IN VIVO NEUROCHEMICAL EFFECTS OF ANTIDEPRESSANT TREATMENTS STUDIED BY MICRODIALYSIS

by

GEORGE GOULIELMOS NOMIKOS

M.D., The University of Athens, 1986

Doctorate, The University of Athens, 1989

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Division of Neurological Sciences

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

November 1990

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Department of Psychiatry

The University of British Columbia
Vancouver, Canada

Date 22-03-91

Abstract

The present experiments investigated the effects of different antidepressant treatments on dopamine (DA) transmission by employing *in vivo* microdialysis in the nucleus accumbens (NAC) and the striatum of freely moving rats. The treatments were: a) the tricyclic antidepressant desipramine (DMI), b) the novel antidepressant drug bupropion, and c) electrically induced seizures (ECS). The following results were obtained:

1) Neither acute (5 mg/kg), nor chronic (5 mg/kg, b.i.d. X 21) DMI influenced basal interstitial concentrations of DA in the NAC or the striatum. Chronic DMI did not influence apomorphine (25 μg/kg, s.c.)-induced decreases in extracellular DA in the NAC. In contrast, d-amphetamine (1.5 mg/kg, s.c.)-induced increases in extracellular DA were significantly enhanced in the NAC (not in striatum) of the chronic DMI group. d-Amphetamine-induced hypermotility was also enhanced in the chronic DMI group.

2) Bupropion (10, 25 and 100 mg/kg, i.p.) increased extracellular striatal DA concentrations in a dose-, time-, and action potential-dependent manner. Bupropion produced similar responses in the NAC. The *in vivo* neurochemical effects of bupropion were compared with the effects of other DA uptake inhibitors such as d-amphetamine, GBR 12909, cocaine, nomifensine, methylphenidate, and benztropine by direct administration of the drugs to the striatum via the perfusion fluid in increasing concentrations (1 to 1000 μM). The rank order of potency of these drugs as determined by the increases in extracellular DA produced by 10 or 100 μM (following correction for dialysis efficiency of the test compounds *in vitro*) was: GBR 12909> benztropine> amphetamine= nomifensine= methylphenidate> cocaine> bupropion. Simultaneous *in vivo* microdialysis in the NAC and striatum was employed to investigate the effects of chronic (10 mg/kg, b.i.d. X 21) bupropion treatment on bupropion (25 mg/kg, i.p.)-induced increases in extracellular DA concentrations. The effect of the challenge bupropion injection was significantly enhanced in the NAC (not in striatum) of

the chronic bupropion group. Bupropion-induced hyperlocomotion was also enhanced in the chronic bupropion group.

3) Following a single ECS (150 V, 0.75 sec) interstitial concentrations of DA in the NAC and striatum increased sharply to 130% and 300%, respectively. The ECS-induced DA increase in the striatum was Ca^{++}-sensitive, partially TTX-independent, and was not influenced by barbiturate-induced anaesthesia. Seizure activity induced by flurothyl did not influence dialysate DA concentrations from the striatum, but increased dialysate DA from the NAC to 150%. These results suggest that the ECS-induced DA release in the striatum (not in the NAC) is related to the passage of current and not to the seizure activity. A course of ECS (8 treatments, one every second day) did not influence basal extracellular DA concentrations in the striatum or the NAC, while it significantly increased the DA metabolites in the striatum. Chronic ECS did not influence apomorphine (25 μg/kg, s.c.)-induced decreases in extracellular DA in the NAC. d-Amphetamine (1.5 mg/kg s.c.)-induced increases in extracellular DA were significantly enhanced in the NAC of the chronic ECS group. d-Amphetamine-induced hypermotility was also enhanced in the chronic ECS group.

These results provide *in vivo* neurochemical confirmation that chronically administered DMI or ECS do not produce DA autoreceptor subsensitivity. They also demonstrate that chronic DMI- or chronic ECS-induced increases in the locomotor stimulant effects of d-amphetamine are accompanied by a potentiation of its effects on interstitial DA concentrations in the NAC. Moreover, these results demonstrate that chronic bupropion-induced behavioral sensitization is accompanied by a selective potentiation of its effects on interstitial DA concentrations in the NAC. Taken together, the present data provide direct neurochemical evidence that these antidepressant treatments can increase the functional output of the meso-accumbens dopaminergic system.

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Acknowledgements

The author wishes to thank Danielle Wenkstern, Sandra Sturgeon, Chui-Se Tham, Catriona Wilson, Jamie Day, Erin Brown, and Drs. Geert Damsma, Thanasis Zis, and Chris Fibiger, for their εκ των ων ουκ ανευ contribution to this thesis.

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I. Introduction

(A) Dopamine, depression and antidepressants

Although the original catecholamine hypothesis of depression focussed primarily on noradrenaline (Schildkraut 1965; Bunney and Davis 1965), recent neurochemical and pharmacological observations have implicated dopamine (DA) in the pathophysiology of some forms of depression (Randrup et al. 1975; Willner 1983a,b; Fibiger 1984). The basic premise of the DA hypothesis of depression is that decreased dopaminergic activity is involved in the pathogenesis of some characteristic symptoms of this syndrome, and derives from the following lines of evidence:

(1) Among the core symptoms of major depression are psychomotor retardation (delayed and slowed motor and speech responsiveness), anhedonia (inability to experience pleasure and lack of affective reactivity to positive stimuli), and disinterest (loss of interest in engaging in pleasurable events) (American Psychiatric Association 1987). Infrahuman studies have provided evidence that the mesotelencephalic DA systems are involved in the control of motor function and in the mediation of reward and/or incentive motivation processes (Fibiger 1978; Wise 1978; Mogenson et al. 1980; Fibiger and Phillips 1986; Mogenson 1987).

(2) Some clinical studies have shown that DA turnover is decreased in depressed patients with psychomotor retardation by measuring the concentrations of the DA metabolite homovanillic acid (HVA) in cerebrospinal fluid (CSF) following probenecid administration (Goodwin et al. 1973; Post et al. 1980; Willner 1985).

(3) There is a higher incidence of depression in Parkinson's disease than in other chronic debilitating disorders (Fibiger 1984). Degeneration of the forebrain dopaminergic projections is responsible for the motor deficit in this disease and may also account for the mood impairment (Price et al. 1979).

(4) The clinical effects of DA receptor agonists are consistent with the hypothesis. Therapeutic responses to DA agonists like L-DOPA, piribedil, bromocriptine and amineptine have been reported (Jimerson and Post 1984; Jimerson 1987; Berwich and Amsterdam 1989). Moreover, the DA uptake inhibitors nomifensine and bupropion have been proven effective in the treatment of endogenous depression in large clinical trials (Rudorfer et al. 1984; Blackwell 1987). Treatment with low doses of neuroleptics, which are thought to increase DA neurotransmission, has also been reported to produce beneficial effects in some forms of depression (Randrup et al. 1975; Nelson 1987). Similarly, low doses of sulpiride have been reported to be effective in depression (Jenner and Marsden 1982; del Zombo et al. 1990).

(5) Preclinical studies have indicated that chronic antidepressant treatments enhance central DA function. Behavioral, biochemical and electrophysiological data have suggested two mechanisms that potentially contribute to this effect, these being desensitization of inhibitory DA autoreceptors and sensitization of postsynaptic responses (Willner 1983c, 1985, 1989).

(6) In some animal models of depression (behavioral despair, chronic mild stress) the therapeutic action of antidepressants appears to be mediated by an increase in DA receptor responsiveness (Borsini and Melli 1990; Willner et al. 1990).

(i) Anatomy and function of mesotelencephalic dopamine systems

Dopamine containing neurons in the midbrain innervate a variety of forebrain structures (Dahlström and Fuxe 1964; Lindvall and Björklund 1974). The mesotelencephalic DA system is mainly composed of two major anatomically and functionally distinct subdivisions: 1) a dorsal mesostriatal projection from the substantia nigra to the dorsolateral and caudal striatum; and 2) a ventral mesostriatal (mesolimbic or meso-accumbens) projection from the ventral tegmental area (VTA) to the ventromedial striatum, nucleus accumbens (NAC), and olfactory tubercle (Heimer and Wilson 1975; Ungerstedt 1971;

Björklund and Lindvall 1984). It is commonly thought that the dorsal striatum, which also receives input from the sensorimotor cortex, is particularly important for sensorimotor processes (Mogenson et al. 1980; Mogenson 1987). The ventral striatum-nucleus accumbens, which is interconnected with a variety of limbic structures, is thought to be involved in initiating movements in response to emotionally or motivationally powerful stimuli (Mogenson et al. 1980; Heimer et al. 1982), and in mediating reward functions as well as other aspects of motivated behavior (Fibiger and Phillips 1986; Wise 1989). Specifically, the mesolimbic DA system seems to play a fundamental role in the rewarding effects of brain stimulation and psychomotor stimulants (Fibiger and Phillips 1986; Wise and Rompre 1989). An involvement of meso-accumbens DA system in the rewarding or hedonic effects of natural stimuli such as food and sex has also been proposed (Wise et al. 1978; Fibiger and Phillips 1986; Wise and Rompre 1989). Recently, data obtained with *in vivo* procedures (microdialysis and electrochemistry) underline the selective activation of the meso-accumbens DA neurons in response to food in both food deprived (Radhakishun et al. 1988; Damsma et al. 1989; Holmes 1990) and non-deprived rats (Nomikos et al. in preparation). Selective mesolimbic activation has also been reported during copulation in male rats (Pfaus et al. 1990). The exact nature of the dopaminergic participation in incentive motivation or reward is not clear. However, based on data obtained from behavioral studies and considering the central placement of the NAC between the limbic and motor systems, it appears that DA neurons may not have a specific behavioral role; rather these neurons appear to facilitate or initiate a variety of processes and may be an integral part of an amplification system of emotional states (Robbins and Koob 1980; Fibiger and Phillips 1986; Louillot et al. 1987). Dysfunction of the meso-accumbens DA system could, therefore, be responsible for the retardation of cognitive, emotional, and pychomotor processes that is characteristic of clinical depression. These considerations raise the possibility that some antidepressant treatments may alleviate depression by enhancing the function of this system. The effects of antidepressant treatments on mesolimbic DA function have been assessed by

behavioral (locomotor activity, intracranial self-stimulation - ICSS), biochemical (measurements of tissue concentrations of DA and its metabolites), and neurophysiological (extracellular recordings of neurons) methods. Although these approaches have provided valuable information, an integrated analysis of the functional status of the mesolimbic DA system following chronic antidepressant treatments is imperative. *In vivo* microdialysis is ideally suited to address the question as to whether chronic antidepressant treatments influence the basal and evoked release of DA from the meso-accumbens and mesostriatal projections in freely behaving animals. The effects of three pharmacologically distinct antidepressant treatments were examined in the present thesis: 1) the prototypical tricyclic antidepressant drug desmethylimipramine (desipramine-DMI); 2) the atypical antidepressant drug bupropion; and 3) electrically induced seizures (electroconvulsive shock-ECS). A common feature of the antidepressant treatments that were studied in this thesis is that they are effective in depressions characterized by psychomotor retardation (Bielski and Friedel 1976; Nelson and Charney 1981; Willner 1985).

(B) In vivo microdialysis

Brain microdialysis is a sampling technique that has given a new dimension to neurochemical research (Ungerstedt 1984; Imperato and Di Chiara 1984; Westerink et al. 1987a; Benveniste 1989). The method permits direct *in vivo* monitoring of neurotransmitters and their metabolites in the interstitial space of discrete brain regions of awake freely moving animals. In brain microdialysis a fine, hollow dialysis fibre (dialysis tube) is stereotaxically implanted into the brain region of interest. At varying postimplantation intervals a physiological solution is pumped through the membrane at a slow rate. Low molecular weight compounds in the extracellular fluid migrate along their concentration gradient into the fibre. The dialysate then is collected and analysed; in the present

experiments the constituent elements were separated by HPLC and quantified by electrochemical detection.

Brain microdialysis has rapidly become a routine research method with a wide range of applications. It has been used as a method to study the *in vivo* release of DA (Zetterström et al. 1983; Imperato and Di Chiara 1984), acetylcholine (Damsma et al. 1987), serotonin (Kalèn et al. 1988) and noradrenaline (L'Heureux et al. 1986). Certain criteria have been established to evaluate the neuronal origin of the sampled neurotransmitter. These include the application of the neurotoxin tetrodotoxin (TTX) directly through the perfusate (Westerink et al. 1987c), omission of Ca^{++} ions from the perfusion solution (Westerink et al. 1989), local infusion of high concentrations of K^{+} (Imperato and Di Chiara 1984), electrical stimulation (Imperato and Di Chiara 1984; L'Heureux et al. 1986), and specific pharmacological manipulations (recent reviews by Di Chiara 1990a,b). By applying some of the above criteria it has become apparent that in acute microdialysis experiments where analyses are conducted 12-18 h postsurgery a portion of the dialysate concentrations of the neurotransmitters is not directly derived from neuronal activity (Westerink and De Vries 1989; Benveniste 1989). This is not the case at later intervals (24-72 h). The postimplantation interval is therefore, of considerable importance.

In the present experiments the analysis of the dialysate was conducted on-line; this methodology employs a direct connection between the outflow of the dialysis tube and the analytical system (Johnson and Justice 1983; Wages et al. 1986; Westerink and Tuinte 1987; Damsma et al. 1987). This connection reduces the time-lag between sample collection and analysis, improves analytical reproducibility, and removes the necessity of adding preservatives to the samples; most importantly, the on-line analysis allows simultaneous determination of the behavior of the freely moving animal during brain microdialysis.

II. In vivo neurochemical effects of desipramine

(A) Introduction

Many early studies provided substantial *in vivo* and *in vitro* evidence that the tricyclic antidepressants (TCAs) are less potent inhibitors of DA than of noradrenaline (NA) or serotonin uptake (Carlsson et al. 1966; Glowinski et al. 1966; Hamberger 1967; Ross and Renyi 1967; Fuxe and Ungerstedt 1968; Horn et al. 1971). In subsequent studies, however, several groups of workers placed a greater emphasis on the possible clinical importance of the ability of these drugs to inhibit DA uptake (Halaris et al. 1975; Friedman et al. 1977; Randrup and Braestrup 1977). The basic argument was that although TCAs are very weak DA uptake inhibitors when administered acutely, it is possible that the necessary concentrations may be reached in the CNS following chronic regimens. In addition, it was deemed possible that biochemical adaptations of DA neurons or of postsynaptic neuronal elements may occur that potentially contribute to the therapeutic response. Several preclinical studies have attempted to address this hypothesis.

When administered chronically, some antidepressant treatments appear to increase the functional output of central DA systems. For example, in animal models of depression (behavioral despair, learned helplessness, chronic mild stress) the efficacy of antidepressants seems to involve an increase in transmission through DA synapses, particularly in the mesolimbic system (Borsini et al. 1984, 1985, 1988; Willner 1985; Delini-Stula et al. 1988; Muscat et al. 1990). Fibiger and Phillips (1981) have shown that chronic administration of DMI, a prototypical tricyclic antidepressant, enhances ICSS obtained from electrodes in the VTA, the origin of the DA neurons that project to the NAC. Several neurophysiological studies have also indicated that there are increases in the number of spontaneously active dopaminergic neurons in the VTA and the substantia

nigra, zona compacta after chronic treatment with DMI (Chiodo and Bunney 1983; White and Wang 1983). However, *ex vivo* studies on the synthesis and turnover of DA after TCAs indicate that these variables are unaffected by chronic antidepressant treatments (Neff and Costa 1967; Leonard and Kafoe 1976; Sugrue 1980).

Two mechanisms have been proposed to account for antidepressant-induced increases in the functional activity of the mesolimbic dopaminergic system: 1) desensitization of presynaptic inhibitory autoreceptors, and 2) sensitization of postsynaptic responses. Behavioral, electrophysiological and neurochemical studies on the effects of antidepressants on DA autoreceptors have generated conflicting results. Serra et al. (1979) first reported that the sedative effect of low doses of apomorphine was reduced by chronic treatment with the TCAs imipramine and amitriptyline and the atypical antidepressant mianserin. This result was not confirmed by Spyraki and Fibiger (1981) who studied the effects of imipramine and desipramine. Neurophysiological and biochemical studies have also produced conflicting results with respect to the DA autoreceptor subsensitivity hypothesis (Chiodo and Antelman 1980a; MacNiell and Gower 1982; Welch et al. 1982; Holcomb et al. 1982; reviews by Willner 1983c, 1985).

Possible changes in postsynaptic receptor mechanisms after chronic antidepressants have been studied behaviorally using directly (apomorphine) or indirectly (d-amphetamine, nomifensine) acting DA agonists. The locomotor stimulant and stereotypy effects of these compounds are thought to be mediated by the meso-accumbens and mesostriatal DA systems, respectively (Kelly and Iversen 1976; Moore and Kelly 1978). Chronic treatment with several antidepressants (DMI, imipramine, mianserin, iprindole) enhances the locomotor stimulant effects of apomorphine, d-amphetamine and nomifensine (Serra et al. 1979; Spyraki and Fibiger 1981; Willner and Montgomery 1981; Martin-Iverson et al. 1983; Maj et al. 1984; Maj 1986). Such treatments also enhance locomotor activity elicited by direct injections of DA or d-amphetamine into the NAC (Maj 1986). Stimulant-induced stereotyped behavior is

either enhanced (Willner and Montgomery 1981; Willner et al. 1984) or not influenced by chronically administered TCAs (Delini-Stula and Vassout 1979; Maj et al. 1979; Spyraki and Fibiger 1981).

The *in vivo* microdialysis procedure permits the dynamics of neurotransmitters to be monitored by repeated sampling of the interstitial fluid in discrete brain regions of conscious animals. Microdialysis has been used to assess the functional activity of dopaminergic neurons after acute and chronic treatment with psychostimulants such as amphetamine, cocaine and methamphetamine (Robinson et al. 1988; Kazahaya et al. 1989; Akimoto et al. 1989; Pettit et al. 1990) or with neuroleptics such as haloperidol (Hernandez and Hoebel 1989; Zhang et al. 1989). The purposes of the present experiments were: (1) to determine whether acute or chronic treatment with DMI affects DA transmission by monitoring the basal interstitial (Note 1) concentrations of DA and metabolites in the NAC and striatum; (2) to assess *in vivo* the neurochemical effects of a DMI challenge on DA efflux in the NAC of chronic DMI or saline pretreated animals; (3) to test the hypothesis that chronic administration of DMI results in DA autoreceptor subsensitivity, by examining the effect of a low dose of apomorphine on interstitial concentrations of DA in NAC; and (4) to determine the effect of chronic DMI treatment on amphetamine-induced increases in DA release in NAC and striatum; simultaneous measurement of locomotor activity permitted correlations to be established between the amphetamine-evoked biochemical and behavioral responses.

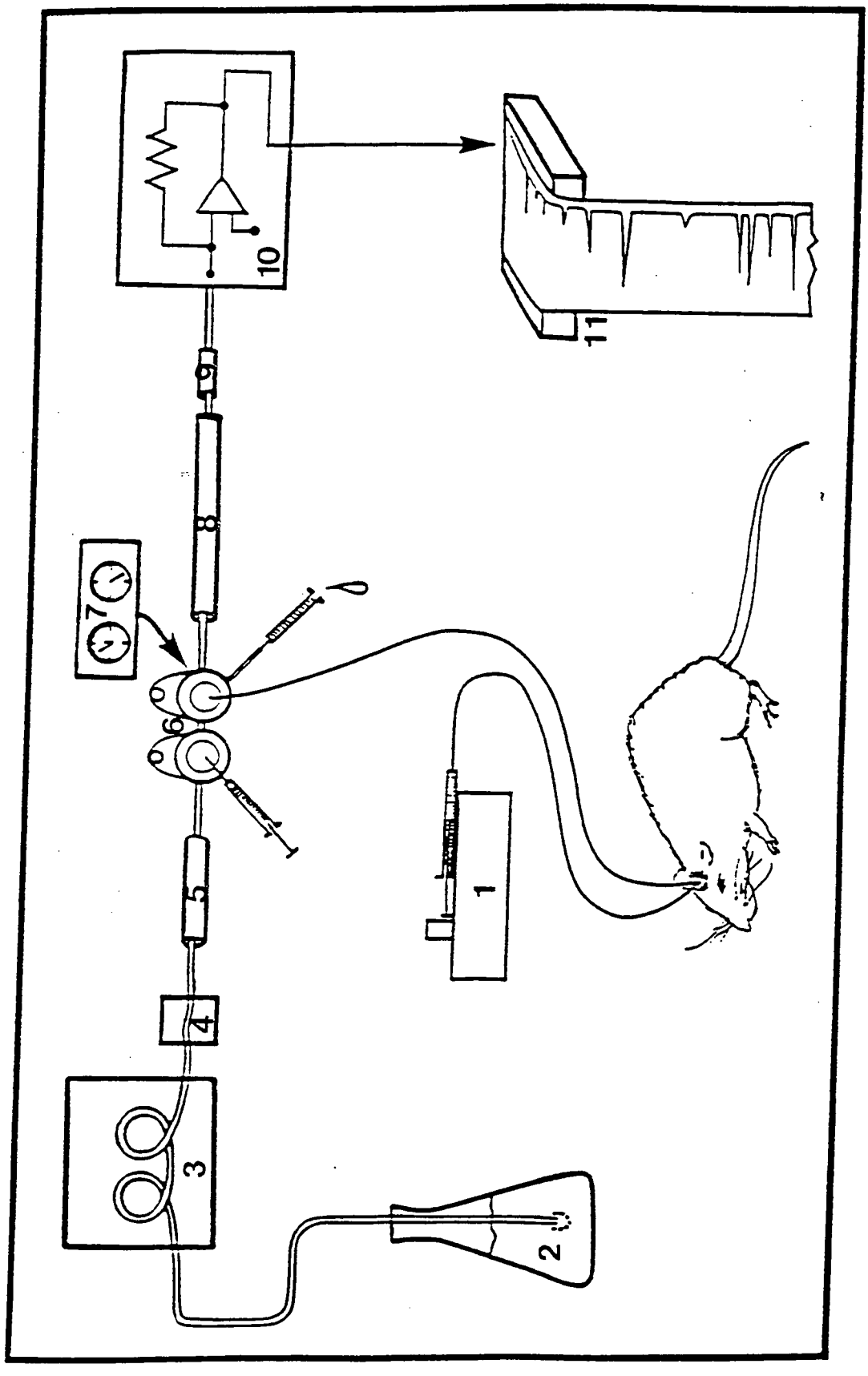
(B) Materials and methods

(i) Acute effects of desipramine

The subjects used in the present studies were male Wistar rats weighing 250-325 g (unless otherwise indicated) on the day of surgery. The rats were housed individually in a 12 h light cycle (lights on at 07:00 h); and temperature (23^{o}C) controlled room with food and water available *ad libitum*. Desipramine hydrochloride (Sigma), dissolved in saline, was injected at dose 5 mg/kg/2ml i.p.

Microdialysis and subsequent chemical analysis were performed essentially as described elsewhere (Imperato and Di Chiara 1984; Westerink and Tuinte 1986; Damsma et al. 1989, 1990) using a fully automated on-line sample injection system (Fig. 1). In brief, rats were anaesthetized (sodium pentobarbital, 50-70 mg/kg, i.p.), mounted in a stereotaxic frame (Kopf) and implanted with a horizontal probe (Note 2) through striatum (V:-4.75, A:+0.7) or nucleus accumbens (V:-7.5, A:+1.7) according to the brain atlas of Paxinos and Watson (1986). The dialysis device consisted of a semipermeable hollow fibre (saponified cellulose ester, 10,000 Daltons, Cordis Dow Medical), a stainless steel cannula glued (epoxy) to the inlet, and a tungsten wire (O.D.=0.15 mm) inserted through the hollow fibre for support during the implantation. The tungsten wire protruded 2 mm beyond the outlet of the dialysis fiber (attached with epoxy glue). The steel cannula was held in the micromanipulator of the stereotaxic instrument in a lateral direction and the fibre was slowly inserted in the brain through holes in the temporal bones. The tip of the tungsten wire and a small part of the fiber glued at the tip were then clipped and the wire was gently removed. The free end of the dialysis tube was glued (epoxy) to a second stainless steel cannula. Both steel cannulae were fixed to the parietal bones with acrylic dental cement anchored by skull screws; the ends were closed with removable plastic (tygon) cups. The outer diameter of the fibre increases from 0.18 mm in dry condition (during implantation) to 0.27 mm in wet condition (during perfusion). The dialysis fiber was covered with silicone glue, except for the two areas measured to correspond to dorsal striatum (2x3.5 mm), or the nucleus accumbens (2x2 mm). The recoveries of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic

Figure 1. Configuration of microdialysis perfusion and automated on-line analysis. 1: microperfusion pump; 2: mobile-phase reservoir; 3: HPLC pump; 4: pulse-dampener; 5: precolumn; 6: sample injector; 7: valve sequence programmer (timer); 8: analytical column; 9: enzyme reactor (for acetylcholine analysis); 10: electrochemical detector; 11: chart-recorder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acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured *in vitro* (Ungerstedt 1984). For this purpose a probe was placed in a beaker containing DA and the acid metabolites (1 μM) dissolved in the perfusion solution at 37^{o} C. The probe was then perfused at the standard flow rate of 5 μl/min, as in the in *vivo* experiments. Recovery was calculated as the ratio between the concentration of recovered substance in the dialysate compared with the beaker concentration and expressed as percentages. Recoveries (mean±S.E.M., n=5) obtained *in vitro* for a 2x3.5 mm membrane in this manner were 15.3±1.5 (DA), 15.1±1.4 (DOPAC), 13.8±1.4 (HVA), 13.4±1.4 (5-HIAA).

After surgery, rats were housed individually in Plexiglas cages (35x35x40 cm) where they remained throughout the experimental period and had free access to food and water. All perfusion experiments were carried out 18 to 48 h (Note 3) after surgery in conscious animals during the light phase of the daily cycle. The steel cannulae (after removal of the plastic cups) were connected by means of polyethylene tubing (I.D.=0.28 mm, length 80 cm) to a microperfusion pump (Carnegie Medicin or Harvard Apparatus) and to a 100 μl sample loop of the electrically actuated injector (sample valve, Valco C10W). The dialysis tube was perfused with a Ringer's solution (147mM NaCl, 4 mM KCl and 2.1 mM $CaCl_2$, pH 6-6.2, Note 4) at 5 μl/min (this flow rate was used in all the perfusion experiments of the present studies). The dialysate (Note 5) was loaded directly into the sample valve; the injector was held in the load position for 20 min and switched to the inject position for 10 sec after which the cycle was repeated. The alternate modes of the sample valve were controlled by an adjustable timer (Valco).

DA, DOPAC, HVA, and 5-HIAA were quantified by HPLC in conjunction with electrochemical detection (Westerink and Mulder 1981; Damsma et al. 1989, 1990). The mobile phase was delivered by a pump (LKB 2150 or BIO-RAD 1350) at 1.5 ml/min. A pulse-dampener (SSI) and a precolumn (50x3 mm, Nucleosil 5 μm, C18) were placed between the pump and injector. DA and metabolites were separated by reverse phase liquid chromatography (250x4.8 mm, Nucleosil 5 μm, C18). Detection was achieved by

setting the glassy carbon working electrode at +700 mV against the Ag/AgCl reference electrode of the electrochemical detector (LC4B, Bioanalytical Systems). The chromatograms were recorded on a two pen chart recorder (Kipp & Zonnen, BD41). The mobile phase consisted of 0.1 M acetic acid adjusted to pH 4.1 with solid sodium acetate, 0.5-0.9 mM octanesulfonic acid (Kodak), 0.01 mM Na_2EDTA, and 100-150 ml methanol/l. The detection limit of the assay was about 5 fmol/injection for DA, DOPAC and 5-HIAA, and about 20 fmol/injection for HVA.

At the end of the experiment the animal was sacrificed, the brain was removed, sliced (50 μm) on a microtome cryostat and microscopically examined for the position of the dialysis fibre.

Basal dialysate concentrations were expressed in fmol/min (legend of Fig. 2, Note 6). For the purpose of graphic representation the average of 3 baseline samples immediately preceding treatment was defined as 100%. All subsequent measures were related to these values, and the mean percentages were calculated for each 20 min sample across the rats in each group. For statistical evaluation of the data, the percent changes (last baseline plus 6 post-treatment samples) were used. Data were analysed by two-way (region X time) ANOVA with repeated measures followed by Newman-Keuls tests for multiple comparisons.

(ii) Chronic effects of desipramine

Subjects were male Wistar rats weighing 200-250 g at the beginning of the experiment. Chronic and acute injections of desipramine HCl as well as vehicle (saline) were administered to rats according to the following schedule: *chronic DMI*: 5 mg/kg injected i.p. in a volume of 2 ml/kg twice a day (09:00 and 18:00 h) for 21 days; *acute DMI*: 2ml/kg saline i.p. twice a day for 19 days followed by 5 mg/kg DMI (i.p., twice a

day) for two days; *control*: 2 ml/kg saline i.p. twice a day for 21 days. Body weights of the rats receiving DMI were monitored throughout the course of treatment and did not differ significantly from the control group. During microdialysis experiments DMI, apomorphine HCl (Sigma) or d-amphetamine sulfate (BDH) were dissolved in saline and administered in doses 5 mg/kg (i.p.), 25 μg/kg (s.c.) or 1.5 mg/kg (s.c.), respectively.

Twenty to 24 h following the last injection of DMI or saline, rats were implanted stereotaxically with a vertical microdialysis probe aimed at the NAC (coordinates of the probe tip relative to bregma were AP:+4.0 mm; ML:±1.5 mm; DV:-8.2 mm according to the atlas of Pellegrino et al. 1979) or striatum (AP:+1.2 mm; ML:±2.7 mm; DV:-7.0 mm according to the atlas of Paxinos and Watson 1986). The microdialysis probe (Damsma et al. in preparation) was a variant of the concentric design (Church and Justice 1987; Robinson and Whisaw 1988). Briefly, the dialysis device consisted of a semipermeable hollow fiber (cellulose, O.D.=0.25 mm, 6,000 Daltons, Spectra Por), 2 lengths of fused silica capillary tubings (I.D.=75 μM, a longer inlet and a shorter outlet), and three stainless steel cannulae (basic, inlet and outlet). The fiber was glued (epoxy) to the basic cannula; the long silica tubing passed through the inlet cannula, the basic cannula and the fiber terminating 0.3 mm from an epoxy plug sealing the tip of the hollow fiber; the short silica tubing passed through the basic and the outlet tubing. Finally, the assembled probe was sealed with epoxy at the junction of the three stainless steel cannulae, and this joint further secured with an outer casing of heatshrink tubing. The desired length of exposed dialysis fiber was achieved by coating the remainder of membrane with epoxy. Dialysis occurred through 2.3 mm (accumbens probe) or 4.2 mm (striatal probe) of the hollow fiber each with an error margin of 0.2 mm. The probes were rinsed with alcohol (80%) for 12-24 h and then for at least 2 h with sterile water prior to implantation. *In vitro* recoveries for this type of dialysis probe were obtained as described above. Recoveries in terms of percentages were (mean±S.E.M., n=5): 1) For the accumbens probes, 3.5±0.4 (DA), 4.0±0.5 (DOPAC), 3.7±0.3 (HVA), 4.3±0.5 (5-

HIAA), and 2) for the striatal probes, 6.6±1.0 (DA), 6.7±1.4 (DOPAC), 6.8±0.9 (HVA), 7.0±0.8 (5-HIAA).

All perfusion experiments were carried out in awake, freely moving animals 40-48 h (day 2) following implantation of the microdialysis probe (approximately 72 h after the last DMI or saline injection) during the light phase of the daily cycle. After a stable baseline was established, each rat with an accumbens probe received an injection of DMI (5 mg/kg, i.p.). Separate groups of rats (chronic DMI, acute DMI and controls with NAC implantations) were injected with apomorphine (25 μg/kg, s.c.). Four hours later, and after DA and metabolites had returned to baseline levels for at least 2 h, the rats were injected with d-amphetamine (1.5 mg/kg, s.c.). Pilot studies indicated that the response of DA and metabolites to d-amphetamine was not influenced by prior exposure (4 h) to apomorphine (see also Kuczenski et al. 1990). Other groups (chronic DMI and control) that were implanted with striatal microdialysis probes were injected with d-amphetamine (1.5 mg/kg, s.c.) 48 h postsurgery.

Microdialysis and subsequent chemical analysis were performed as described above with only minor modifications. The dialysis probe was perfused with a solution containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM sodium phosphate (pH 7.4). The load and inject time intervals were set at 9.8 min and 12 sec respectively.

Upon completion of the experiments, the animals were sacrificed, the brains were removed, sliced on a cryostat (30 μm), stained (Nissl), and examined microscopically for probe placement. Only rats with probes that were verified to be located in the NAC or striatum were included.

During some of the microdialysis experiments a Digiscan Animal Activity Monitor (DAAM, Omnitech Electronics, RXYZCM/16) was used to measure locomotor activity in 10 min blocks corresponding to the 10 min dialysate samples. The animal's cage was placed in the activity monitor frame, which records horizontal photobeam

interruptions. Total distance (cm) was used as an indicator of ambulatory activity (DAAM User Manual, p. 11).

For the purpose of graphic representation the average of 4 baseline samples immediately preceeding treatment was defined as 100%. All subsequent measures were related to these values, and the mean percentages were calculated for each 10 min sample. For statistical evaluation of the data, the percent changes (last baseline plus 6, 9 or 12 post-treatment samples according to experiment) were used. Data were analysed by two-way (treatment X time) ANOVA with repeated measures. The mean absolute baseline values (fmol/min) were evaluated by one-way (treatment: chronic DMI, acute DMI, saline) ANOVA. Locomotor activity measurements following amphetamine administration (twelve 10 min blocks) were also subjected to two-way ANOVA with repeated measures.

(C) Results

(i) Acute effects of desipramine

Acute DMI administration did not influence dialysate concentrations of DA from the NAC or the striatum (Fig. 2). In addition, DMI did not affect the DA metabolites DOPAC and HVA, or the serotonin metabolite 5-HIAA in either structure (data not shown).

(ii) Chronic effects of desipramine

Basal dialysate concentrations of DA, DOPAC, HVA and 5-HIAA from the NAC and striatum of rats given chronic DMI, acute DMI or saline treatment, are presented in

Figure 2. Effect of desipramine HCl (5 mg/kg, i.p.) on dialysate concentrations of dopamine from the striatum (open circles, n=4) or nucleus accumbens (solid circles, n=4). Each point represents mean (± S.E.M.) percent change of baseline. The mean (± S.E.M., fmol/min) baseline values for dopamine are 43±11 (striatum) and 13±3 (nucleus accumbens).

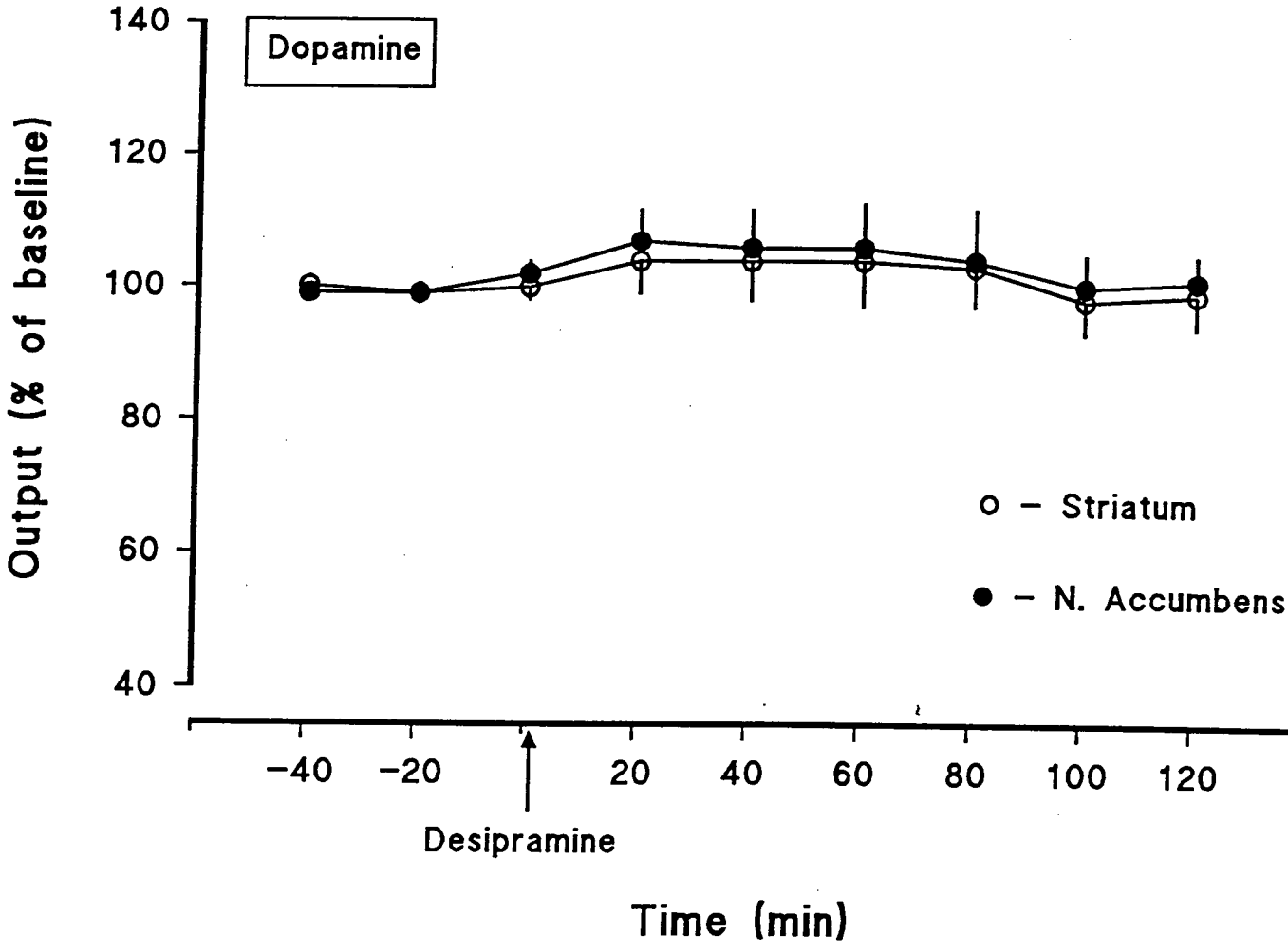

Dopamine
140
120
100
80
60
40
Output (% of baseline)
o – Striatum
● – N. Accumbens
−40
−20
20
40
60
80
100
120
Desipramine
Time (min)

Table I. A one-way ANOVA indicated that neither chronic nor acute DMI treatment significantly influenced the basal concentrations of DA or its metabolites.

Desipramine (5 mg/kg, i.p.) did not influence dialysate concentrations of DA or metabolites (data not shown) in the NAC of rats treated chronically with DMI or vehicle (Fig. 3).

Apomorphine (25 μg/kg, s.c.) reduced interstitial DA concentrations in the NAC to approximately 55% of baseline values within 30 min (Fig. 4). DA gradually returned to pre-apomorphine baseline values within 90 min. Chronic or acute DMI treatment did not influence the apomorphine-induced decrease in DA as revealed by a repeated measures ANOVA ($F_{2,16}$=0.79). There was a significant time effect ($F_{9,144}$=29.8, $p<0.01$) due to significant (Newman-Keuls, $p<0.05$) changes in DA output over time in all three groups. The treatment X time interaction was not significant ($F_{18,144}$=0.98). Apomorphine decreased DOPAC and HVA modestly but significantly as revealed by the respective time effects $F_{9,144}$=3.92, $F_{9,144}$=3.19, both $p<0.05$ (Fig. 5). Apomorphine did not affect 5-HIAA (Fig. 5). Chronic or acute DMI treatment did not significantly influence any of the apomorphine-induced effects on any of these metabolites.

d-Amphetamine (1.5 mg/kg, s.c.) resulted in a pronounced and prolonged increase in NAC interstitial concentrations of DA in all three groups. The effect of amphetamine peaked at 30-40 min. Chronic DMI enhanced the DA response to amphetamine throughout the experimental period, although the temporal pattern was similar (Fig. 6). A repeated measures ANOVA did not indicate a significant treatment effect ($F_{2,16}$=2.67, p=0.09) but revealed a significant time effect ($F_{12,192}$=47.03, $p<0.001$) and treatment X time interaction ($F_{24,192}$=1.68, p=0.02). Post-hoc comparisons with controls showed significantly ($p<0.05$) higher DA responses after amphetamine in the chronic DMI group at the 20-90 min test intervals. Chronic DMI differed

Table I.

Baseline dialysate concentrations of DA and metabolites from nucleus accumbens or striatum of rats following chronic DMI treatment

	DA	*DOPAC*	*HVA*	*5-HIAA*
Nucleus Accumbens				
Saline (n=11)	3.9±0.8	678±79	368±49	287±34
Acute DMI (n=6)	3.6±0.6	592±96	432±63	329±22
Chronic DMI (n=10)	3.2±0.3	712±112	431±54	308±36
Striatum				
Saline (n=6)	5.8±1.2	893±111	581±34	381±40
Chronic DMI (n=6)	6.0±1.5	936±153	627±98	413±64

Mean (± S.E.M.) basal values are expressed in fmol/min. Baseline dialysate concentrations of DA and metabolites from nucleus accumbens were pooled from two separate experiments in which apomorphine and amphetamine or desipramine (DMI) was administered.

Figure 3. Effect of desipramine (5 mg/kg, i.p.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with saline (open circles-Saline, n=4) or desipramine (solid circles-DMI, n=4). Baseline values are indicated in Table I.

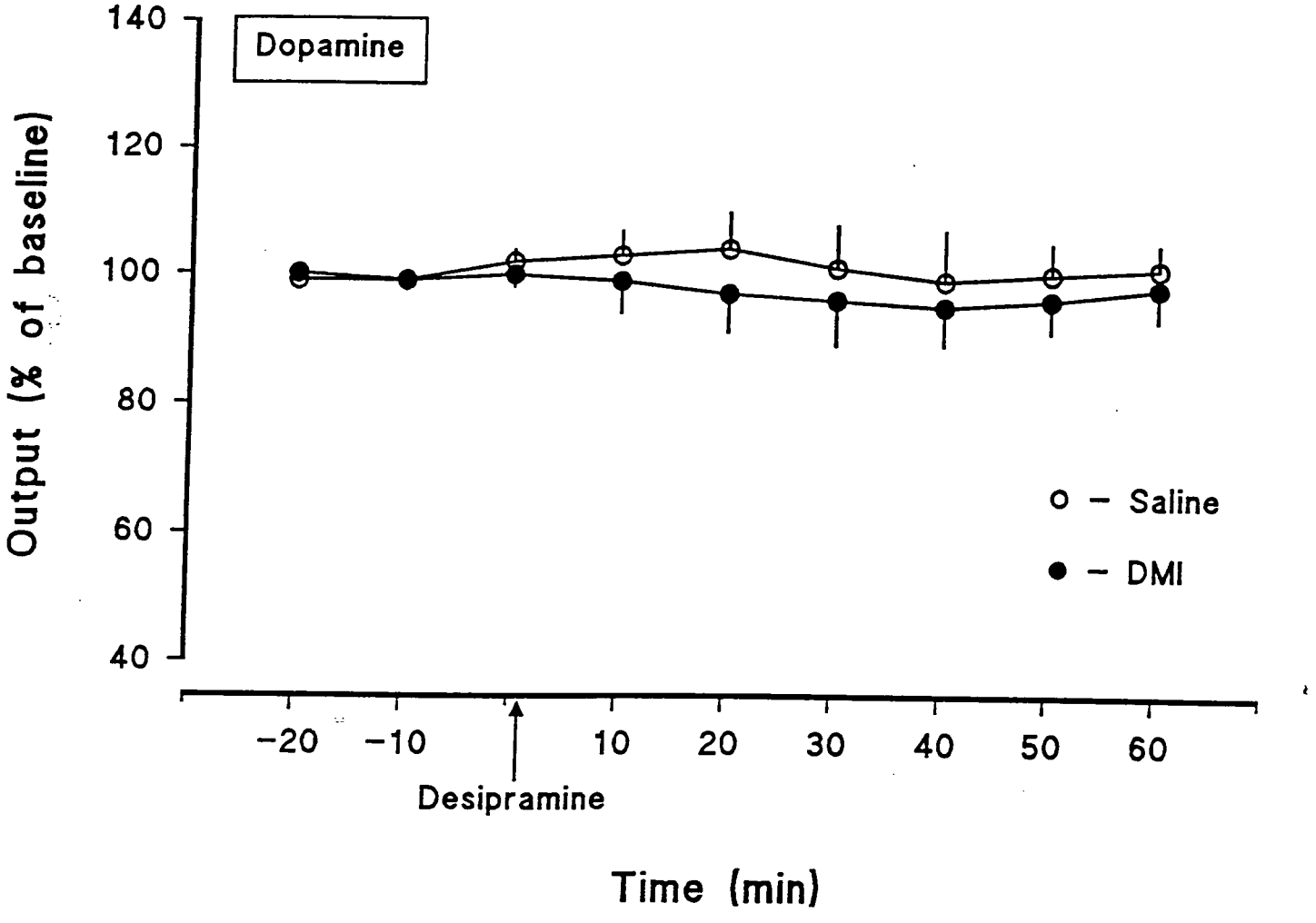

Dopamine
140
120
100
80
60
40
Output (% of baseline)
o – Saline
● – DMI
−20
−10
10
20
30
40
50
60
Desipramine
Time (min)

Figure 4. Effect of apomorphine (25 μg/kg, s.c.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with saline (open squares-Saline, n=7), or desipramine (solid circles-DMI, n=6) or acutely treated with desipramine (open circles-Acute DMI, n=6). Data represent the group mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table I.

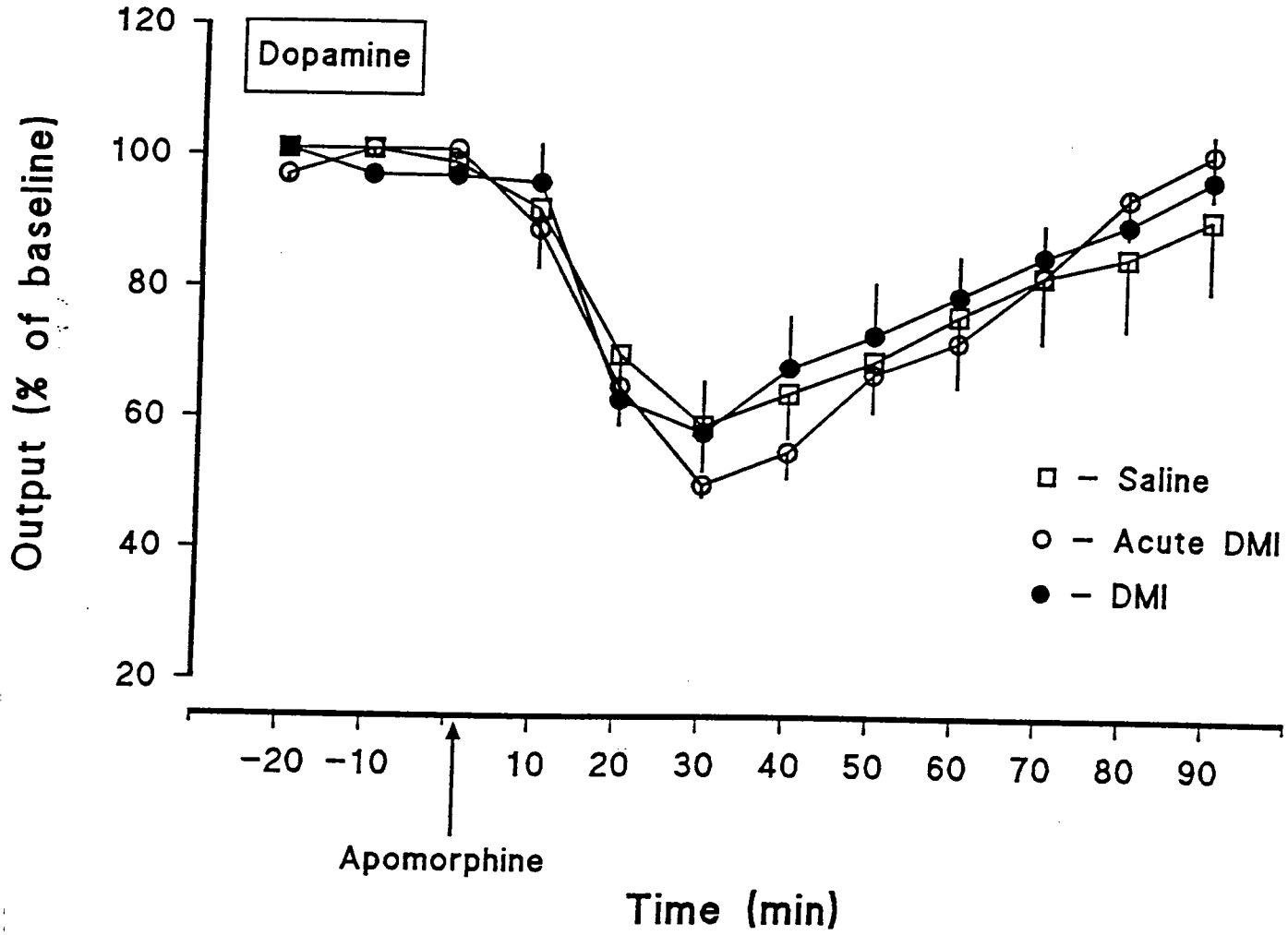
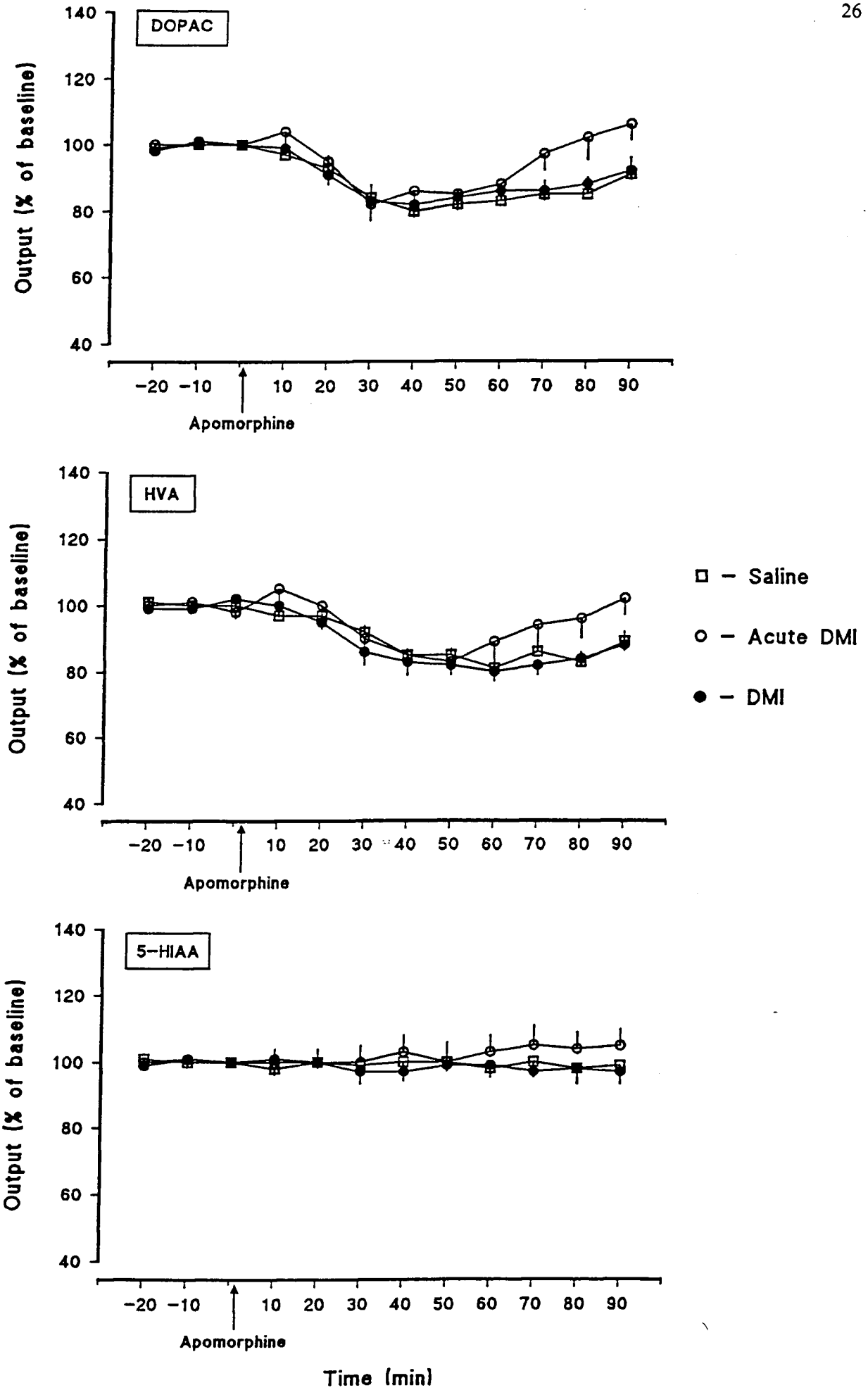

Dopamine
120
100
80
60
40
20
Output (% of baseline)
□ – Saline
○ – Acute DMI
● – DMI
−20 −10
10 20 30 40 50 60 70 80 90
Apomorphine
Time (min)

Figure 5. Effect of apomorphine on interstitial concentrations of DOPAC, HVA and 5-HIAA from the nucleus accumbens of rats chronically treated with saline, DMI or acutely treated with DMI. Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table I.


DOPAC
HVA
5-HIAA
Output (% of baseline)
140
120
100
80
60
40
-20 -10
10 20 30 40 50 60 70 80 90
Apomorphine
Time (min)
□ – Saline
○ – Acute DMI
● – DMI

significantly from the acute DMI group at the 20-50 min time points. The saline and acute DMI groups were not significantly different at any time point (Fig. 6).

d-Amphetamine decreased the output of DOPAC and HVA in the NAC. These effects tended to be more pronounced in rats treated chronically with DMI (Fig. 7). ANOVA performed on the DOPAC data showed a significant time effect ($F_{12,192}$=156.25, p<0.0001), while the treatment effect just failed to reach significance ($F_{2,16}$=3.58, p=0.0505); the treatment X time interaction was not significant ($F_{24,192}$=1.14). ANOVA performed on the HVA data also indicated a significant effect of time ($F_{12,192}$=63.18, p<0.0001) and a significant treatment X time interaction ($F_{24,192}$=1.72, p<0.05). The treatment effect was not significant ($F_{2,16}$=3.03, p=0.07). Post-hoc comparisons were performed and revealed significant differences between the chronic DMI group and both the saline and acute DMI groups (see Fig. 7). d-Amphetamine produced a small but prolonged increase in dialysate concentrations of 5-HIAA (Fig. 7). Pretreatment with DMI (acute or chronic) did not significantly influence this effect. A repeated measures ANOVA revealed only a significant effect of time ($F_{12,192}$=5.32, p<0.001).

d-Amphetamine increased dialysate concentrations of DA from the striatum of both the chronic DMI and saline treated animals (Fig. 8). However, this effect did not differ between the two groups ($F_{1,10}$=0.94, $F_{12,120}$=0.88 for treatment and treatment X time effects, respectively). The effect of time was highly significant $F_{12,120}$=42.12, p<0.0001). The temporal profile and the magnitude of the increase in DA efflux following amphetamine did not differ in rats having striatal or accumbens probes (compare saline groups in Figs. 6 and 8). d-Amphetamine decreased the interstitial concentrations of DOPAC and HVA in the striatum, and modestly increased 5-HIAA (Fig. 9). Chronic treatment with DMI did not affect any of these responses.

Figure 6. Effect of d-amphetamine (1.5 mg/kg, s.c.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with saline (n=7), DMI (n=6) or acutely treated with DMI (n=6). Data points represent group mean (± S.E.M.) percent changes of baseline. Baseline values are indicated in Table I.

*: $p<0.05$, in comparison to Saline group; +: $p<0.05$, in comparison to acute DMI group

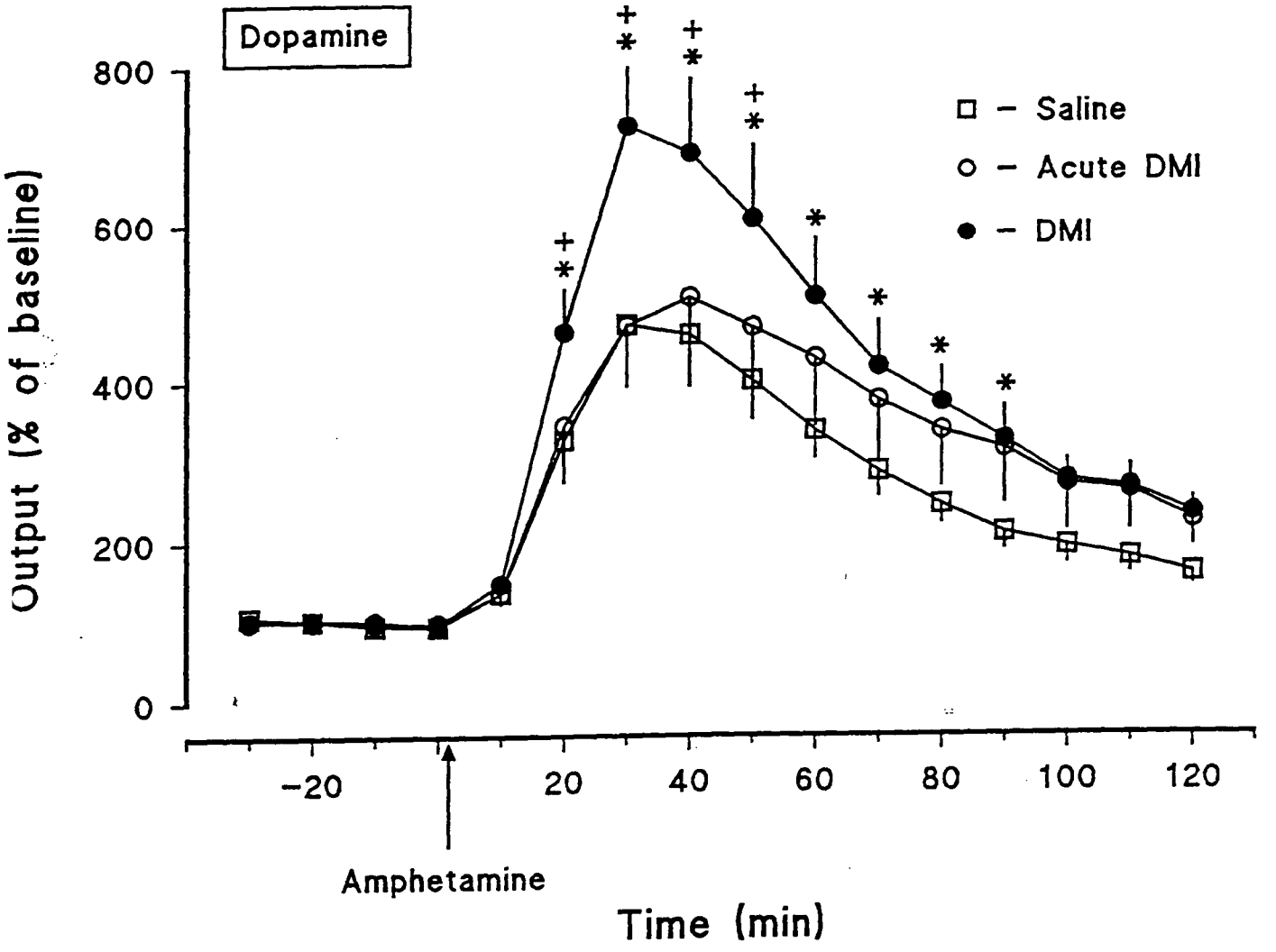

Dopamine
800
600
400
200
0
Output (% of baseline)
□ – Saline
○ – Acute DMI
● – DMI
+
*
−20
20
40
60
80
100
120
Amphetamine
Time (min)

Figure 7. Effect of d-amphetamine on dialysate concentrations of DOPAC, HVA and 5-HIAA from the nucleus accumbens of rats chronically treated with saline, DMI or acutely treated with DMI. Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table I.

*: $p<0.05$, in comparison to Saline group; +: $p<0.05$, in comparison to acute DMI group

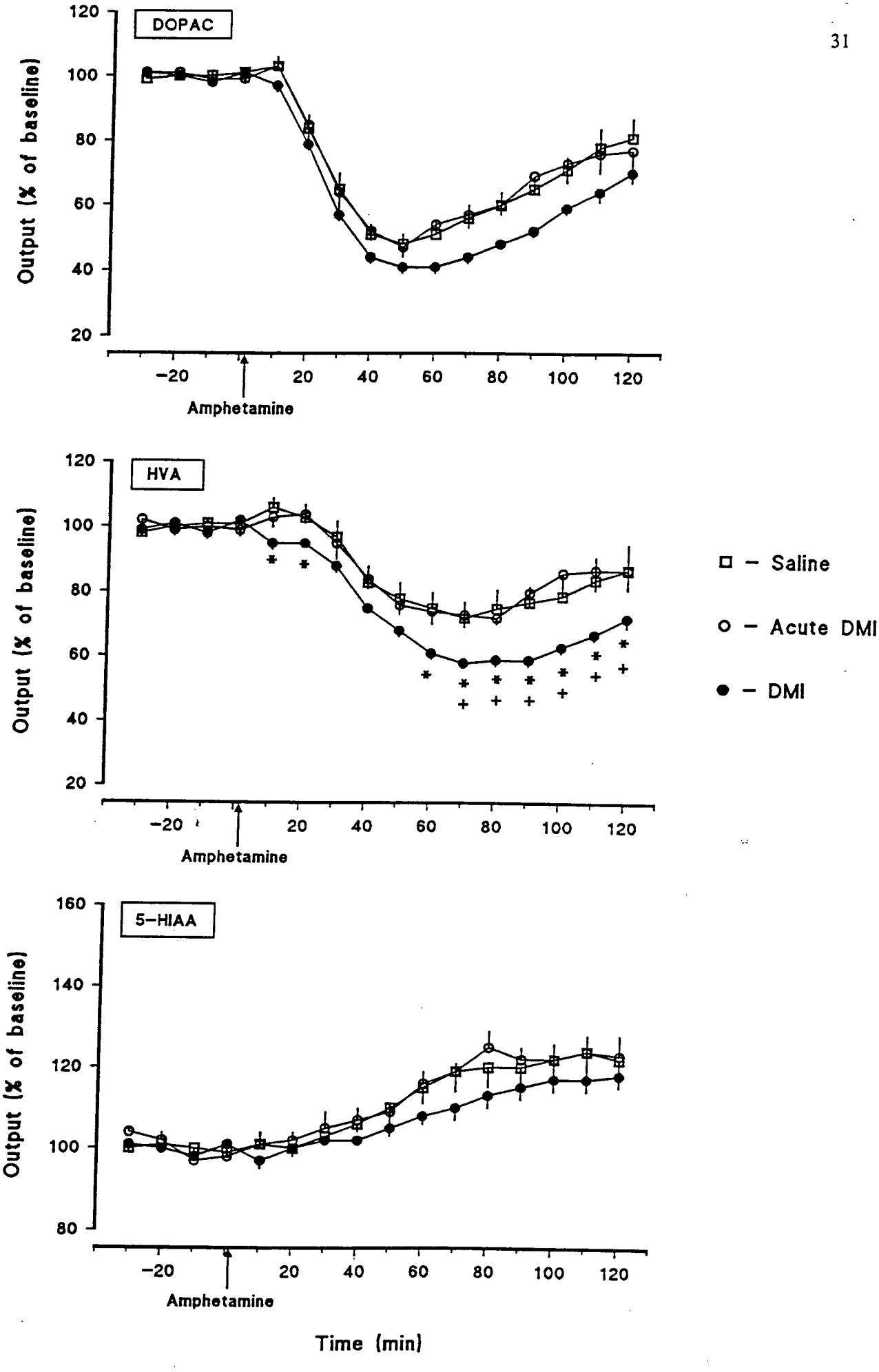

DOPAC
HVA
5-HIAA
Output (% of baseline)
Amphetamine
Time (min)
□ – Saline
○ – Acute DMI
● – DMI

Figure 8. Effect of d-amphetamine (1.5 mg/kg, s.c.) on dialysate concentrations of dopamine from the striatum of rats chronically treated with saline (open circles-Saline, n=6) or desipramine (solid circles-DMI, n=6). Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table I.

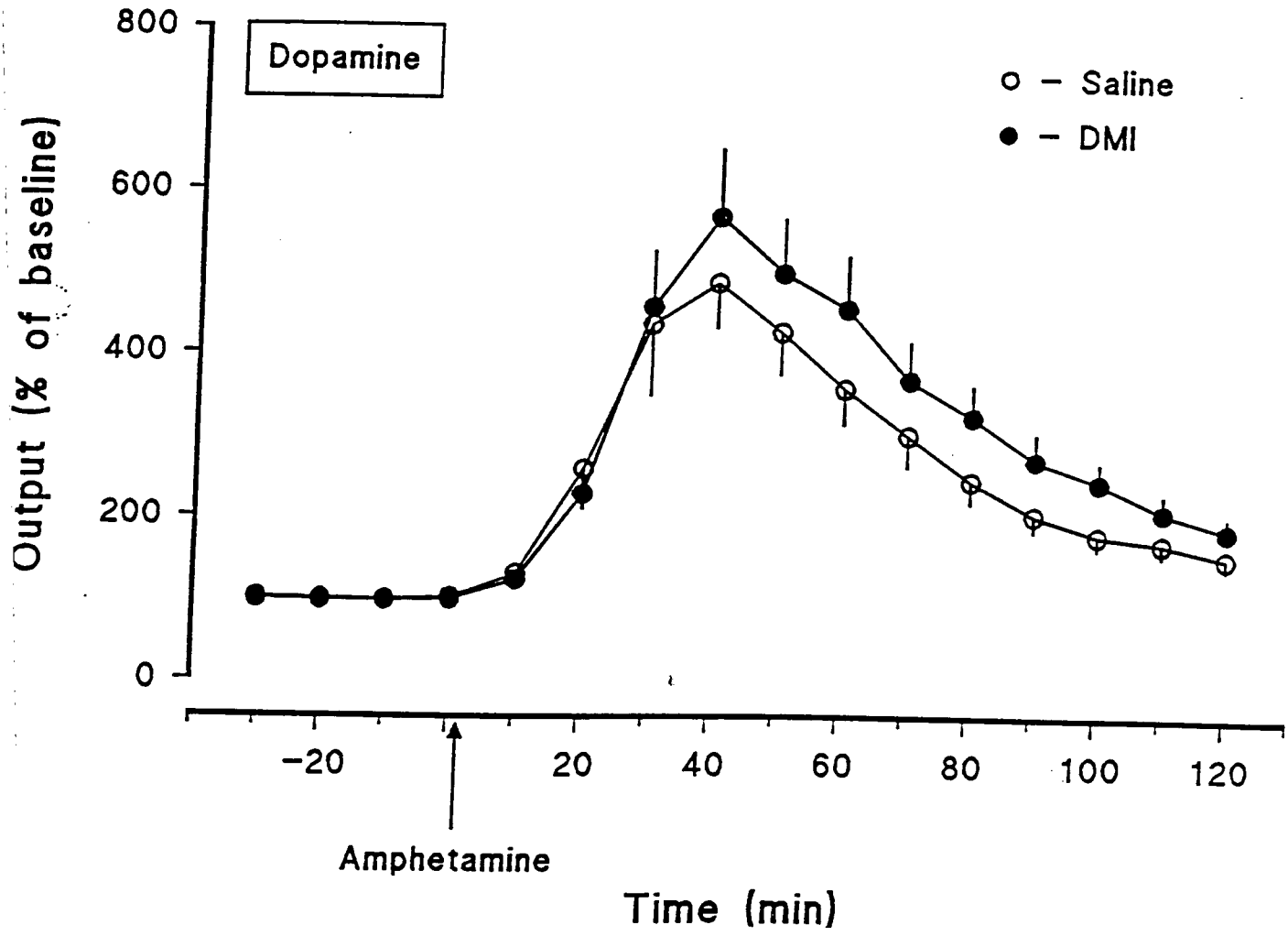
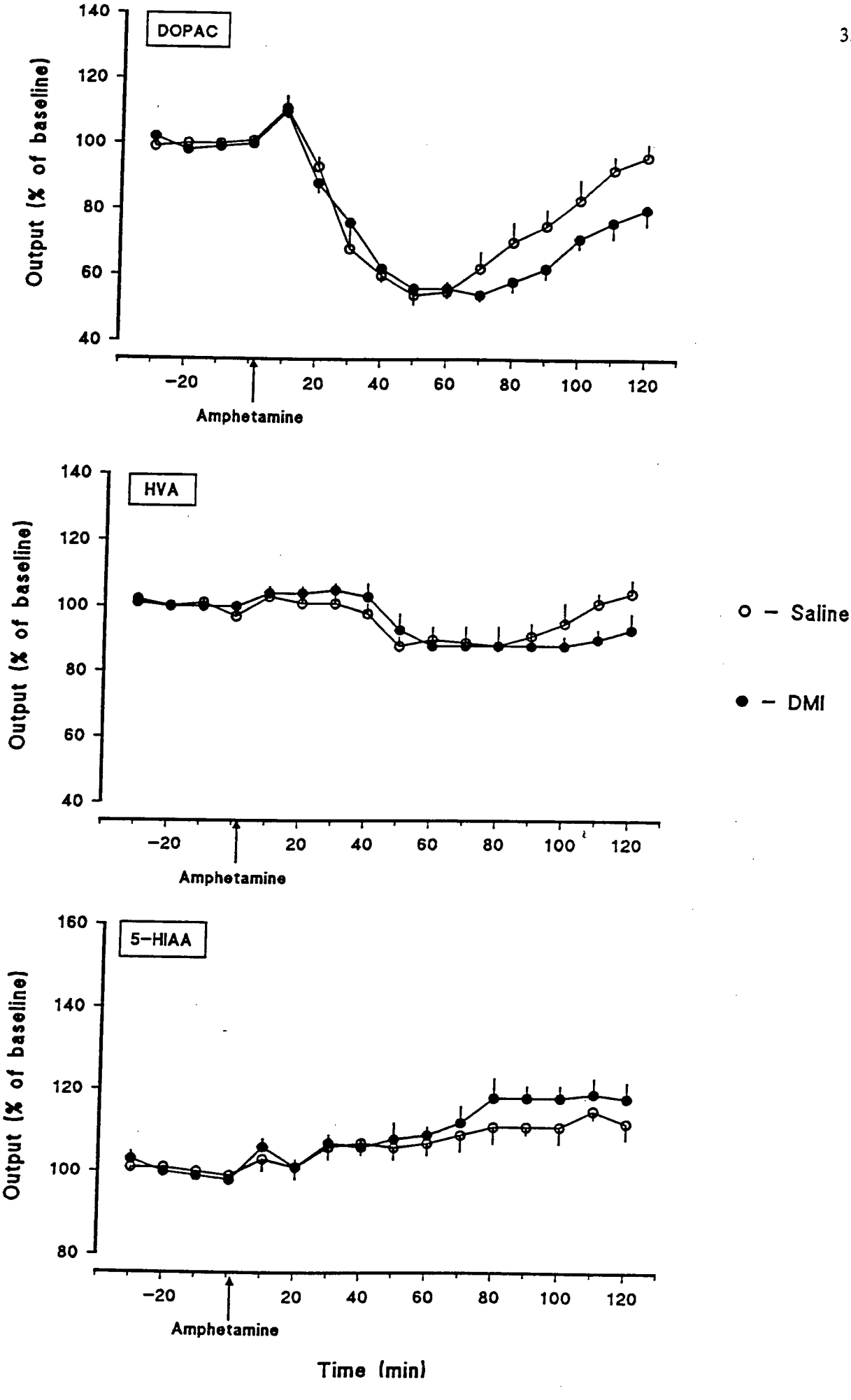

Dopamine
○ – Saline
● – DMI
Output (% of baseline)
800
600
400
200
0
−20
20
40
60
80
100
120
Amphetamine
Time (min)

Figure 9. Effect of d-amphetamine on dialysate concentrations of DOPAC, HVA and 5-HIAA from the striatum of rats following chronic treatment with DMI or saline. Each point represents the mean (± S.E.M.) percent changes of baseline. Baseline values are indicated in Table I.

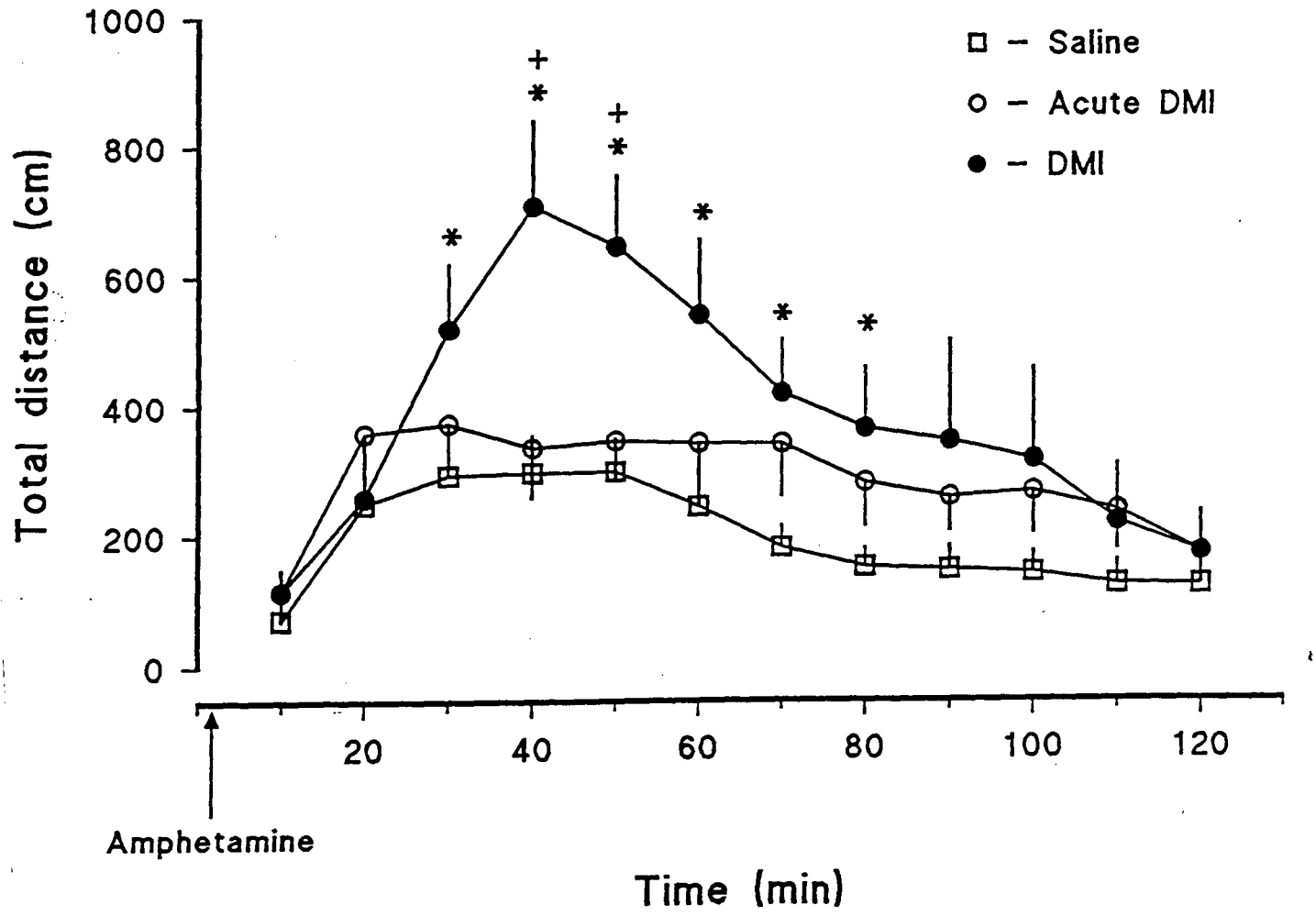

DOPAC
HVA
5-HIAA
Output (% of baseline)
Amphetamine
Time (min)
○ – Saline
● – DMI

Locomotor activity (total distance expressed in cm) following d-amphetamine administration is illustrated in Figure 10. Total distance counts before d-amphetamine or after saline injections did not usually exceed 20 units (data not shown). Behavioral data from animals that were implanted with either accumbens or striatal probes were not different, and were therefore combined for statistical evaluation and graphic representation. Locomotor activity scores peaked within 20-40 min post-injection and then, in contrast to the DA responses, declined rather gradually (compare Figs. 6 and 10). Compared to animals treated acutely with DMI or saline, amphetamine-induced hypermotility was more pronounced in the animals receiving chronic DMI. A repeated measures ANOVA performed on the behavioral data from all three groups following amphetamine indicated significant treatment ($F_{2,28}=3.47$, $p<0.05$), time ($F_{11,308}=10.43$, $p<0.0001$) and treatment X time interaction ($F_{22,308}=2.17$, $p<0.01$) effects. Post-hoc comparisons (Newman-Keuls) revealed significant differences between the three groups (see Fig. 10).

(D) Discussion

In a recent *in vitro* study, De Montis et al. (1990) reported that DMI (and imipramine) inhibited DA uptake in the limbic system (including NAC) with 100-fold higher potencies than in the striatum, and in concentrations that can be reached in the brain by average antidepressant regimens. No evidence for DMI-induced inhibition of DA uptake in the NAC (or the striatum) was obtained in the present acute *in vivo* studies. This confirms previous microdialysis findings by Di Chiara and Imperato (1988) who found that acutely administered imipramine did not affect extracellular concentrations of DA and its metabolites in the NAC. The present results also demonstrate that chronically administered DMI does not influence basal concentrations

Figure 10. Effect of d-amphetamine (1.5 mg/kg, s.c.) on locomotor activity measurements (total distance-cm) of rats following chronic treatment with saline (open squares, n=13) or DMI (solid circles, n=12) or acute treatment with DMI (open circles, n=6). Arrow indicates the injection of amphetamine. Data points represent group mean (± S.E.M.) activity counts over 10 min intervals.


1000
800
600
400
200
0
Total distance (cm)
□ – Saline
○ – Acute DMI
● – DMI
+
*
+
*
*
*
*
20
40
60
80
100
120
Amphetamine
Time (min)

of DA and its metabolites in the interstitial space of the NAC and striatum. This finding suggests that chronic DMI does not change the steady-state synthesis and turnover rates of DA and therefore confirms previous conclusions based on data obtained in *ex vivo* studies (Neff and Costa 1967; Sugrue 1980). The fact that chronic DMI failed to influence the basal extracellular concentrations of DA and its metabolites in the NAC also suggests that potential effects of this treatment on central noradrenergic mechanisms did not significantly influence dopamine neurotransmission in this structure. It is interesting in this regard that Carboni et al. (1990) have demonstrated that DMI increases extracellular concentrations of DA in the prefrontal cortex. These workers presented evidence that uptake into noradrenergic terminals in this structure is a significant mechanism by which DA is removed from the extracellular space. The present results indicate that a similar phenomenon does not occur in the NAC, a structure which also receives significant noradrenergic innervation (Versteeg et al. 1976; Farley and Hornykiewicz 1977).

Apomorphine is thought to decrease interstitial DA concentrations by stimulating inhibitory DA autoreceptors that regulate the firing of DA neurons, and/or the synthesis and release of DA from terminals (Zetterström and Ungerstedt 1984; Radhakishun et al. 1988). Chronic or acute treatment with DMI did not affect the apomorphine-induced decrease in interstitial DA concentrations in NAC, indicating that these treatments did not alter the sensitivity of these autoreceptors. These data are consistent with previous studies which failed to find evidence of DA autoreceptor subsensitivity after chronic antidepressant treatments (Spyraki and Fibiger 1981; MacNiell and Gower 1982; Welch et al. 1982; Willner 1983c; Diggory and Buckett 1984). It should be emphasized, that in the present experiments the effect of apomorphine on interstitial DA concentrations in the NAC probably reflects the combined effects of stimulation of both somatodentritic and presynaptic DA autoreceptors. In this regard, Holcomb et al. (1982) and Towell et al. (1986) found no significant change in DA terminal or DA cell body autoreceptor

sensitivity, respectively, after chronic antidepressant drug treatments. It has been argued that subsensitivity of DA autoreceptors following chronic antidepressants is not a robust phenomenon and can be reliably manifested only following treatment withdrawal bringing into doubt the significance of this effect in the therapeutic response (Towell et al. 1986; Scavone et al. 1986). Nevertheless, in the study by Spyraki and Fibiger (1981) as well as in the present study a three-day withdrawal period was used to assess the behavioral and biochemical effects of apomorphine with negative results.

Amphetamine increases interstitial concentrations of DA by depleting a cytosolic pool of this neurotransmitter from nerve terminals (Imperato and Di Chiara 1984; Zetterström et al. 1986, 1988; Butcher et al. 1988; Robinson et al. 1988). In the present experiments, the effects of amphetamine were selectively enhanced in the NAC of rats receiving chronic DMI. Amphetamine metabolism is not affected 3 days after withdrawal from chronic DMI treatment (Spyraki and Fibiger 1981). Also, in the current study the effect of amphetamine on dialysate concentrations of DA and metabolites from striatum, as well as on dialysate concentrations of 5-HIAA from NAC were not affected by DMI pretreatment. These observations argue strongly against the possibility that altered bioavailability of amphetamine was responsible for the enhanced effect of the drug on DA in the NAC of the chronic DMI treated group. As discussed above, changes in DA metabolism after chronic administration of antidepressants have not been shown in *ex vivo* studies, and no differences in the basal values of DA and its metabolites were detected in the present experiments. These findings make it unlikely that chronic DMI-induced changes in DA metabolism were responsible for the enhanced effect of amphetamine on extracellular concentrations of DA in the NAC.

The effects of chronic antidepressant treatments on DA uptake sites have not been studied systematically. It is possible that the higher extracellular DA concentrations in the NAC that were observed after amphetamine in the chronic DMI group were due to a decrease either in the number or the efficacy of DA uptake sites. Such an effect

would be consistent with demonstrations that chronic antidepressant treatments enhance the behavioral responses to a number of agents that increase extracellular concentrations of DA, including amphetamine, nomifensine, L-DOPA plus an MAO inhibitor, reserpine or dopamine itself (Spyraki and Fibiger 1981; Maj 1986; Willner 1985). Interestingly, both chronic DMI treatment and DA uptake inhibitors increase the rate of responding of ICSS in the VTA (Fibiger and Phillips 1981; Fibiger et al. 1990). However, decreased DA uptake after repeated treatment with antidepressants does not easily explain the enhanced behavioral syndrome produced by direct DA receptor agonists (Spyraki and Fibiger 1981; Maj et al. 1984), and indicate that changes in postsynaptic receptor mechanisms must be involved as well. In this regard, there is evidence that behavioral responses to specific D_1 and D_2 receptor agonists can be affected by chronic antidepressant drug treatments. Thus, various antidepressant drugs increase the behavioral effects of D_2 receptor agonists and reduce D_1 receptor mediated behaviors. Receptor binding studies have detected an increase in the affinity of D_2 receptors for DA agonists after chronic TCAs (Klimek and Maj 1989) but no effect on the number or the affinity of D_2 receptors for DA antagonists in the NAC (Martin-Iverson et al. 1983; Klimek and Nielsen 1987). Furthermore, Klimek et al. (1985) and Klimek and Nielsen (1987) have shown that a variety of chronically administered antidepressant drugs decrease the number of D_1 binding sites in both the striatum and the limbic system (including the NAC).

Chronic DMI may also have facilitated the mechanisms by which amphetamine releases DA from meso-accumbens neurons. The finding that the decreases in DA metabolites (especially HVA) were more pronounced in the chronic DMI group is consistent with this hypothesis. Zetterström et al. (1986, 1988) have suggested that the decreases in DA metabolites produced by amphetamine are due to the depletion of intracellular stores of newly synthesized DA; a further reduction in the concentrations of metabolites might therefore indicate enhanced DA release. This issue is complex,

however, because although the increase in DA release following systemic administration of amphetamine has been reported to be dose-dependent, the decrease of metabolites is not (Zetterström et al. 1986; Butcher et al. 1988).

DMI-induced decreases in the negative feedback mechanisms that regulate DA neurons in the VTA may also have contributed to the potentiation of the amphetamine-induced increase in DA release in the NAC. Neuronal circuits involved in negative feedback control of DA meso-accumbens neurons include neurons in the NAC that project back to the VTA and GABA interneurons in the VTA (Nauta et al. 1978; Walaas and Fonnum 1980; O'Brien and White 1987; Waszczak and Walters 1980), and/or NAC GABAergic neurons that innervate the ventral pallidum which in turn sends GABAergic efferents to the VTA (Jones and Mogenson 1980; Mogenson et al. 1983; Heimer et al. 1982; Penney and Young 1981). Because chronic antidepressant treatments increase the stimulant actions of directly-acting DA receptor agonists, it would be interesting to study the effects of high (postsynaptic) doses of apomorphine on extracellular DA concentrations in NAC after chronic antidepressant treatment; if the hypothesis of decreased negative feedback is correct, a less pronounced decrease in DA would be predicted after this treatment.

In control animals, d-amphetamine did not produce a greater effect on DA output in the NAC than in the striatum. This finding is in agreement with observations by Sharp et al. (1987) and by Robinson and Camp (1990) but not with a study by Di Chiara and Imperato (1988) who claimed that d-amphetamine (and other stimulants) had more potent effects in the NAC. At present, therefore, evidence suggesting that stimulants have preferential actions on DA mechanisms in the NAC is equivocal.

The present studies confirm that chronic administration of DMI can potentiate the locomotor stimulant properties of d-amphetamine (Spyraki and Fibiger 1981; Martin-Iverson et al. 1983). They further indicate that this enhanced behavioral effect is accompanied by significant increases in the ability of this drug to increase the

extracellular concentrations of DA in the NAC. This effect of chronic DMI is relatively selective because a similar potentiation is not observed in the striatum. The mechanisms by which DMI enhances amphetamine-induced increases in extracellular DA in the NAC remain to be determined.

(E) Notes

Note 1: Throughout this thesis the terms interstitial and extracellular (fluid, space, compartment) are interchangeably used. The term extracellular compartment connotes the interstitial comparment plus the vascular space (Hansen 1985; Benveniste 1989). Since the blood brain barrier is restored within 30 min after probe insertion (Benveniste et al. 1984), the vascular compartment is not thought to be sampled in microdialysis experiments. Hence, it seems more appropriate to use the term interstitial. Conventially however, both terms are used (Ungerstedt 1984; Imperato and Di Chiara 1984; Westerink et al. 1987a; Robinson et el. 1988; Benveniste 1989)

Note 2: Two types of probes were used in the present experiments: the transverse and the vertical (concentric). There are advantages and disadvantages of either design (Damsma 1987; Di Chiara 1990a). The major advantage of the horizontal probes is that they have a larger area for dialysis and thus higher recoveries; also, due to the design of the probe the formation of air bubbles is prevented and the flow rate remains constant. A major advantage of the vertical probes is that they can be implanted in symmetrical brain structures (striatum-NAC) for simultaneous dialysis; even more important is the fact that with vertical probes a better anatomical resolution can be achieved in the mediolateral axis than with the horizontal probes.

Note 3: The post-implantation interval is of particular importance in microdialysis experiments (see Introduction). Most of the present microdialysis experiments were performed approximately 48 h postsurgery. Although there is no difference in basal dialysate concentrations of DA between 24 (day 1) and 48 h (day 2), there are indications that after a probe is implanted DA metabolism requires 48 h to return to normal conditions (Reiriz et al. 1989; Zis et al. 1990). It is noteworthy, that to date

the majority of published microdialysis experiments have been performed 24 h (or less) after surgery.

Note 4: The concentration of Ca^{++} in the perfusion fluid influences the dialysate concentrations of DA in a dose-dependent manner (Westerink and de Vries 1988; Moghaddam and Bunney 1989), and it can influence the response of the neurotransmitters to pharmacological manipulations (Moghaddam and Bunney 1989; de Boer et al. 1990). Although Ringers solutions with high concentrations (3-3.4 mM) of Ca^{++} are still used (Hernandez and Hoebel 1989; Carboni et al. 1990), the modified buffered Ringers with low Ca^{++} seems to prevail as the perfusion solution of choice (Benveniste and Hüttemeier 1990). Two types of perfusion solution were used in this thesis: a Ringers and a modified buffered Ringers solution. These solutions contained Ca^{++} in 2.1 and 1.3 mM concentrations, respectively. The baseline dialysate concentrations of DA are higher in the experiments in which the Ringers solution was used. However, the percent changes in DA output were similar when the same pharmacological manipulations were performed (for example, the experiments with acute and chronic DMI and bupropion).

Note 5: The terms perfusate and dialysate have been interchangeably used in the microdialysis literature. In the present studies the word perfusate refers to the perfusion solution that enters the brain, and the term dialysate refers to the perfusion solution that leaves the brain enriched with substances from the interstitial fluid (also, Westerink et al. 1987).

Note 6: The dialysate concentrations of DA and metabolites are by convention expressed in fmol/min (Westerink et al. 1987; Imperato and Di Chiara 1984; Robinson et al. 1988).

Although the concentration/time unit is rather unorthodox, the molarity (or any expression of concentration) of the dialysate solution can be readily calculated.

III. In vivo neurochemical effects of bupropion

(A) Introduction

Bupropion is a novel, atypical antidepressant that has mild stimulant properties. The drug has an antidepressant profile in animal screening tests (Cooper et al. 1980; Soroko and Maxwell 1983) and has been shown to be an effective antidepressant in clinical trials (Chouinard 1983; Feighner et al. 1984, 1986). Bupropion does not seem to affect the release of biogenic amines or the activity of monoamine oxidase (Ferris et al. 1983; Soroko et al. 1977). This compound appears not to have any direct effects on central neurotransmitter receptors (Ferris and Beaman 1983) although an increase in affinity of DA receptors has been reported after acute administration (Bischoff et al. 1984). Furthermore, after chronic treatment with moderate, behaviorally active doses, the drug does not affect ß-adrenergic, a_2-adrenergic, dopaminergic, imipramine, or serotonin (5-HT_2) receptors in the brain, this contrasting with other antidepressant treatments such as monoamine oxidase inhibitors, electroconvulsive shock and tricyclic antidepressants (Ferris and Beaman 1983).

Dopaminergic systems are thought to be important neural substrates for at least some of the effects of bupropion. For example, the drug produces dose-dependent increases in locomotor activity as well as stereotyped behavior at higher doses, and these effects depend on intact dopaminergic but not noradrenergic neurons (Cooper et al. 1980). It is a selective dopamine (DA) uptake inhibitor of moderate potency *in vitro* (Ferris et al. 1983; Richelson and Pfenning 1984). Bupropion also inhibits DA uptake *in vivo*, as measured by the protection of dopaminergic neurons from the neurotoxins 6-OHDA and methamphetamine or the reversal of DA depletion produced by α-methyl-p-tyrosine (Cooper et al. 1980; Marek et al. 1990). *Ex vivo* studies suggest that the drug may also have a DA releasing action (Waldmeier 1982). An intact dopaminergic system is necessary for bupropion's activity in the Porsolt antidepressant screening test (Cooper et al. 1980). However, the doses

required to inhibit DA uptake *in vivo* (>25 mg/kg) are considerably higher than those (8-10 mg/kg) required for activity in this test (Cooper et al. 1980; Butz et al. 1982). These data have brought into question the role of DA uptake inhibition in the antidepressant actions of bupropion. Other evidence for a physiologically relevant dopaminergic mediation of bupropion's activity includes the potentiation of L-DOPA-induced behavioral effects (Ferris et al. 1981), inhibition of prolactin release (Stern et al. 1979), and DA-dependent EEG arousal (Canning et al. 1979).

To date bupropion's clinically relevant mechanism of action remains uncertain (Golden et al. 1988; Berwich and Amsterdam 1989). Due to its DA uptake blockade properties, bupropion has traditionally been compared with other DA uptake inhibitors and indirect DA agonists by *in vitro* and *ex vivo* methods (Soroko et al. 1977; Waldmeier 1982; Richelson and Pfenning 1984; Nielsen et al. 1986; Hyttel et al. 1988; Andersen 1989). The *in vitro* techniques utilize slices or synaptosomal preparations of the rat brain and examine the release or uptake of tritiated DA (Besson et al. 1969; Ferris et al. 1972; Heikkila et al. 1975; Raiteri et al. 1975; Hyttel 1978; Bonnet et al. 1984). In the *ex vivo* procedures the tissue concentrations of DA and its metabolites are assessed following various pharmacological manipulations (Scheel-Krüger 1971; Westerink et al. 1977; Braestrup 1977; Westerink 1979). By employing these techniques the specificity, potency, and differentiation between drug-induced uptake inhibition and drug-induced release of several DA uptake inhibitors have been studied. However, to what extent the *in vitro* and *ex vivo* effects of these compounds can be extrapolated to their *in vivo* effects on DA neurotransmission has not been established.

In vivo studies using the brain microdialysis technique have revealed that different classes of psychoactive drugs which share the property of inhibiting neuronal uptake of DA increase extracellular levels of this neurotransmitter in the rat striatum. Specifically, systemic as well as local administration of the psychostimulants d-amphetamine, methylphenidate and cocaine, and the antidepressant nomifensine increase extracellular

striatal DA concentrations in anaesthetized or freely moving rats (Imperato and Di Chiara 1984; Zetterström et al. 1986, 1988; Butcher et al. 1988; Robinson and Whishaw 1988; Hurd and Ungerstedt 1989; Carboni et al. 1989; Kuczenski and Segal 1989). Systemic injections of the antimuscarinic benztropine and the selective DA uptake inhibitor GBR 12909 also increase extracellular DA concentrations in the striatum of awake or anaesthetized rats (Church et al. 1987; Westerink et al. 1987b). With the exception of d-amphetamine, none of these drugs appear to affect significantly the extracellular concentrations of DOPAC or HVA. In contrast, d-amphetamine decreases DOPAC and HVA (Zetterström et al. 1986, 1988).

The purpose of the present experiments was to characterize *in vivo* the effects of acute and chronic administration of bupropion on DA transmission. Specifically, the following parameters were examined: (1) the acute effects of bupropion on extracellular concentrations of DA and metabolites in striatum of awake freely moving rats in relation to the behavior (stereotypy) that the drug induces; (2) the role of endogenous neuronal activity on bupropion-induced changes in extracellular DA by the application of TTX which blocks the propagation of action potentials and has been used to characterize drug-induced DA release in microdialysis studies (Westerink et al. 1987c); (3) the specificity of bupropion's action on striatal versus NAC dopaminergic nerve terminals; (4) the profile of the *in vivo* effects of bupropion on extracellular concentrations of DA in comparison to other DA uptake inhibitors. The compounds were applied directly to the brain via the microdialysis tube (reverse microdialysis) to circumvent pharmacokinetic factors and the effects of these compounds on non-striatal structures that could indirectly influence striatal DA transmission. The sensitivity of drug-induced effects on DA to topical infusion of TTX was also assessed. In order to determine and compare the potency of the dopamine uptake inhibitors, dialysis efficiency (i.e. the degree of transport of the infused compounds across the dialysis membrane) was determined *in vitro*; (5) the effects of chronically administered bupropion on basal DA transmission by simultaneous sampling of the NAC and striatum;

and (6) the effects of a bupropion challenge on DA efflux in the NAC and striatum of chronic bupropion pretreated animals (concurrent measurement of locomotor activity was conducted to evaluate possible relationships between the bupropion-evoked biochemical and behavioral responses).

(B) Materials and methods

(i) Acute effects of bupropion

Bupropion hydrochloride (Burroughs Wellcome) dissolved in saline, was injected i.p. at doses 1, 10, 25 or 100 mg/kg. Control rats received saline (1 ml/kg, i.p.). In some experiments TTX (Sigma) was directly administered to brain tissue by addition to the perfusion fluid (10^{-6}M).

Stereotypy was rated as follows (Costall and Naylor 1974): 0= lack of locomotor activity (resting or sleeping); 1= continuous locomotor activity with discontinuous sniffing; 2= discontinuous locomotor activity with almost continuous intense "stereotyped" sniffing; 3= sniffing, licking and gnawing interspersed with brief periods of locomotor activity; 4= intense sniffing, licking or gnawing but no exploratory behavior. Behavioral ratings were obtained over 30 sec at the end of every 10 min period after the drug injection.

The animals were implanted with horizontal probes. Microdialysis and subsequent chemical analysis were performed using a fully automated on-line sample injection system basically as described above. All perfusion experiments were carried out 18 to 48 h after surgery. The dialysis tube was perfused with a Ringers solution (147 mM NaCl, 4 mM KCl and 2.1 mM $CaCl_2$; pH=6.0-6.2). The load and inject modes of the injector were set at 20 min and 10 sec, respectively.

The overall percent changes of basal values were used for statistical evaluation by non-parametric analysis of variance (Kruskal Wallis). Stereotyped behavior was represented by the group median of intensity ratings for each 10 min interval.

(ii) In vivo characterization of bupropion

Bupropion hydrochloride, d-amphetamine sulfate, benztropine methane-sulfonate (Sigma), nomifensine (Hoechst), cocaine hydrochloride (BDH), methylphenidate hydrochloride (Ciba-Geigy) and GBR 12909 [1-{2-bis-(4-fluorophenyl)methoxy]-ethyl}-4-(3-phenylpropyl)piperazine dimaleate] (Novo) were dissolved in Ringers solution at 1 mM concentrations. The solutions were sonicated for 10 min, further diluted and administered to the striatum by continuous infusion through the dialysis membrane. After a 60 min baseline period, four concentrations (1, 10, 100 and 1000 μM) of each compound were added sequentially to the perfusion fluid for 60 min each and the experiment was concluded by a further 1 h vehicle condition. In this experiment each animal received only one drug and was used only once. Each drug group consisted of 4 rats.

In a separate experiment various concentrations of each compound (10, 100 and 1000 μM) of each compound were applied locally for 10 min either in the presence or absence of TTX. Different drugs and concentrations were delivered once every 2 h and the order of presentation was counterbalanced between subjects. When TTX was coinfused it was dissolved in the Ringer solution at a concentration of 3×10^{-7} M and applied locally through the dialysis membrane for at least 60 min before the administration of the first drug and continuously thereafter. A total of 14 rats was used in the TTX experiments (7 with TTX and 7 without). Each drug at each dose was tested 3-4 times in separate animals.

To determine the dialysis efficiency of each drug *in vitro* (Ungerstedt 1984) a microdialysis probe was placed in a beaker filled with perfusion solution containing either 10^{-4} or 10^{-3} M concentrations of the test compound. Subsequently, the probe was perfused

with the same solution and flow rate as in the *in vivo* experiments. Recovery was calculated as the percentage of the drug concentration in the dialysate relative to the beaker solution. The drug concentrations in the dialysate and in the beaker were measured by UV spectrometry.

The animals were implanted with a horizontal probe. All perfusion experiments were carried out 18 to 48 h after surgery in conscious animals. The dialysis tube was perfused with a Ringers solution (147 mM NaCl, 4 mM KCl and 2.1 mM $CaCl_2$). In this experiment, the injector was held in the load position for 9.8 min and switched to the inject position for 10 sec.

For purposes of graphic representation (Fig. 15-16) the average of three baseline samples immediately preceding the drug application was defined as 100% and all subsequent measures were related to these values (percent changes). The last baseline measure and the first 10 min sample following administration of the lowest dose of each drug were compared using paired t-tests.

(iii) Chronic effects of bupropion

Subjects were male Wistar rats weighing 200-250 g at the beginning of the experiment. The experiment utilized 3 groups: *chronic bupropion*: bupropion HCl 10 mg/kg injected i.p. in a volume of 2 ml/kg twice a day (09:00 and 18:00 h) for 21 days; *acute bupropion*: 2 ml/kg saline i.p. twice a day for 19 days followed by 10 mg/kg bupropion (i.p., twice a day) for two days; *control*: 2 ml/kg saline i.p. twice a day for 21 days. Body weights of the rats receiving bupropion were monitored throughout and did not differ significantly from the control group at the end of treatment period.

Twenty to 24 h following the last injection of bupropion or saline, rats were implanted bilaterally with vertical microdialysis probes aimed at the NAC and striatum. Dialysis occurred through 2.3 mm (accumbens probe) or 4.2 mm (striatal probe) of a

semipermeable hollow fiber (copolymer of acrylonitrile and sodium methallyl sulfonate, I.D.=0.24 mm, 40,000 Daltons, Hospal). The recoveries in terms of percentages of DA, DOPAC, HVA and 5-HIAA were measured *in vitro* (Ungerstedt 1984); the mean (±S.E.M., n=4) recoveries were for the accumbens probes, 6.8±0.3 (DA), 4.8±0.4 (DOPAC), 4.7±0.4 (HVA), 4.7±0.4 (5-HIAA), and for the striatal probes, 13.1±1.4 (DA), 9.1±0.6 (DOPAC), 9.7±1.3 (HVA), 9.8±1.2 (5-HIAA). All perfusion experiments were carried out in awake, freely moving animals 48 h after surgery (approximately 72 h after the last bupropion or saline injection). Microdialysis experiments were performed simultaneously in the NAC and striatum. After stable baselines were established, each rat was injected with bupropion (25 mg/kg, i.p.).

The dialysis probes were perfused with a solution containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM sodium phosphate (pH 7.4). The perfusate was injected into the analytical system every 10 min (9.8 min for load and 12 sec for injection). Upon completion of the experiments, the animals were sacrificed, the brains were removed, sliced on a cryostat (30 μm), stained (Nissl), and examined microscopically for probe placement. Only rats with probes that were verified to be located in the NAC (plates 12-16, Pellegrino et al. 1979) or the dorsolateral striatum (plates 11-17, Paxinos and Watson 1986) were included.

Electrochemical detection was accomplished using an amperometric detector (BAS, LC4B) with a glassy carbon electrode (+0.7 V vs. a Ag/AgCl reference) and a coulometric detector (5100A Coulochem, ESA, Inc.) with a High Sensitivity Analytical Cell (5011). In the latter system detection of the amines was achieved by the sequential oxidation and reduction of samples (coulometric electrode=+0.4 V; amperometric electrode=-0.2 V). This arrangement allowed DOPAC, 5-HIAA and HVA to be detected at the coulometric cell, and DA at the subsequent amperometric electrode. During the microdialysis experiments locomotor activity was measured using the Digiscan Animal Activity Monitor.

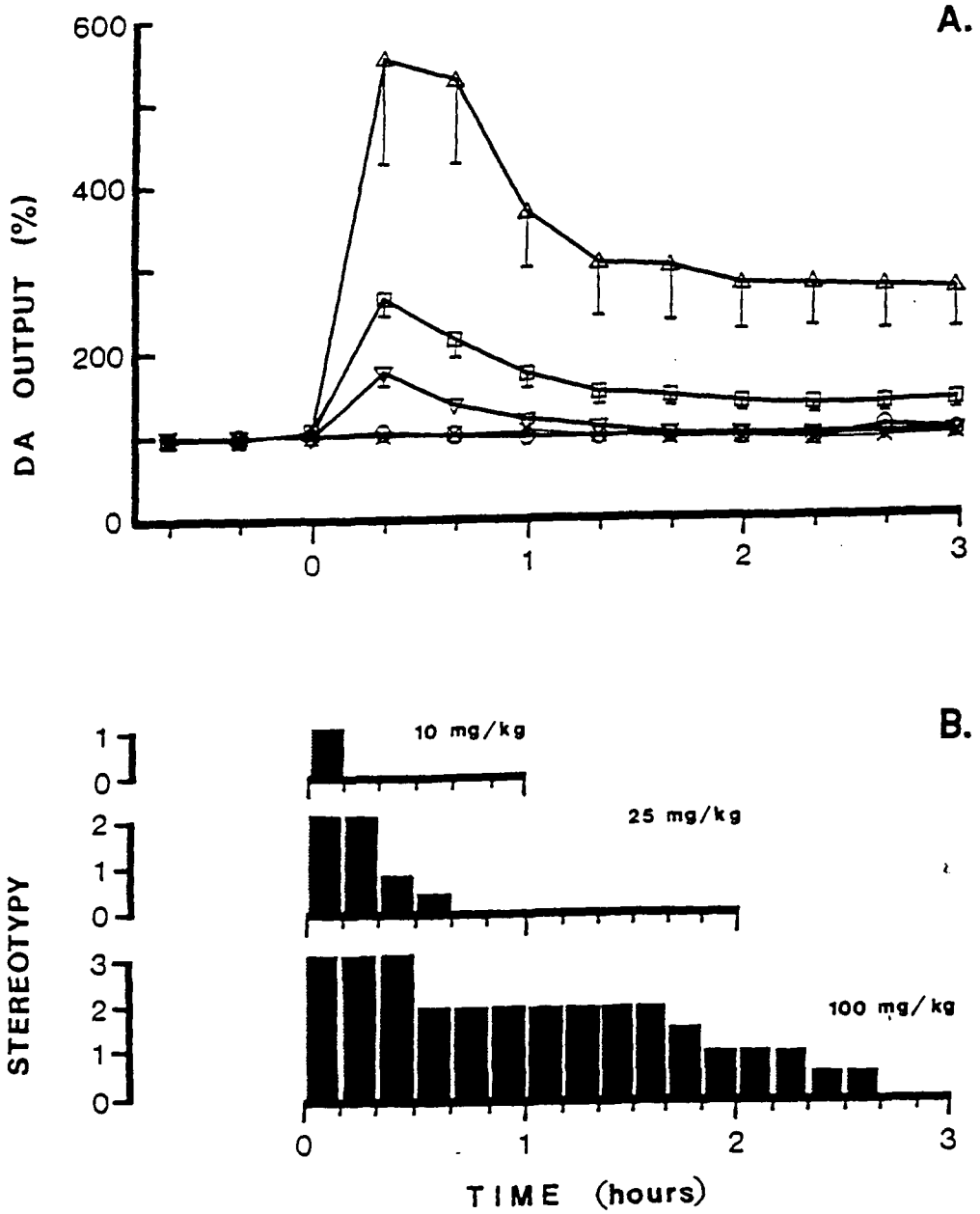
Microdialysis data were expressed in fmol/min. The mean absolute baseline values from NAC or striatum were evaluated by one-way (treatment: saline, acute bupropion, chronic bupropion) analysis of variance (ANOVA). For the purpose of graphic representation (Figures 17-20) absolute values were used. For statistical evaluation of the bupropion data, the absolute values (last baseline plus 12 post-treatment samples) were used. Data were analysed by two-way (treatment X time) ANOVA with repeated measures followed by Newman-Keuls tests for multiple comparisons. Locomotor activity measurements following bupropion administration (twelve 10 min blocks) were also subjected to two-way ANOVA with repeated measures.

(C) Results

(i) Acute effects of bupropion

Bupropion produced a dose- and time-dependent increase in extracellular DA in the striatum (Fig. 11A). This effect was maximal within the first 20 min interval (to 176%, 264% and 543% for 10, 25 and 100 mg/kg, respectively) and then gradually decreased to relatively stable levels for the remainder of the 3 h sampling period (100, 138 and 275%, respectively). Bupropion induced mild stereotypy in a dose- and time-dependent manner (Fig. 11B). During the first hour, the peak DA response was positively associated with the highest stereotypy scores after each dose of bupropion. However, during the stabilization phase (hours 2 and 3 post-injection), the relationship between extracellular DA and stereotyped behavior weakened considerably in the two high dose groups. Specifically, while the behavioral response decreased during this time, extracellular DA remained elevated at a relatively stable level. It is also noteworthy that after bupropion (100 mg/kg)

Figure 11. (A) Effects of bupropion HCl (circle-1 mg/kg, n=4), (inverse triangle-10 mg/kg, n=4), (square-25 mg/kg, n=9), (triangle-100 mg/kg, n=4) or saline (cross-1 ml/kg, n=4) on dialysate concentrations of dopamine from rat striatum. The overall mean (± S.E.M., n=25) baseline values of DA were: 40±6 fmol/min. (B) Effect of bupropion HCl (10 mg/kg, n=3), (25 mg/kg, n=7), (100 mg/kg n=4) on stereotyped behavior (group median scores).


A.
600
400
200
0
DA OUTPUT (%)
0
1
2
3
B.
10 mg/kg
25 mg/kg
100 mg/kg
STEREOTYPY
1
0
2
1
0
3
2
1
0
0
1
2
3
TIME (hours)

extracellular DA during the stabilization phase was higher than the maximal extracellular DA levels response to 25 mg/kg bupropion. Nevertheless, the stereotypy score was lower in the 100 mg/kg group during the stabilization phase than the 25 mg/kg group's peak behavioral response (Fig. 11B).

Figure 12 shows that bupropion tended to decrease extracellular DOPAC concentrations in a dose-independent manner ($H=8.24$, $0.10>p>0.05$). In contrast, the drug induced a significant, dose-dependent increase in 5-HIAA ($H=10.58$, $p<0.05$). Compared to the effect of the drug on DA and its metabolites, its effects on 5-HIAA were considerably delayed. The effect of bupropion on HVA was also statistically significant ($H=10.56$, $p<0.05$); the lower dose of bupropion (10 mg/kg) decreased HVA to 83% of basal values, while 25 mg/kg and 100 mg/kg increased HVA by up to 112% and 140% respectively. The lowest dose of bupropion (1 mg/kg) did not change extracellular DA, DOPAC, HVA or 5-HIAA, nor did it produce significant behavioral effects.

Figure 13 demonstrates that a second injection of bupropion (25 mg/kg), 3 h after the first, produced similar increases in extracellular DA concentrations (first +290%, second +300%). In both cases the DA response peaked 20 min after the injection. In a second group of animals TTX (10^{-6}M) infusions were started 2 h after the first bupropion injection. Forty to sixty min later the extracellular DA concentrations had decreased to about 20% of basal values. Furthermore, TTX blocked the effect of the second injection of bupropion on extracellular DA.

Bupropion (25 mg/kg, i.p.) had similar effects on extracellular DA concentrations in the striatum and the NAC, both in terms of peak response and duration of effect (Fig. 14). The DA increase peaked in both regions 20 min postdrug (+243% for striatum and +286% for NAC) and then declined, neither structure returning to baseline values within 6 h. Although the effects of bupropion on striatal and accumbens DOPAC and 5-HIAA were similar, there was a tendency for a differential effect on HVA, there being a 10% increase

Figure 12. Effect of bupropion HCl on extracellular concentrations of DOPAC, HVA, and 5-HIAA from rat striatum (legends as in Figure 11). Arrows indicate the injection of the drug. The overall mean (± S.E.M., n=25) baseline values are: 2126±190 for DOPAC, 1109±90 for HVA, and 640±44 for 5-HIAA (fmol/min).

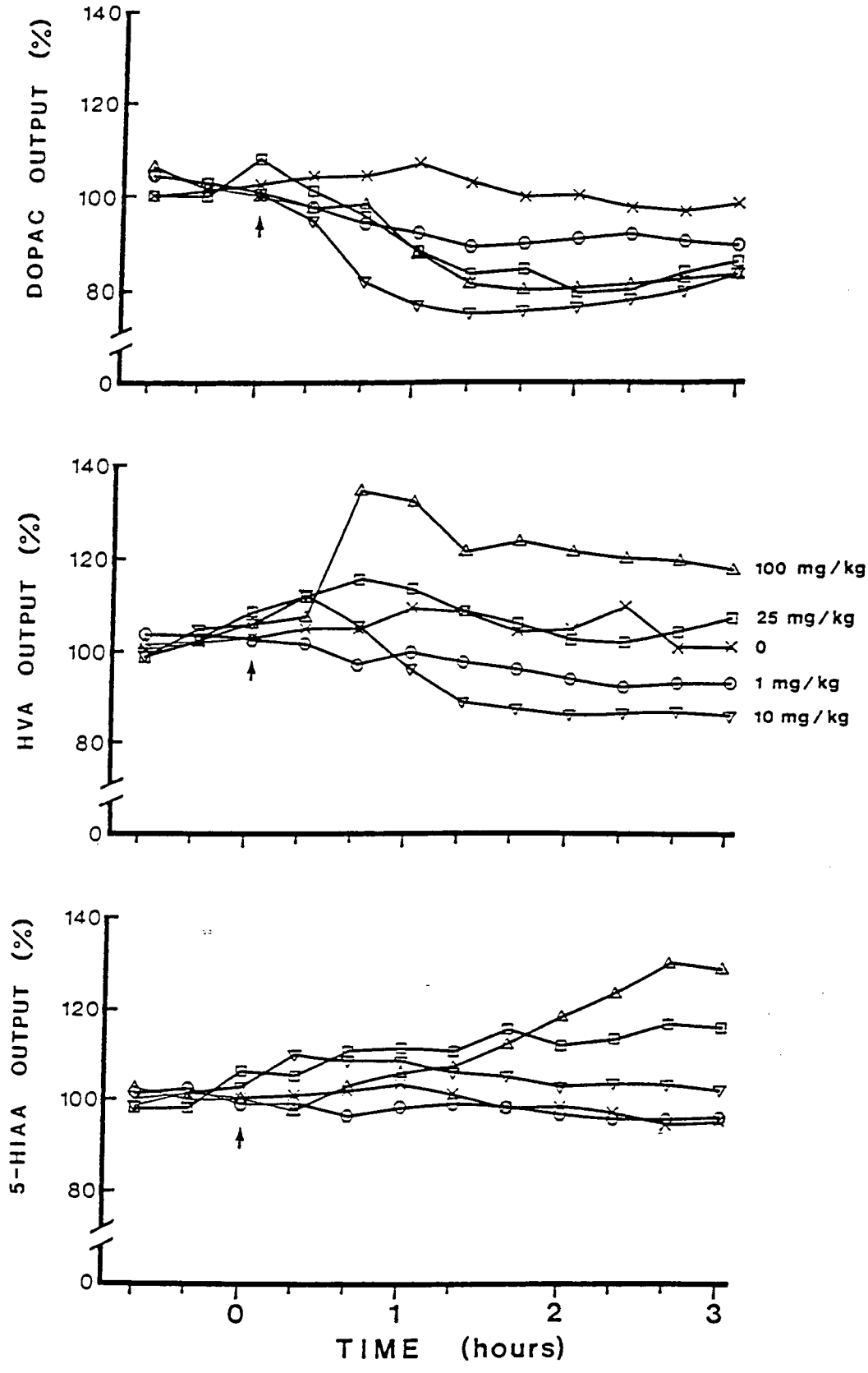

DOPAC OUTPUT (%)
140
120
100
80
0
HVA OUTPUT (%)
140
120
100
80
0
100 mg/kg
25 mg/kg
0
1 mg/kg
10 mg/kg
5-HIAA OUTPUT (%)
140
120
100
80
0
0
1
2
3
TIME (hours)

Figure 13. Effect of consecutive injections of bupropion HCl (25 mg/kg, i.p.) given 3 hours apart on dialysate striatal concentrations of DA (n=7). In one experiment (n=3) TTX (1 μM) was infused (shaded area). Arrows indicate the injection of the drug.

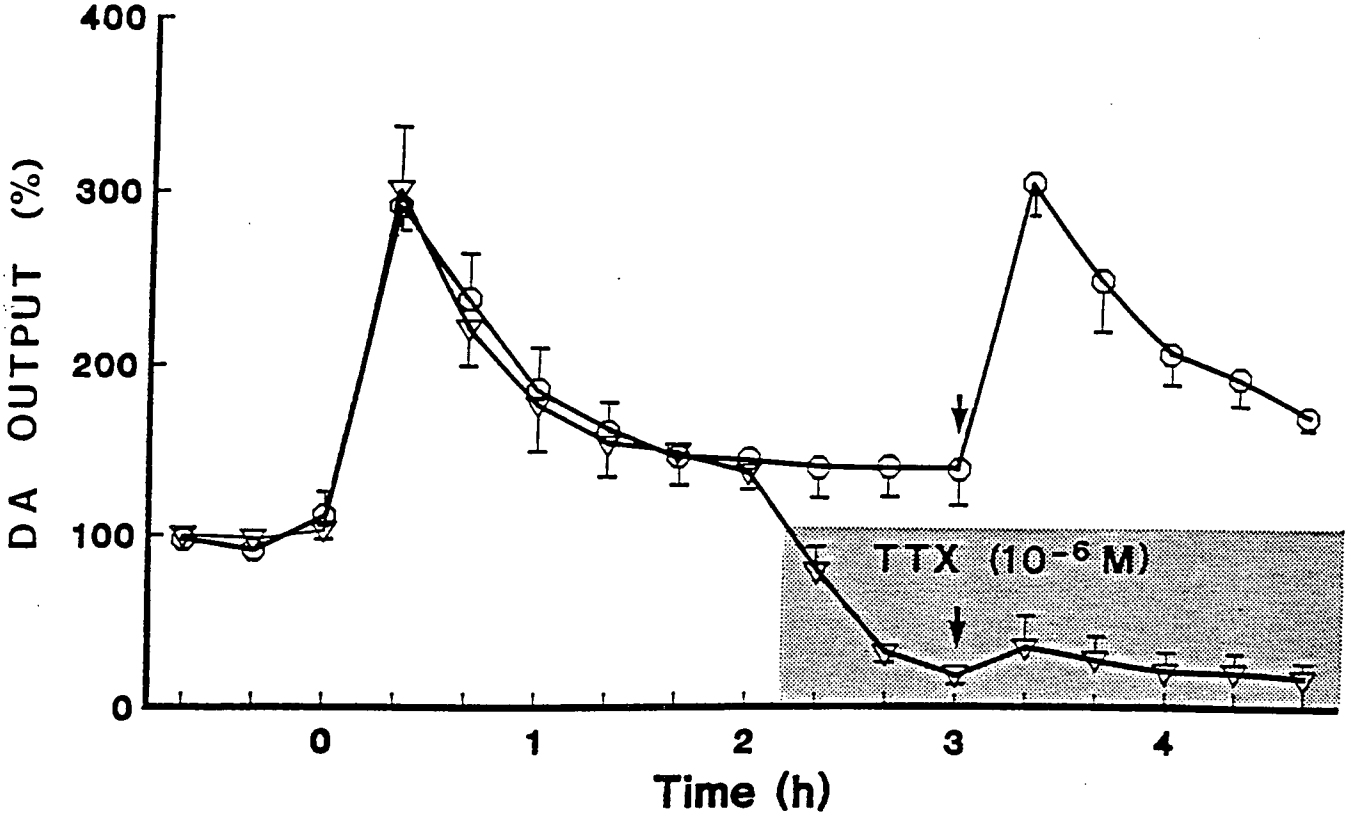
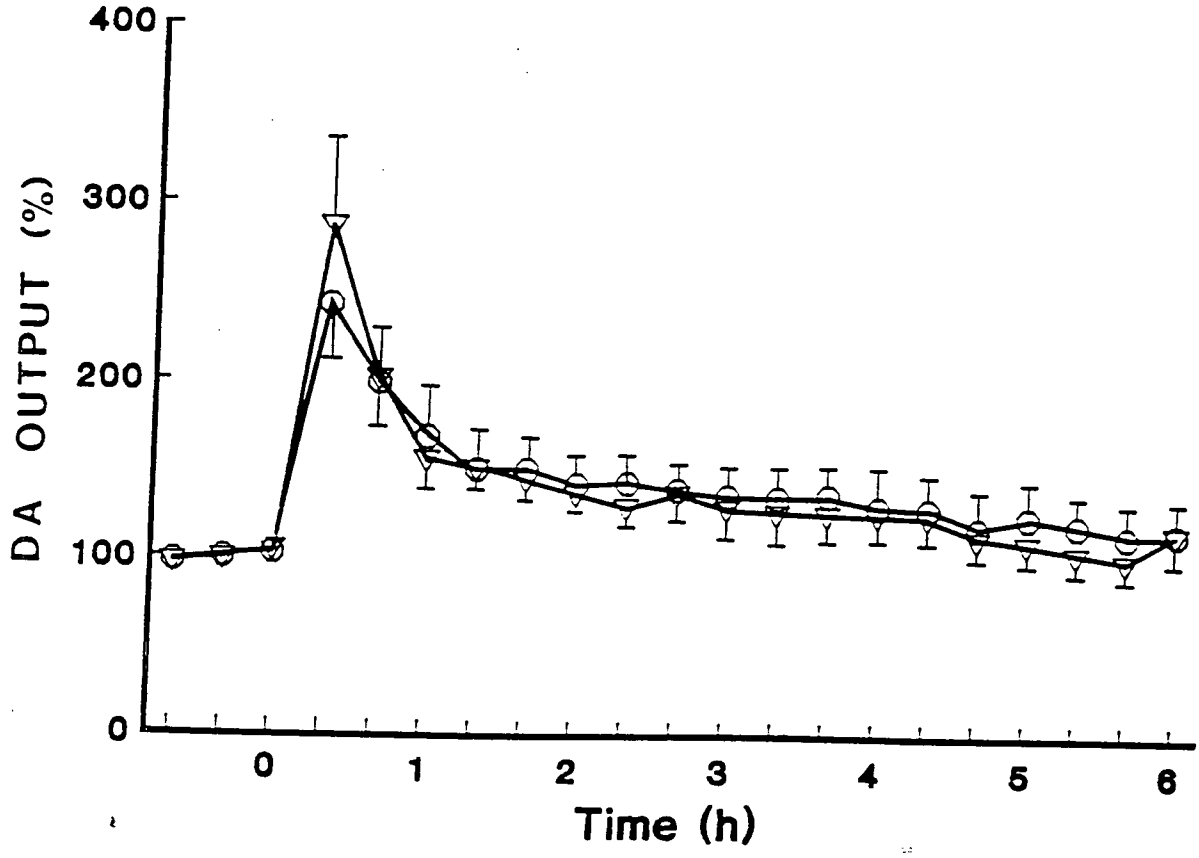

400
300
200
100
0
DA OUTPUT (%)
TTX (10-6 M)
0
1
2
3
4
Time (h)

Figure 14. Effect of bupropion HCl (25 mg/kg, i.p.) on dialysate concentrations of dopamine from the rat striatum (circle) or nucleus accumbens (triangle). The mean (± S.E.M.) baseline values for DA (fmol/min) are: 63±15 (n=5, striatum) and 10±2 (n=4, nucleus accumbens).


400
300
200
100
0
DA OUTPUT (%)
0
1
2
3
4
5
6
Time (h)

in striatum in contrast to a 20% decrease in the NAC ($F_{17,119}$=1.8, 0.10>p>0.05, data not shown).

(ii) In vivo characterization of bupropion

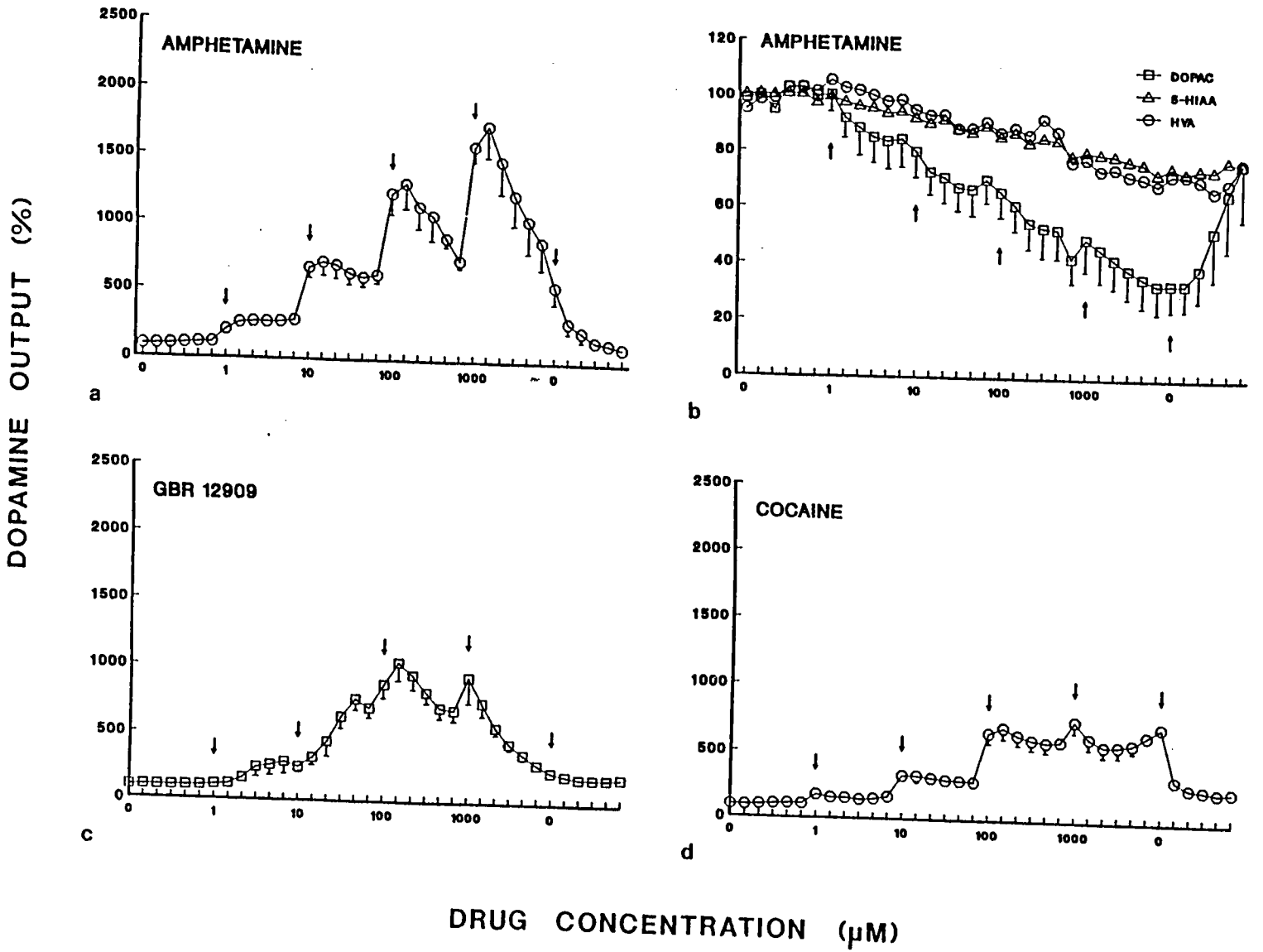
The basal value of striatal DA obtained from all animals used in the first experiment (n=28) was 34.7±2.7 fmol/min. Basal DA values did not differ significantly between any of the drug groups.

Intrastriatal infusion of d-amphetamine produced a dose-dependent increase in DA in the dialysate (Fig. 15a). The lowest dose (1 μM) produced a significant (p<0.05) increase which remained relatively constant throughout the 60 min test period. At 10 μM the DA response peaked during the first two 10 min intervals and decreased gradually thereafter. This pattern, though more pronounced, was also observed during subsequent dose increments. When d-amphetamine was replaced by Ringers solution, DA rapidly decreased and almost returned to baseline levels within 60 min.

Infusion of 1 or 10 μM GBR 12909 increased dialysate DA gradually, with the response peaking 50 minutes after the introduction of the drug into the perfusate (Fig. 15c). The GBR 12909-induced increase was statistically significant at the lowest drug concentration in the first 10 min sample (p<0.05). 100 μM further transiently increased DA in the dialysate, this being followed by a decline after 20 minutes. This pattern was also observed at the highest dose of GBR 12909 (1 mM), though the decline of DA was more pronounced. When the GBR 12909 was withdrawn from the Ringers the decline of DA continued and appeared to stabilize at a level twice that of baseline.

Intrastriatal infusion of 1, 10 and 100 μM cocaine (Fig. 15d) increased the outflow of DA in a dose-dependent manner, and these increases remained relatively stable for the duration of each drug concentration. The increase in DA was already significant (p<0.05) at the 1 μM concentration in the first 10 min sample. Infusion of 1000 μM cocaine produced

Figure 15. a: Effect of local infusion of 1, 10, 100 and 1000 μM d-amphetamine on dialysate concentrations of dopamine from rat striatum. Each point represents the mean (± S.E.M.) percent change of dopamine output during a 10 min sample relative to the drug-free perfusion hour. The first sample after the infusion of each dose is indicated by arrows. The overall baseline values of dopamine are 35±3 (mean±S.E.M., n=28, fmol/min). b: Effect of d-amphetamine on dialysate concentrations of DOPAC, HVA and 5-HIAA. Mean (± S.E.M.) baseline values for DOPAC, HVA and 5-HIAA are 3161±133, 1613±165, 714±47 fmol/min respectively (n=4). c: Effect of local application of 1, 10, 100 and 1000 μM of GBR 12909 on dialysate striatal dopamine concentrations. d: Effect of local application of cocaine on dialysate dopamine concentrations.


DOPAMINE OUTPUT (%)
AMPHETAMINE
a
AMPHETAMINE
DOPAC
5-HIAA
HVA
b
GBR 12909
c
COCAINE
d
DRUG CONCENTRATION (μM)

a further small, transient increase after which the concentration of DA in the dialysate stabilized at the same level produced by a dose that was ten times lower. When cocaine was deleted from the Ringers solution the DA levels declined rapidly, stabilizing at 2.4 times baseline values after 1 h.

Nomifensine (Fig. 16a), methylphenidate (Fig. 16b), bupropion (Fig. 16c) and benztropine (Fig. 16d) increased DA in the dialysates in a dose-dependent manner. The increases were significant ($p<0.05$) at the lowest concentration in the first sample. The lower concentrations (1, 10 and 100 μM) of these compounds increased DA in a relatively constant manner across each 1 h infusion period. However, infusion of 1 mM methylphenidate and benztropine caused the DA outflow to peak in the first sample and this was followed by a marked decline over the next 50 min of the infusion period. When the drug solutions were replaced by the vehicle there was an immediate marked decrease in DA that declined after 1 h to 3, 1.4, 2 and 4.7 times baseline concentrations for nomifensine, methylphenidate, bupropion and benztropine, respectively.

The primary DA metabolite DOPAC decreased as a function of the concentration of d-amphetamine to a final level of 33% of baseline concentrations (Fig. 15b). The secondary DA metabolite HVA and the serotonin metabolite 5-HIAA decreased to 75% of baseline values. Replacement of d-amphetamine with perfusion solution resulted in an gradual return of these metabolite concentrations to baseline values. Intrastriatal infusion of GBR 12909, cocaine, nomifensine, methylphenidate, bupropion and benztropine up to 1 mM failed to change significantly the dialysate concentrations of DOPAC, HVA or 5-HIAA (data not shown).

The mean (±S.E.M.) basal values of DA in the experiment with TTX were 41±4.5 fmol/min (n=14). Addition of TTX ($3x10^{-7}$ M) to the perfusate decreased the concentration of DA to less than 10% of the basal value. The percent increases in DA over the pre-TTX baseline values are presented (Table II) either in the absence (column A) or presence (column B) of TTX. Column C represents the percent TTX-independent effect (B/A). The

Figure 16. Effect of local infusion of 1, 10, 100 and 1000 μM of nomifensine (a), methylphenidate (b), bupropion (c), benztropine (d) on dialysate concentrations of dopamine from rat striatum. Each point represents the mean (± S.E.M.) percent change of dopamine ouput during a 10 min-interval sample.

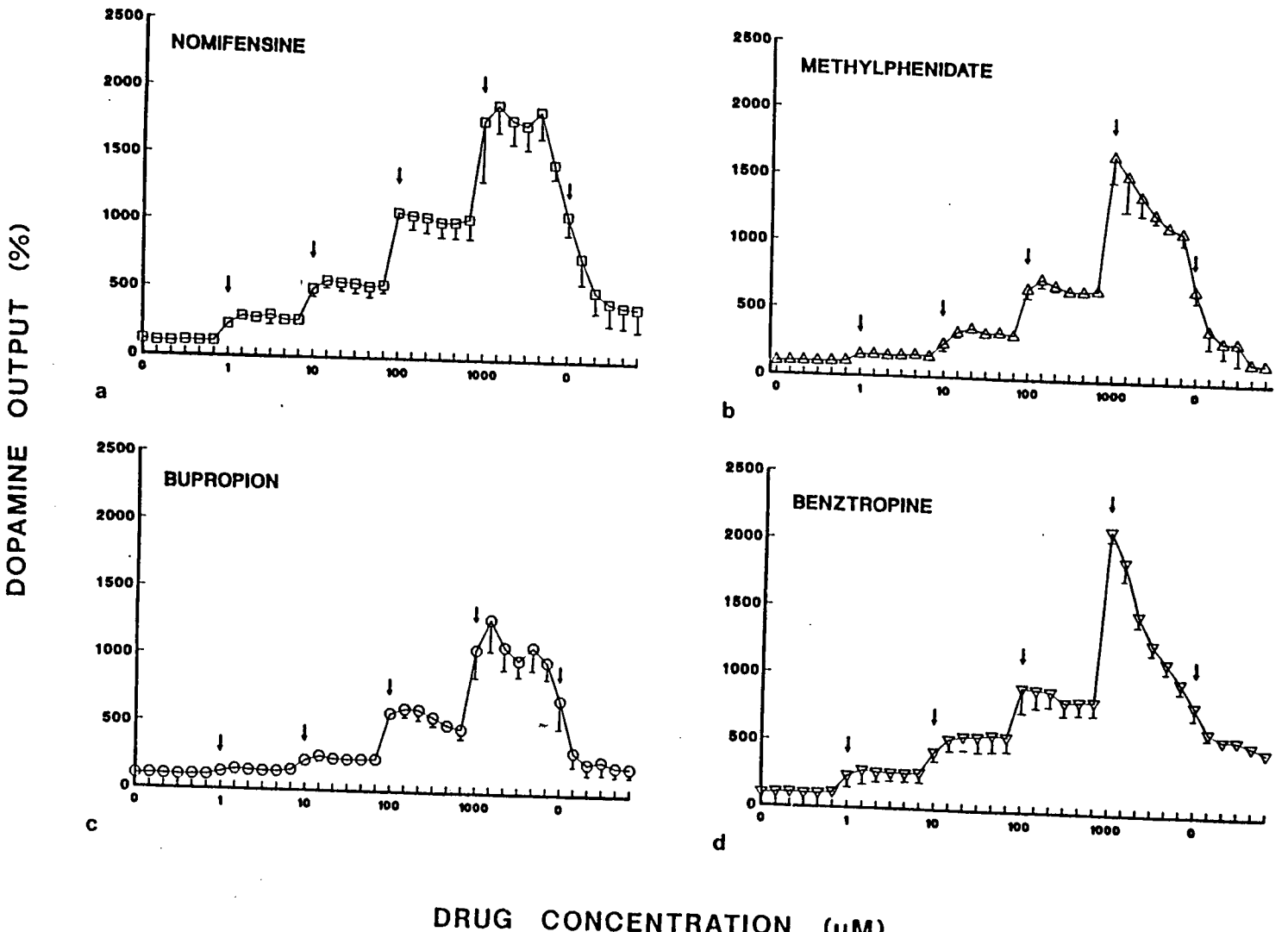

DOPAMINE OUTPUT (%)
NOMIFENSINE
METHYLPHENIDATE
BUPROPION
BENZTROPINE
a
b
c
d
2500
2000
1500
1000
500
0
0
1
10
100
1000
DRUG CONCENTRATION (μM)

Table II.

Effects of different concentrations of dopamine uptake inhibitors, coinfused with TTX, on the release of dopamine

		A	B	C
		Drug	Drug+TTX	TTX-independent
	(μM)	effect	effect	effect (% B/A)
AMPH	100	443±168	416±69	94
GBR	10	110±50	5±2	5
GBR	100	691±161	260±27	38
COC	100	350±40	24±10	7
COC	1000	757±167	57±12	7
NOM	10	440±40	21±9	5
NOM	1000	2117±492	109±31	5
BUPR	100	239±97	40±21	17
BUPR	1000	742±75	87±19	12
MPD	100	333±85	24±2	7
MPD	1000	1613±431	87±20	5
BENZ	10	255±82	36±18	14
BENZ	1000	1712±145	780±231	46

Data are expressed as the percent increase (±S.E.M., n=3-4) relative to the respective baseline DA concentrations measured in the absence of TTX. Column A represents the percent increase in the absence of TTX and column B indicates the percent increase in the presence of TTX. Amphetamine (AMPH), GBR 12909 (GBR), cocaine (COC), nomifensine (NOM), bupropion (BUPR), methylphenidate (MPD) and benztropine (BENZ).

effects of local application of d-amphetamine (100 μM) on extracellular DA was not affected by TTX. The effects of a low dose (10 μM) of GBR 12909 and benztropine were almost completely prevented by the toxin. However, the effects of higher concentrations of GBR 12909 (100 μM) and benztropine (1000 μM) were only partly affected by TTX. The effects of both concentrations of the other compounds (bupropion, cocaine, methylphenidate and nomifensine) were blocked by the coinfusion of the toxin.

The *in vitro* recoveries varied between the drugs under study (Table III, Column B), and were positively correlated (Spearman Rank correlation coefficient $r=0.79$; $p<0.05$) with the molecular weights (Column A). The recovery of each drug did not differ between the 10^{-3} and 10^{-4} M beaker concentrations. In order to determine the potency (Column E) of the uptake inhibitors with respect to increasing extracellular DA, we calculated the ratio between the amount of drug infused (Column C) and the drug-induced increase in DA in the dialysate (Column D). The amount of drug infused (Column C) in pmol/h was calculated using the formula: Drug concentration X Infusion flow rate X Infusion period X Recovery. DA ouput (Column D) was the average drug-induced increase (percent of baseline) over the 1 h infusion period. The drug potency calculation was performed at three different drug infusion concentrations (1, 10 and 100 μM). The rank order potency of the various drugs varied only slightly as a function of drug concentrations, with minor ranking changes taking place between nomifensine, methylphenidate and amphetamine at some concentrations. The rank order of potency in Table III represents data obtained only with the 10 μM drug concentration. When considered across drug concentrations, the relative potencies with respect to increases in extracellular DA were: GBR 12909> benztropine> amphetamine = nomifensine = methylphenidate > cocaine > bupropion.

Table III.

Relative *in vivo* potencies of dopamine uptake inhibitors as determined by microdialysis

Drug	A Molecular weight	B Recovery (%)	C Drug infused (pmol/hr)	D DA output (% baseline)	E Potency (D/C)
BUPR	240	8.3	249	233	0.94
COC	303	7.0	212	309	1.46
MPD	233	5.2	155	318	2.05
NOM	238	8.3	249	528	2.12
AMPH	135	8.6	258	652	2.42
BENZ	307	5.0	150	523	3.49
GBR	450	2.0	60	521	8.68

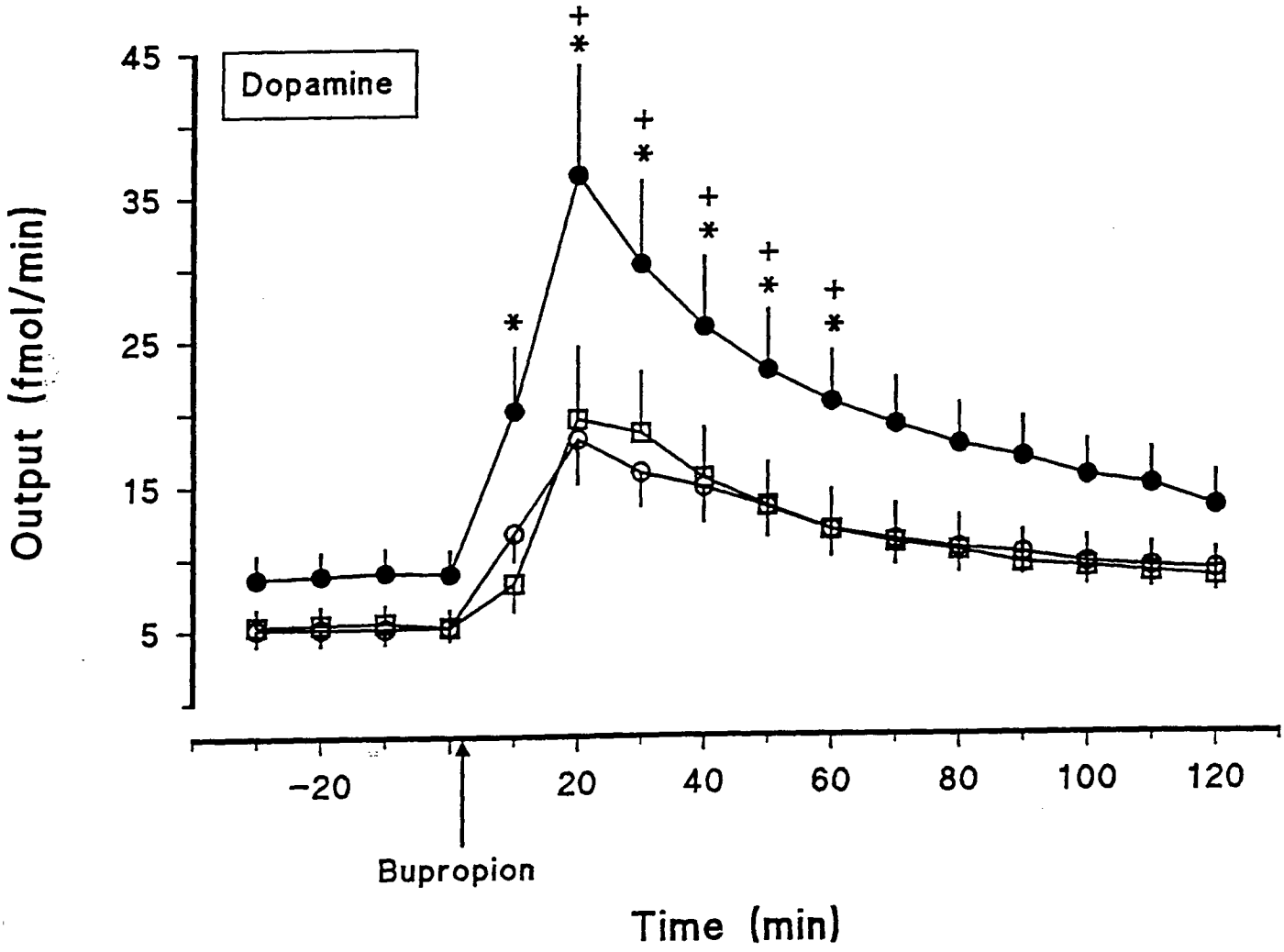
The amount of the drug infused (column C) was calculated by using the formula: Drug concentration x Infusion flow rate x Infusion period x Recovery. Thus, C= 10 μmol/l x 5 μl/min x 60 min x B. The DA output (column D) represents the average drug induced increase of DA over a 60 min infusion period (derived from Figures 1 and 2). Potency is calculated as the ratio between the amount of drug infused and DA output. The abbreviations are as in Table II.

(iii) Chronic effects of bupropion

In toto, 23 rats were implanted with microdialysis probes in the NAC and striatum. Seven were chronically treated with saline, 9 with chronic bupropion, and 7 were acutely treated with bupropion. Due to technical difficulties (leaking or blocked probes, probe misplacement), data from the NAC or the striatum of a few rats were not obtained or were excluded from the analysis. Six saline, 8 chronic bupropion, and 6 acute bupropion rats had viable double implantations; data from two more rats (saline-NAC, acute bupropion-striatum) were included in the analysis. Basal dialysate concentrations of DA, DOPAC, HVA, and 5-HIAA from the NAC and striatum of rats receiving saline, acute bupropion, or chronic bupropion treatment, are presented in Figures 17-20. A one-way ANOVA indicated that neither chronic nor acute bupropion treatment significantly influenced the basal concentrations of DA or its metabolites. There was, however, a tendency for the DA concentrations in the NAC of the chronic bupropion group to be higher (Fig. 17), although this difference did not reach statistical significance ($F_{2,18}$=2.94, $p>0.05$). Nevertheless, because the basal values were 60% higher in the chronic group, it was deemed inappropriate and potentially misleading to present any of the neurochemical data as percent scores.

Bupropion (25 mg/kg, i.p.) produced a marked increase in interstitial concentrations of DA from the NAC of all three groups (Fig. 17). The effect of bupropion peaked at 20 min after which DA gradually declined towards baseline values. Chronic bupropion enhanced the DA response to the challenge bupropion injection throughout the experimental period, although the temporal pattern was similar. A repeated measures ANOVA performed on the absolute values from all three groups, revealed a statistically significant treatment X time interaction ($F_{24,216}$=2.13, $p<0.005$). The values for treatment and time effects were $F_{2,18}$=2.9 ($p>0.05$) and $F_{12,216}$=28.8 ($p<0.001$), respectively. Post-hoc comparisons with controls showed significantly ($p<0.05$) higher DA responses after bupropion in the chronic bupropion group at the 10-60 min post-injection intervals. The chronic bupropion group

Figure 17. Effect of bupropion (25 mg/kg, i.p.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with saline (open squares, n=7), acutely treated with bupropion (open circles, n=6) or chronically treated with bupropion (solid circles, n=8). Group mean (± S.E.M.) baseline values in fmol/min are 5.4±1.3, 4.5±1.1, and 8.8±1.7 for saline, chronic bupropion and acute bupropion groups, respectively. *: $p<0.05$, in comparison to saline group; +: $p<0.05$, in comparison to acute bupropion group


Dopamine
Output (fmol/min)
45
35
25
15
5
-20
20
40
60
80
100
120
Bupropion
Time (min)

differed significantly from the acute group between 20 and 60 min after the injection. The saline and acute bupropion groups did not differ at any time. The average peak increases of interstitial DA concentrations estimated from dialysates (Δ from baseline) after bupropion challenge in all three groups are presented in Table IV. The largest value for each individual animal was used to calculate these differences which in all cases occurred within 30 min post-injection. All values were corrected for probe recoveries (6.8% for NAC and 13.1% for striatum). Although there are certain problems (Wages et al. 1986; Benveniste 1989) in estimating actual interstitial concentrations using *in vitro* recoveries, this procedure allowed direct comparisons of bupropion-induced changes of DA in the NAC and the striatum (Table IV). One-way ANOVA revealed that bupropion-induced increase in interstitial DA concentrations was significantly ($F_{2,18}$=3.73, p=0.04) higher in the NAC of the chronic bupropion group in comparison to saline and acute bupropion groups (both p<0.05, Newman-Keuls).

A challenge dose of bupropion modestly decreased dialysate concentrations of DOPAC, did not influence HVA, and modestly increased 5-HIAA (Fig. 18). Chronic treatment with bupropion did not affect any of these responses. Hence, none of the treatment, time, or treatment X time interaction effects were statistically significant.

Bupropion increased dialysate concentrations of DA from the striata of all 3 groups of animals (Fig. 19). Repeated measures ANOVA performed on the absolute values did not detect statistically significant differences between the groups; the treatment effect and the treatment X time interaction were $F_{2,18}$=1.07 and $F_{24,216}$=0.99, respectively; however, the effect of time was highly significant $F_{12,216}$=19.14 both p<0.0001. The temporal profile and the magnitude of the increase in DA efflux following bupropion did not differ between the striatal and accumbens probes (compare saline groups in Figures 17 and 19). The magnitude of the peak bupropion-induced increases in extracellular DA in the striatum and the NAC did not differ significantly (Table IV). Bupropion did not influence significantly the dialysate concentrations of DOPAC, HVA, 5-HIAA in any of the groups (Fig. 20).

Table IV.

Bupropion-induced increases in interstitial dopamine concentrations in the nucleus accumbens and striatum

	Control	*Acute Bupropion*	*Chronic Bupropion*
Nucleus Accumbens	43.6±9.6	38.2±5.2	81.3±16*
Striatum	32.6±7.6	34.8±11.4	50.1±16.9

Values (nM) represent the difference (Δ) between the last baseline sample and the maximal increase in interstitial concentrations of dopamine following bupropion (corrected for probe recoveries). *: $p<0.05$ in comparison to control and acute bupropion groups. Data represent means (±S.E.M.) of 6-8 animals per group.

Figure 18. Effect of bupropion (25 mg/kg, i.p.) on dialysate concentrations of DOPAC, HVA, and 5-HIAA from the nucleus accumbens of rats chronically treated with saline, bupropion or acutely treated with bupropion. Each point represents the group mean (± S.E.M.) change of absolute values in fmol/min.

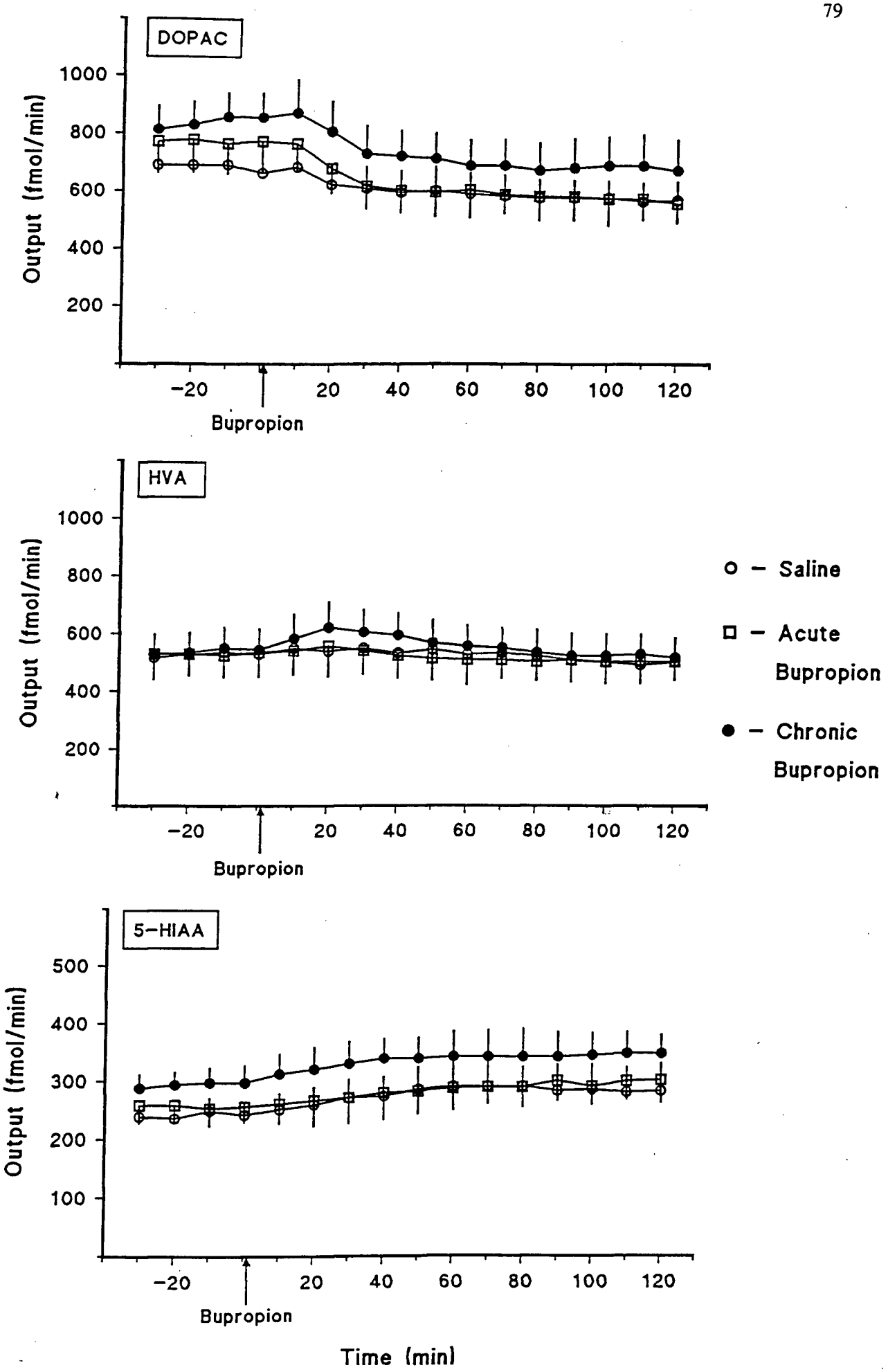

DOPAC
HVA
5-HIAA
Output (fmol/min)
1000
800
600
400
200
500
300
100
-20
20
40
60
80
100
120
Bupropion
Time (min)
o – Saline
□ – Acute Bupropion
● – Chronic Bupropion

Figure 19. Effect of bupropion (25 mg/kg, i.p.) on dialysate concentrations of dopamine from the striatum of rats chronically treated with saline (open squares, n=6), acutely treated with bupropion (open circles, n=7), or chronically treated with bupropion (solid circles, n=8). Group mean (± S.E.M.) absolute values in fmol/min are 9.4±1.9, 7.6±1.6, and 11.5±1.7 for saline, acute and chronic bupropion respectively.

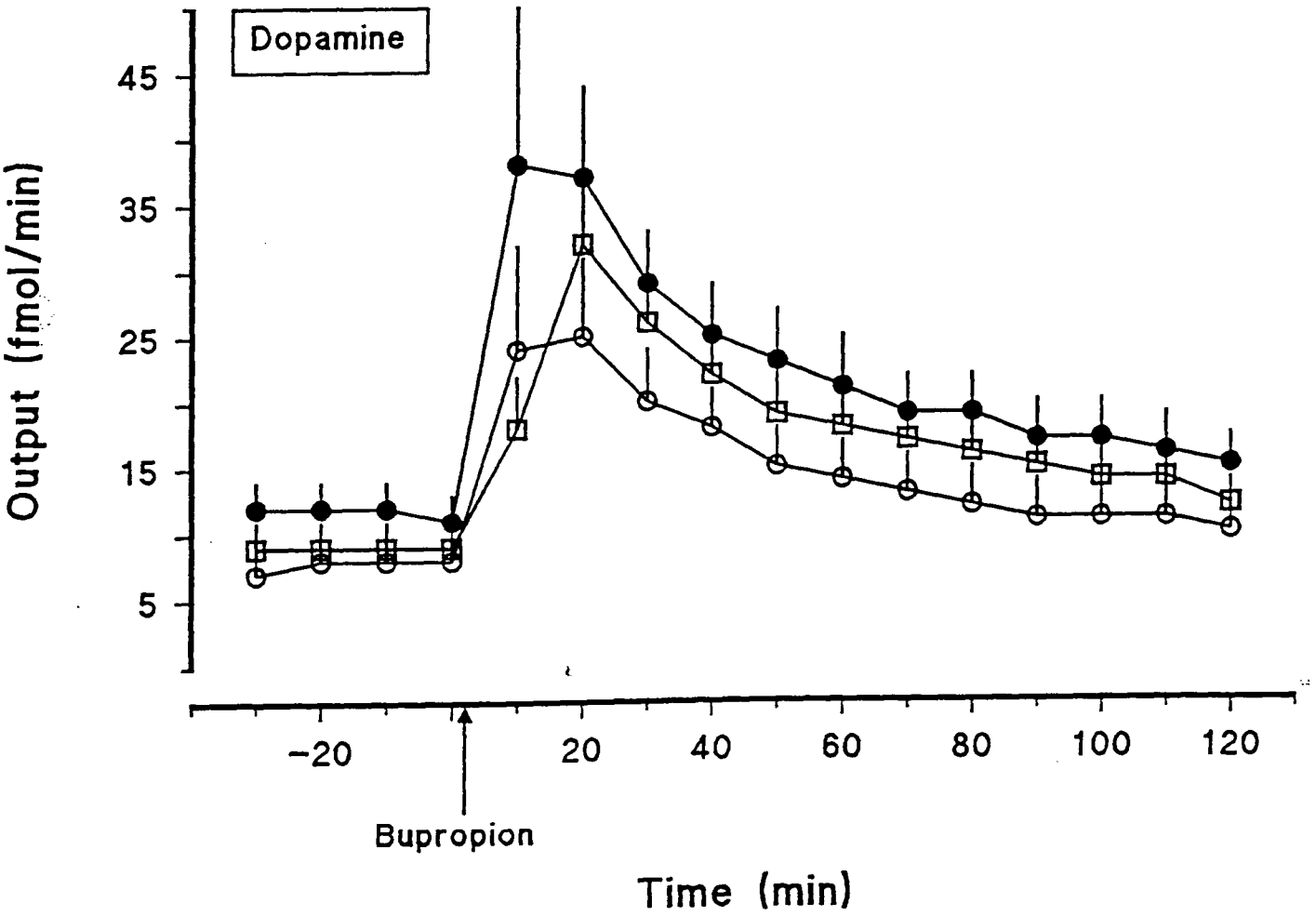
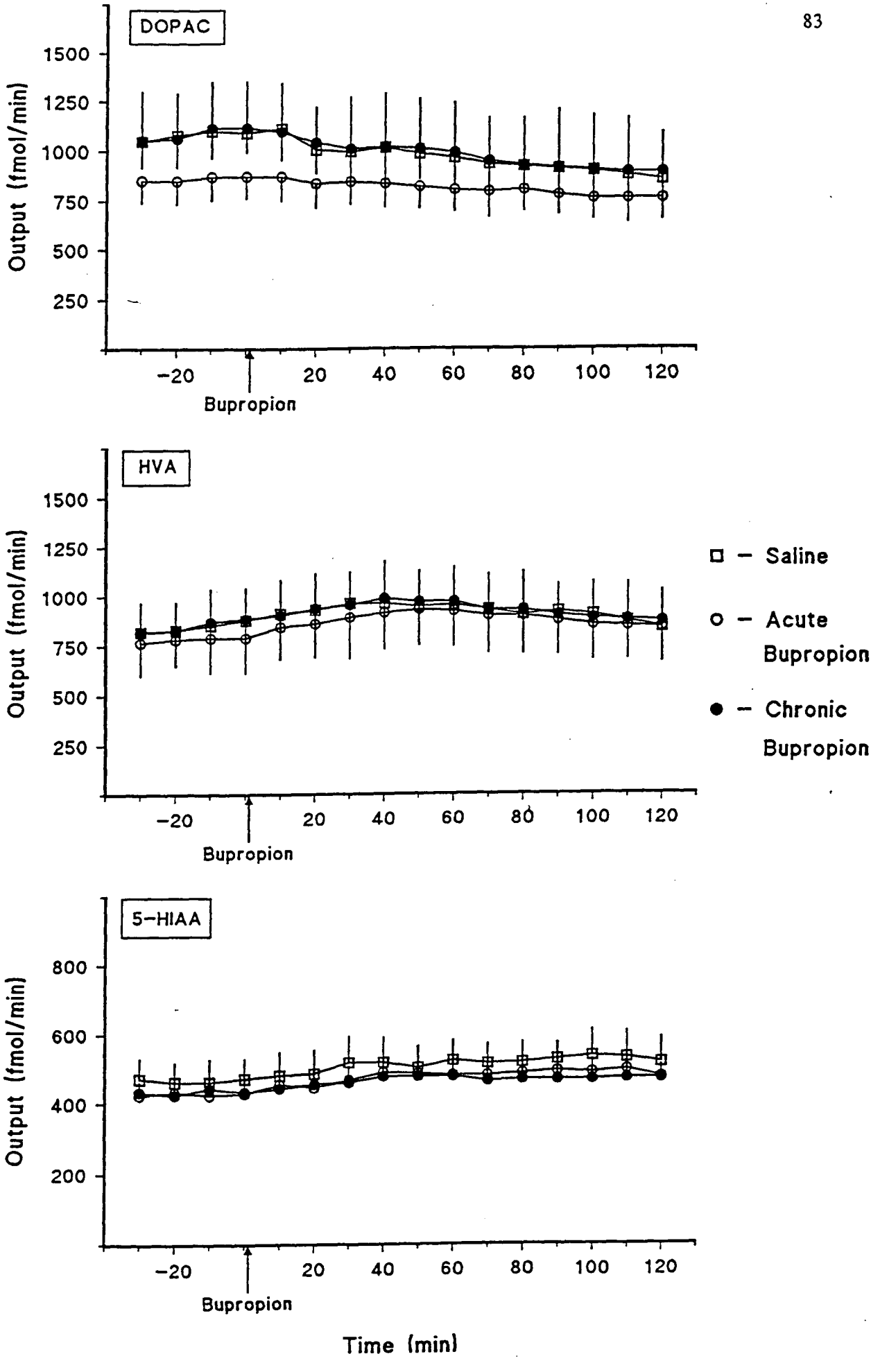

Dopamine
45
35
25
15
5
Output (fmol/min)
-20
20
40
60
80
100
120
Bupropion
Time (min)

Figure 20. Effect of bupropion (25 mg/kg, i.p.) on dialysate concentrations of DOPAC, HVA, and 5-HIAA from the striatum of rats following chronic treatment with saline, bupropion, or acute treatment with bupropion. Each point represents the group mean (± S.E.M.) change of output (absolute values in fmol/min).

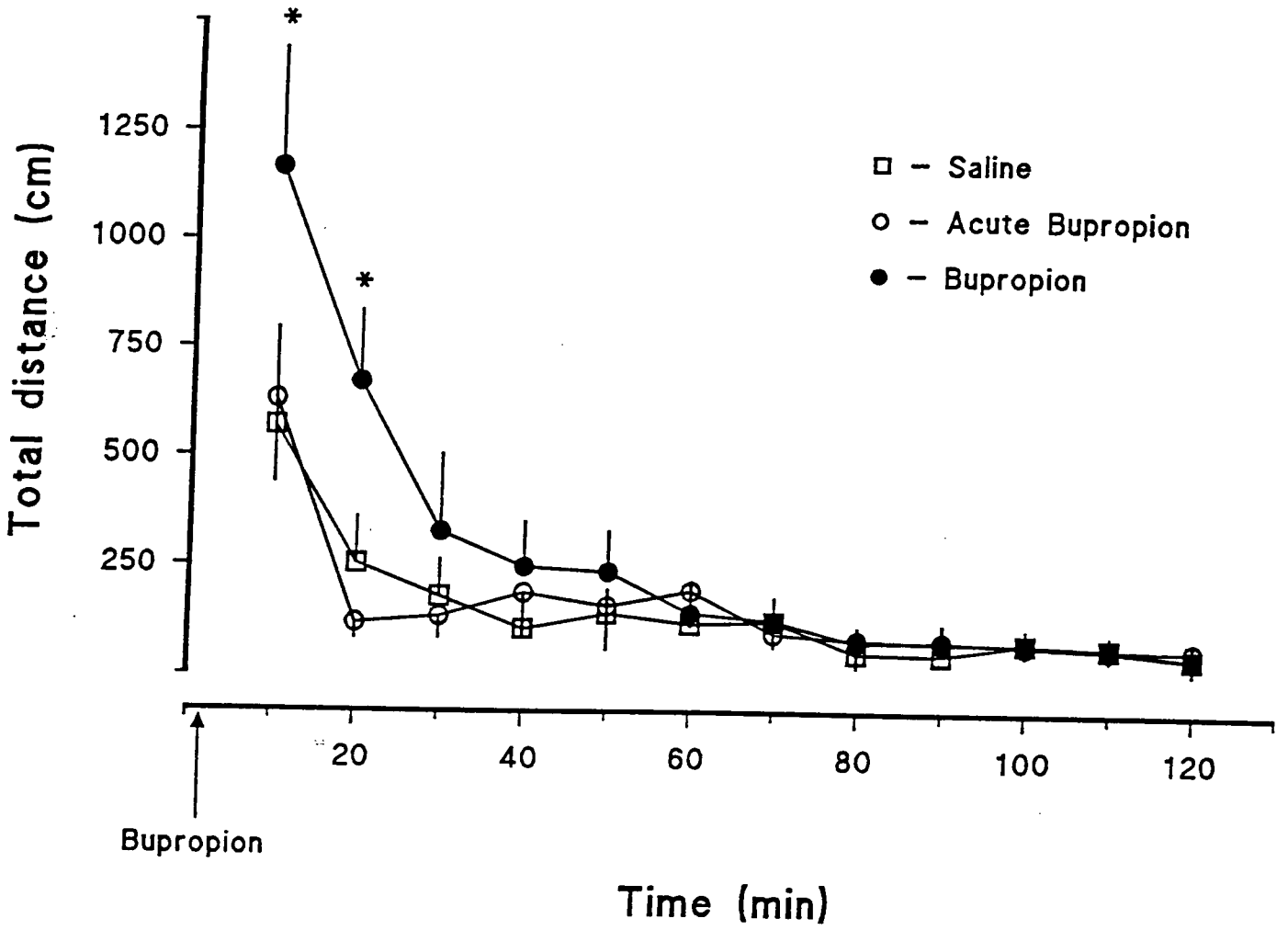

DOPAC
HVA
5-HIAA
Output (fmol/min)
1500
1250
1000
750
500
250
800
600
400
200
-20
20
40
60
80
100
120
Bupropion
Time (min)
□ – Saline
○ – Acute Bupropion
● – Chronic Bupropion

Locomotor activity (total distance expressed in cm) following bupropion administration is illustrated in Figure 21. Locomotor activity scores peaked within 10 min post-injection and then, in contrast to the DA responses, rapidly declined (compare Figs. 17 and 21). Bupropion-induced hypermotility was more pronounced in the chronic bupropion group. A repeated measures ANOVA performed on the behavioral data from all three groups following bupropion indicated significance in time effect ($F_{12,198}$=16.41, p<0.0001) and treatment X time interaction ($F_{22,198}$=2.07, p=0.0049), but not in treatment effect ($F_{2,18}$=1.37, p=0.27). Post-hoc comparisons (Newman-Keuls) revealed significant differences between the three groups in the first 20 min following bupropion administration (see Fig. 21).

(C) Discussion

Bupropion produced a dose-dependent increase in extracellular DA which was associated with drug-induced stereotypy during the first h after the drug injection. The dissociation between biochemical and behavioral response during the late postdrug period suggests that during later stages of the drug action, adaptive, compensatory processes may come into effect that serve to alter the relationship between extracellular DA and behavior. At present, the nature of these compensatory mechanisms is unknown although rapidly adapting postsynaptic mechanisms such as changes in DA receptor affinity or second messenger systems are obvious candidates. A more detailed behavioral analysis is required before firmer conclusions can be reached concerning the relationship between drug-induced extracellular DA changes in the striatum and stereotyped behavior. It is noteworthy, however, that in some instances previous investigators have also noted the absence of simple, linear relationships between drug-induced extracellular DA concentrations and behavior (Sharp et al. 1987; Kuczenski and Segal 1989; Kalivas and Duffy 1990).

Figure 21. Effect of bupropion (25 mg/kg, i.p.) on locomotor activity measurements (total distance-cm) of rats following chronic treatment with saline (open squares, n=7) or bupropion (solid circles, n=8) or acute treatment with bupropion (open circles, n=6). Arrow indicates the injection of bupropion. Data points represent group mean (± S.E.M.) activity counts over 10 min intervals. * $p<0.05$, in comparison to saline and acute bupropion groups


Total distance (cm)
1250
1000
750
500
250
*
*
□ – Saline
○ – Acute Bupropion
● – Bupropion
20
40
60
80
100
120
Bupropion
Time (min)

A comparison of the dose-dependent increase in extracellular DA produced by peripheral injections of bupropion with other DA uptake inhibitors raises a number of questions regarding the mechanism of action of this drug. GBR 12909, a relatively selective and potent DA uptake inhibitor, increases extracellular DA in a gradual manner and has no effect on DOPAC and HVA (Westerink et al. 1987b). Nomifensine increases extracellular concentrations of DA, having a peak effect within 40 min, has no effect on DOPAC, and increases HVA (Butcher et al. 1988). Cocaine induces a short lasting (1 h) DA increase that peaks 20 min after the injection, and has no effect on DOPAC and HVA (Hurd and Ungerstedt 1989). The increase in extracellular DA after d-amphetamine peaks 20-40 min after injection and then stabilizes at a lower level, this occurring with a dose-independent decrease in DOPAC and HVA (Zetterström et al. 1986). Bupropion appears to differ to some extent from all these other compounds that also increase extraneuronal concentrations of DA. As in the case of nomifensine (Butcher et al. 1988), the increase in HVA concentrations after high doses of bupropion may reflect a shift toward an extraneuronal metabolism of DA. The significance of the dose-dependent, delayed increase in 5-HIAA after bupropion is difficult to assess due to the complex relationship between serotonin release and the changes in the concentration of its extracellular metabolite (Kalén 1988). Taking into account possible pharmacokinetic differences bupropion's profile on DA and its metabolites appears to resemble most closely that of nomifensine. However, direct comparisons between these agents, with each compound being tested over a variety of doses, are required before firm conclusions can be reached on this point (see below).

An interesting finding of the present study is that the dose of bupropion which is active in a behavioral test of antidepressant action (10 mg/kg, Cooper et al. 1980) increased extracellular DA to 170% of baseline. Bupropion administered acutely or chronically in this dose range has not been found to affect striatal tissue concentrations of DA or DA synthesis (Nielsen et al. 1986). Also, in other *in vivo* models of inhibition of DA uptake such as inhibition of the neurotoxic effects of 6-OHDA, a dose of 12.5 mg/kg was ineffective

(Cooper et al. 1980). Microdialysis therefore appears to be a more sensitive procedure than the other models that have been used to study the *in vivo* actions of bupropion.

TTX has been used to characterize drug-induced dopamine release in microdialysis studies (Westerink et al. 1987c). This neurotoxin blocks voltage dependent sodium conductance in neurons, thus inhibiting the propagation of action potentials. In the present study the increase in extracellular DA concentrations after bupropion was blocked by TTX and this effect can therefore be considered to be action potential dependent. In this regard, bupropion differs from d-amphetamine and resembles nomifensine since only the effects of the latter drug can be blocked by TTX (Westerink et al. 1987, 1989). While the present data do not exclude the possibility that bupropion possesses both DA releasing and uptake-inhibiting properties, its ability to increase extracellular DA clearly depends on action potential dependent processes in DA neurons. Two injections of bupropion 3 h apart demonstrated that bupropion produced neither acute tolerance nor sensitization with respect to its ability to increase DA transmission.

Using microdialysis it has been shown that a number of drugs having abuse potential for humans more effectively enhance DA transmission in the NAC than in the striatum (Di Chiara and Imperato 1988). The present data provided no evidence for a preferential action of acute bupropion in the NAC; only following chronic administration of bupropion region selective effects became apparent (see below).

In vivo microdialysis provides the option of simultaneous monitoring of the extracellular concentrations of DA and its metabolites, and local administration of drugs via the perfusion medium. This procedure permits these substances to be applied directly to target areas, thus substantially circumventing the pharmacokinetic factors that occur after systemic administration. The continuous infusion of increasing concentrations of bupropion and several other DA uptake inhibitors was used to characterize these indirect DA agonists. The variables examined were : 1) the profile of the change in DA output, 2) the effects on

DA metabolites, 3) the effect of coinfusion of TTX on DA outflow, and 4) the potency of these drugs to increase the interstitial concentrations of DA.

All of the compounds increased extracellular DA in a dose-dependent manner. These increases might not be due solely to DA uptake inhibition since some of these compounds also influence other aspects of monoaminergic function. d-Amphetamine and methylphenidate, for example, are thought to increase DA release from cytosolic and vesicular presynaptic pools, respectively (Moore et al. 1977); benztropine also blocks muscarinic and histaminergic receptors (Richelson 1979); nomifensine, cocaine and d-amphetamine also inhibit the uptake of noradrenaline and serotonin (Richelson and Pfenning 1984). Differences between the drugs with respect to the distance they diffuse in the brain after passing through the dialysis membrane could also influence the results. Interestingly, all the drugs significantly increased interstitial DA when administered at the 1 μM concentration. Taking into consideration the dialysis efficiency of each compound (Table III), the DA increases are achieved in the nM dose-range; in *in vitro* uptake/inhibition release studies the effective concentrations of the tested compounds were also in the nM range (Hyttel 1978; Richelson and Pfenning 1984; Andersen 1989). This indicates that brain microdialysis is among the most sensitive *in vivo* methods for detecting drug actions on central dopaminergic systems.

The patterns of the dose-dependent increases in DA differed between the drugs. Low doses of each drug increased DA outflow to a stable level. With the exception of GBR 12909 this level was reached within 20 min. In contrast, the patterns of the two highest doses of amphetamine, as well as the highest dose of GBR 12909, methylphenidate and benztropine differed in that after the initial increase in DA there was a substantial decrease over the remainder of the one hour period. This biphasic (transient increase followed by a gradual decrease) effect of amphetamine, GBR 12909, methylphenidate and benztropine was apparent when the extracellular concentrations of DA increased by more than eight times. The gradual decrease in DA output may have been due to stimulation of synthesis and

release modulating DA autoreceptors by the high extracellular concentrations of DA (Roth et al. 1987). Arguing against this possibility are the facts that (1) nomifensine increased DA output by up to 20 times without producing any subsequent decreases, and (2) high concentrations of GBR 12909, benztropine and methylphenidate did not affect the extracellular concentrations of the DA metabolites. Another explanation for the biphasic effect on DA output after amphetamine, GBR 12909, benztropine and methylphenidate might be that at high concentrations these compounds stimulate DA release (initial increase) to a degree that cannot be maintained by the rate of DA synthesis, this thereby eventually leading to a gradual decrease (Glowinski 1970). This raises the possibility that the monophasic increase of DA outflow seen at low doses of the test compounds may reflect a selective inhibition of DA uptake, while a combination of both uptake inhibition and stimulated release underlies the biphasic effects observed at higher doses. In this regard, it is noteworthy that on the basis of *in vitro* studies d-amphetamine is thought to release DA even at low concentrations (Raiteri et al. 1975; Bonnet et al. 1984) while some of the other compounds (i.e. methylphenidate and benztropine) only display releasing properties at substantially higher doses (Bonnet et al. 1984).

Data obtained in the presence of TTX, confirmed and extended the results of previous reports (Westerink et al. 1987c; 1989). d-Amphetamine is thought to increase extracellular concentrations of DA by a carrier-mediated process that is independent of neuronal activity (Kuczenski 1983). In accordance with this view, the present results demonstrated that all the DA uptake inhibitors except for d-amphetamine required action potentials to increase DA extracellularly. The only further exceptions were high concentrations of GBR 12909 (100 μM) and benztropine (1000 μM) which were also partly TTX independent, suggesting that at high concentrations these two compounds may in part also enhance DA release by a carrier-mediated mechanism. To date this possibility has not been addressed by other dialysis studies or *in vitro* experiments. It is interesting that TTX sensitivity generally appeared to correlate with biphasic actions of the test compounds, such

that at doses of the test compounds that produced biphasic effects, the TTX sensitivity was reduced. In view of the discussion above, this raises the possibility that the uptake-inhibiting but not the releasing properties of these compounds are TTX dependent. Methylphenidate did not conform to this hypothesis, being TTX sensitive at a dose (1 mM) that produced biphasic effects. However, it should be recognized that methylphenidate produces its effects on DA release via an exocytotic rather than a carrier-mediated mechanism (Moore et al. 1977). Therefore, TTX insensitivity and a biphasic action on extracellular DA concentrations may both be indicative of a carrier mediated, amphetamine-like DA releasing action.

Of the uptake inhibitors tested in this study GBR 12909 and cocaine are considered to be the most selective, requiring very high concentrations to induce release (Van der Zee et al. 1980; Heikkila et al. 1975). Suprisingly the effect of a high concentration of GBR 12909 (100 μM) was partly TTX-independent, suggesting that at this concentration this compound may also have some releasing properties. The plateau of the DA response observed after high doses of cocaine might be due to rapid tolerance, saturation of the DA uptake complex in the DA terminals in the vicinity of the dialysis probe, or to the local anaesthetic effects of this compound which could interfere with DA release.

Zetterström et al. (1988) have proposed that extracellular DOPAC is derived mainly from an intraneuronal pool of newly synthesized DA and that amphetamine-induced release of DA depletes this pool, thereby gradually causing a decrease in DOPAC. In general, the present results are consistent with this hypothesis. For example, d-amphetamine produced clear decreases in DOPAC at doses that increased extracellular DA. In contrast, the other agents, none of which is thought to release DA from the amphetamine-sensitive pool, did not have significant effects on either DOPAC or HVA even at doses that produced up to 20-fold increases in extracellular DA. However, it is noteworthy that high concentrations of GBR 12909 and benztropine may have amphetamine-like effects (see above), and both compounds failed to decrease extracellular DOPAC.

The relative *in vivo* drug potencies determined in the present experiments correspond quite well with previous *in vitro* studies for DA uptake inhibition. At 10 μM the *in vivo* rank order potency of the compounds examined in the present study was GBR 12909> benztropine> amphetamine= nomifensine= methylphenidate> cocaine> bupropion (Table III). Although minor discrepancies exist, the results of a number of *in vitro* studies indicate that bupropion and cocaine are relatively weak DA uptake inhibitors while GBR 12909 is the most potent (Hyttel 1978; Van der Zee et al. 1980; Bonnett et al. 1984; Richelson and Pfenning 1984; Andersen 1989). In further agreement with the present results, the potencies of benztropine, d-amphetamine, nomifensine and methylphenidate are similar, with benztropine showing some consistency in being the most potent among these 4 compounds (Hyttel 1978; Van der Zee et al. 1980; Bonnett et al. 1984; Richelson and Pfenning 1984; Andersen 1989). Given the substantial differences between the *in vivo* and *in vitro* procedures, the degree of agreement between the two approaches is noteworthy. Although the characterization of DA uptake inhibitors by *in vivo* microdialysis can be used to provide useful information about the mechanisms of action and potency of these compounds effects on DA uptake blockade cannot be readily distinguished from DA releasing actions.

Bupropion is similar to other antidepressants in that it requires 5-21 days of treatment and steady-state plasma levels before clinical efficacy can be detected (Peet and Coppen 1979; Maxwell et al. 1981). In contrast to some tricyclic antidepressants, bupropion is rapidly metabolized in rodents (Judd and Ursillo 1975; Schroeder 1983), its half-life in the dose range used in the present study being approximately 1 h (Butz et al. 1982). Furthermore, chronic administration of bupropion induces drug metabolism, including its own (Schroeder 1983). It is likely, therefore, that steady-state was not achieved with the drug regimen used in this study. The dosage of 10 mg/kg b.i.d. was selected for a number of reasons: (1) chronic treatment with this regimen produces behavioral sensitization, i.e. enhanced locomotor activity in response to a challenge injection of bupropion (Nielsen et al. 1986); (2) the ED_{50} of bupropion for reversal of immobility in the Porsolt test is 8 mg/kg,

i.p. (Cooper et al. 1980); (3) acute administration of bupropion at this dose significantly increases interstitial concentrations of DA by 80% as shown above. The 25 mg/kg challenge dose of bupropion was chosen because this dose enhances locomotor activity for a relatively longer period (>30 min) than do lower doses (Cooper et al. 1980; Nielsen et al. 1986); also, this dose reliably increases extracellular DA concentrations 3-fold (see above, Brown et al. submitted).

Chronic bupropion did not significantly influence basal concentrations of DA and its metabolites in the interstitial space of the NAC and striatum, albeit there was a tendency for DA concentrations in the NAC to be higher (↑60%) in the chronic bupropion group. These findings suggest that chronic bupropion does not change the steady-state synthesis and turnover rates of DA and in this regard confirm previous conclusions based on *ex vivo* data (Soroko and Maxwell 1983; Nielsen et al. 1986).

Nielsen et al. (1986) first reported that sensitization to the motor stimulant effects of bupropion occur after chronic administration of this drug. The present data confirm this behavioral finding and indicate that this is accompanied by a selective enhancement of bupropion-induced increases in interstitial concentrations of DA in the NAC. However, a temporal dissociation between the behavioral and biochemical responses was also apparent (Figs. 17 and 21). Chronic bupropion resulted in augmented locomotor activity after a bupropion challenge only in the first 20 min postinjection; in contrast, extracellular DA concentrations in the NAC were significantly enhanced for up to 1 h after the injection. The possibility that there exist rapidly occurring postsynaptic adaptive mechanisms which compensate for the increased extracellular concentrations of DA in the NAC warrants further investigation.

There are several mechanisms which could account for the enhanced behavioral and neurochemical effects of bupropion in the chronic bupropion group. Among these are changes in bupropion metabolism, and alterations in DA release and/or reuptake. With respect to the first, a decrease in bupropion metabolism would result in higher brain

concentrations of the drug. It is noteworthy that cocaine sensitization has recently been attributed in part to higher extracellular concentrations of this drug in chronically treated animals (Pettit et al. 1990). It is unlikely that such a phenomenon contributed to the present findings. First, a 3 day withdrawal period was interposed between the chronic treatment and the challenge injection. Second, bupropion induces its own metabolism (Schroeder 1983). Third, if there were higher circulating concentrations of bupropion in the chronic animals, this should also have been reflected in the striatal data. However, the enhanced DA response was confined to the NAC.

It is difficult to dissociate changes in DA reuptake from changes in DA release (Horn 1979; Fischer and Cho 1979; Raiteri et al. 1979). *In vitro* studies have indicated that bupropion primarily inhibits DA uptake without influencing release (Ferris et al. 1980, 1983). However, the possibility that bupropion influences DA release was not excluded in an *ex vivo* study (Waldmeier et al. 1982). It has not been possible to attribute bupropion-induced increases in interstitial DA concentrations *in vivo* solely to blockade of DA uptake (Stamford et al. 1989; present study). It is possible therefore, that bupropion-induced sensitization is due either to an increase in the ability of bupropion to release DA, or to an enhancement of bupropion-induced inhibition of DA uptake (transporter) mechanisms. Interestingly, Klimek and Nielsen (1987) found a decrease in the density of D_1 receptors in the limbic system (including the NAC) and the striatum following chronic bupropion; this effect was suggested to be the result of biochemical adaptation to overstimulation of D_1 receptors by high interstitial concentrations of DA following chronic bupropion. Chronic administration of other indirect DA agonists such as d-amphetamine and cocaine can also produce behavioral sensitization (Robinson and Becker 1986; Post et al. 1984), and enhanced DA release (Robinson and Becker 1982; Castañeda et al. 1988; Kalivas and Duffy 1988). Recently, Allard et al. (1990) reported that neither chronic cocaine nor chronic amphetamine influence the binding of [^{3}H]GBR-12935 to the DA uptake sites. Taken together, these data suggest that enhanced DA release may be the primary mechanism by which bupropion

produces sensitization. However, at present the possibility that this treatment may also impair DA uptake mechanisms cannot be excluded.

The net effect of DA reuptake inhibitors on interstitial DA concentrations is also influenced by the degree of autoreceptor activation (Cubeddu et al. 1983; Hoffman et al. 1986). Thus, the enhanced extracellular DA concentrations in the NAC of the chronic animals may have been due to a drug-induced subsensitivity of inhibitory DA autoreceptors. In this regard, there is neurophysiological evidence that DA autoreceptor subsensitivity may contribute to the behavioral sensitization produced by antidepressants and psychomotor stimulants (Chiodo and Antelman 1980a; White and Wang 1984). There are many inconsistencies and limitations in the relevant literature, however, and these preclude any firm conclusions from being reached regarding the adequacy of this hypothesis (Willner 1985; Robinson and Becker 1986).

The preferential action of chronic bupropion on meso-accumbens DA neurons has not been shown in previous microdialysis studies in which indirect DA agonists were administered chronically . For example, chronic cocaine treatment enhances cocaine-induced increases in extracellular DA concentrations both in the NAC (Pettit et al. 1990; Kalivas and Duffy 1990) and the striatum (Akimoto et al. 1989). Similarly, sensitization has been reported to occur in both structures after chronic amphetamine or methamphetamine administration (Robinson et al. 1988; Kazahaya et al. 1989). These findings provide further neurochemical evidence that mesolimbic DA neurons are components of the central mechanisms that mediate the actions of antidepressant drugs (Willner 1985; Fibiger and Phillips 1987).

IV. In vivo neurochemical effects of electroconvulsive shock

(A) Introduction

Electroconvulsive therapy was introduced more than sixty years ago, long before the advent of modern psychopharmacology, and although it is still widely used in the treatment of certain psychiatric syndromes its mechanism of action remains unknown (Abrams 1988). A course of six to twelve electroconvulsive shock treatments (ECTs) is effective in depression and is usually administered when pharmacotherapy has failed (Fink 1979; Abrams 1988). Also, the administration of single ECT at monthly intervals has been used in chronic recurrent major depressive disorder to prevent relapse (Decina et al. 1987). Recently, remarkable motoric improvements have been reported after ECT in Parkinson's disease patients (Anderson et al. 1987; Douyon et al. 1989).

Electroconvulsive shock (ECS) affects the neurochemistry of several transmitter systems, including DA, noradrenaline, serotonin, acetylcholine, GABA and histamine (Green and Nutt 1987; Lerer 1987). In view of theories implicating DA in the pathophysiology of affective disorders (Willner 1983a,b; Fibiger 1984, 1990; Swerdlow and Koob 1986; Fibiger and Phillips 1987), the effect of ECS on DA neurotransmission is of particular interest. In addition, the ability of ECT to improve symptoms of Parkinson's disease suggests that dopaminergic mechanisms underlie some of its beneficial effects.

Chronic treatment with ECS appears to increase the functional output of central DA systems. For example, DA-mediated behaviors, including hyperactivity, stereotypy, and circling in rats with unilateral nigrostriatal lesions, are enhanced following a course of ECS (Modigh 1975; Green et al. 1977; Grahame-Smith et al. 1978; Serra et al. 1981; Modigh 1984). Effects on the mesolimbic DA system are underlined by studies in which ECS-treated rats have greater locomotor responses to DA injected directly into the NAC (Heal and Green 1978; Modigh 1984). Receptor binding studies indicate that chronic ECS has no

effect on the number or affinity of D_2 receptors for DA antagonists in the striatum (Bergström and Kellar 1979; Deakin et al. 1981; Lerer et al. 1982). However, it has been shown to decrease the number of D_1 receptors in both the NAC (Klimek and Nielsen 1987; De Montis 1990) and the striatum (Klimek and Nielsen 1987).

There is conflicting evidence that chronic ECS has effects on presynaptic DA mechanisms. *Ex vivo* studies indicate that synthesis and turnover of DA are not affected by this treatment (Evans et al. 1976; Modigh 1976). Serra et al. (1981) reported that repeated ECS reduced the sedative response to a low dose of apomorphine but had no effect on the ability of apomorphine to inhibit DA synthesis in striatum. Acute ECS treatment also failed to influence these effects of apomorphine, both of which are thought to be mediated via presynaptic DA receptors (Serra et al. 1981; Creese et al. 1982). In contrast, neurophysiological studies have indicated that both acute and chronic ECS reduces the ability of low doses of apomorphine to inhibit the spontaneous discharge of nigral DA neurons, suggesting subsensitivity of DA autoreceptors (Chiodo and Antelman 1980b; Tepper et al. 1982; Groves et al. 1990).

The present experiments investigated the effects of acute and chronic ECS on DA transmission using on-line microdialysis in freely moving rats. Specifically, the following parameters were examined: (1) the effect of acute ECS on interstitial concentrations of DA and its metabolites in the striatum of freely moving rats; (2) the effect of ECS on DA in the presence of TTX in the perfusion solution, in the absence of Ca^{++} ions from the perfusion solution, and under anaesthesia (sodium pentobarbital); (3) the effects of a higher intensity ECS and of seizures induced by the convulsant agent flurothyl; (4) the effects of repeated ECS on DA transmission in the striatum; (5) the effects of acute or chronic ECS on interstitial concentrations of DA and its metabolites in the NAC; (6) the effects of a low dose of apomorphine on interstitial concentrations of DA in the NAC of rats chronically treated with ECS in order to test the hypothesis that this results in DA autoreceptor subsensitivity; and (7) the effect of repeated ECS on amphetamine-induced increases in DA

release in NAC. In the latter experiment, concomitant measures of locomotor activity permitted correlations to be established between the amphetamine-evoked biochemical and behavioral responses.

(B) Materials and methods

(i) Acute effects of electroconvulsive shock

A horizontal dialysis probe was implanted through both striata in male Wistar rats (weighing 275-325 g). The perfusion solution consisted of 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM sodium phosphate. The load and inject modes of the injector were set at 9.8 min and 12 sec, respectively. All perfusion experiments were carried out in conscious rats during the light phase of the day-night cycle approximately 48 h after surgery.

ECS was administered via earclip electrodes using a Medcraft B24 III clinical ECT apparatus. Sham treatments consisted of handling and application of earclips. Stimulus parameters unless otherwise specified were 150 V (sinusoidal waveform) for 0.75 sec which induced tonic extension and generalized seizures lasting between 15 and 20 sec. Other animals were given ECS in the presence of a modified perfusion medium in which either TTX (1 μM) was added, or Ca^{++} was omitted (replaced with equimolar Mg^{++}). In some animals ECS was administered under anaesthesia, achieved with sodium pentobarbital (Nembutal, 50-60 mg/kg, i.p.). In order to examine if the operated animals showed differences in the resistance to the applied current due to the implanted probe, the trephined skull, the dental acrylic etc., the amount of current passing between the earclips and through the head was measured. For this purpose, a multimeter (2830 Digital, Dynascan) was set in series with the ECT apparatus; ECS was administered in three groups:

control rats (not operated), rats implanted with a transverse probe, and rats implanted with a vertical probe.

Seizures were also induced chemically by placing the animals in an airtight glass jar with a volume approximately 3 L; flurothyl (0.2 ml, Anaquest) was introduced into the jar; myoclonic jerks were apparent within 20 to 40 sec with generalized seizures beginning within another 5 to 10 sec at which point animals were removed from the jar and observed. The flurothyl-induced generalized seizures lasted 30 to 40 sec. Throughout this experimental phase the analysis of DA and metabolites was determined on-line.

For purpose of graphic representation (Figs. 22-27) the average of three baseline samples immediately preceding treatment was defined as 100% and all measures were related to these values (percent changes). Data were analysed by analysis of variance (one-way ANOVA with repeated measures) followed by the Newman-Keuls test. The Student's t-test for independent and paired data was also used. For the statistical evaluation of the data either percent changes (last baseline plus six post-treatment samples) or the absolute baseline values were used.

(ii) Chronic effects of electroconvulsive shock

Experiments were performed on male Wistar rats weighing 250-300 g at the beginning of the experiment. In order to approximate clinical practice a course of ECS was designed to consist of eight treatments (one every second day). Control animals received sham treatments (chronic sham) consisting of handling and application of earclips. Body weights of the rats receiving either ECS or sham treatments were monitored before the first and after the last treatment.

Twenty to 24 h following the last ECS or sham treatment, rats were implanted with a vertical microdialysis probe aimed at the NAC. Dialysis occurred through 2.3 mm of a semipermeable hollow fiber (copolymer of acrylonitrile and sodium methallyl sulfonate,

I.D.=0.24 mm, 40,000 Daltons, Hospal). All perfusion experiments were carried out in awake, freely moving animals 48 h following implantation of the microdialysis probe (approximately 72 h after the last ECS or sham administration). After a stable baseline was established, each rat was subjected to ECS (150 V, 0.75 sec). Concurrent measurements of the amount of current passing between the earclips and through the animal's head were achieved by a multimeter set in series with the ECT apparatus.

Other groups of rats (chronic ECS and sham) were injected with apomorphine (25 μg/kg, s.c.). Four hours later and after DA and metabolites had returned to baseline levels for at least 2 h, the rats were injected with d-amphetamine (1.5 mg/kg, s.c.).

The dialysis probe was perfused with a solution containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM sodium phosphate. The perfusate was automatically injected to the analytical system every 10 min (9.8 min for load and 12 sec for injection). Upon completion of the experiments, the animals were sacrificed, sliced on a cryostat (30 μm), stained (Nissl), and examined microscopically for probe placement. Only rats with probes that were verified to be located in the NAC were included. During some of the microdialysis experiments the Digiscan Animal Activity Monitor was used to measure locomotor activity in 10 min blocks corresponding to the 10 min dialysate samples.

The mean absolute baseline values (fmol/min) from each experiment were evaluated by Student's t-test (independent variables: chronic ECS and sham). For the purpose of graphic representation (Figs. 27-39) the average of 4 baseline samples immediately preceding treatment was defined as 100%. All subsequent measures were related to these values, and the mean percentages were calculated for each 10 min sample across the rats in each group. For statistical evaluation of the data, the percent changes (last baseline plus 6, 9 or 12 post-treatment samples according to the experiment) were used. Data were analysed by two-way (treatment X time) analysis of variance (ANOVA) with repeated measures followed by Newman-Keuls tests for multiple comparisons. Locomotor activity measurements following

amphetamine administration (twelve 10 min blocks) were also subjected to two-way ANOVA with repeated measures.

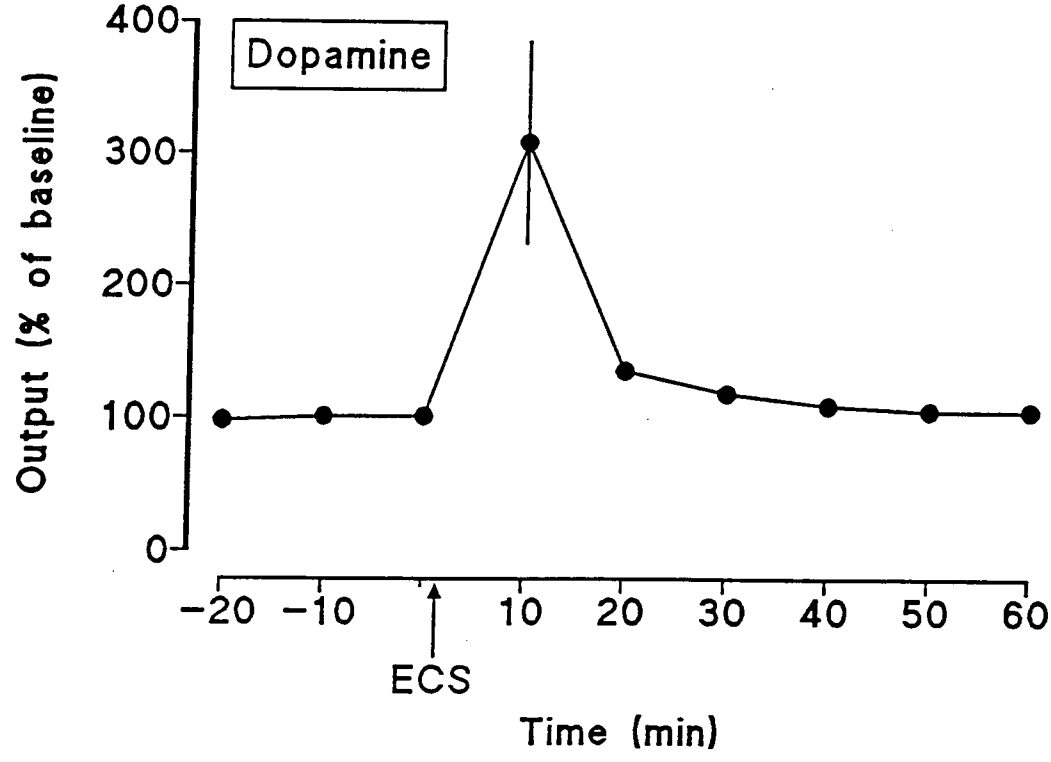
(C) Results

(i) Acute effects of electroconvulsive shock

Extracellular DA concentrations in the striatum increased substantially (+ 200% of baseline) within 10 min following a single ECS. They rapidly declined in the second 10 min sample but remained above baseline for an additional 30 to 40 min (Fig. 22). ECS also induced a more modest but sustained increase in dialysate concentrations of DOPAC and HVA (peak effects: 134% and 137%), though the time courses were different compared to DA (Fig. 22). These increases were statistically significant (peak increase compared to the last baseline sample $p<0.01$, paired t-test). Dialysate concentrations of 5-HIAA were also increased significantly by ECS ($p<0.01$) but were modest compared to those recorded for the DA metabolites. Sham ECS did not influence significantly the dialysate concentrations of either DA or the metabolites (data not shown).

Infusion of 1 μM TTX through the dialysis fiber resulted in a gradual decrease in dialysate DA concentrations from the striatum to 10% of basal values ($p<0.01$, Fig. 23). Thereafter, administration of ECS increased DA output to almost 110% of the initial basal values. Although this increase was approximately 50% of the ECS-induced DA increase in non-TTX conditions (see Fig. 22), a statistically significant difference was not detected ($p>0.05$, Student's t-test for independent samples comparing the peak ECS-induced DA increases in the two conditions). TTX did not influence significantly basal dialysate concentrations of the metabolites, although a tendency for a decrease was apparent in

Figure 22. Effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine (solid circles, upper panel), DOPAC (solid circles), HVA (open circles), and 5-HIAA (solid squares) from the striatum. Each point represents the mean (± S.E.M.) percentage changes of baseline. The mean (± S.E.M., N=8) baseline values (fmol/min) for dopamine, DOPAC, HVA, and 5-HIAA are 15.1±1.5, 1531±167, 903±126, and 645±70, respectively.


Dopamine
Output (% of baseline)
400
300
200
100
0
−20 −10
10 20 30 40 50 60
ECS
Time (min)

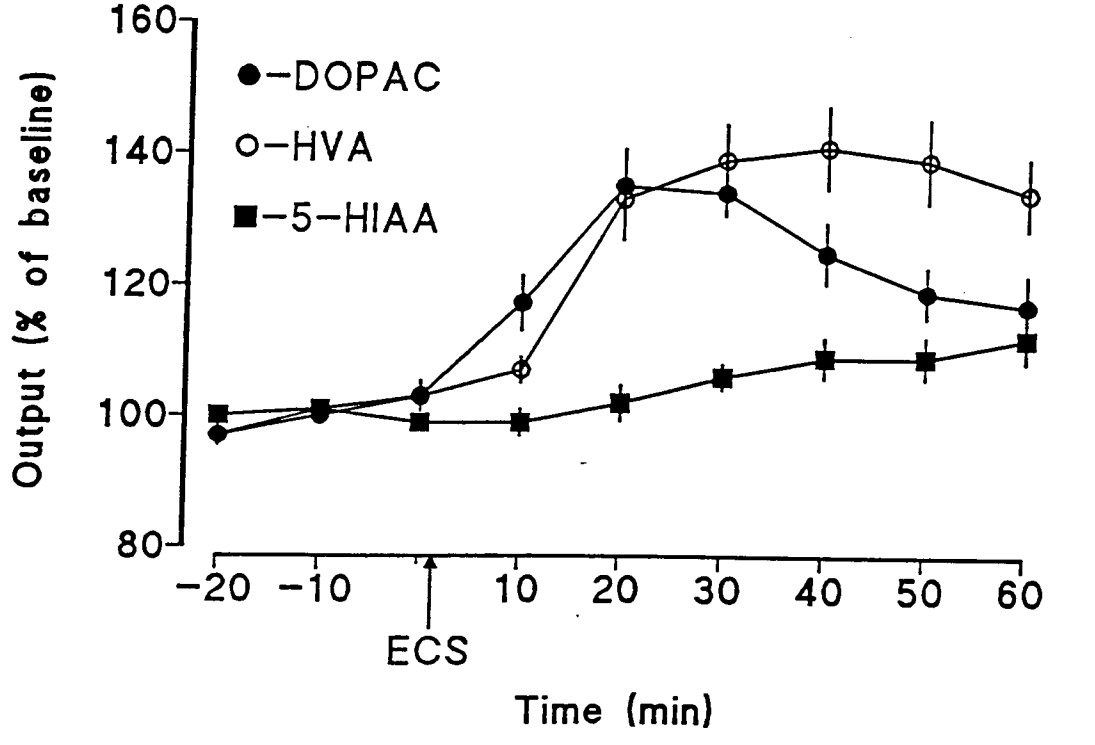
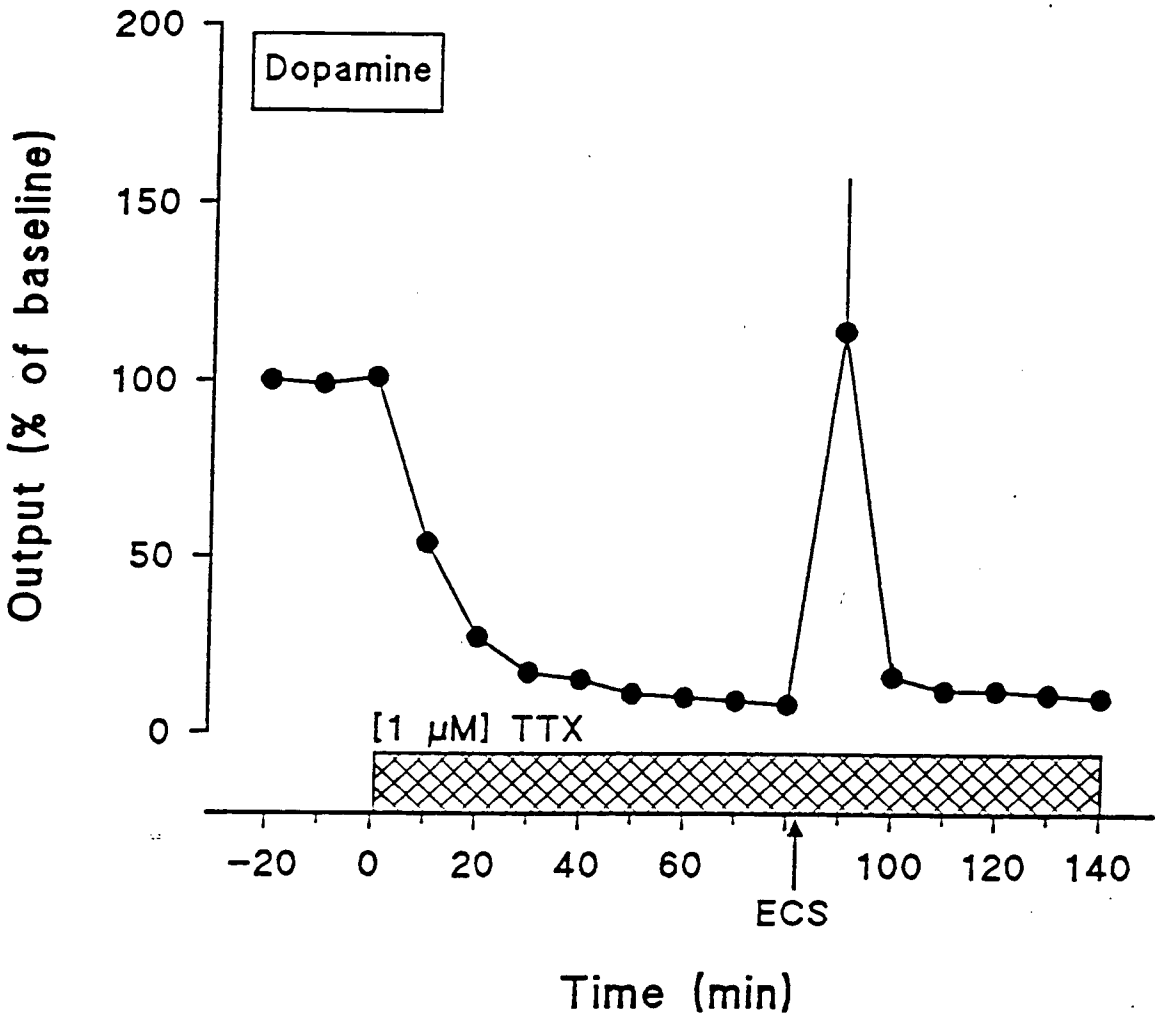

●–DOPAC
○–HVA
■–5–HIAA
Output (% of baseline)
160
140
120
100
80
−20 −10
10 20 30 40 50 60
ECS
Time (min)

Figure 24. Effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine from rat striatum perfused with a Ca^{++}-free solution (hatched area). Each point represents the mean (± S.E.M.) change of baseline. Mean (± S.E.M., n=5) basal values of dopamine in fmol/min are 20.1±2.8.


Dopamine
200
150
100
50
0
Output (% of baseline)
[1 μM] TTX
−20
0
20
40
60
ECS
100
120
140
Time (min)

DOPAC and HVA (data not shown); ECS produced significant ($p<0.01$) increases in dialysate concentrations of the metabolites (comparable to the effects illustrated in Fig. 22).

Omission of Ca^{++} from the perfusion solution and replacement with Mg^{++} gradually decreased dialysate concentrations of DA to 26% of the basal values ($p<0.01$ compared to the last baseline value, Fig. 24) but had no effect on basal concentrations of DOPAC, HVA, or 5-HIAA (data not shown). Subsequently, ECS caused a non significant ($p>0.1$ compared to the last sample before the administration of ECS) increase to 40%. In contrast, ECS significantly ($p<0.01$) increased DOPAC, HVA and 5-HIAA; the peak effects and the time courses were similar to those of the previous experiment (Fig. 22).

Administration of sodium pentobarbital (Nembutal) resulted in a gradual decrease of DA to 67% of basal values ($p<0.05$, Fig. 25). ECS was given while the rats were in deep anaesthesia (non-responsive to tail-pinch). Under these conditions ECS induced only tonic extension (5 sec) without generalization. ECS increased dialysate concentrations of DA to almost 210% of the initial basal values. This effect did not differ significantly from the ECS-induced DA release in awake freely moving animals (Fig. 22). Nembutal significantly increased dialysate concentrations of DOPAC and HVA to 160% and 180%, respectively (both $p<0.05$, data not shown). In contrast to the awake animals, administration of ECS to the anaesthetized rats did not increase dialysate concentrations of DOPAC and HVA.

A higher voltage and longer duration stimulus (170 V for 1 sec vs. 150 V for 0.75 sec) tended to produce more prolonged seizures (30 to 40 sec vs. 15-20 sec) and a greater DA release (Fig. 26). However, the latter difference did not reach statistical significance ($F_{1,11}=2.2$, $p=0.2$). There were no differences in DOPAC, HVA or 5-HIAA dialysate concentrations between the two treatments (data not shown).

The mean (±S.E.M., n=6 for each group) current was 127±16, 135±24, 119±21 mA for control non-operated animals, and animals implanted with transverse or vertical probes, respectively (no significant difference).

Figure 23. Effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine from rat striatum perfused with 1 μM TTX (hatched area). Each point represents the mean (± S.E.M.) percent change of baseline. Basal values of dopamine are 16.6±1.2 fmol/min (mean±S.E.M., n=6).

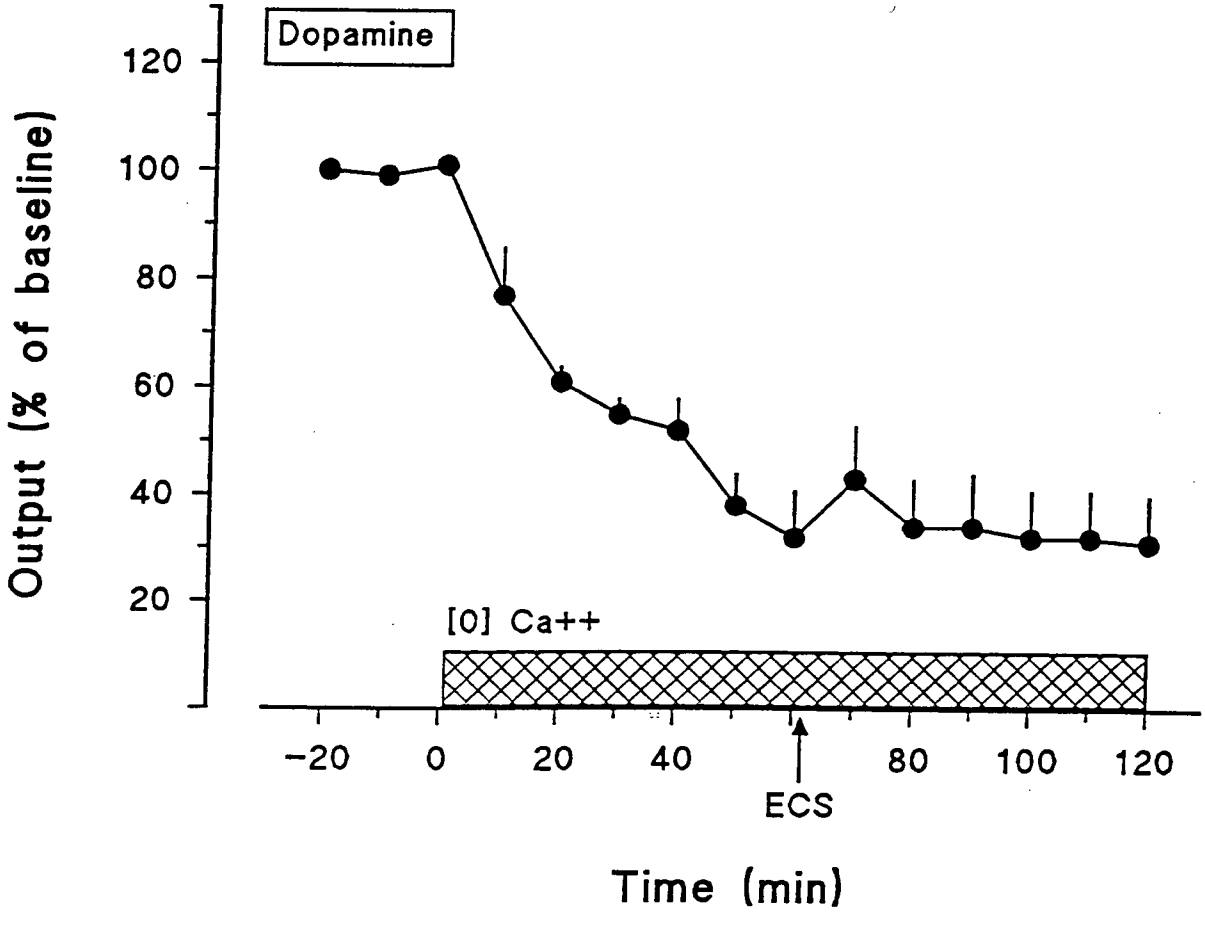

Dopamine
120
100
80
60
40
20
Output (% of baseline)
[0] Ca++
-20
0
20
40
ECS
80
100
120
Time (min)

Figure 25. The effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine from the striatum of anaesthetized rats (nembutal: sodium pentobarbital 50-60 mg/kg i.p.). Each point represents the mean (± S.E.M.) percent change of baseline. Basal values of dopamine are 18.2±2.9 fmol/min (mean±S.E.M., n=7).

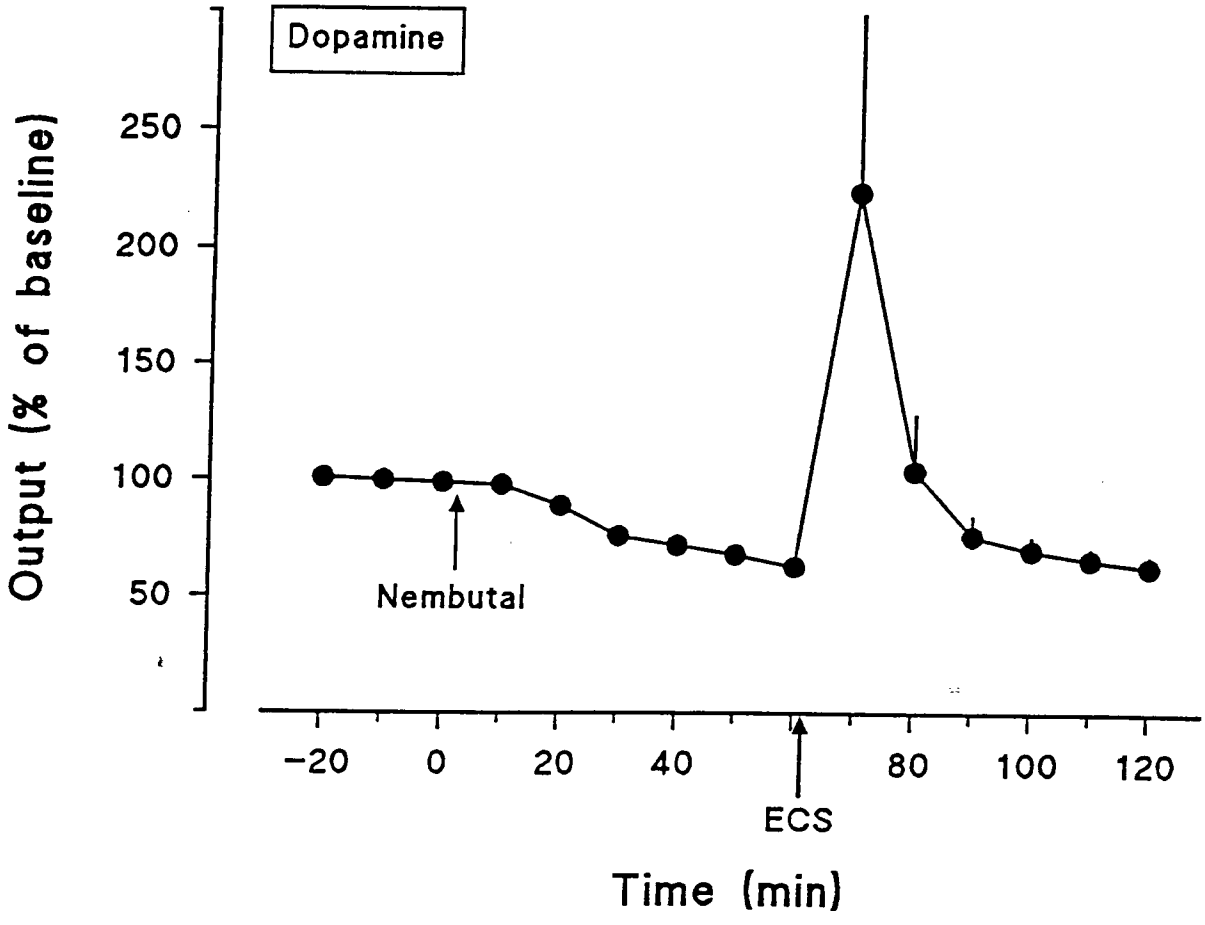
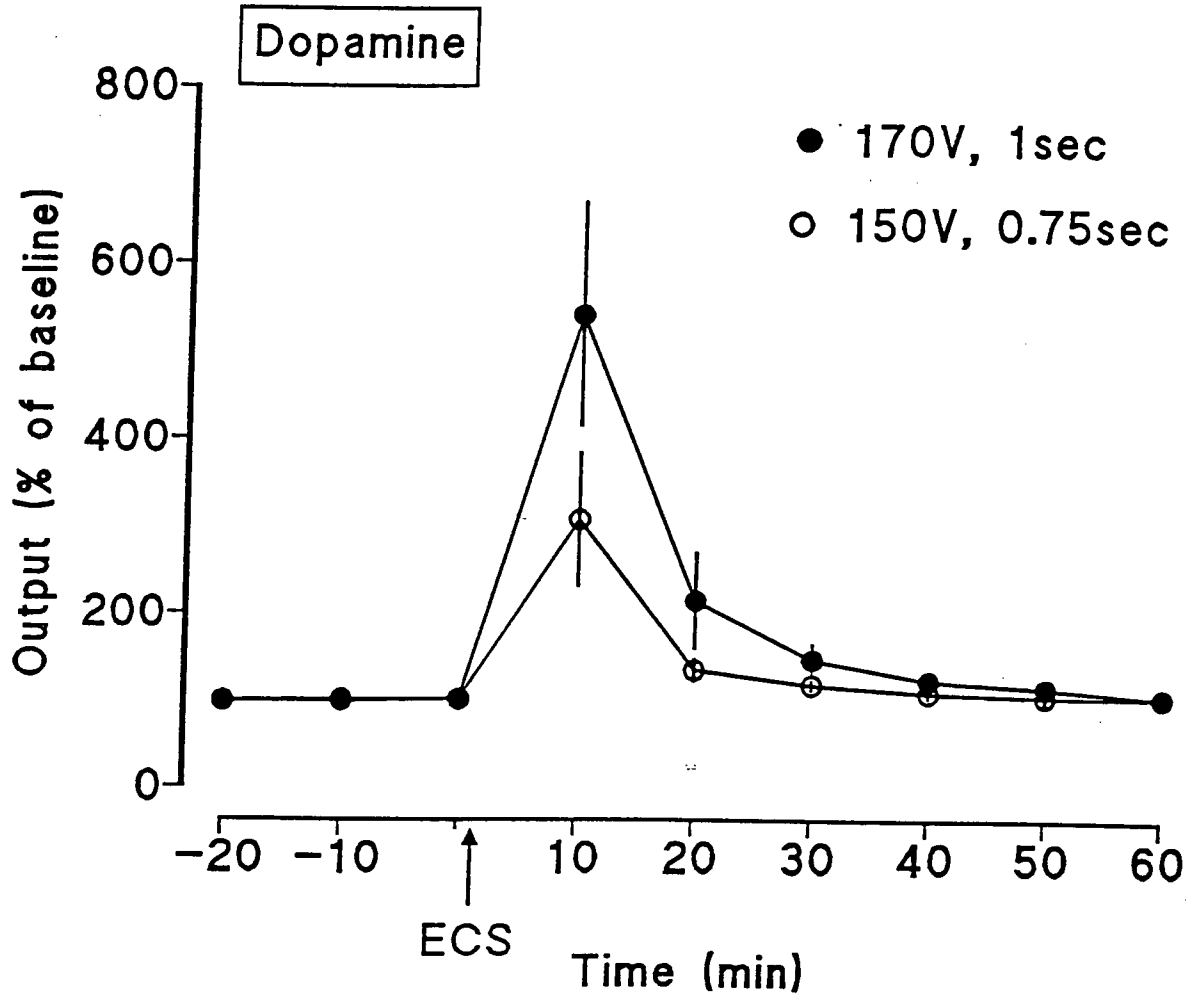

Dopamine
Output (% of baseline)
250
200
150
100
50
Nembutal
−20
0
20
40
ECS
80
100
120
Time (min)

Figure 26. Effect of electroconvulsive shock (ECS) on rat striatal dialysate concentrations of dopamine from the striatum. The stimulus parameters are 170 V for 1 sec (solid circles, n=5) or 150 V for 0.75 sec (open circles, n=8) and the mean (± S.E.M.) baseline values in fmol/min are 13.6±1.6 and 15.1±1.5, respectively.


Dopamine
800
600
400
200
0
Output (% of baseline)
● 170V, 1sec
○ 150V, 0.75sec
−20
−10
10
20
30
40
50
60
ECS
Time (min)

The results of the flurothyl experiment are presented in Figure 27. Although repeated measures ANOVA revealed that the effect of flurothyl-induced seizures on dialysate DA was significant ($F_{6,30}$=8.2, p<0.001), the increase in DA dialysate concentrations was insignificant and was followed by a significant (p<0.05) decrease below baseline values. Significant increases were detected for DOPAC ($F_{6,30}$=12.1, p<0.001), HVA ($F_{6,30}$=7.6, p<0.001), and 5-HIAA ($F_{6,30}$=9.8, p<0.001). Although more modest, the changes in the metabolite concentrations were nevertheless comparable to those observed following a single ECS. Flurothyl-induced seizures significantly ($F_{6,30}$=19.8, p<0.001) increased dialysate concentrations of DA from the NAC of rats implanted with a vertical probe (Fig. 34); for comparison, the flurothyl-induced effects on striatal DA were also included in this figure. ANOVA performed on data from both structures revealed that the effect of flurothyl was more pronounced in the NAC ($F_{1,12}$=10.6, p<0.05). Significant increases were also detected for DOPAC (peak effect: 140% of baseline) and HVA (peak effect: 143%) from the NAC (data not shown); these effects were more pronounced in the NAC in comparison to the striatum.

(ii) Chronic effects of electroconvulsive shock

As shown in Figure 28, a second ECS (ECS2) 2 h following the first (ECS1) produced an increase in striatal DA concentrations which was significantly smaller than the increase that occurred after the first treatment (paired t=2.83, df=10, p<0.05). However, when the second ECS (ECS2) was administered 24 h after the first, the DA response was similar to that observed after the first ECS (ECS1). DOPAC, HVA and 5-HIAA concentrations after ECS2 were not significantly different from those obtained in response to ECS1, irrespective of the time interval between the two treatments (2 or 24 h). The baseline concentrations of DA and its metabolites before the administration of the second ECS did not differ from those reported in the legend of Figure 22.

Figure 27. Effect of flurothyl-induced seizures on rat striatal dialysate concentrations of dopamine (upper panel), DOPAC, HVA and 5-HIAA. Mean (± S.E.M, n=6) baseline values in fmol/min are 14.9±1.9, 1389±150, 967±75 and 721±121 for DA, DOPAC, HVA and 5-HIAA, respectively.

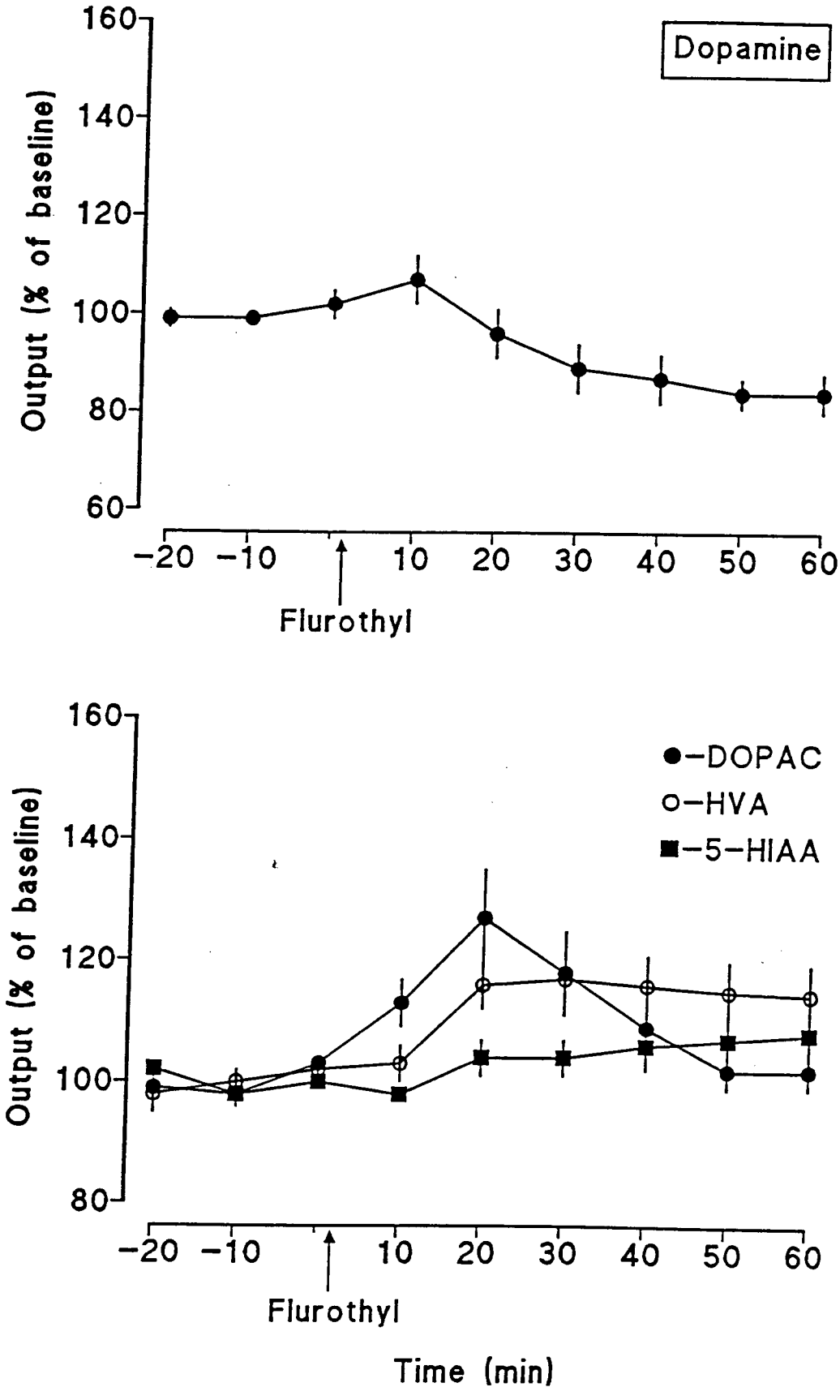
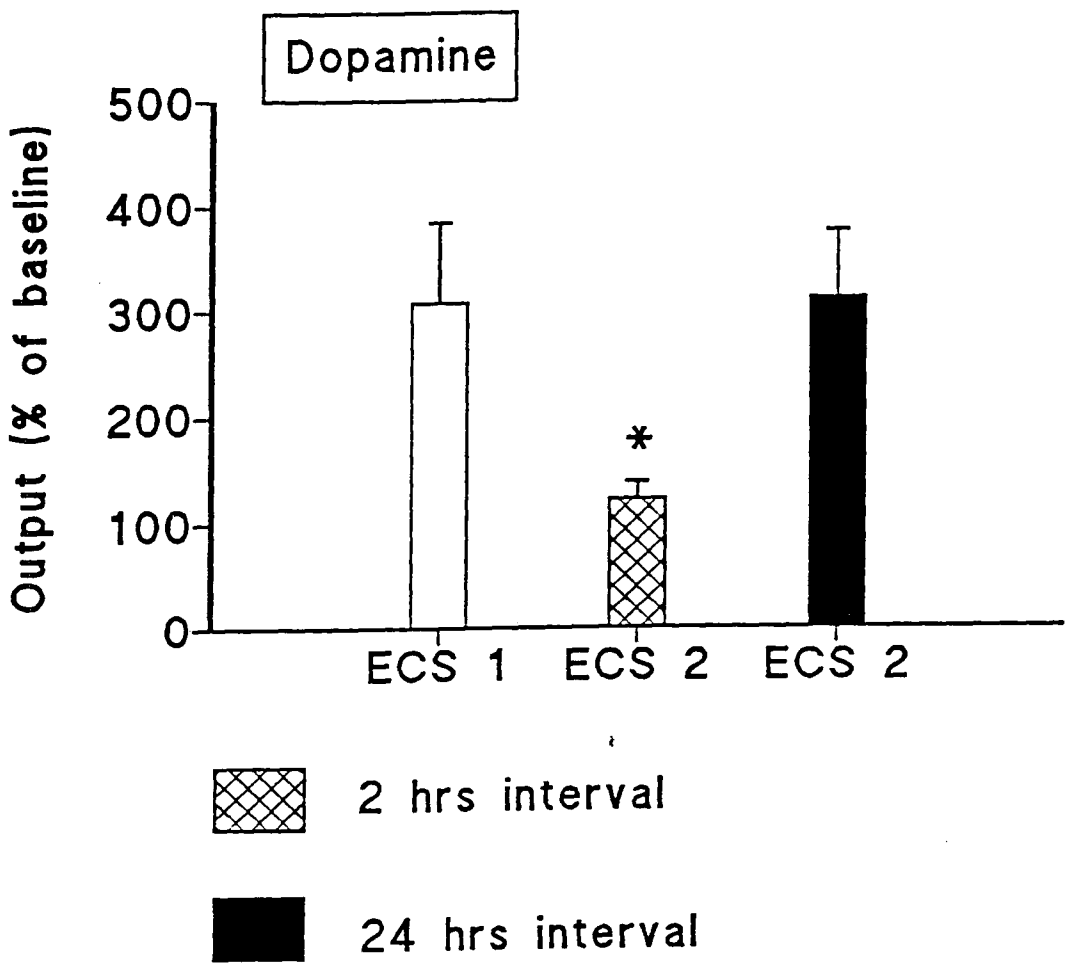

Dopamine
Output (% of baseline)
160
140
120
100
80
60
−20 −10
10 20 30 40 50 60
Flurothyl
●–DOPAC
○–HVA
■–5–HIAA
Output (% of baseline)
160
140
120
100
80
−20 −10
10 20 30 40 50 60
Flurothyl
Time (min)

Figure 28. Peak effects of a second electroconvulsive shock (ECS2) administered 2 (n=6) or 24 (n=8) hours after the first (ECS1) on rat striatal concentrations of dopamine, DOPAC, HVA and 5-HIAA. *: $p<0.05$ vs. ECS1.


Dopamine
Output (% of baseline)
500
400
300
200
100
0
*
ECS 1
ECS 2
ECS 2
2 hrs interval
24 hrs interval

As shown in Table V, baseline DOPAC and HVA dialysate concentrations from the striatum were significantly elevated following a course of ECS (t=2.9 and 2.4, respectively, df=10, $p<0.05$). There was also a trend for increased baseline concentrations of DA and 5-HIAA after chronic ECS but these differences did not reach statistical significance. In contrast, basal dialysate concentrations of DA, DOPAC, HVA and 5-HIAA from the NAC of all rats given chronic ECS did not differ significantly from the sham-treated animals (Table V).

ECS administered to animals 72 h (48 h post-operative) following completion of a course of ECS increased the concentration of DA in the dialysate samples from the striatum (Fig. 29). This increase was blunted compared to the ECS-induced increase observed in animals that were submitted to a course of sham treatments. Thus, a repeated ANOVA revealed a significant group effect ($F_{1,10}=5.9$, $p<0.05$), a significant time effect ($F_{6,60}=22.8$, $p<0.001$) and a significant group X time interaction ($F_{6,60}=4.4$, $p<0.025$). The DOPAC, HVA and 5-HIAA increases were also attenuated in the animals given repeated ECS but these differences did not reach statistical significance (Fig. 30).

Acute ECS increased the dialysate concentrations of DA from the NAC of the control (chronic-sham) animals to 127% of baseline within 20 min and then gradually declined over the next 40 min (Fig. 31). The ECS-induced DA increase appeared somewhat blunted in the chronic ECS animals. A repeated measures ANOVA did not, however, reveal significant treatment ($F_{1,13}=1.77$, $p=0.2$) or treatment X time interaction ($F_{6,78}=1.09$, $p=0.37$) effects. The time effect was statistically significant ($F_{6,78}=14.71$, $p<0.001$) due to significant (Newman-Keuls, $p<0.05$) increases in DA efflux in both groups over time in comparison to the last baseline sample. The mean (±S.E.M.) current was 147±14 and 142±13 mA for chronic ECS and sham groups, respectively (no significant difference). The amount of current did not correlate significantly with the ECS-induced increase in extracellular concentrations of DA.

Table V.

Baseline dialysate concentrations of dopamine and metabolites from the nucleus accumbens and the striatum of rats following chronic ECS treatment

	DA	*DOPAC*	*HVA*	*5-HIAA*
Nucleus Accumbens				
Chronic Sham (n=14)	4.2±0.7	863±116	413±54	302±31
Chronic ECS (n=15)	4.0±0.5	744±62	451±64	338±19
Striatum				
Chronic Sham (n=6)	13.2±2.6	1343±125	844±103	597±91
Chronic ECS (n=6)	16.9±1.9	1948±171*	1381±200*	870±123

Mean (± S.E.M.) basal values are expressed in fmol/min. Baseline dialysate concentrations of DA and metabolites from nucleus accumbens were pooled from two separate experiments in which electroconvulsive shock (ECS) or apomorphine and amphetamine was administered. *: $p<0.05$ in comparison to Chronic Sham

Figure 29. Effect of electroconvulsive shock (ECS) on dialysate dopamine concentrations from the striatum of rats treated with chronic sham (solid circles, n=6) or chronic ECS (open circles, n=6). Each point represents mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.

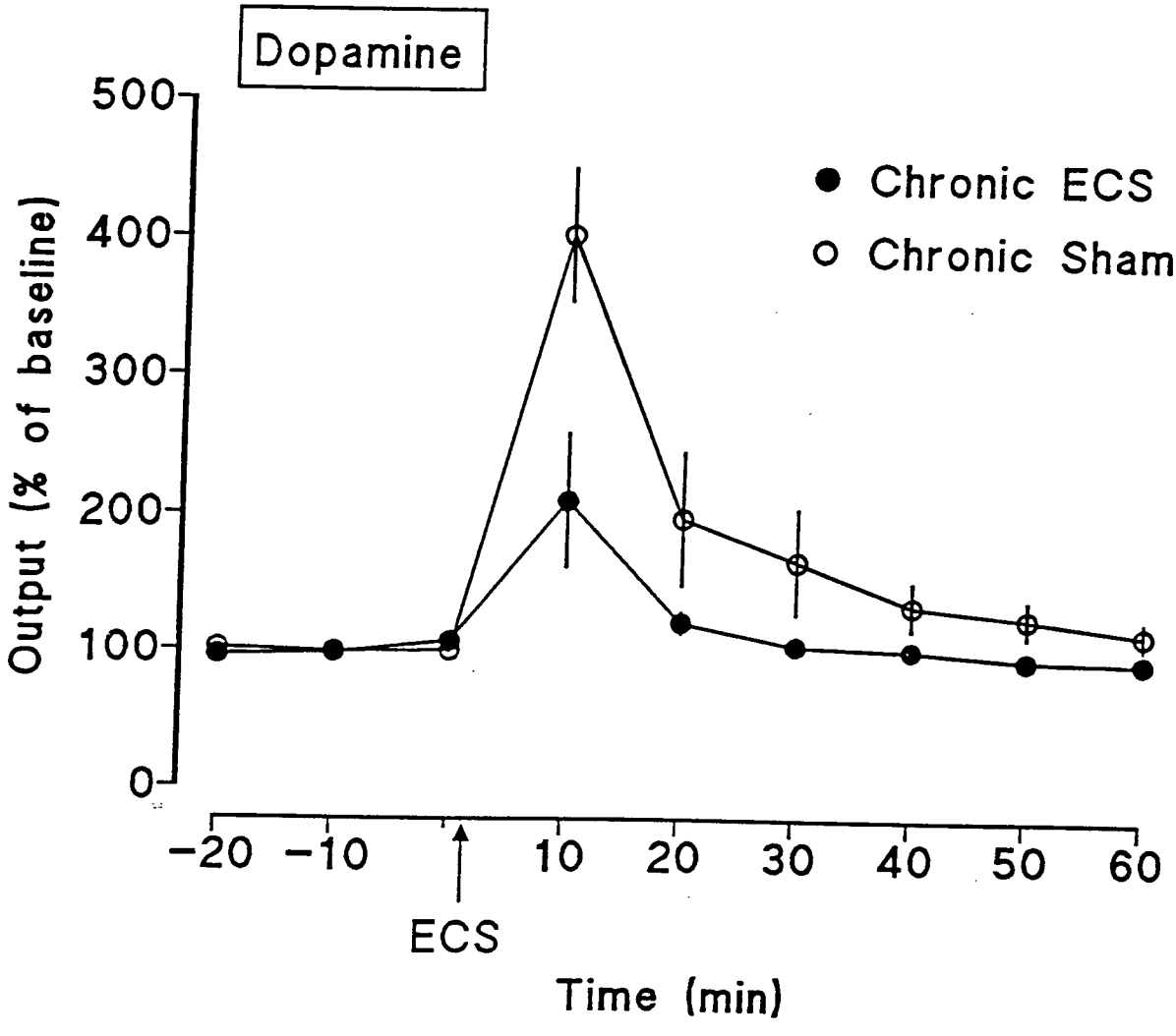

Dopamine
Chronic ECS
Chronic Sham
Output (% of baseline)
500
400
300
200
100
0
−20
−10
10
20
30
40
50
60
ECS
Time (min)

Figure 30. Effect of electroconvulsive shock (ECS) on rat striatal concentrations of DOPAC, HVA and 5-HIAA after chronic sham (solid circles) or chronic ECS (open circles) treatment. Each point represents mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.

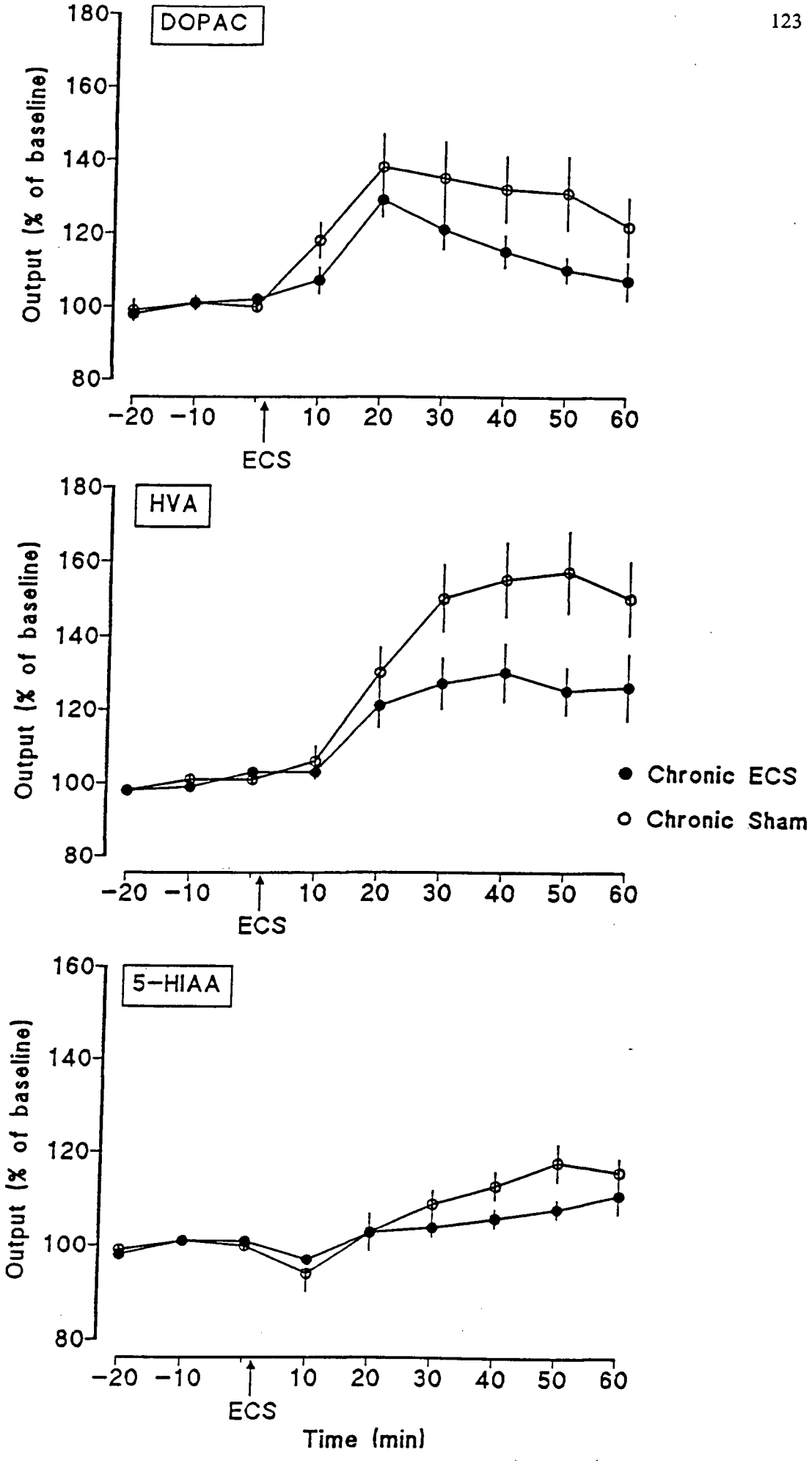
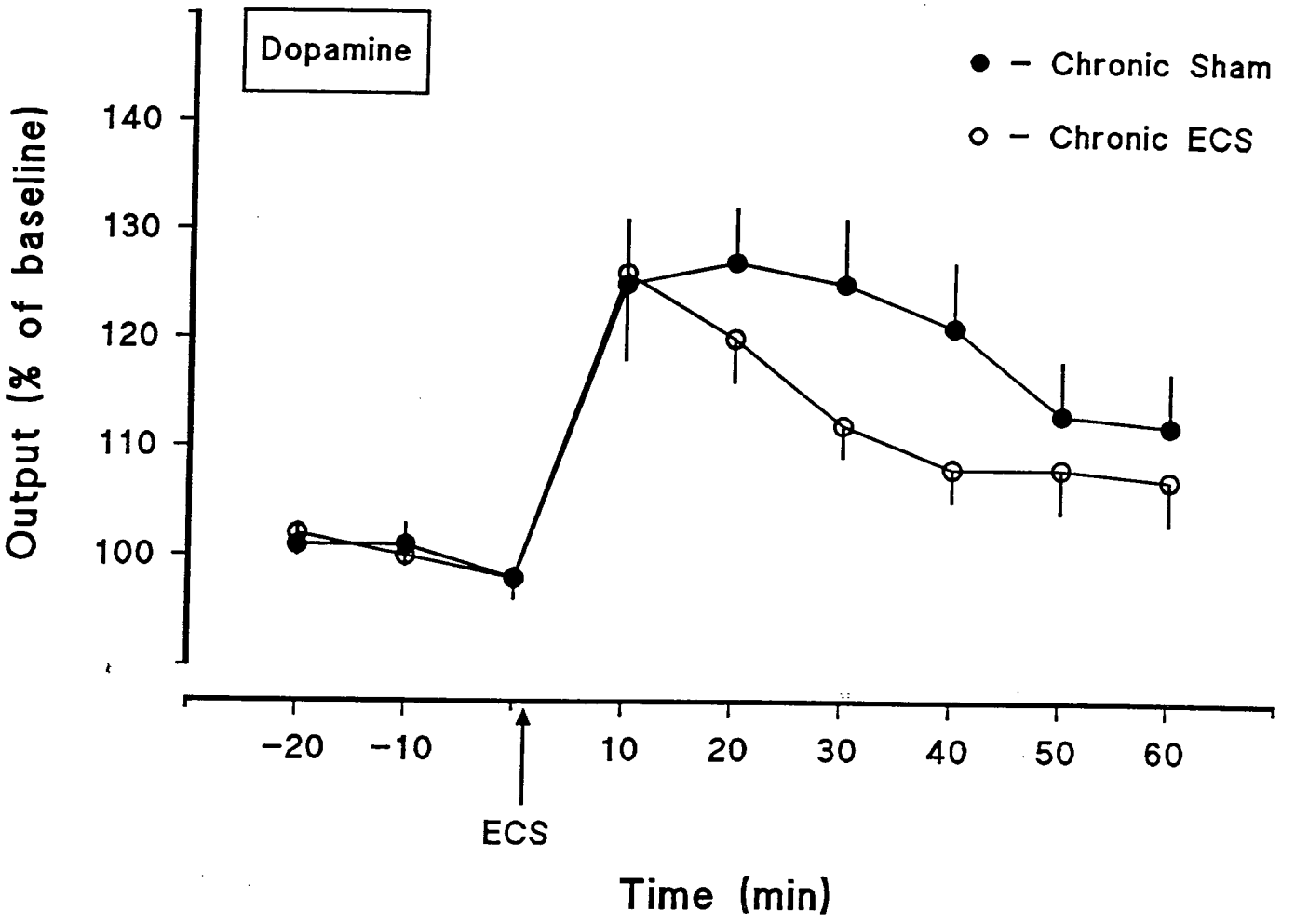

DOPAC
HVA
5-HIAA
Output (% of baseline)
180
160
140
120
100
80
−20 −10 10 20 30 40 50 60
ECS
Time (min)
Chronic ECS
Chronic Sham

Figure 31. Effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with ECS (open circles, n=8) or sham (solid circles, n=7). Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.


Dopamine
● – Chronic Sham
○ – Chronic ECS
Output (% of baseline)
140
130
120
110
100
−20
−10
ECS
10
20
30
40
50
60
Time (min)

Acute ECS increased interstitial concentrations of DOPAC, HVA and 5-HIAA in the NAC (Fig. 32). ANOVA revealed significant time effects ($F_{6,78}$=33.7, $F_{6,78}$=29.19, $F_{6,78}$=23.75, all p<0.001, for DOPAC, HVA and 5-HIAA). The ECS-induced increases in DA metabolites were less pronounced in the animals given repeated ECS. ANOVA performed on the DOPAC data indicated significance in treatment effect ($F_{1,13}$=12.25, p=0.004) but not in treatment X time interaction ($F_{6,78}$=0.42). A significant treatment X time interaction ($F_{6,78}$=2.5, p=0.024) was obtained for HVA, although the treatment effect did not reach statistical significance ($F_{1,13}$=1.87, p>0.05). Post-hoc analyses of the HVA data indicated significance (p<0.05) in the 50-60 min test interval. The ECS-induced increase in 5-HIAA output was also attenuated in the chronic ECS group but this difference did not reach statistical significance.

In order to compare the ECS-induced increases in dialysate concentrations of DA from NAC and striatum, the data from Figs. 22 and 29 were combined in Fig. 33. ECS resulted in a significantly higher ($F_{1,13}$=7.8, p<0.05) DA increase in the striatum than in the NAC. The ECS-induced increases in the metabolites from the NAC and the striatum did not differ significantly.

Apomorphine (25 μg/kg, s.c.) reduced interstitial DA concentrations to approximately 50% of baseline values within 30 min (Fig. 35). Chronic ECS did not influence the apomorphine-induced decrease in DA as revealed by a repeated measures ANOVA ($F_{1,13}$=0.84 and $F_{9,117}$=0.92 for the respective treatment and treatment X time interaction effects). There was a significant time effect ($F_{9,117}$=24.3, p<0.001) due to significant (Newman-Keuls, p<0.05) changes in DA output over time in both groups. Apomorphine decreased DOPAC and HVA modestly but significantly as revealed by the respective time effects $F_{9,117}$=3.02, $F_{9,117}$=2.64, both p<0.05 (Fig. 36). Apomorphine did not influence 5-HIAA concentrations. Chronic ECS did not significantly affect any of the apomorphine-induced effects on these metabolites.

Figure 32. Effect of electroconvulsive shock (ECS) on dialysate concentrations of DOPAC, HVA and 5-HIAA from nucleus accumbens of rats chronically treated with ECS or sham. Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.

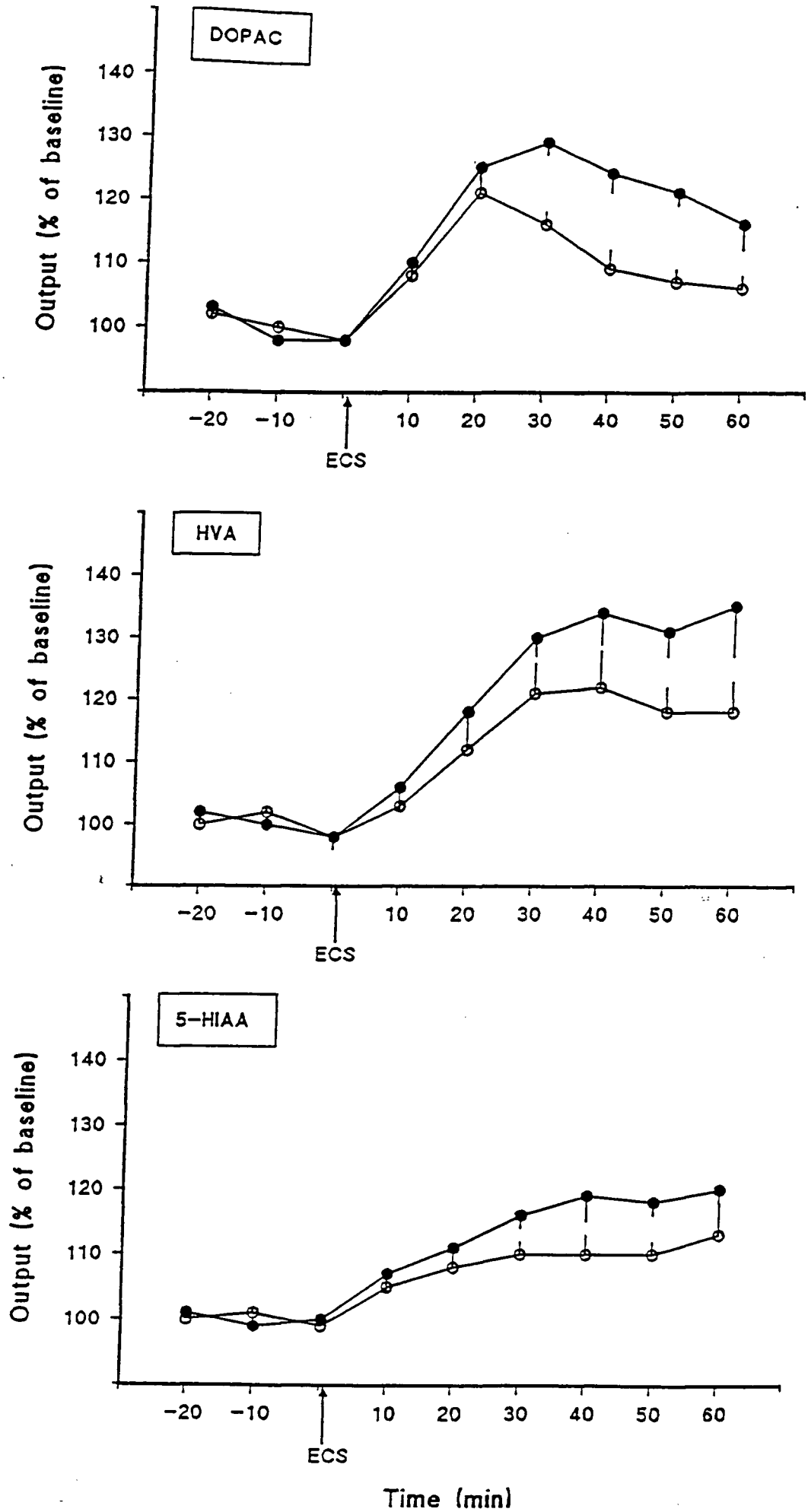

DOPAC
HVA
5-HIAA
Output (% of baseline)
140
130
120
110
100
-20
-10
ECS
10
20
30
40
50
60
Time (min)

Figure 33. The effect of electroconvulsive shock (ECS) on dialysate concentrations of dopamine from the striatum or the nucleus accumbens (combined data from Figs. 22 and 31).

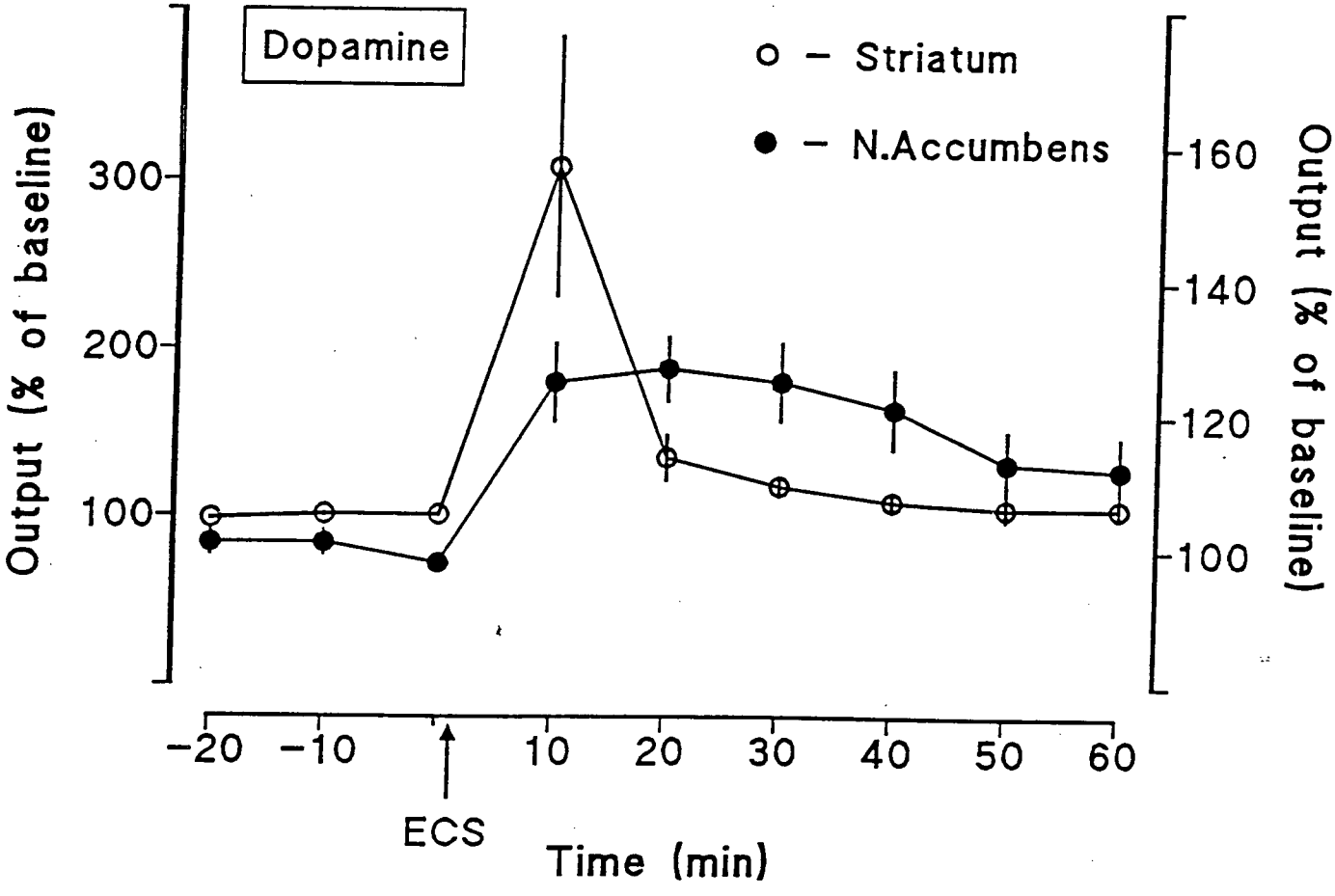

Dopamine
o – Striatum
● – N.Accumbens
Output (% of baseline)
300
200
100
160
140
120
100
Output (% of baseline)
−20
−10
10
20
30
40
50
60
ECS
Time (min)

Figure 34. The effect of flurothyl-induced seizures on dialysate concentrations of dopamine from the striatum (presented in Fig. 27) or the nucleus accumbens. Basal concentrations of dopamine from the nucleus accumbens are 4.8±1.3 (mean±S.E.M., fmol/min, n=6).

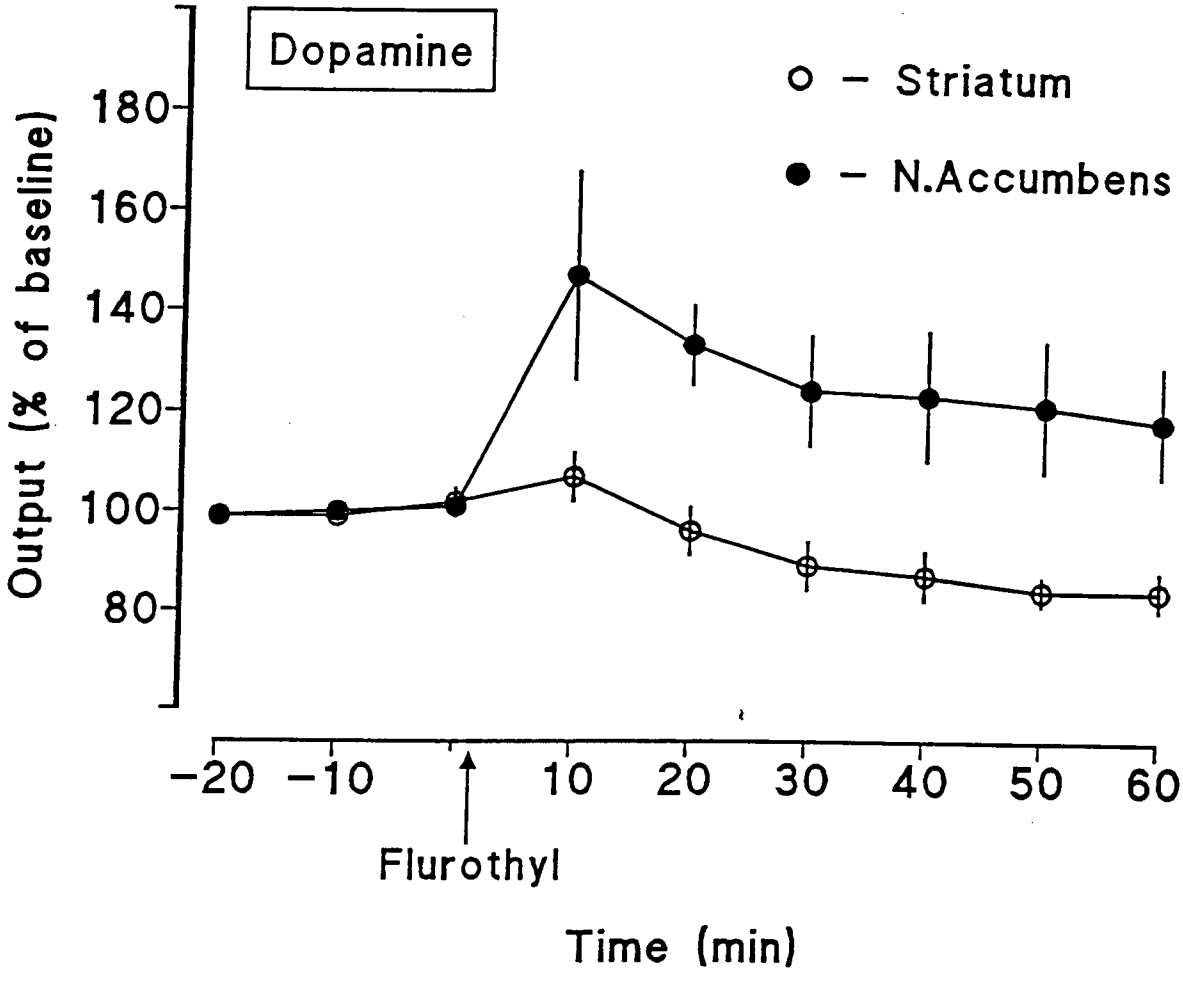

Dopamine
○ – Striatum
● – N.Accumbens
Output (% of baseline)
180
160
140
120
100
80
−20
−10
10
20
30
40
50
60
Flurothyl
Time (min)

Figure 35. Effect of apomorphine (25 μg/kg, s.c.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with ECS (solid circles, n=7) or sham (open circles, n=7). Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.

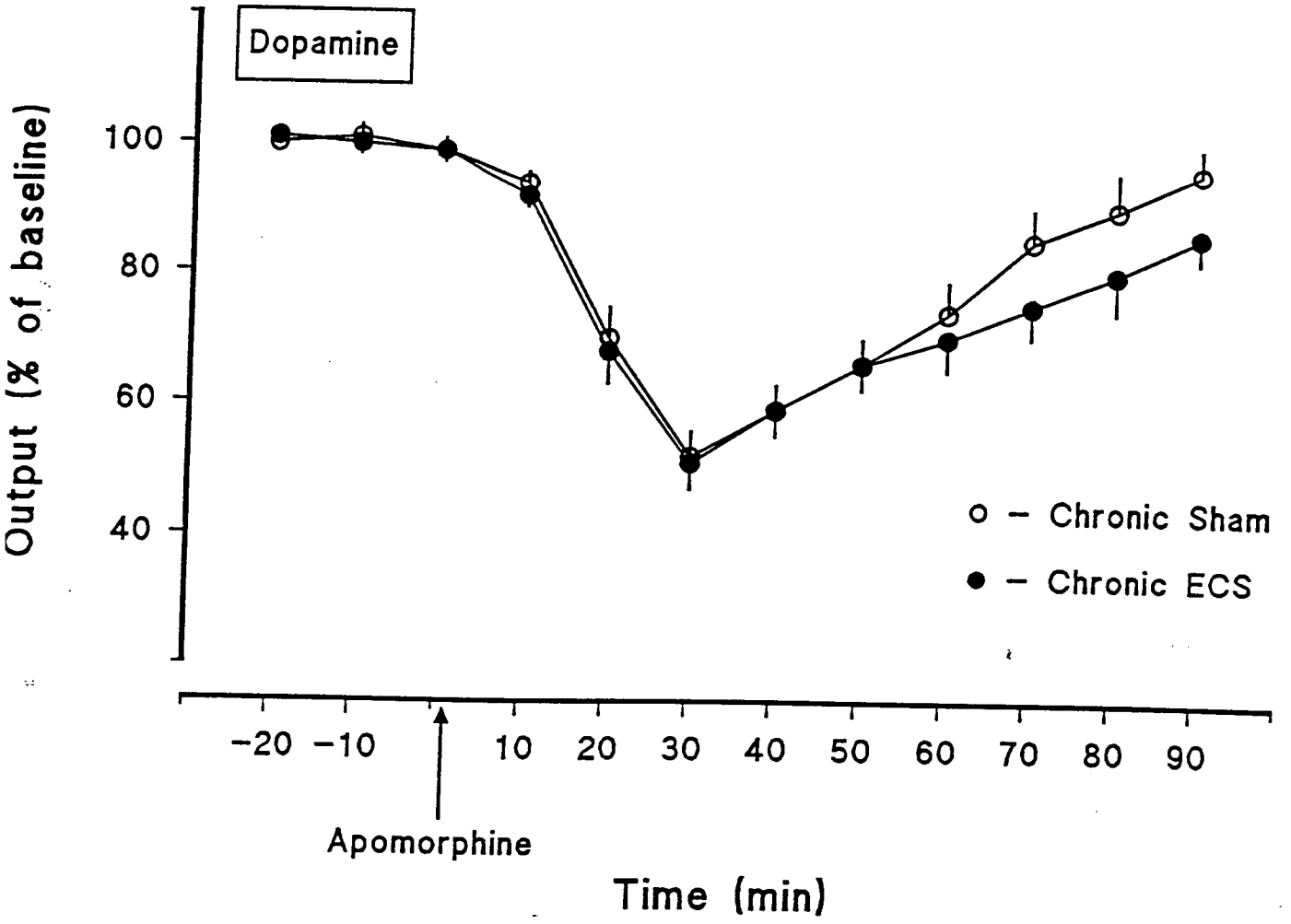
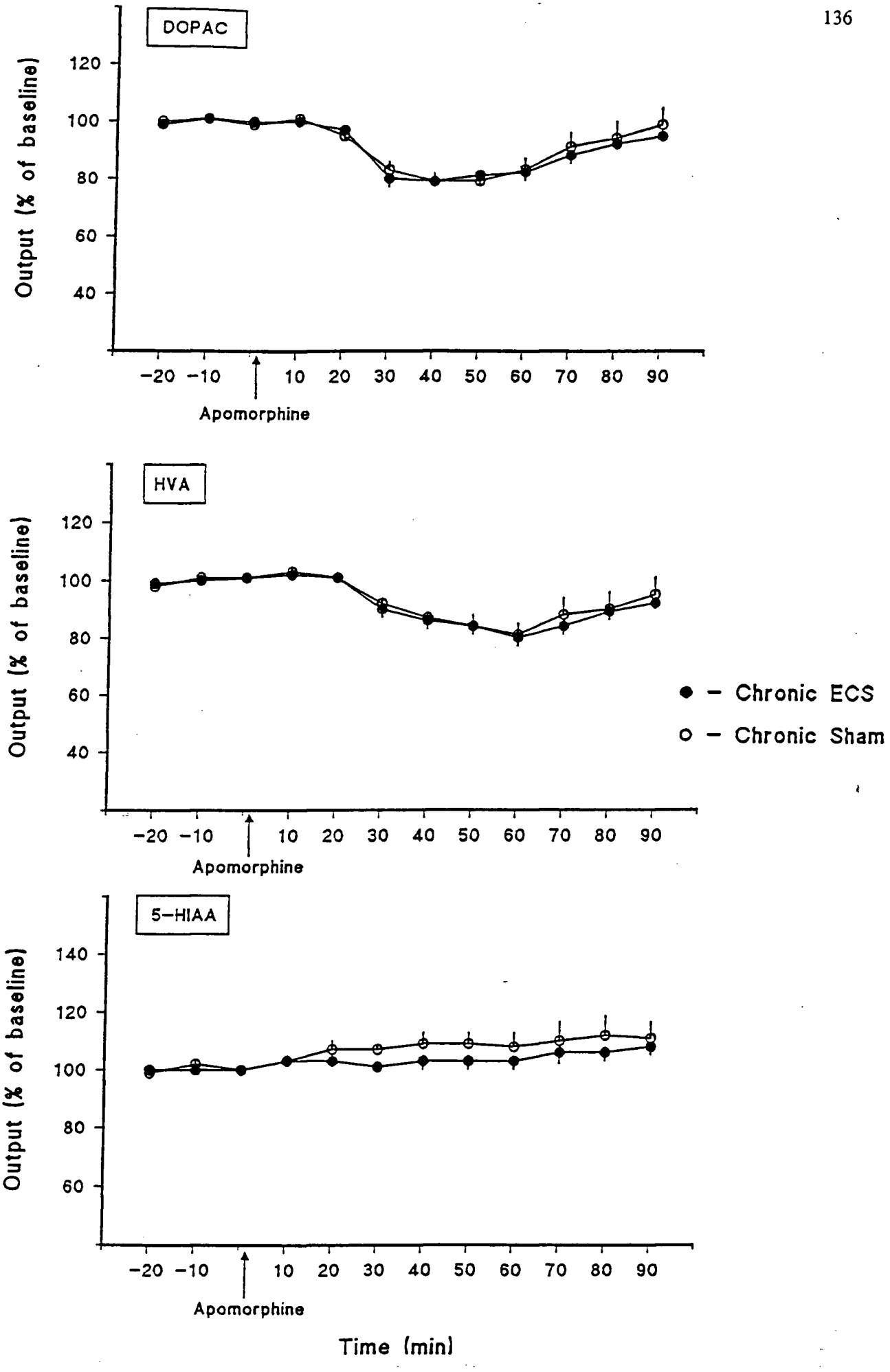

Dopamine
Output (% of baseline)
100
80
60
40
o – Chronic Sham
● – Chronic ECS
-20 -10
10 20 30 40 50 60 70 80 90
Apomorphine
Time (min)

Figure 36. Effect of apomorphine on dialysate concentrations of DOPAC, HVA and 5-HIAA from the nucleus accumbens of rats chronically treated with ECS or sham. Each point represents the mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.


DOPAC
Output (% of baseline)
120
100
80
60
40
-20 -10
10 20 30 40 50 60 70 80 90
Apomorphine
HVA
Output (% of baseline)
120
100
80
60
40
-20 -10
10 20 30 40 50 60 70 80 90
Apomorphine
● – Chronic ECS
○ – Chronic Sham
5-HIAA
Output (% of baseline)
140
120
100
80
60
-20 -10
10 20 30 40 50 60 70 80 90
Apomorphine
Time (min)

d-Amphetamine (1.5 mg/kg, s.c.) produced significant increases in the interstitial concentrations of DA in both the chronic ECS and sham groups (Fig. 37). The amphetamine-induced DA increase peaked at nearly 500% within 30-50 min in both groups, but remained elevated longer in the chronic ECS group. A repeated measures ANOVA did not indicate a significant treatment effect ($F_{1,13}$=1.33, p=0.27) but revealed a significant time effect ($F_{12,144}$=20.14, p<0.001) and a treatment X time interaction ($F_{12,144}$=2.76, p=0.002). Post-hoc comparisons with controls showed significantly (p<0.05) higher DA responses after amphetamine in the chronic ECS group at the 60-90 min test intervals. In addition, the rate at which d-amphetamine increased extracellular concentrations of DA in the NAC of the chronic ECS animals appeared to be somewhat slower than in the controls (Fig. 37). d-Amphetamine decreased the interstitial concentrations of DOPAC and HVA to a similar extent in the chronic ECS and control groups (Fig. 38). ANOVA performed on the DA metabolite data showed significant time effects ($F_{12,144}$=34.82 (DOPAC), $F_{12,144}$=15.25 (HVA), both p<0.001) but not in the treatment or treatment X time interaction effects. d-Amphetamine produced a small but prolonged increase in dialysate concentrations of 5-HIAA (Fig. 38). Repeated ECS did not significantly influence this effect and ANOVA revealed only a significant effect of time ($F_{12,\ 144}$=6.6, p<0.001).

After repeated ECS the animals became irritable and difficult to handle. Also, compared to sham treated rats, they gained less weight over the course of ECS. The mean (±S.E.M.) weight gain was 68±5 and 110±7 g for chronic ECS and sham groups, respectively (p<0.001). Locomotor activity (total distance expressed in cm) following d-amphetamine administration is illustrated in Figure 39. Locomotor activity scores peaked within 30-40 min post-injection and then gradually declined. Compared to sham-treated animals, amphetamine-induced hypermotility was more prolonged in the animals receiving chronic ECS, although the peak effects did not differ. These behavioral effects resembled the amphetamine-induced DA responses in the chronic ECS and sham groups (compare Figs. 37 and 39). A repeated measures ANOVA performed on the behavioral data from both groups

Figure 37. Effect of d-amphetamine (1.5 mg/kg, s.c.) on dialysate concentrations of dopamine from the nucleus accumbens of rats chronically treated with ECS (solid circles) or sham (open circles). Each point represents mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V. *: $p<0.05$

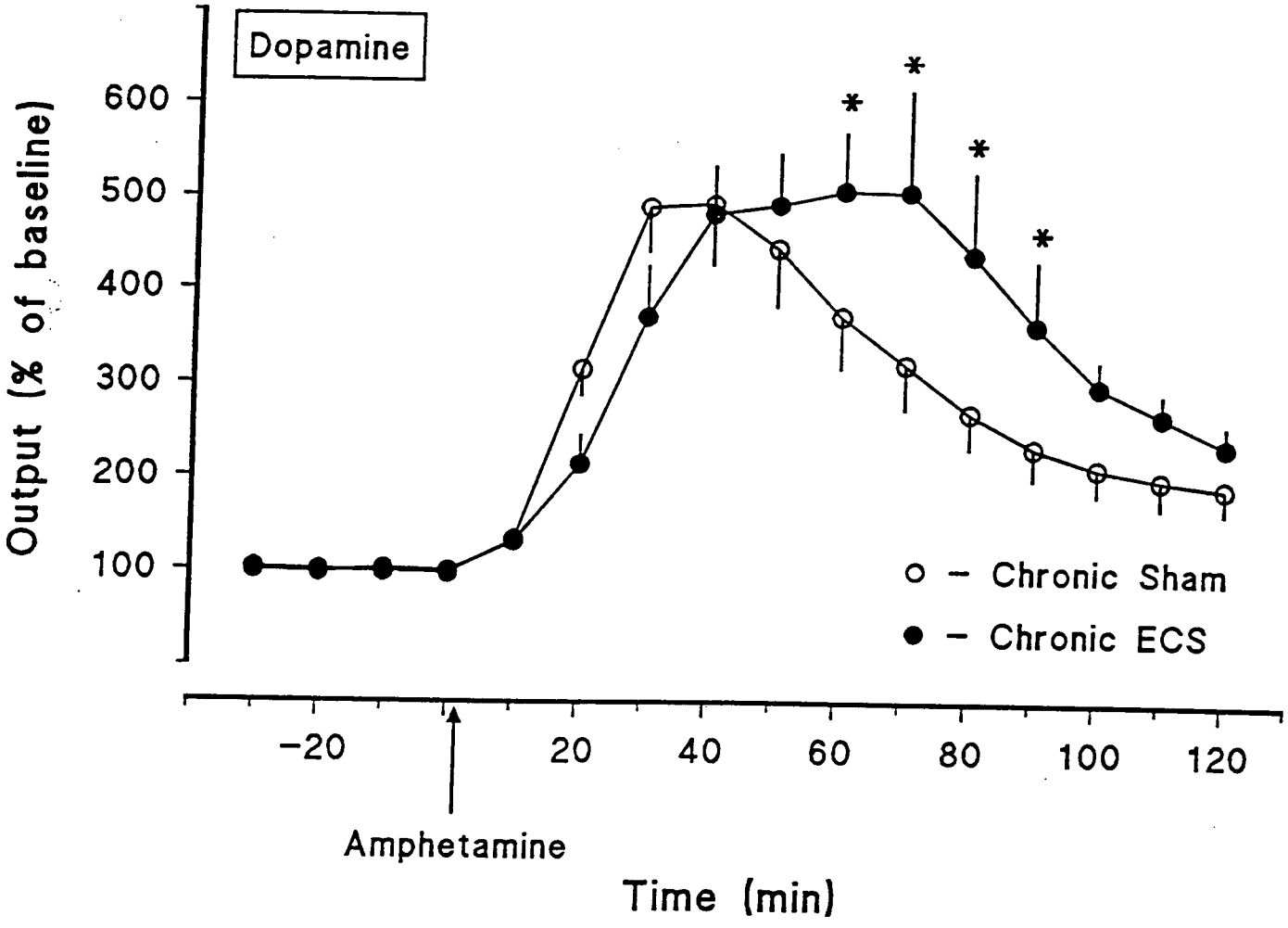

Dopamine
Output (% of baseline)
600
500
400
300
200
100
*
*
*
*
○ – Chronic Sham
● – Chronic ECS
−20
20
40
60
80
100
120
Amphetamine
Time (min)

Figure 38. Effect of electroconvulsive shock (ECS) on dialysate concentrations of DOPAC, HVA and 5-HIAA from the nucleus accumbens of rats chronically treated with ECS or sham. Each point represents mean (± S.E.M.) percent change of baseline. Baseline values are indicated in Table V.

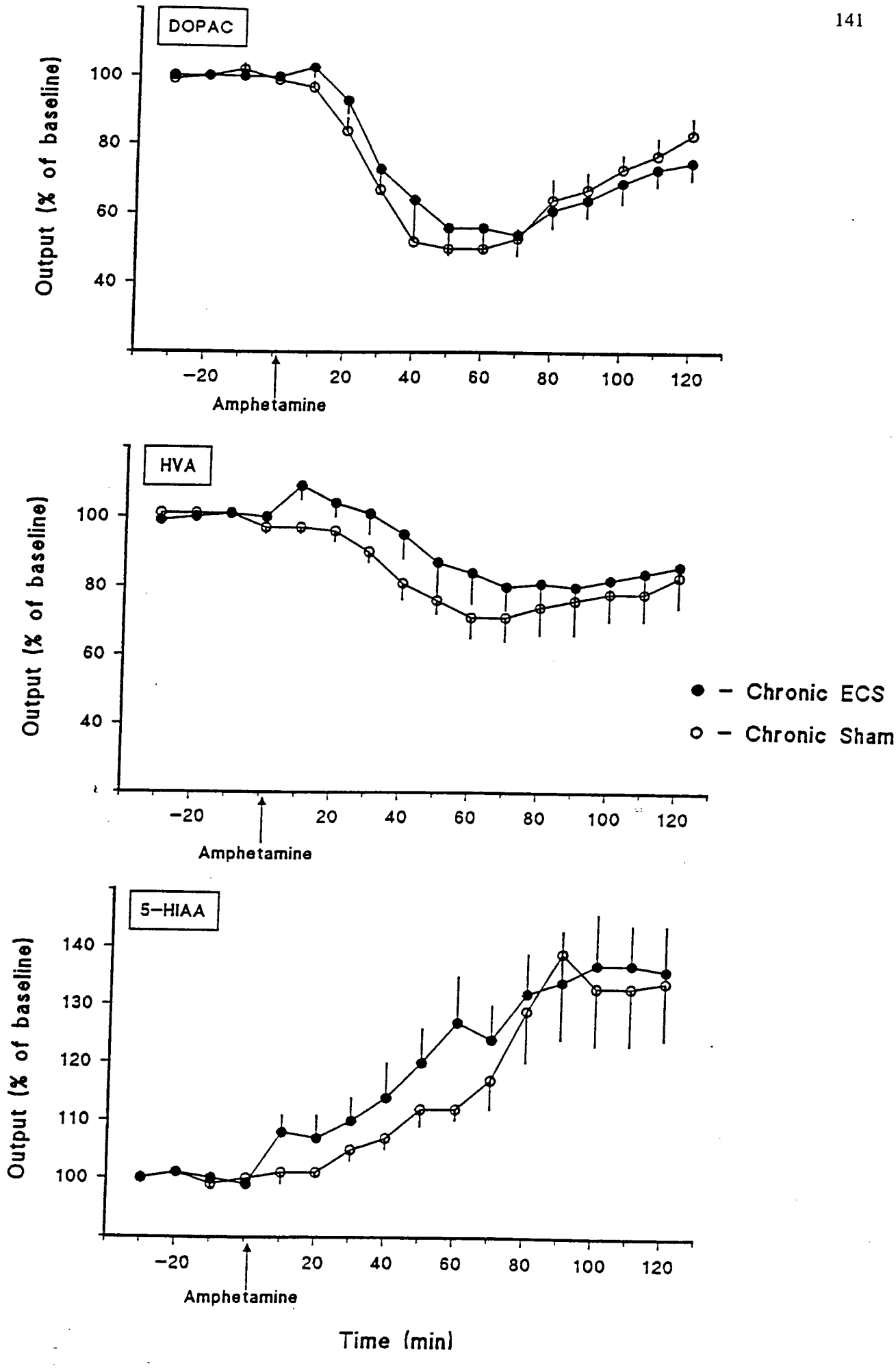
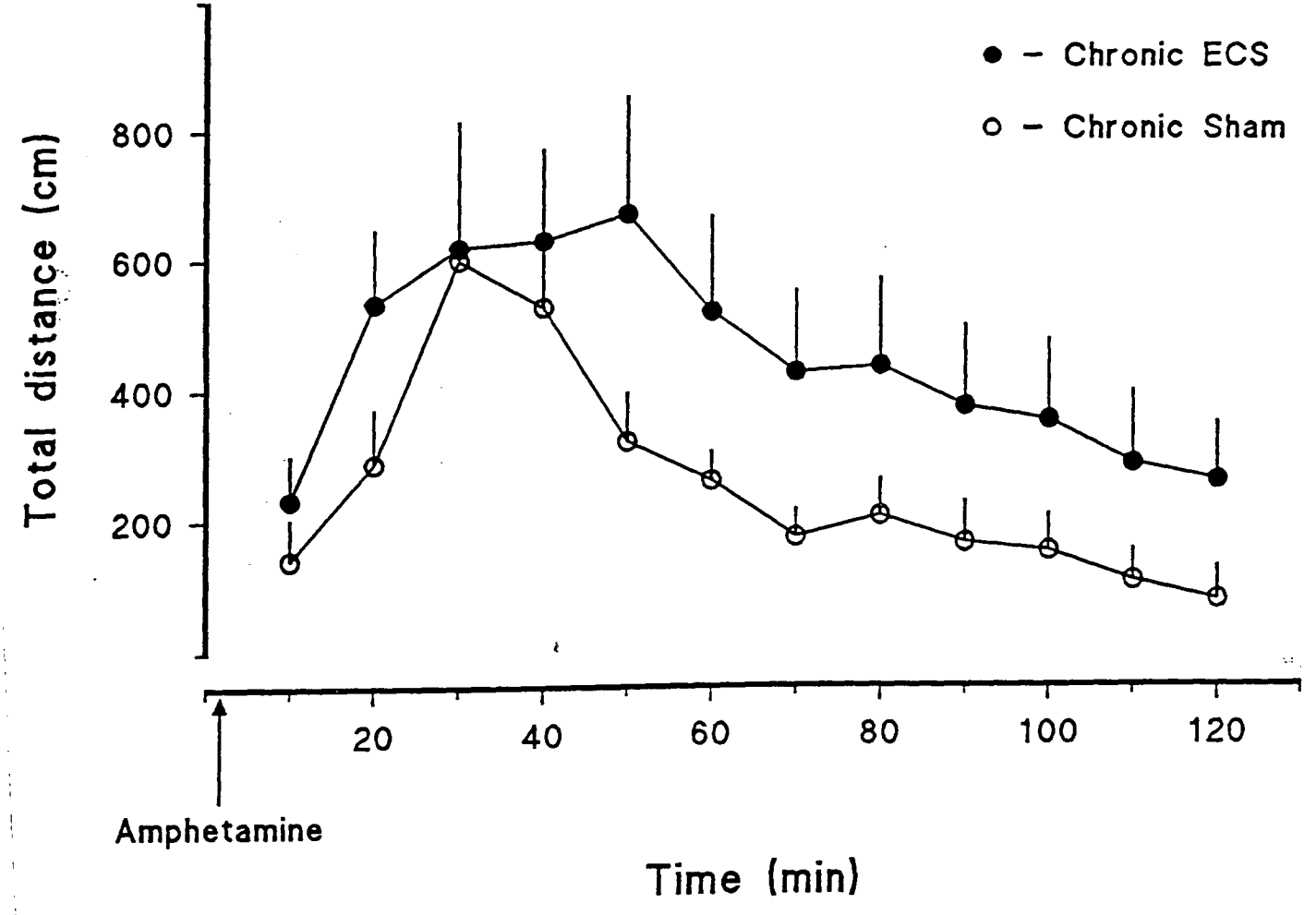

DOPAC
Output (% of baseline)
100
80
60
40
−20
20
40
60
80
100
120
Amphetamine
HVA
Output (% of baseline)
100
80
60
40
−20
20
40
60
80
100
120
Amphetamine
● – Chronic ECS
○ – Chronic Sham
5–HIAA
Output (% of baseline)
140
130
120
110
100
−20
20
40
60
80
100
120
Amphetamine
Time (min)

Figure 39. Effect of d-amphetamine (1.5 mg/kg, s.c.) on locomotor activity measurements (total distance-cm) of rats following chronic treatment with ECS (solid circles, n=7) or sham (open circles, n=7). Arrow indicates the injection of amphetamine. Data points represent group mean (± S.E.M.) activity counts over 10 min intervals.


● – Chronic ECS
○ – Chronic Sham
Total distance (cm)
800
600
400
200
20
40
60
80
100
120
Amphetamine
Time (min)

following amphetamine indicated significant treatment ($F_{1,12}$=4.98, p=0.04) and time ($F_{11,132}$=9.84, p<0.001) effects. The treatment X time interaction was not significant ($F_{11,132}$=0.46).

(D) Discussion

The present data demonstrate that a single ECS produces a short-lasting, large increase in interstitial concentrations of DA in the striatum, suggesting that this procedure results in an acute release of this neurotransmitter. Transient increases in the DA metabolites DOPAC and HVA were also observed, supporting the conclusion that striatal dopaminergic transmission was increased by ECS. The present results also demonstrate that acutely administered ECS increases interstitial concentrations of DA and its metabolites DOPAC and HVA in the NAC. Compared to the striatum where identical ECS parameters produce a 300% increase in extracellular DA the increase in the NAC is more modest. This comparison indicates that ECS has a regionally selective action on DA release. The reasons for this regional diversity are not known but could be due to differences in distribution of current and/or in the excitability of DA neurons in the two structures. Interestingly, the magnitudes of the effects of ECS on DOPAC and HVA in NAC and striatum are comparable. ECS-induced increases in extracellular concentrations of the DA metabolites might indicate increased DA metabolism and/or synthesis. Arguing for the latter, acute ECS has been shown to increase brain tyrosine hydroxylase activity (Masserano et al. 1981), and HVA concentrations in striatal tissue (Engel et al. 1968). ECS also increased interstitial concentrations of 5-HIAA in NAC. A similar 5-HIAA response to ECS is seen in hippocampus where this treatment also produces a large increase in interstitial concentrations of 5-HT (Zis et al. in preparation). Although the relationship between 5-HT and its

metabolite in the extracellular space is complex (Kalén et al. 1989), it is possible that the ECS-induced 5-HIAA increase reflects changes in serotonin release in NAC.

The observation that ECS increases interstitial DA concentrations in the rat striatum and NAC is at variance with a recent report by Glue et al. (1990). According to these authors, DA remained unchanged in the rat striatum, decreased in the NAC while DOPAC and HVA concentrations increased in both regions after a single ECS. Methodological differences probably account for this discrepancy since Glue et al. (1990) conducted their experiments less than 2 h after probe implantation. There is evidence that at short intervals after probe implantation DA collected in dialysate samples is not dependent on neuronal activity (i.e. TTX-insensitive, Westerink and de Vries 1988). Probe implantation causes considerable perturbation of neural tissue and a recovery period of 12-24 h is necessary before dialysis experiments can be performed under stable conditions (Westerink et al. 1987a; Benveniste 1989). Glue et al. (1990) also used a perfusion solution which contained a higher concentration of Ca^{++} and performed their experiments in anaesthetized rats.

Basal dialysate concentrations of DA dramatically decreased in the presence of TTX or after calcium depletion (Note 1). The ECS-induced increase of DA in striatum was partly TTX-independent but completely calcium-dependent. These findings suggest that ECS releases DA by an action-potential independent mechanism that involves an exocytotic process (Westerink et al. 1989; Augustine et al. 1987). A similar mechanism has been indicated for K^+-stimulated DA release (Imperato and Di Chiara 1984; Westerink et al. 1989). It is possible, therefore, that the direct passage of current causes massive depolarization of the nerve terminals and release of the neurotransmitter into the extracellular fluid.

Barbiturate anaesthetics elevate the seizure threshold and decrease the duration of seizures (Fink 1979; Miller et al. 1985). In the present study, Nembutal-induced anaesthesia indeed decreased the duration of the seizure but only marginally affected the ECS-induced increase in interstitial DA concentrations. This finding suggests that the ECS-mediated DA

release in striatum is the result of the direct passage of current rather than the spreading of seizure activity from the cortex.

Flurothyl-induced seizures were used for a period of time as an alternative to electrically induced seizures for the treatment of depression (Fink 1979). Although the clinical experience with flurothyl is not as extensive as that with ECT, it was considered comparable to ECT both in effectiveness and in side-effects (Laurell 1970). Differences between electrically and flurothyl-induced seizures are reflected in the EEG-patterns, the seizure duration, and the focus of seizure onset (Small et al. 1968; Laurell and Perris 1970; Small and Small 1975). Flurothyl-induced ictal activity was eventually abandoned as an alternative to ECT because it was difficult to titrate the dosage adequately in terms of induction and duration of seizures. In the present experiments flurothyl-induced seizures were of longer duration than those obtained with ECS. Since there was a large increase (+200 %) in extracellular DA in the striatum associated with electrically induced seizures but not with flurothyl-induced seizures, it can be concluded that the ECS-induced DA release in this structure was related to the passage of current and not to the seizure activity. In the NAC, however, seizures produced either by ECS or by flurothyl resulted in comparable increases in DA (+30 and +50 %, respectively). These findings indicate that the mesostriatal and meso-accumbens DA neurons differ in respect to chemically or electrically induced seizures. It is interesting that both flurothyl- and ECS-induced seizures increased the concentrations of the DA metabolites in both structures. In spite of the fact that flurothyl produced a slightly higher effect in nucleus accumbens DOPAC and HVA, these data suggest that the synthesis and the metabolism of DA increased to a similar extent in response to seizure activity in both brain regions. Administration of a higher energy electrical stimulus resulted in greater DA release in the striatum; however this difference was not significant thus arguing neither for nor against the hypothesis that the ECS-induced DA release is due to the direct passage of current. In order to test this hypothesis further it would be necessary to obtain a full electrical dose-DA response curve.

Significant improvement in the motor function of Parkinson's disease patients has been reported to occur early in the course of ECT therapy (Douyon et al. 1989). It seems plausible therefore, that the transient large increases in DA transmission in the striatum after acute ECS may contribute to the therapeutic response. The relationship of the transient small increases in DA transmission in the NAC during ECS- and flurothyl-induced seizures to the therapeutic effect of ECT in depression is not known. In animal studies chronic ECS has been shown to induce behavioral supersensitivity to DA agonists (Modigh 1975, 1984; Cowen et al. 1980; Grahame-Smith 1984). Whether repeated short-lasting enhancement of DA transmission contributes to this behavioral sensitization remains to be determined. In this regard, it is of interest that repeated administration of drugs which increase DA release or block reuptake can also produce behavioral supersensitivity (Robinson and Becker 1986) and that even a single ECS can enhance the response to a combination of L-DOPA and a monoamine oxidase inhibitor (Cowen et al. 1980), and decrease the inhibitory action of apomorphine to DA neuron firing (Chiodo and Antelman 1980). In practice however, a single ECS is rarely clinically effective and a course of six to twelve treatments is required for the treatment of depression (Fink 1979; Abrams 1988; American Psychiatric Association-A Task Force Report 1989).

The DA response to ECS appears refractory to a second stimulus delivered 2 h but not 24 h after the first. The biochemical basis of this phenomenon is not readily apparent. It is well established that seizure threshold increases with the number of ECTs administered in a given treatment course (Sackeim 1987a,b; Abrams 1988). It is unlikely however that the decreased DA response is associated with a change in seizure threshold since a concomitant decrease in seizure duration was not detected.

A significant increase in baseline striatal dialysate concentrations of DOPAC and HVA was observed in the repeated ECS group and there was also a trend for an increase in baseline DA concentrations. These results are consistent with the findings of Karum et al. (1986) and Mussachio et al. (1969), who report an increase in HVA rate of formation in the

caudate nucleus and in tyrosine hydroxylase (TH) activity, respectively, in response to chronic ECS. In contrast, Modigh (1976) and Masserano et al. (1981) did not find changes in striatal TH activity after repeated ECS. Chronically admininistered ECS did not influence the basal extracellular concentrations of DA and its metabolites in the interstitial space of the NAC, suggesting that chronic ECS does not change the steady-state synthesis and turnover rates of DA in this brain region. This finding confirms previous conclusions based on data obtained in *ex vivo* studies (Modigh 1976), and represents further evidence of regionally selective effects of chronic ECS on mesotelencephalic DA systems.

The ECS-induced increase in DA, DOPAC and HVA were attenuated after exposure to repeated ECS in both the NAC and the striatum. It is well established that seizure threshold increases with the number of ECTs administered in a given treatment course (Sackeim et al. 1987a,b). However, the decreased responses of DOPAC and HVA cannot readily be attributed to changes in seizure threshold since concomitant decreases in seizure duration were not observed. Also, differences in the dynamic impedance between the chronic ECS and control rats did not account for the decreased DOPAC and HVA responses since the amount of applied current was similar in both groups (see Results). Given the increase in baseline concentrations (in the striatum) this result should be interpreted with caution. Additional studies are required to elucidate the biochemical mechanisms and biological significance of this observation.

Chronic ECS did not affect the apomorphine-induced decrease in interstitial DA concentrations in the NAC, indicating that this treatment did not alter the sensitivity of DA autoreceptors. These data are consistent with the biochemical but not the behavioral results of Serra et al. (1981). The present findings are not compatible with electrophysiological evidence for subsensitivity of nigral DA autoreceptors following chronic ECS (Chiodo and Antelman 1980b; Tepper et al. 1982). It should be noted, however, that in the present study the meso-accumbens rather than the mesostriatal DA system was studied, and as mentioned before, the effect of apomorphine on interstitial DA concentrations in the NAC probably

reflects the combined effects of stimulation of both DA somatodendritic and presynaptic autoreceptors.

In the present experiments, the effects of amphetamine were found to be prolonged in the NAC of the chronic ECS group. In agreement with previous studies, this treatment caused the animals to become irritable and gain less weight than the controls (Pryor 1974; Modigh 1975). It is possible that the chronic exposure to ECS decreased food intake, and thereby altered the bioavailability of amphetamine and/or produced neurochemical changes which in turn influenced the amphetamine responses (Campbell and Fibiger 1971). It is unlikely however, that the altered neurochemical responses were the result of weight-loss-induced changes in d-amphetamine pharmacokinetics because food deprivation increases d-amphetamine-induced hypermotility in the first 30 min (Campbell and Fibiger 1971) whereas the enhanced DA response was only seen at later intervals in the present experiment (Fig. 37). It is also noteworthy in this regard that ECS-treated rats show increased hypermotility after direct injections of DA into the NAC (Heal and Green 1978; Modigh 1984). Furthermore, chronic treatment with the convulsant agent flurothyl results in potentiation of DA agonist-induced hyperlocomotion without influencing weight gain (Green et al. 1978).

The effects of chronic ECS on DA uptake sites have not been studied systematically. Modigh (1976) found that ECS does not affect DA uptake in whole brain. It is possible that the higher extracellular DA concentrations in the NAC that were observed after amphetamine in the chronic ECS group were due to a decrease in the number or the efficacy of the uptake sites in this stucture. Such an effect would be consistent with demonstrations that repeated ECS enhances the behavioral responses to a number of agents that increase extracellular concentrations of DA, including amphetamines, nomifensine, L-DOPA plus an MAO inhibitor or DA (Evans et al. 1976; Green et al. 1977; Heal and Green 1978; Deakin et al. 1981; Wielosz 1981; Modigh 1984). However, decreased DA uptake after repeated ECS could not account for the enhanced behavioral syndrome produced by direct

DA receptor agonists (Modigh 1975; Bhavsar et al. 1981; Green et al. 1983). Recently, direct evidence (albeit contradicting) for changes in postsynaptic receptor mechanisms has been obtained and therefore, these mechanisms may be involved as well. In this regard, chronic ECS decreases the number of D_1 receptors in the limbic system (Klimek and Nielsen 1987; De Montis et al. 1990) and the behavioral response to a D_1 receptor agonist (Hao et al. 1990). In contrast, repeated ECS increases D_1 receptor-mediated activity of adenylate cyclase in the limbic forebrain (Newman and Lerer 1989) and the behavioral syndrome induced by specific D_1 receptor agonists (Sharp et al. 1990). Furthermore, it has been suggested that intact presynaptic DA function is not required for the enhanced DA-mediated behavioral responses that are observed after ECS. For example, haloperidol administration shortly before each ECS failed to prevent enhanced DA-mediated behaviors (Green et al. 1980), and injection of dibutyryl cyclic-AMP directly into the NAC resulted in increased behavioral responses in ECS-treated animals (Heal and Green 1978); this latter compound acts beyond the DA receptor and increases locomotor activity even in the presence of haloperidol (Heal et al. 1978). On the other hand, pretreatment with the catecholamine synthesis inhibitor α-methyl-p-tyrosine has been shown to attenuate the DA behavioral supersensitivity induced by repeated ECS although this could be due to effects on noradrenergic transmission (Green et al. 1980).

ECS-induced decreases in the negative feedback mechanisms that regulate DA neurons in the VTA (Mogenson 1987; Oades and Halliday 1987) may also have contributed to the potentiation of the amphetamine-induced increase in extracellular DA in the NAC. The mechanism by which chronic ECS might decrease negative feedback to DA neurons in the VTA are unkown but could involve changes in GABAergic systems. In this regard, there is evidence that changes in GABAergic transmission contribute to increases in DA-mediated behaviors after ECS treatment (Green et al. 1982; Green and Nutt 1987).

The present studies confirm that repeated ECS can potentiate the locomotor stimulant properties of amphetamine (Green et al. 1977; Wielosz 1981). They further indicate that this

enhanced behavioral effect is accompanied by significant increases in the ability of d-amphetamine to increase the extracellular concentrations of DA in the NAC. While the mechanisms by which ECS lengthens amphetamine-induced increases in extracellular DA in the NAC remain to be determined, these experiments provide further evidence that chronic antidepressant treatments such as ECT can influence both pre- and post-synaptic mechanisms in the meso-accumbens dopaminergic system.

(E) Notes

Note 1. "Calcium ions play a fundamental role in the process of excitation-secretion coupling in neurochemical transmission. Release of transmitter occurs as a result of the arrival of an action potential at the nerve terminal. The depolarization phase of the action potential results in an opening of voltage-dependent Ca^{++} channels in the presynaptic membrane. The influx of Ca^{++} down its electrochemical gradient through calcium channels leads to a transient rise in intracellular Ca^{++} which triggers a transient release of the transmitter. The Ca^{++} entry into the presynaptic terminal can be abolished directly by Mn^{++} and Co^{++}, and indirectly by TTX which blocks sodium-dependent voltage-operated channels and thus blocking the opening of voltage-dependent Ca^{++} channels. Consequently, TTX, inorganic Ca^{++} blockers or calcium depletion inhibit transmitter release" (p. 204, Benveniste and Hüttemeier 1990).

V. General Discussion

In animal studies chronic antidepressant treatments have been shown to facilitate DA-mediated responses. For example, repeated treatment with several antidepressant drugs or ECS enhances the behavioral stimulation induced by various direct or indirect DA receptor agonists (Spyraki and Fibiger 1981; Martin-Iverson et al. 1983; Maj et al. 1984; Arnt et al. 1984; Modigh 1984; Willner 1985). Chronic DMI treatment also increases ICSS rates obtained from electrodes in the VTA (Fibiger and Phillips 1981). In animal models of depression dopaminergic mechanisms also appear to mediate normalization by TCAs of some "pathological" behaviors. This condition is best exemplified by Willner's animal model of depression in which rats chronically exposed to mild stress reduce their consumption of a highly preferred sucrose solution; this anomalous behavior is restored by repeated administration of TCAs and this "therapeutic" effect of the antidepressants is abolished by DA receptor antagonists (Willner et al. 1987, 1990; Muscat et al. 1990).

Most antidepressant drugs do not influence extracellular concentrations of DA when administered acutely. It is probable, however, that with chronic regimens these compounds may reach steady-state plasma levels and brain concentrations (in the low μM range) that are sufficient to inhibit the uptake of DA and thereby increase extracellular DA (Randrup et al. 1975; Vetulani et al. 1976). The present studies indicate that ECS and acute administration of bupropion increases extracellular concentrations of DA. At present it is unclear if and how the increase of extracellular DA under drug steady-state conditions, or immediately after ECS contributes to the facilitation of certain behaviors. This issue has been addressed previously, by others. First, pretreatment with haloperidol before each ECS does not influence the enhancement of hyperlocomotion induced by combined administration of an MAO inhibitor and L-DOPA (Green et al. 1980). One interpretation of this finding is that the chronic ECS-induced enhancement of the behavior does not require changes to occur at the DA synapse but rather beyond the DA receptor. Because haloperidol does not block the ECS-induced DA

release, an alternative hypothesis is that adaptations may occur at the presynaptic level, and thus stimulated DA release is augmented. These presynaptic changes could involve down-regulation of DA uptake sites, increased DA release, or subsensitivity of inhibitory autoreceptors as has been suggested by Serra et al. (1979, 1980) and by Chiodo and Antelman (1980 a,b). Second, α-methyl-p-tyrosine administration during a course of ECS abolishes the enhancement of DA-mediated response (Green et al. 1980). Daily injections of α-methyl-p-tyrosine also prevent the chronic imipramine-induced effects both in [^{3}H]SCH 23390 binding and in the responsiveness of adenylate cyclase to DA (De Montis et al. 1990). These studies suggest that transmission through DA synapses is necessary for the changes in dopaminergic function produced by the chronic antidepressant treatment. Third, chronic ECS potentiates the apomorphine-induced rotation in unilaterally 6-OHDA-lesioned animals (Green et al. 1977). This observation indicates that the denervation-induced supersensitivity of DA receptors is increased by chronic ECS, thus resulting in the enhanced response. The data in Green et al. (1977) strongly suggest that the chronic ECS-induced increases in postsynaptic DA receptor sensitivity are independent of the activity of DA neurons. Taken together, the above findings suggest that both presynaptic and postsynaptic DA mechanisms may contribute to the facilitated DA-mediated responses following chronic antidepressant treatments.

The hypothesis that intact DA transmission is required to detect enhanced DA-elicited responses is supported by several studies in which the potentiated DA responses after chronic antidepressants were blocked by pretreatment with DA receptor antagonists or DA depleting agents. For example, blockade of D_2 receptors antagonizes quinpirole-induced enhanced locomotor activity in chronic imipramine treated rats (Maj 1990; Serra et al. 1990). Similarly, antagonism can be achieved by high doses of the D_1 antagonist SCH 23390 (Serra et al. 1990). Combined administration of reserpine and α-methyl-p-tyrosine before the injection of quinpirole also abolishes the D_2 agonist-induced potentiation of locomotor activity in animals treated with chronic antidepressants (Serra et al. 1990). The activating effects of antidepressants in the behavioral despair test may also be mediated by their potentiating effects on DA

function. For example, a number of studies have shown that low doses of DA receptor antagonists, which had no effect when administered alone, blocked the anti-immobility effects of chronic antidepressant treatments (Borsini et al. 1984, 1985; Pulvirenti and Samanin 1986; Bereterra et al. 1986). Direct application of sulpiride in the NAC blocked the anti-immobility behavior induced by chronic DMI or imipramine (Cervo and Samanin 1987). Arguing against an involvement of DA, however, are the findings that intra-accumbens DA or L-DOPA administration do not exert anti-immobility effects (Plaznik et al. 1985a; Borsini et al. 1988). In the chronic mild stress animal model of depression administration of selective D_1 or D_2 antagonists prevent sucrose consumption in stressed animals chronically treated with TCAs (Willner et al. 1990). There are also reports that clearly point to an increase in the sensitivity of structures postsynaptic to DA neurons. Pretreatment with reserpine does not influence the repeated ECS-induced enhancement of the locomotor stimulant effects of apomorphine (Modigh 1975, 1984). Similarly, enhanced responsiveness to DA agonists produced by REM sleep deprivation remains unaffected after inhibition of DA synthesis (Tufic 1981). The anti-immobility effect of DMI is also found to be undiminished by 6-OHDA lesions of the mesolimbic DA projection (Plaznik et al. 1985b). This latter finding indicates that antidepressants potentiate postsynaptic DA responses by influencing directly the postsynaptic elements. Taken together, the above data suggest that for the expression of the facilitated DA behaviors either presynaptic or postsynaptic elements of the DA synapse may be involved.

The data presented in this thesis indicate that chronic antidepressants facilitate the behavioral and neurochemical effects of indirect DA agonists. One of the foremost methodological issues that needs to be addressed is the timing of the experimental tests. In the present studies a three day interval was interposed between the time of the last drug injection and the time of the perfusion experiments. Many studies of chronic antidepressant administration have allowed a period of drug withdrawal before testing for biochemical or behavioral changes. The rationale is that following this washout period the antidepressants would be eliminated (Bicker and Wedel 1968), and the interference by these drugs in a

biochemical assay, or the pharmacokinetic interactions in a functional study would be avoided. Nevertheless, the use of a washout period introduces the possibility that the observed changes are withdrawal effects. In clinical practice antidepressant withdrawal can precipitate a number of symptoms, including psychic and behavioral activation, mood elevation and hypomania (Dilsaver and Greden 1984). In animal studies however, the possibility that chronic antidepressants potentiate DA-mediated behaviors due to drug withdrawal has been excluded by direct application of DA agonists into the NAC shortly after the last drug administration (Maj 1986; Modigh 1984). Microdialysis studies can be extremely useful in this aspect as local application of the DA agonists can be achieved through the dialysis fibre.

With the exception of striatal DOPAC and HVA concentrations after chronic ECS, changes in basal extracellular concentrations of DA or the metabolites were not detected. These findings suggest that the synthesis and turnover rates of DA do not change after chronic antidepressants, this being in accordance with most of the results obtained by *ex vivo* biochemical measurements (Neff and Costa 1967; Modigh 1976; Leonard and Kafoe 1976; Sugrue 1980). In contrast, neurophysiological studies have indicated a higher number of spontaneously firing DA neurons in the VTA and the substantia nigra following chronic treatment with antidepressants (Chiodo and Bunney 1983; White and Wang 1983). It appears, therefore, that increased basal levels of DA produced by an increase in the number of active DA neurons is not detected by *ex vivo* or *in vivo* biochemical assays. The finding that chronic antidepressants do not influence the basal concentrations of DA argues against the possibility of sustained effects of these treatments on presynaptic DA elements, at least under resting conditions. In this regard, it is interesting that in the well established phenomenon of psychostimulant sensitization, changes in the basal concentrations of DA are also not observed (Robinson and Becker 1986). It is only after stimulation of DA release that changes in extracellular concentrations of DA become apparent (Robinson et al. 1988; Kalivas and Duffy 1990). Similarly, it is possible that chronic antidepressants enhance the stimulated DA release. This hypothesis was tested in a study by Nurse et al. (1986) who found that chronic DMI

treatment does not influence the K^+-stimulated release of DA from NAC slices. The reasons for the discrepancy between the *in vitro* study by Nurse et al. and the present *in vivo* studies with d-amphetamine are not clear, but an interesting issue is raised. Is it the augmentation of the amphetamine-induced increase in extracellular concentrations of DA in the NAC by chronic DMI that causes the behavioral supersensitivity, or vice versa? In psychostimulant-induced sensitization, the biochemical effects appear to be expressed independent of the behavioral effects, as stimulated DA release has been shown in NAC tissue preparations from sensitized animals (Kalivas and Duffy 1988, Castañeda et al. 1988). *In vivo* microdialysis provides an opportunity to address directly this issue. As shown in this thesis, local infusion of indirect DA agonists produce a dose-dependent increase in extracellular concentrations of DA without influencing the behavior of the rats. If the DA increase facilitated by chronic antidepressants is independent of the facilitated behavior, topical administration of amphetamine should shift the dose-response curve to the left.

Decreased DA uptake could account for the enhanced extracellular concentrations of DA in response to the DA agonist challenge. The functional status of DA uptake sites in the NAC following chronic antidepressants has not been assessed. In an early study by Modigh (1976) the DA uptake processes in whole brain were not influenced by a course of ECS. Theoretically, a decrease in the uptake capacity of DA neurons in the NAC following chronic antidepressants could not be easily explained by homeostatic mechanisms; namely, an increase in the function of uptake sites should be expected as a result of biochemical adaptation to the increased extracellular concentrations of DA during the chronic antidepressant treatments (see above). However, the principal of homeostasis is of very limited value in understanding how antidepressants exert their actions (Willner 1989). Decreased negative feedback of DA neurons in the VTA could also explain the biochemical findings of the present experiments. This hypothesis would require changes in the multisynaptic circuits involved in the feedback mechanisms that regulate DA release in the NAC. Although the exact components of such neuronal connections are not known, the participation of GABA neurons has been indicated

(Nauta et al. 1978; Jones and Mogenson 1980). Changes in the function of GABA neurons have been suggested to mediate the enhancement of DA related behaviors by repeated ECS (Green et al. 1982; Bowdler et al. 1983). In addition, a common GABAergic mechanism of action of antidepressant drugs and ECS-mediated via $GABA_B$ synapses has been proposed (Lloyd et al. 1985).

Chronic antidepressant-induced subsensitivity of DA autoreceptors could also potentially account for the present results. Subsensitivity of inhibitory DA autoreceptors would result in a decreased inhibition of the firing of DA neurons, and thus, in an increase in synthesis and release of DA. This would produce high extracellular DA concentrations and an increase in DA function. However, in the relevant literature this issue is controversial with almost equal numbers of positive and negative findings (Willner 1985). The present data do not support the hypothesis of desensitization of DA autoreceptors by chronic antidepressant treatments. It must be emphasized however, that in the present experiments where apomorphine was administered peripherally, the functional status of DA autoreceptors both in the somatodendritic and the terminal regions would be evaluated simultaneously. A more definitive microdialysis study could involve the local application of apomorphine or selective D_2 agonists in the NAC or VTA during measurements of DA efflux in the NAC. In this manner, a more direct comparison with the neurophysiological studies by Chiodo and Antelman (1980 a,b) could be made, thereby providing a more detailed analysis of the role of DA autoreceptors in the mechanisms of action of antidepressants.

Increased DA function following chronic antidepressant treatments could be the result of increased sensitivity of postsynaptic DA receptors in the NAC. Recently, with the use of selective ligands evidence for changes in postsynaptic mechanisms has been indicated. For example, chronic treatment with several antidepressants and ECS decreases the density of D_1 receptors in the limbic system (including the NAC) and striatum (Klimek and Nielsen 1987; De Montis et al. 1990). Furthermore, repeated administration of imipramine or mianserin enhances the affinity of D_2 receptors for DA agonists in the NAC (Klimek and Maj 1990). The activity

of adenylate cyclase in response to DA stimulation appears to decrease following chronic treament with imipramine (De Montis et al. 1990). In contrast, chronic ECS has been reported to increase D_1 agonist-stimulated adenylate cyclase activity in the striatum and the limbic forebrain (Newman and Lerer 1989). Changes in these postsynaptic elements could possibly reflect biochemical adaptations to altered DA transmission during the chronic exposure to the antidepressants. In this regard, pretreatment with α-methyl-p-tyrosine has been found to prevent the decrease in the density of D_1 receptors, and in the activity of adenylate cyclase following chronic treatment with imipramine (De Montis et al. 1990). Taken together, these results suggest that chronic antidepressant treatments affect both presynaptic and postsynaptic elements of the DA synapse in the NAC. Using microdialysis, it would be worthwhile to examine the effects of direct DA agonists on extracellular concentrations of DA in the NAC following chronic antidepressants. Peripheral or topical administration of direct DA agonists decrease extracellular DA concentrations in the terminal regions by stimulating negative feedback processes (Imperato and Di Chiara 1988; Imperato et al. 1988). Chronic antidepressant treatment might result in a less pronounced effect of the direct DA agonists on extracellular DA in the NAC, thus contributing to the enhanced DA function. Another issue that needs to be addressed by *in vivo* microdialysis studies is the functional status of the mesolimbic DA system in animal models of depression. There is evidence that chronic exposure of animals to uncontrollable aversive events can produce significant effects on behavior. It would be interesting to examine the neurobiological correlates of the antidepressant-induced reversal of these abnormal behaviors (immobilized postures, decreased consumption of preferable solution).

In the present experiments the amphetamine-induced increases in extracellular concentrations of DA were prolonged in the chronic ECS group with no difference in the peak response. Also, the DA metabolite concentrations were selectively enhanced in the striatum after chronic ECS. These effects differ somewhat from the effects obtained with chronic DMI. It is likely that antidepressants may act through a variety of neurochemical mechanisms to produce their effects. In this regard, most studies show that chronic treatment with

antidepressants does not influence stereotyped behavior induced by high doses of DA agonists (Delini-Stula et al. 1979; Maj et al. 1981; Spyraki and Fibiger 1981; but see also Willner et al. 1984), while chronic treatment with ECS increases these stereotypic behaviors (Modigh 1976, 1984; but see also Wielosz 1981). Destruction of central NA neurons has been reported to abolish the facilitation of DA function by ECS (Green and Deakin 1980). However, NA depletion did not prevent the facilitation of DA function by DMI (Martin-Iverson et al. 1983). Thus, although the effects of ECS may be mediated indirectly through a primary action on NA neurons, the effect of DMI appears to be independent of NA. An additional difference is that chronic treatment with antidepressant drugs usually decreases the density of 5-HT_2 receptors (Peroutka and Snyder 1980), while chronic ECS results in an increase (Vetulani et al. 1981).

The data presented in this thesis suggest that at least a part of the ECS-induced DA release in striatum is related to the direct passage of current at the DA terminals. In contrast, due to the fact that both chemically and electrically-induced seizures increase DA in the NAC, it is likely that spreading of seizure activity from cortical areas is responsible for this effect in the NAC. It would be worthwhile to study the TTX sensitivity and Ca^{++} dependency of the ECS-induced DA release in the NAC. In this regard, it is interesting that ECS increases serotonin release in hippocampus in a TTX-sensitive manner (Zis et al. in preparation). The above findings support the hypothesis formulated by Baldessarini (1975) that each ECS acutely influences the release of monoamines in the CNS, contributing in some way to the therapeutic efficacy (and possibly the cognitive side-effects) of this treatment. The differentiation between seizure and current-induced release of neurotransmitters by ECS is of particular importance since it has recently been shown that the amount of energy used to induce seizures influences the therapeutic outcome of the ECT (Robin and De Tissera 1982). This finding challenges the view, that induction of seizure (not the current *per se*) is a necessary and sufficient prerequisite for the antidepressant efficacy of the treatment (Cronholm and Ottoson 1960; Fink 1979). In view of the data presented in this thesis, it would be interesting to study the effects of various

stimulus parameters of ECS on DA release in the NAC and striatum. This might have important implications for the effect of ECT in depression and Parkinson's disease.

Measurements of HVA concentrations in the CSF has provided one of the most direct means for assessing brain DA function in clinical studies (Post et al. 1980; Jimerson and Berrettini 1985). Low levels of HVA in the CSF have been consistently indicated in retarded depression (Post et al. 1980); also, a favorable clinical response to some DA agonists has been predicted by low pretreatment levels of HVA in CSF (Jimerson and Post 1984). In general, however, studies of CSF HVA levels in depressed patients treated with a range of antidepressant drugs have shown no reliable changes following drug treatment (Jimerson 1987). Several workers have investigated presynaptic dopaminergic effects of ECT by studying CSF HVA before and after a course of ECT; almost all failed to demonstrate significant changes in HVA (Abrams 1988). Although these results are perhaps disappointing, it has to be noted that CSF DA metabolite levels mostly reflect the activity of nigrostriatal neurons (Sourkes 1973).

Microdialysis can successfully be utilized to provide important information about the mechanisms of action of drugs that influence dopaminergic transmission. In these studies several compounds that share the ability to inhibit the uptake of DA were characterized, and a rank order of potency of these drugs to increase extracellular concentrations of DA was obtained. Amongst these DA uptake inhibitors are drugs that are effective antidepressants (bupropion, nomifensine), drugs that have mood elevating properties in humans (amphetamine, methylphenidate), and drugs that are addictive in humans (cocaine, amphetamine, methylphenidate) (Willner 1985; Rudorfer and Potter 1989; Jimerson 1987). Moreover, all these substances show abuse potential in animal studies (Fibiger and Phillips 1986; Koob et al. 1987; Carr et al. 1989; Nielsen and Andersen 1990). Although the neurochemical consequences of chronic administration of these compounds differentiate their actions, it is striking that all these substances enhance the function of the mesotelencephalic dopaminergic system. Transmission through DA synapses is also enhanced by natural rewards like food and sex (Radhakishun et al. 1988; Holmes 1990; Pfaus et al. 1990; Nomikos et al. in preparation), and by chronic

antidepressant treatments (Willner 1985; Fibiger and Phillips 1987; present study). By drawing these parallels, the central position of the brain dopamine activity in the neurobiology of affect and its disorders is becoming increasingly apparent.

VII. References

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