Effects of Repeated Stress on Mesocorticolimbic Dopaminergic Neurons: In Vivo Voltammetric Studies

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ABSTRACT

The effects of repeated, once daily exposure to either tail pinch or restraint stress on extracellular DA levels in nucleus accumbens (NAcc), prefrontal cortex (PFC), and striatum (STR) was monitored in conscious rats using high-speed chronoamperometry, an electrochemical detection technique. The first exposure to either stress reliably and consistently elevated DA in the extracellular space of NAcc, PFC, and STR, the increases observed in PFC were of greater magnitude than those observed in NAcc and STR. These data are consistent with those of previous studies suggesting a higher responsiveness of the meso-PFC system to stress. However, with repeated exposure increases in DA levels elicited by restraint became progressively larger in NAcc, and to a lesser extent also in STR, but not in PFC. Apomorphine, injected at autoreceptor selective doses, attenuated tail pinch and restraint stimulated increases in DA levels in NAcc but not in PFC, a finding consistent with the drug's action on impulse-modulating receptors of meso-NAcc DA neurons and with the known absense of such receptors on meso PFC DA neurons. That DA was the primary contributor to the electrochemical signals was confirmed by the potentiating effect of GBR-12909, a selective DA uptake inhibitor, on restraint-elicited electrochemical responses in PFC and NAcc.

Taken together, the results of the present study indicate that with repeated exposure the meso-NAcc DA response to subsequent exposure to stress is enhanced. The data indicate that this pathway, which is thought to mediate the positive reinforcing effects of rewards, is also activated during behaviors motivated by aversive stimuli.

CONDENSE

Les changements dans la concentration extracellulaire de dopamine induit par l'application quotidienne répétée de deux stresses exogènes, soit une contention de 15 minutes ou un pincement de la queue de 10 minutes ont été mesurés au niveau du cortex préfrontal, du noyau accumbens et du striatum chez le rat vigilant au moyen de la chronoampérométrie. Les données demontrent qu'à la première application, les deux stresses induisent une augmentation de la dopamine extracellulaire dans les trois régions, mais que la réponse du cortex préfrontal est relativement plus grande que celles du noyau accumbens et du striatum. Par contre, les données démontrent aussi une sensibilisation progressive de la réponse à la contention au niveau du noyau accumbens et du striatum avec chaque application quotidienne, tandis que la réponse du cortex préfrontal demeure relativement constante. Dans une deuxième série d'expériences, il a été démontré que l'injection d'apomorphine, à des doses qui stimulent sélectivement les auto-récepteurs dopaminergiques somatodendritiques, inhibe au niveau du noyau accumbens, mais non au niveau du cortex préfrontal la libération accrue de dopamine produit par l'application de ces stresses ; ces données sont expliquées par l'absence de récepteurs somatodendritiques sur les neurones dopaminergiques mésocorticaux. Enfin, une contribution importante de la dopamine a été confirmée par l'effet facilitateur du GBR-12909, un inhibiteur sélectif de récapture dopaminergique, sur la libération de la dopamine au niveau du cortex préfrontal et du noyau accumbens induit par la contention. L'ensemble de ces résultats démontrent que la reponse des neurones dopaminergiques qui innervent le noyau accumbens est sensibilisée par l'application répétée d'un stresse exogène, phénomène qui s'apparente à

celui produit par l'administration répétée de drogues psychostimulantes telles que l'amphétamine et la cocaïne. Au niveau théorique, les résultats de cette étude suscitent un certain questionnement concernant l'hypothèse voulant que les neurones dopaminergiques qui innervent le noyau accumbens soutendent de façon spécifique le renforcement positif

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PREFACE

Section 1 of this thesis is an introduction to the area of dopaminergic systems and their response to stress, and includes an extensive survey of the literature on the effects of both acute and repeated exposure to stress. Section 2 presents the rationale for studying the effects of repeated stress on ascending DA systems. The methodology and results of the experiments are outlined in section 3 and 4. Section 5 is a general discussion of the results in relation to previous literature, as well as in regards to its clinical implications. All in vivo voltammetric experiments were performed by myself.

1 INTRODUCTION

We are all subjected to varying levels of stress from a number of different sources. For most of us, brief exposures to moderate levels of environmental stress has arousing, performance-enhancing effects. Chronic stress or repeated exposure to intense stress however, can have debilitating effects. Under these conditions, stress can become a precipitating factor in the development of mental disorders such as depression (Breslau and Davis, 1986; Willner 1985; Anisman, 1984, Anisman and Zacharko, 1982), and schizophrenia (Nicholson and Neufeld, 1992, Brier 1989). Stressful events have effects on a number of central neurochemical systems including those that contain norepinephrine (Abercrombie and Jacobs, 1987; Adell, Garcia Marquez, Armario and Gelpi 1988; Roth, Mefford and Barchas, 1982), serotonin (Joseph and Kennett, 1983, Thierry, Javoy, Glowinski and Kety, 1968) and dopamine (Abercrombie, Keefe, DiFrischia and Zigmond, 1989; Deutch, Tam and Roth, 1985; Dunn and File, 1983). The effect of stress on central dopamine (DA) pathways in particular is receiving an increasing amount of attention. It is also the focus of the present study.

There are three major ascending DA systems in the mammalian central nervous system. The nigrostriatal system comprises DA cells of the substantia nigra compacta in the ventralateral mesencephalon (Oades and Halliday, 1987). This pathway innervates striatum (STR) and is generally thought to play an important role in sensory motor integration (Wickens, 1990, Beninger, 1983; Stricker and Zigmond, 1976). Degeneration of the nigrostriatal DA pathway also underlies the hypokinetic symptoms of Parkinson's disease (Hornykiewicz and Kish, 1986).

Medial to the substantia nigra is the ventral tegmental area (VTA) where DA neurons

form the mesocortical and mesolimbic systems. The mesocortical system projects to prefrontal, cingulate, entorhinal and piriform regions of cortex. Functionally, the meso-PFC system has been implicated in attentional processes (Simon and Le Moal, 1987); depletion of PFC DA, for example, has been shown to lead to spatial alternation deficits in rats and monkeys (Simon, Scatton and LeMoal, 1980; Brozovski, Brown, Rosvold and Goldman, 1979). The meso-PFC DA pathway is also increasingly being implicated in the pathophysiology of schizophrenia (for review see Grace, 1992).

The mesolimbic DA system innervates a number of subcortical regions including the amygdaloid and septal nuclei, as well as the olfactory tubercule and the nucleus accumbens (NAcc). Mesolimbic DA neurons, in particular those that project to NAcc have been the focus of much attention. A dysfunction of the meso-NAcc DA system is generally thought to underlie the positive symptoms of schizophrenia (See Grace, 1992, for review). An impressive amount of empirical evidence has also implicated the meso-NAcc DA system as an integral part of the so called brain reward circuitry (Koob, 1992; Rompre and Wise, 1989, Wise, 1989). These DA neurons are thought to regulate behaviors motivated by a number of rewarding stimuli, including naturally occurring rewards such as food and a sexually receptive mate (Mitchell and Stewart, 1989, 1990; Hamilton and Bozarth, 1988; Gratton and Wise, 1988, Jenck, Gratton and Wise, 1986; Geary and Smith, 1985; Eibergen and Caggiula, 1973), and drugs commonly abused by humans such as amphetamine and cocaine (Wise, Bauco, Carlezon and Trojniar, 1992, Koob, 1992, Wise and Bozarth, 1987; Yokel and Wise, 1975), opiates (Ettenberg, Petit, Bloom and Koob, 1982, Bozarth and Wise, 1981), and ethanol (Weiss, Hurd, Ungerstedt, Marcou, Plotsky and Koob, 1992, Pfeffer and Samson, 1988). In addition to acting as positive reinforcers, these and a variety of other rewarding stimuli have in common the property of stimulating

the meso-NAcc DA pathway. For example, increased DA release in NAcc is elicited by presenting animals with food or sex-related cues (Mitchell and Gratton, 1991, 1992, Mitchell and Stewart, 1990), and by injections of cocaine (Kalivas and Duffy, 1989; Dichiara and Imperato, 1988), amphetamine (Robinson and Camp, 1990, Hernandez, Lee and Hoebel, 1987), opiates (Kalivas, Duffy, Abhold and Dilts, 1988) and ethanol (Weiss, Hurd, Ungerstedt Markou, Plotsky and Koob, 1992) Accordingly, DA depleting lesions of the NAcc as well as local injection of DA receptor blockers will inhibit feeding and male copulatory behaviors (Foreman and Hall, 1987, Blundell and Latham, 1978, Ungerstedt, 1971) as well as intravenous self-administration of cocaine (Roberts and Koob, 1982, Roberts, Koob, Klonoff and Fibiger, 1980), amphetamine (Lyness, Friedle and Moore, 1979) and heroin (Spyraki, Fibiger and Phillips, 1983) It is from these and other similar lines of evidence that the DA hypothesis of reward emerged In its simplest form the hypothesis posits that increased meso-NAcc DA neurotransmission is an important, if not necessary, central event for positive reinforcement of behavior (Koob, 1992, Wise, 1989, Wise and Bozarth, 1987; Fibiger and Phillips, 1986, Wise, 1978). Although this hypothesis has gained acceptance over the past 10 years there has been a growing number of discordant findings that have called its validity into question One particularly troublesome finding is that stressful stimuli (eg footshock, restraint), which do not exhibit positive reinforcing properties, also stimulate meso NAc · DA neurotransmission (Abercrombie et al., 1989; Claustre, Rivy, Dennis and Scatton, 1986, Deutch et al , 1985; Watanabe, 1984; Fadda, Argiolas, Melis, Tissari, Onali and Gessa, 1978)

Initial evidence suggested that the meso-PFC DA system was comparatively more responsive to activation by acute stress than other DA pathways (Thierry, Tassin, Blanc and Glowinski, 1976). Animals in this study were exposed to intermittent footshock, then

immediately decapitated after which tissue levels of DA and its primary metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) were measured. The authors reported a 60% decrease in tissue levels of DA in PFC and a 25% decrease in NAcc DA levels, indicating an increase in DA utilization in these regions. These authors later reported that exposure to footshock stress selectively increased meso-PFC DA utilization as reflected by an increase in the DOPAC/DA ratio, which the authors argued to be a better index of DA turnover (Lavielle, Tassin, Thierry, Blanc, Herve, Barthelemy and Glowinski, 1978). This finding was taken to suggest that stress selectively activates the meso-PFC DA system. Evidence of selective activation of the meso-PFC DA pathway has been obtained under a variety of other stress conditions. The anxiogenic beta-carboline compound FG 7142 (a partial benzodiazepine inverse agonist) which is thought to cause an anxiety-like state in rat (McGregor and Atrens, 1990; Dorow, Horowski, Paschelke, Amin and Braestrup, 1983), has been shown to selectively increase DA turnover in PFC (Bradberry, Lori and Roth, 1991; Tam and Roth, 1985; although Bradberry et. al. did not look at NAcc), while food deprivation stress has been reported to result in increased DOPAC levels only in PFC (Carlson, Herrick, Baird and Glick, 1987). A selective increase in meso-PFC DOPAC and homovanillic acid (HVA) levels has also been observed in animals that were merely exposed to other animals receiving footshock, suggesting the meso-PFC system can be activated by psychogenic stress (Kaneyuki, Yokoo, Tsuda, Yoshida, Mizuki, Yamada and Tanaka, 1991).

The idea that the meso-PFC system is the only DA pathway responsive to stress has been questioned, however, by a number of investigators (Roth, Tam, Ida, Yang and Deutch, 1988; Deutch, Bean, Bissette, Nemeroff, Robbins and Roth, 1987; Deutch et. al., 1985). These authors suggested that while only the meso-PFC DA system is activated by mild stresses, increased neurotransmission in the meso-NAcc and nigrostriatal systems can

also occur when animals are subjected to more severe stresses. For example, Deutch et al (1985, 1987) found that while footshock intensities of 0.2 to 0.45 mA elevated DOPAC levels only in PFC, footshock intensities above 0.45 mA increased DOPAC levels in both NAcc and PFC (but not in STR). The idea of regional differences in the threshold for activation by footshock stress is supported by a good number of other studies (Sorg and Kalivas, 1991; Dunn, 1988; Claustre et. al., 1986; Deutch et al., 1985, Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier and Le Moal, 1982; Reinhard, Bannon and Roth, 1982; Fadda et. al., 1978; Tissari, Argiolas, Fadda, Serra and Gessa, 1979; Lavielle et. al., 1978) While Dunn (1988) and Deutch et al., (1985) observed selective activation of the meso-PFC DA system at very low shock intensities (0.18 and 0.2mA, respectively), Sorg and Kalivas (1991) reported increased DA release in NAcc of animals subjected to stronger (0.55 mA) footshock and increased DA utilization in NAcc (and PFC but not STR) was observed by others only when considerably more severe footshock (1.5-2.0 mA) was used. The results of these studies suggest that meso NAcc DA neurons are responsive to footshock, and presumably other stressors, but at intensities considerably greater than necessary to activate the meso-PFC DA pathway.

In the studies discussed above the effects of stress on central DA systems were determined with post-mortem measures. While this approach has provided useful information, the extent to which reliable measurements of extracellular levels of transmitters or transmitter release can be derived from post-mortem assays of intracellular levels of transmitters and metabolites has always been a matter of some speculation (Soares-de Silva, 1987). Some have challenged the contention that alterations in the ratio of intracellular transmitters to metabolite levels can accurately reflect changes in monoaminergic neurotransmission (Commissiong, 1985) and it has been suggested by

others that these and similar types of post mortem measures are relatively poor indices of stress induced increases in DA activity (Imperato, Puglisi-Allegra, Zocchi, Scrocco, Casolini and Angelucci, 1990; Abercrombie et al., 1989). There are, indeed, a number of problems in interpreting data from post-mortem studies. Lavielle et. al. (1978), for example, observed a footshock induced increase in the DOPAC/DA ratio in PFC but not in NAcc or STR where both DOPAC and DA levels increased by equal proportions. These authors interpreted their data as indicating a selective increase in DA turnover in PFC by stress. While an elevation of intracellular DOPAC levels relative to those of DA is usually associated with increased DA release, the contention by these authors that concomittant elevations of both DA and DOPAC, as was found in NAcc, are physiologically irrelevant is debatable. Interpretation of results of post mortem studies is made even more problematic by evidence of massive release of DA and other monoamines immediately following death (van Veldhuizen, Feenstra, Boer and Westerink, 1990; Gonzalez-Mora, Maldment, Guadalupe and Mas, 1989). Since the interval between decapitation and freezing of brain tissue ranges from 1-3 minutes (Palkovitz, 1973), it is more than likely that intracellular levels of transmitter and metabolite have already been significantly altered before the tissue can be properly stored. These and similar other considerations more than anything else, have caused the effects of stress on central DA systems to be re-examined with newly developed in vivo techniques.

Microdialysis and voltammetric techniques allow quantitative and, in the case of voltammetry, rapid measurement of extracellular transmitter and metabolite levels in conscious animals. Overall, the results of in vivo studies have revealed that stress causes a more generalized activation of ascending DAergic systems, including the nigrostriatal DA system than was suggested by the data from post-mortem studies (Abercrombie et. al.,

1989; Knott, Brannan, Andrews, Togasaki, Young, Maker and Yahr, 1986, Keller, Stricker and Zigmond, 1983, but see Heyes, Garnett and Coates, 1988) However, in agreement with post-mortem experiments the data obtained from awake animals also indicate that the meso-PFC DA system is comparatively more responsive to stress than the meso-NAcc and nigrostriatal DA pathways (Imperato, Puglisi Allegra, Casolini and Angelucci, 1991; Abercrombie et al , 1989) Nonetheless, as was the case with post-mortem studies, inconsistent findings have been reported in studies using in vivo detection methods. For example, Bertolucci-D'Angio, Serrano and Scat. (1990) and D'Angio, Serrano, Rivy and Scatton (1987) reported electrochemical evidence of increased extracellular DOPAC levels in NAcc but not in PFC during tail pinch stress, and of a comparatively greater increase in DOPAC levels in NAcc than in PFC during immobilization stress. In contrast, tail pinch was recently reported in a microdialysis study to elevate DA levels in PFC but to not affect those in NAcc (Cenci, Kalen, Mandel and Bjorklund, 1992) Thus, while results derived from in vivo detection methods argue more strongly for a stimulant effect of stress on meso-NAcc and nigrostriatal DA systems than do those from post-mortem studies, they are by no means compelling.

The majority of previous studies have characterized the response of central DA pathways to a single exposure to stress In reality, however, we are more often than not exposed to the same stressful conditions repeatedly. Thus, the important question would be if and how the stress response of DA pathways changes with repeated exposure There is increasing evidence that the response of meso-NAcc DA neurons to a wide range of stimuli is plastic. Mitchell and Gratton (1991, 1992), for example, showed that DA release elicited in NAcc is progressively enhanced with each daily exposure to sexually relevant stimuli. Stimulant drugs such as cocaine and amphetamine and opiates such as morphine

and heroin share with stress the ability to stimulate forward locomotion and increase DA release in NAcc (Sorg, 1992; Leyton and Stewart, 1990; Bradberry and Roth, 1989; Kalivas, Duffy, Abhold and Dilts, 1988; Peris and Zahniser, 1987, Robinson, Angus and Becker, 1985; Kuzcenski, 1983), and a number of studies have shown that the acute stimulant effect of these drugs is enhanced with repeated, intermittent administration Thus when injected every day, or every other day the acute locomotor stimulant effect of cocaine and amphetamine, for instance, increases or sensitizes. Behavioral sensitization to drugs has been shown to be long lasting, if not permanent, sensitized responses are still observed after months of withdrawal from the drug (Kalivas and Duffy, 1990; Petit, Pan, Parsons and Justice, 1990, Robinson, Jurson, Bennett, and Bengten, 1988; Peris and Zahniser, 1987, Stewart and Vezina, 1987, Robinson and Becker, 1986; Herman et al., 1984, Antelman and Eichler 1979 Segal and Mandel, 1974). Furthermore neurochemical studies have provided strong evidence that behavioral sensitization is accompanied by an increased sensitivity of the meso-NAcc DA system to the stimulant action of these drugs; DA release elicited in NAcc by cocaine, amphetamine or morphine is potentiated with repeated administration of these compounds (Sorg, 1992; Kalivas and Stewart, 1991; Kalivas and Duffy, 1990); Robinson, Jursen, Bennett and Bentgen 1988; Robinson and Becker, 1986).

Interestingly, it has been shown that repeated exposure to stress later sensitizes animals to the action of stimulant drugs on behavior and DA release in NAcc (for reviews see Kalivas and Stewart, 1991; Robinson, 1988, Stewart and Vezina, 1988; Robinson and Becker, 1986). For example, MacLennan and Maier (1983) found enhanced locomotor activity in response to cocaine in previously stressed rats, while Sorg (1992) reported that repeated footshock sensitized both NAcc and PFC DA release elicited by cocaine administration. Repeated stress has been shown to later enhance amphetamine-induced

locomotion (Hahn, Zacharko and Anisman, 1986; Herman et. al., 1984; Antelman and Chiodo, 1983; Antelman and Eichler, 1979), rotation (Robinson et. al., 1985), and stereotypy (Anisman, Hahn, Hoffman and Zacharko, 1985), and an enhancement of amphetamine induced DA release has been observed, *in vitro*, from striatum of stressed animals (Wilcox, Robinson and Becker, 1986). There is also evidence that repeated administration of stimulant drugs will enhance the DA activating properties of an acute exposure to stress. Kalivas and Duffy (1989) reported that previous treatment with repeated cocaine resulted in enhanced DA metabolism in PFC and NAcc in response to acute footshock. A similar enhancement of stress elicited increases in DA turnover has been observed following repeated amphetamine administration (Robinson, Becker, Young, Akil and Castenada, 1987). Others have shown that a single exposure to stress is sufficient to alter the DAergic response to later administration of pharmacological agents known to possess DA agonist or antagonist properties (Antelman, Caggiula, Kocan, Knopf, Meyer, Edwards and Barry, 1991; Antelman and Caggiula, 1990) Finally, Kalivas and Duffy (1989) found that repeated, once daily exposure to footshock stress resulted in an greater increase in DA turnover in the PFC and the NAcc (but not the STR) upon subsequent application of footshock when compared to animals that had not previously been shocked. Taken together, the results of these studies suggest that the effects of behaviorally relevant stimuli on meso-NAcc DA neurotransmission become stronger with repeated presentation Thus, while stressful stimuli may initially only elicit a small increase in meso-NAcc DA activity, these findings suggest that the response of this system progressively increases with each subsequent exposure to stress. More importantly, it appears that the sensitizing effect of repeated exposure to stress generalizes to other stimuli.

2 RATIONALE

The extent to which repeated exposure to a stress can influence behavioral responses to subsequent stress or to other types of stimuli has important implications. The interaction between drugs of abuse and stress at the level of the meso-NAcc DA system suggests that repeated exposure to stressful conditions may play an important role in the development of compulsive drug taking by humans, and that long-term use of illicit drugs leads to profound changes in the behavioral response to environmental stresses. There is clinical evidence suggesting that exposure to stress may precipitate or exacerbate pathological behaviors associated with DA dysfunction. The positive symptoms of schizophrenia, for example, are generally considered to reflect an hyperresponsiveness of mesolimbic DA neurons, and it appears that schizophrenics not only have an abnormal, or exaggerated, response to stress (Nicholson and Neufeld, 1992; Falloon, 1986), but that the risk of relapse is correlated with the heightened responsivity of these patients to stress (Nicholson and Neufeld, 1992, Gruen and Baron, 1984). Stressful life events are also thought to be both predisposing and precipitating factors in depression: previous stressful events have been shown to result in an increased susceptibility to depression, or acute stress-induced depression (Breslau and Davis, 1986; Anisman, 1984; Anisman and Zacharko, 1982).

The present study may also shed more light on our current understanding of meso-NAcc DA function. This DA system has been strongly implicated in the control of behaviors motivated by rewards (for reviews see Wise et al., 1992; Koob, 1992, Wise, 1989, 1978; Rompre and Wise, 1989). In its simplest form, the prevailing hypothesis is that the ability to elicit increased DA release in NAcc (and forward, explorative locomotion) is a defining property of rewarding stimuli. That aversive stimuli such as footshock or restraint seem to

also have this property has obvious important theoretical implications and therefore requires further study.

In the present study we characterized the effects on extracellular DA levels in NAcc, PFC and STR of repeated once daily exposure to either tail pinch or restraint stress in freely-behaving animals using high speed chronoamperometry, an electrochemical detection technique. Apomorphine, a mixed D1/D2 receptor agonist, and GBR-12909, a selective DA uptake inhibitor, were used to confirm that stress-elicited increases in electrochemical signals were due to increases in extracellular DA concentration

3 METHODS

Animals and surgery:

Male Long-Evans rats (300-350g, Charles River, St. Constant, Quebec) were each implanted, under sodium pentobarbital anesthesia (50mg/kg i.p.) with a voltammetric electrode aimed at either the NAcc (n=30), STR (n=10) or PFC (n=30) and with a Ag/AgCl reference electrode and a stainless steel ground wire. With the incisor bar adjusted to maintain the skull horizontal and using bregma and the cortical surface as reference points, the stereotaxic coordinates were as follows: NAcc: A.P.=+1.6mm, Lat.=1.6mm, D.V.=7.4mm; STR: A.P.=+1.6mm, Lat.=2.0mm, D.V.=4.5mm, PFC: A.P.=+3.4mm, Lat.=0.8mm, D.V.=4.6mm (Paxinos and Watson, 1986). The reference and ground wires were implanted in the ipsilateral and contralateral parietal cortex, respectively. The miniature pin connectors soldered to the electrochemical and reference electrodes and to the ground wires were inserted into a plastic strip connector which was secured and anchored with acrylic dental cement to five stainless steel skull screws embedded in the cranium. The animals were housed singly on a 12hr light/dark schedule (lights on at 0800hrs), with food and water available ad libitum.

In vivo electrochemical methods

The voltammetric electrode consisted of three 30μm diameter carbon fibers that extended 50-100μm beyond the tip of a pulled glass capillary. The carbon fiber bundle was fixed in the capillary with a drop of Epoxylite and was coated with Nafion (Aldrich), a polymer that reduces the contribution to the electrochemical signal of anions such as ascorbic acid (AA) and the primary DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). The electrodes were calibrated prior to implantation for their sensitivity to DA and for their selectivity for DA against AA. All calibrations were performed at 25°C in 0.1M phosphate buffered

saline (pH=7.4) containing a fixed concentration (250μm) of AA to mimic brain extracellular concentrations. Only electrodes exhibiting a minimum DA to AA ratio of 1000:1 and a highly linear response (r >.997) to increasing concentrations of DA were used. Electrochemical measurements were performed using a microcomputer controlled high-speed chronoamperometric apparatus (IVEC-5, Med Systems Corp., Greenvale, NY). An oxidation potential of +0.55 V, with respect to the reference electrode, was applied to the electrode for 100ms at a rate of 5Hz. The sum of 5 digitized oxidative cycles was graphically displayed on a video monitor at a rate of 1 Hz. The reduction current generated when the potential was returned to resting level (0.0 V for 100ms) was digitized and summed in the same manner and served as an index to identify the electroactive species undergoing oxidation. With the Nafion-coated carbon fiber electrodes used in the present study and at a sampling rate of 5 Hz, the magnitude of the reduction current for DA is 60-80% of the oxidation current (red:ox 0.6-0.8) (Gratton, Hoffer and Gerhardt, 1989). Previous work has shown that the oxidation of AA is virtually irreversible (red:ox -0.0), whereas that of DOPAC is almost entirely reversible (red.ox=0.9-1.0) while the reduction to oxidation ratios for norepinephrine and serotonin are 0.4-0.5 and 0.1-0.2 respectively. Thus the simultaneous monitoring of both the oxidation and reduction currents associated with the electrochemical reaction provides an on-line method of determining the neurochemical identity of the predominant electroactive species contributing to the signal.

Testing procedures:

Two to three days after surgery, the animals were placed in the recording chamber and connected to the recording apparatus via a shielded cable and a low impedance multi channel commutator (Airflyte, Bayonne, NJ). The primary signal amplifier was connected directly onto the animal's electrode connector to minimize extraneous electrical interference

Following a 1hr period during which baseline electrochemical data were obtained, each animal was subjected to either tail pinch or restraint stress. Tail pinch stress was induced by placing a wooden clothespin 2cm from the base of the tail for 10 mins, while restraint stress was induced by immobilizing the animal for 15mins in a polyethylene foam padded jacket made of wire mesh held together by Velcro straps. Each animal was subjected to only one type of stress once a day for 5 consecutive days. Electrochemical recordings were performed during and after the period of stress until the electrochemical signals returned to baseline levels. Additional animals were implanted with electrodes within NAcc or PFC to test the effects of apomorphine, a mixed D1/D2 receptor agonist, and GBR-12909, a selective DA uptake blocker. Animals of the first group were subjected on four different days to stress alone, stress 15 minutes following administration of apomorphine (50 and 100μg/kg, s.c.) and of an equal volume of saline (1ml/kg). Animals of the second group received on different days GBR-12909 (5 and 10mg/kg, i.p.) and vehicle 60 minutes prior to the period of stress (tail pinch or restraint). The order of the treatments was counter-balanced to reduce the effect of repeated daily stress. At the end of the experiments all animals were deeply anesthetized with sodium pentobarbital (75mg/kg i.p.) and transcardially perfused with 10% formalin. The brains were removed and were later sliced in 40μm sections for verification of electrode placements

4 RESULTS

Effects of Repeated Stress

The changes in the electrochemical signals recorded in PFC, NAcc and STR during each of 5 consecutive once-daily exposures to restraint and tail pinch stress are summarized in Figure 1. The electrochemical data are expressed as mean changes in DA concentration as a function of each minute of restraint or tail pinch stress. As can be seen, the peak amplitude of the electrochemical signals elicited by restraint in NAcc became progressively larger with each daily exposure. A similar, but less orderly daily enhancement of restraint elicited increases of electrochemical signals was also recorded in STR. No such enhancement was evident in PFC during the restraint period. However upon release from the restraint apparatus a further increase in the electrochemical signal was observed in PFC, this delayed increase was rarely observed in NAcc and STR. Figure 2 shows the mean changes in signals recorded in PFC on day 1 and day 5 during the 15 min restraint period as well as during the 15 min immediately following termination of restraint. As can be seen, the amplitude of the delayed increase is greater on day 5 when compared to day 1.

In comparison to restraint, tail pinch elicited considerably smaller increases in the electrochemical signals in NAcc and STR, but similar increases in PFC. No obvious day-to-day changes in tail pinch-elicited signals were observed, although signals recorded in PFC on day 1 tended to be smaller than those recorded on subsequent test days, whereas the amplitude of tail pinch elicited signals in STR tended to decrease across test days.

Figure 3 summarizes the statistical analysis of restraint- and tail pinch induced changes in signals recorded within NAcc, PFC and STR. Differences between regions (PFC, NAcc, STR) and test day (Day 1 versus Day 5) in the amplitude and duration of stress elicited signals were examined using two-way analyses of variance (ANOVA) with

repeated measures, and Tukey's post-hoc test. Measures of signal amplitude and duration were obtained directly from each electrochemical record. Peak amplitude was defined as the maximal increase above baseline of the electrochemical signal during the 10 min (tail pinch) or 15 min (restraint) period of stress, whereas duration was defined as the time the signal remained above baseline. The results of the analyses of the maximum amplitude and the duration of restraint and of tail pinch-induced increases in electrochemical signals are summarized in Figure 3. These results revealed a significant effect of test day (F 1, 12 = 9.36, $p < 0.01$), on the amplitude of restraint-elicited signals but no significant effect of region (F 2, 12 = .361, $p > 0.05$) or region by day interaction (F 2, 12 = .073, $p > 0.05$). Careful examination of the data suggests, however, that much of the difference between day 1 and day 5 is due to the increase in amplitude of signals recorded in NAcc. The ANOVA performed on the duration of restraint-elicited signals reinforces this impression revealing a significant region by days interaction F 2, 12 = 15.36, $p < 0.01$, and significant main effects of region, (F 2, 12 = 5.71, $p < 0.01$), and of test day, (F 1, 12 = 14.45, $p < 0.01$). Tukey's post-hoc tests revealed that restraint-elicited increases recorded in PFC on day 1 were significantly longer lasting than those recorded in NAcc and STR. However, on day 5 the duration of increases recorded in the 3 regions did not differ. Post-hoc tests also revealed that the duration of restraint-elicited increases in NAcc were significantly longer on day 5 than on day 1; there were no significant differences between day 1 and day 5 increases in PFC and STR.

Two-way ANOVA performed on the amplitude of tail pinch-elicited signals revealed a significant region by test day interaction (F 2, 12 = 4.22, $p < 0.05$), and a significant main effect of region (F 2, 12 = 8.34, $p < 0.01$). Tukey's post-hoc test indicated a significantly greater increase of tail-pinch elicited signals in PFC as compared to NAcc and STR on day

1, and a significant decrease in the amplitude of signals elicited in STR on day 5 compared to day 1. Analysis of the duration of tail pinch elicited signals revealed no significant main effects of region (F 2, 12 = .655, $p > 0.05$), test day (F 1, 12 = .488, $p > 0.05$) and no significant region by test day interaction (F 2, 12 = 3.64, $p > 0.05$. Representative electrochemical records obtained during repeated restraint stress in NAcc and PFC are shown in Figure 4.

Effects of Apomorphine

Two-way ANOVAs with repeated measures were performed to compare the effects of APO (50 and 100 μg/kg) and vehicle (treatment) on the maximum amplitude and duration (Figure 5) of tail pinch- and restraint-elicited signals in NAcc and PFC (region). The analysis revealed a significant region by treatment interaction, (F 2, 16 = 39.27, $p < 0.01$), and significant main effects of treatment (F 2, 16 = 45.31, $p < 0.01$), and region (F 1, 8 = 56.44, $p < 0.01$) on the amplitude of restraint elicited signals. A Tukey post hoc test indicated that APO at both doses (50 and 100 μg/kg) significantly attenuated the amplitude of the electrochemical signals recorded in NAcc when compared to the saline control. The post-hoc analysis also revealed that APO at both doses significantly attenuated the amplitude of signals recorded in NAcc when compared to those recorded in PFC. The analysis of the duration of restraint-elicited signals recorded in NAcc and PFC revealed a significant region by treatment interaction (F 2, 16 = 3.82, $p < 0.05$), and a significant main effect of treatment, (F 2, 16 = 6.35, $p < 0.01$) and of region (F 1, 8 = 5.88, $p < 0.05$). Results of the post hoc test indicated that APO pretreatment (50μg and 100μg/kg) caused a significant reduction in the duration of increases in NAcc but not in PFC when compared to saline, and that both doses produced a significantly greater effect in NAcc

when compared to PFC. The analysis of the effects of APO on the amplitude of tail-pinch elicited increases in NAcc and PFC revealed a significant main effect of treatment (F 2, 16 = 7.94, $p < 0.01$), but no effect of region or treatment by region interaction. Analysis of simple effects indicated a significant effect of APO at the high but not the low dose; the collapsed mean amplitude of signals recorded in NAcc and PFC following injection of 100μg/kg of APO was significantly lower than the corresponding collapsed means in the saline condition. Visual inspection of the data suggests, however, that increases in NAcc were more strongly attenuated than those in PFC. Similar results were obtained from the analysis of APO effects on the duration of tail pinch elicited increases. There was a significant effect of treatment (F 2, 16 = 9.86, $p < 0.01$) but not of region and no region by treatment interaction; simple effects analysis revealed an effect of APO at the high but not the low dose. Here again, it appears that the treatment effect is due mostly to an attenuation of signals recorded in NAcc. Figure 6 shows representative electrochemical records of the effects of APO in NAcc and PFC (Figure 6).

Effects of GBR 12909

The effects of GBR 12909 pretreatment on stress-induced electrochemical signals in NAcc and PFC are summarized in Figure 7. Two-way ANOVAs were performed to test the effects of the drug pretreatment on the amplitude and duration of restraint induced increases in electrochemical signals recorded in NAcc and PFC (region). Significant main effects of drug treatment were found on both the amplitude (F 2, 14 = 5.37, $p < 0.05$), and the duration (F 2, 14 = 9.59, $p < 0.01$) of restraint elicited signals at the 10mg/kg dose. However no significant effect of region and no region by treatment interaction were found at this dose on either amplitude (region; F 2, 14 = 1.99, $p > 0.05$, interaction; F 2, 14 =

1.94, $p > 0.05$) or duration (region; F 2, 14 = 1 98, $p > 0$ 05, interaction; F 2, 14 = 1.82, $p > 0.05$). Representative electrochemical records of stress elicited increases following GBR 12909 administration are presented in Figure 8.

Figure 9 shows the average ratio of the reduction and oxidation currents recorded in NAcc, PFC and STR during restraint on days 1, 3, and 5. As can be seen, the between days variations in red:ox ratios of recordings in NAcc and STR range from approximately 0.6 to 1.0, while those in PFC range from 0 8 to 1 .0. The scale on the right side of the figure serves to indicate the range of red:ox ratios for electroactive species that can be oxidized at the applied potential used.

Figure 10 shows the results of the histological analysis. The damage produced by the tip of voltammetric electrodes implanted in NAcc was confined to the area medial to the anterior commissure whereas striatal placements were located in the medial and central aspect of this structure. PFC placements were located medially to the corpus callosum at the level of the infralimbic cortex.

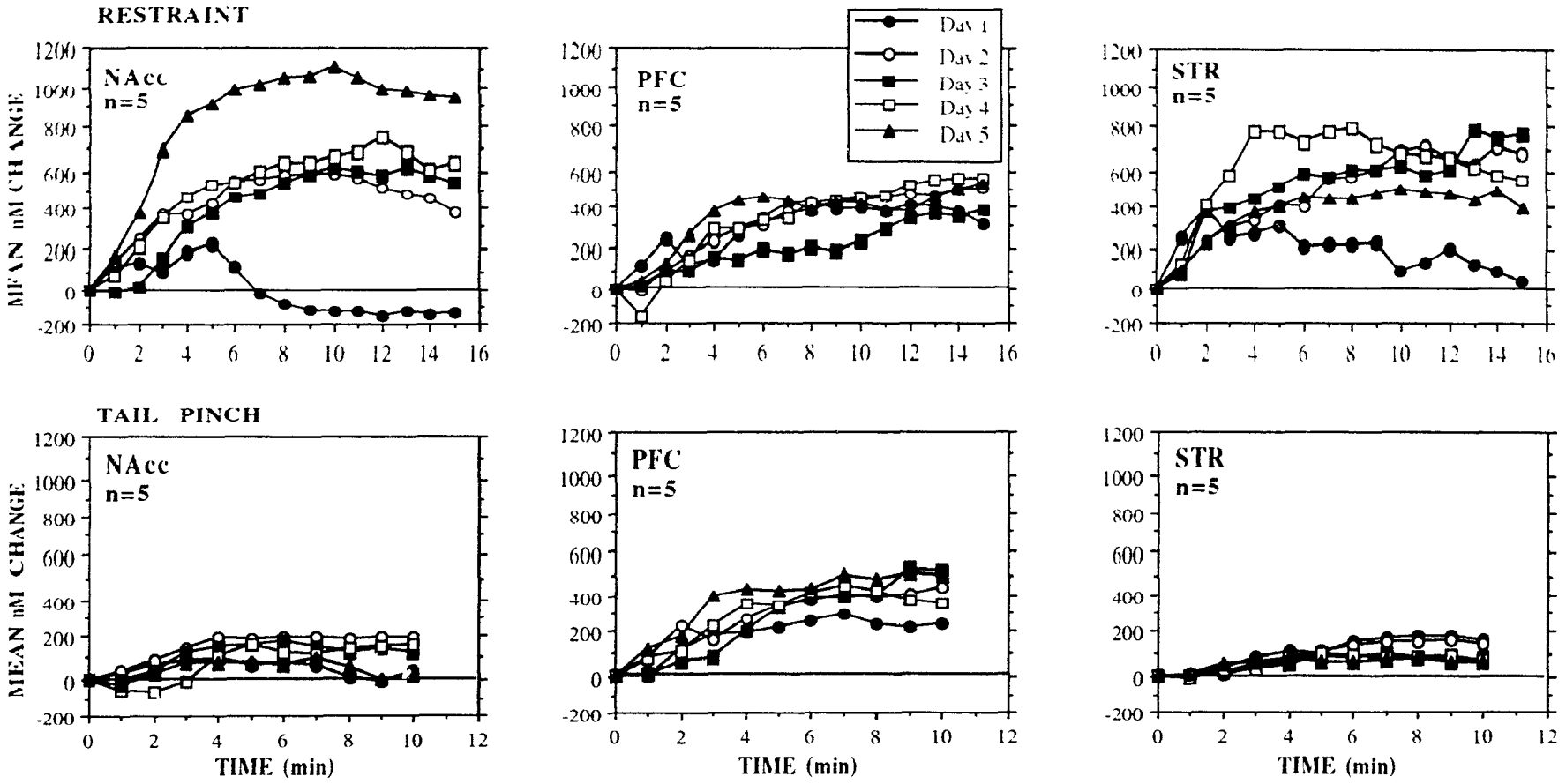

Fig. 1. Mean changes in the amplitude of the electrochemical signals recorded in NAcc, PFC and STR as a function of each minute of restraint and tail pinch across 5 consecutive days of testing. Five rats were tested in each group. The data are expressed as nM changes in DA levels using the in vitro calibration factor (see Methods).

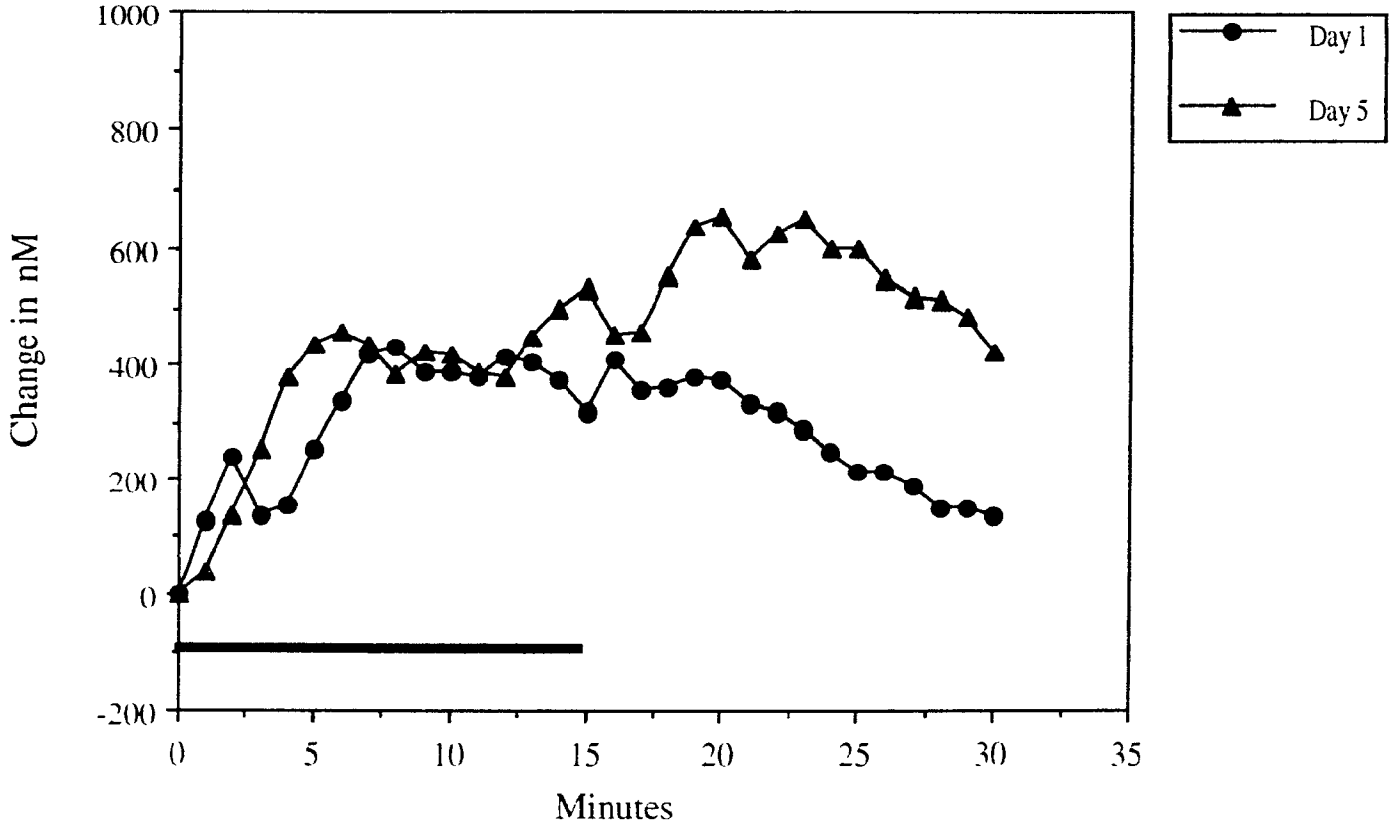

Fig 2 Mean (n=5) changes in the amplitude of electrochemical signals recorded in PFC on day 1 and day 5 during (horizontal bar) and after the period of restraint. Note the slight increase in DA levels upon termination of restraint stress on day 1, and the enhancement of this effect on day 5.

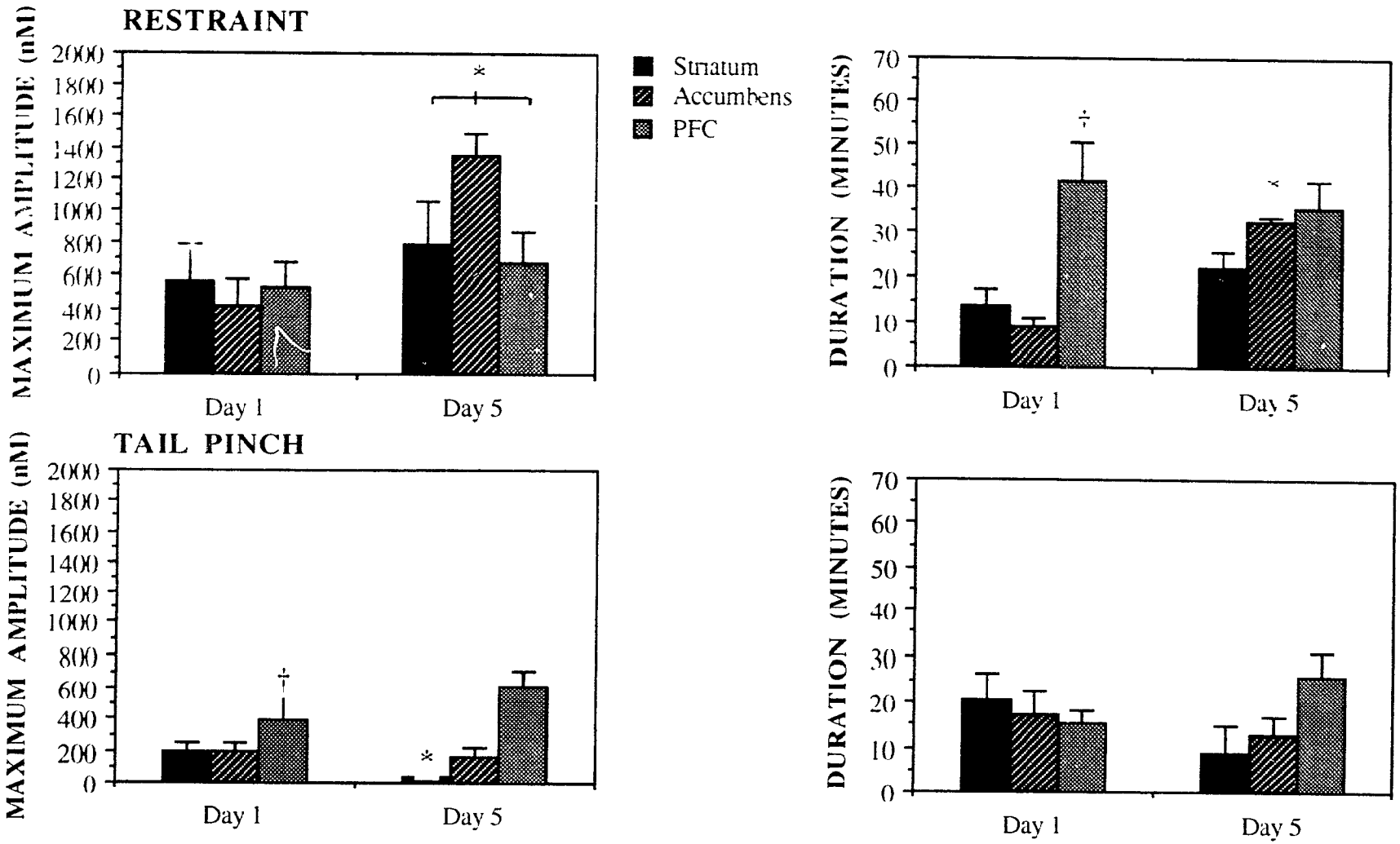

Fig. 3. Between-day and between-region comparison of mean peak increase and mean duration (+1 S E. M.) of electrochemical signals elicited by the 1st and 5th exposure to restraint and tail pinch stress. Asterisks (*) denote significant differences ($P < 0.01$) within region when compared to day 1. Crosses (†) denote significant differences ($P < 0.01$) between regions on same day. Asterisks (*) above horizontal bar denote significant differences between the collapsed means of the regions on day 5 when compared to day 1.

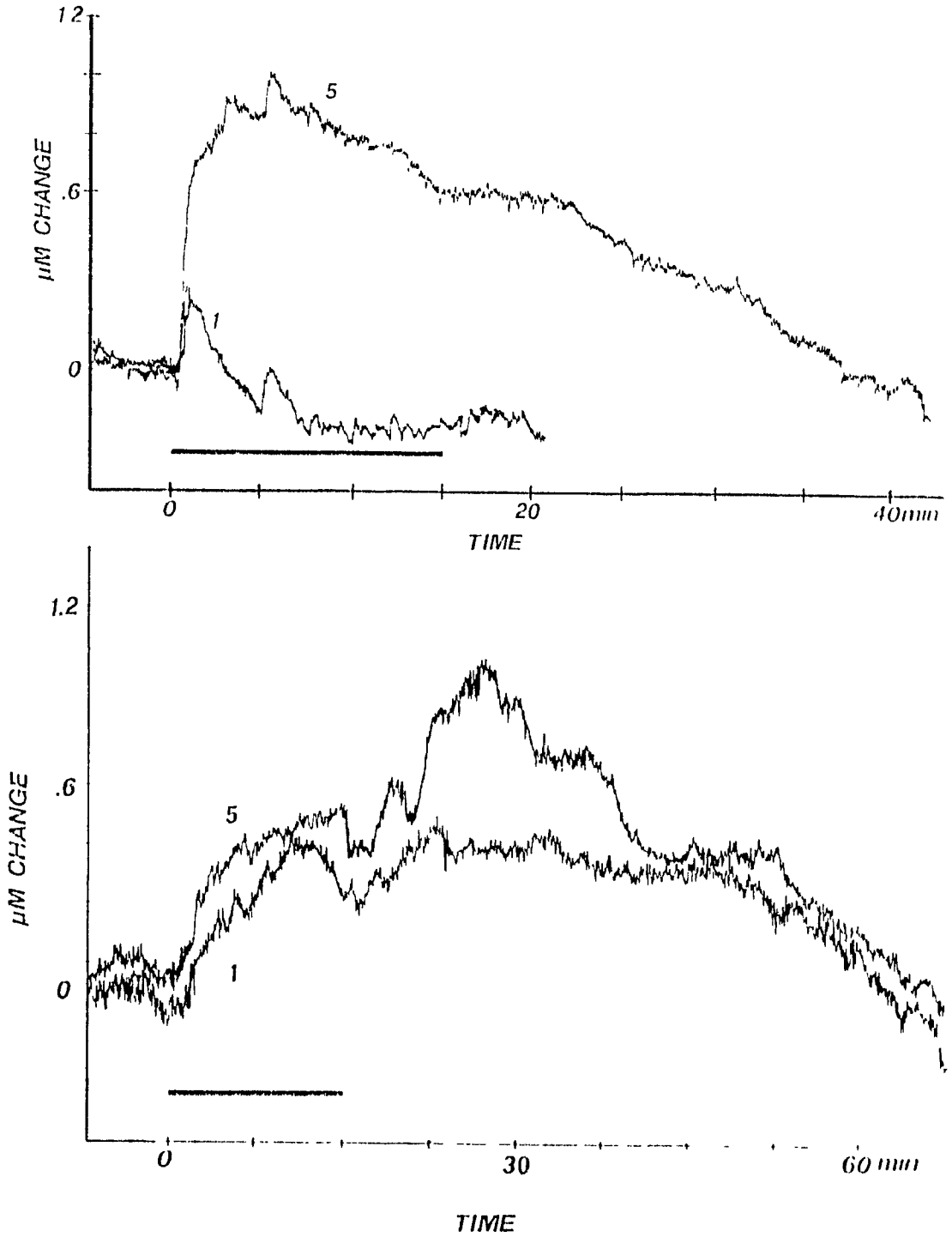

Fig. 4. Examples of chronoamperometric signals recorded in NAcc (top) and in PFC (bottom) during the 1st and 5th exposure to restraint. The data are expressed as micromolar changes in DA concentration. The length of the horizontal bar under the record corresponds to the duration of restraint.

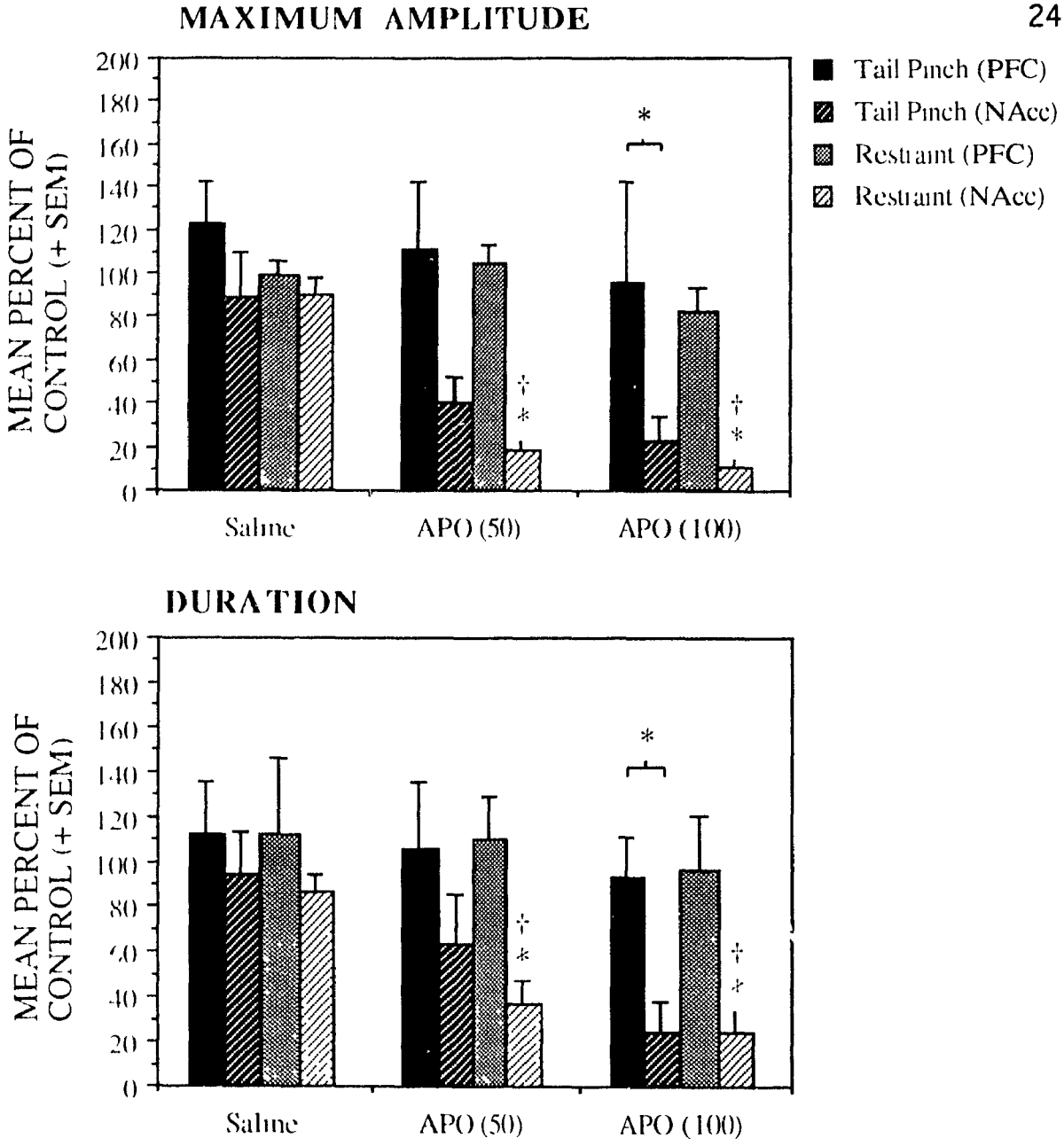

Fig 5 Mean percent change (+1 S. E. M.) from baseline in the magnitude (top) and duration (bottom) of increases in signals in NAcc and PFC elicited by tail pinch (n=6 and 5, respectively) or restraint (n=5 and 5) 15 minutes after injection of apomorphine (APO; 50 and 100 μg/kg s. c.) and saline. Asterisks (*) denote a significant effect of APO ($P < 0.01$) within a recording site when compared to saline. Crosses (†) denote significant differences ($P < 0.05$) between PFC and NAcc to the effects of APO. Asterisks (*) above horizontal bar indicate a significant effect of APO on the collapsed means of NAcc and PFC groups for APO (100 μg/kg) and saline.

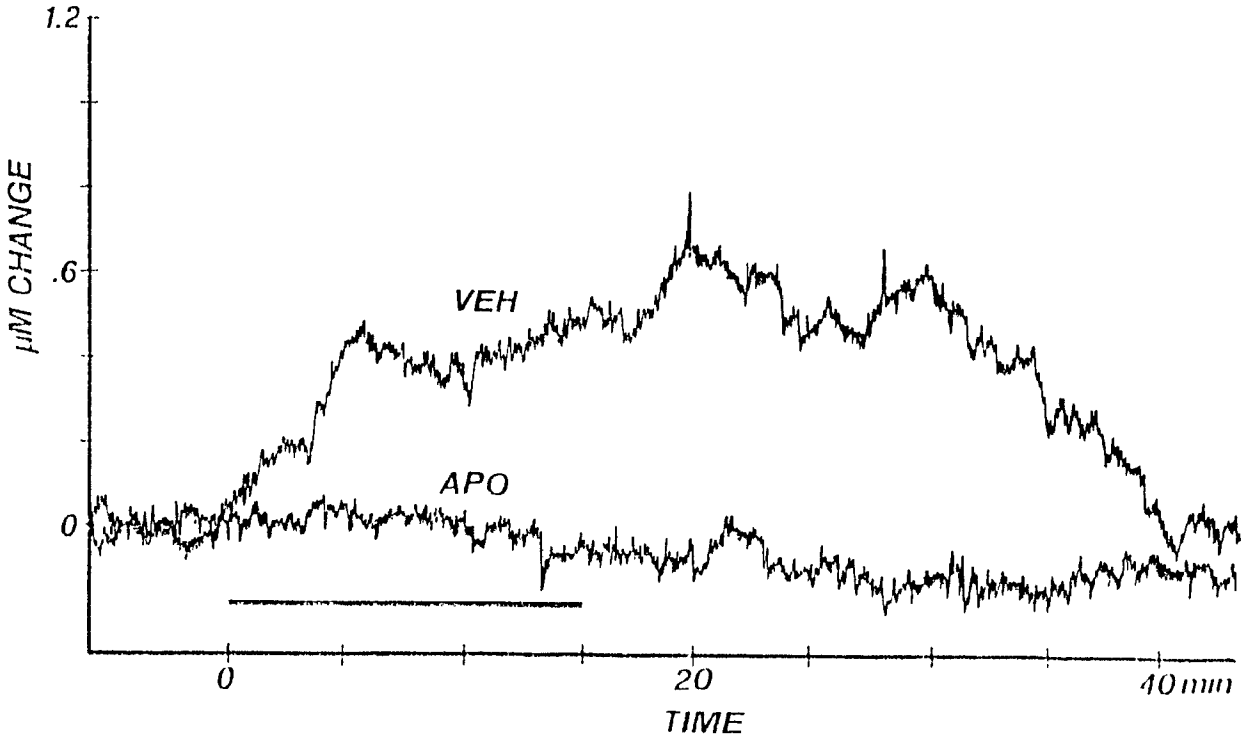

Fig. 6. Example of chronoamperometric signals recorded in NAcc of one animal during restraint 15 min after injection of apomorphine (APO) (100μg/kg s. c.) or of vehicle (VEH); 24 hours separated the APO and VEH injections. Data are expressed as micromolar changes in DA concentration. Length of horizontal bar corresponds to duration of stress.

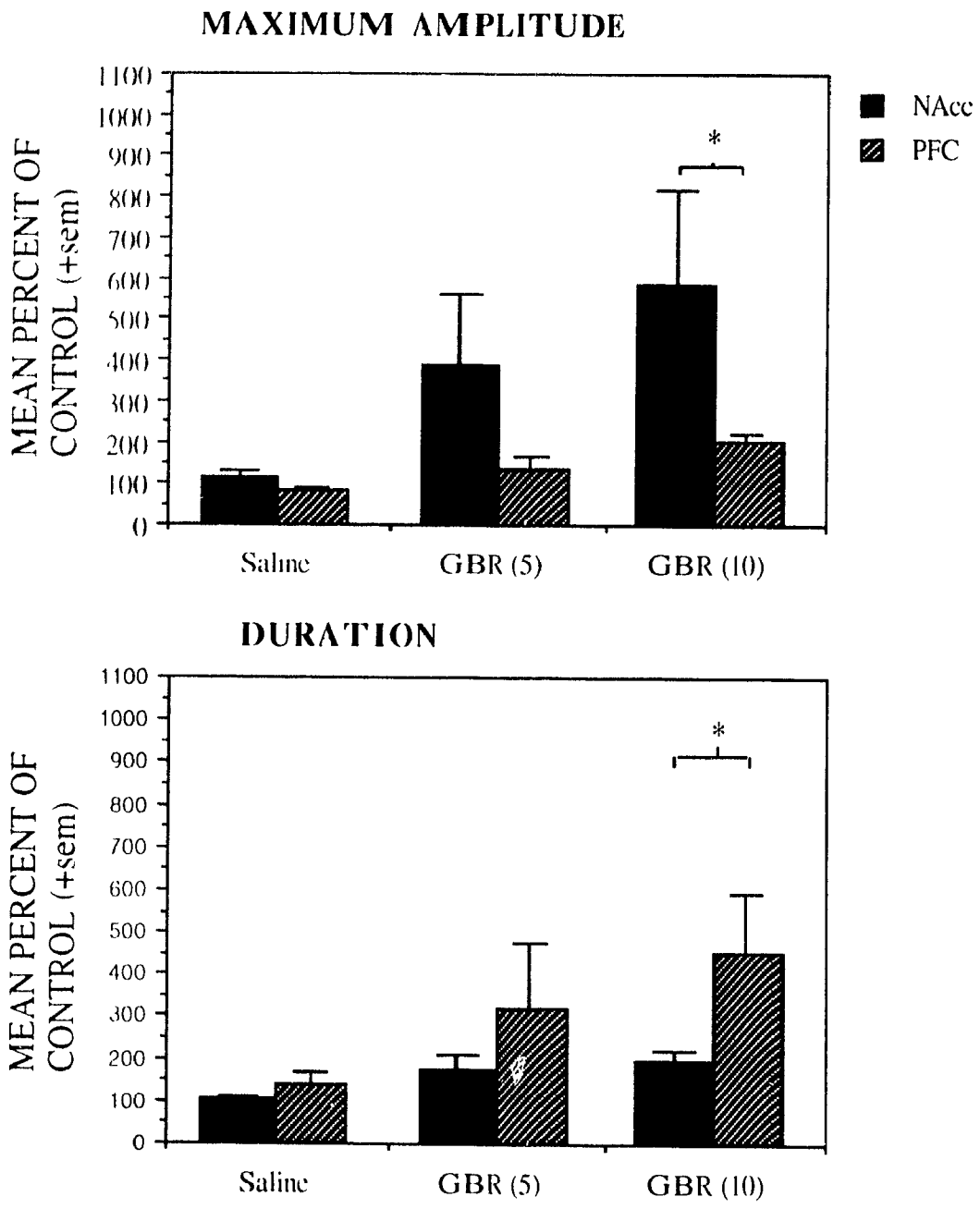

Fig. 7 Mean percent changes (+1 S. E. M.) from baseline in the magnitude (top) and duration (bottom) of electrochemical signals in NAcc (n=5) and PFC (n=4) elicited by restraint stress following pretreatment with GBR-12909 (GBR: 5 and 10 mg/kg i. p.) and saline. Asterisks above horizontal bars indicate significant differences of the collapsed means (of PFC and NAcc) at 10mg/kg GBR-12909 as compared to saline.

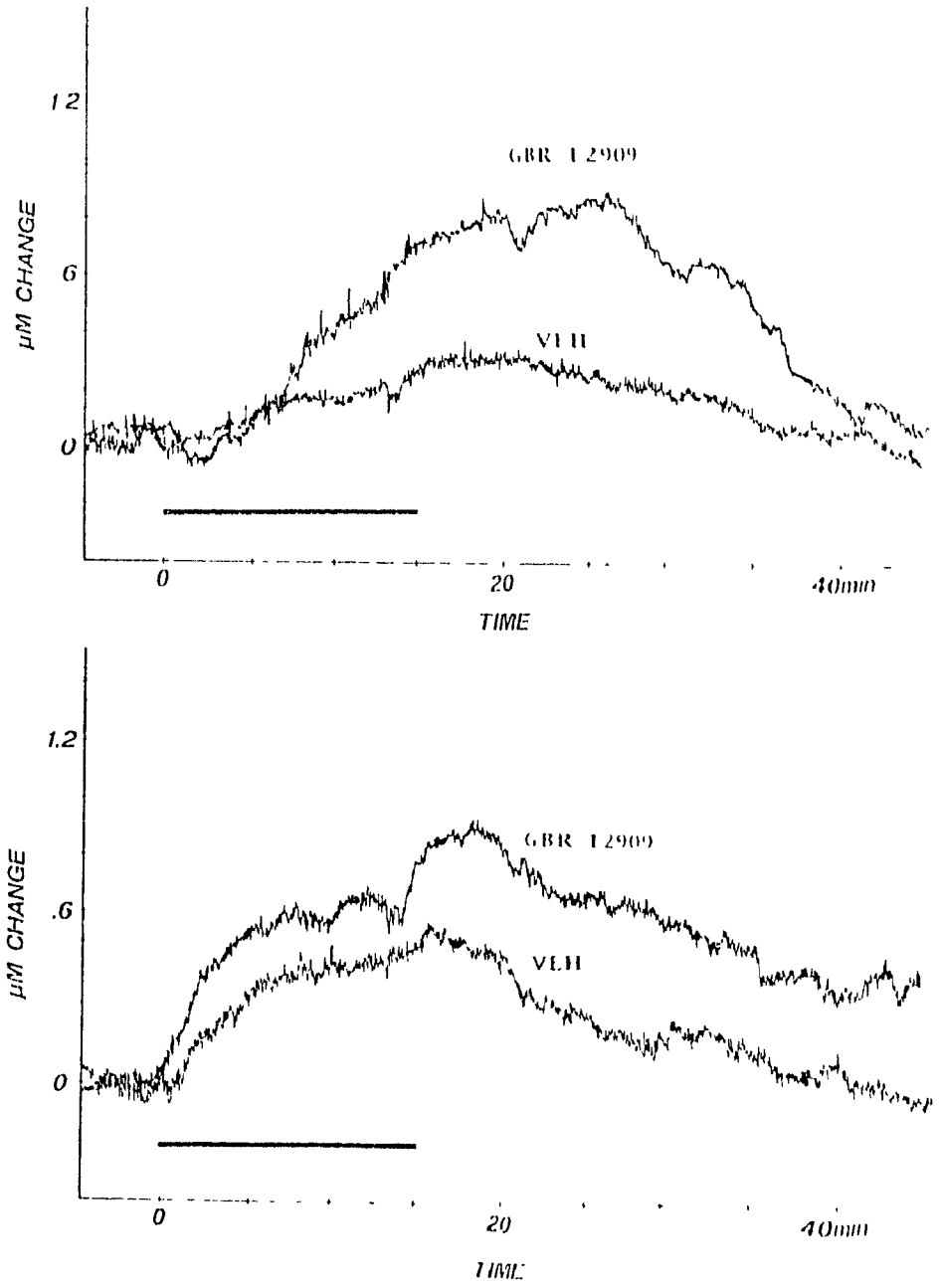

Fig. 8. Examples of chronoamperometric signals recorded in NAcc of one animal and in PFC of another animal exposed to restraint stress 60 min after injection of GBR 12909 (10mg/kg i. p.) or vehicle (VEH); 24 hours separated GBR-12909 and VEH injections. Data are expressed as micromolar changes in DA concentration. Length of horizontal bar corresponds to duration of restraint.

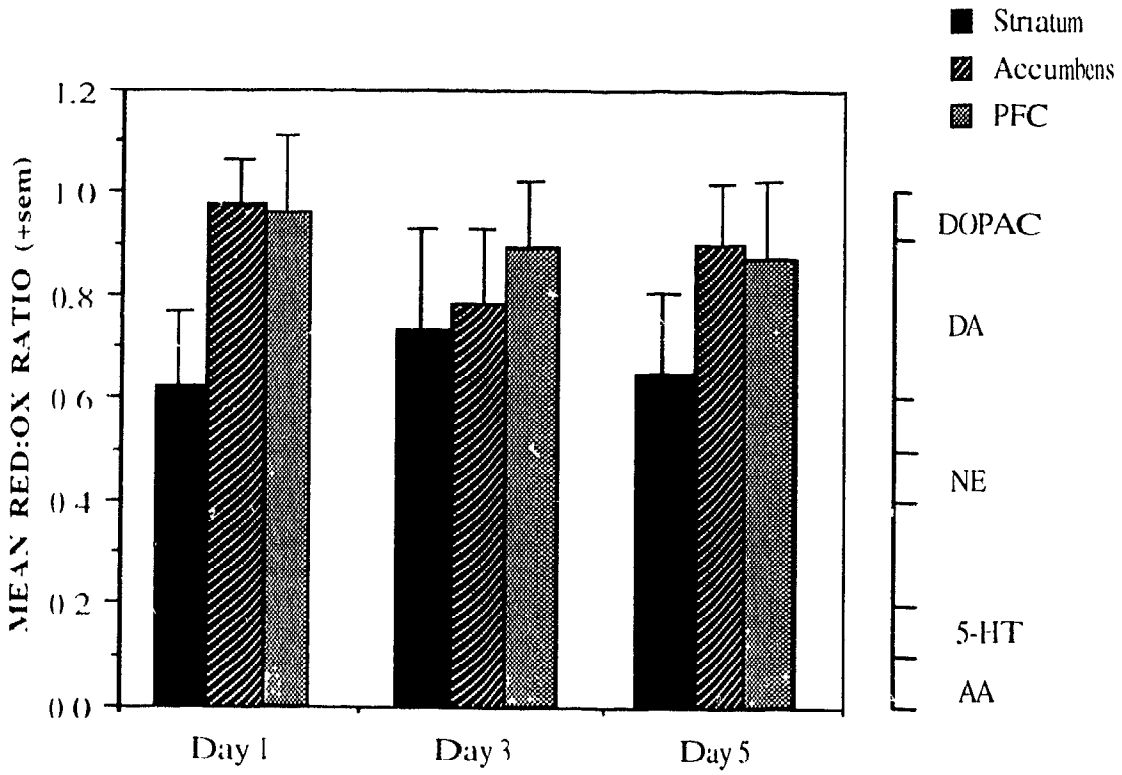

Fig. 9 Average (+ 1 S.E.M.) ratios of reduction and oxidation currents recorded in NAcc (n=5), PFC (n=5) and STR (n=5) of animals subjected to repeated daily restraint. Scale on the right side indicates the range of red:ox ratios for electroactive species that are oxidizable at the applied potential used in the present study. Note that across five days of testing the ratios remain within the range associated with the oxidation of DA and its metabolite DOPAC

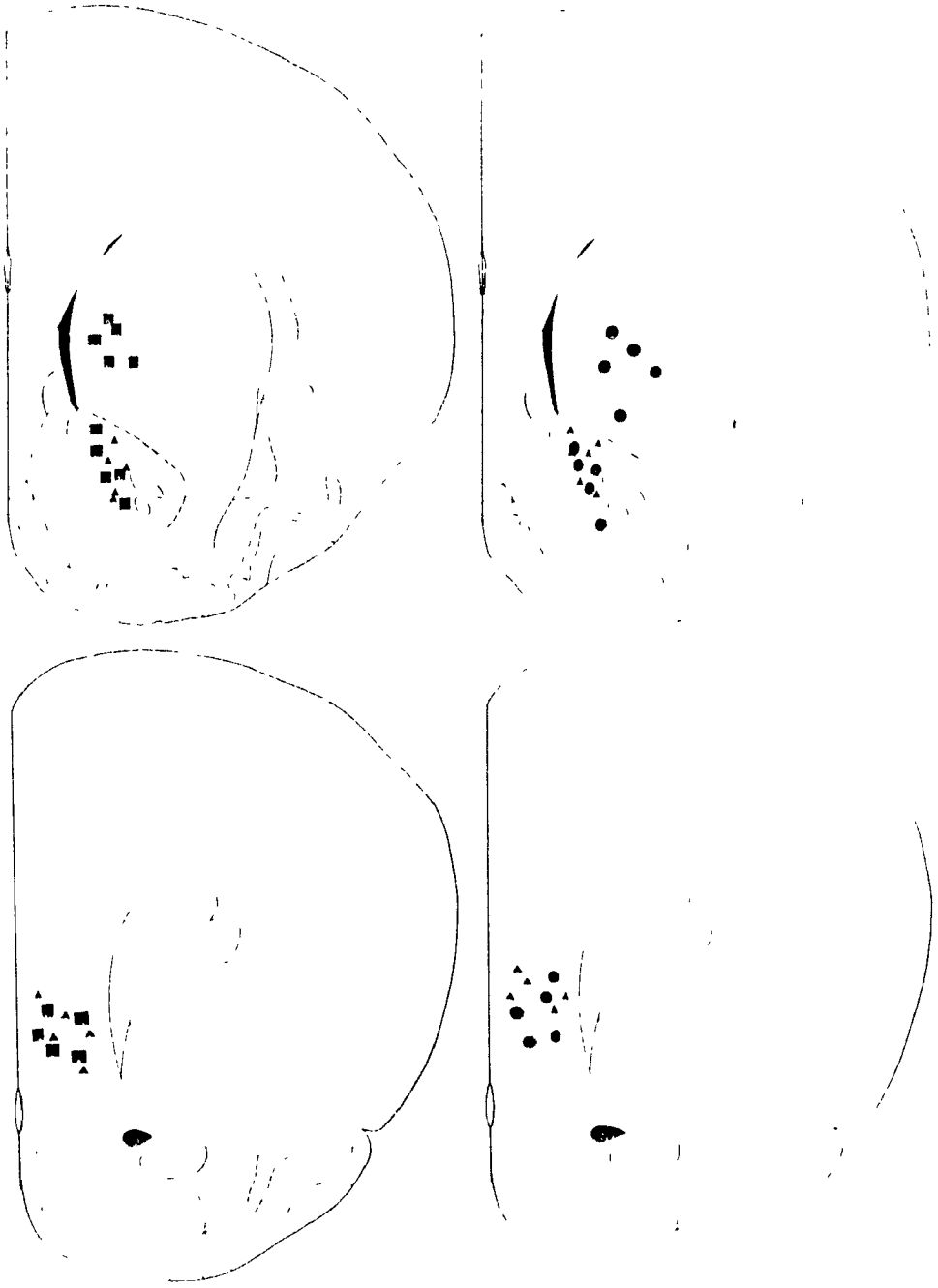

Fig. 10 Histological reconstruction of voltammetric electrode placements in NAcc and STR (top), and PFC (bottom) of animals repeatedly subjected to tail pinch (filled circles) or restraint (filled squares) stress. Triangles represent NAcc and PFC electrode placements of animals used to test the effects of apomorphine on tail pinch (right) and restraint elicited (left) signals. Electrode placements in GBR-12909 experiments were found to be within the areas indicated by other placements illustrated here, and therefore for clarity are not shown.

5 DISCUSSION

The results of the present study indicate that stress stimulates the accumulation of an electroactive species in the extracellular space of NAcc, PFC and STR, and that the magnitude of this accumulation depends to a significant extent on the number of previous exposures to stress and on the type of stress used. In vivo electrochemical techniques do not allow the electroactive species contributing to the signal to be positively identified. However, the present data are consistent with a major contribution of dopamine (DA) to the stress-elicited increases in electrochemical signals recorded in these areas. There are several lines of evidence to support this.

With the chronoamperometric technique used in the present study, the potential between the recording and reference electrodes is momentarily increased to a level at which the neurochemical of interest is oxidized, and then returned to resting level. The rapid change in potential thus allows the measurement of both oxidation and reduction currents of the predominant electroactive species in the extracellular space. While several constituents of the extracellular space are oxidizable at the applied potential used, these all differ in the extent to which they are reduced, thus the magnitude of the reduction current in relation to that of the oxidation current (reduction oxidation ratio, or red.ox ratio) is unique for each compound. It has been shown previously (Gratton et al., 1989) that red.ox ratios of 0.6-0.8 are typically obtained when DA is the primary contributor to the electrochemical signal. The oxidation of dihydroxyphenylacetic acid (DOPAC), the primary metabolite of DA, is almost entirely reversible (red:ox = 0.9 to 1.0), whereas that of ascorbic acid is virtually irreversible (red:ox = 0.0). Serotonin (5-HT) and norepinephrine (NE), two other electroactive species present in NAcc and STR at relatively lower concentrations than DA have red:ox ratios of 0.1-0.2 and 0.4-0.5, respectively. In the present study, the red:ox

ratios measured during the maximum response to stress ranged from 0.6-1.0, indicating that the electrochemical signals elicited by stress reflect an increased availability of DA and probably also of DOPAC at the surface of the voltammetric electrode. Experiments in anesthetized (Gratton et al., 1988; Gerhardt et al., 1986) and recently freely behaving animals (Mitchell and Gratton, 1991, 1992) have confirmed the usefulness of red ox ratios in identifying the species being measured

That the electrochemical signals reflected the oxidation of DA is also suggested by the fact that apomorphine (APO), a mixed D1/D2 receptor agonist, significantly attenuated the increases in signals elicited in NAcc by tail pinch and restraint stress. The inhibitory effect of APO is most likely due to the drug's action at DA autoreceptor sites. Meso NAcc DA neurons possess three types of autoreceptors; release and synthesis modulating receptors located on nerve terminals, and impulse-regulating receptors on DA cell bodies and dendrites (Chiodo, Bannon, Grace, Roth and Bunney, 1984). These autoreceptors provide negative feedback control over DA neurotransmission, increased DA at autoreceptor sites results in compensatory decreases in firing, release and synthesis, whereas decreases in extracellular DA cause compensatory increases in these processes. At doses similar to those used in the present study, APO has been shown to have a higher affinity for somatodendritic than post-synaptic DA receptors, and thus to selectively inhibit spontaneous DA cell firing (Skirboll, Grace and Bunney, 1979). Administration of similar low doses of APO can also cause a decrease in the amount of DA released with each action potential (Timmerman, Dubocovich, Westerink, De Vries, Tepper and Horn, 1989), presumably by an action of the drug at release modulating receptors on DA terminals (Skirboll et. al., 1989). Thus these findings suggest that the inhibitory effect of APO on stress-elicited increases in the electrochemical signals reflects a reduction in the excitability

and firing rate of meso-NAcc DA cells, and possibly also a compensatory decrease in the amount of axonal DA release.

Unlike NAcc, stress-elicited signals in PFC were not attenuated by APO. The differential effects of APO on the meso-PFC and meso-NAcc DA neurons likely reflects known physiological differences between these systems. Although meso-PFC DA neurons possess release modulating receptors, unlike meso-NAcc neurons they do not appear to have impulse-regulating receptors (Wolf, Galloway and Roth, 1986). Thus stress-elicited increases in meso PFC DA cell firing would not be expected to be affected by APO. Although it remains to be tested directly, the fact that APO failed to attenuate stress-induced signals in PFC also indicates that impulse- rather than release-modulating receptors mediated the inhibitory effects of the drug on the stress response in NAcc; suggesting, albeit indirectly, that stress increases DA release by increasing the firing rate of DA neurons. There are few studies on the effects of stress on DA cell firing. Stress-induced changes in firing rates of A9 DA neurons have been observed in anesthetized rats (Chiodo, Antelman, Caggiula and Lineberry, 1979), in response to tail pinch 50% of identified DA neurons decreased their firing rate, while 50% showed an increased firing rate. Trulson and Preussler (1984) showed increased firing in identified A10 DA neurons in cats in a conditioned emotional response (CER) paradigm, while Kiaytkin (1988) reported increased firing in DAergic and non-DAergic A10 cells with a brief exposure to tail pinch.

Alternatively, the failure of APO to block the stress response in PFC may indicate that an electrochemical species other than that of DA was responsible for the electrochemical signals observed in this region. However, this explanation could not account for the fact that GBR 12909, a highly selective DA uptake inhibitor, dose-dependently potentiated restraint elicited signals in NAcc as well as PFC. Taken together, these results strongly

suggest that tail pinch- and restraint-elicited increases in signals recorded in NAcc, PFC and STR reflect primarily increases in extracellular DA levels. These findings are generally consistent with previous reports of restraint stress-induced increases in extracellular DA levels in NAcc and PFC (Imperato et. al., 1992, 1991; Watanabe, 1984), as well as with those of increased DA neurotransmission in response to other types of stressors (Sorg, 1992; Abercrombie et al., 1989, Roth et al., 1988, Deutch et. al., 1985, 1987, Claustre et. al., 1986; Knott et al., 1986; Keller et. al., 1983, for examples).

The present data indicate that, initially, meso PFC neurons are more responsive to the stimulant effect of stress than are meso NAcc or nigrostriatal DA neurons, the first exposure to either restraint or tail pinch stress caused a greater increase in DA levels in PFC than in NAcc or STR levels of DA. The duration of restraint-induced increases in PFC DA levels on day 1, for example, was more than three times greater than those elicited in NAcc and STR, and the amplitude of the first tail pinch-elicited DA release in PFC was two times greater than those recorded in NAcc or STR. These results are in general agreement with those of most previous in vivo (Imperato et al., 1992, 1991, Abercrombie et al., 1989) and post-mortem (Roth et. al., 1988; Deutch et. al., 1985; Lavielle et al., 1978; Thierry et al., 1976, for examples) studies indicating a higher responsivity of the meso-PFC DA system to a variety of stressful stimuli. We can only speculate as to the reasons why the meso PFC DA system appears initially to be more responsive to stress than the meso NAcc and nigrostriatal DA systems. It may reflect, as some have suggested, the differences in autoreceptor regulation between these systems (Bannon, Freeman, Chiodo, Bunney and Roth, 1987). Stress-induced release of somatodendritic DA would activate impulse regulating receptors on meso-NAcc and nigrostriatal DA neurons, resulting in a compensatory reduction of firing and a dampened response to continued exposure to stress.

If this is, in fact, what is occurring then the higher responsiveness of meso-PFC DA neurons to stress may arise from their lack of impulse-regulating receptors. Indeed, the higher basal firing rates and increased DA turnover seen in meso-PFC neurons has been attributed to their lack of impulse-regulating receptors (Bannon et al., 1987). These differences may also underlie the different potentiating effects of GBR-12909 Although DA reuptake inhibition enhanced the restraint-induced increases in extracellular DA in PFC and NAcc, the amplitude of these signals was potentiated to a greater extent in NAcc (600%) than in PFC (200%) while the reverse was true for the duration of these signals (PFC=500%, NAcc 200%). The reasons for this difference are not clear Nonetheless GBR 12909 would be expected to also increase the levels of dendritically-released DA, inhibiting firing of meso NAcc but not meso-PFC DA neurons through activation of impulse regulating receptors, thus reducing the duration of the stress-elicited signals in NAcc relative to those in PFC

In the present study DA levels in NAcc and STR usually returned to baseline levels before the end of the stress period on day 1, whereas those in PFC remained above baseline levels long after the end of the stress period Furthermore, upon termination of restraint stress a further increase in DA levels was recorded in PFC; this effect, however, was observed on only one occasion in one rat in from both NAcc and STR. Other in vivo studies have reported similar post-stress increases in extracellular DA in PFC as well as in NAcc (Imperato et.al., 1992, 1991). This finding suggests that the idea of 'preferential' or 'selective' activation of PFC by stress actually reflects the limitation of post-mortem measurements of tissue DA levels; post-mortem assays would more readily detect the long-lasting increases in DA levels in PFC than the transient increases in NAcc and STR.

The increase in striatal DA levels in response to the first exposure to restraint stress and to a lesser extent, tail pinch stress in the present study is not surprising; increases in striatal

DA have been previously reported by investigators using in vivo techniques (Abercrombie et. al., 1989; Knott et. al., 1986, Keller et. al., 1983, but see Heyes et al., 1988) However, in the present experiment no obvious differences were observed between the NAcc and STR responses to the 1st exposure to either tail pinch or restraint, whereas recent microdialysis experiments reported either a significantly smaller stress response in STR than in NAcc (Abercrombie et al., 1989) or no response in STR at all (Imperato et al., 1989, 1991). We can only speculate as to the reasons for this difference. One possibility is the fact that the density of DA transporter sites is higher in STR than in NAcc (Cass, Gerhardt, Mayfield, Curella, Zahniser, 1992; Scatton, Dubois, Dubocovich, Zahniser and Foge, 1985). It may be that the greater number of uptake sites for DA in STR limits the time for recently released DA to diffuse across the membrane of the dialysis probe. In support of this hypothesis, Keefe, Stricker, Zigmond and Abercrombie (1990) observed greater stress-induced increases in extracellular striatal DA in animals with partial 6 hydroxydopamine lesions than in sham-lesioned animals. The authors attributed this difference to the reduced number of uptake sites in the denervated striatum and to the resulting decrease in clearance of DA from the extracellular space. The high density of uptake sites in the STR, however, would presumably be less of a factor when rapid detection techniques, such as chronoamperometry are used to monitor extracellular DA levels.

The present results indicate that the magnitude of restraint-elicited elevations in DA levels in NAcc, and to a lesser extent in STR, increases with daily testing. The reduction:oxidation ratios of signals recorded in NAcc and STR were found to remain within the range expected for DA across the five days of testing (figure 9), indicating that it was the extracellular accumulation of DA which was progressively enhanced with each

daily exposure to restraint, and not that of another electroactive species. Sensitization of meso-NAcc neurotransmission with repeated stress has been reported previously (Kalivas and Duffy, 1989). That the effects of restraint stress were more potently enhanced in NAcc than in STR with repeated testing is consistent with recent voltammetric evidence showing that repeated presentation of a naturally-occuring rewarding stimulus enhances DA overflow into the extracellular space in NAcc but not in STR, where the amplitude of released DA tended to decrease with repeated testing (Mitchell and Gratton, 1991). The effects of repeated restraint on extracellular levels of DA in NAcc are also consistent with those observed following repeated administration of a number of drugs that enhance DA neurotransmission. Dopamine release and DA-dependent locomotor activity elicited by opioids or psychostimulants are sensitized with repeated administration of these drugs (Sorg and Kalivas, 1991; Kalivas et al., 1988; Kraytkin, 1988; Robinson and Becker, 1986; Hahn et al. 1986; Antelman, Eichler, Black and Kocan, 1980). In addition, cross-sensitization occurs between stress and opiates (Leyton and Stewart 1990, Kalivas and Abhold, 1987), cocaine (Sorg and Kalivas, 1991) and amphetamine (Robinson, 1988; Herman et al., 1984). Repeated exposure to stress later enhances meso-NAcc DA release as well as locomotor activity elicited by cocaine, opiates and psychostimulants (Sorg, 1992; Leyton and Stewart, 1990; Hahn et al., 1986; Robinson et al., 1985; Herman et. al., 1984), and repeated administration of these drugs sensitizes meso-NAcc DA function to the stimulant effect of subsequent stress (Sorg, 1992; Kalivas and Duffy, 1989). Taken together the present results and those of previous studies suggest that with repeated administration, stress shares with a number of drugs abused by humans the ability to increase the responsivity of the meso-NAcc system to further stimulation.

In contrast to the results of this and other studies, Imperato et. al. (1992) recently

reported a progressive decrease in DA release in NAcc in response to repeated once daily restraint for 60 minutes; these authors failed to observe any increase in the DAergic response to the third and all subsequent exposures to restraint stress. The reasons for the difference between the present results and those of Imperato et al (1992) remain, for the moment, a matter of speculation. It is possible that the different durations of restraint (60 versus 15 minutes) contributed to the discordant findings. However, a similar enhancement was observed when animals were restrained once daily for 30 minutes (data not shown). A troubling aspect of the study by Imperato et al (1992) is that each daily response to stress was monitored with the same microdialysis probe. It has been well documented that the recovery of neurotransmitters, including DA, diminishes dramatically when the probe has been in the brain for more than three-four days (Camp and Robinson, 1992). In their study, Imperato et. al attempted to address this issue by showing that haloperidol, a DA receptor antagonist, produces similar, virtually identical, increases in DA, DOPAC, and HVA on the 1st, 5th and 10th days after implantation, suggesting that DA recovery by the probe does not diminish over several days in the brain. However, recent studies have shown that a single injection of saline, a stressful stimulus, enhanced haloperidol-induced catalepsy 14 days later (Antelman, Caggiula, Kocan, Meyer, Edwards and Barry, 1991), and that ten days after a single short-lasting exposure (10 minutes) to a novel environment, another well known stressor, DA release elicited in NAcc by haloperidol is enhanced (Antelman, Kocan, Knopf, Edwards and Caggiula, 1992). Further, a single injection of haloperidol resulted in an enhanced cataleptic response to subsequent haloperidol injection 10 days later (Antelman, Kocan, Edwards, Knopf, Perel and Stiller, 1986). These findings suggest that a brief exposure to stress, including that produced by an intraperitoneal injection, or injection of haloperidol itself, is capable of

increasing the response of meso-NAcc neurons to future administration of haloperidol. Thus, the lack of any change in responsiveness to intermittent systemic injections of haloperidol reported by Imperato et al. (1992) is at odds with data indicating an enhanced DA responsiveness with a similar treatment. It may be, then, that the results obtained by Imperato and colleagues do in fact reflect a diminished DA recovery by the microdialysis probe, rather than support the notion of its long-term patency in situ.

Unlike what was observed in NAcc, repeated restraint stress did not result in a progressive enhancement of extracellular DA levels in PFC during the 15 minute stress interval. However, a further increase in signals in PFC was observed upon termination of the restraint period, and it was these post-stress increases that achieved greater, although not statistically significant, amplitudes across the five days of testing. The few studies that have examined the effects of repeated stress on PFC DA release suggest that repeated or chronic stress may sensitize meso-PFC DA neurotransmission. Long-term isolation stress has been shown to increase tyrosine hydroxylase activity in PFC (Toru, 1982), and Richardson (1984) found a significant increase in post-mortem levels of forebrain DA in animals repeatedly exposed to restraint stress (one hour daily for five days) compared to those receiving acute (one day) restraint.

It is possible that the day-to-day enhancement of post-stress increases of meso-PFC DA neurons seen in the present study may actually reflect, in part, a increasing contribution of norepinephrine (NE) rather than DA. There is a substantial noradrenergic innervation in the PFC (Glavin, 1985), and higher cortical NE levels have been reported in stressed animals (Rossetti, Portas, Pani, Carboni and Gessa, 1990, Irwin, Ahluwalia and Anisman, 1986), and some evidence suggests a sensitization of cortical NE activity after chronic stress (Adell, Garcia-Marquez, Armario and Gelpi, 1988; Irwin et. al, 1986). To what extent NE may be contributing to the signal is not clear; the red:ox ratios though, suggest it is

minimal. As in NAcc and STR, the redox ratios remained within the range associated with DA across the five days of testing. Furthermore, restraint-elicited increases were found to be markedly potentiated by selective DA uptake blockade.

In contrast to restraint, repeated exposure to tail pinch did not result in an enhancement of electrochemical signals in NAcc; rather, there was a tendency towards a decrease in stress elicited signals with repeated testing. This trend was more evident in STR where the day to day decrease in the magnitude of tail pinch-elicited signals achieved statistical significance. While other investigators have reported that mild tail pinch sensitizes their animals to the locomotor stimulant effect of amphetamine, and vice versa (Antelman, Eichler, Black and Kocan, 1980), it appears that this type of stress is not sufficiently potent to sensitize DA release on its own.

The exact mechanisms that control the development of sensitization of DA neurons to a given stimuli are presently unknown, although increasing evidence points to the VTA as a critical site for the induction of sensitized behavioral responses to drugs that facilitate meso-NAcc DA neurotransmission (see review by Kalivas and Stewart, 1991). Another potentially important site is PFC. It has also been shown that depletion of PFC DA will result in enhanced sensitization produced by repeated administration of pharmacological and environmental stimuli. Mitchell and Gratton (1992), for example, found that while sensitization of meso-NAcc activity occurred with repeated exposure to sexual olfactory cues, this sensitization was further enhanced in animals previously depleted of DA in PFC. Furthermore, they reported that repeated daily presentation of a palatable food resulted in relatively similar increases across testing days of extracellular DA in NAcc, but that the food-elicited DA release was progressively enhanced in animals depleted of DA in PFC. Dopamine depletion in PFC has also been shown to enhance both the locomotor response

after repeated amphetamine (Banks and Gratton, 1992; Bannon and Roth, 1983), as well as extracellular DA levels in NAcc (Banks and Gratton, 1992). The data of Mitchell and Gratton (1992) are particularly interesting, in the context of the present study. The lack of sensitized DA release with repeated food presentation they observed in intact animals is similar to the lack of sensitization to tail pinch in the present experiment, suggesting that repeated tail pinch could sensitize NAcc DA neurotransmission in PFC-lesioned animals.

These data taken together suggest that alterations in meso-PFC DA activity or a system whose activity is dependent on meso PFC DA, may underlie in part the development of sensitizatized meso NAcc responses to environmental and pharmacological stimuli. One such output pathway from PFC may involve excitatory amino acids (EAA). Neuroanatomical and tract tracing studies have found evidence for an EAA input from PFC projecting to the NAcc as well as the VTA (Sesack, Deutch, Roth and Bunney, 1989; Christie, Bridge, James and Beart, 1985; Christie, James and Beart, 1985, Walaas, 1981). There is evidence that EAAs can stimulate the meso-NAcc pathway at the level of the cell bodies in the VTA (Kalivas, Duffy and Barrow, 1989) and terminals in NAcc (Jones, Snell and Johnson, 1987) Bilateral injection of amphetamine into PFC, for example, decreased the locomotor hyperactivity produced by intra-NAcc amphetamine, while injection of the D1 antagonist, SCH-23390, into PFC further enhanced the increased locomotor response to intra NAcc amphetamine (Vezina, Blanc, Glowinski and Tassin, 1991). Dopamine depleting lesions of the PFC have been shown to potentiate the increased DA turnover in NAcc produced by footshock stress (Deutch, Clark and Roth, 1990) The mechanism by which the inhibitory influence of PFC on DA release in NAcc is reduced with repeated stress has yet to be identified. The ability of PFC activity to modulate subcortical activity may decrease as a result of a reduction in the effectiveness of the meso-PFC DA pathway;

this possibility is suggested by lesion studies of the PFC DA innervation. However, since results of the present study indicate that the DA response in PFC to restraint does not change with repeated daily testing, while that in NAcc increases, it seems likely that the development of sensitization in NAcc reflects a change in the meso-PFC DA system; the present data however do not rule out the possibility of a decrease in post synaptic DA receptor affinity or density in PFC. Conversely the meso-NAcc terminals may themselves become more responsive to a descending excitatory input from PFC during sensitization.

In conclusion the findings reported in this thesis have a number of implications. The fact that meso-NAcc DA neurotransmission sensitizes to the stimulant effect of some types of stresses as it does to that of drugs commonly abused by humans implicates environmental stresses in the development of drug addiction and in the frequent relapse experienced by former addicts during the initial period of abstinence.

The present results also raises important questions about the idea that the meso NAcc DA pathway is a critical component of the circuitry that controls behaviors motivated by rewards. The fact that the meso-NAcc DA system is activated by rewarding as well as aversive stimuli suggests that it mediates behaviors motivated by any behaviorally relevant stimuli.

REFERENCES

Abercrombie, E. D. and Jacobs, B. L. (1987). Single-unit response of noradrenergic neurons in the locus coeruleus of freely-moving cats. I. Acutely presented stressful and non-stressful stimuli. J. Neurosci., 7, 2837-2843.

Abercrombie, E. D., Keefe, K. A., DiFrischia, D. S. and Zigmond, M. J. (1989). Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem., 52, 1655-1658.

Adell, A., Garcia-Marquez, C., Armario, A. and Gelpi, E. (1988). Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. J. Neurochem., 50, 1678-1681.

Anisman, H., Hahn, B., Hoffman, D. and Zarcharko, R. M. (1985). Stressor invoked exacerbation of amphetamine-elicited perseveration. Pharmacol. Biochem. Behav., 23, 173-183.

Anisman, H. (1984). Vulnerability to depression: Contribution of stress. In: R. M. Post and J. C. Ballenger (Eds.), Neurobiology of Mood Disorders. New York: Williams and Wilkins, pp. 204-238.

Anisman, H. and Zacharko, R. M. (1982). Depression: The predisposing influence of stress. Behav. Brain Sci., 5, 89-137.

Antelman, S. M. and Caggiula, A. R. (1990). The effects of acute exposure to stressors and drugs grow with the passage of time. In: S. Puglisi-Allegra and A. Oliviero (Eds.), Psychobiology of Stress, pps. 161-170. Kluwer, Dordrecht.

Antelman, S. M., Caggiula, A. R., Kocan, S., Meyer, D., Edwards, D. J. and Barry, N. 3rd. (1991). One experience with 'higher' or 'lower' intensity stressors respectively enhances or diminishes responsiveness to haloperidol weeks later: implications for understanding drug variability. Brain Res., 566, 276-283.

Antelman, S. M. and Chiodo, L. A. (1983). Amphetamine as a stressor. In: I. Creese (ed.), Stimulants: Neurochemical, Behavioral and Clinical Perspectives. Raven Press, New York, pp. 269-299.

Antelman, S. M. and Eichler, A. J. (1979). Persistent effects of stress on dopamine related behaviors: Clinical implications. In E. Usdin, I. J. Kopin and J. Barchas (Eds.), Catecholamines: Basic and Clinical Frontiers. Pergammon Press, New York, pp. 1759-1761.

Antelman, S. M., Eichler, A. J., Black, C. A. and Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. Science, 207, 329-331.

Antelman, S. M., Knopf, S., Caggiula, A. R., Kocan, D., Lysle, D. T. and Edwards, D. J. (1988). Stress and enhanced dopamine utilization in the frontal cortex: The myth and the reality. Ann. N. Y. Acad. Sci., 537, 262-271.

Antelman, S. M., Kocan, D., Edwards, D. J., Knopf, S., Perel, J. M. and Stiller, R. (1986). Behavioral effects of a single neuroleptic treatment grow with the passage of time. Brain Res., 385, 58-67.

Antelman, S. M., Kocan, D., Knopf, S., Edwards, D. J. and Caggiula, A. R. (1992). One brief exposure to a psychological stressor induces long lasting, time dependent sensitization of both the cataleptic and neurochemical responses to haloperidol. Life Sci., 51, 261-266.

Banks, K. E. and Gratton, A. (1992). Involvement of prefrontal cortex in amphetamine induced sensitization of mesolimbic dopamine neurons. Soc. Neurosci. Abstr., 18, 321.

Bannon, M. J., Freeman, A. S., Chiodo, L. A., Bunney, B. S. and Roth, R. H. (1987). The electrophysiological and biochemical pharmacology of the mesolimbic and mesocortical dopamine neurons. In Iversen, Iversen and Snyder (eds) Handbook of Psychopharmacology vol 19: New Directions in Behavioral Pharmacology, Plenum, New York.

Bannon, M. J. and Roth, R. H. (1983). Pharmacology of mesocortical dopamine neurons. Pharmacol. Rev., 35, 53-68.

Beckstead, R. M. (1979). An autoradiographical examination of corticocortical and sub cortical projections of the medial dorsal projection (prefrontal cortex) in the rat. J. Comp. Neurol., 184, 43-62.

Beninger, R.J. (1983). The role of dopamine in locomotor activity and learning. Brain Res. Rev., 6, 173-196.

Bertolucci D'Angio, M , Serrano, A and Scatton, B. (1990). Differential effects of forced locomotion, tail pinch, immobilization, and methyl-ß-carboline carboxylate on extracellular 3,4-dihydroxyphenylacetic acid in the rat striatum, nucleus accumbens, and prefrontal cortex An in vivo voltammetric study J Neurochem., 55, 1208-1215.

Blundell, J E and Latham, C J (1978). Pharmacological manipulation of food and water intake In L L Iversen, S D Iversen and S. H Snyder (Eds.), Chemical Influences on Behavior, pps 201-254 London: Academic

Bozarth, M A and Wise, R A (1981) Intra-cranial self-administration of morphine into the ventral tegmental area of rats Life Sci., 28, 551-555.

Bradberry, C W , Lory, J D. and Roth, R H. (1991). The anxiogenic beta-carboline FG 7142 selectively increases dopamine release in rat prefrontal cortex as measured by microdialysis. J Neurochem , 56, 748-752

Bradberry, C W and Roth, R H (1989). Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. Neurosci Lett , 103, 97-102

Breslau, N and Davis, G C (1986). Chronic stress and major depression. Arch. Gen. Psychiat., 43, 309 314

Brier, A (1989) Experimental approaches to human stress research: Assessment of neurobiological mechanisms of stress in volunteers and psychiatric patients. Biol. Psychiat , 26, 438-462

Brozowski, T J , Brown, R. M., Rosvold, M. E and Goldman, P. S. (1979). Science, 205, 929-931

Camp, D M and Robinson, T. E (1992). On the use of multiple probe insertions at the same site for repeated intracerebral microdialysis experiments in the nigrostriatal dopamine system of rats J Neurochem , 58, 1706-1715

Carlson, J N Herrick, K F , Baird, J. L and Glick, S. D. (1987). Selective enhancement of dopamine utilization in the rat prefrontal cortex by food deprivation. Brain Res , 400, 200 203

Carlsson, A (1975) Dopaminergic autoreceptors In: O Almgren, A Carlsson and J. Engels (Eds.), Chemical Tools in Catecholamine Research, Vol II. North Holland Publishing Co., Amsterdam, pp 219-225

Cass, W. A., Gerhardt, G. A Mayfield, R D Curella, P and Zahniser, N. R (1992). Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. J Neurochem , 59, 259-266.

Cenci, M. A., Kalen, P . Mandel, R J and Bjorklund, A (1992) Regional differences in the regulation of dopamine and noradrenalin release in medial frontal cortex, nucleus accumbens and caudate-putamen: a microdialysis study in the rat Brain Res , 581, 217 228.

Chiodo, L. A., Bannon, M. J., Grace, A. A , Roth, R H and Bunney B. S (1984). Evidence for the absence of impulse-regulating somatodendritic and synthesis modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. Neurosci., 1, 1-16

Chiodo, L. A., Caggiula, A. R., Antelman, S M and Lineberry, C G. (1979). Reciprocal influences of activating and immobilizing stimuli on the activity of nigrostriatal dopaminie neurons Brain Res , 176, 385-390

Christie, M. J. Bridge, S , James, L B and Beart, P M (1985) Excitotoxic lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. Brain Res , 333, 169-172

Christie, M. J., James, L. B and Beart, P. M. (1985). An excitant amino acid projection from medial prefrontal cortex to anterior part of nucleus accumbens in the rat J Neurochem , 45, 477-482

Claustre, Y., Rivy, J P , Dennis, T and Scatton, B. (1986) Pharmacological studies on stress-induced increase in frontal cortical dopamine release in rat J. Pharmacol Exp. Ther., 238, 693-700

Commissiong, J. W (1985). Monoamine metabolites: Their relationship and lack of relationship to monaminergic neuronal activity Biochem Pharmacol 34, 1127 1131

D'Angio M., Serrano, A., Rivy, J. P. and Scatton, B (1987). Tail pinch stress increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the rat nucleus accumbens but not frontal cortex: antagonism by diazepam and zolpidem Brain Research,

409, 169-174.

Deutch, A Y, Bean, A. J., Bissette, G., Nemeroff, C B, Robbins, R. J. and Roth, R. H. (1987) Stress-induced alterations in neurotensin, somatostatin and corticotropin-releasing factor in mesotelencephalic dopamine system regions. Brain Res, 417, 350-354.

Deutch, A Y, Clark W. A and Roth, R. H (1990) Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. Brain Res, 521, 311-315

Deutch, A Y and Roth R. H. (1990) Determinants of stress-induced activation of the prefrontal cortical dopamine system In H. B. M. Uylings, C. G. van Eden, J. P. C de Bruin, M. A Corner and M P G. Feenstra. (Eds), The Prefrontal Cortex: Its Structure, Function, and Pathology, (Progress in Brain Research, vol. 85). Elsevier, Amsterdam, pp. 357-393

Deutch, A Y, Tam S Y. and Roth, R H. (1985). Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra Brain Research, 333, 143-146

Di Chiara, G and Imperato, A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl Acad Sci (USA), 85, 5274-5278

Dorow, R, Horowski, R, Paschelke, G, Amin, M. and Braestrup, C. (1983). Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors. Lancet ii, 98-99

Dunn, A. J (1988) Stress related activation of cerebral dopaminergic systems. Ann. NY Acad Sci, 537, 188-205.

Dunn, A J and File S E. (1983). Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiol. Behav, 31, 511-513

Fibergen, R D and Caggiula, A R. (1973). Ventral midbrain involvement in copulatory behavior of the male rat Physiol Behav, 10, 435-441.

Esposito, R U, Motola, A H. D and Kornetsky, C. (1978). Cocaine: acute effects on reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle.

Pharmacol. Biochem. Behav., 8, 437-439.

Ettenberg, A, Pettit, H. O., Bloom, F. E. and Koob, G. F. (1982) Heroin and cocaine intravenous self-administration in rats: Mediation by separate neural systems Psychopharmacology, 78, 204-209.

Fadda, F., Argiolas, A., Melis, M. R., Tissari, A. H., Onali, P. C. and Gessa, G. L. (1978). Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in nucleus accumbens: reversal by diazepam Life Sci., 23, 2219-2224.

Fallon, J. H. and Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain IV. Topography of the dopamine projection to the basal forebrain and neostriatum J. Comp. Neurol., 180, 545-580

Falloon, I. R. H. (1986) Family stress and schizophrenia Psychiat. Clin. North Am., 9, 165.

Fibiger, H. C. (1978) Drugs and reinforcement: A critical review of the catecholamine theory. Ann. Rev. Pharmacol. Toxicol., 18, 37-56

Fibiger, H. C. and Phillips, A. G. (1986) Reward, motivation, cognition: Psychobiology of the mesotelencephalic dopamine systems In F. E. Bloom and S. R. Geiger (Eds), Handbook of Physiology The Nervous System, Vol IV, pps 647-675 American Physiological Society, Bethesda Md.

Foreman, M. M. and Hall, J. L. (1987) Effects of D2 dopaminergic receptor stimulation on male rat sexual behavior. J. Neural Transm., 68, 153-170

Friedman, M. J., Stolk, J. M. Harris, P. Q. and Cooper, T. B. (1984) Serum dopamine-beta-hydroxylase activity in depression and anxiety. Biological Psychiat., 19, 557-571.

Geary, N. and Smith, G. P. (1985) Pimozide decreases the positive reinforcing effect of sham-fed sucrose in the rat. Pharmacol. Biochem. Behav., 22, 787-790

Gerhardt, G. A., Oke, A. F., Nagy, G., Moghaddam, B. and Adams, R. N. (1984) Nafion-coated electrodes with high sensitivity for CNS electrochemistry Brain Res., 290, 390-394.

Gerhardt, G A , Rose, G M. and Hoffer, B. J. (1985). Release of monoamines from striatum of rat and mouse by local application of potassium: Evaluation of a new electrochemical technique. J. Neurochem , 46, 842-850.

Glavin, G B (1985) Stress and brain noradrenaline: A review. Neurosci. Biobehav. Rev., 9, 233 243

Glick, S D and Hinds, P A. (1984) Sex differences in sensitization to cocaine-induced rotation Eur. J Pharmacol , 99, 119-121

Gonzales-Mora, J L , Maidment, N T., Guadalupe, T. and Mas, M. (1989). Post-mortem dopamine dynamics assessed by voltammetry and microdialysis Brain Res. Bull., 23, 323 327.

Grace, A A (1992). The depolarization block hypothesis of neuroleptic action: implications for the etiology and treatment of schizophrenia. J. Neural Transm., 36, 91-131.

Gratton, A , Hoffer, B. J. and Gerhardt, G. A (1989) In vivo electrochemical studies of monoamine release in the medial prefrontal cortex of the rat Neuroscience, 29, 57-64.

Gratton, A. and Wise, R A (1988). Comparisons of connectivity and conduction velocities for medial forebrain bundle fibers subserving stimulation-induced feeding and brain stimulation reward Brain Res , 438, 264-270

Gruen, R and Baron, M (1984). Stressful life events and schizophrenia: Relation to illness onset and family history Neuropsychobiology, 12, 206

Hahn B., Zacharko, R M and Anisman, H. (1986) Alterations of amphetamine elicited perseveration and locomotor excitation following acute and repeated stressor application. Pharmacol. Biochem. Behav., 25, 29-36.

Hamilton, M E and Bozarth, M A. (1988). Feeding elicited by dynorphin 1-13 microinjections into the ventral tegmental area in rats Life Sci., 43, 941-946.

Herman, J -P , Guillonneau, D , Dantzer, R , Scatton, B., Semerdjian-Rouquier, L. and LeMoal, M. (1982). Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat Life Sci , 30, 2207-2214

Herman, J. -P., Stinus, L. and LeMoal, M. (1984). Repeated stress increases locomotor response to amphetamine Psychopharmacol., 84, 431-435

Hernandez, L., Lee, F. and Hoebel, B. G. (1987) Simultaneous microdialysis and amphetamine infusion in the nucleus accumbens and striatum of freely moving rats: Increase in extracellular dopamine and serotonin Brain Res. Bull., 19, 623-628

Heyes, M. P., Garnett, E. S. and Coates, G. (1988) Nigrostriatal dopaminergic activity is increased during exhaustive exercise stress in rats Life Sci., 42, 1537-1542

Hornykiewicz, O. and Kish, S. J. (1986). Biochemical pathophysiology of Parkinson's disease. Adv. Neurol., 45, 19-34

Imperato, A., Angelucci, L., Casolini, P., Zocchi, A. and Puglesi-Allegra, S. (1992) Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res., 577, 194-199

Imperato, A., Puglisi-Allegra, S., Casolini, P. and Angelucci, L. (1991) Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis Brain Res., 538, 111-117

Imperato, A., Puglisi-Allegra, S., Casolini, P., Zocchi, A. and Angelucci, L. (1989) Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: Role of corticosterone. Eur. J. Pharmacol., 165, 337-338

Imperato, A., Puglisi-Allegra, S., Zocchi, A., Scrocco, M. G., Casolini, P. and Angelucci, L. (1990) Stress activation of limbic and cortical dopamine release is prevented by ICS 205-930 but not by diazepam Eur. J. Pharmacol., 175, 211-214

Irwin, J., Ahluwalia, P. and Antsman, H. (1986) Sensitization of norepinephrine activity following acute and chronic footshock Brain Res., 379, 98-103

Jenck, F., Gratton, A. and Wise, R. A. (1986) Opposite effects of ventral tegmental and periaqueductal gray morphine injections on lateral hypothalamic induced feeding Brain Res., 399, 24-32.

Jones, S. M., Snell, L. D. and Johnson, K. M. (1987) Inhibition by phenycyclidine of excitatory amino acid-stimulated release of neurotransmitters in nucleus accumbens.

Neuropharmacology, 26, 173-179

Joseph, M H and Kennett, G A. (1983). Stress-induced release of 5-HT in the hippocampus and its depen 'ence on increased tryptophan availibility: An in vivo electrochemical study Br a Res., 270, 251-257.

Kalivas, P W and Abhold, R. (1987) Enkephalin release into the ventral tegmental area in response to stress: modulation of mesocorticolimbic dopamine. Brain Res., 414, 339-348.

Kalivas, P W and Duffy, P. (1987) Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons J. Pharmacol Exp Ther., 241, 204-212.

Kalivas, P W. and Duffy, P (1988) Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. J. Neurochem. 50, 1498-1504.

Kalivas, P W and Duffy, P (1989) Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biol Psychiat . 25, 913-928.

Kalivas, P W and Duffy, P (1990). The effects of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens J Neurosci 10, 2940-2949.

Kalivas, P W , Duffy, P , Abhold R and Dilts, R. P (1988) Sensitization of mesolimbic dopamine neurons by neuropeptides and stress. In P. W Kalivas and C. D. Barnes, (Eds), Sensitization in the Nervous System. Telford Press, Caldwell, New Jersey, pp 175 205

Kalivas, P W Duffy, P. and Barrow, J. (1989) Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J Pharmacol Exp Ther., 251, 378-387.

Kalivas, P. W and Stewart, J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Rev., 223-244

Kaneyuki, H , Yokoo, H , Tsuda, A , Yoshida, M , Misuki, Y., Yamada, M. and Tanaka, M (1991) Psychological stress increases dopamine turnover selectively in mesoprefrontal dopamine neurons of rats: Reversal by diazepam. Brain Res , 557, 154-161

Keefe, K. A., Stricker, E. M., Zigmond, M. J. and Abercrombie, E. D. (1990) Environmental stress increases extracellular dopamine in striatum of 6-hydroxydopamine-treated rats: In vivo microdialysis studies. Brain Res., 527, 350-353.

Keller, R. W., Stricker, E. M. and Zigmond, M. J. (1983) Environmental stimuli but not homeostatic challenges produce apparent increases in dopaminergic activity in the striatum: an analysis by in vivo voltammetry. Brain Research, 279, 159-170.

Kiaytkin, E. A. (1988). Functional properties of presumed dopamine containing and other ventral tegmental neurons in conscious rats. Int. J. Neurosci., 42, 21-43.

Knott, P. J., Brannan, T. S., Andrews, C. D., Togasaki, D., Young, I. G., Maker, H. and Yahr, M. (1986) Drug, stress, and circadian influences on dopaminergic neuronal function in the rat studied by voltammetry and chronoamperometry. Ann. N. Y. Acad. Sci., 533, 493-495.

Koob, G. F. (1992). Neural mechanism of drug reinforcement. Ann. N. Y. Acad. Sci., 654, 172-191.

Kuzcenski, R. (1983). Biochemical actions of amphetamine and other stimulants. In I. Creese (Ed), Stimulants: Neurochemical, Behavioral and Clinical Perspectives, pps. 31-61. Raven Press, New York.

Lavielle, S., Tassin, J.-P., Thierry, A.-M., Blanc, G., Herve, D., Barthelemy, C. and Glowinski, J. (1978). Blockade by benzodiazepines of the selective high increase in dopamine turnover induced by stress in mesocortical dopamine neurons in the rat. Brain Res., 168, 585-594.

Leyton, M. and Stewart, J. (1990) Preexposure to footshock sensitizes the locomotor response to subsequent systemic morphine and intra-nucleus accumbens amphetamine. Pharmacol. Biochem. Behav., 37, 97-110.

Loullot, A., LeMoal, M. and Simon, H. (1986) Differential reactivity of dopaminergic neurons in the nucleus accumbens in response to different behavioral situations. An in vivo voltammetric study in free moving rats. Brain Research, 397, 395-400.

Lyness, W. H., Friedle, N. M. and Moore, K. E. (1979) Destruction of dopaminergic nerve terminals in nucleus accumbens: effects of d-amphetamine self-administration.

Pharmacol Biochem Behav., 11, 553-556

MacLennan A J and Maier, S. F (1983) Coping and the stress-induced potentiation stimulant stereotypy in rats. Science, 219, 1091-1093.

Marwaha, J., Palmer, M R., Woodward, D J., Hoffer, B. J. and Freedman, R. (1980). Electrophysiological evidence for presynaptic actions of phencyclidine on noradrenergic transmission in rat cerebellum J Pharmacol Exp. Ther., 215, 606-613.

McGregor, I S and Atrens D. M (1990) Stressor-like effects of FG-7142 on medial prefrontal cortex self stimulation Brain Res 516, 170-174.

Mitchell, J B and Gratton, A (1991) Opioid modulation and sensitization of dopamine release elicited by sexually relevant stimuli a high speed chronoamperometric study in freely-behaving rats Brain Res., 551, 20-27

Mitchell, J B and Gratton, A (1992) Partial dopamine depletion of the prefrontal cortex leads to enhanced mesolimbic dopamine release elicited by repeated exposure to naturally reinforcing stimuli J Neurosci., 12 3609-3618

Mitchell, J B and Stewart, J (1989). Effects of castration, steroid replacement, and sexual experience on mesolimbic dopamine and sexual behaviors in the male rat. Brain Res., 491, 116 127

Mitchell, J B and Stewart, J. (1990). Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. Pharmacol. Biochem. Behav., 35, 643-649

Nicholson, I R. and Neufeld, R. J W. (1992). A dynamic vunerability perspective on stress and schizophrenia. Amer. J Orthopsychiat., 62, 117-130

Oades, R D and Halliday, G M. (1987). Ventral tegmental (A10) system: Neurobiology 1 Anatomy and connectivity. Brain Res Rev., 12, 117-165.

Palkovitz, M (1973) Isolated removal of hypothalamic nuclei or other brain nuclei of the rat Brain Res., 59, 449-450

Paxinos, G. and Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Peris, J. and Zahniser, N. (1987). One injection of cocaine produces a long lasting increase in [3H] dopamine release. Pharmacol Biochem and Behav , 27, 533-535

Pettit, H. O., Pan, H T., Parsons, L H and Justice, J B (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J Neurochem , 55, 798-804

Pfeffer, A O and Samson H. H (1988) Haloperidol and apomorphine effects on ethanol reinforcement in free-feeding rats Pharmacol. Biochem Behav , 29, 843 850

Phillipson, O. T. (1978). Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: A horseradish peroxidase study in the rat J Comp Neurol , 187, 117-114.

Rao, T. S , Kim, H. S., Lehmann, J , Martin, L. L and Wood, P. L (1989) Differential effects of phenycyclidine (PCP) and ketamine on mesocortical and mesostriatal dopamine release in vivo. Life Sci , 45, 1065 1072

Reinhard, Jr , J R Bannon, M. J and Roth, R H (1982) Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam Naunyn Schmiedeberg's Arch Pharmacol , 308, 374-377

Reith, M E A , Meisler, B E , Sershen, H and Lajtha, A (1986) Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior Biochem Pharmacol , 35, 1123 1129

Richardson, J. S (1984). Brain part monoamines in the neuroendocrine mechanisms activated by immobilization stress in the rat. Int J Neurosci 23, 57 67

Roberts, D. C. S and Koob, G. F (1982) Disruption of cocaine self administration following 6-OHDA lesions of the ventral tegmental area in rats Pharmacol Biochem Behav , 17, 901-904

Roberts, D. C. S., Koob, G F Klonoff, P. and Fibiger, H C (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens Pharmacol Biochem Behav , 12, 781 787

Robinson, T. E. (1984). Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female

rats. Psychopharmacol., 84, 466-475.

Robinson, T. E. (1988). Stimulant drugs and stress: Factors influencing individual differences in the susceptibility to sensitization. In: P. W. Kalivas and C. Barnes (Eds.), Sensitization in the Nervous System, pps. 145-173. Telford Press, Caldwell, N. J.

Robinson, T. E., Angus, A. L. and Becker, J. B. (1985b). Sensitization to stress: The enduring effects of prior stress on amphetamine-induced rotational behavior. Life Sci., 37, 1039-1042

Robinson, T. E. and Becker, J. B. (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine pyschosis. Brain Res. Rev., 11, 157-198

Robinson, T. E. Becker, J. B., Young, E. A., Akil, H. and Casteneda, E. (1987). The effects of footshock stress on regional brain dopamine metabolism and pituitary ß-endorphin release in rats previously sensitized to amphetamine. Neuropharmacol., 26, 679-691

Robinson, T. E. and Camp, D. M. (1990). Does amphetamine preferentially increase the extracellular concentration of dopamine in the mesolimbic system of freely moving rats? Neuropyschopharmacology, 3, 163-173.

Robinson, T. E., Jurson, P. A., Bennett, J. A. and Bengten, K. M. (1988). Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (-) amphetamine: a microdialysis study in freely moving rats. Brain Res., 462, 211-222

Rompre, P. P. and Wise, R. A. (1989). Opioid-neuroleptic interaction in brainstem self-stimulation. Brain Res., 477, 144-151.

Rossetti, Z. L., Portas, C., Pani, L., Carboni, S. and Gessa, G. L. (1990). Stress increases noradrenaline release in the rat frontal cortex: prevention by diazepam. Eur. J. Pharmacol., 176, 229-231

Roth, K. A., Mefford, I. M. and Barchas, J. D. (1982). Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. Brain Res., 239, 417-424.

Roth, R. H., Tam, S.-Y., Ida, Y., Yang, J.-X. and Deutch, A. Y. (1988). Stress and

the mesocorticolimbic dopamine systems. Annals NY Acad. Sci., 537, 138-147

Scatton, B., Dubois, A., Dubocovich, M. L., Zahniser, N. R. and Foge, D. (1985). Quantitative autoradiography of [3H] nomifensine binding sites in rat brain. Life Sci., 36, 815-822.

Segal, D. S. and Mandell, A. J. (1974). Long-term administration of amphetamine: progressive augmentation of motor activity and stereotypy. Pharmacol Biochem Behav, 2, 249-255.

Sesack, S. R., Deutch, A. Y., Roth, R. H and Bunney, B. S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp. Neurol., 290, 213-242.

Simon, H. and Le Moal, M. (1987). Mesencephalic dopaminergic neurons: Role in the general economy of the brain. Ann. N. Y. Acad. Sci., 537, 235-253.

Simon, H., Scatton, B. and Le Moal, M. (1980). Nature, 286, 150-151

Skirboll, L. R., Grace, A. A. and Bunney, B. S. (1979) Dopamine auto- and postsynaptic receptors: Electrophysiological evidence for differential sensitivity to dopamine agonists. Science, 206, 80-82.

Soares-de-Silva, P. (1987) Does brain 3, 4-dihydroxyphenylacetic acid reflect dopamine release? J. Pharm. Pharmacol., 39, 127-129

Sorg, B. A. and Kalivas, P. W. (1991). Effect of cocaine and stress cross sensitization on extracellular dopamine release in rat nucleus accumbens. Soc Neurosci Abstr., 16, 132

Sorg, B. A. (1992). Mesocorticolimbic dopamine systems: cross-sensitization between stress and cocaine. Ann N. Y. Acad Sci., 654, 136-144.

Spyraki, C., Fibiger, H. C. and Phillips, A. G. (1983). Attenuation of heroin reward in rats by disruption of the mesocorticolimbic dopamine system. Psychopharmacology, 79, 278-283.

Stewart, J. and Vezina, P. (1987) Environment-specific enhancement of hyperactivity induced by systemic or intra-VTA morphine injections in rats preexposed to amphetamine.

Psychobiology, 15, 144-153.

Stewart, J and Vezina, P. (1988) Conditioning and behavioral sensitization. In: P. W. Kalivas and C. D Barnes (eds.), Sensitization in the Nervous System. Telford Press, Caldwell, New Jersey, pp 207-224.

Stricker, E. M. and Zigmond, M. J (1976). Recovery of function following damage to central catecholamine-containing neurons: a neurochemical model for the lateral hypothalamic syndrome In J. M Sprague and A. N. Epstein (Eds.), Progress in Psychobiology and Physiological Psychology, pps 121-189. Academic Press, New York

Tam, S Y and Roth, R H (1985) Selective increase in dopamine metabolism in the prefrontal cortex by the anxiogenic beta-carboline FG 7142. Biochem. Pharmacol., 34, 1595-1598

Thierry, A M., Javoy, F., Glowinski, J and Kety, S. S (1968). Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat I. Modifications of norepinephrine turnover J. Pharmacol. Exp Ther., 163, 163-171.

Thierry, A. M., Tassin, J. P, Blanc, G. and Glowinski, J. (1976). Selective activation of the mesocortical dopaminergic system by stress Nature (London), 263, 242-244.

Timmerman, W , Dubocovich, M. L., Westerink, H C., De Vries, J. B., Tepper, P. G. and Horn, A S (1989) The enantiomers of the dopamine agonist N-0437: in vivo and in vitro effects on striatal dopamine. Eur. J Pharmacol., 166, 1-11

Tissari, A H , Argiolas, A., Fadda, F, Serra, G and Gessa, G L. (1979). Foot-shock stress accelerates non striatal dopamine synthesis without activating tyrosine hydroxylase. Naunyn-Schmiedeberg's Arch. Pharmacol., 308, 155-157.

Toru, M (1982) Increased tyrosine hydroxylase activity in frontal cortex of rats after long term isolation stress Encephale, 8, 315-317.

Trulson, M E and Preussler, D W. (1984). Dopamine-containing ventral tegmental area neurons in freely moving cats: Activity during the sleep-waking cycle and effects of stress. Exper. Neurol , 83, 367-377.

Ungerstedt, U (1971). Aphagia and adipsia after 6-hydroxydopamine induced

degeneration of the nigrostriatal dopamine system Acta. Physiol. Scand. (Suppl), 367, 95-122.

van Veldhuizen, M. J. A., Feenstra, M. G. P., Boer, G. J. and Westerink, B. H. C. (1990). Microdialysis studies on cortical noradrenaline release: basic characteristics, significance of extracellular calcium and massive post mortem release. Neurosci. Lett., 119, 233-236.

Vezina, P., Blanc, G., Glowinski, J. and Tassin, J. P. (1991). Opposed behavioral outputs of increased dopamine transmission in prefrontocortical and subcortical areas: A role for cortical D1 receptor. Eur. J. Neurosci., 3, 1001-1007.

Walaas, I. (1981). Biochemical evidence for overlapping neocortical and allocortical glutamate projections to the nucleus accumbens and rostral caudatoputamen in the rat brain. Neuroscience, 6, 399-405.

Watanabe, H. (1984). Activation of dopamine synthesis in mesolimbic dopamine neurons by immobilization stress in the rat. Neuropharmacol., 23, 1335-1338.

Weiss, F., Hurd, Y. L., Ungerstedt, U., Markou, A., Plotsky, P. M. and Koob, G. F. (1992). Neurochemical correlates of cocaine and ethanol self-administration. Ann. N. Y. Acad. Sci., 654, 220-241.

Wickens, J. (1990). Striatal dopamine in motor activation and reward-mediated learning: steps towards a unifying model. J. Neural. Transm. (Gen.Sect.), 80, 9-31.

Wilcox, R. A., Robinson, T. E. and Becker, J. B. (1986). Enduring enhancement in amphetamine-stimulated striatal dopamine release in vitro produced by prior exposure to amphetamine or stress in vivo. Eur. J. Pharmacol., 124, 375-376.

Willner, P. (1985). Depression: A psychobiological synthesis. John Wiley and Sons, New York.

Wise, R. A. (1973).

Wise, R. A. (1978). Catecholamine theories of reward: A critical review. Brain Res., 152, 215-247.

Wise, R. A. (1989). The brain and reward. In: J. M. Liebman and S. J. Cooper, (Eds.),

The Neuropharmacological Basis of Reward, pps 377-424. Oxford University Press, Oxford

Wise, R. A., Bauco, P., Carlezon, W. A. and Trojnar, W. (1992). Self-stimulation and drug reward mechanisms. Ann. N. Y. Acad. Sci., 654, 192-198

Wise, R. A. and Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. Psychol. Rev. 94, 469-492

Wolf, M. E., Galloway, M. P. and Roth, R. H. (1986). Regulation of dopamine synthesis in the medial prefrontal cortex: Studies in brain slices. J. Pharmaco. Exp. Ther., 236, 698-707

Wolf, M. E. and Roth, R. H. (1986). Dopamine autoreceptors. In: I. Creese, and C. M. Fraser (Eds.), Structure and Function of Dopamine Receptors - Receptor Biochemistry and Methodology, vol 9. Alan R. Liss, Inc., New York

Yokel, R. A. and Wise, R. A. (1975). Increased lever pressing for amphetamine after pimozide in rats: Implications for a dopamine theory of reward. Science, 187, 547-549.