surgery x Drug Treatment	1	12.76	12.76	0.09	.764
Error	88	12356.00	140.41		
Day of Test	1	937.50	937.50	11.73	p<.001
Lesion x Day of Test	1	10.67	10.67	0.13	.716
Surgery x Day of Test	1	522.67	522.67	6.54	.012
Lesion x Surgery x Day of Test	1	160.17	160.17	2.00	.160
Drug Treatment x Day of Test	1	0.10E-01	0.10E-01	0.00	.991
Lesion x Drug Treatment x Day of Test	1	31.51	31.51	0.39	.532

Table 6a cont'd

Source	df	SS	MS	F	SIG
Surgery					
x Drug Treatment					
x Day of Test	1	162.76	162.76	2.04	.157
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test	1	15.84	15.84	0.20	.657
Error	88	7034.40	79.94		
Block of Trials	1	472.59	472.59	20.39	p<.001
Lesion					
x Block of Trials	1	0.10E-01	0.10E-01	0.00	.983
Surgery					
x Block of Trials	1	61.76	61.76	2.66	.106
Lesion					
x Surgery					
x Block of Trials	1	17.51	17.51	0.76	.387
Drug Treatment					
x Block of Trials	1	2.04	2.04	0.09	.767
Lesion					
x Drug Treatment					
x Block of Trials	1	0.67	0.67	0.03	.866
Surgery					
x Drug Treatment					
x Block of Trials	1	37.50	37.50	1.62	.207
Lesion					
x Surgery					
x Drug Treatment					
x Block of Trials	1	9.38	9.38	0.40	.526
Error	88	2040.00	23.18		

Table 6a cont'd

Source	df	SS	MS	F	SIG
Day of Test					
x Block of Trials	1	110.51	110.51	4.46	.038
Lesion					
x Day of Test					
x Block of Trials	1	4.59	4.59	0.19	.668
Surgery					
x Day of Test					
x Block of Trials	1	44.01	44.01	1.78	.186
Lesion					
x Surgery					
x Day of Test					
x Block of Trials	1	31.51	31.51	1.27	.263
Drug Treatment					
x Day of Test					
x Block of Trials	1	0.38	0.38	0.02	.902
Lesion					
x Drug Treatment					
x Day of Test					
x Block of Trials	1	63.38	63.38	2.56	.113
Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trials	1	3.38	3.38	0.14	.713
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trials	1	26.04	26.04	1.05	.308
Error	88	2181.70	24.79		
Total	383	30033.00			

Table 7a

Analysis of Variance for the Mean Escape Response Latency
per Block of 10 Trials in the Shuttle-box as a Function
of Treatment Groups and Block of Trials

Source	df	SS	MS	F	SIG
Lesion	1	8.57	8.57	0.71	.402
Surgery	1	105.67	105.67	8.73	.004
Lesion x Surgery	1	0.46	0.46	0.04	.847
Drug Treatment	1	13.06	13.06	1.08	.302
Lesion x Drug Treatment	1	16.39	16.39	1.35	.248
Surgery x Drug Treatment	1	3.79	3.79	0.31	.577
Lesion x Surgery x Drug Treatment	1	2.13	2.13	0.18	.676
Error	88	1065.60	12.11		
Block of Trials	2	104.61	52.31	10.61	$p < .001$
Lesion x Block of Trials	2	9.48	4.74	0.96	.384
Surgery x Block of Trials	2	22.92	11.46	2.32	.101
Lesion x Surgery x Block of Trials	2	11.28	5.64	1.14	.321
Drug Treatment x Block of Trials	2	3.17	1.59	0.32	.725
Lesion x Drug Treatment x Block of Trials	2	6.24	3.12	0.63	.532

Table 7a cont'd

Source	df	SS	MS	F	SIG
Surgery					
x Drug Treatment					
x Block of Trials	2	0.84	0.42	0.08	.919
Lesion					
x Surgery					
x Drug Treatment					
x Block of Trials	2	15.79	7.90	1.60	.205
Error	176	867.76	4.93		

Table 8a

Analysis of Variance for the Mean Response Latency per Block
 of 20 Trials in the Shuttle-box as a Function of Treatment
 Groups and Day of Test and Block of Trials

Source	df	SS	MS	F	SIG
Lesion	1	168.75	168.75	3.29	.073
Surgery	1	715.72	715.72	13.95	$p < .001$
Lesion x Surgery	1	7.85	7.85	0.15	.692
Drug Treatment	1	405.79	405.79	7.91	.006
Lesion x Drug Treatment	1	31.57	31.57	0.62	.435
Surgery x Drug Treatment	1	2.72	2.72	0.05	.618
Lesion x Surgery x Drug Treatment	1	81.68	81.68	1.59	.210
Error	88	4515.80	51.32		
Day of Test	1	216.14	216.14	6.72	.011
Lesion x Day of Test	1	13.68	13.68	0.43	.516
Surgery x Day of Test	1	1.16	1.16	0.04	.850
Lesion x Surgery x Day of Test	1	0.91	0.91	0.03	.867
Drug Treatment x Day of Test	1	49.93	49.93	1.55	.216
Lesion x Drug Treatment x Day of Test	1	4.76	4.76	0.15	.701

Table 8a cont'd

Source	df	SS	MS	F	SIG
Surgery x Drug Treatment x Day of Test	1	14.53	14.53	0.45	.503
Lesion x Surgery x Drug Treatment x Day of Test	1	22.13	22.13	0.69	.409
Error	88	2830.00	32.16		
Block of Trials	4	1096.80	274.19	78.59	$p < .001$
Lesion x Block of Trials	4	5.40	1.35	0.39	.818
Surgery x Block of Trials	4	69.70	17.43	4.99	$p < .001$
Lesion x Surgery x Block of Trials	4	4.56	1.14	0.33	.860
Drug Treatment x Block of Trials	4	53.19	13.30	3.81	.005
Lesion x Drug Treatment x Block of Trials	4	21.44	5.36	1.54	.191
Surgery x Drug Treatment x Block of Trials	4	12.91	3.23	0.93	.449
Lesion x Surgery x Drug Treatment x Block of Trials	4	6.99	1.75	0.50	.735
Error	352	1228.10	3.49		

Table 8a cont'd

Source	df	SS	MS	F	SIG
Day of Test					
x Block of Trials	4	85.49	21.37	6.75	$p < .001$
Lesion					
x Day of Test					
x Block of Trials	4	14.27	3.57	1.13	.344
,SPB 1					
Surgery					
x Day of Test					
x Block of Trials	4	3.62	0.91	0.29	.887
Lesion					
x Surgery					
x Day of Test					
x Block of Trials	4	9.62	2.40	0.76	.552
Drug Treatment					
x Day of Test					
x Block of Trials	4	4.41	1.10	0.3	.845
Lesion					
x Drug Treatment					
x Day of Test					
x Block of Trials	4	11.79	2.95	0.93	.446
Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trial	4	3.75	0.94	0.30	.881
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trial	4	17.50	4.38	1.38	.240
Error	352	1114.30	3.17		

Table 9a

Analysis of Variance for the Total Number of Conditioned
Avoidance Responses per Block of 20 Trials in the
Shuttle-box as a Function of Treatment Groups
and Day of Test and Block of Trials

Source	df	SS	MS	F	SIG
Lesion	1	960.00	960.00	3.83	.053
Surgery	1	1632.80	1632.80	6.52	.012
Lesion x Surgery	1	30.10	30.10	0.12	.730
Drug Treatment	1	3848.00	3848.00	15.37	p<.001
Lesion x Drug Treatment	1	81.67	81.67	0.33	.569
Surgery x Drug Treatment	1	176.82	176.82	0.71	.403
Lesion x Surgery x Drug Treatment	1	0.71	0.71	0.00	.958
Error	88	22034.00	250.39		
Day of Test	1	3713.10	3713.10	94.82	p<.001
Lesion x Day of Test	1	30.11	30.11	0.77	.383
Surgery x Day of Test	1	73.71	73.71	1.88	.174
Lesion x Surgery x Day of Test	1	8.82	8.82	0.23	.636
Drug Treatment x Day of Test	1	176.83	176.83	4.52	.036
Lesion x Drug Treatment x Day of Test	1	80.50	80.50	2.06	.155

Table 9a cont'd

Source	df	SS	MS	F	SIG
Surgery					
x Drug Treatment					
x Day of Test	1	8.43	8.43	0.22	.644
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test	1	0.27	0.27	0.01	.934
Error	88	3445.90	39.16		
Block of Trials	4	9409.80	2352.50	201.55	p<.001
Lesion					
x Block of Trials	4	13.27	3.32	0.28	.888
Surgery					
x Block of Trials	4	14.72	3.68	0.32	.868
Lesion					
x Surgery					
x Block of Trials	4	19.77	4.94	0.42	.792
Drug Treatment					
x Block of Trials	4	180.00	45.00	3.86	.004
Lesion					
x Drug Treatment					
x Block of Trials	4	17.71	4.43	0.38	.823
Surgery					
x Drug Treatment					
x Block of Trials	4	60.03	15.01	1.29	.275
Lesion					
x Surgery					
x Drug Treatment					
x Block of Trials	4	16.04	4.01	0.34	.848
Error	352	4108.40	11.67		

Table 9a cont'd

Source	df	SS	MS	F	SIG
Day of Test					
x Block of Trials	4	340.73	85.18	8.12	p<.001
Lesion					
x Day of Test					
x Block of Trials	4	77.68	19.42	1.85	.119
Surgery					
x Day of Test					
x Block of Trials	4	174.97	43.74	4.17	.003
Lesion					
x Surgery					
x Day of Test					
x Block of Trials	4	43.40	10.85	1.03	.389
Drug Treatment					
x Day of Test					
x Block of Trials	4	101.35	25.34	2.42	.049
Lesion					
x Drug Treatment					
x Day of Test					
x Block of Trials	4	53.59	13.40	1.28	.279
Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trials	4	14.43	3.61	0.34	.848
Lesion					
x Surgery					
x Drug Treatment					
x Day of Test					
x Block of Trials	4	88.47	22.12	2.11	.079
Error	352	3692.80	10.49		
Total	959	54729.00			

APPENDIX B

Table 1-1

Mean ($\pm$ S.E.M.) Plasma Corticosterone Concentrations
(ug/100 ml plasma) as a Function of Surgery
and Time of Blood Collection

ADX	1400-1700hr	3.304	0.495
ADX	800-1100hr	6.500	0.972
SHAM	1400-1700hr	13.771	1.260
SHAM	800-1100hr	26.608	2.673

* $p < .05$ as determined by the Newman-Keuls test

Table 3-1

The Mean ($\pm$ S.E.M.) Concentration ($\mu\text{g/g}$ tissue) for each Amine in each Brain Part Assayed are Presented for the Eight Treatment Groups

GROUPS	NOREPINEPHRINE				DOPAMINE		SEROTONIN				
	Cx	Hi	Hs	Es	Hs	Ne	Cx	Hi	Hs	Es	Ne
S	.206 $\pm .019$	.228 $\pm .017$	.778 $\pm .053$	.334 $\pm .032$	.118 $\pm .014$	5.195 $\pm .315$	.554 $\pm .024$	.485 $\pm .019$	2.746 $\pm .119$	.793 $\pm .019$	.744 $\pm .025$
MET	.199 $\pm .012$	.223 $\pm .017$	.778 $\pm .057$	.374 $\pm .019$	.077 $\pm .025$	5.131 $\pm .189$	.569 $\pm .009$	.491 $\pm .014$	2.881 $\pm .120$	.717 $\pm .032$	.777 $\pm .026$
ADX	.184 $\pm .019$	.202 $\pm .014$	.632 $\pm .120$	.411 $\pm .018$	.122 $\pm .019$	5.256 $\pm .234$	.546 $\pm .021$	.486 $\pm .611$	2.907 $\pm .119$	.812 $\pm .012$	.739 $\pm .012$
ADX+ MET	.172 $\pm .011$	.231 $\pm .018$	.743 $\pm .048$	.412 $\pm .025$	.128 $\pm .027$	4.908 $\pm .280$	.532 $\pm .012$	.520 $\pm .025$	2.892 $\pm .124$	.774 $\pm .020$	.778 $\pm .027$
DNB	.009 $\pm .002$	.004 $\pm .002$	.197 $\pm .053$	.530 $\pm .031$	.139 $\pm .055$	3.689 $\pm .387$	.521 $\pm .027$	.454 $\pm .017$	2.561 $\pm .085$	.767 $\pm .027$	.613 $\pm .098$
DNB+ MET	.008 $\pm .001$	.005 $\pm .001$	.196 $\pm .040$	.490 $\pm .028$	.145 $\pm .047$	3.400 $\pm .488$	.565 $\pm .032$	.479 $\pm .018$	3.020 $\pm .095$	.759 $\pm .023$	.606 $\pm .096$
DNB+ ADX	.009 $\pm .002$	.003 $\pm .001$	.191 $\pm .032$	.443 $\pm .012$	.113 $\pm .017$	3.454 $\pm .410$	.507 $\pm .040$	.516 $\pm .020$	2.764 $\pm .101$	.746 $\pm .036$	.680 $\pm .016$
DNB+ ADX+ MET	.010 $\pm .001$	.005 $\pm .002$	.224 $\pm .029$	.463 $\pm .022$	.152 $\pm .051$	3.331 $\pm .469$	.535 $\pm .028$	.527 $\pm .016$	3.011 $\pm .109$	.771 $\pm .016$	.700 $\pm .036$

Legend: Cx = Cortex
 Hi = Hippocampus
 Hs = Hypothalamus
 Es = Brainstem
 Ne = Nucleus

Table 3-2

Mean Serotonin Concentration (ug/g tissue) in
the Hypothalamus as a Function of DNB
Lesion and MET Treatment

DNB + MET	3.015 *
DNB	2.665
MET	2.886
SHAM	2.827

* $p < .05$ as determined by the Newman-Keuls test

Table 3-3

Mean Norepinephrine Concentration (ug/g tissue)
in the Brainstem as a Function of DNB
Lesion and Adrenalectomy

DNB + ADX	0.453 *
DNB	0.510
ADX	0.412
SHAM	0.379

* $p < .05$ as determined by the Newman-Keuls test

Table 4-1

Mean Plasma Corticosterone Concentrations
(ug/100 ml plasma) as a Function of
Surgery and Drug Treatment

ADX + MET	5.740
ADX	7.298
MET	20.971
SHAM	49.883

* $p < .05$ as determined by the Newman-Keuls test

Table 6-1

Mean ($\pm$ S.E.M.) Number of Intertrial Crossings
as a Function of Surgery and Day of Test

ADX	First Test Day	4.594	$\pm$ 0.810
ADX	Second Test Day	5.385	$\pm$ 0.628
SHAM	First Test Day	6.156	$\pm$ 0.758
SHAM	Second Test Day	11.615	$\pm$ 1.161 *

* $p < .05$ as determined by the Newman-Keuls test

Table 6-2

Mean Number of Intertrial Crossings per Block
of Trials as a Function of Day of Test
and Block of Trials

First Test Day - Block 1	4.802
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First Test Day - Block 2	5.948
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Second Test Day - Block 1	6.854
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Second Test Day - Block 2	10.146 *
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* $p < .05$ as determined by the Newman-Keuls test

Table 7-1

Mean Escape Response Latency (seconds)
as a Function of Block of Trials

Block 1	9.814	*
Block 2	8.369	
Block 1	9.814	*
Block 3	8.833	
Block 2	8.369	
Block 3	8.833	

* $p < .05$ as determined by the Newman-Keuls test

Table 8-1

Mean Response Latency (seconds) per Block of 20
Trials as a Function of Blocks of Trials

Block 1	7.705	*
Block 2	6.051	
Block 2	6.051	*
Block 3	5.341	
Block 3	5.341	*
Block 4	4.940	
Block 4	4.940	
Block 5	4.761	

* $p < .05$ as determined by the Newman-Keuls test

Table 8-2

Mean Response Latency (seconds) per Block of 20 Trials
as a Function of Surgery and Block of Trials

ADX		Sham	
Block 1	8.790 *	Block 1	6.620 *
Block 2	7.177	Block 2	4.926
Block 2	7.177 *	Block 2	4.926
Block 3	6.320	Block 3	4.363
Block 3	6.320 *	Block 3	4.363
Block 4	5.667	Block 4	4.214
Block 4	5.667	Block 4	4.214
Block 5	5.163	Block 5	4.360

* $p < .05$ as determined by the Newman-Keuls test

Table 8-3

Mean Response Latency (seconds) per Block of 20 Trials
as a Function of Drug Treatment and Blocks of Trials

Metopirone		Vehicle	
Block 1	8.356 *	Block 1	7.054 *
Block 2	7.177	Block 2	4.986
Block 2	7.177 *	Block 2	4.926
Block 3	6.001	Block 3	4.682
Block 3	6.001 *	Block 3	4.682
Block 4	5.460	Block 4	4.421
Block 4	5.460	Block 4	4.421
Block 5	5.116	Block 5	4.407

* $p < .05$ as determined by the Newman-Keuls test

Table 8-4

Mean Response Latency (seconds) per Block of Trials of 20
Trials as a Function of Day of Test and Block of Trials

	Block 1	Block 2	Block 3	Block 4	Block 5
First Test Day	8.736	6.586	5.670	5.172	5.008
	*	*			
Second Test Day	6.674	5.517	5.013	4.709	4.515

* $p < .05$ as determined by the Newman-Keuls test

Table 9-1

Mean Number of Avoidance Responses per Block of
20 Trials, as a Function of Blocks of Trials

Block 1	5.896	*
Block 2	10.688	
Block 2	10.688	*
Block 3	12.984	
Block 3	12.984	*
Block 4	14.000	
Block 4	14.000	
Block 5	14.422	

* $p < .05$ as determined by the Newman-Keuls test

Table 9-2

Mean Number of Avoidance Responses per Block of 20 Trials
as a Function of Drug Treatment and Day of Test

First Test Day	Metopirone	7.200	*
	Vehicle	12.063	
Second Test Day	Metopirone	11.992	*
	Vehicle	15.137	
Metopirone	First Test Day	7.200	*
	Second Test Day	11.992	
Vehicle	First Test Day	12.063	*
	Second Test Day	15.137	

* $p < .05$ as determined by the Newman-Keuls test

Table 9-3

Mean Number of Avoidance Responses per Block of 20 Trials
as a Function of Drug Treatment and Block of Trials

Metopirone		Vehicle	
Block 1	4.042 *	Block 1	7.750 *
2	7.948	2	13.427
2	7.948 *	2	13.427 *
3	10.875	3	15.094
3	10.875 *	3	15.094
4	12.104	4	15.896
4	12.104	4	15.896
5	13.010	5	15.833

* $p < .05$ as determined by the Newman-Keuls test

Table 9-4

Mean Number of Avoidance Responses per Block of 20 Trials
as a function of Day of Test and Block of Trials

First Test Day		Second Test Day	
Block 1	3.000 *	Block 1	8.792 *
2	8.375	2	13.000
2	8.375 *	2	13.000
3	11.042	3	14.927
3	11.042 *	3	14.927
4	12.594	4	15.406
4	12.594	4	15.406
5	13.146	5	15.698

* $p < .05$ as determined by the Newman-Keuls test.

Table 9-5

Mean Number of Avoidance Responses per Block of 20 Trials as
a Function of Surgery and Day of Test and Block of Trials

ADX			Sham		
First Test Day	Block 1	2.063 *	Block 1	3.938 *	
	2	6.792	2	9.958	
	2	6.792 *	2	9.958 *	
	3	9.438	3	12.646	
	3	9.438	3	12.646 *	
	4	10.479	4	14.708	
	4	10.479	4	14.708	
	5	11.479	5	14.813	
Second Test Day	Block 1	6.917 *	Block 1	10.667 *	
	2	11.667	2	14.333	
	2	11.667 *	2	14.333	
	3	14.250	3	15.604	
	3	14.250	3	15.604	
	4	14.854	4	15.958	
	4	14.854	4	15.958	
	5	15.000	5	16.396	

* $p < .05$ as determined by the Newman-Keuls test

Table 9-6

Mean Number of Avoidance Responses per Block of 20 Trials as a
Function of Drug Treatment and Day of Test and Block of Trials

Metopirone			Vehicle		
First Test Day	Block 1	1.292 *	Block 1	4.708 *	
	2	4.938	2	11.813	
	2	4.938 *	2	11.813 *	
	3	8.208	3	13.875	
	3	8.208 *	3	13.875	
	4	10.125	4	15.063	
	4	10.125	4	15.063	
	5	11.438	5	14.854	
Second Test Day	Block 1	6.792 *	Block 1	10.792 *	
	2	10.958	2	15.042	
	2	10.958 *	2	15.042	
	3	13.542	3	16.313	
	3	13.542	3	16.313	
	4	14.083	4	16.729	
	4	14.083	4	16.729	
	5	14.583	5	16.813	

* $p < .05$ as determined by the Newman-Keuls test

END

0 6 0 6 8 3

FIN