1

OF/DE

2

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

32174

NATIONAL LIBRARY
OTTAWA

BIBLIOTHÈQUE NATIONALE
OTTAWA

NAME OF AUTHOR... Matti Juhani Saari
TITLE OF THESIS... Differential Behavioural And Catecholaminergic
Effects of 6-Hydroxydopamine Administered
At Different Ages To Neonatal Rats
UNIVERSITY... Carleton University
DEGREE FOR WHICH THESIS WAS PRESENTED... Ph. D.
YEAR THIS DEGREE GRANTED... 1977

Permission is hereby granted to THE NATIONAL LIBRARY
OF CANADA to microfilm this thesis and to lend or sell copies
of the film.

The author reserves other publication rights, and
neither the thesis nor extensive extracts from it may be
printed or otherwise reproduced without the author's
written permission.

(Signed)... *Matti Saari*

PERMANENT ADDRESS:

R.R. 1,
Lively, Ontario. POM 2E0

DATED... May 19 1977

NL 1 (1C-68)

Supervisor... Dr. Bruce Pappas

National Library of Canada

Cataloguing Branch
Canadian Theses Division

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage
Division des thèses canadiennes

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

**THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED**

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

**LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE**

DIFFERENTIAL CATECHOLAMINERGIC
AND BEHAVIOURAL EFFECTS
OF 6-HYDROXYDOPAMINE
ADMINISTERED AT DIFFERENT AGES TO
NEONATAL RATS

by Matti J. Saari, M.A.

Thesis presented to
the Faculty of Graduate Studies
at Carleton University
in partial fulfillment of the requirements for
the Ph.D. degree

Department of Psychology

Ottawa, May, 1977

© Matti Saari 1977

2

6

The undersigned recommend to the Faculty
of Graduate Studies acceptance of the thesis
"Differential Catecholaminergic and Behavioural Effects of
6-Hydroxydopamine Administered at Different Ages to Neonatal Rats"

submitted by

Matti J. Saari

in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

.....
Thesis Supervisor

.....
Chairman, Department of Psychology

Carleton University

May, 1977

ABSTRACT

The locus coeruleus, which gives rise to the dorsal noradrenergic bundle has been the focus of intensive research in recent years. Despite these efforts no clear definition of the behavioral relevance of this small pontine nucleus of noradrenergic cell bodies has emerged. Some have suggested that it is involved in learning, dreaming, cardiovascular responses, attention, and fear. The neurotoxin 6-hydroxydopamine alters the development of noradrenergic neurons when it is injected into newborn rats. Since the effects of the injections vary with the age of the rat, a comparison of the behavioral and neurochemical effects of early 6-hydroxydopamine treatments was expected to allow the ascription of some behavioral effect(s) to differing patterns of neurochemical change which primarily involve the axons.

The locus coeruleus not only sends axons to the fore-brain via the dorsal noradrenergic bundle but also to the cerebellum and the spinal cord. Consequently, the behavioral tests were chosen with a view to measuring multiple behavioral functions which may involve these neurons.

Two 50 mg/kg, injections of 6-hydroxydopamine, or vehicle, were administered subcutaneously to newborn rats on the first, fifth or tenth day of life. The two injections

were 12 hours apart. Biochemical assays of norepinephrine and dopamine concentrations of 15 brain areas and hearts of the rats in adulthood indicated that the drug effects were primarily limited to the noradrenergic neurons. Areas containing a predominance of cell bodies showed increased transmitter concentrations while areas thought to contain the noradrenergic terminal fields of the locus coeruleus revealed reduced concentrations of transmitter. An interesting exception was the cerebellum where increased transmitter concentrations were observed only after the day one injections. In general, the day one injections were the most effective in altering transmitter levels while the day five and day ten injections often resulted in a lesser but similar alteration and sometimes tended to an opposite alteration in transmitter concentration. An exception to this observation was the heart where the day ten injections resulted in the most, while the day one injections resulted in the least depletion of transmitter.

Behavioral alterations were observed mainly for the day one injected rats. These injections abolished the post-decapitation convulsions which were displayed by the vehicle and day five and ten injected rats. Although the 6-hydroxydopamine injections did not lead to alterations in spontaneous activity levels or in the degree of suppression of activity accompanying a sudden tone, the day one injected rats were deficient in the performance of tasks sensitive to motor co-ordination difficulties. No drug related effects were

observed in a test of hot plate analgesia after morphine injection when appropriate control groups were included. There also was no suggestion of reduced sensitivity to thermal pain. Behavior of day one injected rats was altered in the response to punishment of food reinforced alleyway running and water reinforced licking. On the other hand, they did not differ in extinction of food reinforced alleyway running.

It is suggested that the various behavioral deficits may represent the effect of the 6-hydroxydopamine injections on the three main terminal field areas of the locus coeruleus neurons. The lack of post-decapitation convulsions might be attributed to the effect of the treatment on the spinal noradrenergic terminals; the deficits in motor co-ordination tasks might be attributable to the effects on the cerebellar noradrenergic terminals and the alterations in learning tasks may be a function of the drug injections on the forebrain locus coeruleus terminals.

Most of the biochemical and some of the behavioral alterations following the day one injections of 6-hydroxydopamine were similar to those observed after adult lesions of the dorsal noradrenergic bundle. Both treatments lead to a similar alteration on the response to punishment of food reinforced alleyway running, and no change in activity levels. On the other hand, the neonatal treatments did not lead to

increased distractability nor resistance to extinction as the adult lesions have been reported to do. Furthermore only the day one injections alter the post-decapitation convulsions while the adult lesions do not. The deficit in the motor coordination tasks found after the day one injections suggested that such deficits might also be observed after the adult lesions of the dorsal noradrenergic bundle.

ACKNOWLEDGEMENTS

I wish to thank Dr. Bruce A. Pappas who has been my advisor during my graduate career. In his laboratory, Dr. Pappas was successful in creating an environment that demanded not only personal discipline and original ideas but also a dedication to the ideals of scientific endeavour. I am also grateful for his friendship.

I also wish to thank Ray Ings without whose friendship and dedicated professionalism the path might have been impossible.

Dr. McIntyre and Dr. Dillon both provided important guidance at key stages of the thesis preparation.

And finally, I wish to thank Tim Dugas, who convinced me to go multivariate.

The research was supported by a National Research Council grant to Dr. Pappas.

This thesis is dedicated to Cathy

TABLE OF CONTENTS

	Page
INTRODUCTION	1
METHOD AND PROCEDURE	19
SUBJECT POOL	19
BEHAVIOURAL MEASURES	20
<i>Activity and Reactivity Recording</i>	20
<i>Motor Behaviour Tests</i>	22
- Rotating Rod	22
- Rope Descent	23
- Beam Cross	24
<i>Morphine Analgesia Assessment</i>	25
<i>Water Lick Punishment Procedure</i>	28
- Adaptation Procedure	30
- Acquisition Procedure	31
- Extinction Procedure	31
<i>Runway Extinction and Punishment Procedures</i>	31
- Acquisition Procedure	32
- Extinction Procedure	33
- Punishment Procedure	33
NEUROCHEMICAL MEASURES	34
<i>Dissection and Post-Decapitation Convulsions</i>	34
<i>Assay Procedure</i>	40

	Page
STATISTICAL ANALYSES	44
RESULTS	47
ASSAYS AND POST-DECAPITATION CONVULSIONS	47
BEHAVIOURAL OBSERVATIONS	57
<i>Activity & Reactivity</i>	57
- Body Weight	57
- Activity and Reactivity	58
Motor Behaviour Tests	65
Morphine Analgesia Results	75
Water Lick Punishment Results	83
Runway Performance	93
- Acquisition	93
- Extinction	96
- Punishment	96
- Punishment Extinction	104
DISCUSSION	108
REFERENCES	133

Since the pioneering work of Swedish investigators (Fuxe, 1965; Ungerstedt, 1971) revealed the monoamine pathways of the brain, a great deal of research has been applied toward understanding the role that these pathways play in behavior. A compact group of norepinephrine (Ne) containing neurons, the locus coeruleus (LC), is situated in the dorsal pons. Although the cell bodies of the rat LC are tightly clustered together, their projections are very diffuse and extend to nearly all parts of the forebrain via the so called dorsal Ne bundle (Ungerstedt, 1971) or dorsal tegemental bundle (Lindvall & Bjorklund, 1974) and also to the cerebellum (Olson & Fuxe, 1971), and the spinal cord (Hancock and Fougere 1976). Thus it is not surprising that the suggested functions of the LC have received considerable attention: the mediation of reinforcement and learning (Crow, 1968), attention (Roberts, Price & Fibiger, 1976), fear behavior (Huang et al., 1976), cardiovascular responses (Ward & Gunn, 1976), morphine induced analgesia (Sasa et al., 1975), the initiation of dreaming (Jouvet, 1969).

Since the various monoaminergic neural systems are defined in terms of their neurotransmitter, pharmacological manipulations have become important tools for studying their functions. For example, the drug 6-Hydroxydopamine (6-OHDA) preferentially and permanently depletes Ne nerve

endings of their transmitter, thereby allowing the selective alteration of these neural systems (Kostrzewa & Jacobowitz, 1974). Besides providing a useful technique for lesioning NE terminals in the brain of mature animals, 6-OHDA can also be used to discern the behavioral relevance of these terminals in the developing brain.

An apparently permanent and nearly total peripheral sympathectomy is produced when newborn rats are injected with 6-OHDA (Angeletti, 1971; Angeletti & Levi-Montalcini, 1970). Seven daily injections beginning the day of birth produce massive destruction of all the paravertebral and prevertebral sympathetic ganglia (Angeletti & Levi-Montalcini, 1971). It has been suggested that this effect may be permanent since the affected sympathetic ganglia appear to be destroyed for as long as ten months after treatment (Angeletti, 1971).

Kostrzewa and Jacobowitz (1974), after a lengthy review of the pharmacological, neurochemical and behavioural effects of 6-OHDA, concluded that peripheral neonatal injections of 6-OHDA also have radical effects on brain chemistry. These effects seem to be limited to direct alterations of the development of NE neurons while apparently leaving other transmitter systems relatively unaffected (Taylor, Clark, Laverty & Phelan, 1972; Sachs, 1973; Versteeg, van Ree, Provoost & de Jong, 1975; Jonsson, Pycock, Fuxe and Sachs, 1974;

Scott & Lavery, 1974) if the dose exceeds at least 10 ug/mg (two times) and the total dose is given as a series of injections rather than a single injection (Breese & Traylor, 1972).

These changes in brain chemistry have been observed to be age dependent, possibly because 6-OHDA is limited in its access to the CNS as a function of the maturing blood brain barrier (BBB) of the neonatal rats (Singh & De Champlain, 1972; Sachs, 1973; Pappas et al., 1975). Sachs (1973) has suggested that the BBB for 6-OHDA develops around seven to nine days after birth for the cortex and by day five for the hypothalamus while the BBB for the spinal cord is slower to develop. Liew & Taylor (1972) reported that the cortex and the hippocampus are most vulnerable to intraperitoneal 6-OHDA injections on days one and two after birth while the cerebellum is only vulnerable later (days five to twelve). Kostrzewa & Jacobowitz (1974) summarized the effects of neonatal 6-OHDA treatments on Ne neurons in discrete regions of the brain by noting that regions containing a predominance of Ne terminals (cerebral cortex, hippocampus, cerebellum, spinal cord) tend to show depletion of Ne levels when assayed at a later age while brain regions containing a predominance of Ne cell bodies or nonterminal axons (especially the lower brain stem) show increases in Ne content.

It has generally been observed that some of the long term neurochemical effects of neonatal injections of

6-Hydroxydopa (6-OHDopa) which passes through the BBB are similar to those observed after neonatal 6-OHDA injections (Kostrzewska & Harper, 1974; McLean, Kostrzewska & May, 1976; Zeiher & Jaim-Etcheverry, 1973). This is reasonable since 6-OHDopa derives its pharmacological activity from its metabolic conversion to 6-OHDA and thereby is ultimately taken up by the central Ne neurons. Kostrzewska & Harper (1974) and McLean et al. (1976) found that neonatal 6-OHDopa, like 6-OHDA, produces increases in brain stem and decreases in telencephalic Ne levels. They reported, however, that 6-OHDopa does not produce a marked peripheral sympathectomy as does 6-OHDA.

Kostrzewska & Garey (1975), Sweig et al. (1975) and McLean et al. (1976) have all reported an interesting, previously unnoticed, effect of early 6-OHDopa treatment. They found increased Ne levels in the cerebellum when the drug was injected on the day of birth and reduced Ne levels when it was injected between three and fourteen days. Kostrzewska and Garey also reported increased cerebellar levels of Ne after a single peripheral 6-OHDA injection given on the day of birth. It is not clear why Liew and Taylor (1972) failed to observe these age specific effects on the cerebellum since they also injected the drug on days one and two. All other previous neonatal 6-OHDA studies had used injection schedules which extended beyond the single day one injection.

The intriguing and unexpected regional brain biochemical changes following peripheral neonatal 6-OHDA or 6-OHDopa injections have stimulated speculation concerning the mechanism through which some areas show Ne increases (others show no change and still others show decreases). One of the earliest explanations for the brain stem increase was suggested by Pappas and Sobrian (1972) who speculated that it was a compensatory response to altered peripheral autonomic function following peripheral 6-OHDA sympathectomy. It is clear that this suggestion is incompatible with the results of Kostzewska and Harper (1974) who found that 6-OHDopa injections in neonatal rats led to similar brain changes observed after 6-OHDA, but did not cause a marked peripheral sympathectomy.

Researchers at the Karolinska Institute have made several suggestions regarding these regional changes in Ne levels. They suggested that the selective degeneration of Ne nerve terminals in the forebrain induced by neonatal 6-OHDA injections leads to increased Ne in the hindbrain because the perikaryon is not subject to 6-OHDA degeneration. This may either induce the outgrowth of new nerve terminals in the cell body region or the destruction of the forebrain axon may induce collateral accumulation due to the redistribution of the Ne storage granules to the remaining brainstem nerve terminals (Jonsson, Pycock, Fuxe and Sachs, 1974). As an al-

ternative Sachs and Jonsson (1973) also suggested that since the dorsal Ne bundle, which originates in the LC seems more vulnerable to 6-OHDA injections than the ventral Ne bundle system, it is possible that the ventral system sprouts and re-innervates the vacant synaptic sites left by the degenerated Ne nerve terminals from the LC.

Kostrzewska and Harper (1974) and Jacobowitz and Kostrzewska (1971) suggested that the increased brainstem Ne levels following neonatal 6-OHDA probably reflect a piling-up of Ne of the axons of the terminal regions. Kostrzewska and Garey (1976) also provide several suggestions for the marked rise in cerebellar Ne. Since they found no evidence for an increase in the number of nerve endings in the cerebellum, they argued that the Ne increase is due to an increase in intraneuronal storage depots. This hypothesis rests on the assumption that the LC perikarya are bipolar (Jacobowitz, 1973). They also suggested that the cerebellar Ne terminals regenerate after lesioning and thereby provide this alternate pathway for axoplasmic transport. When the axons ascending rostrally are damaged, the axoplasmic flow of storage granules and enzymes is directed towards the cerebellum.

In contrast to these findings, a reduction in the cerebellar Ne was brought about by 6-OHDA injections any time between three and fourteen days of age (Kostrzewska and

Garey, 1976). A similar effect occurs after intraventricular injections of 6-OHDA (Pappas, Saari and Peters, 1976). This reduction may reflect the elaboration of the Ne lesioning potential of 6-OHDA in the cerebellum due to the maturation of cerebellar terminals at approximately two - three days of age.

Insofar as neonatal 6-OHDA and 6-OHDopa have similar neurochemical effects, it is not surprising that the behavioural effects of these treatments have been reported to be essentially similar (Kostrzewa & Jacobowitz, 1974; Zieher & Jaim-Etcheverry, 1973). At least in some respects these effects may be similar to those occurring after dorsal bundle lesions in the adult rat (Mason & Iversen, 1975). From table 1, which summarizes the effects of neonatal 6-OHDA on different behavioural measures, we can see that while most of the reports on long-term effects of neonatal peripheral 6-OHDA injections have indicated no effects on general activity levels (Pappas & Sobrian, 1972; Pappas et al., 1974; Taylor et al., 1972; Lew & Quay, 1971, Nyakas et al., 1973), some reports have indicated reduced exploratory activity (Nyakas et al., 1973; Clark et al., 1972) and others have suggested differences in activity patterns (Scott and Lavery, 1974). Lesions of the dorsal Ne bundle have also been reported to have no effect on overall activity levels (Roberts, Price & Fibiger, 1976; Crow and Wendlandt, 1976). Although McLean et al. (1976) did find increased open field activity levels after neonatal 6-OHDopa treatment, they remarked that this observation may have been an artifact since the rats were

TABLE 1
BEHAVIOURAL EFFECTS OF
NEONATAL PERIPHERAL 6-OHDA INJECTIONS

MOTOR BEHAVIOURS

Authors	General Activity	Exploratory Behaviour	Motor Co-ordination
Lew & Quay (1971)	no effect		
Pappas & Sobrian (1972)	no effect		
Taylor et al. (1972)	no effect	no effect	no effect
Clark et al. (1972)	reduced	reduced	
Nyakas et al. (1973)	no effect	reduced	
Pappas et al. (1974)	no effect		
Scott & Laverty (1974)	pattern change		
Hicks & D'Amato (1975)			deficit

APPETITIVELY MOTIVATED BEHAVIOURS

Authors	Acquisition	Extinction
Saari & Pappas (1973)	no effect	
Mason & Iversen (1975)	no effect	more resistant to extinction
Thornton et al. (1976)	no effect	more resistant to extinction
Peterson & Laverty (1976)	altered response rates on certain schedules	no effect

AVERSIVELY MOTIVATED BEHAVIOURS

Authors	Acquisition	Retention
Taylor et al. (1972)*	no effect	
Pappas & Sobrian (1972)*	no effect	no effect
Nyakas et al. (1973)*	no effect	
Saari & Pappas (1973)**	reduced	reduced
Lord et al. (1976)*	reduced	

* active avoidance
** punishment

previously exposed to testing procedures with aversive stimulation, and this treatment may have changed responsiveness to shock.

The recently reported deficit in motor co-ordination after neonatal 6-OHDA treatment (Hicks and D'Amato, 1975) is particularly interesting in view of the injection age dependent effects on cerebellar Ne. A deficit was also observed following 6-OHDopa on days one and three which may in fact induce increased cerebellar Ne (Sweig, McLean, Kostrzewa & May, 1975). Motor deficits have not been reported after more extended 6-OHDA injections - regimens which lead to reduced cerebellar Ne levels (Taylor et al., 1972).

The main focus of the research into the behavioural effects of dorsal bundle lesions, which deplete the forebrain areas of Ne has been the effect on learning. It is appropriate to point out that Molliver and Kristt (1975) found that a great proportion of synaptic terminals in the neonatal rat cortex layer four are monoaminergic. This layer of the cortex has classically been characterized as being predominantly receptive, receiving a strong input of thalamic fibers (Brodal, 1969). The fibers which give rise to the above cortical synapses may provide the cortical mechanism assumed by those theorists who suggest that the dorsal Ne bundle might be essential for the learning process because it "stamps in" thalamic input (Crow, 1968; Kety, 1970). Table 1 indicates

that only in aversively motivated learning is there an acquisition deficit after neonatal 6-OHDA, while in appetitively motivated tasks no acquisition differences are typically found. However, in appetitive tasks the treated rats may differ from normals in that they sometimes show increased resistance to extinction of the appetitively motivated operant.

Normal acquisition in appetitively motivated tasks has also been reported after lesions of the dorsal Ne bundle in adult rats (Roberts, Price & Fibiger, 1976; Mason & Iversen, 1975). Like neonatal 6-OHDA treatment, adult dorsal bundle lesions have also been reported to lead to a deficit in the acquisition and retention of an aversively motivated task (Crow & Wendlandt, 1976) and increased resistance to extinction of an appetitively reinforced operant (Mason & Iversen, 1975). Neonatal 6-OHDopa also produced behavioural effects quite similar to those after neonatal 6-OHDA. McLean et al. (1976) used a food reinforced approach-avoidance procedure similar to the water reinforced one used by Saari and Pappas (1973). These procedures revealed similar effects of neonatal 6-OHDA and 6-OHDopa in that the drug-treated rats were more likely to approach positive rewards after punishment than control rats. Pre-shock adaptation training revealed no treatment differences in either experiment.

It is difficult to argue that the above learning deficits are simply deficits in consolidation of the appropriate

response pattern (Kety, 1970) since no clear-cut acquisition differences are found, whereas differences in extinction are reported to occur, especially in appetitive tasks. Nor does the dorsal Ne pathway seem essential for the general reinforcement process as suggested by Ritter and Stein (1973) since again, normal acquisition seems to take place in appetitive tasks. The Crow and Wendlandt (1976) hypothesis that this system of Ne neurons arising from the LC is necessary for initiating the transition from a short to a long term memory however, does seem to account for most of the observed deficits in the learning tasks if one suggests (as they did) that this deficit only occurs in aversively motivated tasks and if one classifies the non-reward during the extinction of an appetitively trained operant as being aversive. This model would predict reduced acquisition and retention of aversively motivated responses while appetitively motivated responses should only be affected during extinction.

In the following series of experiments the main objective was to further the exploration of the behavioral and neurochemical effects of altering developing Ne nerve terminals by injecting 6-OHDA into developing rats. The neurochemical effects were examined using a brain dissection technique developed for our laboratory (Saari, Ings, Pappas and McIntyre, in preparation). This technique allows freehand dissection of the rat brain into fifteen distinct anatomical

parts largely following natural separations in neural tissue. Furthermore, a refined assay technique utilizing a separate comparable tissue blank for each tissue sample was developed and utilized in the assay of the endogenous Ne and dopamine (Da) levels.

The 6-OHDA injection regimen consisted of two subcutaneous (sc) 50 mg/kg injections given at one of three ages: Day 1, Day 5, or Day 10 after birth. This regimen was chosen since the literature suggests that quite distinct patterns of Ne levels may follow 6-OHDA injection at these different ages. The cortex appeared most susceptible to 6-OHDA given on days one and two after birth whereas the cerebellum showed the greatest decreases in Ne levels following 6-OHDA injection on days nine and ten after birth (Liew & Taylor, 1972; Lavery et al., 1973; Sachs, 1973). The cerebellum may even show increased Ne levels when the drug is injected on day one (Kostrzewa & Garey, 1975).

On this injection regimen it was expected that the findings of Liew and Taylor (1972) would be replicated, but with more refinement of age parameters. The Day 1 injections were predicted to elicit maximal destruction of Ne nerve endings in the cerebral cortex and hippocampus, increases in cerebellar Ne levels and the largest increases in the pons-medulla. The Day 10 injections on the other hand, were pre-

3

dicted to cause little hippocampal and cortical depletion while producing decreases in cerebellar Ne levels and relatively smaller increases in the pons-medulla. The Day 5 injections were expected to provide an intermediate pattern of Ne level changes with partial cortical and hippocampal depletion but no cerebellar Ne increases. The small dosage was expected to produce minimal effects on Da levels at all ages.

This age dependent injection schedule was used since it might offer an opportunity to isolate discrete behavioral effects of 6-OHDA as a function of its age dependent neurochemical effects on the LC terminal fields. The schedule might also make it possible to dissociate the peripheral from the central drug effects. In any case, a considerably refined inquiry into the biochemical effects of neonatal 6-OHDA injections on various brain areas would be provided by the combination of the dissection-assay procedure with the injection regimen.

Several investigators have noted the lack of post-decapitation convulsions after neonatal 6-OHDA sympathectomy. Lord et al. (1976) for instance, found that neonatal 6-OHDA treatment abolished the normal postmortem motor activity which involves massive contraction of the trunk muscles following decapitation. Eichbaum et al. (1975) suggested that these convulsions are induced by a 'convulsion centre' situated in the higher spinal cord. The mechanism by which 6-OHDA injec-

tions block these convulsions remains to be explained although it may be due to the effect of the treatment on spinal Ne terminals (Suenaga, 1977). Clearly, it was important to gather data on the onset and duration of the post-decapitation convulsions in the rats since it was likely that the age at the time of injection would determine the vulnerability of the spinal Ne terminals to 6-OHDA. This was expected to allow the evaluation of hypotheses regarding the neural substrate of this reflex.

Conflicting reports have appeared in the literature regarding the effects of neonatal 6-OHDA sympathectomy on the activity levels of rats. Lew & Quay (1971), Pappas & Sobrian (1972) and Taylor et al. (1972) have all reported that neonatal 6-OHDA injections did not alter activity as measured by various means. On the other hand, Clark, Laverty and Phelan (1972) found 6-OHDA treated rats to have lower activity levels than that of the controls. Scott and Laverty (1974) reported that the 6-OHDA treated rats lacked the cyclic bursts of high activity observed in controls. Some recent observations of rats injected neonatally in the lateral ventricles with 6-OHDA suggest that hyperactivity may result (Gallivan, Saari & Pappas, unpublished observations).

Other recent observations suggest that neonatal 6-OHDA injections may lead to other types of alterations of locomotor behaviour. Hicks and D'Amato (1975) observed that around the third week of age,

rats who had been injected sc developed various locomotor deficits. These were characterized by a slovenly positioning of the feet as the animals ran and walked on narrow pathways. The authors argued that these deficits were somewhat like those observed following ablations of parts of the motor sensory cortex, although it should also be noted that the cerebellum is affected by the neonatal 6-OHDA treatments and may play a role in motor learning (Gilbert, 1975).

In the following experiment general activity levels were tested at six different ages and the development of motor coordination was also studied at these times. The motor coordination tasks were adapted from Altman et al. (1971) and Potvin & Fried (1972) and are sensitive to cerebellar damage induced by X-irradiation of the developing cerebellum as well as the effects of drugs on motor behaviour.

Elchisak and Rosencrans (1973) reported that rats treated intracisternally with 6-OHDA at two weeks of age were characterized by reduced morphine antinociception six weeks later when this was measured by hot plate and tail flick tests. Their research was based on the hypothesis that the antinociceptive action of morphine may be mediated through the LC (Korf et al., 1974; Sasa et al., 1975). Price and Fibiger (1975), on the other hand, produced a significant potentiation of morphine analgesia by bilateral 6-OHDA lesions of the dorsal Ne bundle while 6-OHDA lesion of the zona compacta of the

substantia nigra abolished the analgesic response to morphine. The conclusions reached by these researchers may be misleading since they did not include control groups receiving saline in place of the morphine injections. It is possible that the treatment may lead to a differential response to the stress of the hot plate or tail flick tests when repeated measures are taken on the same rat. Therefore the effect of neonatal 6-OHDA on morphine analgesia was reassessed by the hot plate technique, with the above mentioned control groups. Since the LC neurons not only contain a high density of opiate receptors but are also sensitive to morphine in terms of firing rates (Bird & Kuhar, 1977), it was possible that the differential patterns of drug effect on the Ne terminals would be accompanied by a differential pattern of morphine effects. Assessment of sensitivity to thermal pain in the different non-morphine treated groups would also be important for confirmation of previously observed unaltered thresholds to foot shock in neonatal 6-OHDA rats (Saari & Pappas, 1973).

The most consistent behavioral deficit following neonatal 6-OHDA or 6-OHDopa injections has been in the learning of aversively motivated behaviors (Saari & Pappas, 1973; Lord et al., 1976; McLean, Kostrzewa & May, 1976) and in extinction of an appetitively reinforced operant (Mason & Iversen, 1975; Thornton et al., 1976). The more discrete injection schedule

in the following three experiments was expected to potentially allow for the ascription of these behavioral deficits to the critical pattern(s) of neurochemical change.

Two experiments were carried out to examine the effects of the three neonatal 6-OHDA treatments on shock induced lick suppression and the response to punishment of food reinforced runway running. This laboratory has previously reported a deficit in the learning of shock-induced lick suppression after a series of ten 6-OHDA injections spanning the first 22 days after birth (Saari and Pappas, 1973). Since the catecholamine changes induced by neonatal 6-OHDA have been reported to be age dependent, it is possible that this behavioral alteration might be attributable to one age of injection and thereby to a specific pattern of neurochemical change.

An experiment similar to the above lick suppression procedure was recently reported by McLean et al. (1976). They trained rats to run a straight runway for food reward. Once this response was well established they then punished attempts to eat the food. The results were compatible with those of the lick suppression experiment. The neonatally 6-OHDopa injected rats did not differ from controls until the punishment phase when they tended to leave the start box sooner and approach the goal box more closely than did the control

rats. Similarly, Mason and Iversen (1975) found no difference in acquisition of food reinforced maze running among rats with adult lesions of the dorsal Ne bundle, neonatal 6-OHDA injected and control rats. Both the treated groups showed resistance to extinction however. Thornton et al. (1976) also found that the extinction of an appetitively reinforced operant is affected by neonatal 6-OHDA treatments, although Peterson and Laverty (1976) were unable to replicate this result. Thus, reduced effectiveness of punishment of appetitively reinforced operants seems to be a common feature of neonatal 6-OHDA and 6-OHDopa. Furthermore, there was some indication that neonatal 6-OHDA and adult lesions of the dorsal Ne bundle retarded extinction of some responses. In order to clarify these issues the effect of the three neonatal 6-OHDA treatment regimens was evaluated with regard to extinction and to punishment of food reinforced alleyway running.

METHOD AND PROCEDURE

SUBJECT POOL

The subjects (Ss) for the following series of experiments were born from pregnant rats acquired from Bio-Breeding, Ottawa, Ontario. Their pregnancies were timed and all fifteen Wistar litters were born within a three day period. One other set of eight pregnant rats was ordered when required for later experiments. The pups were randomly cross-fostered to the mothers and each litter was culled to five male and five female pups. The litters were then randomly assigned to one of the four treatment groups. The following injection schedule was used:

Treatment Group	Number of Litters	Day 1		Day 5		Day 10	
		Morning	Evening	Morning	Evening	Morning	Evening
6-OHDA-1	6	6-OHDA	6-OHDA	veh.	veh.	veh.	veh.
6-OHDA-5	5	veh.	veh.	6-OHDA	6-OHDA	veh.	veh.
6-OHDA-10	6	veh.	veh.	veh.	veh.	6-OHDA	6-OHDA
Vehicle	6	veh.	veh.	veh.	veh.	veh.	veh.

The dosage injected was 50 mg/kg of 6-OHDA dissolved in .05 ml of 1 mg/ml ascorbic acid/saline vehicle. Each pup was injected twice in a 24 hour period on one of three separate ages: Day 1, Day 5, or Day 10. The two within-day injections times were twelve hours apart.

The injection technique consisted of holding the rat pup in the left hand so that its weight was supported by the middle finger while the thumb and index finger were used to lift up a fold of skin along the back. The needle was inserted into this fold, at about shoulder level, and passed along the back under the skin where the drug was injected.

The pups were weaned at 30 days of age, sexed and housed five to a colony cage, until 60 days of age when they were placed in single cages. They were housed in a room with a reversed twelve hour light/dark cycle (lights off at 8 a.m.).

BEHAVIOURAL MEASURES

Activity and Reactivity Recording

Five males and five females were randomly selected from each treatment group. They were 15 days of age at the time of the first test and were ear marked for the purpose of identification.

Each rat was tested at six different ages: 15, 20, 25, 30, 40 and 60 days of age. On a particular test day the rat was brought to the experimental room and weighed before being placed into the experimental chamber. Ear markings made after the day 15 test allowed identification of the individual rats. Each activity measurement period was 15 minutes,

with duration controlled by logic circuitry; all rats were run in the same random order on each test day. After the test the rats were immediately placed back into their home cages.

On day 60, the sixth and final activity measurement period, the 15 minute test period was followed by a 1.5 second tone produced by an electronic signal (Mallory Sonalert, SC628p). This produced a pulsating 2900Hz tone of 80 db intensity. The rat's activity following the sudden tone onset was recorded for five minutes and comprised the measure of reactivity.

The activity sensor was enclosed in a sound attenuating, dark experimental chamber which was 44 cm deep, 62 cm wide and 58 cm high. The sensor consisted of a 30 cm diameter speaker with the mouth of the speaker cone covered by a thin mylar membrane. Movement of the rat on the membrane displaced the speaker cone in proportion to the force exerted on it. The cone displacement induced current to flow in the coil of the speaker. Voltage changes induced in the speaker coil were displayed on one channel of a Beckman R411 Dynograph, then rectified and integrated on additional channels. One measure of activity consisted of the total number of integrator resets which were recorded on a mechanical counter. An additional measure was the total duration of time that the rat was active. This was recorded by setting a Schmitt

trigger to turn on a cumulating timer when the rectified activity increased slightly above random noise level.

A clear plastic cylinder was placed 2 mm above the mylar covering of the speaker cone in order to restrict the area of the membrane accessible to the rat. A cylinder, 10 cm in diameter was used for the first two tests,, a 15 cm diameter cylinder for the third and fourth tests and one of 30 cm diameter for the last two tests. The cylinders were high enough to restrict the rats to the speaker surface.

Motor Behaviour Tests

The rats tested on these tasks were those which had undergone the activity-reactivity recording procedure.

This battery consisted of the following three tests, selected from Smith et al.* (1974) and Potvin & Fried (1972) because of their apparent sensitivity in detecting the effects of x-ray induced cerebellar damage in infant rats and the effects of drugs on motor behaviour.

Rotating Rod: The aluminum rotating rod was 37 cm long and 6 mm in diameter. Its surface was grooved longitudinally to allow the rats to grip it more easily and it was belt rotated (2 rpm) at one end by a constant speed electric motor. The rod was held in place by an aluminum stand so that the belt drive apparatus was protected while the rest of the rod projec-

ted out from the stand, parallel to the base at a height of 60 cm. The rats were initially placed within 10 cm of the free end of the rod. To initiate a trial, the rat was picked up and its front paws were placed over the rod between the free end of the rod and the 10 cm start line. Timers were started and the rat's time on the rod until falling was recorded. Foam padding in a plastic breeding cage was placed under the rotating rod to prevent injury to the rats when they fell.

Rope Descent: A braided hemp rope about 2.2 cm in diameter and 70 cm long was attached at one end to a wooden support. This left the loose end of the rope hanging free about 2 cm over a foam-padded, plastic breeding cage into which the rat could descend. A line was marked around the rope at 10 cm from the top to denote the placement of the rat at the beginning of the trial. To initiate the test, the rat's abdomen was placed against the rope such that its nose was on the start line. The rat responded to this by grasping the rope with all four paws and was then released. Timers were used to measure the time spent on the rope before a fall or a successful descent. Falls were defined as occasions where the rat lost its grip on the rope and either slipped or fell to the foam below. A successful descent occurred when the rat climbed down the rope either head or tail first, without falling to the foam and in a controlled fashion.

Beam Cross: A plexiglass beam, 1.8 cm wide and 88 cm long was supported at the starting point by a dowel. The goal end led into a standard, wire mesh, rat cage situated on a table. About 50 cm underneath the beam surface lay several layers of foam padding. A "starting" line was drawn 10 cm from the dowel-supported end. Each rat was placed behind this start line facing the goal at the initiation of the trial with its nose lined up with the start line. The time to cross successfully and the time on the beam before falling were recorded. A successful cross was defined as one which ended by the rat's nose entering the opening of the cage at the goal end.

The rats underwent this test battery on the day following activity testing, i.e., on days 16, 21, 26, 31, 41, and 61. The rats were exposed to each of the three different tasks twice each test day in this order: rotating rod, rope descent and beam cross. They were allowed a maximum of three minutes on each of the tasks and a minimum of three hours separated the two exposures to the battery.

A trial was initiated when the rat was placed on the apparatus. If the rat fell off within the first two seconds it was placed back on.

Morphine Analgesia Assessment

Initially it was intended to use fourteen male and fourteen female rats from each treatment group, half of which would receive morphine and the other half saline at 60 days of age. Table 2 shows the numbers of rats per cell retained for analysis and the number of rats rejected from the study. The limited availability of rats in the various treatment groups necessitated initial unequal cell frequencies and it was also decided to reject all rats who failed to paw lick on the first exposure to the hot plate.

The apparatus consisted of a hot plate built by the Science Workshop (Carleton University) which allowed constant temperature control of the surface of a round aluminum plate, approximately 38 cm in diameter and 9 mm in thickness. A thermistor was embedded in the plate allowing circuitry to apply the appropriate current to heating elements on the underside of the plate. The plate temperature ($54 \pm 0.1^{\circ}\text{C}$) was preset by a lockable variable control. An 18.8 cm diameter pyrex cylinder, 8.5 cm high, was placed on the hot plate. The cylinder could be capped by a 26 cm by 20 cm clear plastic plate 3 mm in thickness in order to confine the rat to the surface of the plate. The temperature of the hot plate was monitored by a thermometer with its end dipped in

TABLE 2

2
 SAMPLE SIZES FOR MORPHINE ANALGESIA EXPERIMENT
 AND THE FREQUENCY OF
 REJECTS IN EACH GROUP

Group	Treatments			
	<u>Morphine</u>	<u>Rejected</u>	<u>Saline</u>	<u>Rejected</u>
6-OHDA-1 males	5	1	6	1
females	7	0	7	0
6-OHDA-5 males	5	0	5	0
females	5	0	5	0
6-OHDA-10 males	7	1	6	1
females	5	2	7	0
vehicle males	7	0	4	3
females	5	2	6	1

mercury contained in a pyrex flask placed on top of the hot plate. A digital timer was used to measure paw lick latency and a Yellow Spring model 402 temperature probe was used to measure core temperature.

On the day preceding the experiment each of the rats was weighed and half were randomly assigned to the morphine group. The required morphine sulphate doses were weighed, placed into labelled, covered, glass containers which were refrigerated until the next day when the drug for each rat was dissolved in .2 cc of physiological saline and injected intraperitoneally (ip). On this test day, each rat was carried to the laboratory in its individual cage and its core temperature was taken with a rectal probe inserted by an experimenter who was unaware of the particular treatment received by the rats. Immediately following the measurement of the core temperature, the rat's paw lick latency was recorded. The trial was terminated when the rat licked a paw or after 30 seconds, by taking the cover off the cylinder and lifting the rat out.

After this initial base-line measure, the rat was injected either with morphine (10 mg/kg) or saline ip and placed back into its home cage. At 30 minutes, 60 minutes and 90 minutes after the injection, core temperatures and paw lick latencies were again measured. Core temperature was

always measured before the paw lick latency and the order of testing the treatment groups was counter-balanced across time. Again the observer was not informed as to whether the rat had received saline or morphine.

Water Lick Punishment Procedure

Five male and five female rats were randomly chosen from each of the four treatment groups. They were approximately 70 days old at the beginning of the experiment.

The apparatus consisted of a clear plexiglass experimental chamber which measured 25 cm by 23 cm and was 38 cm high. A microswitch was attached to the top left-hand corner of the door opening which was on the front wall. The microswitch closed when the door was closed. The grid floor of the chamber was raised 12 cm, leaving a space under the chamber for a sliding plastic tray which was used to collect boli. The stainless steel grids which formed the floor of the chamber were 3 mm in diameter and were 1.2 cm apart. A constant current shock source (Science Workshop, Carleton University) was used to deliver a 2 second, .1 ma unscrambled shock through these grids. A hole for a water bottle spout was centre-located in one side of the chamber about 5 cm above the grid floor. A metal drinking tube projected about 1 cm into the chamber through this hole and delivered room tempera-

ture tap water. The water reservoir was weighed before and after each rat in order to determine the amount drunk by the rat while in the apparatus.

The rat's licks were recorded on counters (BRS - Foringer) that were activated by a relay when the rat closed a circuit from the drinking tube to the floor grids; thus allowing a sub-threshold current to pass through it.

The experimental chamber was in turn enclosed within a sound attenuating outer shell. The inside of this outer enclosure was lined with acoustic tile. The rats could be observed through a one way mirror built into the door of this enclosure. Continuous air circulation and masking noise were provided by a small electric fan which circulated the air in the enclosure through sound attenuating, baffled air intake ducts. Illumination in the enclosure was provided by two 24 volt dc light bulbs which were attached to the top corners of the rear wall of the enclosure.

The experiment was carried out in two replications which were run on alternate days. Each rat was placed into the experimental chamber for 15 minutes at the same time of day on 15 alternate days, which comprised the adaptation, acquisition and extinction phases of the experiment. They had been water deprived for 23½ hours prior to each placement into the chamber. Water was available only in the apparatus during

these experimental days, whereas food in the form of Rat Chow pellets was available ad libitum in the home cages. These days were separated by one recovery day when water was available ad libitum in the home cage.

On every second day each rat was carried in its individual home cage to the laboratory, where it was weighed and then placed back into its cage for about 10 minutes prior to being placed in the apparatus. During this time the previous day's home cage water intake was measured for the rats who would be run through the procedure the following day and who were, therefore, being placed on water deprivation. The order that the rats were exposed to the apparatus was randomized with regard to both sex and treatment group and this order was the same throughout the experimental days.

The procedure consisted of three phases: adaptation, shock acquisition and shock extinction.

Adaptation Procedure (Days 1 - 5): After the waiting period in the home cage, the rat was placed into the apparatus. While in the apparatus the latency to complete the first ten and the second ten licks were automatically recorded to the nearest 0.1 second. After 15 minutes the rat was removed and placed back into its home cage. The amount of water consumed by the rat in the apparatus was then recorded.

Acquisition Procedure (Days 6 - 11): After the first five days the procedure was slightly changed in that the electric shock was applied to the grids contingent on the rat's tenth lick. This same procedure was repeated during the next six days.

Extinction Procedure (Days 12 - 15): The procedure during these days was the same as on the adaptation days.

Runway Extinction and Punishment Procedures

A subject pool was prepared as outlined earlier making available ten male and ten female rats from each treatment group: 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle. They were approximately 70 days old at the beginning of the experiment.

The apparatus consisted of a wooden runway 220 cm by 9 cm by 9 cm (inside dimensions) with a red plexiglass ceiling. A trial was initiated by placing a rat into the start box and opening the gate located 30 cm from the beginning of the runway. This enabled the automatic measurement of the time used to emerge from the start box after the gate opening since a timer was stopped by the activation of a photocell sensor when the rat had travelled 30 cm past the gate. At this point a second timer measuring the mid-runway time was initiated. This timer was stopped by a second photocell sen-

sor which was 90 cm down the runway from the first one. A third timer was started here measuring the time spent in the terminal 70 cm of the runway. Goal entry was indicated by a third photocell sensor located 6 cm from the runway end. This sensor also stopped the third timer.

The floor of the runway was wooden except for the goal box area which had a grid floor. The grids were 3 mm in diameter, 18 cm long and .5 cm apart. The circuitry which sensed goal entry was adapted to control shock delivery to these grids. The shock source was the same as in the previous experiment and identical levels and delivery methods were used. The shock could be escaped by leaving the goal box and re-entering after the shock was turned off. Shock duration (2 seconds) and onset were controlled by external logic circuitry.

The subject pool available for this phase of the experiment was divided into two, allowing two groups of rats with each treatment group represented by five male and five female rats. Two separate experiments were then carried out concurrently: a runway extinction experiment and a runway punishment experiment.

Acquisition Procedure (Days 1 to 5): The acquisition procedure was identical for both groups of rats. The rats were initially food deprived for 48 hours and then were allowed to explore the runway with .19 gm Noyes food pellets available

in the goal end of the runway in a 2 cm diameter plastic food cup. Each of the two 5 minute exploration periods were carried out on consecutive days. On the third day each rat was placed into the startbox and a trial was initiated by the experimenter who controlled the remote start gate opening mechanism. The three times acquired for each trial were noted. Five consecutive trials were carried out per rat per day. Goal box emergence, mid-runway and terminal runway times were averaged across the five trials for each rat. After the five trials, each rat was placed back into it's home cage where 20 Noyes pellets were available for 30 minutes. The rats were run in the same order and at the same time of day during acquisition.

Extinction Procedure (Days 6 - 10): The extinction procedure was identical to that for acquisition except that the single pellet per trial was not available.

Punishment Procedure (Days 6 - 10): In the second experiment the five days of acquisition training were followed by five days during which the procedure was the same as for the acquisition phase except that the entry into the goal of the runway was punished by delivery of a single .1 ma electric foot shock. Also, only one trial per day was carried out for five days after which these rats were placed on punishment extinction for five days. The procedure during punishment extinc-

tion was identical to the acquisition procedure.

The data here were the same as in the previous experiment except that the following measures were also taken: the time to re-enter the goal area after shock, the distance that the rat initially retreated from the goal area after shock and the average activity that the rat engaged in as measured by the distance(s) covered in the runway each second following the initial post-shock retreat until the termination of the trial. A trial was terminated on all but the shock trials 5 seconds after goal entry or when any of the timers indicated more than 100 elapsed seconds. The shock trials were terminated at 200 seconds or 5 seconds after goal re-entry.

NEUROCHEMICAL MEASURES

Dissection and Post-Decapitation Convulsions

Each rat was carried to the laboratory in a covered weighing scale basket and weighed. An experimenter, who was unaware of the treatment that any particular rat had received, decapitated the rat at the mid cervical level with a guillotine (Harvard Apparatus Co.) and immediately placed the body into a 4,000 ml pyrex beaker. Two timers were initiated at the moment of decapitation and one was stopped at the initiation of the convulsions. The second timer was stopped when

the convulsions were over, permitting the determination of convulsion duration. The post-decapitation convulsions consisted of violent clonic movements resembling epileptic seizures and were substantially more pronounced in the hindlimbs. Convulsion onset was defined as the first major clonic jerk of the hindlimbs and the timer measuring convulsion duration was stopped when all clonic hindlimb movement was over.

While the convulsions were being observed, the experimenter who had decapitated the rat proceeded to remove the brain from the skull and carried it on a saline soaked filter paper to the table where dissection took place. The dissection was performed by two experimenters, one dissecting the hindbrain and the other, the forebrain. Meanwhile, the experimenter who had been timing the convulsions, opened the thoracic cavity of the rat and removed a sample of spinal cord by first counting down ten ribs and then removing a section of the spinal cord from the seventh to tenth rib. He also removed the heart, exposed its cavities and rinsed it with cold saline solution. All pieces were immediately placed in liquid nitrogen.

Prior to the dissection of the brain, the external vasculature was removed. The dissection took place on saline-rinsed ice-cooled plates and care was taken to divide the brain following natural separations wherever possible. The following tissues were dissected: hippocampus, septum, temporal

neocortex, remaining neocortex, striatum, midbrain, amygdala, olfactory lobe, hypothalamus, thalamus, cerebellum, medulla, dorsal and ventral pons and spinal cord. First the hindbrain was separated from the mid- and forebrain by a coronal cut between the colliculi. Forebrain and hindbrain were then dissected concurrently by the two experimenters.

After placing the forebrain on its ventral surface, the cerebral hemispheres were deflected to expose the corpus callosum (see figure 1). A shallow cut severed callosal fibers, allowing for the retraction of the dorsal cortex and exposure of the subcortical structures. The dorsal cortex was then removed by a section of the deflected cortices rostrally from the most lateral aspects of the corpus striata to the most lateral aspects of the hippocampi caudally. The hippocampi were freed coronally from their fornical fibers, transected longitudinally at the medial margin of the entorhinal cortex, and the entire structure was removed by blunt dissection. After stripping the remaining anterior callosal fibers to expose the septum, this structure was removed by undercutting with a horizontal rostral to caudal sweep immediately dorsal to the anterior commissure, and freed of its fornical fibers. Next the neostriata were removed by intersecting between the internal capsule and each striatum and then carefully rolling each striatum dorsalward, beginning at its ventral margin near the dorsal amygdala and moving superiorly

Figure 1: Photographs of anatomical structures followed to guide dissection of the rat forebrain, as outlined in text. The brain is viewed from the dorsal surface in the top four photographs which show the successive phases of the dissection. The bottom two are ventral views, included only for demonstration purposes.

along the medial border of the external capsule. The mid-brain section consisted of a coronal section of the tissue immediately caudal to the mammillary bodies and included the superior colliculi and the inferior colliculi which were removed from the hindbrain section by the other experimenter. The temporal neocortex was then sectioned by placing the brain fragment on its dorsal surface and separating neocortex from amygdala-pyriform with a longitudinal cut along the rhinal fissure. The tissue containing the amygdala was next removed by separating the amygdala-pyriform lobe from the rest of the forebrain fragment along the fissura choriodea medially and the middle cerebral artery rostrally. At this point, the remaining rostral-pyriform tissue was separated from the diencephalon medially along the optic tracts and placed with the tissue containing the olfactory bulbs, stria and tubercle as well as the preoptic and accumbens nuclei, and was labelled "olfactory lobe." Finally, the diencephalon was separated into hypothalamus and thalamus. The former was blocked laterally along the medial border of the optic tracts, rostrally at the optic chiasm and caudally at the posterior edge of the mammillary bodies. The dorsal extent of the cube was taken as the superior edge of the third ventricle. The remaining thalamic sample also included most of the internal capsule.

The hindbrain was sectioned by removing the cerebellum with a midline bisection in the longitudinal plane and then severing the brachia at the lateral aspect of the pons. This also exposed three indentations on the dorsal surface of the pons as described by Zigmond et al. (1974). A cut was made just posterior to these indentations and the caudal portion, the medulla, was separated for assay. Next, the inferior colliculi were removed by placing the remaining rostral piece of tissue on it's anterior surface and cutting out the inferior colliculi along the border defined by the dorsal border of the pons. Finally, the dorsal pons was separated from the ventral pons by a horizontal section along the central canal.

All samples were stored in liquid nitrogen until assayed. Each separate assay consisted of one equivalent brain part from all the rats. Five male and five female rats were assayed from each treatment group.

Assay Procedure

This method was modified from Maickel et al. (1968), and Laverty and Taylor (1968) and differs from assay methods previously used in this laboratory in that each brain part sampled was used as it's own blank in the fluorescence calculations.

Numbered centrifuge tubes (Nalgene, 28.7 mm X 103 mm), one for each sample being assayed, were supported in crushed ice in a portable basin. To each tube 3.0 ml n-butanol/HCl (100:0.1 v/v) was added. The frozen samples were quickly weighed and these weights and the sample identification marks were recorded along with the corresponding centrifuge tube number and sample number. The sample was then placed in the appropriate tube to thaw. Each assay examined identical brain parts from all rats. The sample weights were equalized to 0.28 grams with distilled water if required.

Each sample was then homogenized (Polytron, type PT 10-20-3500) for 10 seconds, with the homogenizer washed with the previously described cold acidified butanol and ice water between each sample.

The samples were next centrifuged at 5,000 rpm for 10 minutes. 2.50 ml of each sample supernatant was transferred to a labelled tube containing 5.0 ml heptane and 0.50 ml 0.1 N HCl, covered with parafilm and agitated for 10 minutes in a wrist action shaker (Burrell, Model 75) and then centrifuged as above.

Five disposable culture tubes (Canlab, polycarbonate 15 X 75 mm) for each sample being assayed were placed in a test tube rack. The sample extracts (water layer) were

transported to one of these tubes using Pasteur pipettes. Then, 0.10 ml aliquots of these sample extracts were transferred to each of the remaining four tubes, two of which contained 0.60 ml 1 M potassium phosphate buffer (pH 8.0) for the Da assay and it's blank, while the remaining two contained 0.50 ml of the same buffer adjusted to pH 7.0 for the Ne assay and it's blank. The remainder of the sample was discarded. The final four solutions were then mixed on a vortex mixer (Maxi Mix, model M-16715).

Two standards were made by dilution of stock solutions of both Ne and Da to 0.05 ug in 0.10 ml of 1N HCL. Next, 0.05 ml of each diluted standard plus 0.05 ml of 0.1 N HCl were added to the appropriate culture tubes containing the buffer solutions. A solution blank was prepared for each standard consisting of the appropriate pH and volume of buffer and an additional 0.10 ml 1N HCL. Standards and their blanks were all made in duplicate and treated as though they were samples.

The conversion reaction procedure for Da and Ne* was as follows:

- 1) Oxidation reaction: to the sample was added 0.05 ml I_2/NaI (0.25 gm iodine and 0.50 gm sodium iodide)

*brackets, where different from Da.

in 100 ml distilled water). This was then mixed and left to stand for exactly 4 minutes.

- 2) Conversion reaction: to the sample was added 0.25 (0.50) ml $\text{NaSO}_3/\text{EDTA}$ (2.5 gm NaSO_3 plus 1.0 gm Na_2EDTA in 100 ml of NaOH (10:100 w/v)). This was mixed and left to stand for 5 minutes.
- 3) Acidification reaction: to the sample was added 0.10 (0.20) ml glacial acetic acid. This was then mixed.

The same chemicals as above were added to the duplicate (blank) set of tubes but in the reverse order. These tubes, which served as blanks, were therefore identical to their particular sample tube except that the conversion reaction was not carried out in the proper order.

The pairs of Da tubes were heated at 100 degrees C° for 15 minutes, allowed to cool and their fluorescence was read at 330 mu/390 mu/excitation/emission wavelengths respectively on a Aminco-Bowman Spectrophotofluorometer (model D223-62155, slit widths 3.0 mm). The pairs of Ne tubes were left at room temperature for 20 minutes and read at 380/480 millimicrons.

The data collected was entered into the following formula to obtain a value of ug of catecholamine per gram of tissue:

$$X \text{ } \mu\text{g/g} = \frac{\text{Standard Concentration} \times (\text{Sample fluorescence} - \text{Sample Blank fluorescence}) \times 6.280}{(\text{Standard Fluorescence} - \text{Standard Blank Fluorescence})} \div \text{Tissue Weight.}$$

STATISTICAL ANALYSES

Generally the data of these experiments, where within S factors were considered, were analyzed by the method of multiple profile analysis (Harris, 1975). Multivariate techniques were used because univariate techniques require certain highly restrictive assumptions about the nature of the intercorrelations among the Ss' responses to the various within S treatments. In the univariate within S case for example, one must make the unrealistic assumption that the variance-covariance of the difference in response to the treatment levels is the same for all possible pairs of treatment levels, i.e., that the variance-covariance matrices are homogenous. Harris (1975) suggests that these within factors would be best treated as an outcome vector where the measurement at each level of the within factor would make up the outcome vector and that the data would best then be analyzed by multiple profile analysis.

Three statistically independent tests are made by multiple profile analysis and they can easily be described by comparing them to their analogs, the univariate within S

analysis where between S factors are also involved.

The test of the parallelism hypothesis is in effect analogous to the univariate test of the within S factor and between S factor interactions. The parallelism hypothesis is stated as follows: the profiles of the treatment groups are mutually identical and this is tested by conducting a Manova on the Ss' slope scores.

Harris (1975) points out that interpretation of other effects is misleading when the within S factor interaction in the univariate, or the parallelism test in the multivariate case is significant, although the tests of the main effects are statistically independent in the multivariate case.

The flatness test is analogous to a test of the within S factor main effect in the univariate case. It tests the hypothesis that the mean population slope score (i.e., difference score) is equivalent to zero. The third test made in multiple profile analysis is analogous to a between Ss factor main effect in the univariate case. It is actually run as a univariate F test with the sum of the repeated measures for each S constituting the unit of analysis. This levels test examines the hypothesis that the mean sum of the dependent measures of the within factor is equal for all between group population mean sums. In other words, it tests the between factors main effects and interactions.

In some cases, where multivariate procedure were inapplicable because of missing scores (the Carleton Manova computer programme has no missing data option) traditional unweighted means Analysis of Variance procedures were followed. In the multiple profile analysis Manova procedures significance was evaluated by the Wilk's U statistic (Kerlinger & Pedhazur, 1973). All multiple comparisons were carried out by Tukey's HSD test (Kirk, 1968).

RESULTS

ASSAYS AND POST-DECAPITATION CONVULSIONS

The effect of the different neonatal drug treatments on post-decapitation convulsion latency of onset and duration can be seen in figure 2. Significant drug treatment effects were found on analysis of variance of both variables. Analysis of convulsion onset latency revealed no significant drug by sex interactions ($F(3,32) = .08, p > .05$) or sex effects ($F(1,32) = .00, p > .05$). The drug effect was significant ($F(3,32) = 45.37, p < .05$) with the 6-OHDA-1 rats showing significantly longer latencies than any of the other groups which did not significantly differ (Tukey's HSD test, $p < .05$). Only one rat from the 6-OHDA-1 group convulsed (latency = 24.8 seconds) while all but two of the day 5 injected did as did all other rats.

The analysis of the convulsion durations matched the latency analysis. The drug treatment by sex interaction ($F(3,32) = .32, p > .05$) and the sex effect ($F(1,32) = 1.15, p > .05$) were non-significant while the drug treatment effect was significant ($F(3,32) = 12.59, p < .05$). The day 1 injected rats had significantly shorter convulsion durations than all the other drug treatment groups which did not differ among themselves (Tukey's HSD test, $p < .05$).

Figure 2: Average latency of onset and duration of post-decapitation convulsions of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats.

The results of the assay for Da and Ne are summarized in figures 3 and 4. Two separate multivariate analyses were carried out on the catecholamine levels of all the brain parts - one analysis of Da levels and another of the Ne levels.

The multivariate analysis of the Da levels revealed no significant drug treatment by sex interaction, sex effect or drug treatment effect. Univariate F tests on the Da levels in the various brain parts suggested, however, that the amygdala was affected by the drug treatment ($F(3,32) = 3.25, p < .05$). Rats injected with 6-OHDA on Day 1 had significantly higher Da levels than vehicle injected controls.

The analysis of the Ne levels revealed no significant drug treatment by sex interactions or significant sex effects. The drug effect, however, was significant ($U = .0022, m = 29, s = 3.08, v = 23, p < .05$). Most brain areas showed significant drug effects on the univariate tests. Table 3 summarizes the significant multiple comparisons among the four groups in the various brain parts and the MS(Error) associated with their respective univariate Fs. All multiple comparisons were carried out with the Tukey's HSD test ($p < .05$).

From the table we can see that the pattern of results, at least after the Day 1 injections, agrees with the

Figure 3: Average Da concentrations of various tissues of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats.

SIGNIFICANT MULTIPLE COMPARISONS
(TUKEY'S HSD $p < 0.05$)

* from VEN

Figure 4: Average Ne concentrations in various tissues of
the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats.

SIGNIFICANT MULTIPLE COMPARISONS
(TUKEY'S HSD $p < 0.05$)

▲ from VEH
△ from 6-OHDA-1
○ from 6-OHDA-5
◻ from 6-OHDA-10

TABLE 3

MULTIPLE COMPARISONS OF THE DRUG TREATMENT GROUP MEANS AS A
 FUNCTION OF BRAIN PART Ne LEVELS FOR ALL BRAIN PARTS
 REVEALING SIGNIFICANT DRUG TREATMENT EFFECTS

<u>Brain Part</u>	MS (Error)	1<5	1<10	1<V	5<1	10<1	V<1	5<10	10<V	5<V
Cortex: Temporal	.0018	*	*	*						
Cortex: Remainder	.0025	*	*	*				*		*
Olfactory Lobes	.0057	*	*							
Hippocampus	.0019		*	*				*		*
Amygdala	.0022	*	*	*						
Amygdala (Da)	.0145						*			
Hypothalamus	.0139					*				
Mid-Brain	.0044				*	*	*			
Pons: Dorsal	.0230				*	*	*			
Pons: Ventral	.0099				*	*	*			
Medulla	.0725				*	*	*			
Cerebellum	.0020				*	*				
Spinal Cord	.0099	*	*							
Heart	.0035			*		*			*	*

general notion that areas which contain Ne cell bodies (such as dorsal and ventral pons as well as the medulla and mid-brain) tend to show significantly increased Ne levels while areas which are thought to contain Ne terminals tend to demonstrate significant decreases in Ne levels. The cerebellum which also contains Ne terminals showed on the other hand, increased Ne levels after the Day 1 6-OHDA treatment.

The most striking observation was the highly injection-age specific effect of the 6-OHDA injections on Ne concentration in most brain areas. Some areas demonstrated increases or decreases after one injection age, and a tendency towards reversed effects on Ne levels at another treatment age. Notable were areas such as the olfactory lobes, spinal cord and the amygdala where Day 1 injections led to decreased Ne levels while Day 10 injections tended to lead to increased Ne levels. Conversely, increased Ne levels were noted in the cerebellum and midbrain after injection on Day 1, while decreased Ne levels tended to appear after Day 5 and Day 10 injections. Other areas did not demonstrate apparent reversals as a function of age but rather seemed to reflect decreased drug effects as a function of injection age. Notable among these areas were the hippocampal and neo-cortical areas where Ne levels were decreased by 6-OHDA injections, with the greatest decreases occurring at the early injection age. In the medulla and pons, the early injections produced the greatest

effects, which were in this case increased Ne levels.

The peripheral effects of the injections as measured by heart Ne concentrations were again age specific. However, the pattern of the drug effects on heart Ne levels contrasted with the central effects in that the Day 10 injected rats had the lowest Ne levels - significantly lower than both the vehicle and the Day 1 injected rats. Thus the largest peripheral Ne depletions occurred after injections at the latest age used here.

BEHAVIOURAL OBSERVATIONS

Activity & Reactivity

Body Weight: The profile of weight gain for the four drug treatment groups can be seen in figure 5. The multiple profile analysis of the weight gain data revealed no significant drug treatment by sex interaction on the parallelism test ($U = .4041$, $m = 32$, $s = 4.69$, $v = 20$, $p > .05$). The drug effect was significant however ($U = .2684$, $m = 34$, $s = 2.83$, $v = 8$, $p < .05$), there being different weight gain profiles between days 15 and 20 ($F(3,32) = 12.33$, $p < .05$) and between days 40 and 60 ($F(3,32) = 3.12$, $p < .05$). The vehicle injected group displayed a significantly higher rate of weight gain than all the 6-OHDA groups between days 15 and 20, while only the Day 5 injected rats gained significantly less weight than

the vehicle injected rats between days 40 to 60 (Tukey's HSD test, $p < .05$).

The parallelism test of the sex effect was also significant ($F(5,28) = 72.546$, $p < .05$) and reflected faster weight gain by the males than by the females. This effect was most pronounced at later ages; that is, days 30 to 40 ($F(1,32) = 34.89$, $p < .05$) and days 40 to 60 ($F(1,32) = 386.84$, $p < .05$).

As was expected, both the flatness test and the levels test were significant but do not require interpretation since the parallelism tests were significant (Harris, 1975).

Activity and Reactivity: Figures 6 and 7 display the ontogeny of activity in the three 6-OHDA and the vehicle groups. Multiple profile analysis of the data revealed only a significant flatness test for mean total resets ($F(5,28) = 95.14$, $p < .05$) and for time spent active ($F(5,28) = 8.85$, $p < .05$). The significant flatness tests show that for both variables, activity changed as a function of age with the changes differing significantly from zero between every age except days 15 and 20 for resets, and days 40 and 60 for the time spent active. Since the activity sensor was weight sensitive, the apparent high activity levels at the later ages reflect not only activity as measured by area traversed but also the increased body mass of the older rats. No sex or drug effects were revealed in the parallelism or levels tests.

Figure 5: Average body weights of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of age.

Figure 6: Average number of activity sensor integrator resets of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of age.

Figure 7: Average time spent active of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of age.

Figure 8 summarizes the effects of the tone on the activity scores (reactivity) of 60 day old rats. The figure shows mean total integrator resets during the 5 minute period just prior to and after tone presentation. Analysis of variance of these data indicated that all groups reacted to the tone by suppression of activity ($F(1,32) = 83.3, p < .05$). No significant drug main effect or interaction between drug treatment and the within variable was observed.

A reactivity score (R) was also computed for each rat, where

$$R = \frac{\text{number of resets 5 minutes after tone}}{\text{number of resets 5 minutes before tone} + \text{number of resets 5 minutes after tone.}}$$

When the reactivity scores were analyzed by an analysis of variance, only the drug effect approached significance ($F(3,32) = 2.33, p = .09$) with the 6-OHDA-10 rats tending to be most reactive and the 6-OHDA-5 tending to be the least reactive (see Table 4).

Motor Behaviour Tests

Figure 9 summarizes the overall performance of the rats on the three motor co-ordination tasks in terms of the average total number of falls per rat for each testing day and each drug treatment group. Since two test batteries were ad-

Figure 8: Average ($\pm$ se) pre tone (dark bars) and post tone (white bars) activity (integrator resets) of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats.

TABLE 4

MEAN R ($\pm$ se) SCORES FOR THE 4 TREATMENT GROUPS

<u>Group</u>	<u>Reactivity Score</u>
6-OHDA-1	.42 $\pm$.018
6-OHDA-5	.44 $\pm$.017
6-OHDA-10	.36 $\pm$.030
Vehicle	.40 $\pm$.026

Figure 9: Average total number of falls during the motor tests of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of age.

ministered each test day, the maximum possible number of falls recorded per rat per day was 6. The parallelism test of the multiple profile analysis revealed a significant sex effect ($F(5,28) = 4.47, p < .05$). This effect arose mainly from the change in the number of falls between days 41 and 61: the males did not change from their average 3.8 falls on day 41, while the females fell more often on day 41 (2.8 falls) than on day 61 (2.2 falls). The flatness test was also significant ($F(5,28) = 163.92, p < .05$) indicating that the change in the number of falls differed significantly from zero between all days except days 41 and 61. The levels test revealed an interaction between the sex and the drug treatments ($F(3,32) = 3.58, p < .05$). The form of this interaction reveals that generally in the 6-OHDA groups, the males tended to fall more frequently than the females, with both males and females falling less often with increasing age at the time of the 6-OHDA injection. In the vehicle group however, the females tended to fall more often than the males. Comparisons of the means by Tukey's HSD test found that the Day 1 injected male rats fell significantly more often than the Day 10 injected males and females and Day 5 injected females ($p < .05$). Both the drug ($F(3,32) = 3.26, p < .05$) and the sex ($F(1,32) = 8.84, p < .05$) main effects were also significant.

The only significant effect on the rope task was provided by the sex variable (parallelism test: $F(5,28) =$

2.63, $p < .05$). Between days 41 and 61 ($F(1,32) = 5.43$, $p < .05$) the males decreased their descent times from 251.3 seconds to 184.8 seconds respectively while the females increased from 229.5 seconds to 258.5 seconds. It was also evident that the time spent on the rope changed as a function of age. This is reflected by a significant flatness test ($F(5,28) = 21.64$, $p < .05$) with the change scores differing significantly from zero between all ages except days 21 and 26 and days 41 and 61.

The time on the beam provided a clear differentiation between the drug treated and control rats (see figure 10). On the parallelism test only the sex factor proved significant ($F(5,28) = 4.09$, $p < .05$) with the females changing more than the males between days 41 and 61 ($F(2,32) = 12.15$, $p < .05$). The males changed from 222.3 to 234 seconds and the females from 216.4 to 341.3 seconds. The test of the within effect, i.e., the flatness test, was significant ($F(5,28) = 28.68$, $p < .05$) with the change scores differing significantly from zero between all ages except between days 26 and 31. The levels test of the drug effect was significant ($F(3,32) = 3.87$, $p < .05$) with the vehicle injected rats having significantly higher time scores than the 6-OHDA-1 rats (Tukey's HSD test, $p < .05$). Other comparisons were not significant.

The groups were also found to differ in their performance on the rotating rod task. On the parallelism test

Figure 10: Average beam cross task time scores for the
6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats
as a function of age.

both the sex effect ($F(5,28) = 3.99, p < .05$) and the drug treatment effect ($U = .4174, m = 34, s = 2.83, v = 8, p < .05$) were significant. The significant sex effect on the parallelism test arose between days 31 and 41 ($F(1,32) = 6.96, p < .05$) where the males' scores changed from 113.2 to 53.4 seconds while the females changed from 124 to 105.8 seconds. The significant drug effect arose mainly from group differences in score changes between days 16 and 21 ($F(3,32) = 6.30, p < .05$). Multiple comparisons revealed that the control group changed less than each of the drug groups between day 16 and 21 (Tukey's HSD test, $p < .05$ - see figure 11).

Morphine Analgesia Results

Figure 12 shows the mean log (paw-lick latency + 1) of the morphine and saline injected rats as a function of drug treatment group and measurement time. Since the groups contained an unequal number of rats the statistical analyses were carried out by using an unweighted means analysis of variance and adopting the conservative F when assessing the significance of the within S variables (Kirk, 1968).

The analysis of the log paw lick latencies revealed only two significant effects. The measurement-time effect was significant ($F(1,76) = 9.35, p < .05$) demonstrating that the paw lick latencies increased with the number of measurements.

Figure 11: Average rotating rod task time scores for the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of age.

Figure 12: Average hot plate log (paw lick latency + 1) of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of time after morphine or saline injection.

i.e. exposures to the hot plate. Multiple comparisons (Tukey's HSD test, $p < .05$) revealed that the baseline mean paw lick latency was significantly lower than the average latency at 30, 60 and 90 minutes after either the saline or morphine injection. The only other significant main effect was the neonatal drug treatment effect ($F(3,76) = 3.62$, $p < .05$) with the Day 1 injected rats having lower latencies than Day 10 or vehicle injected rats. No other interactions or main effects including the morphine-saline comparison were significant.

As shown in Figure 13, the morphine injections were clearly effective in increasing core temperatures in all groups. Analysis of variance revealed a significant morphine by saline injection by measurement time interaction ($F(1,89) = 61.76$, $p < .05$). The core temperatures of the saline or morphine injected rats did not differ on the pre-injection measurement. However, core temperatures of the morphine injected rats were significantly higher than those of the saline injected rats at 30, 60 and 90 minutes. Furthermore, at 60 and 90 minutes temperatures of morphine injected groups were also significantly higher than at 30 minutes. No neonatal drug treatment main effects or any of the interactions involving them were significant.

The only other significant temperature effect was the sex by measurement time interaction ($F(1,89) = 4.94$,

Figure 13: Average core temperatures of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of time after morphine or saline injection.

$p < .05$). This interaction arose because the males had lower core temperatures than the females in their base line measure but did not differ significantly from the females at any of the post injection measurement times.

Water Lick Punishment Results

The latency of the first ten and second ten licks were the main dependent variables of this experiment. All latencies were transformed to x where $x = \log (\text{latency} + 1)$, for statistical treatment and infinite latencies were scored as 900 seconds. Body weights and water intake both in the apparatus and home cage were analysed.

Figure 14 which shows the latency of the first ten licks during adaptation, shock acquisition and extinction phases as a function of drug treatment suggests that the 6-OHDA-1 group demonstrated less shock induced lick suppression than all other groups. The multiple profile analysis parallelism test revealed no drug treatment by sex interaction and no significant sex effect. The neonatal drug treatment was significant, however ($U = .1085$, $m = 29.5$, $s = 2.97$, $v = 21.5$, $p < .05$). None of these profile effects was significant on the univariate F tests during the adaptation or extinction days. Significant profile effects on the univariate tests were noted only between shock days 7 and 8 ($F(3,32) = 4.38$, $p < .05$) and days 9 and 10 ($F(3,32) = 3.14$, $p < .05$). Between days 7

Figure 14: Average log (latency + 1) of the first ten licks of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of adaptation, shock and extinction days.

2 OF/DE 2

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

and 8, the 6-OHDA-10 group changed to a significantly greater degree than the 6-OHDA-1 and the vehicle groups (Tukey's HSD, test, $p < .05$). Between days 9 and 10, the 6-OHDA-1 group also changed less than the 6-OHDA-10 group and the vehicle group. The 6-OHDA-5 group changed significantly less than the vehicle group between these days as well. As could be expected from figure 14, the flatness test proved significant ($F(14,19) = 35.524$, $p < .05$) with the change scores differing significantly from zero on all days except those between the last two adaptation days and between the last two extinction days. The levels test revealed a significant sex effect ($F(1,32) = 29.68$, $p < .05$) with the females having longer latencies than the males and a significant drug treatment effect ($F(3,32) = 4.96$, $p < .05$) with the 6-OHDA-1 group having shorter total latencies than the 6-OHDA-5 and the vehicle groups.

Figure 15 summarizes the latency of the second ten licks as a function of experimental day and drug treatment group. Multiple profile analysis of the latency of the second ten licks revealed no significant drug treatment by sex interaction or drug treatment effect on the parallelism tests. The sex effect was significant on the parallelism test, however ($F(14,19) = 3.18$, $p < .05$) with the females changing more than the males between days 5 and 6 ($F(1,32) = 10.02$, $p < .05$). That is, the females showed a greater degree of change in lick suppression after shock than the males and between days

Figure 15: Average log (latency + 1) of the second ten licks of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of adaptation, shock and extinction days.

14 and 15 ($F(1,32) = 5.99, p < .05$) they also tended to recover more quickly than the males. The flatness test was again significant ($F(14,19) = 33.88, p < .05$) with the change scores differing from zero between days 1 and 2, and 5 and 6 and between all days from day 8 on. The levels test revealed no significant drug treatment by sex interaction, a drug effect which approached significance ($F(3,32) = 2.46, p = .08$) and a significant sex effect ($F(1,32) = 22.99, p < .05$).

Multiple profile analysis of the treatment group weight profiles as a function of experimental day revealed neither significant drug treatment by sex interactions, nor drug treatment effects on the parallelism tests. A significant sex effect appeared however ($K(14,19) = 14.14, p < .05$) with the females gaining less weight than the males between all days from 2 to 9 as well as during days 12 to 15 during extinction. The flatness test was also significant ($F(14,19) = 145.25, p < .05$) with all rats gaining weight through all the experimental days except between days 1 and 2 and days 9 and 10. The levels test revealed a significant drug treatment by sex interaction ($F(3,32) = 2.97, p < .05$), a significant sex effect ($F(1,32) = 264.78, p < .05$) and a significant drug treatment effect ($F(3,32) = 6.78, p < .05$). The significant drug by sex interaction arose since the Day 5 injected females seemed to be more affected in body weight than the Day 5 injected males. In general, all the males were heavier than the fe-

males and the Day 1 injected rats were lighter than Day 10 and vehicle injected rats. The Day 1 injected males were the lightest of all the males while the Day 5 injected females were the lightest of the females although they differed significantly only from the Day 10 and vehicle injected females. The Day 1 injected females were also significantly lighter than the vehicle injected females (Tukey's HSD test, $p < .05$).

Home cage water intake shown in figure 16 tended to parallel the weight profiles with the heavier rats tending to drink more during recovery days than the lighter rats. Multiple profile analysis of the home cage water intake revealed no significant drug treatment by sex interaction, sex effect or drug treatment effect on the parallelism tests. The flatness test was also not significant suggesting that one could not reject the hypothesis that the change scores were equal to zero. The levels test, on the other hand, revealed significant effects for the drug treatment variable ($F(3,32) = 8.85$, $p < .05$) with the 6-OHDA-10 and vehicle groups drinking significantly more overall than the Day 5 and Day 1 injected groups (Tukey's HSD test, $p < .05$). The sex effect was also significant ($F(1,32) = 26.64$, $p < .05$) with the females drinking less than the males.

Multiple profile analysis parallelism tests revealed no significant drug treatment by sex interaction or

Figure 16: Average home cage water intake during the recovery days of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle rats as a function of adaptation, shock and extinction days.

drug treatment effects on the water intake in the apparatus dependent variable. A significant sex effect ($F(14,19) = 2.74$, $p < .05$) was revealed however. Although none of the univariate tests were significant for the sex variable, the females typically tended to have lower change scores than the males. The flatness test was also significant ($F(14,19) = 7.19$, $p < .05$) with the change scores differing from zero between days 1 and 2 ($F(1,32) = 5.04$, $p < .05$) and between days 7 and 8 ($F(1,32) = 4.51$, $p < .05$).

The levels test revealed only a significant sex effect ($F(1,32) = 18.82$, $p < .05$) with the females drinking less than the males in the apparatus throughout the experiment.

Runway Performance

Acquisition: The performance of the drug treatment groups during the acquisition phase of the runway task is shown in figure 17. Analyses of the data were based on all the rats since the procedure was identical for all the rats to this point. No drug treatment effects were significant on the parallelism or the levels tests on any of the three dependent measures. The only significant parallelism test arose from a sex effect on the start box emergence latencies ($F(4,68) = 3.03$, $p < .05$). This effect was due to the sex dependent change in start box emergence latencies between the last two days of

Figure 17: Acquisition of food reinforced runway behavior. Start box emergence, mid-alley and goal-box entrance latencies are shown for the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle injected rats.

MEAN ADAPTATION-DAY LATENCIES (SEC)

GOAL-BOX
ENTRANCE

MID-ALLEY
DISTANCE

START-BOX
EMERGENCE

6-OHDA-1
6-OHDA-5
6-OHDA-10
VEH

acquisition ($F(1,71) = 7.47, p < .05$) when the males reduced their times from 7.5 to 4.4 seconds while the females increased theirs from 4.9 to 5.2 seconds. As expected the flatness test was significant for the start box emergence ($F(4,68) = 25.63, p < .05$), mid-runway ($F(4,68) = 14.14, p < .05$) and the goal entry ($F(4,68) = 10.81, p < .05$) latencies, simply reflecting the increased runway speeds as the training days progressed.

Extinction: Figure 18 summarizes the performance of the four drug treatment groups during the extinction phase of the experiment. Multiple profile analyses revealed no significant parallelism, levels or flatness tests during the extinction phase of the experiment on any of the dependent variables.

Punishment: The effect of the shock in the goal box on emergence, mid-runway and goal box latencies is summarized in figure 19 as a function of drug treatment group and punishment day. Multiple profile analysis of the emergence latencies revealed no significant parallelism, levels or flatness tests. The parallelism test of the drug effect was significant for mid-runway time ($U = .4473, m = 35, s = 2.65, v = 5, p < .05$). This effect arose between days 9 and 10 ($F(3,32) = 7.03, p < .05$) and between the last day of shock and the first extinction trial ($F(3,32) = 3.12, p < .05$) with the 6-OHDA-10 rats increasing their latencies more than any of the other groups be-

Figure 18: Start box emergence, mid-alley and goal-box entrance latencies during extinction of food reinforced runway behavior.

MEAN NO-SHOCK EXTINCTION LATENCIES (SEC)

GOAL-BOX ENTRANCE
MID-ALLEY DISTANCE
START-BOX EMERGENCE

6-OHDA-1
6-OHDA-5
6-OHDA-10
VEH

Figure 19: Food reinforced runway behavior of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle injected rats under punishment of goal entry conditions.

MEAN SHOCK-DAY LATENCIES (SEC)

START-BOX
EMERGENCE

MID-ALLEY
DISTANCE

GOAL-BOX
ENTRANCE

tween days 9 and 10 and decreasing more than 6-OHDA-5 rats between the final shock day and the first extinction trial. No other multiple comparisons were significant. No other parallelism test on this dependent variable was significant although the flatness test was significant ($F(4,29) = 5.30$, $p < .05$) indicating the latency-increasing effect of the repeated shock over days. The drug treatment effect was also significant on the levels test ($F(3,32) = 3.43$, $p < .05$) with the 6-OHDA-1 rats showing significantly lower overall shock induced mid-runway latencies than did the 6-OHDA-10 rats.

There were no significant effects on the parallelism test on the goal entry latency during the shock days. A significant flatness test ($F(4,29) = 20.65$, $p < .05$) reflected the general latency increasing effect of the shock over days. The levels test revealed a significant drug treatment effect ($F(3,32) = 3.04$, $p < .05$). Multiple comparisons of the drug treatment means indicated that the 6-OHDA-1 rats had significantly shorter goal entry latencies during the shock days than the 6-OHDA-10 rats. No other comparisons were significant.

The effect of the drug treatments on post shock goal re-entry times, post shock activity and retreat distance from the goal area can be seen in figure 20. Multiple profile analysis of the post shock goal re-entry times revealed only a

Figure 20: Runway behavior of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle injected rats following punished goal entry. Distance of retreat from the goal area, time to re-enter the goal area following the punishment and the post-punishment activity prior to trial termination are shown in the upper, middle and lower panels respectively.

significant flatness test ($F(4,29) = 19.04, p < .05$). This was due to the increase in change scores between day 6 and 7 ($F(1,32) = 31.40, p < .05$). Analysis of the initial distance that the rat retreated from the goal area after shock revealed no significant parallelism tests. A significant flatness test ($F(4,29) = 15.32, p < .05$) arose from longer retreats from the goal box area as the shock days progressed, from day 6 to day 7 ($F(1,32) = 4.28, p < .05$) and from day 7 to day 8 ($F(1,32) = 11.19, p < .05$). The drug treatment effect was also significant on the levels test ($F(3,32) = 3.66, p < .05$) with the 6-OHDA-1 rats retreating a significantly shorter distance after the shock than the 6-OHDA-10 rats. No other comparisons were significant. The post shock activity scores averaged across the 5 shock days were based on the average runway distance traversed per second by the rats after their initial post-shock retreat. Analysis of variance of the data revealed no sex or drug treatment effects or interactions.

Punishment Extinction: The data for the shock extinction days is summarized in figure 21. Multiple profile analysis of the start box emergence latencies revealed no significant parallelism or levels tests. A significant flatness test was found however ($F(4,29) = 4.59, p < .05$) with the scores decreasing significantly between the second and third and the last two days of extinction. The mid-runway latency during

Figure 21: Runway behavior of the 6-OHDA-1, 6-OHDA-5, 6-OHDA-10 and vehicle injected rats during punishment extinction. The data illustrated are latencies for start box emergence, mid-alley traverse and goal box entrance.

MEAN POST-SHOCK EXTINCTION LATENCIES (SEC)

START-BOX
EMERGENCE

MID-ALLEY
DISTANCE

GOAL-BOX
ENTRANCE

DAYS

extinction revealed only a significant levels test for the drug treatment variable ($F(3,32) = 3.23, p < .05$). Multiple comparisons revealed no significant pairwise comparisons however. Analysis of the goal entry latency variable indicated no significant parallelism tests. A significant flatness test ($F(4,29) = 9.39, p < .05$) confirmed that all the rats decreased their goal entry speeds as the extinction progressed. A significant levels test of the drug treatment variable ($F(3,32) = 5.36, p < .05$) was followed by multiple comparisons of the means. Significantly longer goal entry times were indicated for the 6-OHDA-10 rats than the 6-OHDA-1 and vehicle injected rats. No other comparisons were significant.

DISCUSSION

The results obtained in this study confirm and extend previous observations of the biochemical and behavioural effects of subcutaneous injections of 6-OHDA administered to newborn rats. Catecholamine assays of the brains of rats injected on Day 1 indicate that brain areas which contain Ne cell bodies show increased Ne levels while areas which contain mainly terminal fields show decreased Ne levels when the brains are assayed after maturation. These results were not obtained after injections administered at either five or ten days of age. Rather there seemed to be four different patterns of neurochemical observations which were differentially related to the age at injection.

The cortical and hippocampal areas showed simple, treatment-age related reductions of Ne concentrations with the 6-OHDA-1 rats being most depleted, the 6-OHDA-10 rats not differing significantly from vehicle injected rats and the 6-OHDA-5 rats demonstrating intermediate degrees of Ne depletion. This pattern of drug effect may best be attributed to differential rates of maturation of the blood brain barrier (BBB) in these brain areas (Jacks et al., 1972; Singh and De Champlain, 1972; Pappas et al., 1975). Specifically, the BBB in the rat neocortex and hippocampus appears to develop

around 7 to 9 days after birth. At this time it limits the access of 6-OHDA to these areas (Sachs, 1973).

The second pattern was characterized by treatment-age related increases in Ne concentrations in the medulla, pons and midbrain, as well as the hypothalamus, all areas which contain Ne cell bodies. Once again the greatest alteration was observed in the 6-OHDA-1 rats. This pattern of results was expected from previous work in this laboratory (Pappas & Sobrian, 1972; Pappas et al., 1974; Saari & Pappas, 1973) and others (Clark et al., 1972; Provoost, Bohus & De Jong, 1974; Liew & Taylor, 1972; Amaral et al., 1975; etc.) and various hypotheses, outlined earlier have been proposed to explain the brain stem Ne increase (Pappas & Sobrian, 1972; Jonsson et al., 1975; Sachs & Jonsson, 1973; Kostrzewa & Harper, 1974).

The third pattern was characterized by an apparent, though statistically non-significant age dependent reversal of the treatment effect. In some brain areas, either a reduction or an increase in Ne levels was noted after Day 1 injections while a tendency to the opposite effect was observed following one or both of the other two injection ages. The cerebellum exhibited increased Ne levels after Day 1 injections and decreased levels after Day 5 and Day 10 injections. Increases in cerebellar Ne concentrations have been reported following early neonatal 6-OHDopa treatment (Kostrzewa

& Garey, 1976; McLean, Kostrzewa and May, 1976; Zieher & Jaim-Etcheverry, 1975) and 6-OHDA treatment (Kostrzewa & Garey, 1975; Pappas, Saari and Peters, 1976) and have been attributed to a collateral accumulation of Ne in the cerebellar axons due to the interruption of neurotubular transport of this amine to the forebrain. The cerebellar axons may regenerate (Kostrzewa & Garey, 1976) and accumulate the transmitter or may, in fact, not suffer ultrastructural damage after very early 6-OHDA treatment. According to this latter hypothesis, the maturational state of these neurons may determine their pattern of susceptibility to the treatment (Pappas, Saari and Peters, 1976; Zieher and Jaim-Etcheverry, 1975).

A decrease in Ne levels following Day 1 injections and an increase following injections at later ages was suggested for the spinal cord, amygdala, olfactory area and possibly the septum, although reversals with respect to the vehicle injected control group were not statistically significant in any of these brain areas when a relatively conservative test of the significant differences between the means was carried out (Tukey's HSD test, $p < .05$). In all of these areas the Day 5 and Day 10 injected rats always had significantly higher Ne concentrations than the Day 1 injected rats. These results might reflect responses of the neuronal processes in these areas to partial destruction. Presumably the 6-OHDA-1 rats

were more vulnerable to the drug since their BBB is less mature than of the 6-OHDA-5 and the 6-OHDA-10 rats. Sachs et al. (1974) and Sachs and Jonsson (1975) have argued that limited damage of the Ne nerve terminals may be followed by a massive regenerative growth while more extensive 6-OHDA induced damage prevents the regrowth process. Devor (1975) has demonstrated such compensatory sprouting in the hamster olfactory neurons after neonatal lateral olfactory tract lesions.

The final pattern was the lack of any effect of the drug on Ne concentrations, age-dependent or otherwise. The Ne levels in the corpus striatum, thalamus and septum appeared to be unaffected by the drug. The lack of effect in the thalamus is very puzzling since there is extensive Ne innervation of the thalamus from the dorsal tegmental bundle. Lindvall et al. (1974) point out, however, that the thalamic area contains the terminal fields of four other catecholaminergic pathways which may neurochemically compensate for the loss of input from the locus coeruleus. Furthermore, the thalamic tissue assayed here was sampled mainly from the medial thalamic structures while the lateral nuclear groups which showed the greatest depletions after bilateral LC lesions (Lindvall et al., 1974) were discarded. It is also possible, however, that the thalamus was unaffected by the treatment because of regional BBB maturation differences.

This lack of thalamic Ne depletions has been noticed by other experimenters but no hypotheses have been presented to explain it (Taylor et al., 1972; Clark et al., 1972). No effects on Ne levels were noted in the septum as well, although there was some suggestion of decreased Ne after Day 1 and increased Ne after Day 5 and 10 injections. The septum was the smallest brain part assayed and these results may reflect the lower bounds of the sensitivity of the assay technique.

The injection regimen limited the direct drug effects only to the Ne neurons since neither the corpus striatum nor the so-called olfactory area which contained the nucleus accumbens showed any alterations in Da concentrations after the treatment. These results agree with those demonstrating the selectivity with which the effects of neonatally administered 6-OHDA can be limited to only the Ne neurons after repeated peripheral injections of relatively small doses (Breese and Traylor, 1972). Higher doses of peripherally injected 6-OHDA into newborn rats have been reported to lead to reductions in brain Da concentrations (e.g. Sachs, 1973) as have intraventricular injections (Creese and Iversen, 1973; Kaplanski et al., 1973; Matsuura et al., 1974; Nyakas and van Delft, 1975; Pappas, Saari and Peters, 1976). Thus the selectivity of the treatment to the Ne neurons can be attributed to the relatively low dose of the drug given in two separate injections, thereby preventing the high local concen-

trations which may be necessary to induce striatal Da depletions.

The significant increase in Da concentrations in the amygdala of the 6-OHDA-1 rats was unexpected and has not been previously noted. It may be significant that reductions in Ne concentrations were observed simultaneously in this structure. Hence the Da increase may reflect a compensatory alteration to the Ne reductions. Tassin et al. (1975) observed a significant increase in Da concentrations accompanying Ne decrease in the striatum following a peripheral injection of 6-OHDA in one-day old rats. In this laboratory a similar increase in Da concentrations in the hypothalamus has been observed when the Ne concentrations were reduced after one intraventricular injection of the drug on the day of birth (Pappas, Saari & Peters, 1976). Alternatively, the increased Da concentration may reflect partial damage effects which cause excessive regeneration of Da terminals (Tassin et al., 1975). It is reasonable to expect alterations in other neural systems when components of the brain Ne systems are inactivated. For example, increased hindbrain serotonin turnover and elevated hypothalamic histamine levels have been observed following extended neonatal 6-OHDA treatment (Peters, Mazurkiewicz-Kwilecki and Pappas, 1974).

The age-dependent effect of 6-OHDA on catecholamine levels in the sample of peripheral tissue (heart) which was

collected was opposite to the central effects in that the Day 1 injected rats had significantly less heart Ne depletion as adults than did the Day 10 injected rats. The latter had the greatest depletion of all the drug treated groups. These observations are in agreement with those on the development of the capacity of rat cardiac tissue to accumulate Ne (Iversen et al., 1967; Mirkin, 1972) and the development of sympathetic nerve supply to the heart as determined by the histofluorescence method (Lipp & Rudolph, 1972). Iversen et al. (1967) also showed that the uptake of radioactive Ne in day old rats is only about 19% of adult controls increasing to about 30% of adult values at day 4, 45% of adult values at day 8 and almost 80% of adult Ne uptake values at 12 days of age. It appears that the Ne uptake mechanisms are not developed in the newborn rat heart and therefore 6-OHDA probably does not affect cardiac tissue until sufficient uptake capability is developed.

In summary, the central effects of the 6-OHDA treatment were age dependent with the 6-OHDA-1 rats being most affected. On the other hand, the least peripheral effect was observed with these rats. The neurochemical aspects of the experiments reported here substantially contribute to the present literature since age dependence of neonatal 6-OHDA treatment effects in a wide variety of brain areas was demonstrated. The dissection and assay procedures developed for this study

provided a much more detailed look at the pattern of central neurochemical changes induced by the treatment. This allowed new data showing a Da level increase in the amygdala. The greatest contribution, however, lies in the demonstration of a potentially highly useful tool for discrete and probably permanent neurochemical alterations of the infant rat brain.

Recent data from this laboratory (Pappas, Fibiger and Saari, unpublished observations) has allowed a direct comparison of the biochemical effects of the 6-OHDA-1 treatment with adult dorsal Ne bundle lesions. The pattern of the neurochemical effects of the dorsal bundle lesions was strikingly similar to the effects of 6-OHDA-1 treatments and agreed in general terms with those reported by Ungerstedt (1971). Both treatments produced profound neocortical and hippocampal Ne depletion, increased brain stem Ne concentration as well as increased cerebellar Ne concentration. Not all areas revealed similar effects, however. Both heart and spinal cord Ne concentrations were reduced in the 6-OHDA-1 rats but they were unaffected by the dorsal bundle lesions. Also increased midbrain Ne concentrations were not seen in the dorsal bundle lesioned rats although the increase was present in the 6-OHDA-1 rats. This difference might be due to diffusion of 6-OHDA from the injection site to affect nearby Ne terminals in the midbrain, which could occur during the dorsal bundle lesioning procedure. The final difference between the biochemical

effects of the two treatments was observed in the thalamus. The dorsal bundle lesions depleted thalamic Ne while the 6-OHDA-1 injections did not. This observation might be explained by an early maturing thalamic BBB to 6-OHDA or a neurochemical compensation by other catecholaminergic pathways to the thalamus as outlined earlier.

The behavioral effects of neonatal injections of 6-OHDA demonstrated an age of injection specificity since, generally, the behavior of the Day 1 injected rats was found to be most altered. This specificity matched the biochemical observations since the Day 1 injected rats also showed the greatest central neurochemical changes. Three general classes of behavior were found to be affected by the treatment: post-decapitation convulsions, motor performance and response to suppression on two punishment tasks.

The most obvious alteration was the absence of post-decapitation convulsions in the 6-OHDA-1 rats. This reflex was present in all other groups. This laboratory has previously noted the absence of post-decapitation convulsions after both subcutaneous and intraventricular injections of 6-OHDA into newborn rats (Pappas, Saari and Gallivan, unpublished observations) as has another (Lord, King & Phister, 1976) but this was the first demonstration of the treatment age dependent nature of this phenomenon. Kamat and Sheth (1971) observed that reserpine abolished and L-Dopa reinstated these

convulsions in adult mice. They consequently postulated that the convulsions are initiated by a sudden severing of a monoaminergic pathway of supraspinal origin which tonically inhibits spinal motor interneurons. The sudden release of inhibition on decapitation causes a short-lived rebound hyperactivity of these interneurons. Suenaga et al. (1977) implicated Ne as being crucial for the mediation of these convulsions. They observed that the level of spinal cord Ne after intraventricular injections of 6-OHDA was negatively correlated with the latency to convulse after decapitation. Further support for the hypothesis that spinal Ne mediates post-decapitation convulsions derives from observations in this laboratory that rats with adult dorsal Ne bundle lesions convulse normally while rats receiving the 6-OHDA-1 treatment do not. As mentioned above, the adult dorsal Ne bundle lesions do not alter spinal Ne concentrations while the 6-OHDA-1 treatment does. Similarly, in this experiment, only the 6-OHDA-1 rats had reduced spinal cord Ne levels and lacked the convulsions, whereas the 6-OHDA-5 and 6-OHDA-10 rats convulsed and did not have reduced spinal Ne concentrations.

Future experiments could be aimed at attempting to reinstate the convulsions in the 6-OHDA-1 treated rats in adulthood through pharmacological manipulations (such as L-Dopa). For example, if L-Dopa reinstated the convulsion, it could be argued that the Ne neurons which are necessary for it are simply hypoactive. On the other hand, failure to

restore the convulsions would be consistent with anatomical degeneration of these neurons. Furthermore, it might be possible to trace the degeneration of these neurons using such a technique. Finally, other types of convulsions should be examined after the 6-OHDA-1 treatment since intraventricular injections of high doses of 6-OHDA into adult rats potentiates both metrazol and 'kindled' convulsions (Corcoran et al., 1974). Thus, while 6-OHDA administered to the adult enhances susceptibility to some types of seizure, neonatal 6-OHDA eliminates the post-decapitation seizure.

The neonatal 6-OHDA injections did not alter general activity levels or the appearance of hyperactivity usually observed in young rats around the third week of life (Moorcroft, Lytle and Campbell, 1971). These observations agree with previous ones from this laboratory (Pappas and Sobrian, 1972; Pappas et al., 1974) and other laboratories (Lew Quay, 1971; Taylor et al., 1972; Nyakas et al., 1973). A lack of effect on activity has also been reported after dorsal Nucleus bundle lesions in the adult rat (Roberts, Price and Fibiger, 1976; Crow and Wendlandt, 1976). Recent observations in this laboratory suggested that intraventricular injections of 6-OHDA on days one and two after birth induce enhanced infant hyperactivity (Pappas, Saari and Dugas, in preparation). It is possible that this alteration in activity levels was caused by the depletion in the striatum since such a result was also

observed when the neonates were selectively depleted of Da. The lack of change in the activity levels of the rats in this experiment thus supported the accumulating evidence for a lack of involvement of the dorsal Ne system in the modulation of activity.

When a loud tone was presented at the end of the day 60 test session all the groups reacted with decreased activity levels. Although the drug treatment effect approached significance no drug group differences in reaction to the tone were observed. From these observations no evidence was found that 6-OHDA injections on the first day of life alter the rat's ability to suppress ongoing basal activity when the environmental stimulus configuration is suddenly altered. The above results are not in support of the hypothesis proposed by Roberts, Price and Fibiger (1976) who suggested that rats with dorsal bundle lesions are more distractable than controls. It is possible, however, that the failure to observe results in agreement with the above hypothesis is due to procedural differences or some differences in the biochemical effects of the two treatments. It could be significant that the adult dorsal Ne bundle lesions reduce thalamic Ne concentrations while the 6-OHDA-1 treatment does not. The role that the thalamic Ne plays in distractability could be examined by intracerebral injections of 6-OHDA into various brain areas, notably the thalamus, in adult rats.

The combination of the three motor tasks revealed a clear deficit in the Day 1 injected rats. These rats fell more often than all the other drug injected rats who did not differ significantly from those who had received vehicle injections. It is difficult to attribute this altered motor behaviour to any specific alteration in brain biochemistry since several areas (cerebellum, motor cortex, spinal cord) which are traditionally thought to play a role in motor behaviour were affected by the drug treatment. This type of abnormality has also been reported following neonatal 6-OHDopa (Sweig et al., 1975) and 6-OHDA injections (Hicks and D'Amato, 1975). The emergence of the locomotor deficit was similar to that reported by Hicks and D'Amato (1975) who noted that the locomotor deficits appeared around the time when mature locomotion normally occurred. They felt that these deficits were reminiscent of locomotor abnormalities that follow ablation of parts of the motor sensory cortex. They also provided evidence that 6-OHDA induced impairment of motor cortex development. However, given that the cortical Ne depletion observed here was accompanied by increased cerebellar and decreased spinal cord Ne in these rats, it is clear that these behavioral effects can be attributed to the changes in any of these brain areas. A fruitful approach to the resolution of this problem might be to test adult dorsal bundle lesioned rats on the same motor tasks. If they show deficits similar

to the 6-OHDA-1 rats, the spinal Ne depletions could be eliminated as being crucial for the expression of this deficit. Intracerebral injections of 6-OHDA could also be used to selectively deplete either cortical or cerebellar Ne. Through such manipulations it would be possible to clarify which pattern of neurochemical change and which structure(s) are necessary for the manifestation of this deficit.

The unusual results of the test on morphine analgesia implied that morphine had no effect on analgesia as tested in this procedure. From the upper portion of figure 12 it appeared that some of the properties of previously reported results after dorsal Ne bundle lesions were obtained (Price & Fibiger, 1975; Elchisak & Rosencrans, 1973) in that the rats injected on Days 1 or 5 apparently displayed reduced morphine analgesia. However, because the vehicle injected rats showed progressively longer latencies than morphine injected rats over repeated trials, it seems illogical to argue that the hot plate test in this experiment was sensitive to morphine analgesia. Perhaps the stress of repeated measures elicited responses which competed with the paw lick response. Morphine, because it attenuated the stress response, may actually have caused lower paw lick latencies. Whatever the explanation, it would seem that experiments which utilize repeated exposures to the hot plate or other painful stimuli (e.g. Elchisak and Rosencrans, 1973; Price and Fibiger, 1975) may

not be interpretable unless saline controls also undergo repeated tests. Clearly this issue should be re-examined using a between Ss design. Finally, regardless of treatment, the 6-OHDA-1 group had the fastest paw lick latencies on this task. This latter result supports earlier data (Saari and Pappas, 1973) where it was shown that neonatal peripheral 6-OHDA treatment did not decrease sensitivity to footshock as measured by startle thresholds. Thus the treatment does not lead to reduced sensitivity to thermal or electrically induced pain.

One of the first demonstrations of a behavioural deficit in the neonatal 6-OHDA injected rat was provided by Saari and Pappas (1973). They demonstrated that this treatment attenuated footshock-induced lick-suppression and the time to initiate licking on the day following shock. In several control experiments they showed that the 6-OHDA treated rats drank less in the apparatus and in the home cage but when their lighter body weights were taken into account, they did not differ from controls. Also the lick latencies in a non-shock situation, although sensitive to various levels of water deprivation did not differ from control animals. Furthermore, the 6-OHDA treated rats did not differ from normals in water reinforced Y-maze learning or in their reactivity to electric shock as measured by their startle thresholds. From these results it was suggested that the treatment may

produce animals that are less "fearful" because they developed without a normal sympathetic nervous system. Alternatively it was argued that these rats may suffer from an inability to inhibit learned responses because of their reduced levels of Ne in the hippocampus, a structure which has been suggested as essential for behavioural inhibition (Douglas, 1967).

The lick suppression experiment reported here, confirmed the above finding for punishment-induced suppression and extended it by showing that the effect is injection age specific. The body weight observations made earlier were replicated as was the accompanying difference in home cage water intake. The 6-OHDA effect on water lick latencies appeared only during shock days and not during adaptation to the situation as in the previous experiment, and the weight and water intake differences also appeared to be injection age specific.

Thornton, Goudie and Bithell (1976) attacked the interpretation of an inhibitory deficit in the 6-OHDA injected rat suggesting instead that the treatment produced an animal with decreased affective states because of its effect on the sympathetic nervous system. The data presented here are clearly incompatible with their hypothesis since the rats which demonstrated the greatest peripheral Ne depletion (6-OHDA-10) did not react on any of the behavioral tasks like

the 6-OHDA-1 rats who exhibited reduced lick suppression. The peripheral Ne depletion observed for the latter was the least of all the drug treated groups and in the lick suppression experiment, the 6-OHDA-10 rats suppressed more than all other groups. Furthermore, although neonatal 6-OHDA injections do not produce a marked peripheral sympathectomy they do attenuate the response to punishment (Kostrzewa & Harper, 1974). It would seem that this deficit in the response to punishment must be attributed to the central and not peripheral neurochemical changes induced by neonatal 6-OHDA treatment.

In an attempt to dissociate the effects of neonatal peripheral sympathectomy from adult sympathectomy Lord et al. (1976) found that rats injected neonatally with 6-OHDA (days 1-5) showed a small but significant overall two way avoidance deficit whereas a two way escape deficit only appeared after repeated escape trials. Effects on two way avoidance have not been confirmed in this laboratory (Pappas, 1974). It is impossible to attribute this relatively small behavioural effect to any central catecholamine alterations since these latter data were not reported by Lord et al. (1976). Also, no data pertaining to performance on motor tasks were presented by Lord et al. (1976). Since one of the consequences of neonatal 6-OHDA reported in this study is a motor performance deficit, it is possible that this may affect performance on avoidance tasks and consequently interpretation of a

learning deficit on the basis of these tasks may be suspect.

An experiment which was quite similar to the lick suppression procedure used here and elsewhere (Saari and Pappas, 1973) was recently reported by McLean et al. (1976). They trained rats to run a straight runway for food reward and then, once this response was well-established, punished attempts to eat the food. Their results were similar to the ones reported here for the punishment experiments. The rats, injected neonatally with 6-OHDopa, did not differ from controls until the punishment phase when the drug injected rats tended to leave the start box sooner and approach the goal box more closely than did the saline injected control rats. In agreement with these data Mason and Iversen (1975) have reported that initial acquisition of food reinforced L maze running did not differ among dorsal N₁ bundle lesioned, neonatally 6-OHDA injected or control rats. Both the treated groups showed high resistance to extinction, however.

The alley-way experiment reported here confirms some aspects of the above results since no drug treatment-related acquisition differences were found. Furthermore, although neither the 6-OHDA-10 nor the 6-OHDA-1 rats differed significantly from the vehicle injected rats during the punishment phase of the experiment they did differ from each other in their goal box approach latency and the retreat distance dur-

ing the punishment trials. The 6-OHDA-10 rats reacted more to the shock than did the 6-OHDA-1 rats. These differences may in part, reflect the relative peripheral and central Ne effects in the respective treatment groups. In the lick suppression experiment the 6-OHDA-10 rats were initially more reactive to the punishment than either the 6-OHDA-1 or the vehicle injected rats. Perhaps the greater peripheral effects of the Day 10 injections resulted in sympathetic hyperactivity because of supersensitivity of the Ne receptors as well as hyperfunction of the adrenal gland. Compensatory adrenal hyperfunction has been reported following systemic neonatal 6-OHDA treatment (Lew & Quay, 1971). This peripheral hyperactivity might have resulted in heightened fear responses in the punishment situation.

No drug treatment related difference in the extinction of the appetitively reinforced runway task was noted. This observation is puzzling because it fails to replicate the recent reports of Mason and Iversen (1977) on dorsal Ne bundle lesioned rats as well as neonatal 6-OHDA injected rats (Mason & Iversen, 1975). There was some suggestion here of faster extinction of goal entry by the vehicle group from the first to the third extinction day but on the fourth and fifth extinction day it appeared as if no extinction had taken place. It might be fruitful to repeat the experiment, but

continue the extinction procedure until a clear criterion of extinction would be reached. It should be noted, however, that a recent report by Peterson and Laverty (1976) failed to find extinction differences between neonatal 6-OHDA treated and control rats, and such differences have not been observed in this laboratory in the extinction of shock induced lick-suppression (Saari and Pappas, 1973).

The results from the two punishment experiments could be interpreted as indicating that early neonatal 6-OHDA injections reduce sensitivity to aversive stimuli. Alternatively, this laboratory has suggested that neonatal 6-OHDA may cause a deficit in response inhibition due to the interception of the input of Ne neurons to the hippocampus (Saari & Pappas, 1973). Consistent with this suggestion, depletion of forebrain Ne has been reported to retard runway extinction (Mason & Iversen, 1975) and increase bar press response rates during extinction (Thornton, Goudie & Bithell, 1976) and on fixed ratio schedules (Peterson & Laverty, 1976). The failure to observe any effect of 6-OHDA on the extinction components of the lick suppression and runway tasks agrees with neither the response inhibition hypothesis nor with these other experiments. It is unlikely that the failure to observe extinction effects here were due to insufficient depletion of brain Ne since post-decapitation convulsions, motor behavior and response to punishment were altered. A third

possibility is that the forebrain Ne projections are essential mainly for normal responses in conflict procedures and that these procedures may tap an as yet unspecified dimension of behavior which is altered when these projections are damaged. Speculatively, this dimension of behavior could be related to arousal. Perhaps the affective or activating response to punishment is reduced although the pain threshold as measured by reflexive behaviors is not increased. A further possibility might be distractability as outlined by Roberts et al. (1976). Perhaps the 6-OHDA-1 rats were less 'distracted' by punishing stimuli than the normals. This is of course the opposite effect to that observed for dorsal bundle lesioned rats, who are more distractable than their controls. Is it possible that the opposite effects of these treatments on thalamic Ne concentrations explain these possibly opposite effects on distractability?

In summary I find that the behavioral and biochemical effects of neonatal 6-OHDA injections are highly age specific. The greatest biochemical and behavioral alterations were induced by injections given within 24 hours after birth. The three types of behavioral alteration may indicate discrete behavioral effects of neurochemical alteration of the various terminal fields of the neurons in the LC. Speculatively, the lack of post-decapitation convulsions in the 6-OHDA-1 rats might be attributed to the effects of the treat-

ment on the spinal terminals, the alteration in motor co-ordination could be due to the effects of the drug on the cerebellar terminals while the altered response to a conflict situation may be due to alteration of the forebrain terminals. This theoretical framework is ultimately testable by observing the effects of discrete alterations of the Ne terminals through local intracerebral injections of 6-OHDA. Thus the functions of the LC are likely multiple and cannot be restricted to a single dimension at this time. It can be argued that the lesions induced by the neonatal peripheral injections of 6-OHDA, accompanied by a battery of behavioral tests is a valid approach to the exploration of the functions of the LC. Research utilizing adult dorsal Ne bundle lesions can be integrated into such an effort. Interpretation of the behavioral effects of neurochemical dorsal Ne bundle lesions must be tempered by an awareness of the multiple neurochemical effects of such lesions. If the behavioral tests following these lesions are limited in scope it is difficult to ascribe an observed behavioral effect to the effect on the forebrain Ne terminals. For example, apparent deficits in learning tasks requiring good motor co-ordination might reflect cerebellar dysfunction rather than the mediation of learning by forebrain Ne terminals. The neonatal treatments are open to further criticism insofar as one must take into account the possibility of neural regeneration and

the obvious reorganization of hindbrain Ne neurons. In addition any behavioral, or neurochemical alteration may be accompanied by, or be due to compensatory adjustments in other neurochemical systems. It is possible that these adjustments are of greater magnitude and/or functional relevance in the developing brain. The value of the approach is clear, however. It has been shown that Ne concentrations can be altered in discrete patterns by simply varying the age at which the drug is injected. Furthermore, a specific pattern leads to various behavioral changes. Future research will allow the definition of the crucial parameters for specific behavioral alterations.

Some final aspects of this research merit comment. The drug treatment variable interacted with the sex variable in a statistically significant fashion in two instances. When the performance on the motor tasks was summarized as the total number of falls per test day it appeared that the males fell more often in the 6-OHDA treated groups while the females fell more often in the vehicle injected rats. This interaction was not significant on the individual analyses of the specific tasks however. The body weights were also found to be reduced by the drug treatment in an apparently sex related fashion but only during adulthood. Since the Ne and Da concentrations were not significantly affected by the 6-OHDA treatments in a sex dependent fashion, no hypotheses can be of-

ferred regarding the possible underlying bases of these interactions. Future experiments should test the reliability of the sex dependent effects of neonatal 6-OHDA treatments as well as provide hypotheses regarding the mechanism of these putative effects.

Sex differences, which were unrelated to the drug treatments were found to be significant only on some of the behavioral measures and may be attributable to other sex related observations. For instance, significant sex differences were found in weight gain with the females gaining weight at a slower rate than the males after 30 days of age. Although no sex related differences in activity, reactivity or post-decapitation behavior were found in these rats the 3 motor tasks all revealed significant sex differences. The males differed from the females between days 41 and 61 on the rope climb and beam cross tasks while the sex related differences appeared on the rotating rod between days 31 and 41. It might be possible to relate these effects to the divergent weight profiles occurring at day 30 between the sexes.

As could be expected, the mean basal core temperature of the male rats was lower than the females prior to the morphine injection. The morphine caused temperature increases in both the sexes, however. The lighter body weights of the females could possibly also explain their increased

sensitivity to the electric shock in terms of the immediate and day to day suppression of licking. Shock sensitivity has been shown to be related to body weight (Paré, 1969). The only significant sex effect found in the runway experiments was faster start box emergence latency on the initial adaption day by the females.

It is impossible to relate the above sex related behavioral effects to the biochemical observations since neither the 6-OHDA treatment effects nor amine concentrations were significantly sex related. One cannot dismiss the possibility that sex related developmental differences occur in the amine concentrations but such data were not collected in this experiment. It is not surprising that sex related behavioral effects were revealed and these may simply demonstrate the sensitivity of the various behavioral tasks to inherent behavioral differences between male and female rats.

REFERENCES

- Altman, J., Anderson, W. J., and Strop, M. Retardation of cerebellar and motor development by focal X-irradiation during infancy. Physiol. Behav., 1971, 8, 57-67.
- Amaral, D. G., Foss, J. A., Kellogg, C., and Woodward, D. J. Effects of sc, administration of 6-OH in neonatal rats on dendritic morphology in the hippocampus. Neuroscience Abstracts, 1975, 1209, 789.
- Angeletti, P. U. Chemical sympathectomy in newborn animals. Neuropharmacology, 1971, 10, 55-59.
- Angeletti, P. U. and Levi-Montalcini, R. Sympathetic nerve cell destruction in newborn mammals by 6-Hydroxydopamine. Proceedings of the National Academy of Science, 1970, 65, 114-121.
- Angeletti, P. U. and Levi-Montalcini, R. Growth regulation of the sympathetic nervous system: Immunosympathectomy and chemical sympathectomy, Rev. Europ. Etudes Clin. et Biol., 1971, XVI, 866-874.
- Bird, S. J., and Kuhar, M. J. Ionophoretic application of opiates to the locus coeruleus. Brain Research, 1977, 122, 523-533.
- Breese, G. R. and Traylor, T. D. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: effects of 6-hydroxydopamine. Brit. J. Pharmacol., 1972, 44, 210-222.
- Brodal, A. Neurological Anatomy. Oxford University Press, New York, 1969.
- Clark, D. W. J., Laverty, R. and Phelan, E. L. Long lasting peripheral and central effects of 6-OH in rats. Brit. J. Pharmacology, 1972, 44, 233-243.

- Corcoran, M. E., Fibiger, H. C., McCraughan, J. A. and Wada, J. A. Potentiation of amygdaloid kindling and metrazol-induced seizures by 6-Hydroxydopamine in rats. Experimental Neurology, 1974, 45, 118-133.
- Creese, I. and Iversen, S. D. Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-OH. Brain Research, 1973, 55, 369-382.
- Crow, T. J. Cortical synapses and reinforcement: a hypothesis. Nature, 1968, 219, 736.
- Crow, T. J. and Wendlandt, S. Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine. Nature, 1976, 259, 42-44.
- Devor, M. Neuroplasticity in the sparing or deterioration of function after early olfactory tract lesions. Science, 1975, 190, 998-1000.
- Di Guisto, E. L. and King, M. G. Chemical sympathectomy and avoidance learning in the rat. Journal of Comparative and Physiological Psychology, 1972, 81, 491-500.
- Douglas, R. J. The hippocampus and behavior. Psychological Bulletin, 1967, 67, 416.
- Eichbaum, F. W., Slemer, O. and Yasaka, W. J. Postdecapitation convulsions and their inhibition by drugs. Experimental Neurology, 1975, 49, 802-812.
- Elchisak, M. A. and Rosencrans, J. A. Effect of central CA depletions by 6-OH on morphine antinociception in rats. Res. Comm. in Chem. Path. and Pharm., 1973, 6(1), 349-352.
- Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. 111. The monoamine nerve terminal. Z. Zellforsch, Mikroskop. Anat., 1965, 65, 573-596.

- Gilbert, P. How the cerebellum could memorize movements. Nature, 1975, 254, 688-689.
- Hancock, M. B. and Fougereousse, C. L. Spinal projections from the nucleus locus coeruleus as demonstrated by retrograde transport of horseradish peroxidase. Brain Research Bulletin, 1976, 1, 229-234.
- Harris, R. J. A Primer of Multivariate Statistics. Academic Press, New York, 1975.
- Hicks, S. P. and D'Amato, C. J. 6-OHDA alters developing cortex and locomotion in rats. Neuroscience Abstracts, 1975, 1209, 788..
- Huang, Y. H., Redmond, D. E. Jr., Snyder, D. R. and Maas, J. W. The locus coeruleus and fearful behavior of the nonhuman primate. Neuroscience Abstracts, 1976, 698, 489.
- Iversen, L. L., DeChamplain, J., Glowinski, J. and Axelrod, J. Uptake, storage and metabolism and norepinephrine in tissues of the developing rat. The J. of Pharmacology and Experimental Therapeutics, 1967, 157(3), 509-516.
- Jacks, B. R., DeChamplain, J. and Cordeau, J. P. Effects of 6-OH on putative transmitter substances in the CNS. European J. of Pharmacology, 1972, 18, 353-360.
- Jacobowitz, D. M. Effects of 6-hydroxydopa. In Frontiers in Catecholamine Research, ed. by E. Usdin and S. Snyder, pp.729-740, Pergamon Press, Oxford, England, 1973.
- Jacobowitz, D. and Kostrzewa, R. Selective action of 6-hydroxydopa on noradrenergic terminals: Mapping of preterminal axons of the brain. Life Sci. Part I Physiol. Pharmacol., 1971, 10, 1329-1341.
- Jonsson, G., Pycock, C., Fuxe, K. and Sachs, C. Changes in the development of central noradrenaline neurons following neonatal administration of 6-OH. Journal of Neurochem., 1974, 22, 419-426.

Jouvet, M. Biogenic amines and the states of sleep. Science, 1969, 163, 32-40.

Kamat, U. G. and Sheth, U. K. The role of central monamines in decapitation convulsions of mice. Neuropharmacology, 1971, 10, 571-579.

Kaplanski, J., Nyakas, C., van Delft, A. M. L. and Smelik, P. G. Effect of central early postnatal 6-OH administration on brain catecholamines and pituitary-adrenal function in adulthood. Neuroendocrinology, 1973/74, 13, 123-127.

Kerlinger, F. N. and Pedhazur, E. J. Multiple regression in behavioral research. Holt, Rinehart and Winston, Inc., New York, 1973.

Kety, S. S. Biogenic amines in the central nervous system: The possible roles in arousal, emotion and learning. In The Neurosciences: Second Study Program, ed. F. Schmitt. New York, Rockefeller University Press, 1970.

Kirk, R. E. Experimental Design: Procedures for the Behavioral Sciences. Wadsworth Publishing Co., Belmont, California, 1968.

Korf, J., Bunney, B. S. and Aghajanian, G. K. Noradrenergic neurons: morphine inhibition of spontaneous activity. Eur. J. Pharmacol., 1974, 25, 165-169.

Kostrzewa, R. M. and Garey, R. E. Investigation of the development of cerebellar noradrenergic neurons after 6-hydroxydopa. Neuroscience Abstracts, 1975, 636, 410.

Kostrzewa, R. M. and Garey, R. E. Effects of 6-hydroxydopa on noradrenergic neurons in the developing rat brain. The J. of Pharmacol. and Experimental Therapeutics, 1976, 197(1), 105-118.

Kostrzewa, R. M. and Harper, J. W. Effect of 6-hydroxydopa on catecholamine containing neurons in brains in newborn rats. Brain Research, 1974, 69, 174-181.

- Kostrzewska, R. M. and Jacobowitz, D. M. Pharmacological actions of 6-hydroxydopamine. Pharmacological Reviews, 1974, 26(3), 199-287.
- Laverty, R. and Taylor, K. M. The fluorometric assay for catecholamines and related compounds; improvements and extensions of the hydroxyindole technique. Anal. Biochem., 1968, 22, 269-279.
- Laverty, R., Liew, M. C. and Taylor, K. M. Selective depletion of brain regional noradrenaline by systemic 6-OH in newborn rats. In Frontiers in Catecholamine Research, eds. E. Usdin and S. H. Snyder. Pergamon Press, Oxford, 1973, 1063.
- Lew, G. M. and Quay, W. B. Noradrenaline contents of hypothalamus and adrenal gland increased by postnatal administration of 6-OH. Research Comm. in Chem. Path. and Pharmacol., 1971, 2(6), 807-812.
- Liew, M. C. and Taylor, K. M. Selective destruction by 6-OH of noradrenergic neurones in the cerebral cortex and cerebellum. Proceedings of the University of Otago Medical School, 1972, 50(3), 58-59.
- Lindvall, O., Bjorklund, A., Nobin, A. and Stenevi, U. The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. J. Comp. Neurol., 1974, 154, 317-348.
- Lipp, J. M. and Rudolph, A. M. Sympathetic nerve development in the rat and guinea pig heart. Biol. Neonate, 1972, 21, 76-82.
- Lord, B. J., King, M. G. and Pfister, H. P. Chemical sympathectomy and two-way escape and avoidance learning in the rat. J. Comp. and Physiol. Psychol., 1976, 90(3), 303-316.
- Maickel, R. P., Cox, R. H., Saillant, J. and Miller, F. P. A method for the determination of serotonin and norepinephrine, in discrete areas of rat brain, Int. J. Neuropharmacol., 1968, 7, 275-281.
- Mason, S. T. and Iversen, S. D. Learning in the absence of forebrain noradrenaline. Nature, 1975, 258(5534), 422-424.

- Mason, S. T. and Iversen, S. D. Effects of selective fore-brain noradrenaline loss on behavioral inhibition in the rat. J. Comp. and Physiol. Psychol., 1977, 165-173.
- Matsuura, T., Nojyo, Y., Inoue, T., Ibata, Y. and Sano, Y. The influence of 6-OHDA upon immature dopamine containing neuron of the central nervous system of the rat. Acta Histochem., Cytochem., 1974, 7(4) 342-352.
- McLean, J. H., Kostrzewa, R. M. and May, J. G. Behavioral and biochemical effects of neonatal treatment of rats with 6-hydroxydopa. Pharmacology, Biochemistry and Behavior, 1976, 4, 601-607.
- Mirkin, B. L. Ontogenesis of the adrenergic nervous system: functional and pharmacological implications. Federation Proceedings, 1972, 31(1), 65-73.
- Molliver, M. E. and Kristt, D. A. The fine structural demonstration of monoaminergic synapses in immature rat neocortex. Neuroscience Letters, 1975, 1, 305-310.
- Moorcroft, W. H., Lytle, L. D. and Campbell, B. A. Ontogeny of starvation induced behavioral arousal in the rat. J. Comp. Physiol. Psychol., 1971, 75, 59-67.
- Nyakas, C., van Delft, A. M. L., Kaplanski, J. and Smelik, P. G. Exploratory activity and conditioned avoidance acquisition after early postnatal 6-OH administration. J. of Neurotransm., 1973, 34, 253-266.
- Nyakas, C. and van Delft, A. M. L. Behavioral and electrocortical activity in rats after neonatal intraventricular 6-OH administration. Pharmacology, Biochemistry and Behavior, 1975, 3, 271-277.
- Olson, L. and Fuxe, K. On the projections from the locus coeruleus noradrenaline neurons: the cerebellar innervation. Brain Research, 1971, 28, 165-171.

- Pappas, B. A. Immunological and chemical sympathectomy in the neonatal rodent: effects on emotional behavior. In Limbic and Autonomic Nervous Systems Research, ed. L. V. DiCara, Plenum Press, New York, 1974.
- Pappas, B. A., Peters, D. A. V., Saari, M., Sobrian, S. K. and Minch, E. Neonatal 6-OH sympathectomy in normotensive and spontaneously hypertensive rat. Pharmacology, Biochemistry and Behavior, 1974, 3, 381-386.
- Pappas, B. A., Peters, D. A. V., Sobrian, S. K., Blouin, A. and Drew, B. Early behavioral and catecholaminergic effects of 6-hydroxydopamine and guanethidine in the neonatal rat. Pharmacology, Biochemistry and Behavior, 1975, 3, 681-685.
- Pappas, B. A., Saari, M. and Peters, D. A. V. Regional Brain Catecholamine levels after intraventricular 6-Hydroxydopamine in the neonatal rat. Research Communications in Chemical Pathology and Pharmacology, 1976, 14(4), 751-754.
- Pappas, B. and Sobrian, S. K. Neonatal Sympathectomy by 6-Hydroxydopamine in the rat: no effects on behavior but changes in endogenous brain norepinephrine. Life Sciences, 1972, 11(1), 653-659.
- Paré, W. P. Age, sex and strain differences in the aversive threshold to grid shock in the rat. J. Comp. and Physiol. Psychol., 1969, 69, 214-218.
- Peters, D. A. V., Mazurkiewicz-Kwilecki, I. M. and Pappas, B. P. 6-OH sympathectomy in the neonatal rat: effects on brain serotonin and histamine. Biochemical Pharmacology, 1974, 23, 369-378.
- Peterson, D. W. and Laverty, R. Operant behavioral and neurochemical effects after neonatal 6-hydroxydopamine treatment. Psychopharmacology, 1976, 50, 55-60.
- Pötvin, R. J. and Fried, P. A. Acute and chronic effects on rats of (-) Δ^1 -trans-tetrahydrocannabinol on unlearned motor tasks. Psychopharmacologia, (Berl.), 1972, 26, 369-378.

- Price, M. T. C. and Fibiger, H. C. Ascending catecholamine systems and morphine analgesia. Brain Research, 1975, 99, 189-193.
- Provoost, A. P., Bohus, B. and De Jong, W. Neonatal chemical sympathectomy: functional control of denervation of the vascular system and tissue Na level in the rat after 6-OH. Naunyn-Schmeideberg's Arch. Pharmacol., 1974, 284, 353-363.
- Ritter, S. and Stein, L. Self-stimulation of noradrenergic cell group (AG) in locus coeruleus of rats. Journal of comparative and Physiological Psychology, 1973, 85(3), 443-452.
- Roberts, D. C. S., Price, M. T. C. and Fibiger, H. C. The dorsal tegmental noradrenergic projection: an analysis of its role in maze learning. Journal of Comparative and Physiological Psychology, 1976, 90(4), 363-372.
- Saari, M. and Pappas, B. A. Neonatal 6-OH sympathectomy reduces foot-shock induced suppression of water licking in normotensive and hypertensive rats. Nature, New Biology, 1973, 244(136), 181-183.
- Sachs, C. Development of the BBB for 6-OH. J. of Neurochem., 1973, 20, 1753-1760.
- Sachs, C. and Jonsson, G. Degeneration of central noradrenaline neurons after 6-OH in newborn animals. Research Communications in Chemical Pathology and Pharmacology, 1972, 4(1), 203-220.
- Sachs, C. and Jonsson, G. Changes in central noradrenaline neurons after systemic 6-OHDA administration. J. of Neurochem., 1973, 21, 1517-1524.
- Sachs, C., Pycock, C., and Jonsson, G. Altered development of central Na neurons during ontogeny by 6-OH. Medical Biology, 1974, 52, 55-65.
- Sasa, M., Munekiyo, K. and Takaori, S. Morphine interference with Na mediated inhibition from the L.C. Life Sci., 1975, 17, 1373-1380.

- Scott, C. and Laverty, R. Diurnal rhythms in brain regional 5-hydroxytryptamine metabolism: effects of age and 6-OH treatment. Proceedings of the University of Otago Medical School, 1974, 52(3), 52-54.
- Singh, B. and DeChamplain, J. Development of central and peripheral innervation in rats after neonatal injections of 6-OH. Can. Fed. Bio. Soc., 1972, 15, 475.
- Smith, R. L., Parks, T. and Lynch, G. A comparison of the role of the motor cortex in recovery from cerebellar damage in young and adult rats. Behavioral Biology, 1974, 12, 177-198.
- Suenaga, N., Yamada, K. and Fukuda, T. Correlation between central catecholamine level and post-decapitation convulsion in rats treated with 6-hydroxydopamine. Brain Research, 1977, 122, 165-169.
- Sweig, R., McLean, J. H., Kostrzewa, R. M. and May J. G. Behavioral and biochemical effects of 6-hydroxydopa injections. Neuroscience Abstracts, 1975, 633, 407.
- Tassin, J. P., Velley, L., Stinus, L., Blanc, G., Glowinski, J. and Thierry, A. M. Development of cortical and nigro-neostriatal dopaminergic systems after destruction of central noradrenergic neurons in foetal or neonatal rats. Brain Research, 1975, 83, 93-106.
- Taylor, K. M., Clark, D. W. J., Laverty, R. and Phelan, E. L. Specific noradrenergic neurons destroyed by 6-OH injections into newborn rats. Nature, New Biology, 1972, 239(25), 247-248.
- Thornton, E. W., Goudie, A. J. and Bithell, V. The effects of neonatal 6-OH induced sympathectomy on response inhibition in extinction. Life Sciences, 1976, 17, 363-368.
- Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. 367, 1971, 1-48.

- Versteeg, D. H. G., Van Ree, J. M., Provoost, A. P. and De Jong, W. Neonatal 6-OH treatment: Noradrenaline levels in vitro ^3H -catecholamine synthesis in discrete brain regions of the adult rats. Life Science, 1975, 15, 2127-2134.
- Ward, D. G. and Gunn, C. G. Locus coeruleus complex: elicitation of a pressor response and a brain stem region necessary for its occurrence. Brain Research, 1976, 107, 401-406.
- Zieher, L. M. and Jaim-Etcheverry, G. Regional differences in the long term effect of neonatal 6-hydroxydopa treatment on rat brain Na. Brain Research, 1973, 60, 199-207.
- Zieher, L. M. and Jaim-Etcheverry, G. 6-Hydroxydopa during development of central adrenergic neurons produces different long term changes in rat brain noradrenaline. Brain Research, 1975, 86, 271-281.
- Zigmond, R. E., Schon, F. and Iversen, L. L. Increased tyrosine hydroxylase activity in the locus coeruleus of rat brain stem after reserpine treatment and cold stress. Brain Research, 1974, 70, 547-552.

END

1111

FIN