EFFECTS OF HYPOXANTHINE UPON DOPAMINE NEURONS:

AN ANIMAL MODEL FOR LESCH-NYHAN DISEASE

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ABSTRACT

In Lesch-Nyhan disease, concentrations of hypoxanthine are elevated especially in the brain and cerebrospinal fluid; dopamine and its metabolites are reduced in the caudate and putamen. Hence we investigated the possibility that hypoxanthine has direct effects on dopamine neurons.

Hypoxanthine, adenine or allopurinol was delivered unilaterally into the rat brain. Behavioural effects were monitored by apomorphine-induced rotation; ipsilateral turning was time and dose-dependent. Turning was competitively blocked by a non-specific DA antagonist, suggesting that dopamine neurons were altered. In hypoxanthine treated animals, a D1 antagonist specifically blocked rotation; catalepsy occurred after caffeine administration.

After two or three weeks treatment all groups had elevated purine levels in the caudate nuclei, while catecholamine levels were variably altered.

LES EFFETS DE L'HYPOXANTHINE SUR LES NEURONES DOPAMINERGIQUES: UN MODÈLE ANIMAL POUR LE MALADIE LESCH-NYHAN

ABSTRAIT

Dans la maladie Lesch-Nyhan, les concentrations d'hypoxanthine sont élevées en particulier dans le parenchyme cérébral et la liquide céphalo-rachidien; la dopamine et ses métabolites sont diminués dans le noyau caudé et le putamen. Nous avons donc étudié la possibilité que l'hypoxanthine a un effet direct sur les neurones de dopaminergiques.

L'hypoxanthine, l'adenine ou l'allopurinol ont été injectés unilatéralement dans le cerveau de rat . Les effets comportementaux ont été évalués par la rotation induite par l'apomorphine. La version ipsilatérale était dépendante du temps et de la dose. La rotation était bloquée de façon compétitive par un blocqueur dopaminergique non-spécifique, suggèrant que les neurones dopaminergique étaient impliqués. Chez des animaux traités avec l'hypoxanthine, un antagoniste D1 bloquait spécifiquement la rotation; une catalepsie a été observée après administration de caféine.

Après un traitement de deux ou trois semaines, tous les groupes avaient des niveaux de purines élevés dans le noyau caudé, alors que les niveaux de catécholamines étaient changés de façon variable.

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IV) LIST OF ABBREVIATIONS

3MT	3-methoxytyramine
6OHDA	6-hydroxydopamine
AD	Adenine
ADO	Adenosine
ADP	Adenosine diphosphate
ALLO	Allopurinol
AMP	Adenosine monophosphate
AMY	Amygdala
APO	Apomorphine
APRT	Adenine phosphoribosyltransferase
ATP	Adenosine triphosphate
BST	Brain stem
C	Carbon
CAF	Caffeine
cAMP	Cyclic AMP
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CON	Control
CSF	Cerebrospinal fluid
CTX	Cortex
DA	Dopamine
DOPA	Dihydroxyphenyl alanine
DOPAC	Dihydroxyphenyl acetic acid
GABA	Gamma-aminobutyric acid
GLUT	Glutamate
GMP	Guanosine monophosphate
GTP	Guanosine triphosphate
GU	Guanine
GUO	Guanosine
HALO	Haloperidol
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
HIAA	Hydroxyindole acetic acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
HX	Hypoxanthine

HYP	Hypothalamus
IM	Intra-muscular
IMP	Inosine monophosphate
INO	Inosine
IP	Inter-peritoneal
LCD	Left caudate nucleus
LND	Lesch-Nyhan Disease
MAO	Monoamine oxidase
Mg	Magnesium
N	Nitrogen
NA	Noradrenaline
NAC	Nucleus Accumbens
PRA	Phosphoribosylamine
PRPP	Phosphoribosylpyrophosphate
RCD	Right caudate nucleus
SC	Subcutaneous
SCH	SCH 22390
S.E.M.	Standard error of the mean
TH	Tyrosine hydroxylase
UA	Uric acid
XAN	Xanthine
XO	Xanthine oxidase

V) INTRODUCTION

A) THESIS QUESTION

Lesch-Nyhan disease (LND) is an inborn error of metabolism resulting from the mutation and subsequent deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). As a consequence, there are increased cellular concentrations of inosine (INO), hypoxanthine (HX), xanthine (XAN) and uric acid (UA) (Rosenbloom et al, 1967; Sorensen, 1970; Kelley and Wyngaarden, 1983).

At the metabolic level, there is a substantial understanding of the relationship between the HGPRT deficiency and gout leading to nephrotoxicity (Seegmiller, 1981). However the compulsive self-mutilatory behaviour remains the conundrum of LND (Nyhan, 1976). To date, there is no effective therapy for this behaviour, in marked contrast to the symptoms of gout and renal disease.

Neurochemically, post-mortem brain samples of LND patients showed a marked reduction of dopamine (DA) and its metabolites in the caudate and putamen (Lloyd et al, 1981).

According to the postulates of Garrod (1908), it should be possible to understand all the pathophysiological consequences of an inborn error of metabolism, within the context of metabolite changes secondary to the primary lesion. Hence this led to the speculation that the high concentrations of one or more metabolites found in LND might

have direct effects on DA-containing neuron terminals. Although a number of oxypurines are substantially increased in plasma and urine of LND patients (Rosenbloom et al, 1967; Kelley and Wyngaarden, 1983), HX has been consistently reported to have a high cerebrospinal fluid (CSF) : plasma ratio (Sweetman, 1968; Edwards et al, 1986). Therefore, using an animal model, this thesis investigated the possibility that high extra-cellular concentrations of HX has direct effects upon DA containing neurons.

To provide a detailed background of LND, the remainder of the introduction shall address genetic, biochemical, neuro-chemical and clinical aspects of this disease. Moreover it will examine aspects of patient treatment and finally give the background of an animal model which will be used to monitor the development of HX treatment effects upon dopaminergic neurons.

B) GENETIC ASPECTS OF LND

As mentioned above, the ultimate cause of LND is a gene mutation resulting in a deficiency of the enzyme HGPRT. Mutations affecting the HGPRT gene are heterogeneous (Wilson et al, 1986) and their effects can include: (1) at the processing level of the gene, elimination of transcription and/or translation; and (2) at the structural level of the enzyme leading to protein instability or alterations of the binding sites for co-factors or substrates. The familial pattern of LND is consistent with a recessive X-linked mode

of inheritance (Hoefnagel et al, 1965; Nyhan et al, 1967). Thus hemizygous males are affected, while heterozygous females are unaffected carriers. Female homozygotes are extremely rare, because affected males do not reproduce (Watts et al, 1982; Watts et al, 1987). Nevertheless, there has been at least one report of a female with the classical clinical and biochemical features of LND (Hara et al, 1982). The prevalence of LND is not known, but estimates of its incidence range from 1:380,000 live births (Crawhill et al, 1972), to 1:10,000 male births (Kelley and Wyngaarden, 1983). The disease is not restricted to specific ethnic or racial groups (Kelley and Wyngaarden, 1983).

C) BIOCHEMISTRY OF PURINE METABOLISM

C. i) General

Purine metabolism consists of nucleotide synthesis and degradation (figure 1). Purine nucleotide synthesis occurs via the coordinated action of the de novo and salvage pathways. A brief review of these respective pathways will be presented for context.

C. i. a) The key intermediate

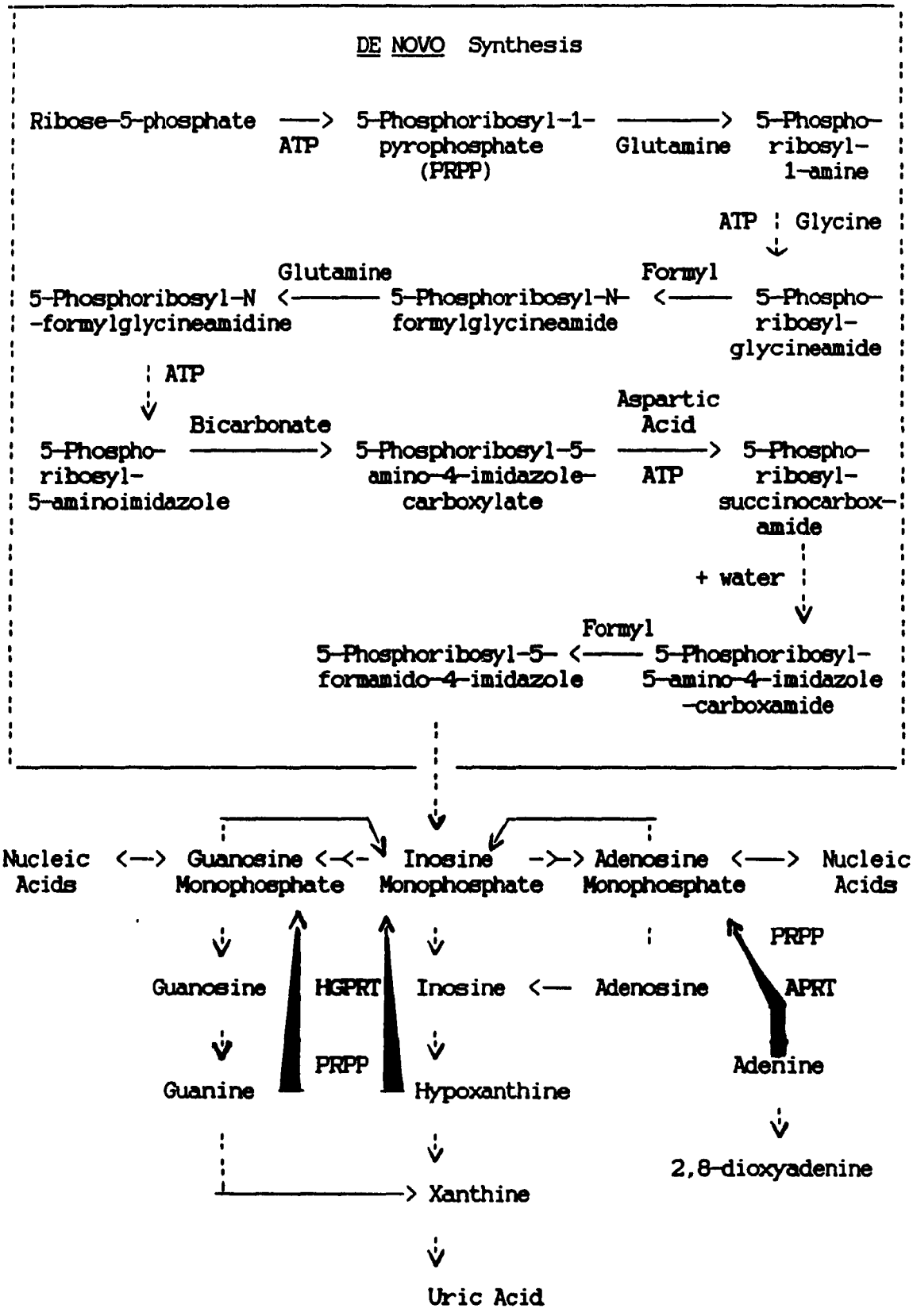
For nucleotide synthesis, the de novo and salvage pathways have the same key intermediate, 5-phosphoribosyl-α-1-pyrophosphate (PRPP). PRPP is formed by a transfer of the terminal pyrophosphate of adenosine triphosphate (ATP) to

carbon 1 of ribose-5-phosphate; the reaction is catalyzed by PRPP synthetase (Kronberg et al, 1955). PRPP synthetase requires either magnesium (Mg) or manganese as a co-factor and inorganic phosphate as an allosteric activator (Fox and Kelley, 1972). This reaction is inhibited by high levels of PRPP, 2,3-diphosphoglycerate, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide or adenosine-, guanosine- and inosine-phosphates with di- and tri- phosphates being more potent inhibitors than mono-phosphates (Fox and Kelley, 1972; Green and Martin, 1973).

PRPP is important for de novo synthesis, because it interacts with L-glutamine as substrate for the first committed reaction in this 10-step pathway (Flaks, Erwin et al, 1957a). In purine salvage, PRPP combines with purine bases to form purine (monophosphate) nucleotides by liberating inorganic pyrophosphate (Flaks, Erwin et al, 1957a).

The metabolic requirement of high energy phosphates differ for these two processes. De novo synthesis requires at least seven high energy phosphate groups to form monophosphate nucleotides. On the other hand, purine salvage requires two high energy phosphate groups, which makes salvage a highly economical means of generating purine nucleotides.

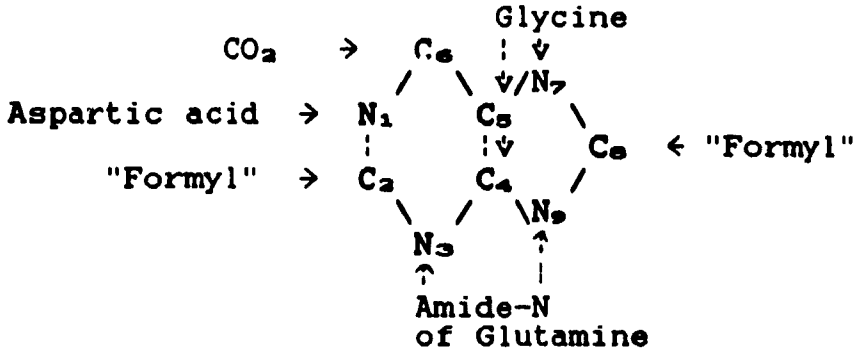
FIGURE 1. Purine metabolism which includes de novo synthesis in the box and the salvage pathways of HGPRT and adenine phosphoribosyltransferase (APRT).



C. ii) De Novo Purine Nucleotide Synthesis

The carbons (C) of the purine ring are derived from glycine [C4 + C5] (Hartman et al, 1956; Hartman and Buchanan, 1958b; Goldthwait et al, 1970), formate [C2 + C8] (Flaks, Warren et al, 1957; Flaks, Erwin et al, 1957b; Warren and Buchanan, 1957; Warren et al, 1957) and carbon dioxide for [C6] (Lukens and Buchanan, 1957); nitrogen (N) is derived from glutamine [N3 + N9] (Mizobuchi and Buchanan, 1968a+b; Mizobuchi and Kenyon, 1968), glycine [N7] (Hartman et al, 1956; Hartman and Buchanan, 1958b; Goldthwait et al, 1970) and aspartic acid [N1] (Lukens and Buchanan, 1957).

Figure 2. Contributors to the purine ring.



The first specific intermediate of the de novo pathway is 5-β-phosphoribosyl-1-amine (PRA), where the α-pyrophosphate group of PRPP is displaced by the amide group of glutamine and there is an inversion of the substituents to yield linkage characteristics of a glycosidic bond (Hartman and Buchanan, 1958a). The amide group contributes the first atom of the purine ring (N9). This irreversible reaction is

catalyzed by amidophosphoribosyltransferase which absolutely requires a divalent cation, preferably Mg (Wyngaarden, 1972). This reaction is a control step for the de novo pathway, because purine nucleotides only regulate this enzyme (Wyngaarden and Kelley, 1978) and not enzymes that catalyze reactions from PRA-glycine to inosine monophosphate (IMP), (Wyngaarden et al, 1958). Allosteric inhibition of amidophosphoribosyltransferase occurs via adenyl- and guanyl-ribonucleotides such that mono- > di- > tri- (Holmes, Wyngaarden et al, 1973). Besides enzymatic regulation, de novo synthesis can be affected at this step by substrate availability (Henderson and Khoo, 1965; Greene and Seegmiller, 1969; Kelley et al, 1970; Raivio and Seegmiller, 1971) and by concentrations of pyrimidine nucleotides (Holmes, McDonald et al, 1973).

In subsequent steps, PRA is converted to a number of intermediates which add the necessary elements of the purine ring. The biosynthesis of the purine ring is complete with the formation of IMP (Flaks, Erwin et al, 1957b). In total six high energy phosphate groups of ATP are used from ribose-5-phosphate to IMP.

IMP is the parent compound for the biosynthesis of adenosine monophosphate (AMP) and guanosine monophosphate (GMP). The formation of AMP requires guanosine triphosphate (GTP) as an energy source to replace IMP's hydroxy group at position 6 with an amino group (Wyngaarden and Greenland, 1963; Van Der Weyden and Kelley, 1974). Hence seven high energy phosphate groups are required to make AMP from

ribose-5-phosphate.

The formation of GMP requires ATP as an energy source for the dehydrogenation and amination at position 2 of the purine ring (Mager and Magasanik, 1960). Forming GMP requires a total of eight high-energy phosphate groups from ribose-5-phosphate. Because synthesis of GMP requires ATP and the synthesis AMP requires GTP, there is a co-regulation of nucleotide synthesis.

De novo purine nucleotide synthesis is not found in all tissues. The liver, for example has a very high de novo activity (Wyngaarden et al, 1969), while bone marrow (Lajtha and Vane, 1958), erythrocytes (Fontenelle and Henderson, 1969), platelets (Holmsen and Rozenberg, 1968) and the brain (Howard et al, 1970; Allsop and Watts, 1982) have relatively low activity. Other tissues like fibroblasts (Rosenbloom et al, 1968), lymphocytes (McKeran and Watts, 1976; Wood and Seegmiller, 1973) and placenta (Wyngaarden et al, 1958) use both the de novo and salvage pathways.

C. iii.) Degradation of Purines

a) Salvage for nucleotide synthesis

In catabolism of purine nucleotides, up to 90% of free purines formed by man are salvaged and recycled (Lehninger, 1979). There are two known mechanisms of salvage. A minor pathway involves two steps, shown in a general form below:

1) Base + ribose-1-phosphate <--> base-ribose + Pi

2) Base-ribose + ATP --> base-ribophosphate + ADP

In the first step, catalyzed by purine nucleotide

phosphorylase, free bases react with ribose-1-phosphate to form ribonucleosides. This phosphorylase is widely distributed in mammalian tissue and actively converts guanine (GU), HX and to a lesser extent XAN, (Fiedkin and Kalckar, 1961). In the following step, kinase reactions phosphorylate the ribonucleosides to ribonucleotides. Kinases phosphorylating INO or guanosine (GUO) are described in animal tissues (Pierre and LePage, 1968), but are absent in human fibroblasts (Friedmann et al, 1969).

Recycling of HX and GU by the nucleoside phosphorylase-nucleoside kinase route is not very active and the most extensive recycling into nucleotide pools occurs via a major salvage mechanism involving two phosphoribosyltransferases, known as HGPRT and adenine phosphoribosyltransferase (APRT), (Wyngaarden and Kelley, 1976). As noted before, HGPRT, the enzyme deficient in LND, catalyzes the conversion of HX to IMP and GU to GMP (Lukens and Herrington, 1957; Henderson et al, 1968). HGPRT requires PRPP and Mg (Benke, Hebert et al, 1973) and is inhibited by its end-products, IMP and GMP, as well as by other purine nucleotides (Henderson et al, 1968; Gutensohn and Guroff, 1972). The importance of HGPRT in embryonic development and cell growth is exemplified by the fact that increases in enzyme activity parallel increasing embryonic weight (Salerno and Giacomello, 1981). In humans (Kelley et al, 1969), monkeys (Krenitsky, 1969) and rats (Howard et al, 1970; Allsop and Watts, 1982), HGPRT has its highest activity in the brain. For man, HGPRT activity is highest within the basal ganglia and other sub-cortical

structures (Kelley et al, 1969). This combined with the finding that the central nervous system (CNS) has low amidophosphoribosyltransferase activity (Howard et al, 1970; Allsop and Watts, 1982), may make the brain dependent upon the salvage pathway for IMP or GMP. APRT catalyzes the conversion of adenine (AD) to AMP (Kronberg et al, 1955). It also requires PRPP as a cofactor and is inhibited by the end-product, AMP (Hori and Henderson, 1966). The turnover rate of AD is greatest in tissues with a high rate of cell division, but overall the total turnover of the AD nucleotide pool is only 0.7% per day (Bartlett, 1977). The brain has a relatively low APRT activity as compared to skin, bone marrow or gut epithelial lining (Bartlett, 1977). Exogenous AD and GU are key for a coordinated and complementary mechanism which ensures that when a cell derives purines from salvage of a purine base, IMP from the de novo pathway will be shunted to the other nucleotide family (Hershfield and Seegmiller, 1976).

C. iii. b) Degradation of terminal end products

Unsalvaged purines are further degraded and excreted. AD and GU are first converted to either HX or XAN and then to UA, (figure 1). The conversion of HX to XAN and XAN to UA is catalyzed by xanthine oxidase (XO), a flavo-protein enzyme containing iron and molybdenum, capable of oxidizing a wide variety of purines, aldehydes and pteridines (De Renzo, 1956). Superoxide radicals are generated as a by product of HX or XAN oxidation (Lehninger, 1979). To prevent damage of

cell membrane's lipid bilayer, these radicals are converted to hydrogen peroxide by superoxide dismutase (Lehninger, 1979). Once UA is formed, the majority of it is excreted in the urine, while a small proportion is eliminated through the gastrointestinal tract (Sorensen, 1959).

D) DEFICIENCY OF HGPRT

D. i) Effects Upon Purine Metabolism

If HGPRT is deficient, then there is dysregulation of purine metabolism. In erythrocytes and fibroblasts from LND patients, HGPRT has a range of activity between 0-5% of normal (Seegmiller et al, 1967; Kelley, 1968; Kelley and Meade, 1971; Sorensen, 1970; Mizuno et al, 1970; de Bruyn et al, 1973). It has been reported that the reduction of HGPRT activity results in an increase of de novo synthesis (Lesch and Nyhan, 1964; Rosenbloom et al, 1968; Sperling et al, 1975; Wood, Becker et al, 1973). The escalation of de novo synthesis was originally thought to be due to an increased availability of the substrate PRPP or to a reduced feedback inhibition by nucleotide end-products of the de novo pathway (Fox and Kelley, 1971; Rosenbloom, 1968; Rosenbloom et al, 1967). However, a thorough study of lymphoblasts from normal and HGPRT deficient individuals reveal their de novo rates to be similar. Thus intra-cellular concentrations of PRPP do not limit the rate of purine de novo synthesis (Hershfield and Seegmiller, 1977).

Nonetheless, LND patients have an accumulation of terminal purine end-products, namely INO, HX, XAN and UA (Rosenbloom et al, 1967; Sorensen, 1970; Kelley and Wyngaarden, 1983). Patients excrete 25-143 mg/kg body weight/day of UA (Michener, 1967; Rosenberg et al, 1968), while a normal child only excretes 18 mg/kg body weight/day (Michener, 1967). High urinary concentrations of HX and XAN were also found in these patients (Balis et al, 1967; Balis, 1969; Harkness et al, 1988). In CSF, HX and XAN were four times greater in LND patients than normouricemic control patients, while UA concentrations remained normal (Rosenbloom et al, 1967), or slightly elevated (Edwards et al, 1986). Furthermore, the CSF/plasma oxypurine concentrations in the LND patient, are three times higher than the normal ratio (Sweetman, 1968).

D. ii) Clinical Presentation

With the excessive production of UA, many patients progress to symptomatic UA nephrolithiasis (Wyngaarden and Kelley, 1976). If untreated this may lead to obstructive uropathy, which is the common cause of death in the first decade (Wyngaarden and Kelley, 1976). With prompt treatment, it is unusual for these patients to develop gouty arthritis (Wyngaarden and Kelley, 1976).

LND patients do not usually present at birth, but follow a developmental pattern of symptoms. At birth they have a normal delivery, but during their first three months some

experience hypotonia and recurrent vomiting (Nyhan, 1968 and 1973). By three to four months of age there is a consistent finding of delayed motor development (Seegmiller, 1980).

When these patients reach eight months to a year of age, extra-pyramidal signs develop and can include athetoid movements of the hands and feet, dystonia and chorea (Seegmiller, 1980). The athetosis is the most common feature and is similar to that noted with asphyxia, birth injury and hyperbilirubinemia (Dreifuss et al, 1968). At one year of age there is cortico-spinal tract involvement with features that include hyperreflexia, sustained ankle clonus, extensor plantar responses and scissoring of the legs (Kelley and Wyngaarden, 1983).

One of the features noted in most reports of LND patients is severe mental retardation. Nonetheless, there are cases where patients have normal intelligence (Bull and La Vecchio, 1978; Christie et al, 1972; Scherzer and Ilson, 1969). In this clinical population, standardized tests may underestimate intelligence due to the severe motor dysfunction and related speech impairment (Scherzer and Ilson, 1969).

Between the ages of two and sixteen years, there is development of compulsive self-mutilation. This type of behaviour occurs in other clinical populations such as schizophrenic children (Green, 1968; Shodell and Reiter, 1968), institutionalized mentally retarded persons (Maisto et al, 1978; Schroeder et al, 1978) and Gilles de la Tourette's patients (Van Woert et al, 1976). The distinction

between the LND patient and the other groups is that self-mutilatory behaviour in the LND patient occurs with a higher incidence and greater severity. Most LND patients bite their fingers, lips and buccal mucosa, with a preference for biting their fingers (Nyhan, 1968). The self-mutilation typically becomes so severe that it is necessary to keep elbows in extension with splints or have the hands wrapped (Nyhan, 1968). Mutilation of the lips can only be controlled by extraction of teeth (Wyngaarden and Kelley, 1976). Patients will plead with caretakers to maintain their restraints; if restraints are removed, patients become extremely upset and cry during their self-mutilation (Nyhan, 1968).

Pain sensitivity testing (Anderson et al, 1977) and observations of crying during self-mutilatory episodes suggest there is no sensory deficit . Yet this pain does not detract them from biting, head butting or placing their extremities in dangerous places (Kelley and Wyngaarden, 1983). It is not uncommon for these patients to exhibit aggressiveness towards others (Nyhan, 1968) and in stressful situations to respond with increased agitation, episodes of opisthotonic posturing and attempts at self-mutilation (Wyngaarden and Kelley, 1976).

In addition to these features there can also be seizures due to hypocalcemia (Marks et al, 1968) or hypoglycemia (Smith et al, 1983).

Moreover, megaloblastic anemia may also be present in some LND patients (van der Zee et al, 1968)

D. iii.) Pathological Findings

a) General autopsies

A number of autopsies upon LND patients have been reported (Hoefnagel et al, 1965; Sass et al, 1965; Partington and Hennen, 1967; Hoefnagel, 1968; Munsat et al, 1968; Crussi et al, 1969; Mahnovski et al, 1975; Mizuno et al, 1976; Berger et al, 1977; Basserman et al, 1979; Lloyd et al, 1981; Warzok et al, 1982; Watts et al, 1982; Rassin et al, 1982; Ogawa et al, 1985; Watts et al, 1987). Age at death ranged from 4-30 years. In general there was evidence of growth retardation; microcephaly; self-mutilation; bilateral shrunken kidneys with deposits of monosodium urate and UA; testicular atrophy (Watts et al, 1987); and no distinct gross or cellular morphological changes of the brain (Hoefnagel et al, 1965; Watts et al, 1982).

b) Neurochemical findings

Some feel spinal fluid determination of biogenic amines is an accurate reflection of CNS neurotransmitter metabolism (Moir et al, 1970). Hence a number of reports upon LND patients, measured the concentration of DA and serotonin metabolites in CSF. One of the first reports which measured CSF catecholamine concentrations in LND patients found low concentrations of homovanillic acid (HVA) and 5-hydroxy-indoleacetic acid (HIAA) in a 2 week old LND patient (Castells et al, 1979). Interpretation of this study was difficult, because there were no age-matched controls. Another study made serial determinations of CSF HVA and HIAA

in four young LND patients over a five year period with age-matched controls (Silverstein et al, 1985). This study found an inverse relationship between age and both HVA and HIAA in controls and LND patients with HVA being consistently lower in the LND patient than the control. Moreover these LND patients had a significant inverse relationship between XAN and either HVA or HIAA throughout the five year period and an inverse correlation between HX and HVA or HIAA in the neonatal period (Edwards et al, 1986). These results suggest that LND patients have an alteration of DA metabolism with HX and especially XAN accumulation accompanying catecholamine depletion.

Direct neurochemical analysis of post-mortem brain tissues from three LND patients aged 13, 14 and 27, revealed a significant alteration of neurotransmitter concentrations (Lloyd et al, 1981). As compared to controls, these patients' brains had a significant reduction of DA and its metabolites in the striatum, but not in the DA cell bodies of the substantia nigra. This anatomical localization of DA depletion is not characteristic of generalized cell death, but consistent with a dishomeostatic process initiated at the terminals. Other neurotransmitter levels such as serotonin were increased in the putamen, while choline acetyltransferase activity was decreased. Gamma-aminobutyric acid (GABA) and noradrenaline (NA) remained at normal levels.

Besides the striatum, post-mortem brain samples of a LND patient also had a significant depletion of DA and its metabolites in the amygdaloid body (AMY), (Seegmiller and

Palmour, personal communication).

E) A REVIEW OF NEUROCELLULAR CHEMISTRY AND ANATOMY

To facilitate a greater appreciation of the neurochemical findings in LND, a review of general neurocellular aspects will be presented with emphasis upon DA containing neurons. This will include DA synthesis, degradation and the categorization of dopaminergic neuronal tracts.

E. i) DA Metabolism

DA is a catecholamine, i.e. its structure is based upon an amine group and a catechol ring (Cooper et al, 1982). DA is synthesized within the cytosol of the neuron and its initial substrate is tyrosine (Coyle and Snyder, 1981). Tyrosine is present in the whole brain at levels of approximately 1.2mg/100g fresh weight of tissue (Sourkes, 1981). Tyrosine hydroxylase (TH) is an enzyme located uniquely in tissues that synthesize catecholamines and is the initial and the rate-limiting enzyme in this biosynthetic pathway (Shiman et al, 1971). TH uses molecular oxygen and tyrosine as substrates and requires iron and tetrahydropteridine as co-factors to form 3,4-dihydroxy-L-phenylalanine (Coyle and Snyder, 1981), (figure 3).

Dihydroxyphenylalanine (DOPA) is readily decarboxylated to DA (3,4-dihydroxyphenylethylamine) by a pyridoxine-dependent enzyme, aromatic L-amino-acid decarboxylase

(Christenson et al, 1970). This enzyme is widely distributed throughout the body and can decarboxylate 5-hydroxy-tryptophan, the precursor of serotonin, as well as other amino acids (Coyle and Snyder, 1981).

For a neuron to contain NA, DA would need to be converted inside synaptic vesicles by dopamine-β-hydroxylase (Craine et al, 1973).

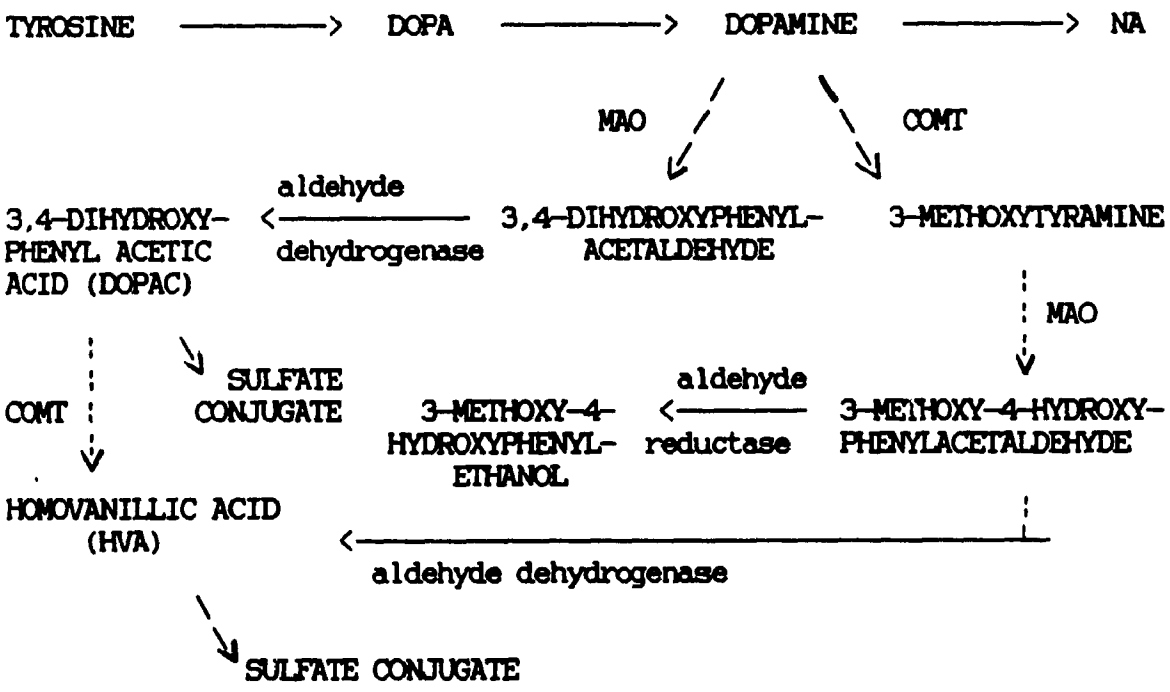
Upon release from synaptic vesicles, the DA molecule can interact with receptors on the post-synaptic membrane. DA has at least two receptor subtypes (Kebabian and Calne, 1979). The D1 subtype is positively coupled to an adenylate cyclase second messenger system, while the D2 receptor subtype is negatively coupled to this system. Thus activation of D1 receptors increases intra-cellular cyclic AMP (cAMP), while activation of D2 receptors is typically accompanied by a decrease in intra-cellular cAMP.

After DA has bound to one of its receptors and initiated the appropriate response in the post-synaptic cell, it can be taken up by the pre-synaptic terminal (Coyle and Snyder, 1969). This re-uptake mechanism involves endocytosis and chemical recycling, so that DA can be stored once more in synaptic vesicles or degraded.

DA can be degraded either by monoamine oxidase (MAO) or by catechol-O-methyltransferase (COMT). MAO is located within the neuron on the outer membrane of the mitochondria (Costa and Sandler, 1972), while COMT is primarily located on the outer membrane of the neuron and therefore acts upon extra-neuronal catecholamines (Nikodejevic et al, 1970). The

major degradative metabolites of DA are 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA), (figure 3). When investigating the turnover of DA in the striatum, a good indicator in humans is HVA (Sourkes, 1981), while in the rat, DOPAC more adequately reflects the flow of dopaminergic impulses (Cooper et al, 1982).

FIGURE 3. Dopamine metabolism. Adapted from figure 6-13, Cooper et al, 1982.



E. ii) Dopaminergic Systems

No function is carried out by a single neuron, rather behaviour is generated by many cells; some perform roughly the same types of operations, while others carry out related aspects of sensory analysis or motor integration. Moreover,

specific functions or aspects are located to particular regions or set of regions within the brain. With this in mind, according to Cooper et al (1982), the dopaminergic neuronal pathways can be classified into three major categories in terms of length of the efferent DA fibers.

E. ii. a) Ultrashort systems

Examples include: the inter-plexiform amacrine-like neurons, which link the inner and outer plexiform layers of the retina; and the periglomerular DA cells of the olfactory bulb, which link together mitral cell dendrites in separate glomeruli.

E. ii. b) Intermediate-length systems

These systems include: the tuberohypophysial DA cells (also known as the tuberoinfundibular system), which project from arcuate and periventricular nuclei into the intermediate lobe of the pituitary and into the median eminence; the incertohypothalamic neurons, which link the dorsal and posterior hypothalamus with the dorsal anterior hypothalamus and lateral septal nuclei; and the medullary periventricular group, which includes DA cells in the perimeter of the dorsal motor nucleus of the vagus nerve, the nucleus tractus solitarius and cells scattered in the tegmental radiation of the periaqueductal grey matter.

E. ii. c) Long-length systems

These systems are the projections which link the ventral

tegmental and substantia nigra DA cells with three principal sets of targets: the neostriatum (principally the caudate and putamen); the limbic cortex (medial prefrontal, cingulate and entorhinal areas); and other limbic structures (the regions of the septum, olfactory tubercle, nucleus accumbens septi, AMY and piriform cortex). The latter two groups are also known as the mesocortical and mesolimbic DA projections.

It is the dysfunction of these long-length systems that is of particular interest with respect to aberrant motor behaviour seen in LND. For example, characteristics of neostriatal disorders can include involuntary movements (dyskinesia), poverty and slowness of movement and disorders of muscle tone and postural reflexes (Sourkes et al, 1975). These abnormal movements can encompass the following: tremor; athetosis; chorea; and ballism. Besides LND, other major disorders of the basal ganglia include Parkinsonism (Hornykiewicz, 1973), Huntington's disease (Bird and Paulson, 1971), ballism (Kase et al, 1980), Wilson's disease (Sourkes et al, 1975) and Gilles de la Tourette's syndrome (Shapiro and Shapiro, 1980). The majority of these disorders have a homeostatic alteration of at least one neurotransmitter found within the basal ganglia or its associated structures.

The AMY also receives DA innervation and when lesioned shows some aberrant behaviour patterns similar to LND. Specifically, bilateral destruction of the temporal lobe results in at least the compulsive tendency to place objects

in the mouth (Terzian and Dalle Ore, 1955). Both behavioural and neurochemical findings in the LND patient may suggest that the AMY plays a role in the neurological manifestations of LND.

F) TREATMENT

Based upon the clinical features of LND, therapy is primarily directed at (1) the excessive UA production and (2) the CNS dysfunction; while prevention of LND is obtained through prenatal diagnosis.

F. i) Excessive UA Production

To minimize kidney damage from the large UA load, LND patients receive a drug which inhibits the formation of UA, rather than an uricosuric drug therapy. The drug of choice at present is allopurinol (ALLO).

ALLO is a XO inhibitor, similar in structure to HX (Lorz and Hitching, 1956). Not only does ALLO act as a competitive inhibitor for XO, but it can also be a substrate for the enzyme (Elion et al, 1964). The oxidized product, allo-xanthine also acts as an inhibitor of XO (Elion et al, 1964). Allo-xanthine plays an important role in the inhibition of this enzyme since ALLO is rapidly oxidized in vivo and the remaining circulating ALLO is promptly excreted by the kidney (Elion et al, 1966). Allo-xanthine remains in the system for a longer period and for long term therapy is

the major contributor to enzyme inhibition (Elion et al, 1966). In experimental animals, ALLO and its metabolites were found throughout the body, with the brain having half the concentration of blood (Elion et al, 1966).

ALLO is indicated for the prevention of UA stone formation, UA and urate nephropathy, gouty arthritis and the development of tophi (Wyngaarden and Kelley, 1976). If these findings are present, administration of this drug will halt the progression of kidney damage; some reversal maybe obtained (Wyngaarden and Kelley, 1976). There is some evidence suggesting that commencement and maintenance of this therapy shortly after birth, will not affect the progression of the CNS dysfunction (Marks et al, 1968).

F. ii) CNS Dysfunction

Unfortunately attempts to control the CNS progressive dysfunction of LND have not been successful. Many problems confront the development of an adequate treatment. First, although the neurological dysfunction may be due to a striatal DA deficiency, effective treatment and/or control of self-mutilatory behaviour remains elusive. Furthermore if a treatment were to be devised, subtle changes in neurologic function might not be noticed. Moreover, treatment which addresses prevention, may begin too late to reverse the disease's evolution. And finally, to illustrate our lack of knowledge concerning psychological components, LND patients seem to have a better prognosis when placed in an

environment where care and attention is freely given (Wyngaarden and Kelley, 1976). Nevertheless experimental trials have been performed and some are described below.

One therapeutic approach was directed towards the replacement of the presumed deficiency of either GMP or IMP. Administration of GMP or AMP was attempted without evidence of clinical improvement (Rosenberg et al, 1968). GUO and INO have also been tried without success (Kelley et al, 1969; Berman et al, 1969).

Administration of AD was attempted on the rationale that AD could be converted by APRT and then inter-converted to increase IMP and GMP pools. This therapy received mixed results with respect to improvement of neurological behaviour (Watts et al, 1974; van der Zee et al, 1970; Demus et al, 1973; Nissim et al, 1974). The therapy in all cases had to be discontinued due to the nephrotoxicity by the AD metabolite, 2,8-dioxyadenine. This conversion was catalyzed by XO (Phillips et al, 1952; Wyngaarden and Dunn, 1957), despite the use of ALLO.

Following observations that HGPRT-deficient fibroblasts grew readily with AD and folic acid supplementation (Felix and DeMars, 1969), one patient was started on AD and folic acid at birth (Benke, Herrick et al, 1973). Unfortunately there was no delay of the progressive abnormal neurological development . Likewise, folic acid supplements did not have any beneficial neurological effects in older patients (Wyngaarden and Kelley, 1976).

Based upon observations that aggressive behaviour in rats

could be eliminated with L-5-hydroxytryptophan, four patients were treated with this amino acid. All four obtained control of their self-mutilation, which manifested itself again upon termination of the therapy (Mizuno and Yugari, 1974). However in double blind studies, this treatment had no therapeutic benefit (Wyngaarden and Kelley, 1976).

Other agents which have been found to be of little therapeutic benefit include orotic acid (Wyngaarden and Kelley, 1976), L-tryptophan (Mizuno and Yugari, 1974), tetrabenazine, thiopropazate and chlorpromazine (Watts et al, 1974).

Drugs which appear to be useful with regard to the movement disorder include diazepam, haloperidol (HALO) and phenobarbital (Wyngaarden and Kelley, 1976). Recently it was shown that a relatively potent DA D1 antagonist, fluphenazine, attenuated the self-mutilatory behaviour of the LND patient better than the non-specific DA antagonist, HALO (Goldstein et al, 1985).

F. iii) Prevention: Prenatal diagnosis

At the present time, the enzyme deficiency cannot be corrected by drug and/or diet therapy as in the case of other inborn errors of metabolism (Scriver, 1982). Moreover gene therapy is still at the research stage (Anderson, 1985; Caskey, 1987). Hence this makes prenatal diagnosis of LND and the subsequent termination of HGPRT deficient

fetuses the only means of prevention. Prenatal testing can detect a HGPRT deficient fetus before 20 weeks of gestation. At 15-16 weeks of the gestation an amniocentesis can obtain a sample of amniotic cells and the HGPRT activity determined (Boyle et al, 1970; Singh et al, 1976; Hosli et al, 1977; Halley and Heukels-Dully, 1977; Burkhardt et al, 1978; Shin-Buehring et al, 1980; de Bruyn, 1983). More recently, chorionic villi can be obtained at 8-9 weeks of gestation and HGPRT activity assayed (Gibbs et al, 1984). These tests are usually performed on women who have had a previous child with LND or on a woman with a positive family history of LND and a heterozygote carrier of the HGPRT deficiency, as determined by hair root (Silvers et al, 1972), peripheral blood T cell (Kamatani et al, 1984) or skin fibroblast analysis (de Bruyn, 1983).

G) ADDRESSING THE THESIS QUESTION

In addressing the thesis question, does high extracellular concentration of HX effect DA containing neurons, a key point is that the LND patient's neurological dysfunction occurs in a developmental fashion. Thus if high levels of HX effect DA neurons, then these affects could also follow a developmental pattern. Therefore any animal model used would have to measure not only the biochemical alterations of purine and catecholamine homeostasis, but also measure dopaminergic dysfunction resulting from pre- or post-synaptic effects. An animal model which can study both

neurochemical alterations and dopaminergic dysfunction is the Ungerstedt rotational model (Ungerstedt and Arbuthnott, 1970).

Ungerstedt's Rotational Model is based upon observations that dopaminergic nerve fibers can be selectively destroyed by a local unilateral intracerebral injection of 6-hydroxy-dopamine (6OHDA), which is a chemical analogue of the catecholamine transmitter (Ungerstedt, 1971; Uretsky and Iversen, 1970). Rats which received 6OHDA intra-ventricularly as neonates were found, as adults, to have virtually total depletion of forebrain DA and substantially reduced forebrain NA levels (Creese and Iversen, 1972). Apomorphine (APO), which is a direct central DA receptor stimulating agent (Seeman, 1978), produced locomotion and stereotyped behaviour in these animals at dose levels which were ineffective in normal rats. These behaviours were only induced when animals were challenged with APO and baseline behaviours did not significantly differ between 6OHDA lesioned and control animals. Furthermore, unilateral 6OHDA lesions that induce degeneration of the ascending DA pathways from the mesencephalon, produce animals which exhibit circling movements upon APO challenge (Ungerstedt and Arbuthnott, 1970). In theory, unilateral 6OHDA lesioning results in the super-sensitization of DA post-synaptic receptors on the lesioned side due to the loss of their pre-synaptic nerve-endings (Ungerstedt, 1971). This super-sensitivity is thought to be due to a lack of stimulation by transmitter release (Ungerstedt et al, 1981). Hence when an

unilaterally DA denervated rat is treated with APO, the action of the agonist is considerably enhanced on the denervated DA post-synaptic receptors resulting in a contralaterally directed rotation (Ungerstedt et al, 1981). Moreover this rotational behaviour is highly reproducible within a single animal and quantitatively related to the degree of DA denervation when comparing different individuals. Conversely, if a treatment results in a desensitization of the DA post-synaptic receptor, then upon APO challenge, the animal will rotate ipsilaterally to the treated side.

The Ungerstedt model can be applied to the thesis question by unilaterally treating rats with HX. If HX treatment results in super-sensitization of the DA post-synaptic receptor within the caudate nucleus on the treated side, then the animal should rotate the contralaterally (to the lesioned side). If HX treatment resulted in an APO-induced ipsilateral rotation, then this would suggest that the DA post-synaptic receptors on the treated side are desensitized. Because the effect upon DA neurons can be monitored quantitatively by the number of APO-induced rotations, neurochemical measures of both purines and catecholamines over the post-implantation period can be obtained at different developmental stages of the HX lesion.

VI) METHODS

A) SUBJECTS

Sprague-Dawley male rats (175-200g) were obtained from Charles River Laboratories (St. Constant, Quebec), housed four per clear plastic cage (36 X 48 cm) with wood chip bedding, maintained in environmentally controlled rooms (6:00 AM light, 6:00 PM dark cycle; temperature, 23-25 Celsius) and provided with Purina Laboratory Chow and water ad libitum.

B) SURGICAL MATERIALS AND PROCEDURES

B. i) Cannulae

Stainless steel, 21 gauge cannulae were 1 cm in length. In preliminary experiments, the cannulae were used as guides for Hamilton syringe injections, while in later experiments the cannulae were packed with powdered HX, AD or ALLO.

B. ii) Drugs

For the anesthetic a mixture of nine parts 100mg/ml ketamine to one part 10 mg/ml xylazine was administered intra-muscularly (IM) at 0.1ml/100g of body weight. Any additional injections did not exceed 0.1ml IM.

In preliminary experiments, a group of animals were pre

treated with intraperitoneal (IP) injections of 15mg/kg desmethylimipramine (Merrell, Cincinnati, OH) 30 minutes prior to an intracranial injection of 6OHDA-hydrobromide (Sigma, St. Louis, Mo.) dissolved in 0.9% saline containing ascorbic acid (1 mg/ml) as an antioxidant (Breese and Traylor, 1970). The dose used was 8ug of 6OHDA in 2ul of solution.

B. iii) Implantation Sites

Anesthetized animals were placed in the stereotactic apparatus and cannulae were implanted into either: the left caudate nucleus of the corpus striatum (LCD),[stereotactic coordinates, Bregma -0.2mm, Lateral 3.0mm, Vertical -4.8mm]; or the left lateral ventricle,[Bregma -0.2mm, Lateral 1.5mm, Vertical -3.0mm]. All stereotactic coordinates were derived from the atlas of Konig and Klippel (1967).

The cannula implants were anchored with skull screws, fixed in place with dental cement and the incision was closed with Michel Clips.

C) THE POST-OPERATIVE PERIOD

C. i) General

In preliminary experiments animals implanted with cannula guides received daily injections beginning on the first post-operative day. Manual injections into the LCD were

performed for five groups of animals: three of the groups received 5mM HX in volumes of 5ul, 10ul or 20ul; one group received 10ul of 5mM AD; and the last group received 10ul of the vehicle (0.9% saline). These injections continued for at least two weeks before behavioural evaluation.

The animals with packed cannulae implants or a single intracranial injection of 6OHDA were allowed to recover until the first behavioural testing, which was no sooner than the fourth post-operative day.

C. ii.) Behavioural Testing

a) General

All animals were challenged with subcutaneous (SC) injections of APO-HCL (Sigma, St Louis Mo.) and their induced rotational response recorded. APO was dissolved in 0.1 M ascorbic acid in concentrations of 5, 10 or 15mg/kg body weight. Animals with LCD cannulae implants or unilateral intracranial 6OHDA injections were evaluated on post-operative days fourteen and twenty-one. Animals with left lateral ventricle cannulae implants, were evaluated behaviourally at post-operative day four, seven, eleven, fourteen, eighteen and day twenty-one.

Rotational scoring of individual animals was carried out in open cages (36 X 48 cm), using the focal scoring method; animals were observed for a one minute interval every five minutes over a ninety minute period with the number of observed rotations summed. A rotation was defined as a complete 360° turn.

C. ii. b) Blockade of rotational response

Once an animal had a consistent rotational response to APO challenge, it was selected for DA antagonist testing. Animals implanted with HX, ALLO or HX plus ALLO packed cannulae were pre-treated with vehicle, HALO (McNeil Pharmaceutical, 0.6mg/kg IP), (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SCH 22390, Schering Corporation, 0.1mg/kg SC) or caffeine (33mg/kg IP) and challenged with APO at a dose of 5, 10 or 15mg/kg. Behavioural observations were performed as outlined above.

Levels of other APO-induced behaviours were recorded. Behaviours such as locomotion, sniffing, licking and gnawing were scored on a semi-quantitative scale of 0 to 5 (Kelly et al, 1975; Cresse and Iversen, 1973), with 5 representing continuous performance of the behaviour and 1 representing intermittent occurrence.

C. iii.) Biochemical Analysis

a) Sacrificing animals

After post-operative day seven, fourteen or day twenty-one, animals were stunned and sacrificed by decapitation. Their brains were rapidly removed, put in ice and then frozen at -70C. At a later date, they were partially thawed and dissected on ice. In preliminary experiments, animals that received injections, 6OHDA or packed cannulae into the LCD had both caudates removed, weighed, placed in mini-vials and refrozen at -70C. Animals that received packed cannulae

into the left lateral ventricle had the following areas removed, weighed, placed in mini-vials and refrozen at -70C: both AMY; the hypothalamus (HYP); the frontal cortex (CTX); the LCD; the right caudate nucleus (RCD); both nuclei accumbens (NAC); and the brain stem (BST).

C. iii. b) Catecholamine and purine analysis

Dissected brain regions were homogenized in 0.1 M perchloric acid at a final concentration of 25mg brain tissue/ml. Following centrifugation, a lab technician analyzed 20ul aliquots by high performance liquid chromatography (HPLC) with electrochemical detection using conditions similar to those described by Westerink and Mulder (1981). The concentration of DOPAC, DA and HIAA were measured in all brain region samples, while NA was only measured in the LCD, RCD and NAC. Purine concentrations of UA, HX, XAN, INO and adenosine (ADO) were determined on the same samples from the AMY, LCD and RCD using HPLC and UV detection (Hartwick et al, 1979).

C. iv) Statistical Analysis

Since the data did not meet the assumptions for analysis of variance, the non-parametric Mann-Whitney U-test was used (Sokal and Rohlf, 1981). One-tailed tests were used for behavioural results, while two-tailed tests were used for the biochemical results. For n_1 greater than 20, t values were obtained to approximate the correct probability value.

VII) RESULTS

The results shall be divided into three sections. The first section will report upon preliminary experiments to establish maximal delivery of HX into the brain of a rat. Utilizing both behavioural and neurochemical analysis, the following segment will build upon the preliminary findings to examine the thesis question. The final section will further examine the behavioural aspects of HX treatment and begin to elucidate the effects upon DA neurons.

Please note that the primary data are tabulated in Appendix I.

A.) PRELIMINARY EXPERIMENTS

The purpose of these preliminary experiments was to devise an effective method of delivering high concentrations of HX into the brain of a rat. Two trial experiments are presented in this section.

A. i) Delivery of HX by Micro-Injection into the LCD

The first method of delivery, was daily micro-injections of 5mM HX solution into the LCD. Unilateral injections were performed so that Ungerstedt's Rotational Model could be used for rapid screening of behavioural effects.

There were two control groups for this experiment. One group of animals received saline injections (10ul/day), to

control for the vehicle in which HX was dissolved.

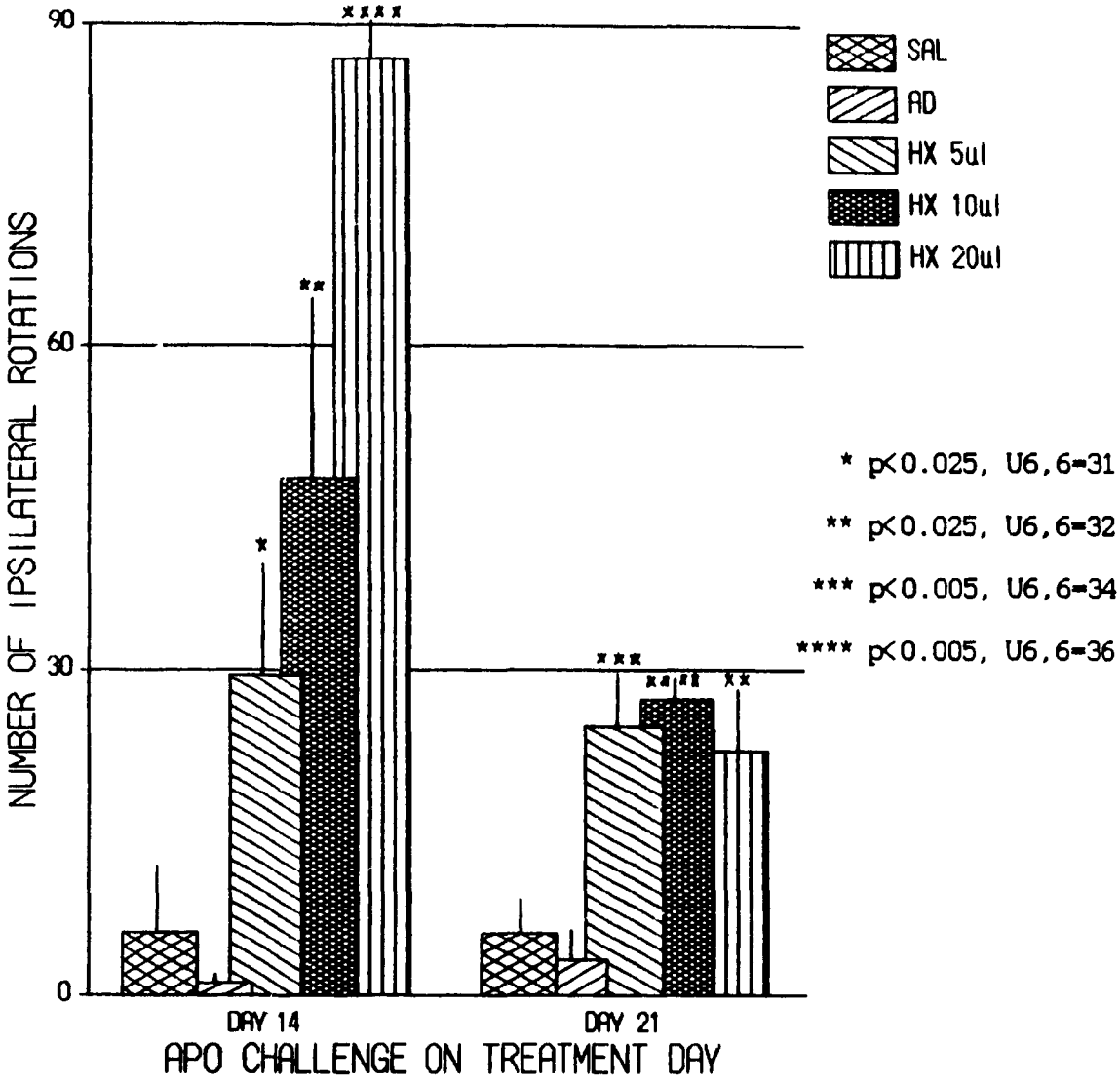
The other control group received 10ul/day of 5mM AD to control for possible affects of PRPP depletion, since PRPP is a necessary cofactor for both HGPRT and APRT. By delivering HX or AD to the brain of these animals, there might be increased activity of the respective enzymes and the subsequent depletion of PRPP (see figure 1 in Introduction).

Animals treated with 5mM HX injections were grouped according to the daily volume they received, either 5, 10 or 20ul.

Injections for all treatment groups continued for a three week period.

At day 14 and day 21 of treatment, all animals were challenged with 10mg/kg APO and their rotational responses recorded. APO did not induce consistent rotation for the saline or AD treated groups. However, the HX treated animals responded to the challenge with consistent ipsilateral rotation to the treated (left) side which was significantly different from the rotational response of the saline treated animals (figure 4). According to the Ungerstedt model, APO-induced ipsilateral rotation to the treated left side suggests that HX treatment is accompanied by desensitization of DA post-synaptic receptors within the LCD.

FIGURE 4. The mean number of ipsilateral rotations observed after APO challenge on day 14 and day 21 of treatment for animals (n=6) which received daily injections of saline (SAL), 5mM AD or one of three volumes of 5mM HX (HX 5ul; HX 10ul; or HX 20ul). * indicates significantly different from SAL and error bars represent S.E.M.



At two and three weeks, a sample of animals was sacrificed. Upon examination of the treated (left) side, all treatment groups, including saline treated animals, had gross morphological brain damage. This suggested that the delivery vehicle (saline), had non-specific necrotic effects

which would make it difficult to evaluate the specific effects of HX. Repeated injection was thus judged to be an unacceptable mode of delivery, despite apparent behavioural differences between groups.

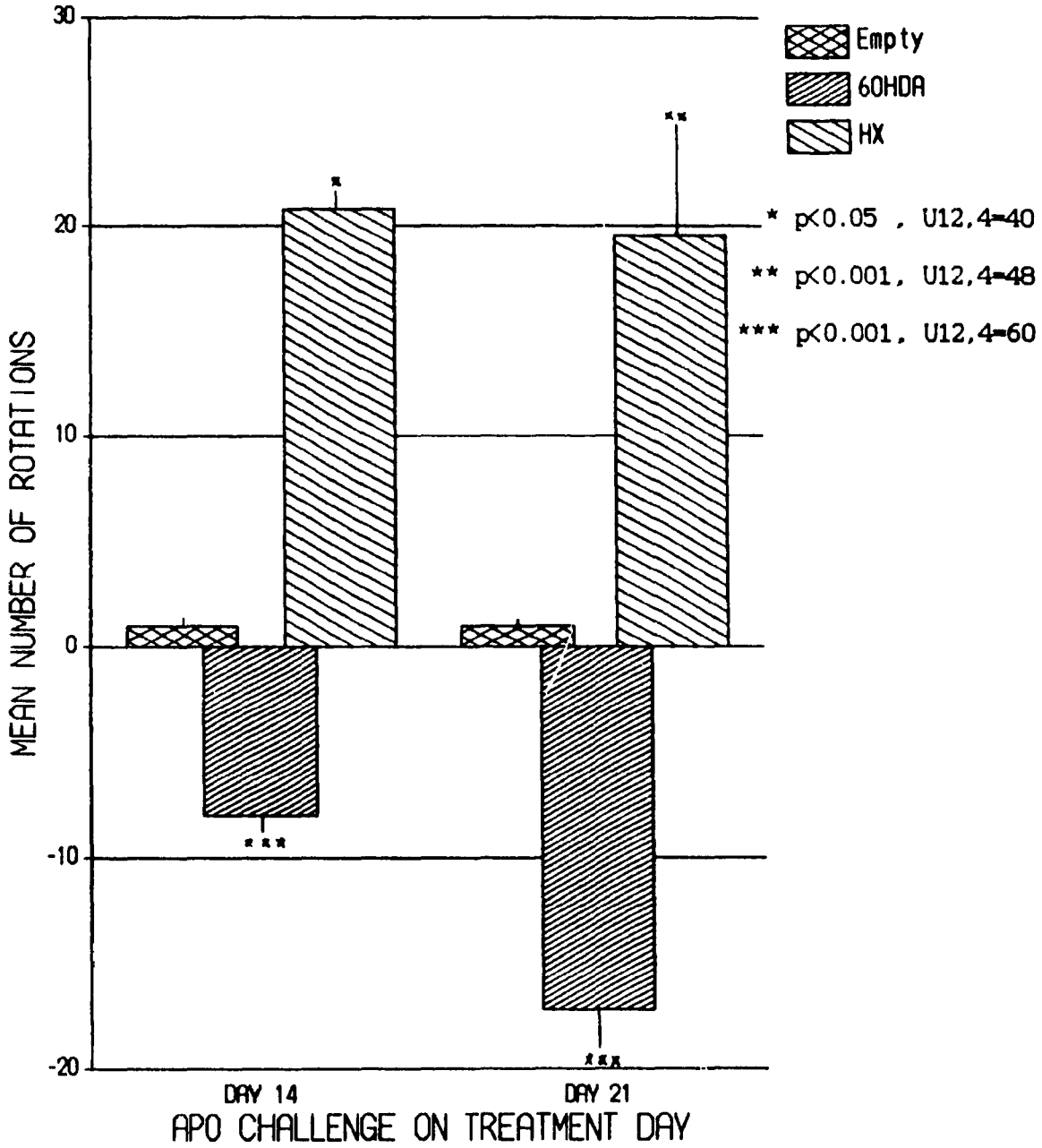
A. ii) HX Packed Cannulae Implanted into The LCD

To eliminate damage caused by injection and to increase the efficacy of HX delivery, the next trial utilized cannulae packed with powdered HX and implanted into the LCD. By localizing the HX treatment to the LCD, Ungerstedt's Rotational Model could be utilized. To control for mechanical damage resulting from cannulae implantation, a group of animals received empty cannulae implants. A further control group of animals received a single unilateral injection of 6OHDA into the LCD.

At post-operative day 14 and day 21, animals were challenged with 10mg/kg APO. Animals implanted with empty cannulae did not exhibit any APO-induced rotation, while the 6OHDA treated animals responded with consistent contra-lateral rotation and the HX treated animals with consistent ipsilateral rotation to the treated (left) side (figure 5). The APO-induced rotation from the HX and 6OHDA treatments were significantly different from animals implanted with empty cannulae on both challenge days. Again Ungerstedt's model would indicate that ipsilateral rotation suggests DA post-synaptic receptor desensitization, while contralateral rotation following 6OHDA treatment classically implies

super-sensitization of the DA post-synaptic receptor.

FIGURE 5. The mean number of rotations observed after APO challenge on post-implantation day 14 and day 21 from animals implanted with empty cannulae (Empty) [n=12], animals treated with 6OHDA [n=5] and animals implanted with HX packed cannulae (n=4). A positive number of rotations indicates ipsilateral rotation, while a negative number indicates contralateral rotation to the treated (left) side. * indicates significantly different from Empty and error bars represent S.E.M.

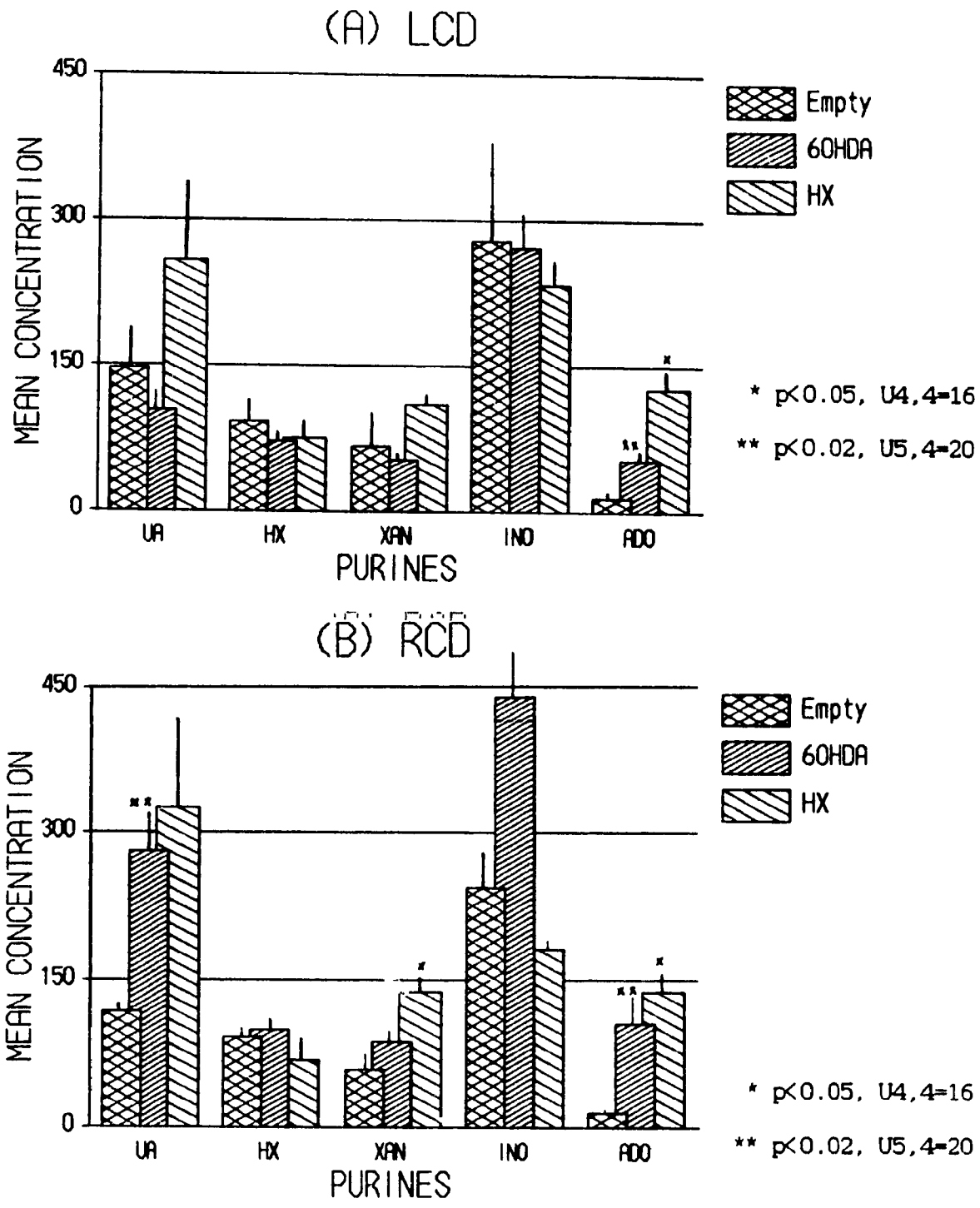


In theory, the potential delivery of HX via packed cannulae is much greater than 5mM HX injections, since a cannula can hold up to 3mg of HX, while injections would only deliver 150ug over a three week period. Yet HX injections resulted in a greater APO-induced rotational response. Dissolving a powder within tissue may limit the distribution of HX within the caudate and therefore it is possible that this method of delivery may not yield maximal behavioural effects.

To evaluate the effectiveness of HX delivery, purine concentrations were measured in the caudate nuclei after post-operative day 21. As compared to animals implanted with empty cannulae, animals that received 6OHDA treatment had significant changes of UA in the RCD and ADO in the LCD and RCD, (figure 6). Furthermore, RCD levels of UA and ADO were about twice the concentrations found in the LCD.

Animals implanted with HX packed cannulae had as compared to animals with empty cannulae implants, an overall increase in both caudate nuclei of UA, XAN, INO and ADO, with XAN in the RCD significantly increased and ADO significantly increased in the LCD and RCD. HX levels remained unchanged from those seen in animals implanted with empty cannulae, suggesting that HX delivered into the LCD is converted to other products within the purine metabolic pathway. Accumulation of ADO is present in both caudate nuclei and is similar to the ADO levels seen in the 6OHDA treated animals.

FIGURE 6. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) LCD and (B) RCD for animals implanted with empty cannulae (Empty) [n=4], animals treated with 6OHDA [n=5] and animals implanted with HX packed cannulae [n=4] into the LCD after post-operative day 21. * indicates significantly different from Empty and error bars represent S.E.M.



In addition, catecholamine concentrations were measured in the caudate nuclei after post-operative day 21. As compared to the animals' RCD, LCDs implanted with empty cannulae had no significant changes, while LCDs which received 6OHDA had a significant reduction of DA and HX implants resulted in significant reductions of DOPAC and DA (figure 7).

FIGURE 7. The mean concentration (ng/mg of tissue) of NA, DOPAC and DA in the LCD (treated) and RCD (control) for animals implanted with (A) empty cannulae [n=12], (B) 6OHDA treated animals [n=5] and (C) animals implanted with HX packed cannulae [n=4] into the LCD for three weeks. * indicates significantly different from RCD and error bars represent S.E.M.

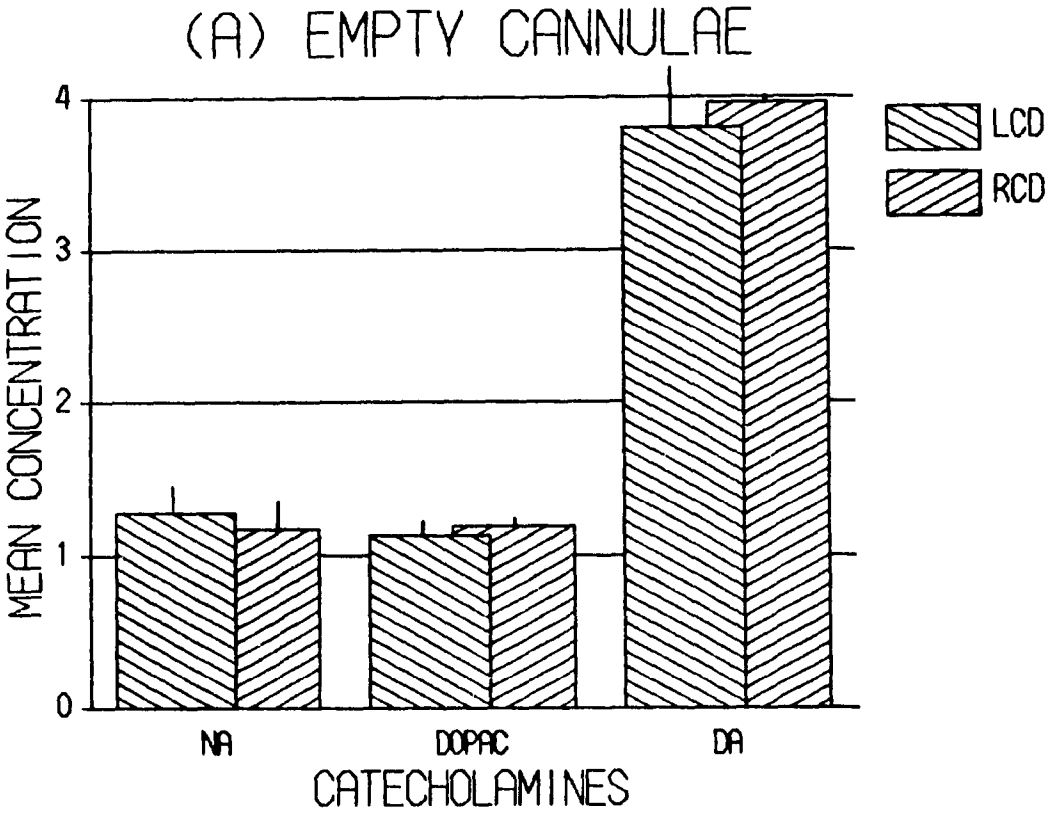
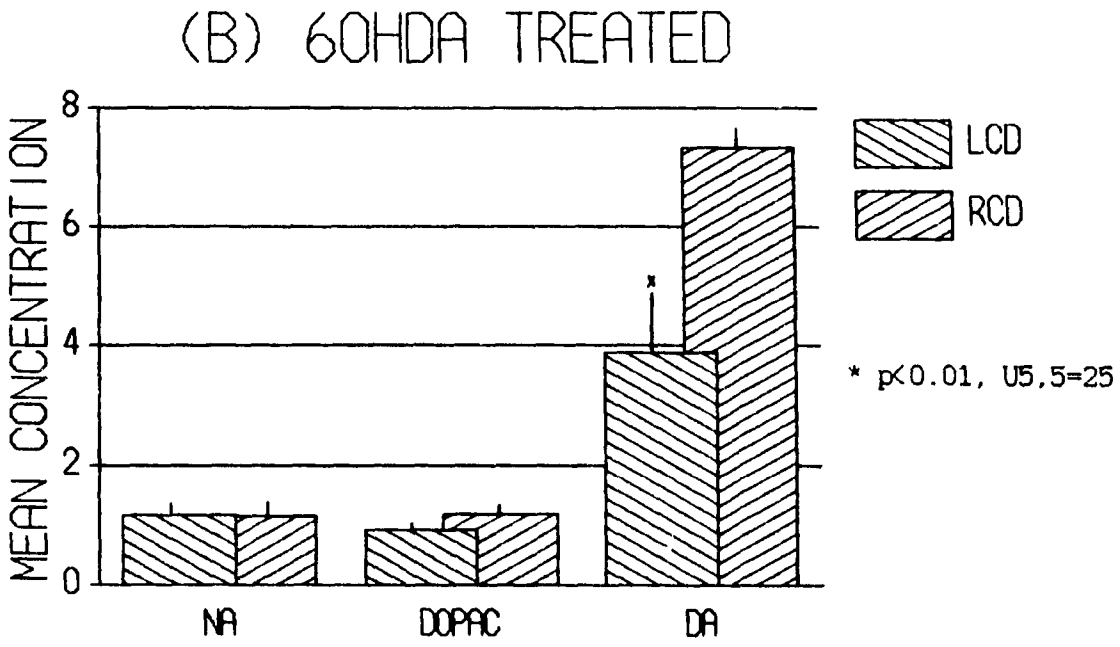
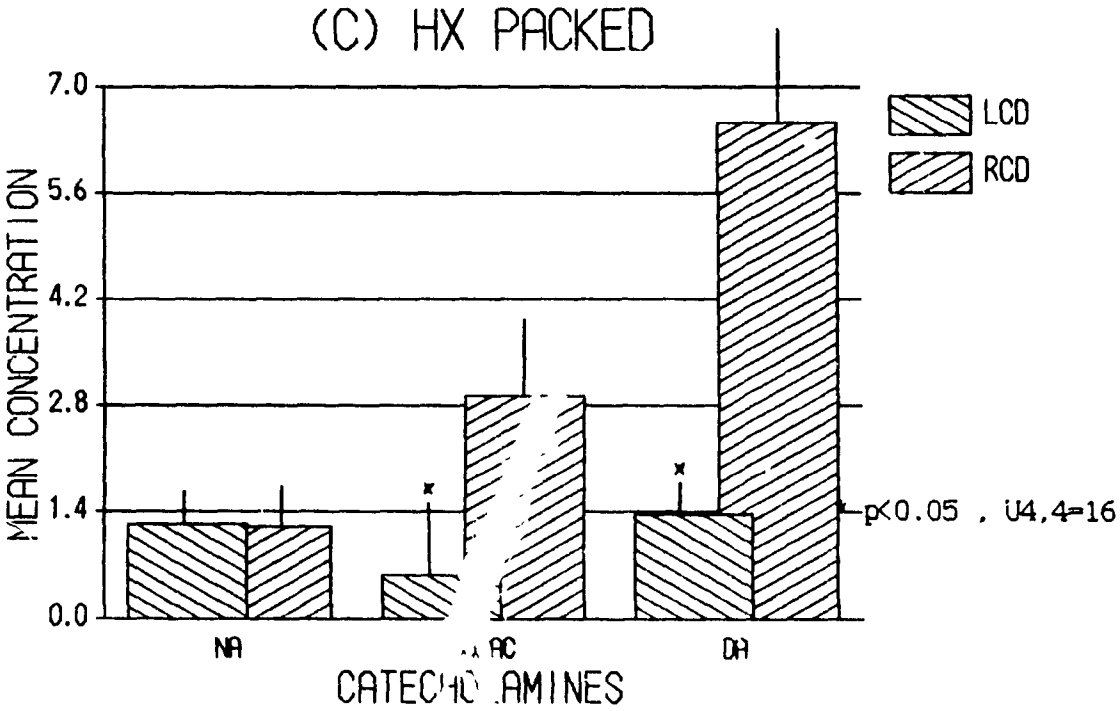


FIGURE 7 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC and DA in the LCD (treated) and RCD (control) for animals implanted with (A) empty cannulae [n=12], (B) 6OHDA treated animals [n=5] and (C) animals implanted with HX packed cannulae [n=4] into the LCD for three weeks. * indicates significantly different from RCD and error bars represent S.E.M.





From the results thus far, packed powdered HX cannulae are a preferable method of delivery, as compared to HX injections. The most advantageous feature of the packed cannulae is its specificity of tissue damage. Its short-coming is the reduced response to APO. The reduced behavioral response might be due to the site of implantation. Powdered HX may not be maximally delivered within tissue, because the powder may not be uniformly dissolved or distributed. To overcome this problem, the next trial will examine the effects of cannulae implanted within a lateral ventricle.

B) IMPLANTATION INTO THE LATERAL VENTRICLE

On the basis of preliminary experiments, HX was administered via unilateral packed cannulae implants into the left lateral ventricle of a rat's brain. Lateral ventricular placement would theoretically maximize delivery of HX and the lateralization of treatment would allow the use of Ungerstedt's Rotational model for behavioural scoring.

To examine the effects of HX upon DA neurons, this section is subdivided into three parts. One will report the effects of implanting HX packed cannulae. Another will examine the effects of implanting AD packed cannulae and the last part will examine the effects of implanting packed cannulae containing ALLO alone or ALLO plus HX. The control

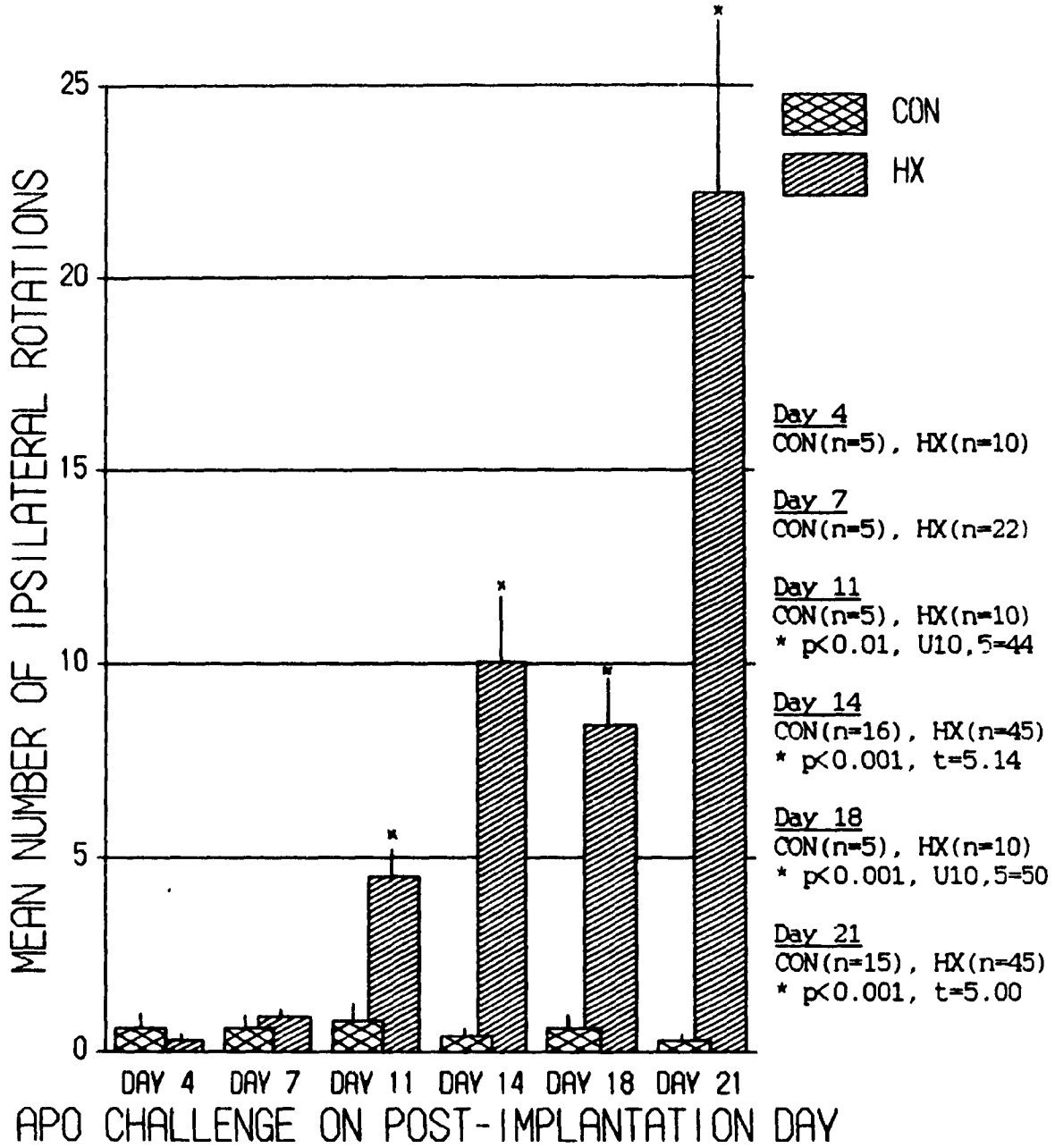
(CON) for all packed cannulae treatments was empty cannulae implants. At scheduled intervals over the post-implantation period, each treatment group was challenged with APO to monitor the developmental pattern of their rotational response.

B. i.) Implantation of HX Packed Cannulae

a) Rotational response to APO

Animals with left ventricular HX packed cannulae implants were challenged with 10 mg/kg APO on post-implantation day 4, 7, 11, 14, 18 and day 21 and their rotational response recorded (figure 8). The CON animals did not rotate in either direction, while HX treated animals responded with consistent ipsilateral rotation to the treated (left) side. According to Ungerstedt's model, these results suggest that desensitization of the DA post-synaptic receptor within the LCD was apparent at post-implantation day 11.

FIGURE 8. The mean number of ipsilateral rotations observed after challenge with APO on post-implantation day 4, 7, 11, 14, 18 and day 21 from CON and animals implanted with HX packed cannulae into the left lateral ventricle. * indicates significantly different from CON and error bars represent S.E.M.



B. i. b.) Biochemical Analysis

i) Purine concentrations

As compared to CON at two weeks post-implantation (figure 9), HX treated animals had elevated levels of UA, XAN, INO and ADO in the AMY and both caudate nuclei, with ADO being significantly increased in the RCD. HX remained at CON levels, suggesting the delivered HX was being converted to other metabolites within the purine metabolic pathway.

At three weeks post-implantation, the HX treated animals continued to have elevated purine levels with ADO significantly elevated in the AMY, LCD and RCD.

These results suggest high levels of ADO in at least the RCD are associated with APO-induced ipsilateral rotation.

FIGURE 9. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with HX packed cannulae [n=6] for two and three weeks. * indicates significantly different from CON and error bars represent S.E.M.

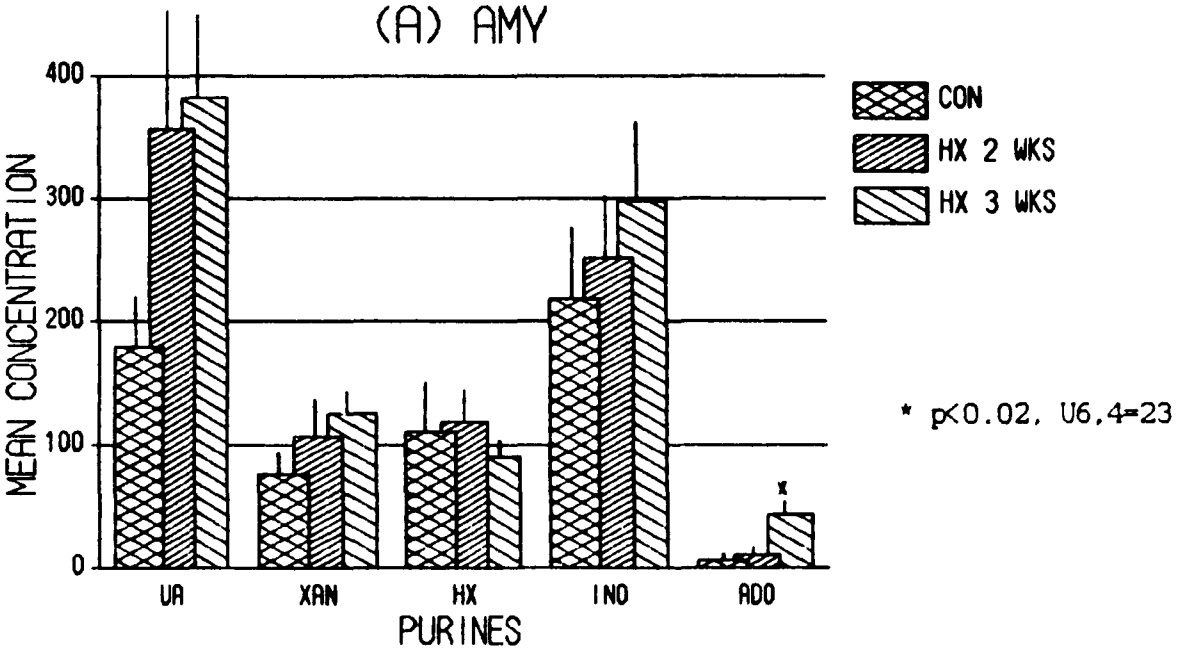
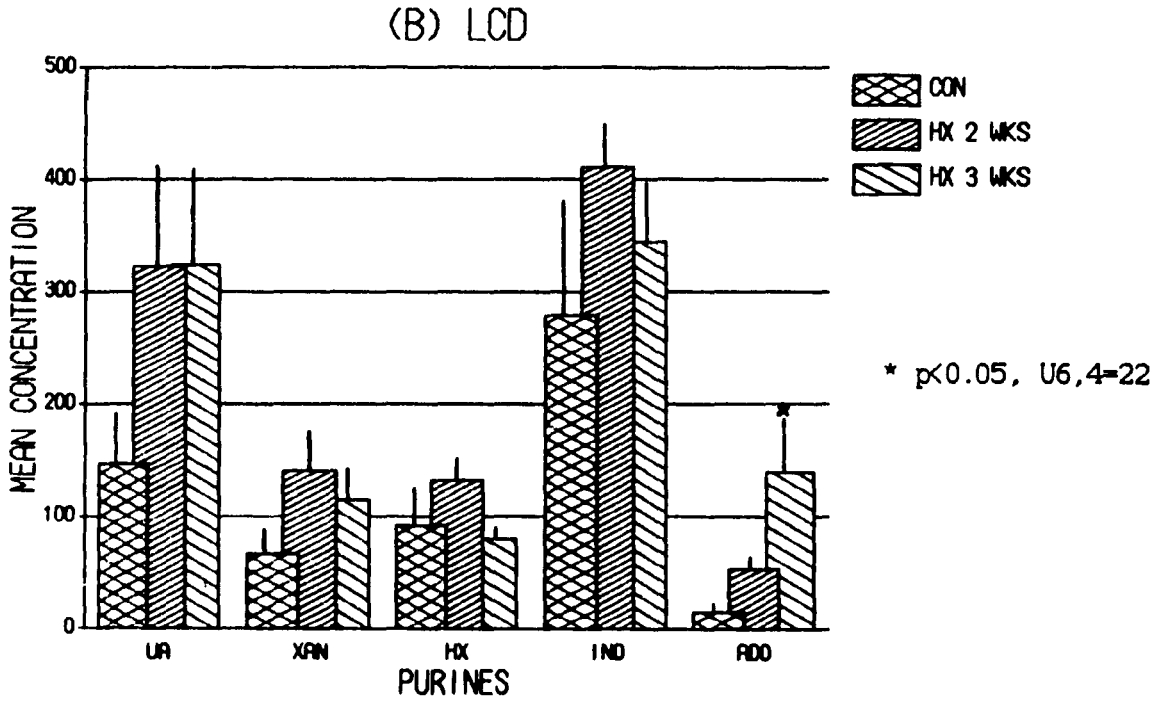
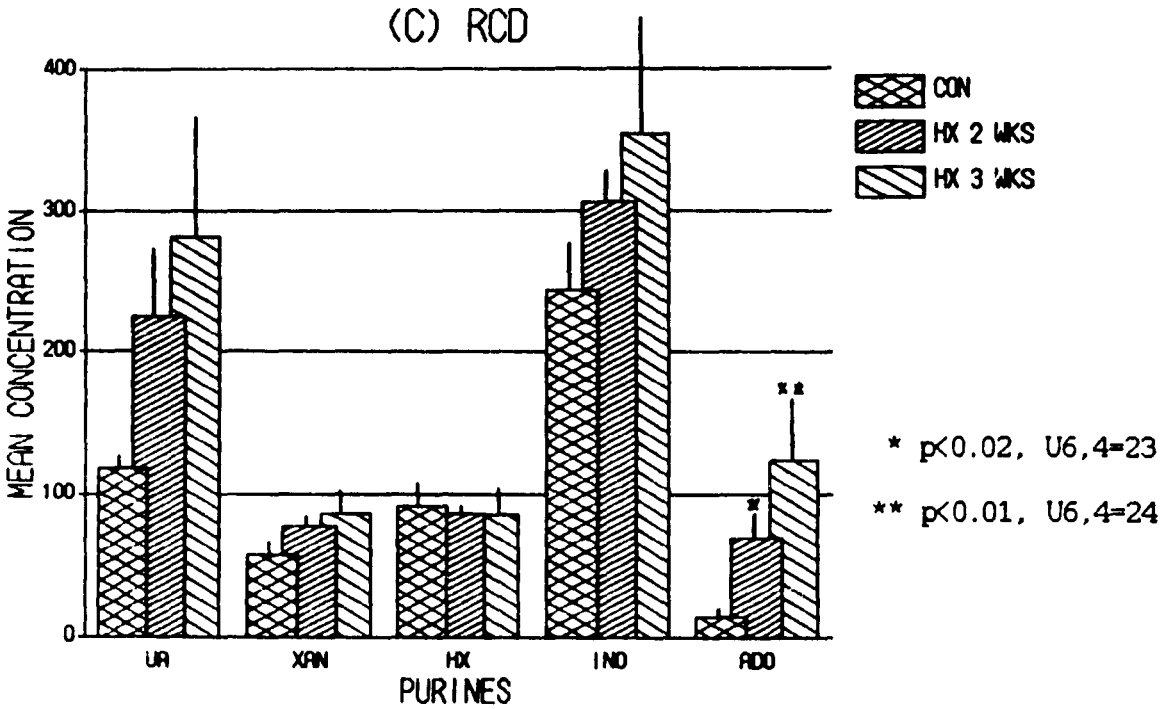


FIGURE 9 continued. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with HX packed cannulae [n=6] for two and three weeks. * indicates significantly different from CON and error bars represent S.E.M.





B. i. b. ii) Catecholamine concentrations

As compared to CON at one week post-implantation, HX treated animals showed significant changes in the levels of DOPAC in the AMY, CTX, LCD and BST; and HIAA in all seven measured brain regions (figure 10).

At two weeks post-implantation, fewer differences were noted and include changes in the levels of NA in the NAC; DOPAC in the HYP; and HIAA in the HYP and BST.

At three weeks post-implantation, changes were noted in the levels of DOPAC in the RCD; and HIAA in the AMY and BST.

Despite time-dependent increases in APO-induced ipsilateral rotation, these results suggest that catecholamine concentrations are altered early in treatment, but normalize as the implantation period progresses.

FIGURE 10. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A)AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON (n=12) and animals implanted with HX packed cannulae for 1 (n=6), 2 (n=6) and 3 (n=21) weeks. * indicates significantly different from CON and error bars represent S.E.M.

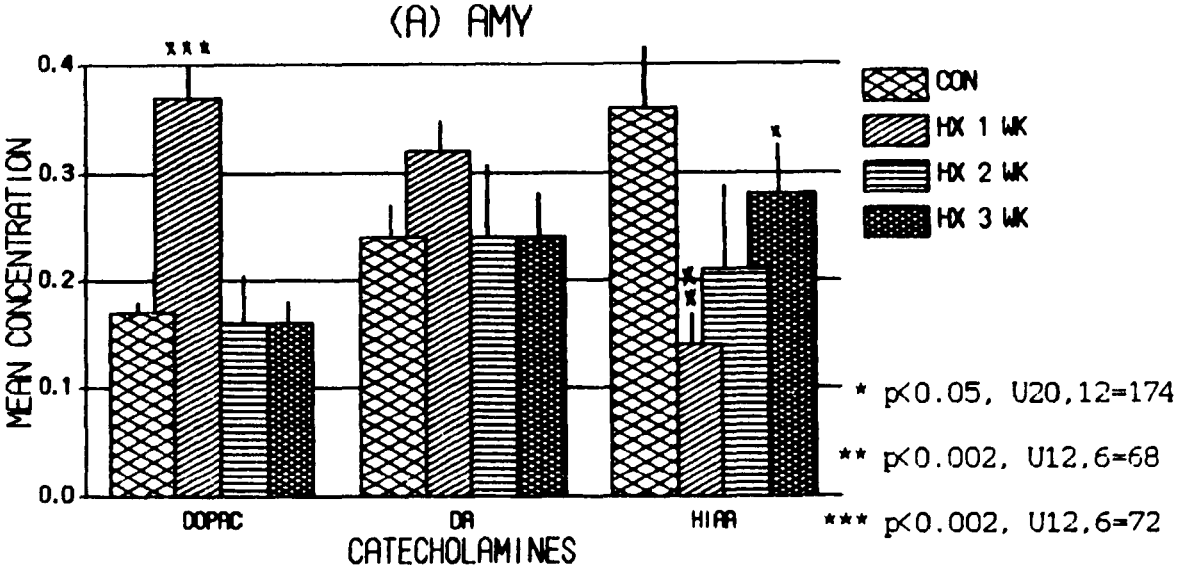
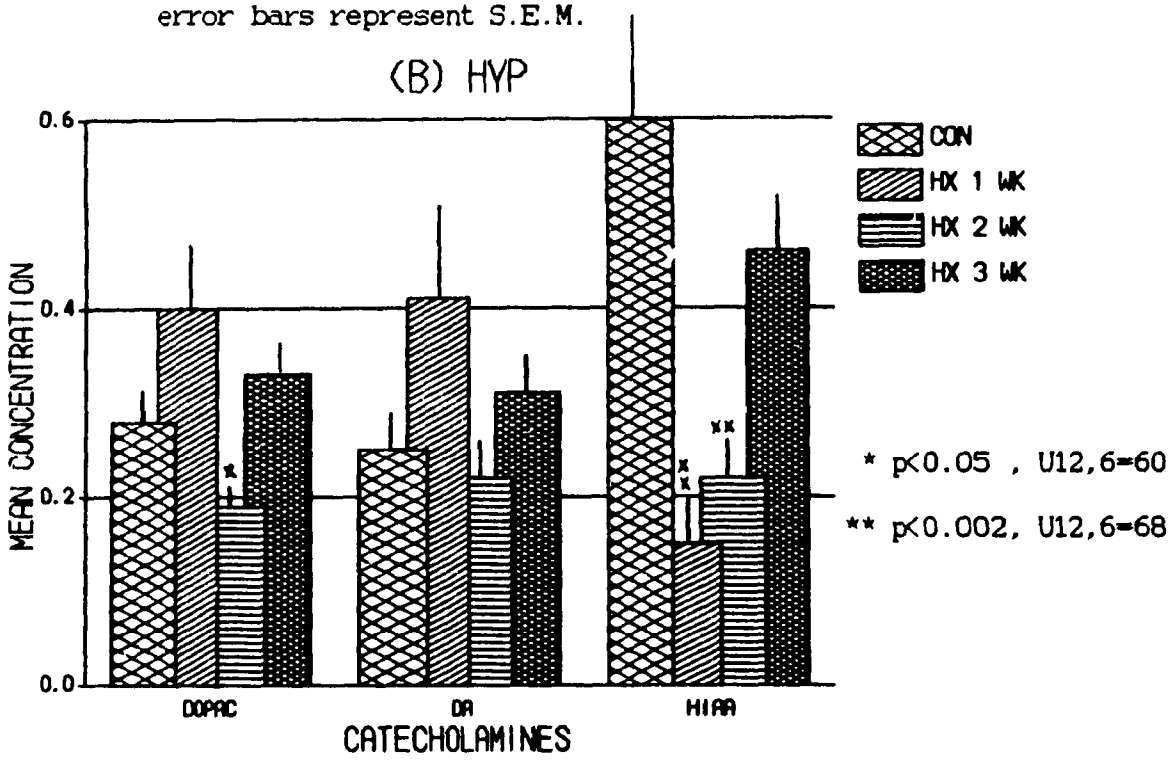


FIGURE 10. continued The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A)AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON (n=12) and animals implanted with HX packed cannulae for 1 (n=6), 2 (n=6) and 3 (n=21) weeks. * indicates significantly different from CON and error bars represent S.E.M.



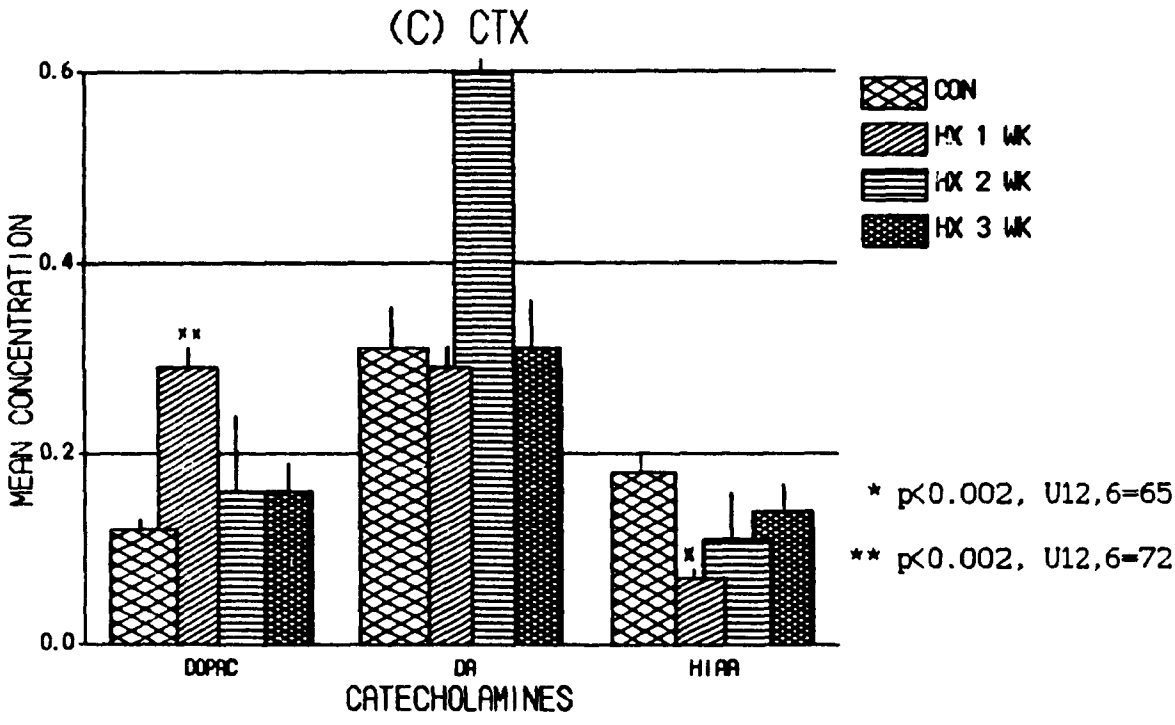
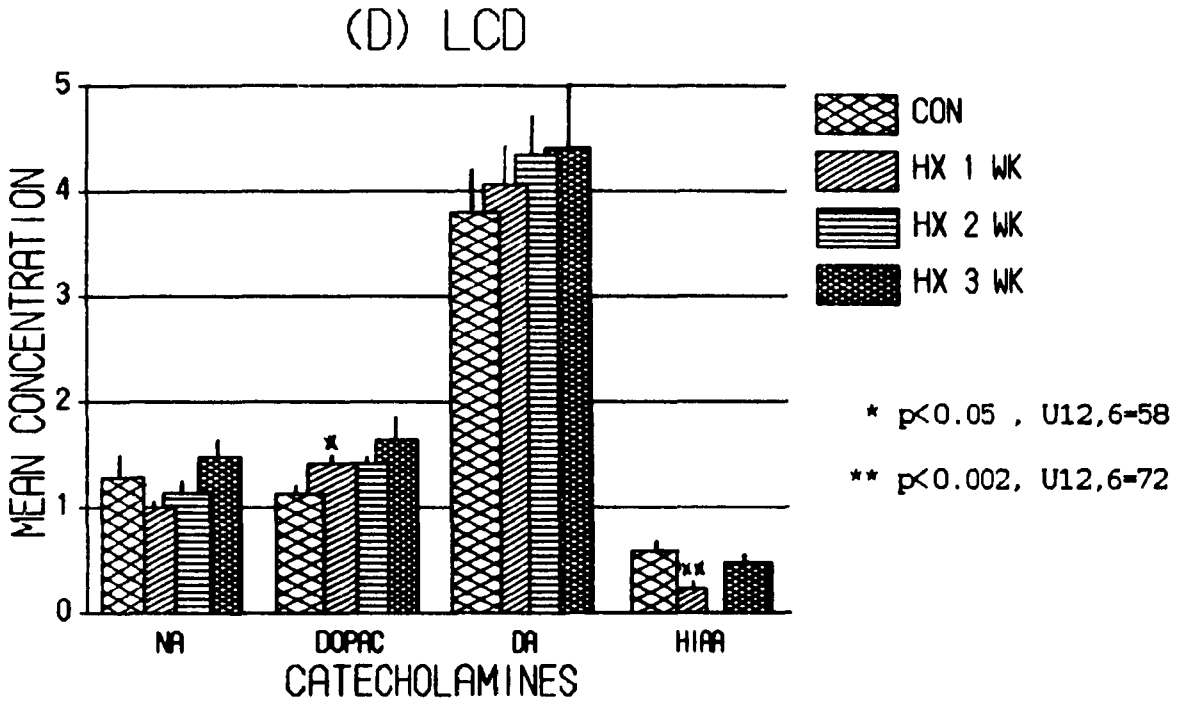


FIGURE 10. continued The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A)AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON (n=12) and animals implanted with HX packed cannulae for 1 (n=6), 2 (n=6) and 3 (n=21) weeks. * indicates significantly different from CON and error bars represent S.E.M.



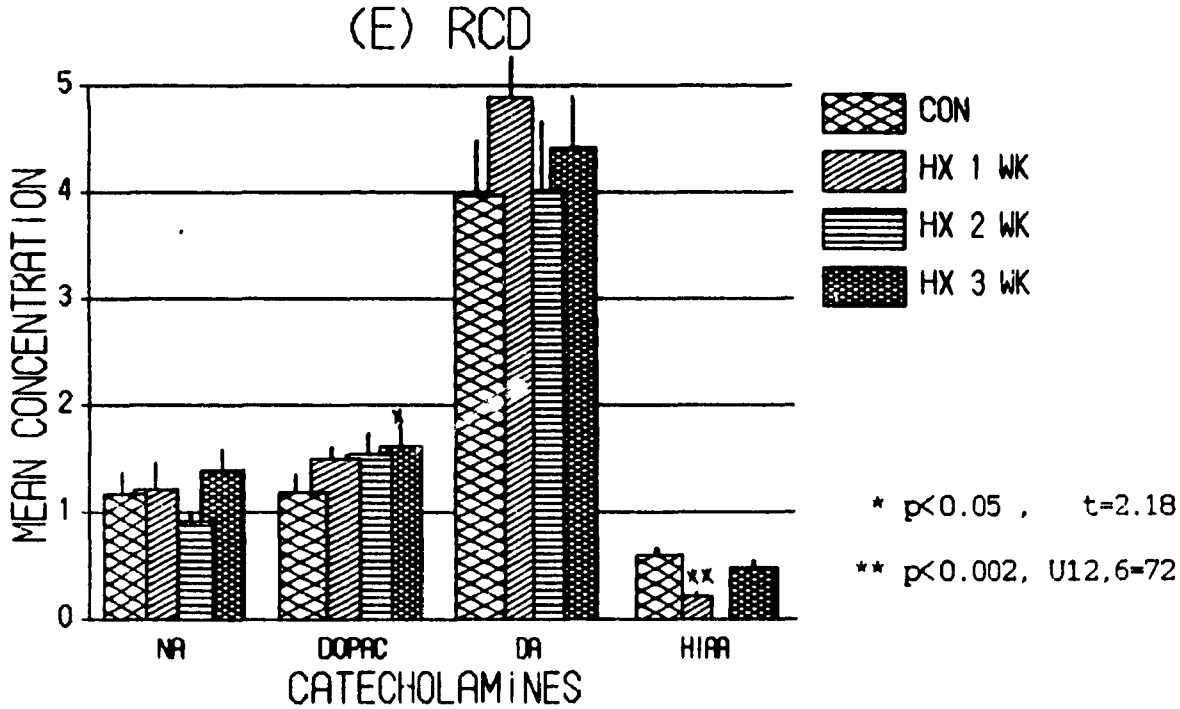
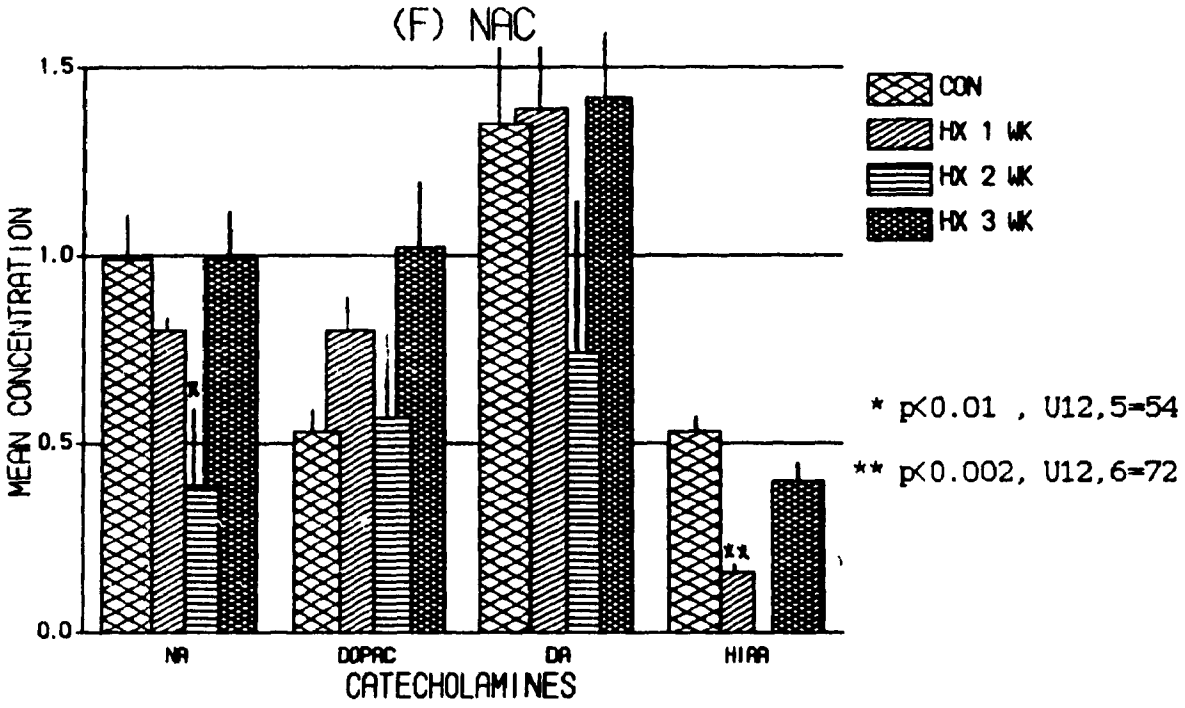
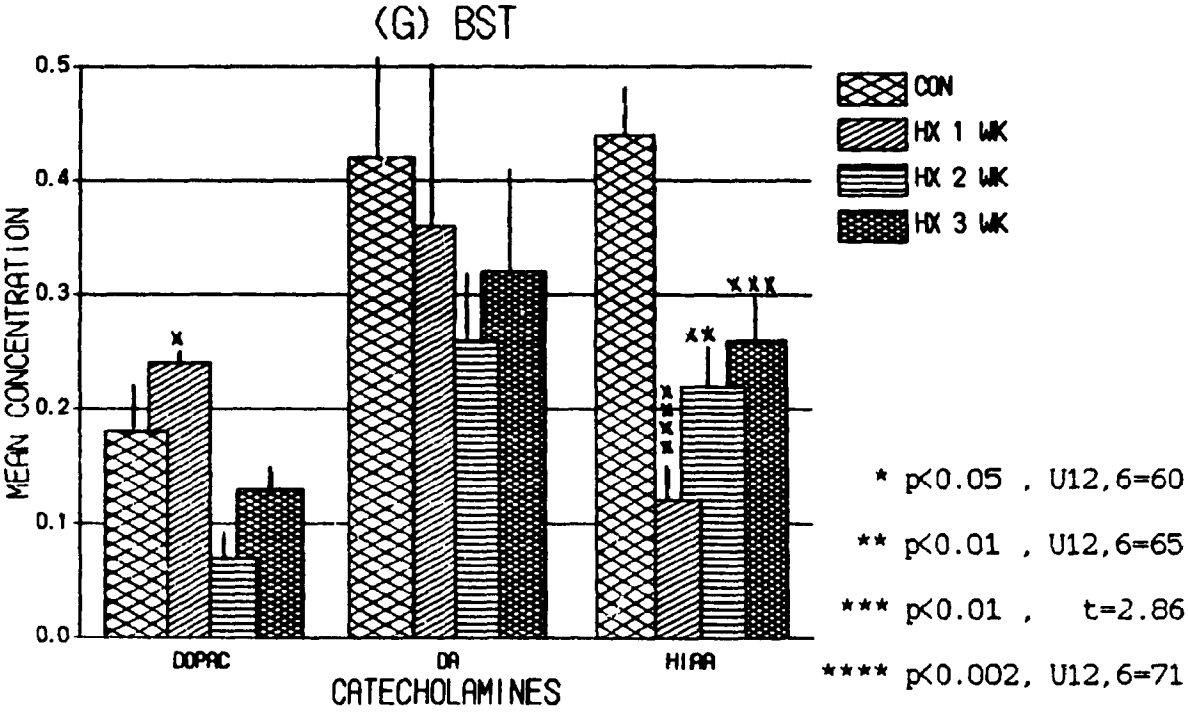


FIGURE 10. continued The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A)AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON (n=12) and animals implanted with HX packed cannulae for 1 (n=6), 2 (n=6) and 3 (n=21) weeks. * indicates significantly different from CON and error bars represent S.E.M.





In summary, implantation of HX packed cannulae resulted in a time-dependent increase of APO-induced rotation. This suggests that HX treatment is accompanied by DA post-synaptic receptor desensitization within the LCD. Furthermore, purine analysis suggests that rotational behaviour is associated with high purine levels, especially ADO within at least the RCD. Finally catecholamine analysis reveals transient alteration of catecholamine metabolism early in the treatment period.

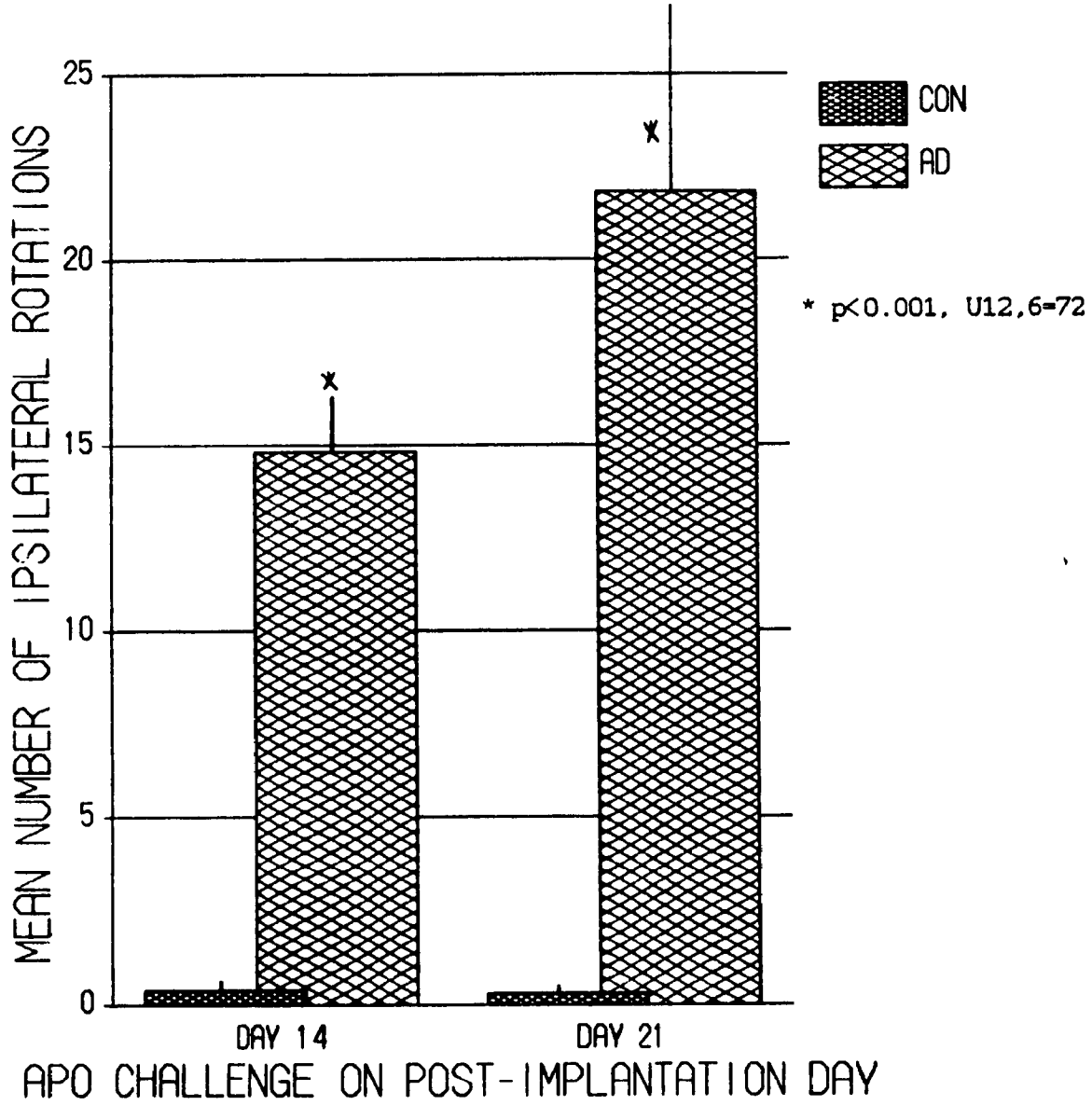
B. ii.) Implantation of AD Packed Cannulae

Animals implanted with AD packed cannulae were used to CON for the possible effects of PRPP depletion. Compared to injections of AD, the potential delivery of AD in packed cannulae is at least 10 times greater. Over the three week treatment period, the total injected amount of AD delivered was 135ug, while a cannula can hold up to 3mg of AD.

a) Rotational response to APO

As compared to CON, animals implanted with AD packed cannulae had a significant increase of APO-induced ipsilateral rotation to the treated (left) side (figure 11), on post-implantation day 14 and day 21, suggesting that AD treatment is accompanied by DA post-synaptic receptor desensitization within the LCD.

FIGURE 11. The mean number of rotations observed after APO challenge on post-implantation day 14 and day 21 for CON [n=12] and animals with AD packed cannulae [n=6] implanted into the left lateral ventricle. * indicates significantly different from CON and error bars represent S.E.M.



B. ii. b.) Biochemical analysis

i) Purine concentrations

No formal purine analysis was performed upon these treated animals. Nevertheless, inspection of the cannulae determined that an average of 0.9 mg of AD was delivered

over the three week period. Furthermore, the more AD delivered, the higher the rotational response to APO (table 1). This suggests that either AD or one of its metabolites, may be associated with DA post-synaptic receptor desensitization within the LCD.

TABLE 1. A comparison between the number of rotations observed after APO challenge and the amount of AD delivered (mg) for individual treated animals for a 3 week period

	Treated Animals					
	AD 2	AD 1	AD 4	AD 5	AD 6	AD 3
# of Rotations	44	35	29	10	7	6
Amount of AD Delivered (mg)	1.2	1.0	1.0	0.7	0.2	0.2

B. ii. b. ii) Catecholamine concentrations

As compared to CON at three weeks post-implantation, the AD treated animals showed changes in the levels of NA in the NAC; DOPAC in the AMY, CTX and BST; and DA in the BST (figure 12).

These results are important when compared to the changes of catecholamine concentrations found with HX treatment. These data suggest that the two treatments do not effect catecholamine metabolism in a similar manner, despite producing similar APO-induced rotational behaviour.

FIGURE 12. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) CTX, (C) NAC for CON [n=12] and animals implanted with AD packed cannulae [n=6] for 3 weeks. * indicates significantly different from CON and error bars represent S.E.M.

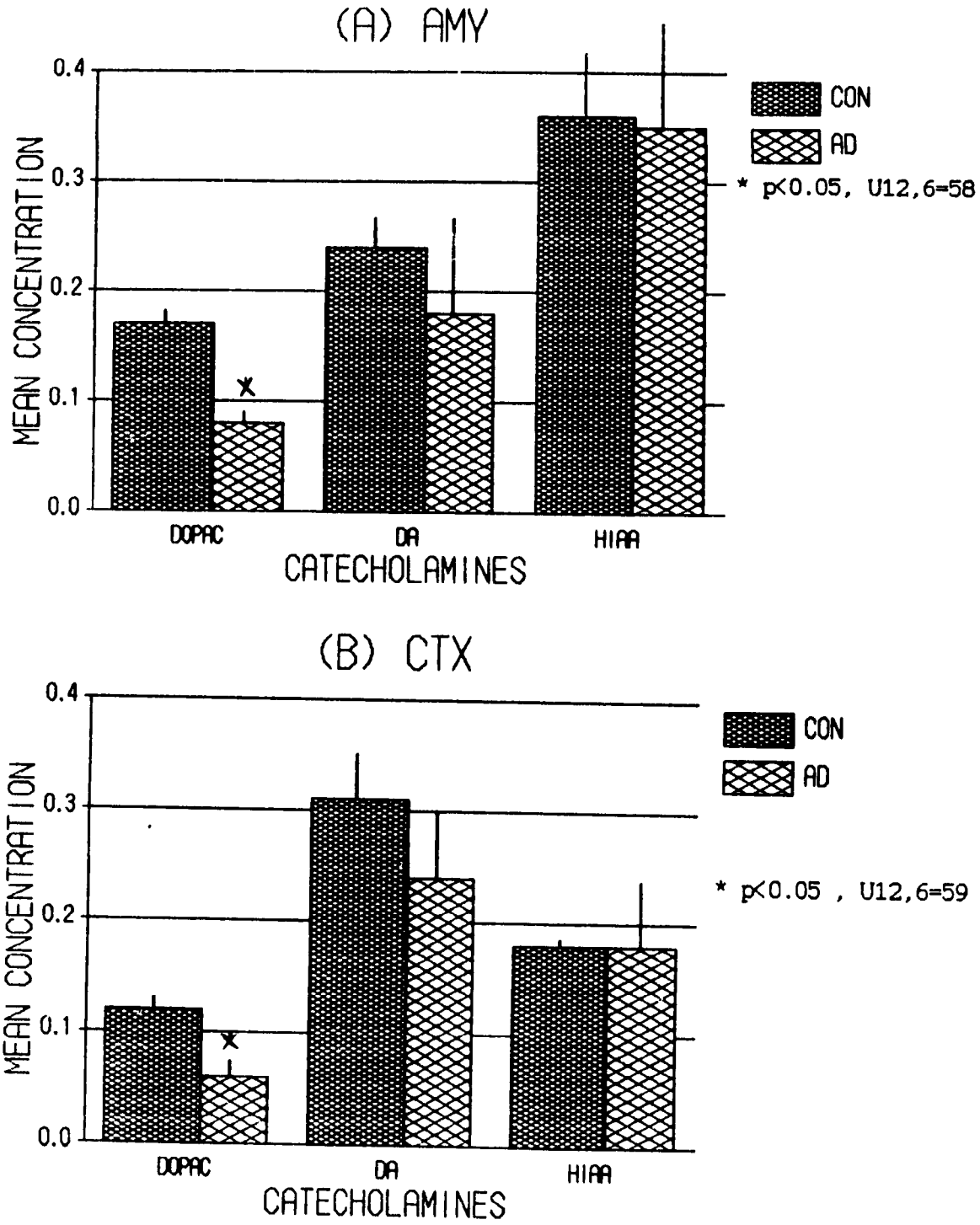
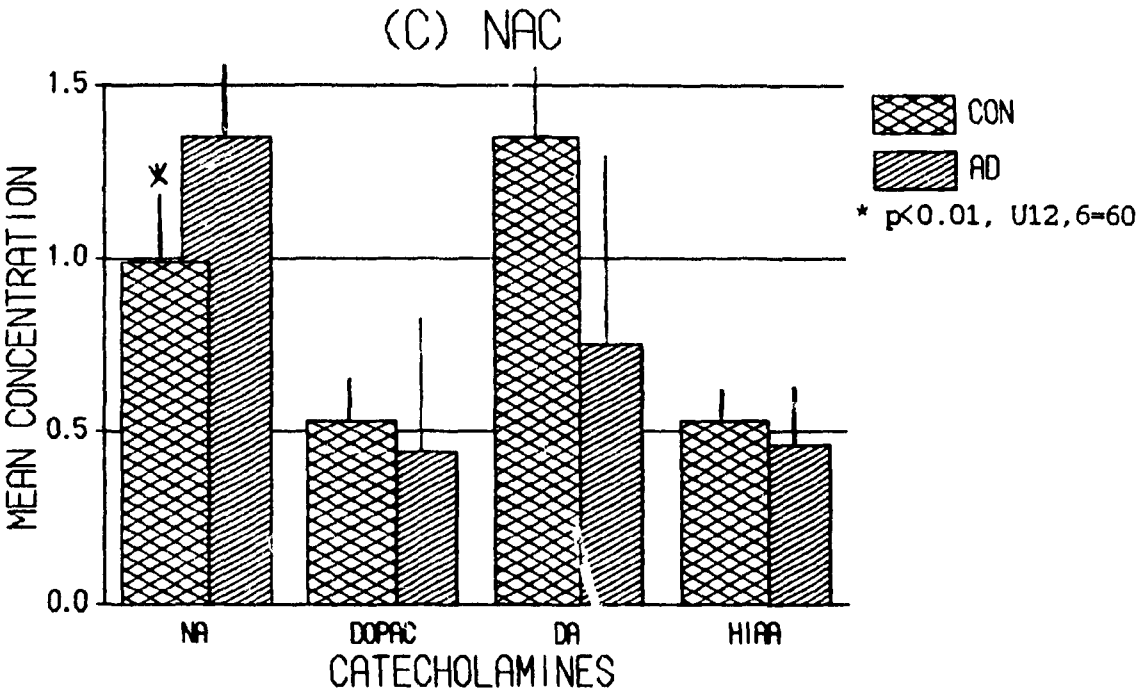


FIGURE 12 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) CTX, (C) NAC BST for CON [n=12] and animals implanted with AD packed cannulae [n=6] for 3 weeks. * indicates significantly different from CON and error bars represent S.E.M.



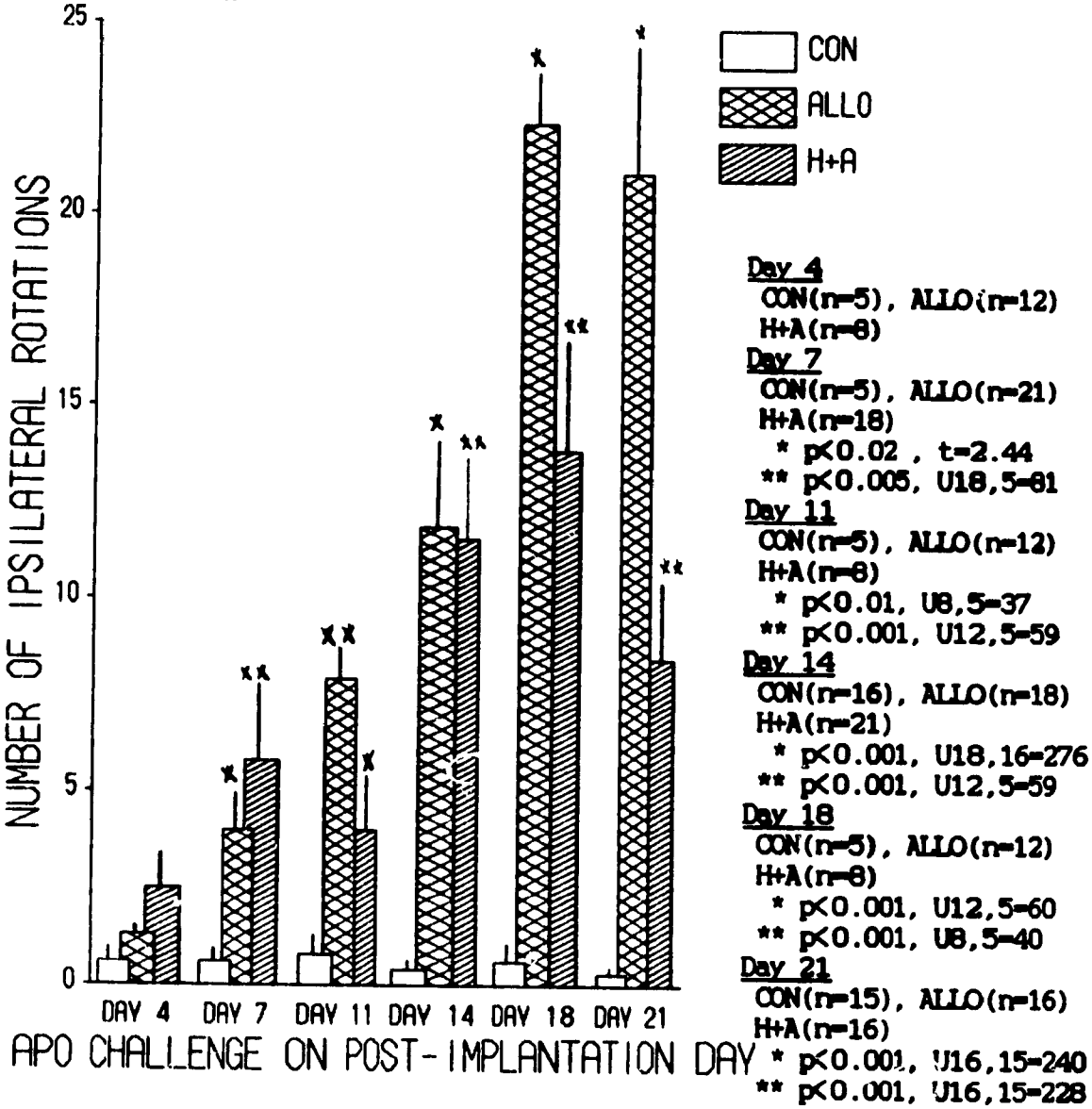
B. iii.) Implantation of ALLO or ALLO Plus HX Packed Cannulae

Elevation of UA and XAN were noted two weeks after HX implantation. Superoxide radicals are known to be generated when HX is converted to XAN and UA (Lehninger, 1979). If this process is responsible for DA post-synaptic receptor desensitization it should be blocked by ALLO, an inhibitor of XO. ALLO was thus packed into cannulae and implanted alone or with HX into the left lateral ventricle.

B. iii. a) **Rotational response to APO**

As compared to CON, both treatment groups showed significant increases of APO-induced ipsilateral rotation (figure 13). This suggests that there is a DA post-synaptic receptor desensitization within the LCD.

FIGURE 13. The mean number of ipsilateral rotations observed in response to APO challenge on post-implantation day 4, 7, 11, 14, 18 and day 21 from CON and animals implanted with ALLO alone or HX plus ALLO (H+A) packed cannulae. The * indicates significantly different from CON and error bars represent S.E.M.



B. iii. b.) Biochemical Analysis: i.) Purine concentrations

.i) ALLO packed cannulae

As compared to CON at one week post-implantation, treated animals showed significant changes in the levels of UA, XAN and ADO in the AMY, LCD and RCD (figure 14).

Because of a small sample size at two weeks post-implantation, changes in the levels of UA and ADO only approached significance in the AMY, LCD and RCD.

At three weeks post-implantation, treated animals had no purine levels significantly different from CON.

Comparing the effects of ALLO and HX treatments upon purine metabolism suggests they are dissimilar, especially for the effects upon ADO.

FIGURE 14. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=6] weeks. * indicates significantly different from CON, + approaches significance and error bars represent S.E.M.

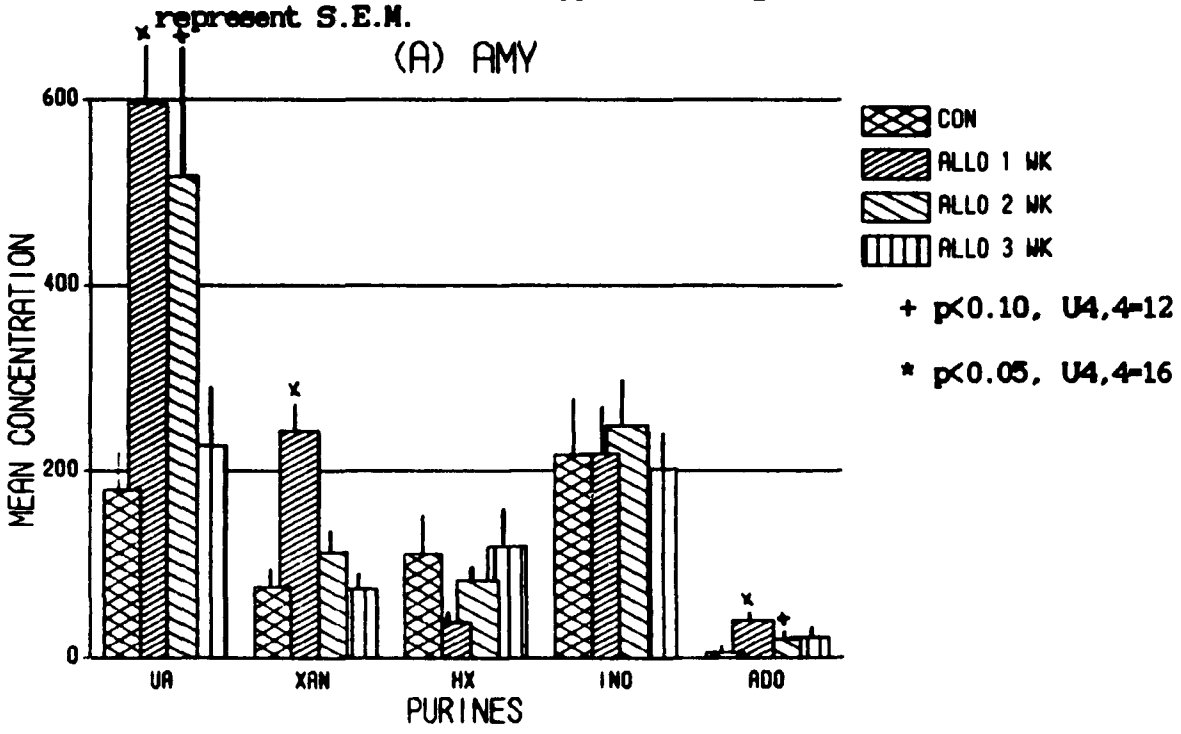
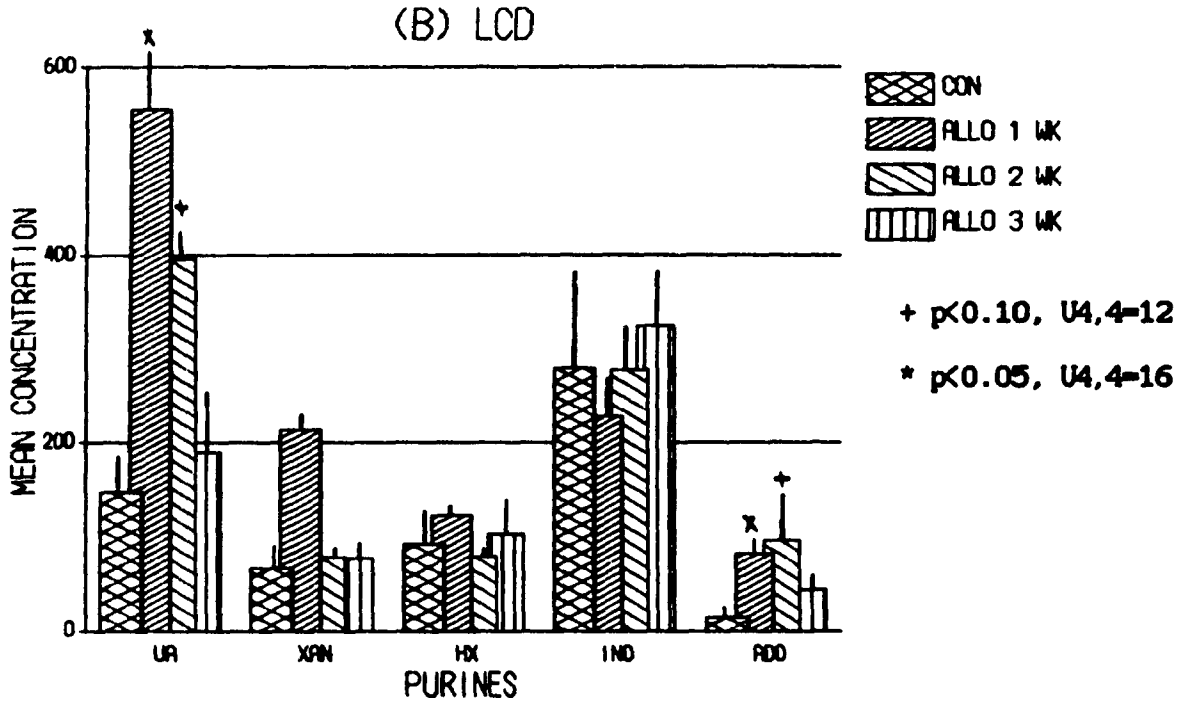
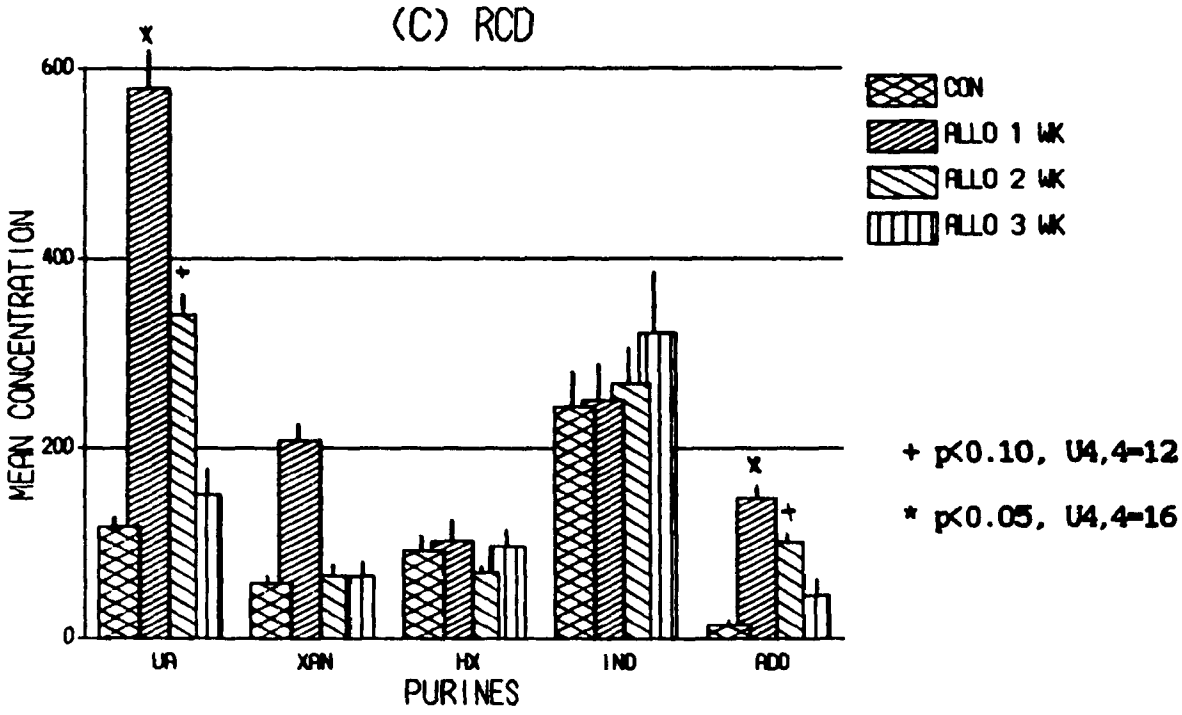


FIGURE 14 continued. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=6] weeks. * indicates significantly different from CON, + approaches significance and error bars represent S.E.M.





B. iii. b. i. ii) ALLO plus HX packed cannulae

As compared to CON at one week post-implantation, treated animals showed a significant increase in the levels of UA in the AMY, LCD and RCD; XAN in the LCD and RCD; and ADO in the AMY and RCD (figure 15).

At two weeks post-implantation, treated animals had significant increases of UA, XAN and ADO only in the RCD.

At three weeks post-implantation, only ADO in the LCD and RCD were significantly increased in treated animals.

These results show similar patterns of effect when compared to the individual treatments of ALLO or HX.

FIGURE 15. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.

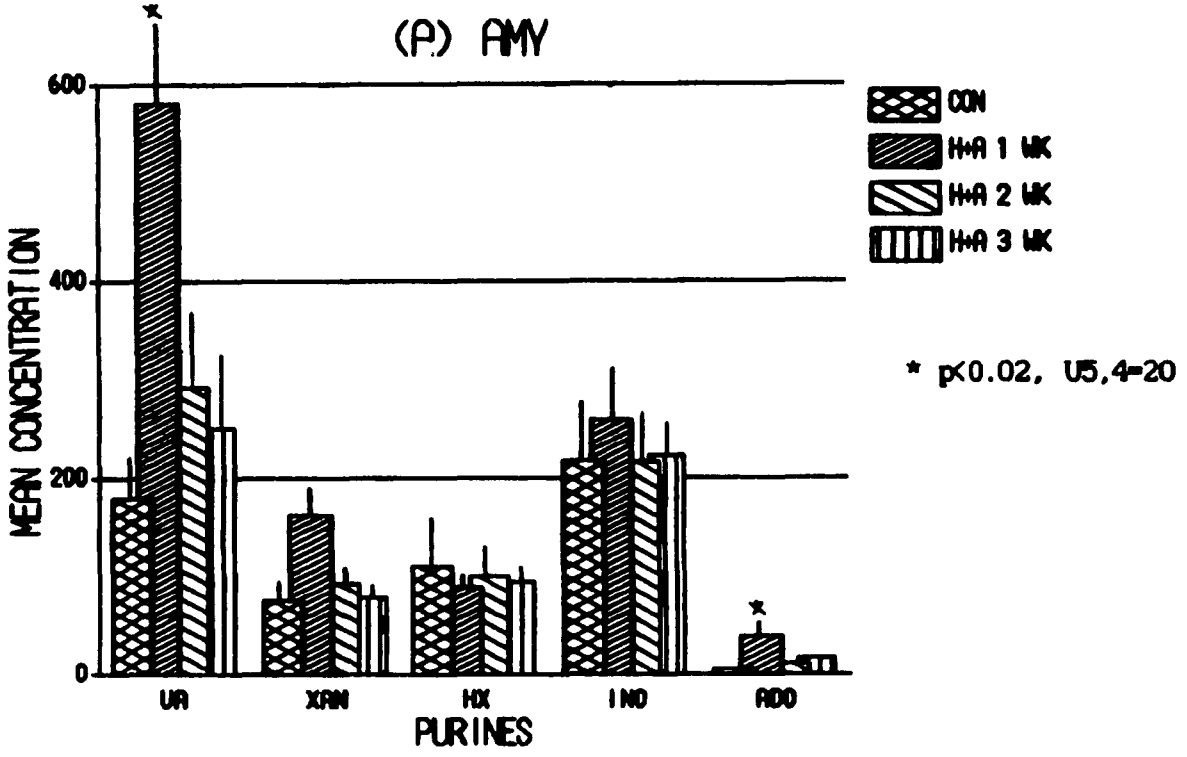
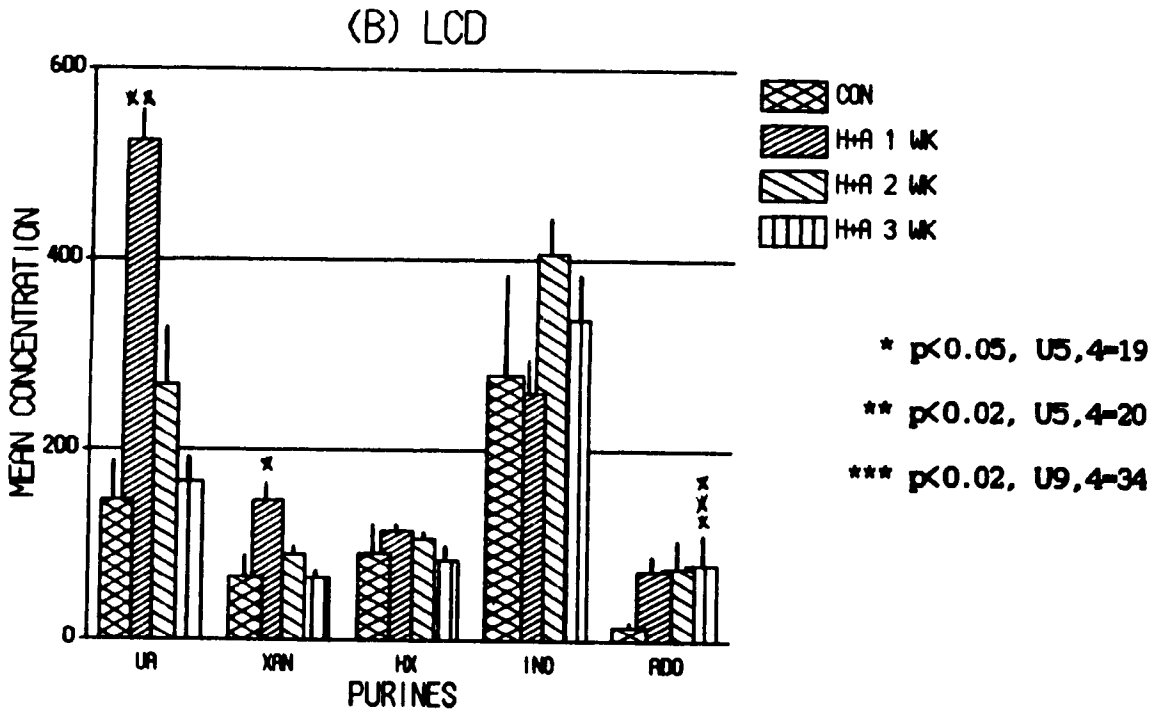
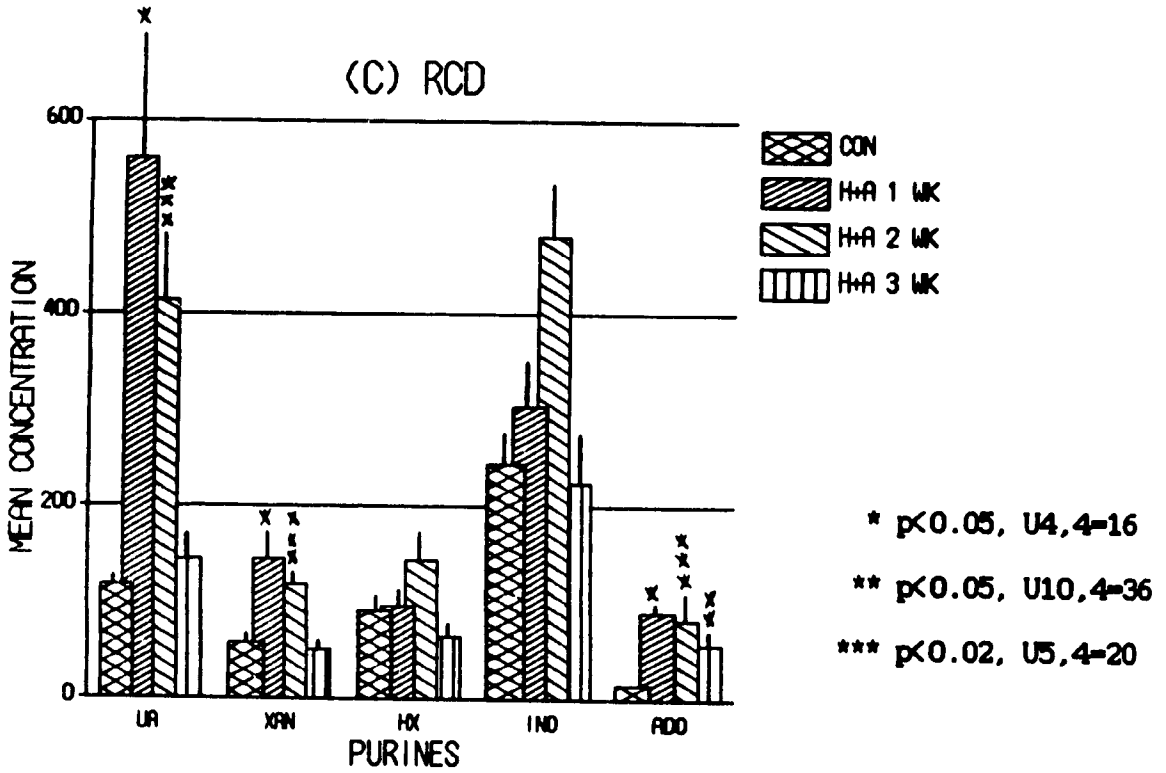


FIGURE 15 continued. The mean concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the (A) AMY, (B) LCD and (C) RCD for CON [n=4] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.





B. iii. b. ii.) Catecholamine concentrations

i) ALLO packed cannulae

As compared to CON at one week post-implantation, treated animals showed significant decreases in the levels of DOPAC in the HYP; DA in the AMY; and HIAA in the CTX and BST (figure 16).

At two weeks post-implantation, there were significant decreases of DOPAC in the BST; and HIAA in the LCD and NAC.

At three weeks post-implantation, significant changes were noted in levels of NA in the LCD, RCD and NAC; DOPAC in the HYP and CTX; and HIAA in all seven brain regions.

These results suggest that ALLO treatment does not produce a developmental pattern of catecholamine alterations which parallel APO-induced rotation.

FIGURE 16. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=7] weeks. * indicates significantly different from CON and error bars represent S.E.M.

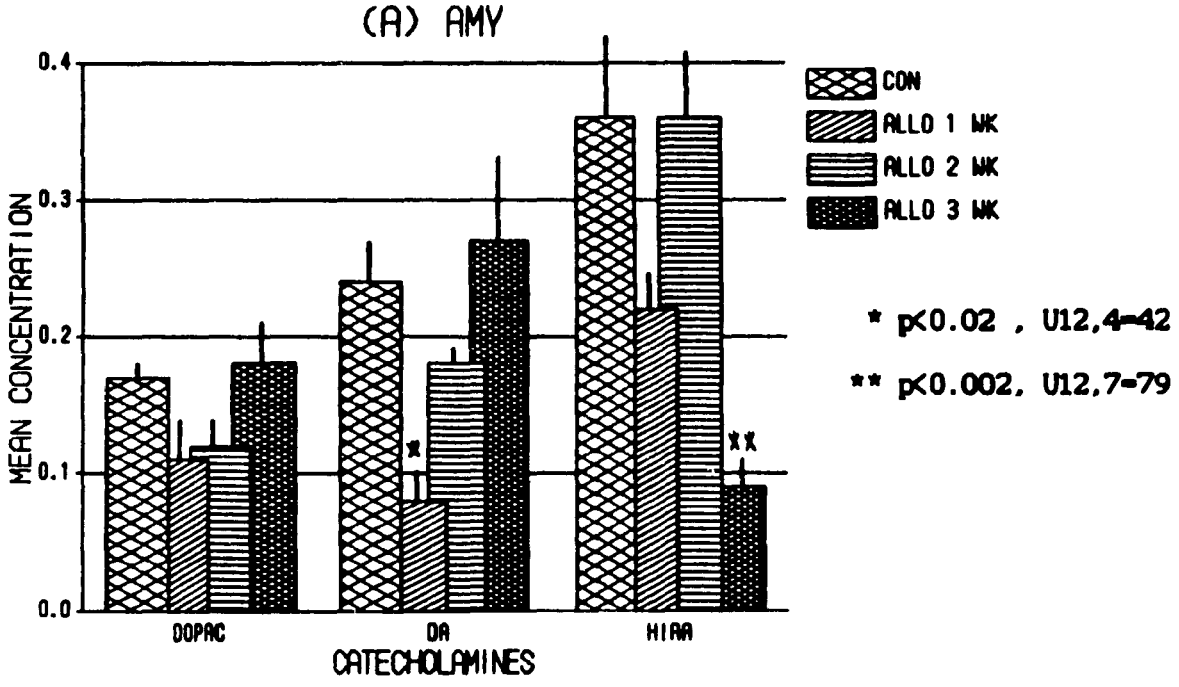
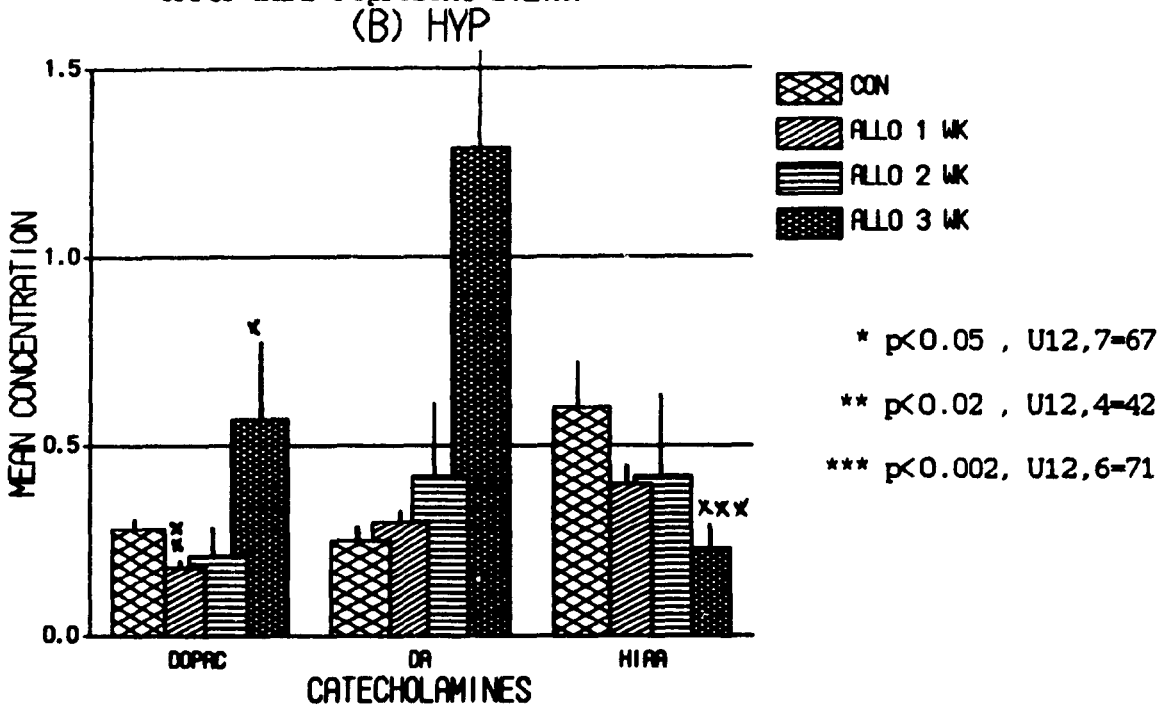


FIGURE 16 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=7] weeks. * indicates significantly different from CON and error bars represent S.E.M.



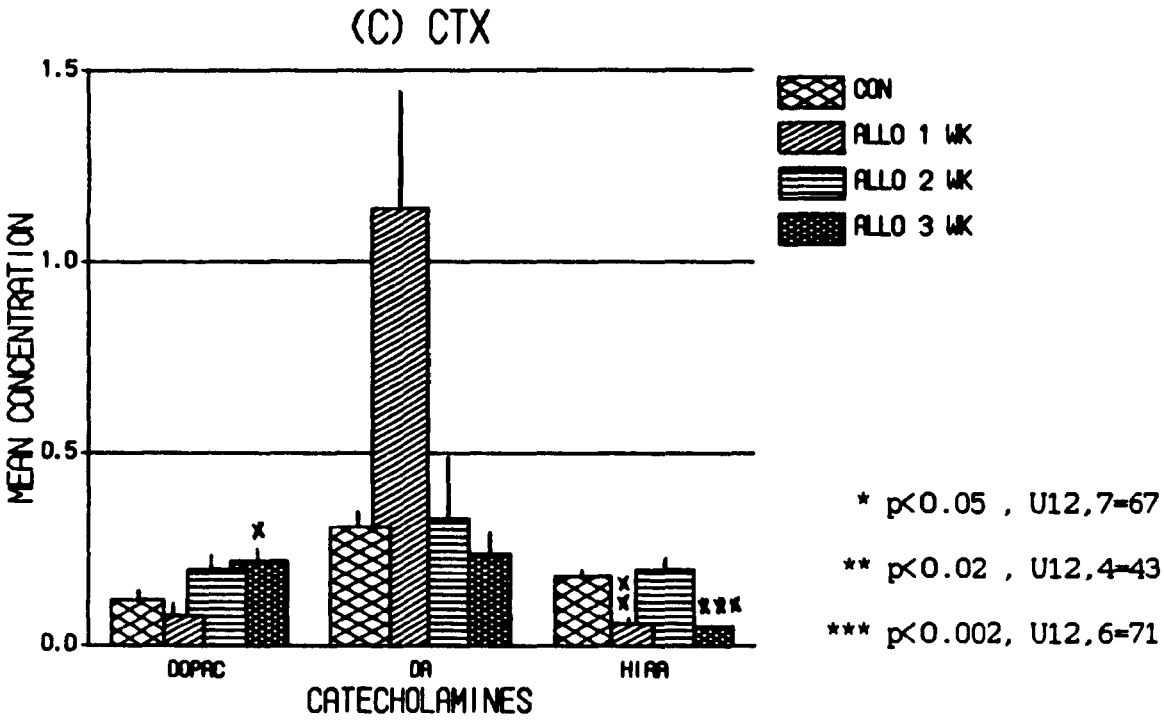
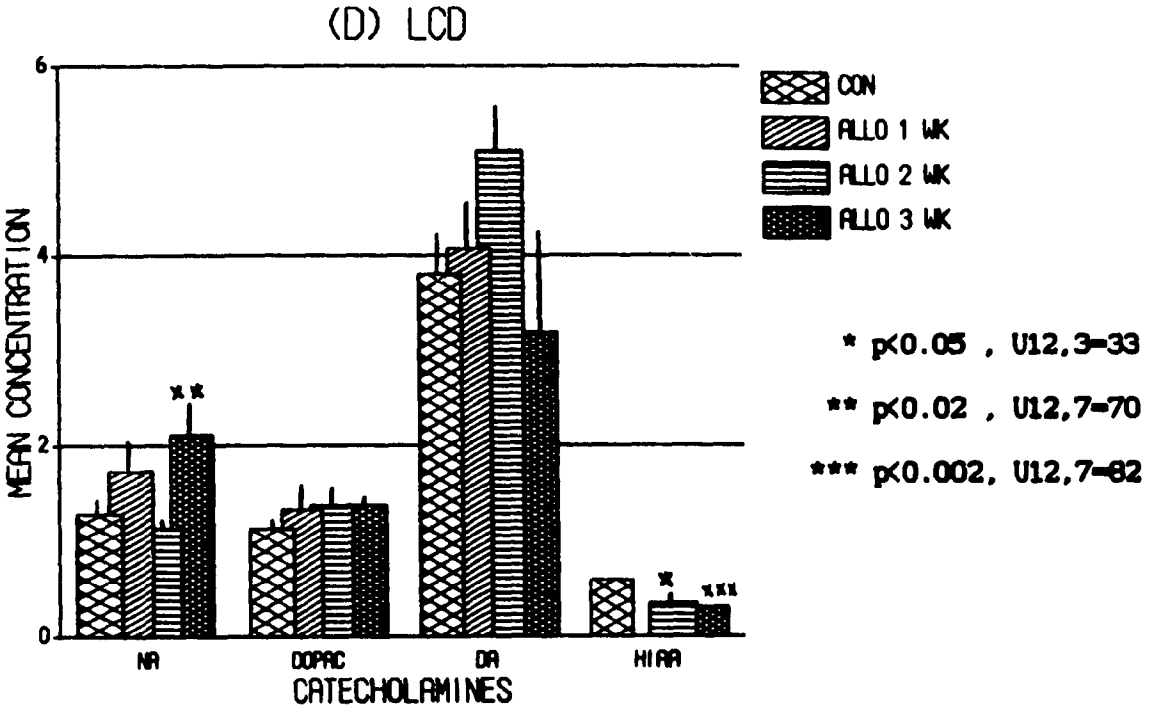


FIGURE 16 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=7] weeks. * indicates significantly different from CON and error bars represent S.E.M.



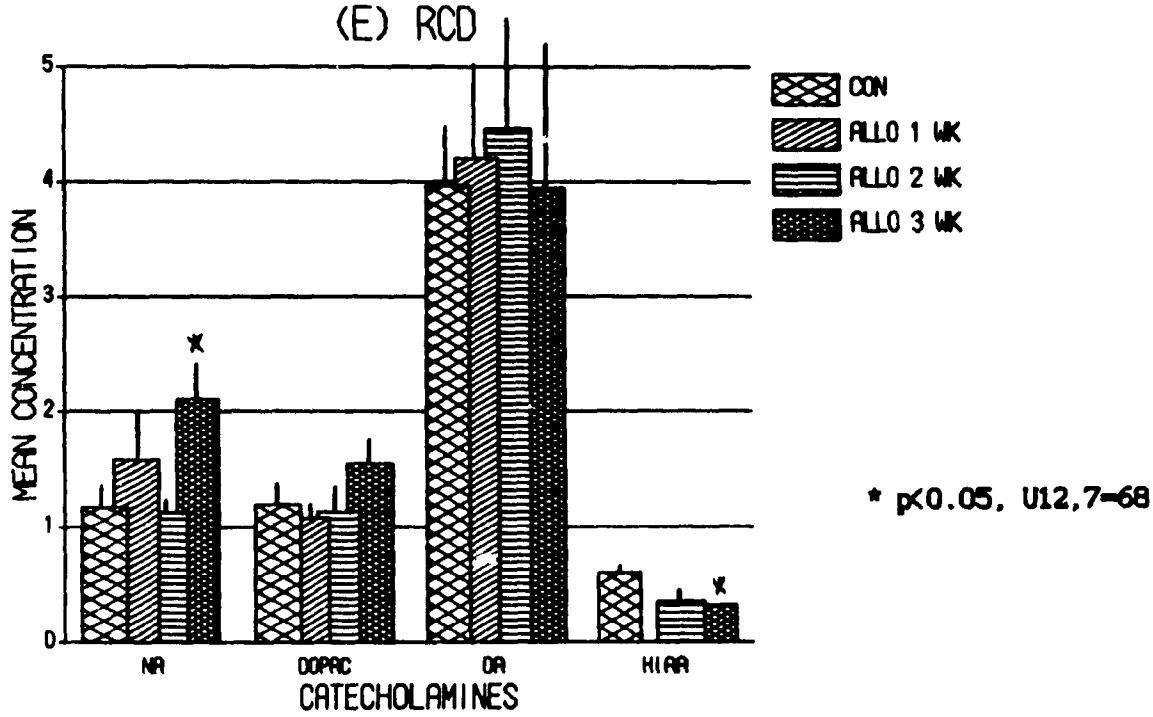
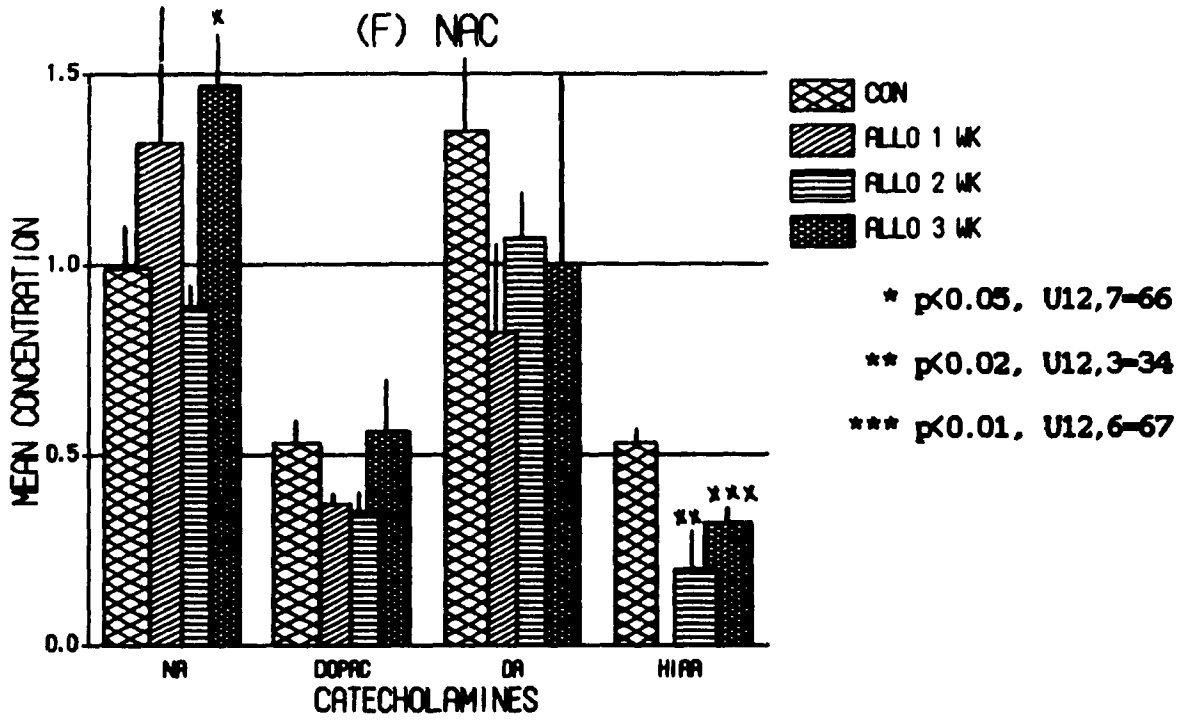
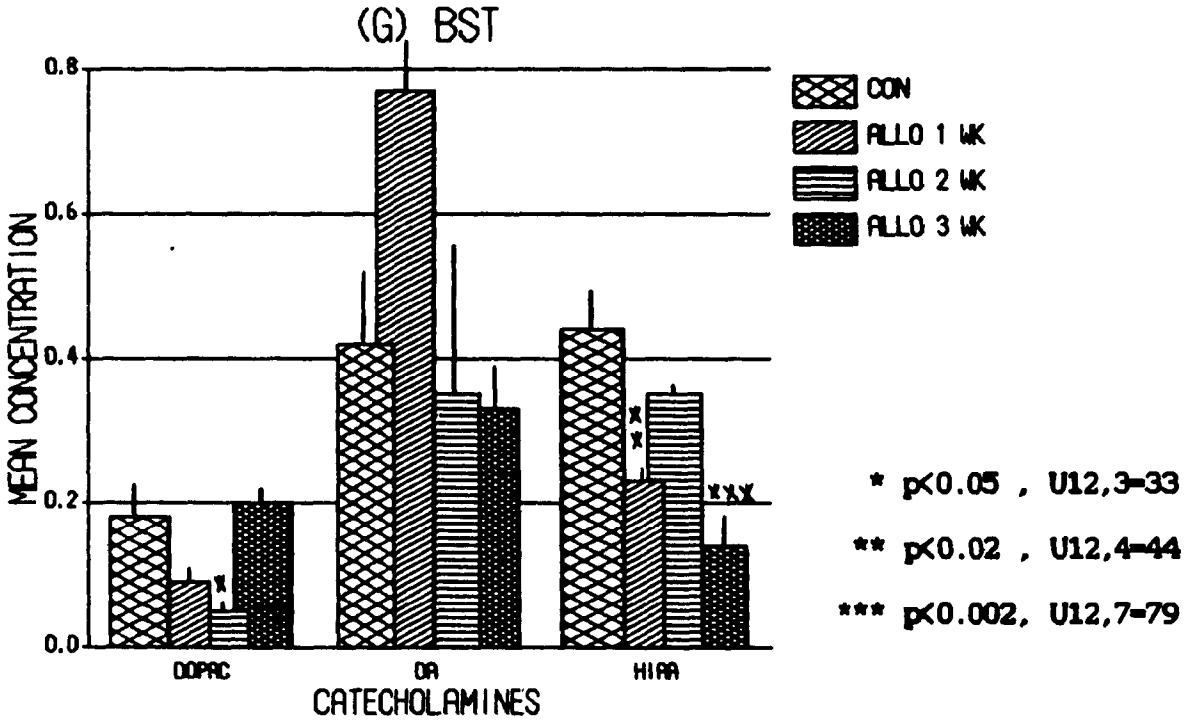


FIGURE 16 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO packed cannulae for 1 [n=4], 2 [n=3] and 3 [n=7] weeks. * indicates significantly different from CON and error bars represent S.E.M.





B. iii. b. ii. ii) ALLO plus HX packed cannulae

As compared to CON at one week post-implantation, treated animals showed significant changes in levels of DOPAC in the RCD; DA in the CTX and RCD; and HIAA in the BST (figure 17).

At two weeks post-implantation, there were significant alterations in the levels of NA in the NAC; DOPAC in the AMY and LCD; and HIAA in the LCD, RCD and NAC.

At three weeks post-implantation, treatment resulted in significant changes in the levels of NA in the LCD, RCD and NAC; DOPAC in the AMY, HYP, CTX and LCD; and HIAA in all measured brain regions except the RCD.

FIGURE 17. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.

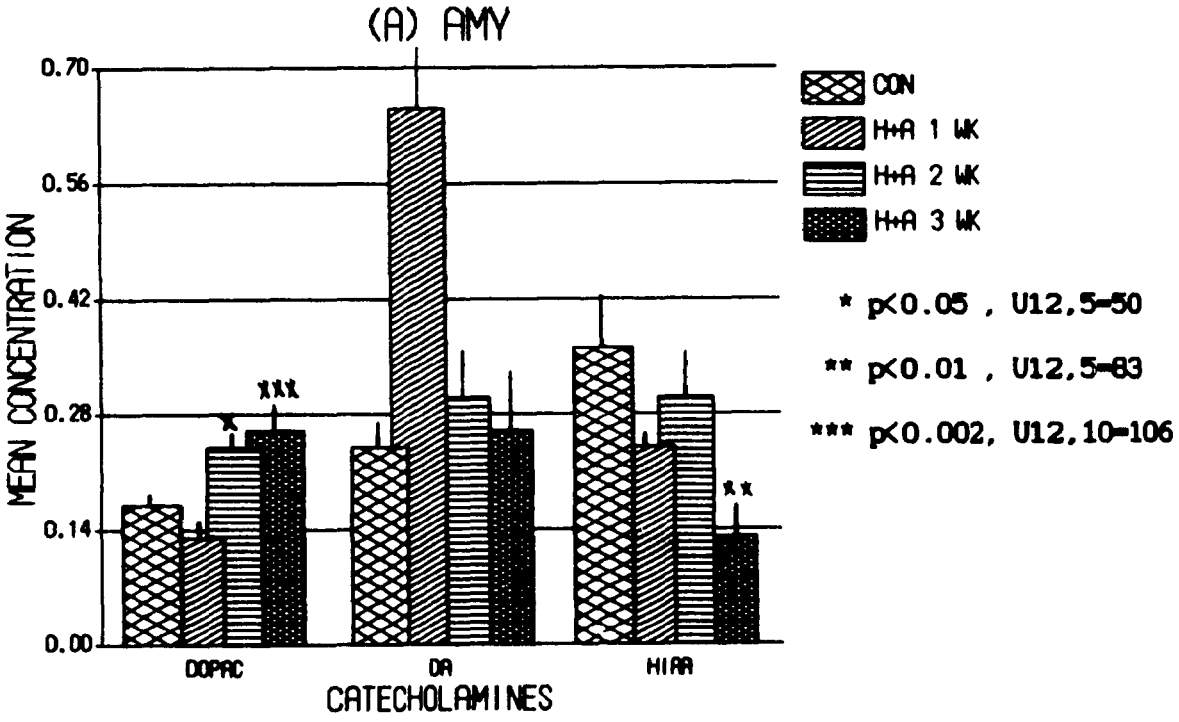
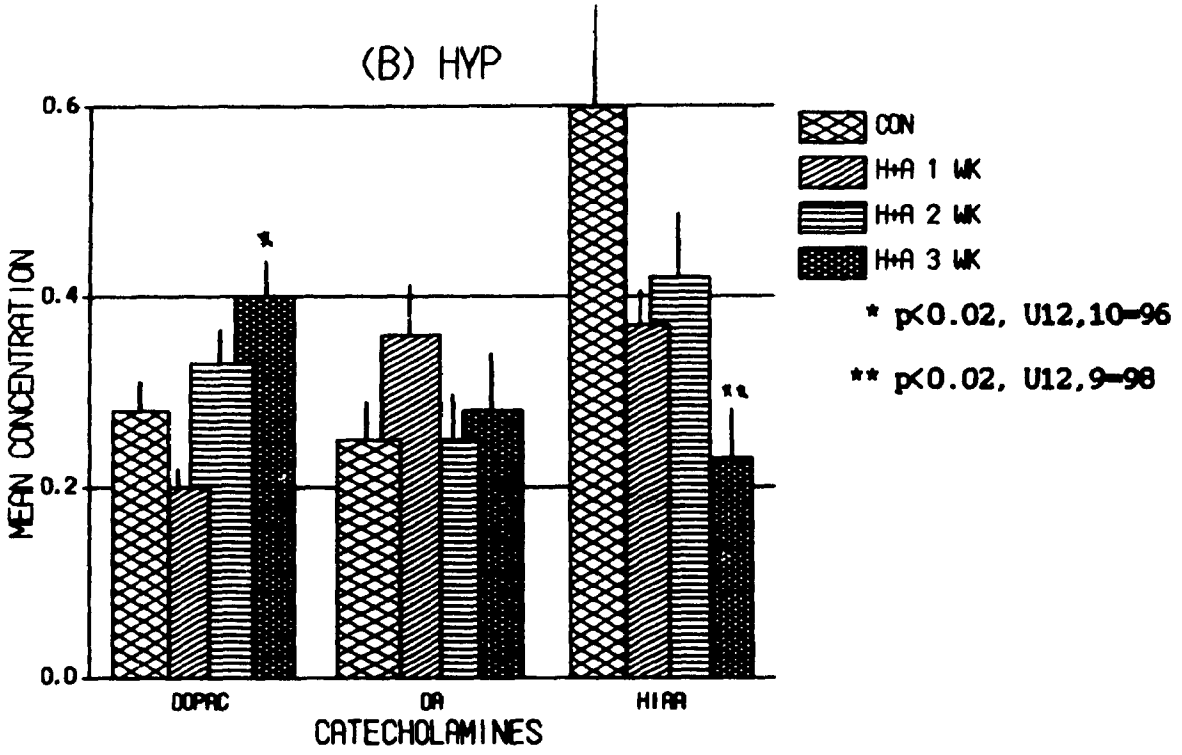


FIGURE 17 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.



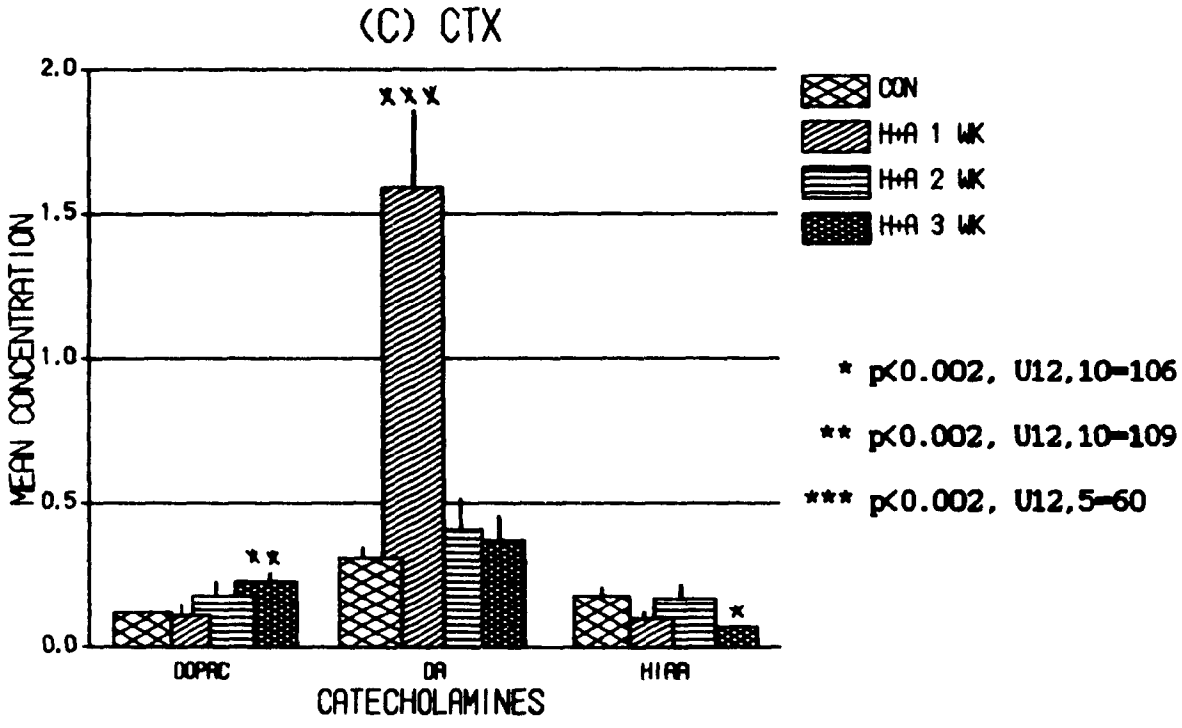
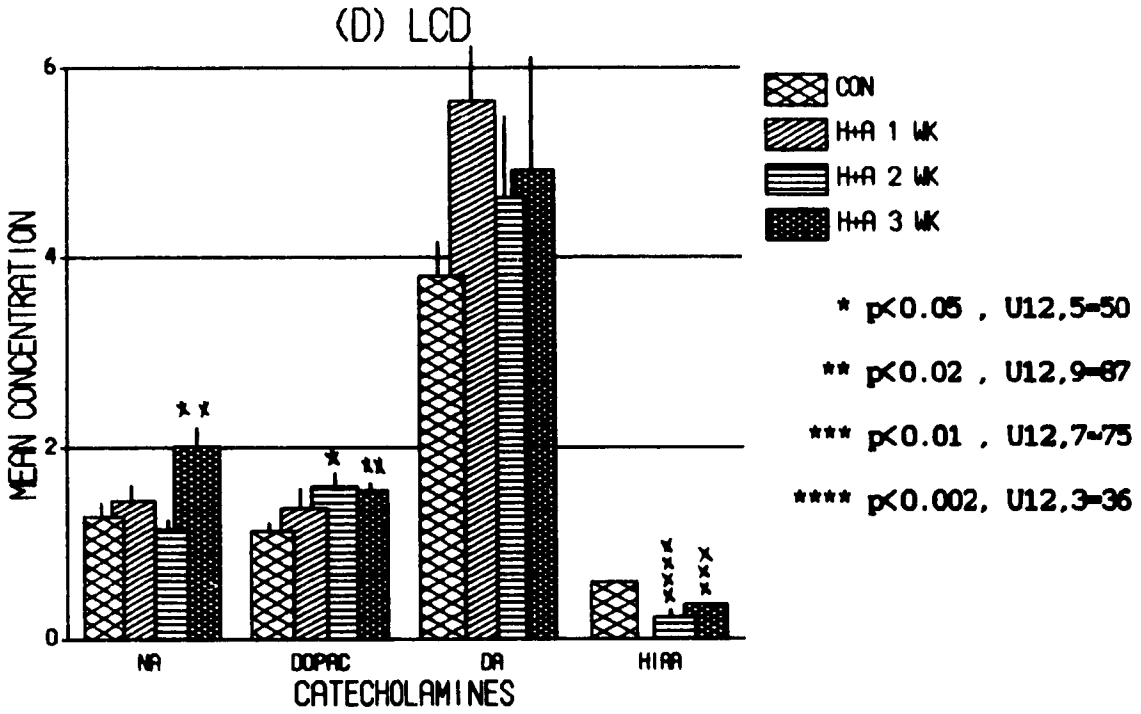


FIGURE 17 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.



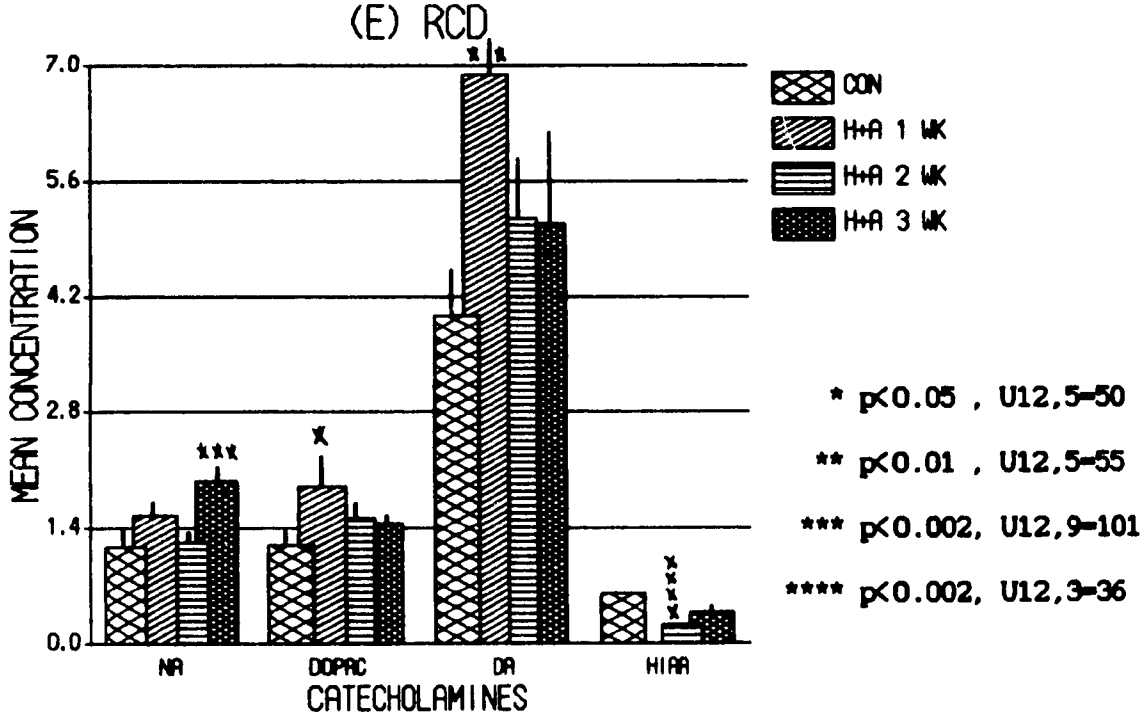
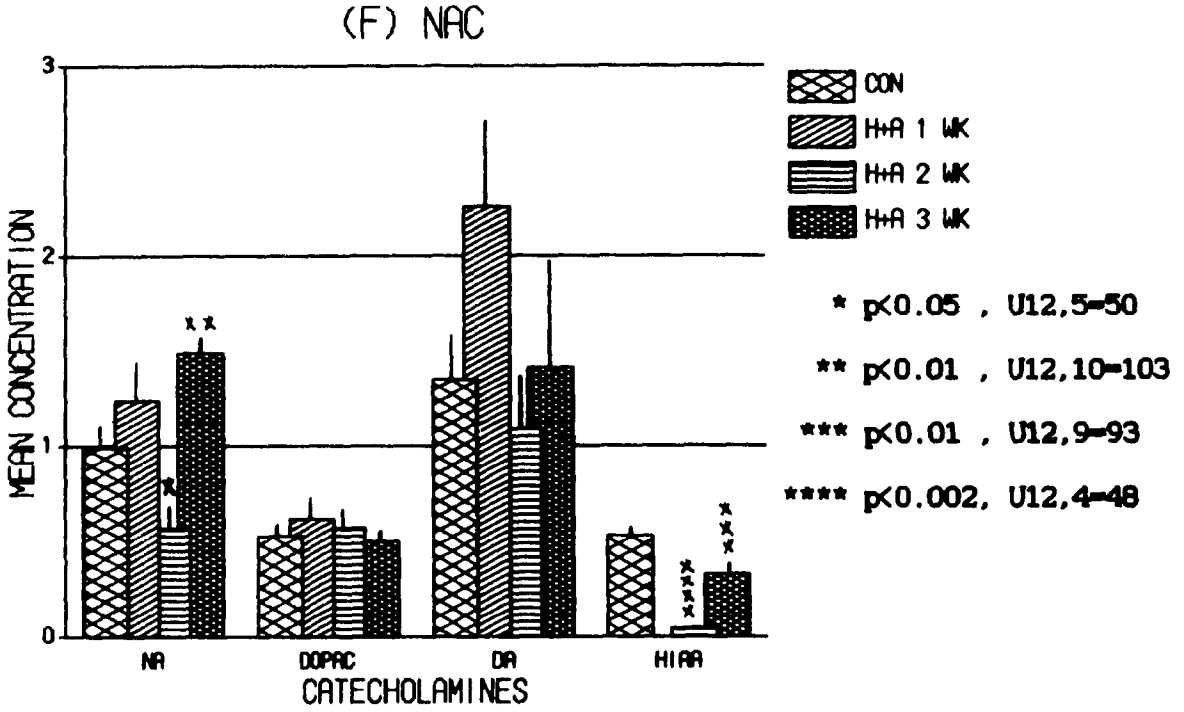
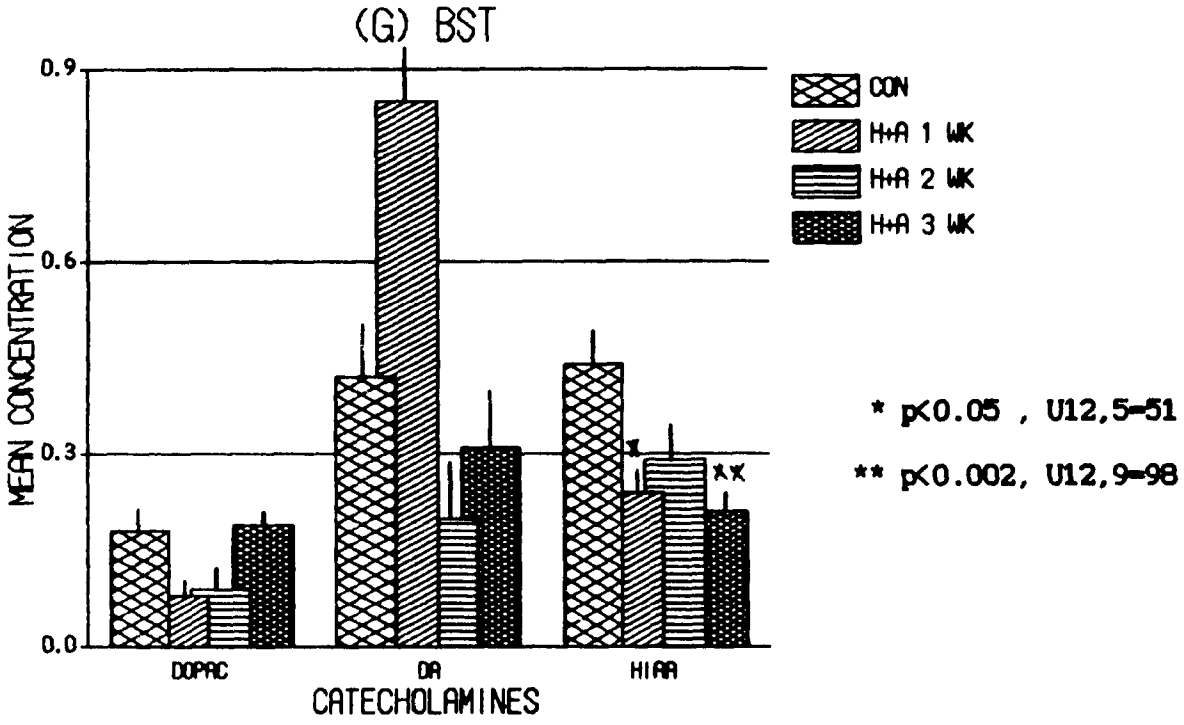


FIGURE 17 continued. The mean concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the (A) AMY, (B) HYP, (C) CTX, (D) LCD, (E) RCD, (F) NAC and (G) BST for CON [n=12] and animals implanted with ALLO plus HX packed cannulae (H+A) implanted into the left lateral ventricle for 1 [n=5], 2 [n=5] and 3 [n=10] weeks. * indicates significantly different from CON and error bars represent S.E.M.





In summary, the ALLO treatment alone and ALLO plus HX treatment resulted in a time-dependent increase in APO induced rotations suggesting that DA post-synaptic receptor desensitization occurs in a similar fashion to that of HX treatment. High levels of UA and XAN are seen early in both treatments and ADO is not associated with APO-induced rotation for the ALLO treated animals. Furthermore, catecholamine analysis for both treatment groups does not reveal any trends in the alteration of catecholamines which might parallel rotational behaviour.

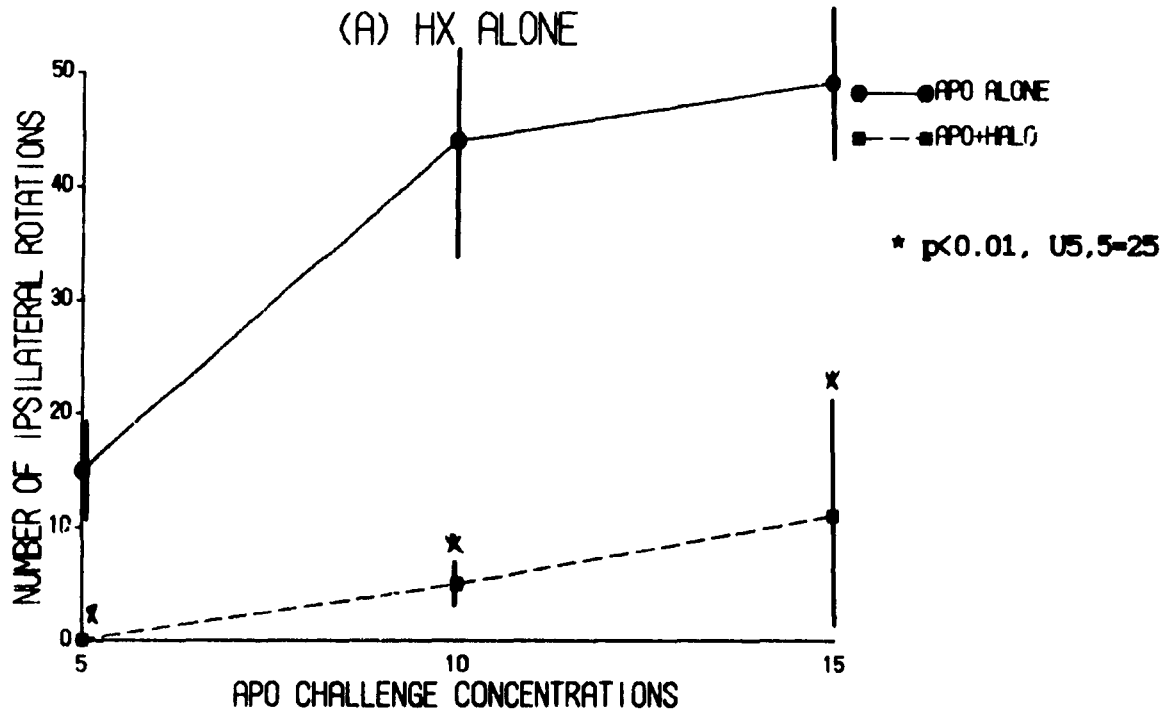
C) ADDITIONAL BEHAVIOURAL STUDIES OF TREATED ANIMALS

To confirm the effects of treatment upon DA post-synaptic receptors as suggested by the Ungerstedt model, DA antagonists were used to block APO-induced rotation.

C. i) Blockade of Rotational Response by DA Antagonist Pretreatment

Confirmatory studies were carried out with a non-specific DA antagonist HALO upon animals which received implanted cannulae packed with HX, ALLO or HX plus ALLO. As compared to the rotational response of animals that received APO alone (control), animals pretreated with HALO showed reductions of APO-induced ipsilateral rotation (figure 18). This blockade suggests that induced rotational behaviour is related to actions at the DA receptor.

FIGURE 18. The mean number of observed rotations after challenge with one of three doses of APO [5mg/kg, 10mg/kg or 15mg/kg] alone (Control) or pretreatment with HALO for animals implanted with packed cannulae containing (A) HX alone [n=5], (B) ALLO alone [n=5], or (C) HX+ALLO [n=5]. * indicates significantly different from control and error bars represent S.E.M.



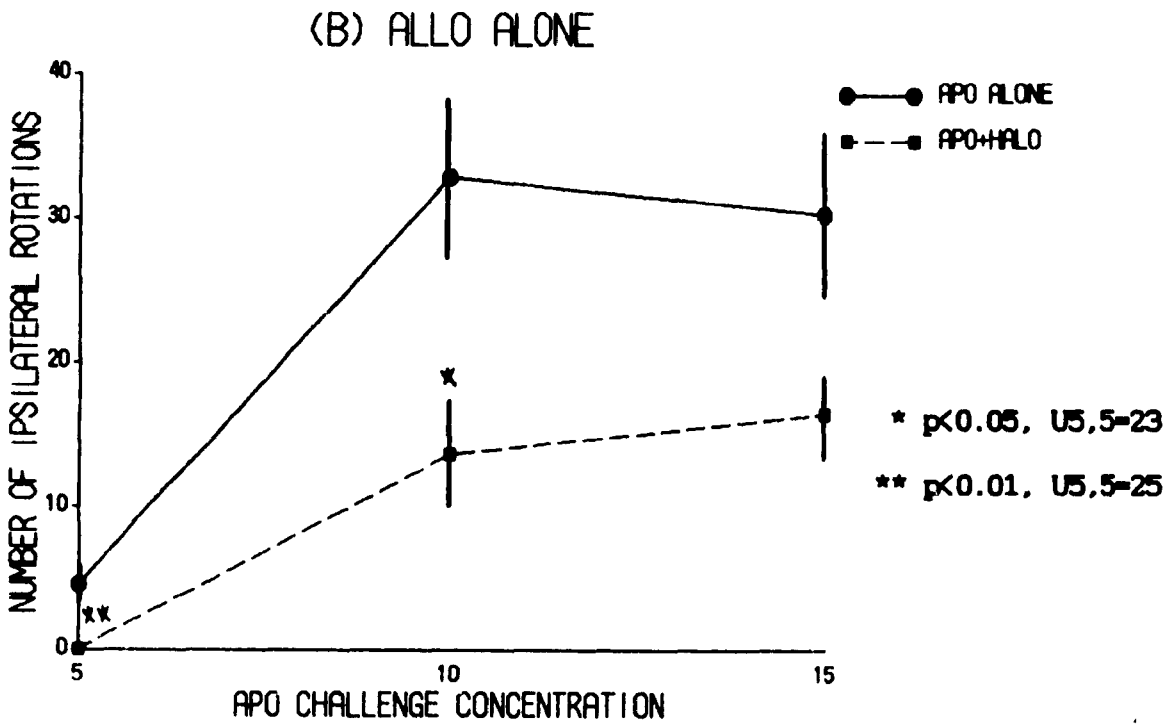
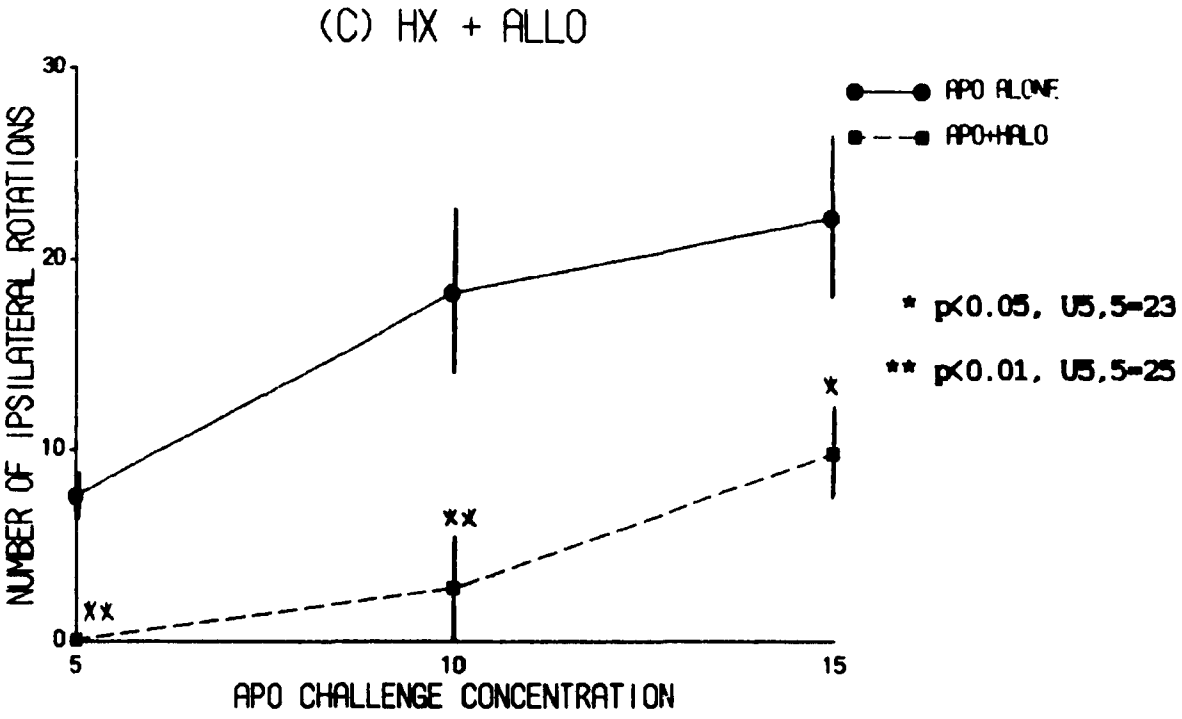


FIGURE 18 continued. The mean number of observed rotations after challenge with one of three doses of APO [5mg/kg, 10mg/kg or 15mg/kg] alone (Control) or pretreatment with HALO for animals implanted with packed cannulae containing (A) HX alone [n=5], (B) ALLO alone [n=5], or (C) HX+ALLO [n=5]. * indicates significantly different from control and error bars represent S.E.M.



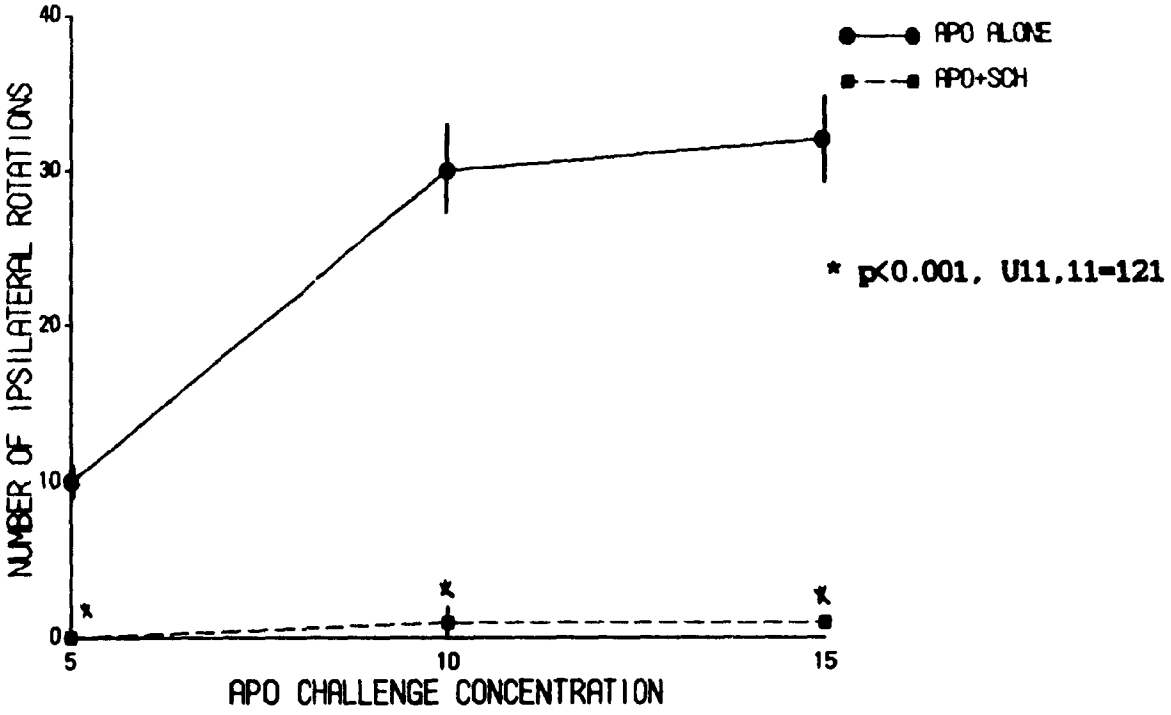
C. ii) DA Antagonist Specificity Upon Reduction of Rotational Behaviour

The next question to ask is whether HX treatment acts specifically on a particular dopamine receptor subtype.

As compared to control, animals pretreated with SCH 23390 (SCH), a specific DA D1 antagonist, had significant reductions of APO-induced ipsilateral rotations (figure 19). This is consistent with the hypothesis that D1 DA post

synaptic receptors are preferentially affected by HX.

FIGURE 19. The mean number of rotations observed for animals with HX packed cannulae [n=11] after challenge with one of three doses of APO alone [5mg/kg, 10mg/kg or 15mg/kg body weight] or with a pretreatment of SCH. * indicates significantly different from APO alone and error bars represent S.E.M.



To address the question of DA D1 post-synaptic receptor specific involvement in rotational behaviour, other APO induced behaviours were scored (figure 20). For HX treated animals that received HALO pretreatment, there was a significant reduction of all measured activities. On the other hand, HX treated animals that received SCH pretreatment, did not have significantly altered activity

levels and suggests that SCH blockade of D1 DA receptors specifically affects APO-induced rotation.

FIGURE 20. The activity level of animals implanted with HX packed cannulae for (A) motor, (B) sniffing and (C) gnawing/licking behaviours when challenged with APO alone or pretreated with SCH [n=11] or HALO [n=5]. * indicates significantly different from APO alone and error bars represent S.E.M.

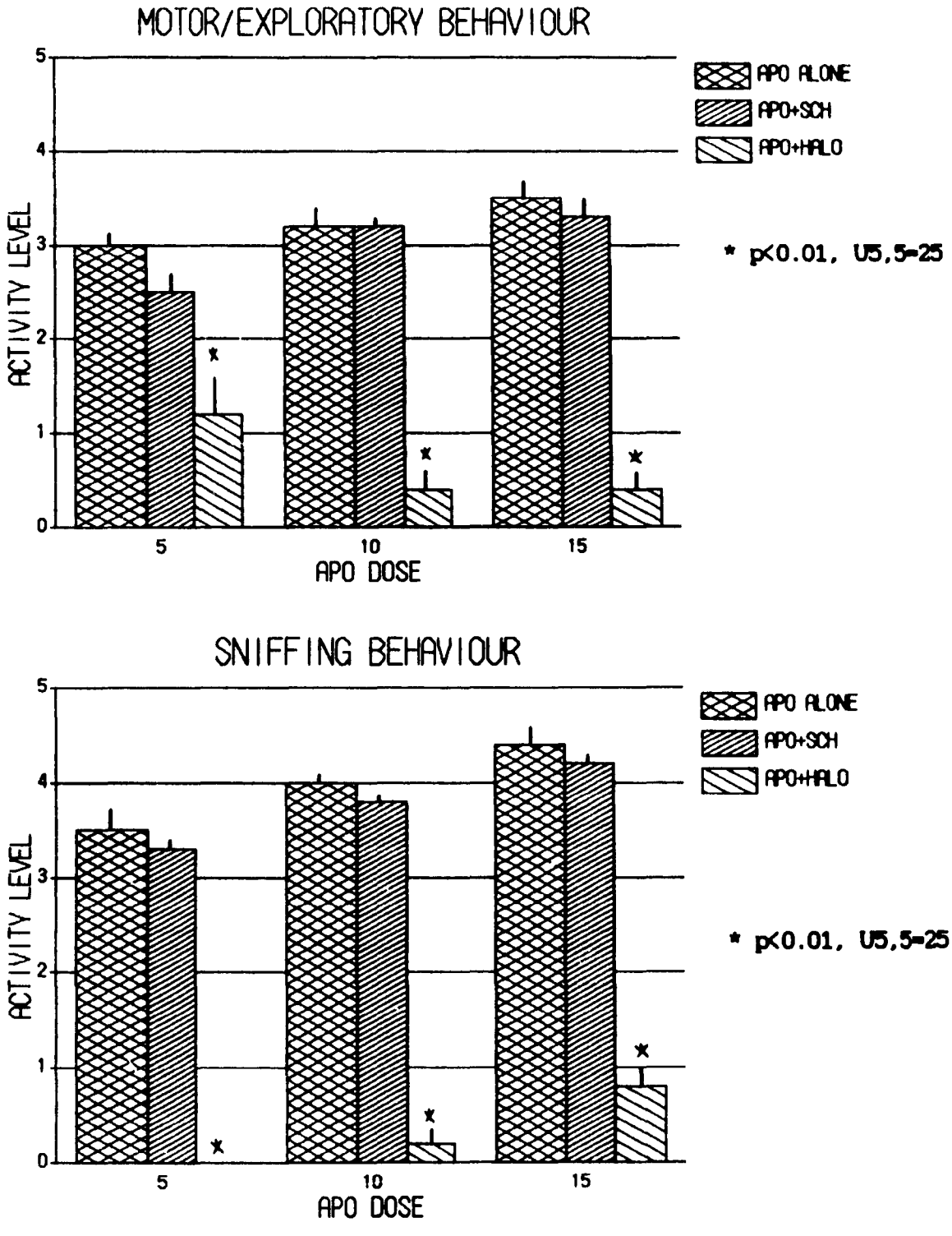
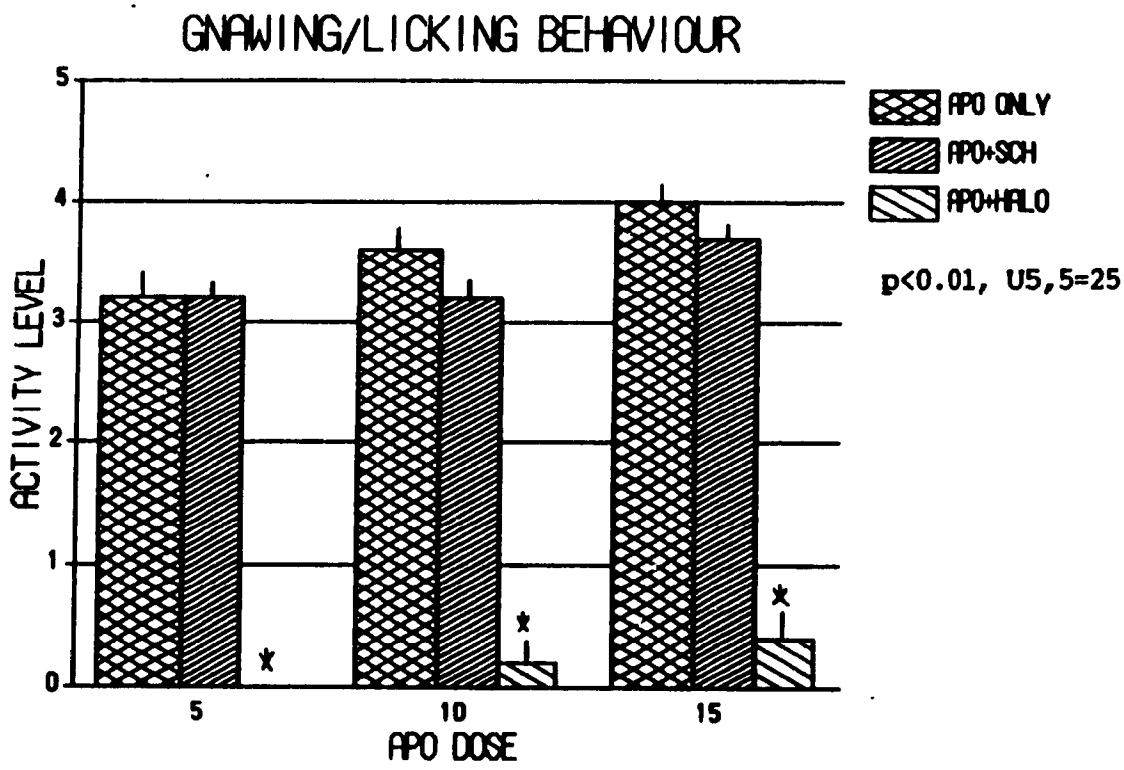


FIGURE 20 continued. The activity level of animals implanted with HX packed cannulae for (A) motor, (B) sniffing and (C) gnawing/licking behaviours when challenged with APO alone or pretreated with SCH (n=11) or HALO (n=5). * indicates significantly different from APO alone and error bars represent S.E.M.



C. iii) Purine-Dopamine Interactions

A sample of HX treated animals were pretreated with CAF and then challenged with APO. All animals exhibited cataleptic behaviour, suggesting that blockade of ADO receptors in lesioned animals obliterates APO-induced rotational behaviour.

VIII) DISCUSSION

A) CONCLUSIONS

These experiments were designed to evaluate the possibility of a direct biochemical relationship between HX accumulation and DA depletion in the brain of the LND patient. These experiments are preliminary in nature and many detailed experiments are needed to further resolve this question. Nevertheless prolonged local administration of HX did have the following behavioural and biochemical effects:

(1) There was an elaboration of DA receptor-specific behaviours infrequently observed in normal rats.

(2) These behaviours were stimulated by a DA agonist and blocked by DA antagonists. The stimulation of these behaviours were time and dose dependent suggesting that number of DA neurons altered by this treatment increased over time.

(3) The data are consistent with the hypothesis that DA receptors, probably of the D1 subtype, were functionally altered.

(4) Associated with an increase of DA receptor-specific behaviours was a significant increase of ADO in at least the untreated RCD.

(5) The effects of HX treatment were specific to DA and DOPAC, with some effect upon serotonin metabolites and little affect upon NA concentrations in the brain.

(6) Overall there was a time-dependent developmental

sequence of HX effects on DA neurons, just as there is a clinical sequence of expression of the behavioural phenotype in LND.

To discuss the significance of these findings, the discussion shall be partitioned into two subsections. The first will discuss tne validity of the results and draw attention to possible errors attributable to experimental design or technique. The other section will examine the implications of these results and suggest future experiments to further explore this complex problem.

B) VALIDITY OF CONCLUSIONS

i) Behavioural Data

When scoring the rotational behaviour, there was a consistent response to APO for all of the treatment groups. CON animals did not rotate upon APO challenge, which seems to indicate that mechanical damage due to cannulae implantation has little affect upon DA neurons.

B. ii.) Neurochemical Analysis

i) Preparation of tissue

Precision errors may exist in the preparation of tissues. If these brains were not rapidly removed and dissected efficiently, then there could be a depletion of both purine and catecholamine levels within the examined brain regions. Moreover, samples were frozen and thawed more than once,

which could lead to significant alterations. However, since CON brain samples were handled in the same manner as samples from treated animals, the CON values may still serve as a good reference point.

ii) **Measurement of purine concentrations**

Similar purine concentrations within the AMY and caudate nuclei are found when comparing the values from CON animals and untreated controls studied in other experiments (table 2). The only exception is ADO concentrations which are underestimated in the CON animals. Nevertheless, HX treatment still produced ADO levels far above either value. Hence any error as a result of technique does not seem to affect purine concentrations in these examined brain regions.

TABLE 2. The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY and caudate nuclei (CAUD) from animals implanted with empty cannulae (CON) and other animals drawn from other experiments (Oth). The values are mean and ± S.E.M.

Brain Region + Group		UA	HX	INO	ADO
AMY	CON	180 ± 40	111 ± 40	218 ± 59	6 ± 2
	Oth	103 ± 33	113 ± 7	238 ± 53	69 ± 29
CAUD	CON	130 ± 40	60 ± 16	255 ± 100	15 ± 4
	Oth	74 ± 11	158 ± 15	223 ± 45	50 ± 27

A problem which affected the statistical significance of purine concentrations between treated animals and CON was the number of analyzed CON animals. Because of the large variance and small sample size, some purine differences only

approached significance.

An unexpected result was seen with the ALLO treated animals. The high concentrations of UA and XAN should not have occurred if ALLO affects XO in the brain as it does in the periphery. It is possible that the peaks registered on HPLC were not of UA and XAN, but rather ALLO metabolites. It is known that ALLO can be converted by XO to allo-xanthine (Elion et al, 1966) and it may be this metabolite or others which were being measured. To determine if these substances are UA and XAN, one could add XO to the homogenized brain sample and see a decrease in the XAN level with a subsequent increase in the UA level. Moreover the addition of uricase would eliminate the UA peak. The shifts in these levels to baseline due to added enzymes should only occur if these substances are UA and XAN.

B. ii. iii) Measurement of catecholamine concentrations

When experimental control concentrations were compared to that reported in the literature we find that the experimental control values for DA within the caudate nuclei are 50% lower than other reports (Altar et al, 1987). Moreover, comparing untreated animals from other experiments within our laboratory show that DA within AMY for these experimental controls are also reduced by 50%. This suggests that precision errors exist in the handling of these samples which resulted in a depletion of catecholamine concentrations. Since the tissues for the treated animals were handled the same way as the experimental control

the relative values between treated and control animals can be assumed to be due to treatment, provided that catecholamines are not differentially reduced as a function of their initial values. The absolute values are, of course, inaccurate.

C) IMPLICATIONS OF THE RESULTS

These results have significance for both basic neuroscience and for the clinical management of the LND patient.

At the basic neuroscience level, a number of points are of note. First, the APO-induced rotation found in the HX treatment group clearly indicates a developmental pattern of treatment effects. According to the Ungerstedt model, significant desensitization or hypoactivity of DA post-synaptic receptors within the treated LCD became apparent at post-implantation day 11. There are a number of possible explanations for this apparent hypoactivity.

Although the Ungerstedt model reveals effects due to alterations of the DA post-synaptic receptor (Snyder, 1979), the DA post-synaptic receptor might not be the primary site of HX action. As is the case for 6OHDA treatment (Ungerstedt et al, 1981), the primary target for HX may be at the pre-synaptic level. One possible explanation for the hypoactivity of DA post-synaptic receptors is that HX treatment is affecting neurotransmitter release. If a depletion of DA and a subsequent decrease of DA release can cause DA post-synaptic receptor super-sensitization

(Ungerstedt et al, 1981), then an increased release of DA could over stimulate the post-synaptic receptor and cause down-regulation (Harden, 1983). An increase of striatal DOPAC was seen in lateral ventricle implants of HX; this could suggest an increased DA turnover(Roth et al, 1976 ;Wood et al, 1982) and might be responsible for the DA post-synaptic receptor desensitization. DOPAC can also be elevated following a variety of treatments which block impulse flow (Altar et al, 1984), because it is formed both inside and outside the dopaminergic neuron (Schoepp and Azzaro, 1982). It is thought, therefore, that striatal DOPAC concentrations reflect the recent history of DA biotrans-formation rather than the extent of DA release (Altar et al, 1987). In contrast, the formation of 3-methoxytyramine (3MT) from DA occurs solely ex ra-neuronally (Zumstein et al, 1981) and indicates the level of DA in the synaptic cleft (Westerink, 1979; Westerink and Spaan, 1982). Hence future experiments should examine the striatal 3MT concentrations of these HX treated animals. Elevated concentrations of 3MT within th LCD would be consistent with the hypothesis that HX treatment increases the impulse flow of DA. This would be consistent with the hypothesis that HX treatment increase the release of DA which would be one possible mechanism for DA post-synaptic receptor desensitization.

Contrary to this hypothesis was the observation that animals implanted with HX packed cannulae within the LCD had a depletion rather than an increase of DOPAC when APO-induced rotation was at its maximum. The increase of DOPAC

and DA found in animals implanted with HX packed cannulae into lateral ventricle and the depleted levels found in animals implanted with HX packed cannulae into the LCD may represent different windows in a developmental process. Although the purine levels of these two treated groups are similar late in the treatment period, the present data does not examine purine and catecholamine concentrations early in the post-implantation period. Initially with this packed powder delivery system, one would expect a great release of HX. If the HX was implanted into the LCD, then the potential delivery would be extremely tissue specific and could result in a complete lesion of dopaminergic neurons. On the other hand, lateral ventricle HX implants are not solely affecting the LCD as demonstrated by alterations of catecholamine concentrations in other brain regions. Moreover, CSF concentrations of HX adjacent to the LCD may contain high levels of HX, but nonetheless not all of the LCD may be exposed to these increased levels. Thus for the lateral ventricle implanted animals, progression to the hypothesized end stage (ie DA and DOPAC depletion) may take more time to evolve. Indeed the lateral ventricle implanted animals have a striatal concentration profile of DA and DOPAC similar to those seen in rats (Hefti et al, 1980; Melamed et al, 1980; Zigmond et al, 1984), humans (Rinne et al, 1971; Bernheimer et al, 1973) and nonhuman primates (Sharman et al, 1967) with a partial lesion of the nigrostriatal pathway. Therefore further experiments should examine the concentrations of purines and catecholamines at earlier time points for the

LCD implanted animals and at extended time points for the lateral ventricle implanted animals. These additional time points may indicate the same developmental process, but occurring at different progression velocities.

As an alternative to a pre-synaptic affect, the primary effects of HX treatment could be directed towards the DA post-synaptic receptors. This may be accomplished by destruction and/or alteration of the receptor; by direct interaction with the receptor's binding site or with second messenger systems; or by stimulation of other neuromodulator receptor systems which influence the DA post-synaptic receptors. The conversion of HX to other purine metabolic products may be responsible for destruction/alteration of the DA receptor and subsequently affect DA striatal output. For example, a conversion of HX to XAN and UA by XO generate superoxide radicals which can affect the cell membrane (Lehninger, 1979). Both UA and XAN are elevated in these HX treated animals and could produce enough superoxide radicals to damage DA receptors. For a direct DA receptor binding site interaction, there must be a continual supply of whatever is blocking the receptor's binding site. This could be accomplished if there was non-competitive inhibition of the DA receptor. The irreversible binding of a substance to the DA receptor would lower the physiological response induced by DA containing neurons and give the appearance of hypoactivity. However, this is unlikely to be the mechanism of HX action on DA neurons, because HX levels are at normal levels when APO-induced rotation reaches its maximum. To

explore the possibility that HX treatment directly affects D1 DA post-synaptic receptors, receptor studies using ligand binding and adenylate cyclase assays could be performed on these brain samples. Alteration of D1 receptor affinity, a reduction in the maximum response to DA or a decrease in receptor number may give the appearance of receptor desensitization within the LCD. Since animals with super-sensitive DA post-synaptic receptors typically have an increase in receptor number (Creese et al, 1978), HX treated animals could have a decrease in the number of D1 receptors.

Alternatively, HX treatment could affect the transduction of the signal by the receptor's second messenger system. Because D1 receptors are known to be coupled with adenylate cyclase activity, HX or another purine metabolite might directly affect the formation of cAMP. ADO, which is significantly elevated within the caudate nuclei of HX treated animals, has receptors which are also known to be coupled to adenylate cyclase (Van Calker et al, 1979). Evidence from other groups suggests that administration of ADO or its analogs results in a dose-dependent inhibition of adenylate cyclase stimulation mediated by D1 DA receptors (Abbracchio et al, 1987; Porter et al, 1988). Moreover evidence from our own laboratory suggests that chronic HX administration modulates ADO receptor function in cultured neuroblastoma cells. Hence both DA and ADO receptors should be analyzed in the HX treated brains as it has been suggested that ADO acts as a neuromodulator through both pre- and post-synaptic actions upon dopaminergic mechanisms

in 6OHDA lesioned rats (Criswell et al, 1988).

A final possibility for the primary site of HX effects is not DA neurons themselves, but rather the neurons with which they synapse such as cholinergic, opiatergic, serotonergic, GABAergic or glutamatergic/aspartatergic neurons. Although choline acetyltransferase (Lloyd et al, 1981) and opiate activity (Gillman and Sandyk, 1985) are altered in the LND patient it is unclear if these are antecedents or consequences of DA depletion. Furthermore for this animal model, alteration of these two neurotransmitters has not been demonstrated to induce rotation resulting from APO challenge. Serotonin is altered in the striatum of LND patients (Lloyd et al, 1981) and in the HX treated animal its metabolite HIAA, had variable levels in different brain regions. Although unilateral alteration of serotonin concentrations can induce rotation (Jacobs et al, 1977), the type of rotation produced is different from that produced by 6OHDA lesions (Ungerstedt et al, 1981) and HX treatment. Although GABA concentrations were not affected in the LND patient (Lloyd et al, 1981) it is known that local injections of potent and specific GABA agonists into the striatum can induce locomotor and stereotypic behaviours which resemble behaviours induced by systemic injections of DA agonists (Cools and Van Rossum, 1976, 1980; Costall and Naylor, 1975, 1976; Costall et al, 1977). Furthermore HX, XAN and INO have been shown to bind to the mammalian brain benzodiazepine receptor (Asano and Spector, 1979; Skolnick et al, 1980) and inhibit in vitro both the GABA receptor

binding and the GABA-stimulated [^{3}H]benzodiazepine binding (Marangos, Paul et al, 1981; Marangos, Trams et al, 1981; Ticku and Burch, 1980). With increased levels of XAN and INO in these HX treated rats, GABA neurons may be functionally inhibited. However to induce rotation, GABA agonists must be injected into the substantia nigra, zona reticulata and not the striatum (Scheel-Krüger et al, 1977). This combined with the fact that D1 DA receptors have been shown to be present in the striatum on intrinsic neurons (Creese et al, 1978) suggests that although GABA containing neurons may be affected by HX treatment, they are probably not the primary lesion site. Glutamatergic/aspartatergic neurons need to be considered, because of increasing evidence that in suitable concentrations glutamate (GLUT) and other dicarboxylic amino acids are neurotoxins (Olney, 1978). In infants, a large number of GLUT receptors are expressed in the basal ganglia in the perinatal period (Barks et al, 1988). Moreover during complete brain ischemia, GLUT release (Benveniste et al, 1984; Hagberg et al, 1985) is coupled with the accumulation of ADO, INO, HX and XAN (Kleihues et al, 1974). If HX accumulates as in the LND patient, then it is possible that this accumulation may be coupled with GLUT release and DA neuronal damage in the perinatal period. Since the neonatal density and innervation of DA terminals within the striatum is not complete until three months of age in the humans (Dobbings, 1970; Loizou and Salt, 1970; Loizou, 1972), it is possible that this HX-GLUT accumulation may be responsible for the developmental alteration of DA neuronal function. In

LND patients, Rassin et al (1982) noted that GLUT was unchanged from age matched controls. It is important to note that the post-mortem tissues analyzed by Rassin were from patients above the age of 12. By this proposed mechanism of GLUT action, tissue damage due to GLUT release has already occurred many years prior. Some studies upon the impact of perinatal hypoxic-ischemic insult show that regions with high GLUT innervation pre-insult (ie striatum) had post-insult reductions in GLUT receptor binding (Silverstein et al, 1987), but not in the level of GLUT (Girault et al, 1986). These studies suggest that GLUT-induced tissue damage occurred in the area of GLUT receptors, but the level of GLUT post-insult remains at normal levels. Hence it is possible to have GLUT involved in the primary events of DA dishomeostasis found in LND, but decrease to normal levels in the older LND patients. In this animal model, GLUT involvement is possible since the type of rotational behaviour exhibited by HX treated animals is similar to those treated with kianic acid, an analog of GLUT (Schwarz et al, 1979). The high concentrations of ADO in this hypothesized mechanism might be present to exert an anti-excitotoxic effect, since ADO attenuates the physiological release of GLUT and aspartate (Dolphin and Archer, 1983; Corradetti et al, 1984), while the administration of theophylline, an ADO receptor antagonist, enhances ischemic injuries (Wieloch et al, 1986). To test if GLUT is involved in the effects upon DA neurons amino acid analysis should be performed early in the post-implantation period and compared

to values at later time points.

By exploring the purine-DA interactions of this LND animal model, these experiments also shed new light upon the classical Ungerstedt model. Both 6OHDA and HX treated animals had significant increases of ADO within the untreated RCD. If ADO can inhibit D1 DA receptor function, then both the 6OHDA and HX treated animals could have desensitized DA receptors within their RCD. The following hypothesis may explain why 6OHDA and HX treated animals do not rotate without a DA agonist challenge.

At the neuronal level, if an animal received 6OHDA within the LCD, the majority of the LCD's DA-containing neurons would be destroyed (Snyder, 1979). Even with the development of post-synaptic super-sensitization, the physiological response by the post-synaptic receptor to the depleted endogenous DA would be diminished and overall these DA receptors could have the functional appearance of being desensitized. That 6OHDA animals do not rotate unchallenged suggests that the physiological response of the DA post-synaptic receptor within the untreated RCD compensates to maintain an equilibrium between normal and lesioned caudate nuclei. Maintenance of this reduced physiological response to DA within the RCD may be mediated by ADO since its concentra-tions within the RCD are about twice that of the LCD. Upon APO challenge, the LCD's super-sensitive DA post-synaptic receptors now have an increased availability of a DA agonist. This agonist-receptor interaction dramatically increases the striatal output of the LCD as compared to the

RCD and this disequilibrium results in a contralateral rotation to the right.

For the HX treated animals, treatment results in an apparent desensitization of DA post-synaptic receptors within the LCD. So at the neuronal level, to compensate for the reduced physiological response to DA by the post-synaptic receptors within the LCD, the untreated RCD lowers its striatal output. Again the RCD may achieve this hypoactivity by the presence of high ADO concentrations. HX treated animals do not rotate unchallenged as the physiological response to DA in both the LCD and RCD are equivalent. Upon APO challenge, DA post-synaptic receptors within the treated LCD cannot respond to the DA agonist, while the DA receptors in the untreated RCD can respond resulting in a greater physiological response and an ipsilateral rotation to the left.

Basically this hypothesis suggests that there is some form of homeostatic communication between the two caudate nuclei and/or nigrostriatal pathways to maintain an equivalent response to DA. This communication results in the alteration of neurochemicals within the untreated RCD to compensate for the treated LCD's altered neurochemistry. It is suggested that the RCD reduces its physiological response to DA to that of the LCD, by increasing the levels of ADO. The maintenance of this equilibrium may prove to be the reason why unilaterally treated animals do not rotate unchallenged. Upon challenge with a DA agonist, the equilibrium is lost and a rotation results.

Since ADO has been hypothesized to act as a major contributor to APO-induced rotation, are these HX packed cannulae treatment effects related to HX or solely ADO? Futhermore is the experimental condition found in this animal model comparable to the neurochemical state found in LND? For the first question, the present data cannot determine what events initiated this apparent D1 DA post-synaptic receptor desensitization. It is uncertain whether the high levels of ADO are responsible for the initiation of the process or are responsible for the maintenance of this hypoactivity. Definitely ADO is affecting both caudate nuclei since cataleptic behaviour was induced following CAF administration. To assess ADO's involvement in the initiation and/or maintenance of these DA alterations, one needs to sample animals which have had HX packed cannulae implants for a very short period of time. Samples at 24, 48 and 72 hours post-implantation may reveal alterations in DA and purine levels which might be responsible for initiating DA post-synaptic receptor desensitization. In addition DA and ADO receptor studies at these time points may prove useful. These suggestions are based on the fact that 24 hours after receiving 6OHDA treatment, animals develop increased DA levels, which then decrease (Anden et al, 1972). Similarly DA post-synaptic receptor super-sensitivity develops rapidly during the first six days after denervation and then the sensitivity levels off (Ungerstedt et al, 1981).

Additional studies must be performed on animals which had

bilateral caudate implants of HX packed cannulae. If ADO is responsible for the reestablishment of a striatal output equilibrium, then bilateral caudate treatment would affect both caudate nuclei and eliminate the need for ADO to lower the striatal output of an untreated nucleus. Moreover this bilateral delivery might allow other treatment effects to surface. This suggestion is based on the observation that animals bilaterally lesioned with 6OHDA had no significant difference of ADO within the caudate nuclei (Criswell et al, 1988).

Does HX treatment address the conditions found in the LND patient? One of the big difficulties with this animal model is the fact that these rats have active HGPRT enzyme in their brains. Therefore when HX was delivered, it could be rapidly converted to IMP and then interconverted to other metabolites in the purine metabolism. This routing of HX may explain the high concentrations of ADO present in these rats. Since HGPRT is not present in the brain of LND patients, a similar routing of HX does not occur. Recently HGPRT deficient mice have become available by the use of recombinant DNA techniques (Kuehn et al, 1987; Hooper et al, 1987). Studying the neuronal development of these animals may prove to be a more adequate reflection of the situation present in the LND patient. Nevertheless, when HGPRT+ (normal) rats are unilaterally implanted with HX packed cannulae, approximately 25% show evidence of self-mutilatory behaviour on the contralateral hind limb by post-implantation day 7. Furthermore animals implanted with HX

packed cannulae in both caudate nuclei and challenged with amphetamine exhibit extreme self-mutilatory behaviour on the forepaws (data not shown). This response to amphetamine is similar to animals with bilateral 6OHDA caudate lesions (Breese et al, 1984a,b,c) and it has been suggested that these 6OHDA lesioned animals may serve as pharmacological model for LND. The HX treated animal model may represent the condition found in the LND patient since delivery is of an accumulating endogenous substance found in these patients (ie HX), rather than a chemical lesion. Furthermore unilateral 6OHDA and HX treatments produce equal yet opposite APO-induced rotations, suggesting different effects upon the DA post-synaptic receptor. These rotational data imply that the situation created by 6OHDA may not be homologous to the HX treatment or to, the LND patient. In any event, future experiments should examine the blockade of amphetamine-induced self-mutilatory behaviour in the HX treated animal by D1 and D2 DA antagonists.

A very interesting result of these experiments came from the ALLO treated animals. The results suggest that ALLO is better than HX for stimulating APO-induced rotation and implies that ALLO has effects upon DA containing neurons. Is this affect the same as HX treatment? Different purine and catecholamine concentration profiles and a different response to HALO blockade of APO-induced rotation suggests ALLO effects may be different from HX effects. Nevertheless this data seems to suggest ALLO does play a role in altering DA-containing neurons and should be re-evaluated in its wide-

spread use for LND patients. Further study at the DA receptor level is important in establishing ALLO's affects upon DA-containing neurons.

In summary these studies show that an alteration of extra-cellular purine concentrations within the brain of a rat affect DA homeostasis. This is the initial step in the definition of a new model of chemical lesioning using endogenous substances. Future study of this animal model may allow the better understanding of purine-dopamine interactions.

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X) APPENDIX

C) Additional Behavioural Studies

A.) PRELIMINARY EXPERIMENTS

A. i) HX Delivery By Micro-Injection Into The LCD

TABLE A1.

The mean number of ipsilateral rotations observed after APO challenge on day 14 and day 21 of injection treatment from animals that received one of the five injection treatments. The number of animals/group = 6, ± S.E.M. and * indicates significantly different from saline treated animals.

APO Challenge on Treatment Day	Saline (10ul/day)	AD (5mM) (10ul/day)	HX (5mM) 5ul/day	HX (5mM) 10ul/day	HX (5mM) 20ul/day
Day 14	5.8 ± 5.8	1.2 ± 0.8	29.5 * ± 10.2	47.7 * ± 16.9	86.7 * ± 22.4
Day 21	5.7 ± 3.2	3.3 ± 2.8	24.8 * ± 5.0	27.3 * ± 1.8	22.5 * ± 5.7

A. ii.) HX Packed Cannulae Into The LCD

a) Rotational data

TABLE A2.

The number of rotations observed after APO challenge on post-operative day 14 and day 21 from animals implanted with HX packed cannulae [n=4], animals treated with 6OHDA [n=5] and animals implanted with empty cannulae (Empty)[n=4] into the LCD. A positive rotation number indicates ipsilateral rotation, a negative number indicates contralateral rotation to the treated (left) side. The numbers are mean, ± S.E.M. and * indicates significantly different from CON.

Treatment Group	APO Induced Rotations on Post-Implantation Day DAY 14	DAY 21
Empty	+1.0 ± 0.4	+1.0 ± 0.3
6OHDA	−8.0 ± 0.8 *	−17.2 ± 0.9 *
HX	+20.8 ± 7.3 *	+19.5 ± 5.4 *

A. ii. b) Purine concentrations

TABLE A3.

The purine concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the LCD and RCD from animals implanted with empty cannulae (Empty) [n=4], animals treated with 6OHDA [n=5] and animals implanted with HX packed cannulae [n=4] into the LCD for 3 weeks. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Treatment Groups	LCD					RCD				
	UA	XAN	HX	INO	ADO	UA	XAN	HX	INO	ADO
Empty	147 ± 40	67 ± 22	92 ± 33	279 ± 103	15 ± 5	118 ± 9	58 ± 10	92 ± 16	244 ± 35	15 ± 3
6OHDA	103 ± 8	53 ± 4	72 ± 7	272 ± 34	54* ± 11	281* ± 42	99 ± 11	87 ± 13	440 ± 52	106 ± 28
HX	258 ± 80	110 ± 16	75 ± 10	234 ± 28	127* ± 18	325 ± 93	138* ± 24	68 ± 15	181 ± 8	138* ± 17

A. ii. c) Catecholamine concentrations

TABLE A4.

The concentration (ng/mg of tissue) of NA, DOPAC and DA in the LCD (treated) and RCD (control) from animals implanted with empty cannulae (Empty) [n=12], animals treated with 6OHDA [n=5] and animals implanted with HX packed cannulae [n=4] into the LCD for 3 weeks. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Treatment Groups	LCD (Treated)			RCD (Control)		
	NA	DOPAC	DA	NA	DOPAC	DA
Empty	1.28 ± 0.18	1.13 ± 0.08	3.80 ± 0.42	1.17 ± 0.18	1.19 ± 0.17	3.97 ± 0.52
6OHDA	1.17 ± 0.07	0.93 ± 0.11	3.89 ± 0.98	1.15 ± 0.04	1.18 ± 0.17	7.34 ± 1.05
HX	1.24 ± 0.02	0.58 ± 0.27	1.37 * ± 0.43	1.20 ± 0.01	2.93 ± 0.43	6.53 ± 1.34

B.) IMPLANTATION INTO THE LATERAL VENTRICLE

B. i.) HX Packed Cannulae

a) Rotational data

TABLE A5.

The number of rotations observed in response to APO challenges on post-implantation days 4, 7, 11, 14, 18 and day 21 from controls and animals implanted with HX packed cannulae into the left lateral ventricle. The number of animals challenged per group/day is shown in brackets beside each mean, ± S.E.M. and * indicates significantly different from control.

Group	Number of APO Induced Rotations on Post-Implantation Days					
	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21
Control	0.6 (5) ± 0.4	0.6 (5) ± 0.4	0.8 (5) ± 0.5	0.4 (16) ± 0.2	0.6 (5) ± 0.4	0.3 (15) ± 0.1
HX Treated	0.3 (10) ± 0.1	0.9 (22) ± 0.1	4.5 (10) ± 0.7	10.0 (45) ± 1.8	8.4 (10) ± 1.2	22.2 (32) ± 4.4

B. i. b) Purine concentrations

TABLE A6.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with HX packed cannulae (n=6) into the left lateral ventricle for 2 and 3 weeks. period. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Brain Regions and Purines Measured		Control (n=4)	HX Treatment Period 2 Weeks (n=6)	3 Weeks (n=6)
AMY	UA	180 ± 40	357 ± 105	383 ± 74
	XAN	76 ± 19	107 ± 32	125 ± 20
	HX	111 ± 40	119 ± 28	90 ± 11

TABLE A6 continued.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with HX packed cannulae (n=6) into the left lateral ventricle for 2 and 3 weeks. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Brain Regions and Purines Measured		Control (n=4)	HX Treatment Period 2 Weeks (n=6)	3 Weeks (n=6)
AMY	INO	218 ± 59	252 ± 52	298 ± 63
	ADO	6 ± 2	11 ± 4	44 * ± 12
LCD	UA	147 ± 40	322 ± 90	324 ± 84
	XAN	67 ± 22	140 ± 34	115 ± 26
	HX	92 ± 33	132 ± 17	80 ± 11
	INO	279 ± 103	411 ± 39	344 ± 54
	ADO	15 ± 5	53 ± 13	139 * ± 49
RCD	UA	118 ± 9	225 ± 47	281 ± 84
	XAN	58 ± 10	78 ± 6	87 ± 17
	HX	92 ± 16	87 ± 7	86 ± 19
	INO	244 ± 35	306 ± 22	354 ± 83
	ADO	15 ± 3	70 * ± 17	124 * ± 43

B. i. c) Catecholamine concentrations

TABLE A7.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with HX packed cannulae into the left lateral ventricle for 1, 2 or 3 weeks. The number of animals per sample is either indicated at the top of the column or beside each mean in brackets, ± S.E.M., - means not measured and * indicates significantly different from control.

Brain Region + Catecholamines		Control (n=12)	HX Treatment Period 1 Week (n=6)	2 Weeks	3 Weeks
AMY	DOPAC	0.17 ± 0.01	0.37 * ± 0.03	0.16 (6) ± 0.04	0.16 (21) ± 0.02
	DA	0.24 ± 0.03	0.32 ± 0.03	0.24 (6) ± 0.07	0.24 (21) ± 0.04
	HIAA	0.36 ± 0.06	0.14 * ± 0.03	0.21 (5) ± 0.08	0.28 (21)* ± 0.05
HYP	DOPAC	0.28 ± 0.03	0.40 ± 0.07	0.19 (6)* ± 0.02	0.33 (21) ± 0.03
	DA	0.25 ± 0.04	0.41 ± 0.10	0.22 (6) ± 0.04	0.31 (21) ± 0.04
	HIAA	0.60 ± 0.12	0.15 * ± 0.05	0.22 (6)* ± 0.04	0.46 (20) ± 0.06
CTX	DOPAC	0.12 ± 0.01	0.29 * ± 0.02	0.16 (6) ± 0.08	0.16 (19) ± 0.03
	DA	0.31 ± 0.04	0.29 ± 0.02	0.60 (6) ± 0.16	0.31 (19) ± 0.05
	HIAA	0.18 ± 0.02	0.07 * ± 0.01	0.11 (6) ± 0.05	0.14 (19) ± 0.03
LCD	NA	1.28 ± 0.18	1.00 ± 0.06	1.14 (6) ± 0.11	1.48 (21) ± 0.17
	DOPAC	1.13 ± 0.08	1.42 * ± 0.09	1.42 (6) ± 0.10	1.64 (21) ± 0.22

TABLE A7 continued.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with HX packed cannulae into the left lateral ventricle for 1, 2 or 3 weeks. The number of animals per sample is either indicated at the top of the column or beside each mean in brackets, ± S.E.M., - means not measured and * indicates significantly different from control.

Brain Region + Catecholamines		Control (n=12)	HX Treatment Period 1 Week (n=6)	2 Weeks	3 Weeks
LCD	DA	3.80 ± 0.42	4.06 ± 0.39	4.35 (6) ± 0.39	4.42 (21) ± 0.65
	HIAA	0.59 ± 0.04	0.23 * ± 0.03	-	0.48 (19) ± 0.04
RCD	NA	1.17 ± 0.18	1.22 ± 0.26	0.92 (6) ± 0.07	1.39 (21) ± 0.20
	DOPAC	1.19 ± 0.17	1.50 ± 0.12	1.55 (6) ± 0.20	1.62 (21)* ± 0.21
	DA	3.97 ± 0.52	4.89 ± 0.29	4.02 (6) ± 0.66	4.42 (21) ± 0.48
	HIAA	0.60 ± 0.06	0.22 * ± 0.03	-	0.48 (19) ± 0.06
NAC	NA	0.99 ± 0.11	0.80 ± 0.03	0.39 (5)* ± 0.20	0.99 (21) ± 0.13
	DOPAC	0.53 ± 0.06	0.80 ± 0.09	0.57 (5) ± 0.26	1.02 (21) ± 0.18
	DA	1.35 ± 0.24	1.39 ± 0.18	0.74 (5) ± 0.40	1.42 (21) ± 0.22
	HIAA	0.53 ± 0.04	0.16 * ± 0.01	-	0.40 (21) ± 0.05
BST	DOPAC	0.18 ± 0.04	0.24 * ± 0.01	0.07 (6) ± 0.02	0.13 (21) ± 0.02
	DA	0.42 ± 0.09	0.36 ± 0.14	0.26 (6) ± 0.06	0.32 (21) ± 0.09
	HIAA	0.44 ± 0.05	0.12 * ± 0.03	0.22 (6)* ± 0.04	0.26 (21)* ± 0.04

B. ii.) AD Packed Cannulae

a) Rotational data

TABLE A8.

The mean number of observed rotations for animals challenged with APO on post-implantation day 14 and day 21 for controls and animals implanted with AD packed cannulae into the left lateral ventricle. The number of animals observed are given in brackets beside the mean, ± S.E.M and * indicates significantly different from control.

Group	Number of APO Induced Rotations on Post-Implantation Day	
	DAY 14	DAY 21
Control	0.4 (16) ± 0.2	0.3 (15) ± 0.1
AD Treated	14.8 (6) * ± 1.5	21.8 (6) * ± 6.6

c) Catecholamine concentrations

TABLE A9.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls [CON] (n=12) and animals implanted with AD packed cannulae [AD] (n=6) into the left lateral ventricle for 3 weeks. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Brain Region + Catecholamines		CON (n=12)	AD (n=6)	Brain Region + Catecholamines		CON (n=12)	AD (n=6)
AMY	DOPAC	0.17 ± 0.01	0.08 * ± 0.01	HYP	DOPAC	0.28 ± 0.03	0.26 ± 0.04
	DA	0.24 ± 0.03	0.18 ± 0.09		DA	0.25 ± 0.04	0.16 ± 0.01
	HIAA	0.36 ± 0.06	0.35 ± 0.10		HIAA	0.60 ± 0.12	0.54 ± 0.07
CTX	DOPAC	0.12 ± 0.01	0.06 * ± 0.02	NAC	NA	0.99 ± 0.11	1.35 * ± 0.12

TABLE A9 continued.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls [CON] (n=12) and animals implanted with AD packed cannulae [AD] (n=6) into the left lateral ventricle for 3 weeks. The numbers are mean, ± S.E.M. and * indicates significantly different from control.

Brain Region + Catecholamines		CON (n=12)	AD (n=6)
CTX	DA	0.31 ± 0.04	0.24 ± 0.06
CTX	HIAA	0.18 ± 0.02	0.18 ± 0.06
LCD	NA	1.28 ± 0.18	1.41 ± 0.18
	DOPAC	1.13 ± 0.08	0.85 ± 0.22
	DA	3.80 ± 0.42	3.28 ± 1.45
	HIAA	0.59 ± 0.04	0.44 ± 0.12
BST	DOPAC	0.18 ± 0.04	0.04 * ± 0.00
	DA	0.42 ± 0.09	0.12 * ± 0.02
	HIAA	0.44 ± 0.05	0.31 ± 0.05
NAC	DOPAC	0.53 ± 0.06	0.44 ± 0.21
	DA	1.35 ± 0.24	0.75 ± 0.30
	HIAA	0.53 ± 0.04	0.46 ± 0.09
RCD	NA	1.17 ± 0.18	1.46 ± 0.13
	DOPAC	1.19 ± 0.17	1.01 ± 0.15
	DA	3.97 ± 0.52	3.87 ± 0.83
	HIAA	0.60 ± 0.06	0.42 ± 0.08

B. iii.) ALLO Alone Or ALLO Plus HX Packed Cannulae

a) Rotational data

TABLE A10.

The mean number of rotation observed in response to APO challenges on post-implantation day 4, 7, 11, 14, 18 and day 21 from controls and animals implanted with ALLO packed cannulae or ALLO plus HX packed cannulae. The number of animals observed/day is indicated in brackets, ± S.E.M. and * indicates significantly different from control.

Group	Number of APO Induced Rotations on Post-Implantation Days					
	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21
Control	0.6 (5) ± 0.4	0.6 (5) ± 0.4	0.8 (5) ± 0.5	0.4 (16) ± 0.2	0.6 (5) ± 0.4	0.3 (15) ± 0.1
ALLO	1.3 (12) ± 0.2	4.0 (21) * ± 1.0	7.9 (12) * ± 0.8	11.8 (18) * ± 2.2	22.3 (12) * ± 1.3	21 (16) * ± 3.3
ALLO Plus HX	2.5 (8) ± 1.0	5.8 (19) * ± 2.1	4.0 (8) * ± 1.4	11.5 (21) * ± 2.2	13.8 (8) * ± 2.8	8.4 (16) * ± 2.1

B. iii. b.) Purine concentrations:

i) ALLO packed cannulae

TABLE A11.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with ALLO packed cannulae into the left lateral ventricle for 1 (n=4), 2 (n=3) and 3 (n=6) weeks. The numbers are mean, ± S.E.M., * indicates significantly different from control and # indicates approaching significance ($p<0.10$).

Brain Regions and Purines Measured		Treatment Period			
		Control (n=4)	1 Week (n=4)	2 Weeks (n=3)	3 Weeks (n=6)
AMY	UA	180 ± 40	597 * ± 61	518 # ± 137	228 ± 60

TABLE A11 continued.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with ALLO packed cannulae into the left lateral ventricle for 1 (n=4), 2 (n=3) and 3 (n=6) weeks. The numbers are mean, ± S.E.M., * indicates significantly different from control and # indicates approaching significance ($p<0.10$).

Brain Regions and Purines Measured		Treatment Period			
		Control (n=4)	1 Week (n=4)	2 Weeks (n=3)	3 Weeks (n=6)
AMY	XAN	76 ± 19	243 * ± 28	113 ± 23	74 ± 15
	HX	111 ± 40	38 ± 9	83 ± 16	120 ± 39
	INO	218 ± 59	219 ± 46	249 ± 47	202 ± 38
	ADO	6 ± 2	40 * ± 5	21 # ± 5	23 ± 10
LCD	UA	147 ± 40	555 * ± 48	396 # ± 21	190 ± 44
	XAN	67 ± 22	213 * ± 18	78 ± 15	77 ± 19
	HX	92 ± 33	123 ± 10	79 ± 7	103 ± 33
	INO	279 ± 103	228 ± 32	277 ± 43	324 ± 59
	ADO	15 ± 5	82 * ± 16	96 # ± 44	44 ± 14
RCD	UA	118 ± 9	580 * ± 42	341 # ± 24	151 ± 26
	XAN	58 ± 10	208 * ± 18	66 ± 12	66 ± 17
	HX	92 ± 16	103 ± 22	70 ± 6	97 ± 14
	INO	244 ± 35	250 ± 3	268 ± 39	321 ± 65

TABLE A11 continued.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with ALLO packed cannulae into the left lateral ventricle for 1 (n=4), 2 (n=3) and 3 (n=6) weeks. The numbers are mean, ± S.E.M., * indicates significantly different from control and # indicates approaching significance ($p<0.10$).

Brain Regions and Purines Measured		Control (n=4)	Treatment Period 1 Week (n=4)	2 Weeks (n=3)	3 Weeks (n=6)
RCD	ADO	15 ± 3	147 * ± 14	102 # ± 2	46 ± 21

B. iii. b. ii) ALLO plus HX packed cannulae

TABLE A12.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with ALLO plus HX packed cannulae into the left lateral ventricle for 1, 2 and 3 weeks. The number of animals per sample is indicated at the top of each column or in brackets beside each mean, ± S.E.M. and * indicates significantly different from control.

Brain Regions and Purines Measured		Control (n=4)	Treatment Period 1 Week	2 Weeks (n=5)	3 Weeks
AMY	UA	180 ± 40	581 (5)* ± 85	293 ± 76	252 (10) ± 70
	XAN	76 ± 19	163 (5) ± 27	94 ± 23	79 (10) ± 13
	HX	111 ± 40	90 (5) ± 18	101 ± 30	95 (10) ± 15
	INO	218 ± 59	260 (5) ± 53	217 ± 48	223 (10) ± 33
	ADO	6 ± 2	39 (5) * ± 14	11 ± 4	17 (10) ± 5
LCD	UA	147 ± 40	525 (5)* ± 33	268 ± 57	167 (9) ± 22

TABLE A12 continued.

The concentration (ug/mg of tissue) of UA, XAN, HX, INO and ADO in the AMY, LCD and in the RCD from controls (n=4) and animals implanted with ALLO plus HX packed cannulae into the left lateral ventricle for 1, 2 and 3 weeks. The number of animals per sample is indicated at the top of each column or in brackets beside each mean, ± S.E.M. and * indicates significantly different from control.

Brain Regions and Purines Measured		Control (n=4)	Treatment Period 1 Week	2 Weeks (n=5)	3 Weeks
LCD	XAN	67 ± 22	147 (5)* ± 16	90 ± 9	66 (9) ± 10
	HX	92 ± 33	115 (5) ± 9	107 ± 7	84 (9) ± 15
	INO	279 ± 103	260 (5) ± 36	406 ± 37	337 (9) ± 50
	ADO	15 ± 5	74 (5) ± 17	77 ± 29	81 (9) * ± 28
RCD	UA	118 ± 9	561 (4)* ± 140	414 * ± 68	145 (10) ± 26
	XAN	58 ± 10	145 (4)* ± 28	119 * ± 15	52 (10) ± 9
	HX	92 ± 16	97 (4) ± 20	144 ± 30	65 (10) ± 13
	INO	244 ± 35	304 (4) ± 46	480 ± 58	224 (10) ± 49
	ADO	15 ± 3	90 (4) * ± 11	83 * ± 28	57 (10) * ± 13

B. iii. c.) **Catecholamine concentrations:**

i) **ALLO packed cannulae**

TABLE A13.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with ALLO packed cannulae into the left lateral ventricle for 1 (n=4), 2 (n=3) and 3 (n=6) weeks. The numbers are mean, ± S.E.M., - means not measured and * indicates significantly different from control.

Region + Catecholamines		Control (n=12)	Treatment Period 1 Week (n=4)	2 Weeks (n=3)	3 Weeks (n=7)
AMY	DOPAC	0.17 ± 0.01	0.11 ± 0.03	0.12 ± 0.02	0.18 ± 0.03
	DA	0.24 ± 0.03	0.08 * ± 0.02	0.18 ± 0.01	0.27 ± 0.06
	HIAA	0.36 ± 0.06	0.22 ± 0.03	0.36 ± 0.05	0.09 * ± 0.02
HYP	DOPAC	0.28 ± 0.03	0.18 * ± 0.02	0.21 ± 0.08	0.57 * ± 0.21
	DA	0.25 ± 0.04	0.30 ± 0.03	0.42 ± 0.20	1.28 ± 1.10
	HIAA	0.60 ± 0.12	0.40 ± 0.06	0.42 ± 0.21	0.23 * ± 0.06
CTX	DOPAC	0.12 ± 0.01	0.08 ± 0.03	0.20 ± 0.04	0.22 * ± 0.03
	DA	0.31 ± 0.04	1.14 ± 0.35	0.33 ± 0.16	0.24 ± 0.06
	HIAA	0.18 ± 0.02	0.06 * ± 0.02	0.20 ± 0.03	0.05 * ± 0.01
LCD	NA	1.28 ± 0.18	1.73 ± 0.32	1.13 ± 0.09	2.11 * ± 0.31
	DOPAC	1.13 ± 0.08	1.33 ± 0.23	1.38 ± 0.17	1.38 ± 0.08

TABLE A13 continued.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with ALLO packed cannulae into the left lateral ventricle for 1 (n=4), 2 (n=3) and 3 (n=6) weeks. The numbers are mean, ± S.E.M., - means not measured and * indicates significantly different from control.

Region + Catecholamines		Control (n=12)	Treatment Period 1 Week (n=4)	2 Weeks (n=3)	3 Weeks (n=7)
LCD	DA	3.80 ± 0.42	4.07 ± 0.48	5.11 ± 0.47	3.19 ± 1.09
	HIAA	0.59 ± 0.04	-	0.35 * ± 0.09	0.31 * ± 0.03
RCD	NA	1.17 ± 0.18	1.58 ± 0.31	1.12 ± 0.11	2.10 * ± 0.31
	DOPAC	1.19 ± 0.17	1.08 ± 0.14	1.13 ± 0.21	1.55 ± 0.19
	DA	3.97 ± 0.52	4.20 ± 0.81	4.46 ± 1.14	3.95 ± 1.32
	HIAA	0.60 ± 0.06	-	0.35 ± 0.08	0.32 * ± 0.04
NAC	NA	0.99 ± 0.11	1.32 ± 0.39	0.89 ± 0.05	1.47 * ± 0.14
	DOPAC	0.53 ± 0.06	0.37 ± 0.02	0.35 ± 0.05	0.56 ± 0.14
	DA	1.35 ± 0.24	0.82 ± 0.23	1.07 ± 0.12	1.00 ± 0.49
	HIAA	0.53 ± 0.04	-	0.20 * ± 0.10	0.32 * ± 0.03
BST	DOPAC	0.18 ± 0.04	0.09 ± 0.02	0.05 * ± 0.01	0.20 ± 0.02
	DA	0.42 ± 0.09	0.77 ± 0.19	0.35 ± 0.20	0.33 ± 0.06
	HIAA	0.44 ± 0.05	0.23 * ± 0.02	0.35 ± 0.01	0.14 * ± 0.04

B. iii. c. ii) ALLO Plus HX Packed Cannulae

TABLE A14.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with ALLO and HX packed cannulae into the left lateral ventricle for 1, 2 and 3 weeks. The number of animals per sample is indicated at the top of each column or in brackets beside each mean, ± S.E.M., - means not measured and * indicates significantly different from control.

Brain Region + Catecholamines		Control (n=12)	Treatment Period 1 Week (n=5)	2 Weeks	3 Weeks
AMY	DOPAC	0.17 ± 0.01	0.13 ± 0.02	0.24 (5) * ± 0.02	0.26 (10)* ± 0.03
	DA	0.24 ± 0.03	0.65 ± 0.42	0.30 (5) ± 0.05	0.26 (10) ± 0.07
	HIAA	0.36 ± 0.06	0.24 ± 0.02	0.30 (5) ± 0.05	0.13 (8) * ± 0.04
HYP	DOPAC	0.28 ± 0.03	0.20 ± 0.02	0.33 (5) ± 0.04	0.40 (10)* ± 0.04
	DA	0.25 ± 0.04	0.36 ± 0.05	0.25 (5) ± 0.05	0.28 (10) ± 0.06
	HIAA	0.60 ± 0.12	0.37 ± 0.04	0.42 (5) ± 0.07	0.23 (9) * ± 0.05
CTX	DOPAC	0.12 ± 0.01	0.11 ± 0.02	0.18 (5) ± 0.04	0.23 (10)* ± 0.03
	DA	0.31 ± 0.04	1.59 * ± 0.26	0.41 (5) ± 0.10	0.37 (10) ± 0.07
	HIAA	0.18 ± 0.02	0.10 ± 0.02	0.17 (5) ± 0.06	0.07 (10)* ± 0.01
LCD	NA	1.28 ± 0.18	1.45 ± 0.17	1.15 (5) ± 0.07	2.01 (9) * ± 0.23
	DOPAC	1.13 ± 0.08	1.38 ± 0.21	1.60 (5) * ± 0.16	1.55 (9) * ± 0.11
	DA	3.80 ± 0.42	5.66 ± 1.26	4.63 (5) ± 0.84	4.92 (9) ± 1.22

TABLE A14.

The concentration (ng/mg of tissue) of NA, DOPAC, DA and HIAA in the AMY, HYP, CTX, LCD, RCD, NAC and in the BST from controls (n=12) and animals implanted with ALLO and HX packed cannulae into the left lateral ventricle for 1, 2 and 3 weeks. The number of animals per sample is indicated at the top of each column or in brackets beside each mean, ± S.E.M., - means not measured and * indicates significantly different from control.

Brain Region + Catecholamines		Control (n=12)	Treatment Period 1 Week (n=5)	2 Weeks	3 Weeks
LCD	HIAA	0.59 ± 0.04	-	0.23 (3) * ± 0.07	0.36 (7) * ± 0.05
RCD	NA	1.17 ± 0.18	1.55 ± 0.14	1.23 (5) ± 0.10	1.97 (10)* ± 0.20
	DOPAC	1.19 ± 0.17	1.90 * ± 0.37	1.52 (5) ± 0.18	1.45 (10) ± 0.13
	DA	3.97 ± 0.52	6.90 * ± 0.53	5.14 (5) ± 0.72	5.08 (10) ± 1.12
	HIAA	0.60 ± 0.06	-	0.22 (3) * ± 0.06	0.38 (8) ± 0.07
NAC	NA	0.99 ± 0.11	1.24 ± 0.21	0.57 (5) * ± 0.12	1.49 (10)* ± 0.08
	DOPAC	0.53 ± 0.06	0.62 ± 0.11	0.57 (5) ± 0.11	0.50 (10) ± 0.05
	DA	1.35 ± 0.24	2.26 ± 0.48	1.09 (5) ± 0.28	1.41 (10) ± 0.56
	HIAA	0.53 ± 0.04	-	0.05 (4) * ± 0.01	0.33 (9) * ± 0.05
BST	DOPAC	0.18 ± 0.04	0.08 ± 0.02	0.09 (5) ± 0.03	0.19 (10) ± 0.02
	DA	0.42 ± 0.09	0.85 ± 0.29	0.20 (5) ± 0.09	0.31 (10) ± 0.09
	HIAA	0.44 ± 0.05	0.24 * ± 0.04	0.29 (5) ± 0.06	0.21 (9) * ± 0.03

C) ADDITIONAL BEHAVIOURAL STUDIES

C. i) HALO Pretreatment

TABLE A15.

The mean number of observed rotations after challenge with APO alone (Control) or pretreatment with HALO for the 3 treatment groups (number of animals/group = 5), ± S.E.M. and * indicates significantly different from control.

APO Dose (mg/kg body weight)	Number of Rotations					
	HX Treated		ALLO Treated		HX + ALLO Treated	
	APO alone	APO+ HALO	APO alone	APO+ HALO	APO alone	APO+ HALO
5	11.2 ± 4.3	0.0 ± 0.0	4.6 ± 0.8	0.0 ± 0.0	7.6 ± 1.2	0.0 ± 0.0
10	43.6 ± 10.8	5.0 ± 1.8	32.8 ± 5.7	13.6 ± 3.9	18.2 ± 4.3	2.8 ± 1.5
15	48.6 ± 6.7	10.8 ± 3.4	30.2 ± 5.8	16.4 ± 2.9	22.2 ± 3.6	9.8 ± 2.0

C. ii) D1 Antagonist Pretreatment

TABLE A16.

The mean number of rotations observed after challenge with APO alone (Control) or with a pretreatment of SCH plus APO for animals treated with HX packed cannulae (n=11), ± S.E.M. and * indicates significantly different from control.

APO Dose (mg/kg body weight)	HX Treated	
	APO alone	APO+SCH
5	10.0 ± 1.0	0.0 ± 0.0
10	30.0 ± 3.0	1.0 ± 1.0
15	32.0 ± 3.0	1.0 ± 0.33

C. iii) Activity Level of Other APO-Induced Behaviours

TABLE A17.

Activity level of animals challenged with either APO alone [5, 10 or 15 mg/kg body weight] (n=16) or APO plus pretreatment with a DA antagonist (HALO [n=5] or SCH [n=11]) for animals implanted with HX packed cannulae. Activity levels are mean, ± S.E.M., and * indicates significantly different from APO alone.

APO Dose	Motor/Exploratory Behaviour			Sniffing Behaviour			Gnawing/Licking Behaviour		
	APO alone	APO + HALO	APO + SCH	APO alone	APO + HALO	APO + SCH	APO alone	APO + HALO	APO + SCH
5	3.0 ±0.1	1.2 ± 0.4	2.5 ± 0.2	3.6 ± 0.2	0.0 ± 0.0	3.3 ± 0.1	3.2 ± 0.2	0.0 ± 0.0	3.2 ± 0.1
10	3.2 ± 0.2	0.4 ± 0.2	3.2 ± 0.1	4.0 ± 0.1	0.2 ± 0.2	3.8 ± 0.1	3.7 ± 0.2	0.2 ± 0.2	3.2 ± 0.1
15	3.4 ± 0.2	0.4 ± 0.2	3.3 ± 0.2	4.4 ± 0.2	0.8 ± 0.2	4.2 ± 0.1	4.0 ± 0.1	0.4 ± 0.2	3.7 ± 0.1