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The Involvement of Ventral Tegmental Opioid Receptors in Mediation of Opiate-Reward and in Modulation of Mesolimbic Dopamine: Behavioural and Neurochemical Analyses

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ABSTRACT

The Involvement of Ventral Tegmental Opioid Receptors in Mediation of Opiate-Reward and in Modulation of Mesolimbic Dopamine: Behavioural and Neurochemical Analyses

Darragh P. Devine
Concordia University, 1993

Intracranial self-administration was used to evaluate the involvements of ventral tegmental area (VTA) μ and ∂ opioid receptors in the rewarding effects of opiates. VTA microinfusions of DAMGO (a μ agonist) and DPDPE (a ∂ agonist) were each effective in establishing and maintaining lever-pressing habits in rats. Lever-presses were extinguished when vehicle was substituted for drug, and reinstated when drug reward was re-established. Thus, it appears that VTA μ and ∂ opioid receptors may each participate in the habit-forming effects of opiates. The effective dose of DAMGO was 100 times lower than the effective dose of DPDPE, suggesting that the major contribution of VTA mechanisms to opiate self-administration involves μ opioid receptors.

Neurochemical concomitants of opiate administration were evaluated using microdialysis and high pressure liquid chromatography (HPLC). VTA microinjections of DAMGO and DPDPE

each produced dose-orderly increases in ventral striatal dopamine (DA) and DA metabolite concentrations. The doses of DPDPE that increased these concentrations were 100-fold higher than the equi-effective doses of DAMGO. Therefore, the receptor selectivities of tne actions of DAMGO and DPDPE were examined. Pretreatment with VTA microinjections of naltrindole (a ∂ antagonist) antagonized the effects of DPDPE, but failed to antagonize the effects of DAMGO. Pretreatment with VTA microinjections of CTOP (a μ antagonist) antagonized the effects of DAMGO, but failed to antagonize the effects of DPDPE. Thus, DAMGO appears to increase mesolimbic DA neurotransmission concentrations through selective actions on μ receptors, and DPDPE appears to increase mesolimbic DA neurotransmission through selective actions on ∂ receptors in the VTA.

The role of VTA κ opioid receptors in the DA-modulating actions of opiates was also examined. Microinjections of U-50,488H (a κ agonist) failed to alter basal DA and metabolite concentrations. Thus, VTA κ receptors appear not to participate in modulation mesolimbic DA neurotransmission.

These findings indicate that VTA μ and ∂ receptors each appear to participate in the actions of endogenous opioid peptides in the VTA. Further, the corresponding dose-differentials between DAMGO and DPDPE in the behavioural and neurochemical tests suggest an important role for dopamine in mediation of the rewarding effects of opiates after VTA administration.

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Chapter 1

General Introduction

1.1 Overview

During the past thirty years, research using animal models of drug addiction has significantly advanced our understanding of the environmental, pharmacological and neurobiological variables that contribute to drug abuse in humans (for reviews see Schuster and Thompson, 1969; Johanson, 1978; Schuster and Johanson, 1981; Brady, 1991). Accordingly, a great deal has been learned about the mechanisms of action of commonly abused drugs. These drugs exert their effects in animals through a variety of actions on neurons in the central nervous system (CNS). Some abused drugs (e.g. amphetamine, cocaine) increase the synaptic or extracellular concentrations of endogenous neurotransmitters by increasing the release of a transmitter or by blocking its reuptake into presynaptic neurons (Moore *et al.*, 1977; Fischer and Cho, 1979; McMillen *et al.*, 1980). Other abused drugs (e.g. opiates) exert their effects by binding to the receptors at which endogenous neurotransmitters act, and "mimicking" the effects of the endogenous transmitters (Hughes, 1975; Hughes and Kosterlitz, 1977). Still others (e.g. caffeine) act by blocking the receptors at which endogenous neurotransmitters normally act,

antagonizing the actions of the endogenous transmitters (Fredholm, 1980, 1985).

Analyses of the alterations in neuronal activity that accompany drug administration have helped to identify neural circuits that participate in the rewarding effects of drugs. In fact, it seems that the habit-forming effects of opiates and other drugs result from the drugs' abilities to increase neural activity that is ordinarily activated by natural rewards such as food and sex (Johanson, 1978; Wise, 1987; Di Chiara and North, 1992). Thus, animal models of drug addiction offer a potentially valuable tool for studying the neural mechanisms that underlie motivated behaviour.

The neurobiology of opiate reward is one of the better understood examples of drug reward. This thesis is an examination of some of the neural mechanisms that are involved in the rewarding effects of opiates.

1.2 Animal Models of Opiate Addiction

1.2.1 Types of Animal Models

Four animal models have been used to study the neurobiology of opiate addiction. Each of the experimental paradigms used in these animal models focuses on a different aspect of the rewarding properties of opiates (see Wise and Hoffman, 1992), and each has contributed to our understanding of the neurobiology of opiate reward.

1.2.1.1 Opiate Self-Administration

Opiates function as operant reinforcers (see Skinner, 1938). As such, administration of any of these drugs will increase the probability that a particular behaviour will occur when drug delivery is paired with that behaviour. This property of opiates is assessed in the drug self-administration paradigm. Animals will learn to emit an arbitrary behaviour such as a lever-press when that behaviour is rewarded with the administration of any of a variety of opiates. Initiation and maintenance of drug self-administration is taken as an indicator of the habit-forming potential or reinforcing efficacy of the drug.

Intravenous opiate self-administration has been demonstrated in a variety of animal species including rats (e.g. Weeks, 1961, 1962; Weeks and Collins, 1964), mice (Criswell, 1982), and monkeys (e.g. Thompson and Schuster, 1964; Deneau *et al.*, 1969; Schuster, 1970; Bonese *et al.*, 1974; Johanson, 1978; Mello and Mendelson, 1978), and under a variety of experimental conditions and reinforcement schedules (for review see Schuster and Johanson, 1981).

When animals self-administer opiates, they appear to regulate their intake, maintaining a constant titer of drug. If a consistent dose of opiate is administered for each response, the rate of self-administration is influenced by the size of the dose. Self-administration response rates are higher when low doses of drug are administered than when higher doses are administered (Weeks, 1962; Weeks and Collins, 1964). Additionally, for any

particular drug dose, response rates are higher at the beginning of a session, when little drug has been administered, than they are later in the session, when more drug has been administered (Downs and Woods, 1974; Bozarth and Wise, 1981a). It has been proposed that these within-session changes in response rates result from the differing requirements of establishing and maintaining satiating concentrations of drug at reward-relevant receptors in the CNS (Bozarth and Wise, 1981a). Animals work to attain an optimal level of receptor occupancy by opiates, and then "titrate" their responses to maintain this level of receptor occupancy.

Further evidence that animals titrate their responding to maintain an adequate receptor occupancy has been obtained using the variable dose per infusion (VDI) paradigm. In this paradigm, the animal is rewarded with randomly varying doses of drug per response. When rats are tested for intravenous self-administration of heroin on a VDI schedule, the duration of the pause between one response and the next is a function of the dose of heroin that was administered after the earlier response (Gerber and Wise, 1989). Larger doses of heroin produce longer pauses between responses.

Opiate self-administering animals also appear to regulate their intake in response to treatment with opioid receptor antagonists. Treatment with low doses of selective antagonists such as naloxone and naltrexone produce increased rates of opiate self-administration (Weeks and Collins, 1964; Schuster, 1970; Ettenberg *et al.*, 1982), while higher doses of these antagonists

extinguish responding for opiates (Schuster, 1970). The increased rates of opiate self-administration that are seen after administration of low doses of opiate antagonists appear to result from competition between the opiate and the antagonist for binding with opioid receptors. Animals increase their rate of opiate intake, thereby overcoming the competitive antagonism of opioid receptors, and reestablishing adequate receptor occupancy by the opiate. The decreased responding after treatment with high doses of antagonists appears to result from an overwhelming blockade of the pharmacological actions of opiates on opioid receptors. In this case, animals are unable to overcome the receptor blockade through increased opiate intake, and they quit responding.

1.2.1.2 Reinstatement of Drug Self-Administration

A second property of opiates is that they will reinstate behaviour that was previously rewarded with contingent administration of an abused drug. When testing for reinstatement of drug-rewarded behaviour, an animal is first trained to self-administer a drug. The drug-rewarded response is then extinguished during a period in which the drug is not administered. Under extinction conditions (i.e. no response-contingent drug injections), a non-contingent "priming" injection of the drug is administered. If the animal then displays the original trained response, then the priming injection is said to reinstate the

response habit, in the absence of response-contingent drug reward.

Reinstatement of response habits have been demonstrated with a variety of commonly abused drugs. Lever-pressing that was trained with heroin is reinstated by priming injections of morphine (de Wit and Stewart, 1983; Stewart and Wise, 1992), heroin, amphetamine, and apomorphine (de Wit and Stewart, 1983), even though the reinstated lever-presses do not produce further drug injections. Further, lever-pressing that was trained with cocaine is reinstated by priming injections of cocaine, amphetamine, apomorphine, and morphine (Gerber and Stretch, 1975; de Wit and Stewart, 1981). Thus, it appears that a variety of commonly abused drugs will reinstate response habits that were established by another abused drug. It has been proposed that the ability of one drug to reinstate response habits established by another drug is a consequence of the activation of common motivational processes by the two drugs (de Wit and Stewart, 1981, 1983; Stewart, 1983).

1.2.1.3 Conditioned Place Preference

The conditioned place preference paradigm is based upon the observation that animals will approach stimuli that have previously been associated with rewarding drug injections (Beach, 1957). In this paradigm, the amount of time an animal spends in proximity to "drug-associated" stimuli is quantified. Experimentally naive animals are first given free access to two

different environments, and the amount of time spent in each environment is measured. Then, on subsequent days, drug injections are paired with one of these two environments and vehicle injections are paired with the other. Usually, drug and vehicle injections are repeatedly paired with the appropriate environments. The animals are later tested under drug-free conditions; they are given free access to the two environments, and the amount of time spent in each environment is measured. An increase in time spent in the drug-paired environment (relative to the time spent during the pre-drug trial) is interpreted as a drug-conditioned preference for that environment (see Hoffman, 1989).

The ability of opiates to produce conditioned place preferences has been extensively characterized. Conditioned place preferences are observed after systemic (i.e. intravenous, subcutaneous, or intraperitoneal) injections of a variety of opiates, including morphine, heroin, fentanyl, sufentanil, β-endorphin, and the enkephalin analogue D-Ala^2-methionine enkephalin (DALA: Schwartz and Marchok, 1974; Rossi and Reid, 1976; Phillips and LePiane, 1980, 1982; Sherman *et al.*, 1980; Bozarth and Wise, 1981b; Mucha *et al.*, 1982; Spyraki *et al.*, 1983; Mucha and Iversen, 1984; Mucha and Herz, 1985; Amalric *et al.*, 1987; Leone and Di Chiara, 1987; Shippenberg and Herz, 1987, Bals-Kubik *et al.*, 1988, 1990a; Shippenberg *et al.*, 1988, 1989). Further, these opiate-conditioned place preferences are prevented by pretreatment with naloxone (Phillips and LePiane, 1980, 1982; Bozarth and Wise, 1981b; Mucha and Iversen, 1984). Thus, it

seems that opiate-conditioned place preferences are mediated by drug actions on opioid receptors.

1.2.1.4 Facilitation of Brain Stimulation Reward

A fourth method that has been used to study the rewarding effects of opiates is based upon the findings that animals will self-administer electrical stimulation of the medial forebrain bundle (MFB: Olds and Milner, 1954), and that the reinforcing efficacy of this stimulation is potentiated by opiates. Potentiation of the reinforcing efficacy of brain stimulation reward has been inferred from the finding that morphine increases the frequency of responding for electrical stimulation of the MFB (Broekkamp *et al.*, 1976, 1979a). Further, morphine-induced potentiation of brain stimulation reward has been demonstrated in the curve-shift paradigm (Rompré, 1986). In this paradigm, the animal is allowed to self-administer electrical stimulation over a range of stimulation frequencies (or intensities). The response rate is measured at these various levels of stimulation, generating a function that relates the level of stimulation to the response rate (similar to a drug dose-response curve: see Liebman, 1983). Then, the drug is injected, and the animal is retested for intracranial self-stimulation across the range of stimulation levels. The drug is considered to increase the rewarding impact of electrical brain stimulation if it produces a leftward shift in the function that relates the response rate to the stimulation level, so that lower stimulation frequencies (or

intensities) maintain responding (for discussion, see Wise and Rompré, 1989).

1.2.1.5 Summary

In summary, opiates exert reward-relevant actions in all four animal models. They reinforce behaviours that result in their intravenous administration. They reinstate responding that previously resulted in rewarding injections of the drug. They elicit approach to drug-associated stimuli, and they facilitate electrical brain stimulation reward.

1.3 Localization of the Rewarding Effects of Opiates in the CNS

Modifications of the four previously described animal models have been used to localize the CNS sites at which opiates exert their rewarding effects. Opiate self-administration has been investigated with intracranial injections of very small volumes of opiates targetted at specific CNS sites (see Bozarth and Wise, 1980; Welzl *et al.*, 1985). Self-administration of an opiate into a particular site is considered to implicate neurons in the vicinity of the injection in mediation of the reinforcing effects of the opiate. CNS systems involved in opiate reward have also been characterized with intracranial injections of quaternary opioid receptor antagonists in animals that self-administer intravenous opiates. (These antagonists diffuse

out of the brain more slowly than lipophillic opioid antagonists such as naloxone and naltrexone: see Schroeder *et al.,* 1991.) Alterations in rates of intravenous self-administration (i.e. compensatory rate increases or extinction of responding) are taken as evidence that neurons in the vicinity of the site of the antagonist injection are involved in mediation of the reinforcing effects of the self-administered opiate. Intracranial injections of opiates have also been used in the reinstatement of self-administration, conditioned place preference, and facilitation of brain stimulation reward paradigms. However, it must be noted that many of these studies have not reported controls for the possibility that drugs may diffuse after intracranial injections, exerting actions at sites distal to the injection site (see Wise and Hoffman, 1992).

To date, studies of self-administration, reinstatement, conditioned place preference, and facilitation of brain stimulation reward have resulted in the identification of at least five CNS sites that may be involved with opiate reward (for reviews see Wise and Bozarth, 1982; Bozarth, 1983; Bozarth and Wise, 1983; Koob *et al.,* 1986; Wise, 1988, 1989; Koob, 1992). However, opiate actions in some of these sites have not been rigorously characterized. Further, conflicting evidence has been found when the different animal models have been employed to examine the involvements of some of these sites in the rewarding effects of opiates.

When intracranial injections are used, evidence from all four animal models have implicated ventral tegmental area (VTA)

opioid receptors in the mediation of opiate reward. Rats will learn to self-administer morphine (Bozarth and Wise, 1981a; Welzl *et al.*, 1989) or the opiate fentanyl (van Ree and de Weid, 1980) when these opiates are microinjected directly into the VTA. Further, in animals that self-administer intravenous heroin, intracranial microinjections of quaternary opiate antagonists into the VTA produce compensatory increases in the rates of heroin self-administration (Britt and Wise, 1983; Vaccarino *et al.*, 1985). VTA microinjections of morphine reinstate intravenous self-administration of heroin (Stewart, 1984), and VTA microinjections of morphine (Phillips and LePiane, 1980; Bozarth, 1987a), DALA (Phillips and LePiane, 1982; Phillips *et al.*, 1983; Glimcher *et al.*, 1984), or the enkephalinase inhibitor thiorphan (Glimcher *et al.*, 1984) each produce conditioned preferences for the drug-associated environments. VTA microinjections of morphine (Broekkamp *et al.*, 1976, 1979a; Rompré, 1986; Jenck *et al.*, 1987; Bauco *et al.*, 1991) and DALA (Broekkamp *et al.*, 1979a) each facilitate electrical self-stimulation of the MFB. Further, intracranial microinjections of morphine fail to produce place preferences (Bozarth, 1987a) or to facilitate brain stimulation reward (Broekkamp *et al.*, 1979a) when the drug is injected into sites surrounding the VTA, demonstrating anatomical specificity of these effects. Thus, actions on opioid receptors in the VTA appear to be involved in the rewarding effects of opiates.

Evidence has also been found that nucleus accumbens (NAcc) opioid receptors may participate in opiate reward. Rats will self-administer microinjections of morphine (Olds, 1982) or DALA

(Goeders *et al.*, 1984) into the NAcc, and microinjections of quaternary opiate antagonists into the NAcc produce compensatory increases in responding for systemic heroin injections (Vaccarino *et al.*, 1985; Corrigall and Vaccarino, 1988). Further, microinjections of morphine into the NAcc produce conditioned preferences for the drug-associated environment (Van der Kooy *et al.*, 1982), and NAcc microinjections of enkephalin analogues facilitate intracranial self-stimulation (West and Wise, 1989). On the other hand, NAcc microinjections of morphine fail to reinstate intravenous heroin self-administration (Stewart and Vezina, 1988), and the doses of morphine that are required to produce place preferences when the drug is microinjected into the NAcc exceed the doses that are required when the drug is microinjected into the VTA (see Wise, 1989). Further, anatomical controls have not been reported for any of these studies with opiate microinjections into the NAcc. Thus, it is possible that the opiate-effects were produced by diffusion into distal sites. The anatomical specificity of the rewarding effects of NAcc opiate microinjections requires further study.

Hippocampal opioid receptors may also be involved in opiate reward. Rats will self-administer low doses of morphine (Grauer *et al.*, 1989) and dynorphin A (Stevens *et al.*, 1991) into the CA3 region of the hippocampus, and hippocampal microinjections of high doses of morphine produce conditioned place preferences (Corrigall and Linesman, 1988). These observations raise the possibility that opioid receptors in this brain region are also involved in opiate reward. However, as in the case of studies

involving the NAcc, appropriate anatomical controls have not been done for opiate microinjections into the hippocampus. Further investigations are required to confirm the possibility that hippocampal opioid receptors are involved in the rewarding effects of opiates.

It has been suggested that the lateral hypothalamus (LH) may be involved in the rewarding effects of opiates. Lateral hypothalamic self-administration of morphine (Olds, 1979) and DALA (Olds and Williams, 1980) has been reported, and lateral hypothalamic microinjections of morphine facilitate intracranial self-stimulation of the MFB (Broekkamp *et al.*, 1976, 1979a). However, the animals in the self-administration studies were pretrained to respond for electrical stimulation of the LH. Experimentally naive rats failed to self-administer morphine into the LH (Bozarth and Wise, 1982), and kainic acid lesions of the LH failed to alter the rate of intravenous self-administration of heroin in rats (Britt and Wise, 1981). Further, Broekkamp *et al.* (1979a) have suggested that the facilitory effects of LH morphine microinjections on brain stimulation reward actually occurred due to diffusion of the drug to the VTA. Thus, the evidence for an involvement of the LH in opiate reward is not compelling.

The periaqueductal grey (PAG) has also been suggested as a site at which opiates produce their rewarding effects. Rats exhibit compensatory increases in their rates of intravenous heroin self-administration after PAG microinjections of a quaternary opiate antagonist (Corrigall and Vaccarino, 1988). However, rats failed to self-administer morphine into the PAG

(Bozarth and Wise, 1982; 1984), and microinjections of morphine into the PAG failed to produce conditioned place preferences (Phillips and LePiane, 1980). Further, microinjections of morphine into the PAG inhibited electrical self-stimulation of the MFB (Broekkamp *et al.*, 1976). Thus, an involvement of the PAG in opiate reward appears unlikely.

In summary, it appears that the VTA, NAcc, and hippocampus may each contribute to the mediation of reward produced by systemic administration of opiates. The VTA has been the most extensively characterized of these sites, and it is the only site for which appropriate anatomical controls have been done. Further, when low doses of opiates are used, injections into the VTA have consistently yielded results that implicate VTA opioid receptors in opiate reward. Results from low dose injections into other CNS sites have not yielded such clearly consistent results.

1.4 The Involvement of Specific VTA Opioid Receptors in the Rewarding Effects of Opiates

1.4.1 Opioid Receptor Types in the VTA

An important, but largely unexplored issue in determining the neuronal mechanism of opiate actions in the VTA is the identification of which opioid receptor types are involved in these actions. There are at least three major types of opioid receptors (μ, ∂, and κ: Gilbert and Martin, 1976; Martin *et al.*, 1976; Lord

et al., 1977; Paterson *et al.*, 1983) which are differentially distributed in the CNS, and could potentially be involved in opiate reward.

A dense population of μ opioid receptors has been identified in the VTA (Quirion *et al.*, 1983; Mansour *et al.*, 1987, 1988; Dilts and Kalivas, 1989; Speciale *et al.*, 1989), but the data for ∂ receptors have been less clear. The tritiated ∂ agonist [^{3}H]DPDPE failed to label opioid receptors in the VTA (Mansour *et al.*, 1987, 1988), but iodinated DPDPE ([^{125}I]DPDPE: Dilts and Kalivas, 1990) and tritiated DSTBULET: ([^{3}H]DSTBULET: Delay-Goyet *et al.*, 1990) each labels a sparse population of VTA ∂ receptors. The ineffectiveness of [^{3}H]DPDPE may result from the fact that the VTA is rich in myelinated fibres, which may quench the relatively weak signal produced by [^{3}H]DPDPE (see Herkenham and Sokoloff, 1984; Kuhar and Unnerstall, 1985; Dawson *et al.*, 1986; Dilts and Kalivas, 1990). Adequate resolution of VTA ∂ receptors may require the use of iodinated compounds, which provide a "hotter" (i.e. more energetic) radioactive signal that is resistant to myelin quenching (Kuhar and Unnerstall, 1985; Dawson *et al.*, 1986; Dilts and Kalivas, 1990). Thus, it seems that there is actually a sparse population of ∂ receptors in the VTA. On the other hand, no significant κ receptor binding has been found in the VTA (Mansour *et al.*, 1987, 1988; Tempel and Zukin, 1987; Jomary *et al.*, 1988), despite the use of an iodinated ligand (Jomary *et al.*, 1988).

1.4.2 The Involvement of VTA μ, ∂, and κ Receptors in the Rewarding Effects of Opiates

VTA μ and ∂ receptors may each participate in the rewarding effects of opiates. Intracerebroventricular (icv: Bals-Kubik *et al.*, 1990a) or VTA (Bals-Kubik *et al.*, 1990b) microinjections of the μ agonist DAMGO each establish conditioned preferences for environments associated with prior drug exposure. Further, VTA microinjections of DPDPE facilitate responding for electrical stimulation of the MFB (Jenck *et al.*, 1987), and conditioned place preferences are established by icv microinjections of the ∂ agonist DPDPE (Shippenberg *et al.*, 1987; Bals-Kubik *et al.*, 1990a). In this latter case, it is possible that the rewarding effects of DPDPE occurred through diffusion to the ∂ receptors in the VTA. Conditioned place preferences have not been evaluated with VTA microinjections of a ∂-selective agonist.

On the other hand, VTA κ receptors may be uninvolved, or may mediate effects that are opposite to the rewarding effects produced by activation of μ and ∂ receptors. VTA injections of the κ agonist U-50,488H fail to alter responding for rewarding electrical stimulation of the MFB (Jenck *et al.*, 1987), but repeated systemic (Shippenberg and Herz, 1987; Shippenberg *et al.*, 1988), icv (Bals-Kubik *et al.*, 1989) or VTA (Bals-Kubik *et al.*, 1990b) administration of either of the κ agonists U-69,593 or U-50,488H produces conditioned aversion to the drug-paired environment.

In summary, it appears that VTA μ opioid receptors are involved in the rewarding actions of opiates. VTA ∂ receptors may also participate, but this possibility has not been confirmed. The existence of κ receptors in the VTA is questionable, and the role that these receptors might play is unresolved. If VTA κ receptors are involved in opiate actions, they are likely to mediate an aversive input.

1.5 Locomotor Activating Effects of Opiates

1.5.1 Effects of Systemic Administration of Opiates on Locomotion

In addition to the rewarding effects produced by opiate injections, these drugs exert biphasic effects on locomotor activity. Low to moderate doses of opiates produce an initial locomotor inhibition, followed by hyperlocomotion (Shuster *et al.*, 1975; Segal *et al.*, 1977; Amalric and Koob, 1985). Muscular rigidity and catalepsy are seen after administration of high doses (Segal *et al.*, 1977).

1.5.2 Localization of the Locomotor Activating Effects of Opiates in the CNS

While systemic administration of opiates produces biphasic effects on locomotion, opiate actions in the VTA appear to

produce only locomotor activation. Unilateral VTA microinjections of morphine produce contraversive circling (i.e. forward locomotion that is directed toward the side opposite to the side of the drug injection: Holmes *et al.*, 1983; Holmes and Wise, 1985), while injections into surrounding sites produce little or no circling (Holmes and Wise, 1985). Bilateral VTA microinjections of morphine (Joyce and Iversen, 1979), DALA (Broekkamp *et al.*, 1979b), β-endorphin (Stinus *et al.*, 1980), and thiorphan (Kalivas and Richardson-Carlson, 1986) each produce forward locomotion. Further, VTA microinjections of a quaternary opioid antagonist fail to block the initial locomotor depression produced by subcutaneous heroin injections, but these antagonist microinjections block the subsequent locomotor activation produced by the heroin injections (Amalric and Koob, 1985). Thus, neurons in the VTA appear to be implicated in the locomotor activating effects of opiates.

Opiates may produce both locomotor activation and inhibition through actions in the region of the NAcc. Microinjections of morphine into the NAcc produce an initial motor inhibition, followed by hyperlocomotion (Cunningham and Kelley, 1992). NAcc microinjections of a quaternary opioid antagonist block both the initial locomotor depression and the subsequent locomotor activation produced by subcutaneous heroin injections (Amalric and Koob, 1985). However, anatomical controls have not been reported, so the precise localization of the neural substrates mediating these effects are not clear.

Injections of opiates into a variety of other CNS sites, including the PAG (Jacquet and Marks, 1976), hypothalamus (Tseng *et al.*, 1980), and nucleus raphe pontis (Broekkamp *et al.*, 1984) produce akinesia. Thus, the locomotor activating and inhibiting effects of opiates appear to be (at least partially) anatomically dissociable. Opioid receptors in the VTA appear to be involved in only the locomotor activating effects. Opioid receptors in the vicinity of the NAcc may be involved in both the activating and inhibitory effects of opiate injections, but the precise neuroanatomical substrates have not been identified. Various other CNS sites may participate in the locomotor inhibiting effects of opiates.

1.5.3 Involvement of VTA Opioid Receptor Types in the Locomotor Activating Effects of Opiates

VTA μ and ∂ opioid receptors each appear to be involved in opioid-induced locomotion. Microinjections of DAMGO and DPDPE administered icv (Michael-Titus *et al.*, 1989; Mickley *et al.*, 1990), or bilaterally into the VTA (Latimer *et al.*, 1987), each produce forward locomotion, while unilateral VTA microinjections of DPDPE produce contraversive circling (Jenck *et al.*, 1988). Similarly, VTA microinjections of DTLET, DSLET or BUBU (selective ∂ agonists) each produces forward locomotion in rats (Calenco-Choukroun *et al.*, 1991).

VTA κ receptors do not appear to participate in the locomotor activating effects of opiates. In fact, κ receptors appear to participate in an inhibitory effect on locomotion, and this inhibitory effect appears not to involve actions in the VTA. VTA microinjections of U-50,488H do not elicit locomotor activity (Bhattacharya *et al.*, 1988). On the other hand, systemic U-50,488H decreases locomotor activity in mice (von Voightlander *et al.*, 1983), while systemic administration of the κ agonists U-50,488H, bremazocine, and trifluadom each decrease locomotor activity in rats (Di Chiara and Imperato, 1988a).

In summary, VTA μ and ∂ opioid receptors each appear to participate in the locomotor activating effects of opiates. If κ receptors exist in the VTA, they appear not to participate in the locomotor activating effects of opiates. However, there does seem to be a κ receptor-mediated inhibitory effect on locomotion that results from actions in an area other than the VTA.

1.6 The Focus of the Thesis

The principal focus of this thesis is the involvement of VTA μ and ∂ opioid receptors in the neurophysiology of reward. Rats were tested for intracranial self-administration of selective opioid agonists into the VTA. During self-administration,

locomotor activity was measured as an additional indication of opiate actions in the VTA. Neurochemical concomitants of opiate actions in the VTA were evaluated using intracranial microdialysis.

Chapter 2

Self-administration of Selective μ and ∂ Opioid Agonists into the VTA

2.1 Introduction

While it is known that rats will self-administer morphine into the VTA (Bozarth and Wise, 1981a, 1984; Welzl *et al.*, 1989), the specific types of opioid receptors that mediate this behaviour have not been identified. The present experiments were designed to evaluate the relative involvements of VTA μ and ∂ opioid receptors in the rewarding effects of self-administered opiates.

Independent groups of rats were tested for self-administration of morphine, DAMGO, and DPDPE into the VTA. Intracranial self-administration of morphine was demonstrated at a dose that has previously been reported (Bozarth and Wise, 1981a, 1984). The relative contributions of VTA μ and ∂ opioid receptors to VTA opiate self-administration were then examined. Doses of DAMGO and DPDPE that were just sufficient to initiate and maintain VTA self-administration were identified. The rates of self-administration that were produced by morphine, DAMGO, and DPDPE were then compared.

VTA self-administration of a κ agonist was not examined. It is likely that rats would not self-administer a κ agonist into the

VTA, as VTA microinjections of κ agonists do not facilitate responding for electrical stimulation of the MFB (Jenck *et al.*, 1987), and they produce conditioned aversions to drug-associated environments (Bals-Kubik *et al.*, 1990b).

2.2 Methods

2.2.1 Animals and Surgery

Twenty-eight experimentally naive male Long-Evans rats (Charles River, Boston, MA) weighing 360-425 grams were individually housed with lights set on a reverse cycle (lights off at 8:00 a.m.; lights on at 8:00 p.m.). Each rat was implanted under sodium pentobarbital anaesthesia (65 mg/kg, ip) with a unilateral 22 gauge guide cannula terminating 1.0 mm above the VTA (3.6 mm posterior to bregma, 2.2 mm lateral to the midsagittal suture, and 7.6 mm ventral to dura). Surgery was performed with the incisor bar set 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. (Measurements in the dorsal-ventral plane refer to distances along the track at 10° from the vertical.) Stainless steel obturators (28 gauge) extending 1.1 mm beyond the tip of each guide cannula were put in place at the time of surgery and removed for the duration of each test session.

2.2.2 Drugs

Morphine sulphate (mixed opioid agonist) was provided by the Canadian Department of Health and Welfare and was dissolved in Ringer's solution. DAMGO ([D-Ala^2,N-Me-Phe^4-Gly^5-ol]-enkephalin; a selective μ opioid agonist: Handa *et al.,* 1981; Goldstein and Naidu, 1989; Hruby and Gehrig, 1989) and DPDPE ([D-Pen^2,D-Pen^5]-enkephalin; a selective ∂ opioid agonist: Mosberg *et al.,* 1983, 1987; Akiyama *et al.,* 1985; Hruby *et al.,* 1991) were purchased from Sigma Chemical Co. DAMGO and DPDPE were each dissolved in artificial extracellular fluid (aECF) that more closely approximates concentrations found in brain than does commercial Ringer's solution (Moghaddam and Bunney, 1989). This aECF was composed of a 2.0 mM Sorensen's phosphate buffer, containing 145 mM Na^+, 2.7 mM K^+, 1.0 mM Mg^{2+}, 1.2 mM Ca^{2+}, 150 mM Cl^-, and 0.2 mM ascorbate, at pH 7.4.

2.2.3 Apparatus

Each rat was tested in a 27 cm x 27 cm x 27 cm operant chamber with a 5 cm x 1.5 cm lever mounted in one wall, 6 cm above a grid floor. A wooden block was mounted under the lever to prevent the animals from inadvertently depressing the lever with their electrode cable while attending to the floor beneath the lever. A small white cue light was mounted in the wall of each chamber 9 cm above the lever. The operant chambers were

contained in sound-attenuating boxes, which were dimly illuminated with red lights.

Drugs or vehicle solutions were delivered via electrolytic microinfusion transducers (EMITs: Plastics One, Roanoke, VA). These EMIT systems were similar to those previously described (Criswell, 1977; Bozarth and Wise, 1980; Bozarth, 1983). Briefly, each EMIT consisted of 2 platinum electrodes in a plastic reservoir, attached to a 28 gauge injector cannula (see Fig. 2.1). These EMIT systems were connected via flexible lead wires and mercury commutators to a constant-current generator (Mundl, 1981). Contingent upon lever-presses, a 200 μA current was delivered to the electrodes for 5 seconds, generating gas from the aqueous solution contained in the reservoir. This gas displaced a 120 nl infusion from the injector tip. Lever-presses that occurred during the drug infusions did not result in additional drug infusions.

Circling was detected by a rotometer that detected 360° rotations of the electrode cable at the level of the commutator. Circling and lever-presses were monitored with a microprocessor.

2.2.4 Intracranial Self-Administration Procedure

The rats were allowed to recover from surgery for at least 10 days. The rats were then tested for intracranial self-administration of opioids in four-hour sessions on each of eight consecutive days. At the start of each of the first five sessions, each EMIT system was loaded with one of the drug

solutions and the injector cannula was inserted through the guide cannula and anchored with its tip in the VTA. The rats were then allowed to lever-press for microinfusions into the VTA. The rats were not given any priming infusions, and lever-pressing was not shaped by successive approximations. During the sixth session (extinction session), each EMIT system contained vehicle rather

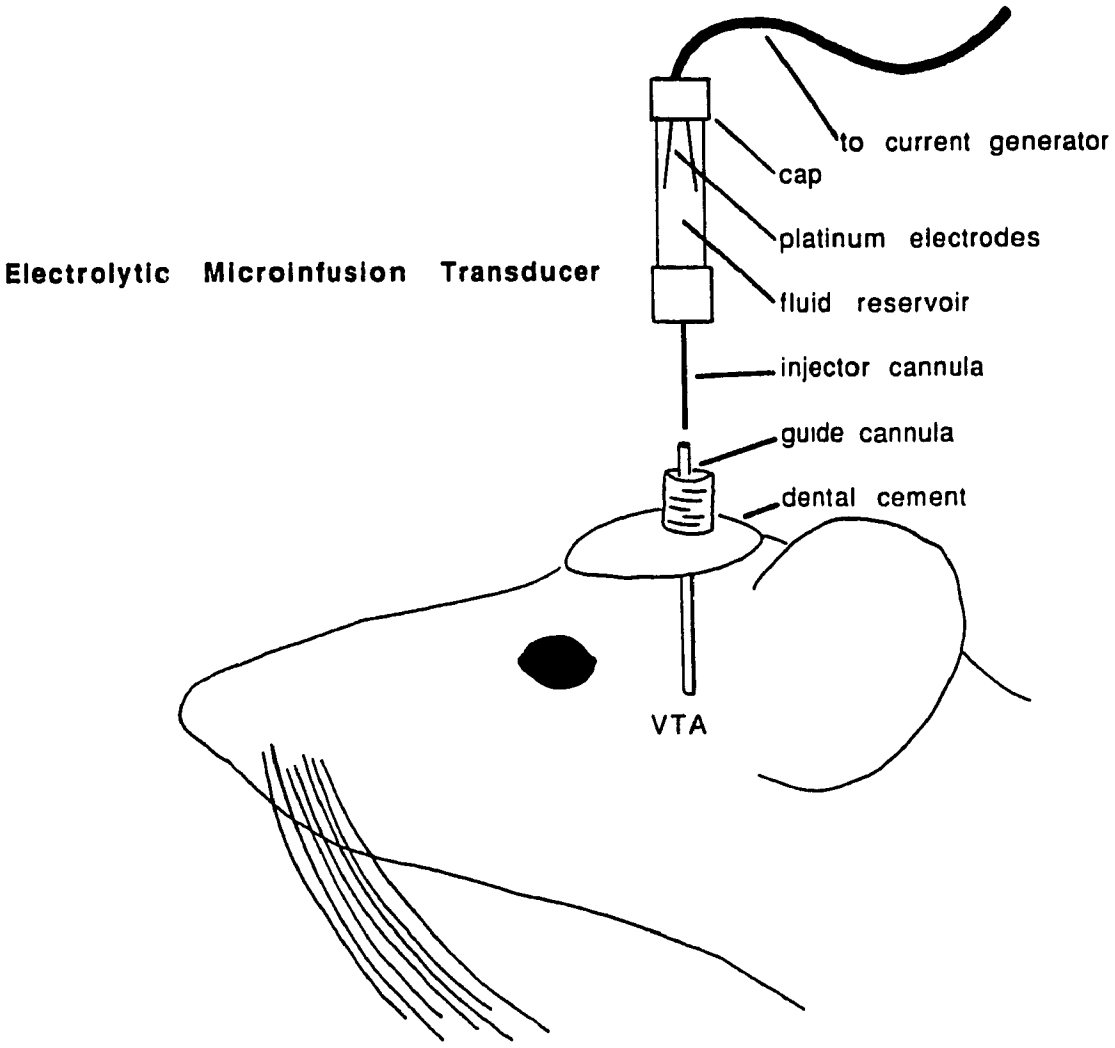

Fig. 2.1 A schematic representation of an EMIT system for intracranial microinfusions.

than drug, and rats were tested as in the previous sessions. During the seventh and eighth sessions (reinstatement sessions), each EMIT system again contained the drug solution at the original concentration, and the rats were tested as in previous sessions. Circling was monitored throughout each session.

2.2.4.1 Preliminary Investigation

Two groups of five rats each were tested. The rats in Group 1 were tested for intracranial self-administration of morphine (dissolved in Ringer's solution: 2.64 mM) and the rats in Group 2 were tested for self-administration of the Ringer's vehicle alone. Each lever-press resulted in delivery of 3.17×10^{-10} moles of morphine (or Ringer's solution) into the VTA.

After the eighth session, each rat was anesthetized with chloral hydrate and perfused with physiological saline followed by 10% formalin. Each brain was removed immediately, stored in formalin for one or more days, frozen and sliced in 40 μm coronal sections, and stained with thionin for localization of VTA cannula tracks.

2.2.4.2 Intracranial Self-administration of μ and ∂ Agonists

Three groups of six rats each were tested. The rats in Group 3 were tested for intracranial self-administration of DAMGO (dissolved in aECF: 0.0264 mM). Each lever-press resulted

in delivery of 3.17 x 10^{-12} moles of DAMGO into the VTA. The rats in Group 4 were tested for intracranial self-administration of DPDPE (dissolved in aECF: 2.64 mM). Each lever-press resulted in delivery of 3.17 x 10^{-10} moles of DPDPE into the VTA. The rats in Group 5 were tested for intracranial self-administration of aECF vehicle solution in each of the eight test sessions.

After the eighth session, each rat was anesthetized with chloral hydrate and perfused with a cold glyoxylic acid solution. Each brain was removed immediately, frozen on dry ice, and prepared for glyoxylic acid catecholamine histofluorescence, according to the method of Battenberg and Bloom (1975). The brains were sliced in 20 µm coronal sections, and VTA cannula tracks were localized relative to the fluorescent DA cell bodies of the VTA.

2.2.5 Statistics

2.2.5.1 Preliminary Investigation

Group differences in daily rates of intracranial self-administration were evaluated using a 2 x 8 repeated-measures analysis of variance (ANOVA). Significant effects were further analysed with Tukey's tests, comparing values from the Ringer's and morphine groups during each of the daily sessions.

The mean hourly rates of intracranial self-administration were calculated, based on the fourth and fifth days of testing.

Group differences in hourly rates of intracranial self-administration were evaluated using a 2 x 4 repeated-measures ANOVA. Significant effects were further analysed with Tukey's tests. Similarly, group differences in mean hourly rates of intracranial self-administration during the extinction session were analysed using a 2 x 4 repeated-measures ANOVA and Tukey's tests.

2.2.5.2 Intracranial Self-administration of μ and ∂ Agonists

Group differences in daily rates of intracranial self-administration were evaluated using a 3 x 8 repeated-measures ANOVA. Significant effects were further analysed with Tukey's tests. Values from the aECF group and the DAMGO or DPDPE groups were compared during each of the daily sessions. Similarly, group differences in daily rates of circling were analysed using a 3 x 8 repeated-measures ANOVA and Tukey's tests.

The mean hourly rates of intracranial self-administration and circling were calculated, based on the fourth and fifth days of testing. Group differences in hourly rates of intracranial self-administration were evaluated using a 3 x 4 repeated-measures ANOVA. Significant effects were further analysed with Tukey's tests. Similarly, group differences in mean hourly rates of circling were analysed using a 3 x 4 repeated-measures ANOVA and Tukey's tests. Group differences in

mean hourly rates of intracranial self-administration during the extinction session were analysed using a 3 x 4 repeated-measures ANOVA and Tukey's tests.

The relationships between rates of intracranial self-administration and contraversive circling in the DAMGO and DPDPE groups were evaluated with simple logarithmic regression analyses.

2.3 Results

2.3.1 Preliminary Investigation

The rats that were rewarded with VTA microinfusions of morphine (3.17×10^{-10} moles per infusion) lever-pressed infrequently at first, but then more regularly and rapidly over the course of the first and subsequent sessions. Stable elevated lever-pressing rates were established by the fourth and fifth sessions. In contrast, the rats whose lever-presses produced VTA microinfusions of Ringer's vehicle solution exhibited constant low daily rates of lever-pressing throughout the eight test sessions (Fig. 2.2). The daily rates of lever-pressing did not differ significantly between the morphine and Ringer's groups during session 6 (extinction session), but these rates rapidly increased again in the rats that were given response-contingent morphine during sessions seven and eight (reinstatement sessions).

The pattern of hourly lever-pressing rates for morphine and Ringer's (measured in the fourth and fifth sessions) is illustrated

in Fig. 2.3. The rats that were rewarded with VTA microinfusions of morphine responded rapidly in the early portion of these sessions and then stabilized at a lower but reliable rate of responding. In contrast, the rats infused witn the Ringer's vehicle solution exhibited constant low rates of responding throughout

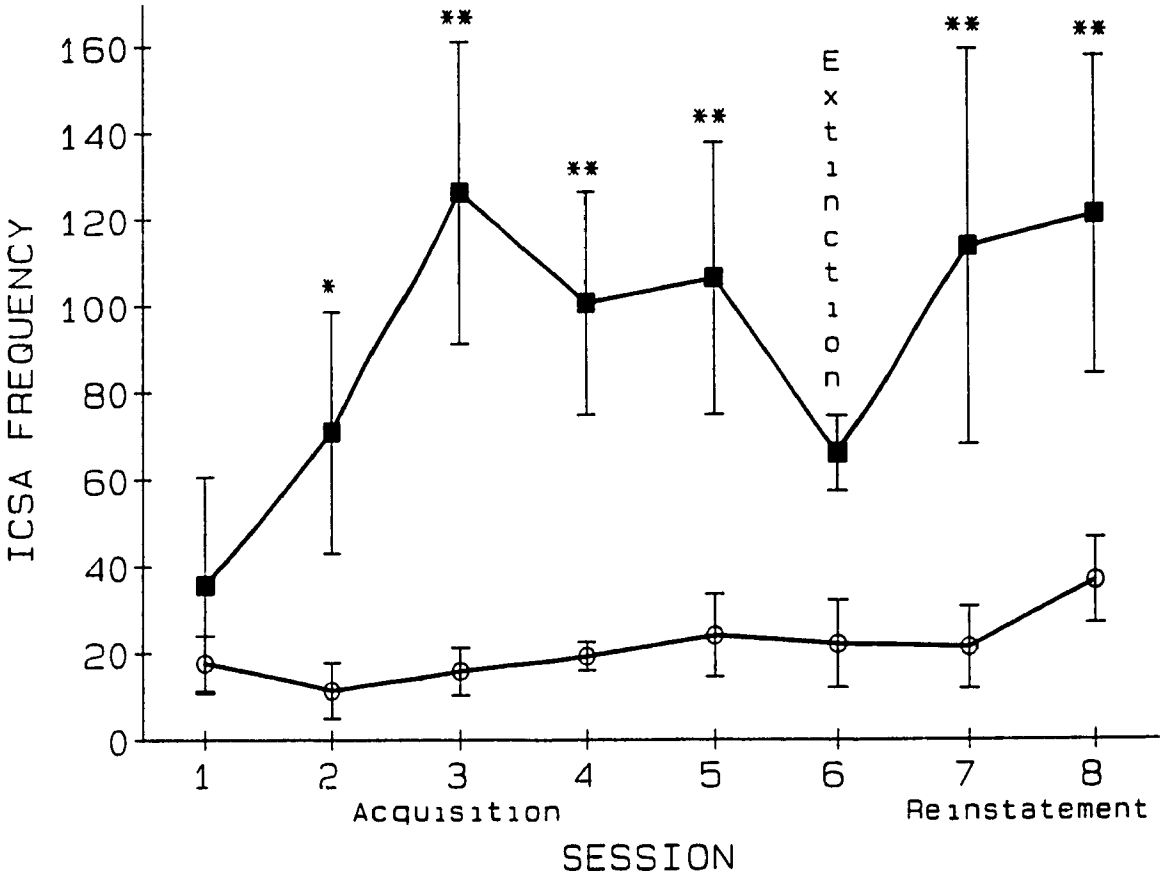

Fig. 2.2 Daily rates of lever-pressing responses in the rats given access to VTA morphine (■) or Ringer's vehicle (○). The rats given access to morphine exhibited significantly higher daily lever-pressing frequencies than did the rats given access to Ringer's solution ($F_{(1,8)} = 8.644$, $p < .05$). Values expressed are group means ± SEM. Significant differences in daily lever-pressing frequencies between the rats in the morphine group and the rats in the vehicle control group for each daily session (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$.

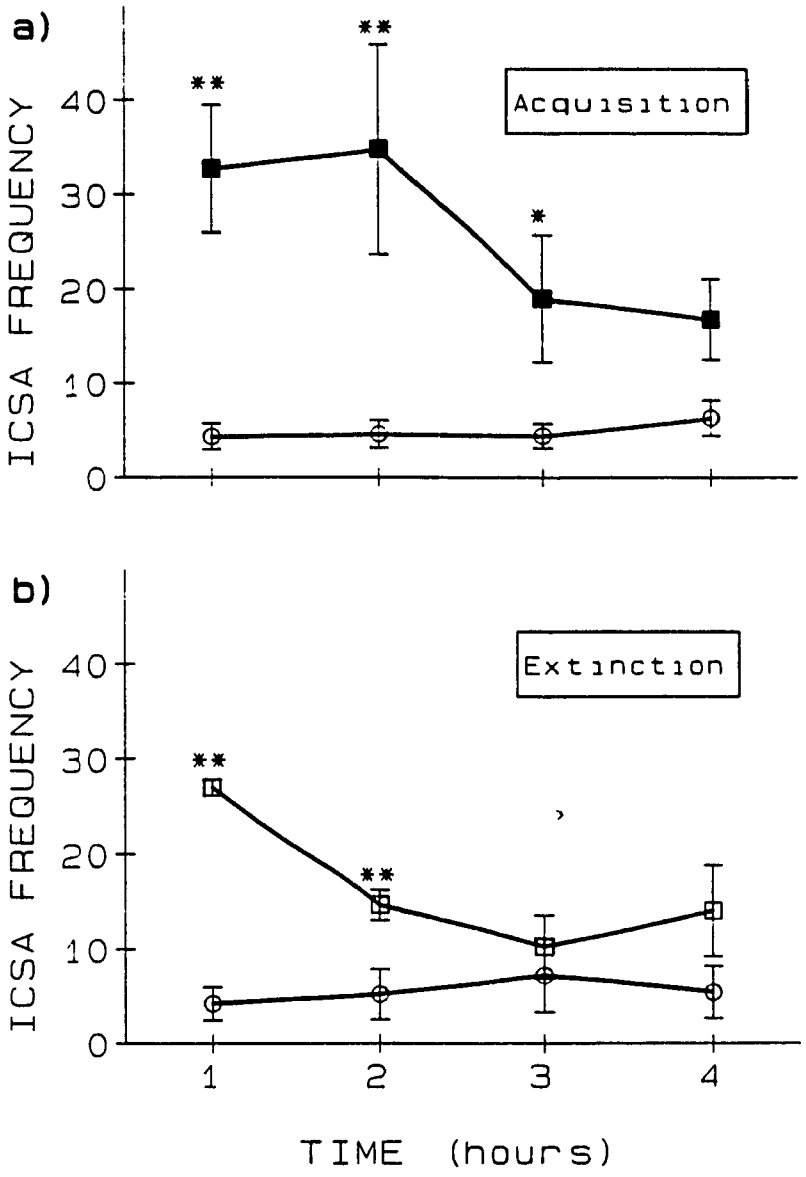

Fig. 2.3 Hourly rates of lever-pressing responses in the rats given access to VTA morphine (■) or Ringer's vehicle (○). **a)** The rats given access to morphine exhibited significantly higher hourly rates of lever-pressing, averaged across the fourth and fifth days of testing, than did the rats given access to Ringer's solution ($F_{(1,8)} = 10.27$, $p < .05$). **b)** There was a significant group x trial interaction on the extinction day, indicating that the rats given access to morphine exhibited significantly higher hourly rates of lever-pressing during the first two hours of testing than did the rats given access to Ringer's solution ($F_{(3,24)} = 7.903$, $p < .01$). Values expressed are group means ± SEM. Significant differences in hourly lever-pressing frequencies between the rats in the morphine group and the rats in the vehicle control group for each hour (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$.

these sessions (Fig. 2.3a). During the extinction session, the rats that had previously been rewarded with morphine responded rapidly at first but infrequently after the first hour (Fig 2.3b). The rates of lever-pressing in these rats did not differ from the rates in the Ringer's control rats during the latter half of the extinction session (Fig. 2.3b).

2.3.2 Intracranial Self-administration of μ and ∂ Agonists

The rats that were rewarded with VTA microinfusions of DAMGO (3.17×10^{-12} moles per infusion) or DPDPE (3.17×10^{-10} moles per infusion) lever-pressed infrequently at first, but then more regularly and rapidly over the course of the first and subsequent sessions. Stable elevated lever-pressing rates were established by the fourth and fifth sessions. This pattern of lever-pressing in the rats that received response-contingent DAMGO or DPDPE was similar to the pattern of responding in the rats that received morphine. In contrast, the rats in the aECF control group did not alter their daily response rates throughout the eight test sessions (Fig. 2.4). The daily rates of lever-pressing did not differ significantly between the DAMGO, DPDPE and aECF groups during the extinction session, but daily lever-pressing rates increased rapidly in both groups of rats that were rewarded with the opioids during the reinstatement sessions. In early studies, rats failed to initiate

self-administration of lower doses of DAMGO and DPDPE (data not shown).

The pattern of hourly lever-pressing rates for DAMGO, DPDPE and aECF (measured in the fourth and fifth sessions) is illustrated in Fig. 2.5. The rats that were rewarded with VTA microinfusions

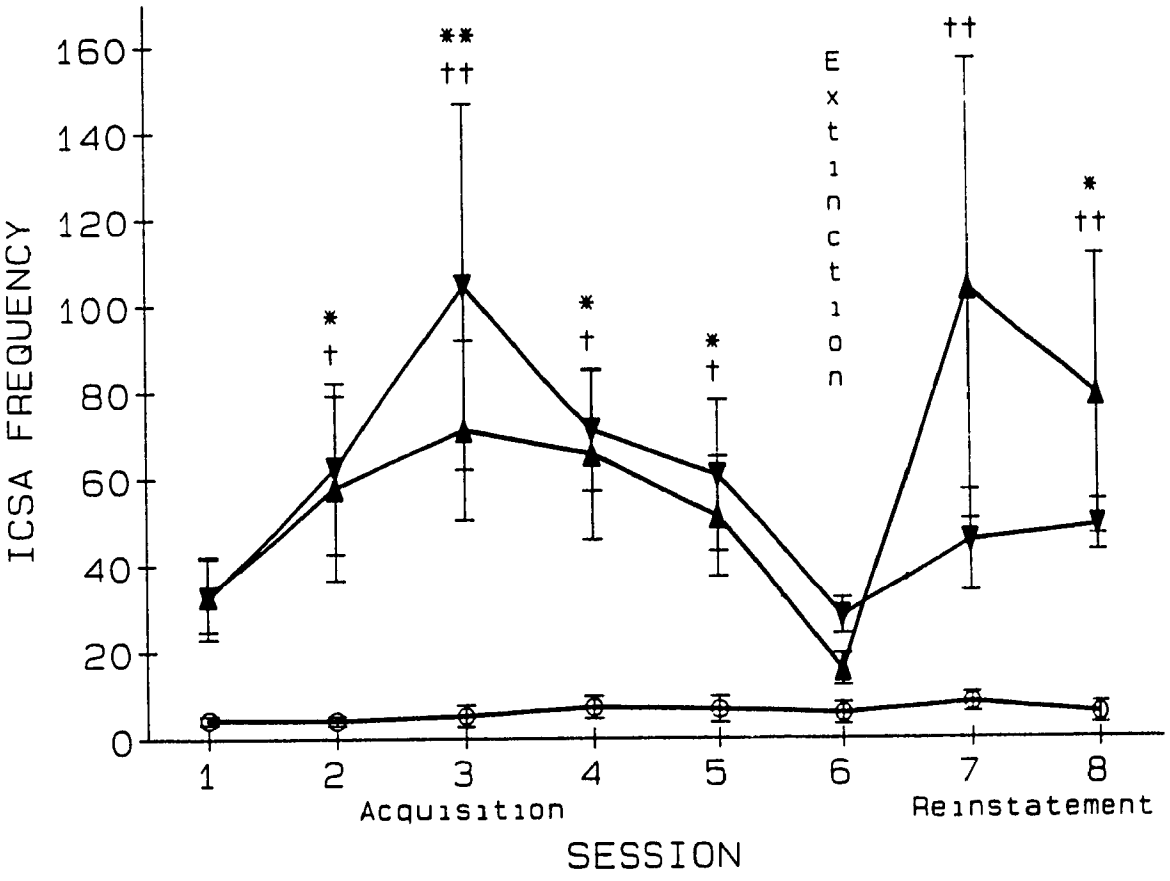

Fig. 2.4 Daily rates of lever-pressing responses in the rats given access to VTA DAMGO (▼), DPDPE (▲), or aECF vehicle (○). The rats given access to DAMGO, and the rats given access to DPDPE exhibited significantly higher daily lever-pressing totals than did the rats given access to aECF ($F_{(2,15)} = 10.09$, $p < .01$). Values expressed are group means ± SEM. Significant differences in daily lever-pressing frequencies between the rats in the aECF group with the rats in the DAMGO and DPDPE groups for each daily session (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for DAMGO; † $p < .05$, †† $p < .01$ for DPDPE.

of DAMGO or DPDPE each responded rapidly in the early portion of these sessions and then stabilized at lower but reliable rates of responding. Again, the pattern of responding in these rats was similar to the pattern of responding in the morphine-rewarded rats. In contrast, the rats infused with aECF exhibited constant low rates of responding throughout these sessions (Fig. 2.5a). During the extinction session, the rats that had previously been rewarded with DAMGO or DPDPE each responded rapidly at first but infrequently after the first hour (Fig. 2.5b).

Self-administered DAMGO induced contraversive circling (Fig. 2.6), and this circling was significantly correlated with the daily rate of lever-pressing ($r = .4051$, $F_{(1,46)} = 9.031$, $p < .01$). The rats that self-administered DPDPE also exhibited contraversive circling, but this circling was not statistically significant (Fig. 2.6). The daily rates of lever-pressing and circling were significantly correlated in the rats that self-administered DPDPE ($r = .6572$, $F_{(1,46)} = 34.98$, $p < .01$). Rates of circling did not differ significantly between the DAMGO, DPDPE and aECF groups during the extinction session, but daily contraversive circling rates were again elevated in the rats that were rewarded with DAMGO or DPDPE during the reinstatement sessions.

All injector tips were located in within the boundaries of the DA-containing cell group of the VTA, as confirmed by fluorescence histochemistry. Placements of VTA injector tips are depicted in Fig. 2.7. There were no apparent between-groups differences in the locations of VTA injector tracks.

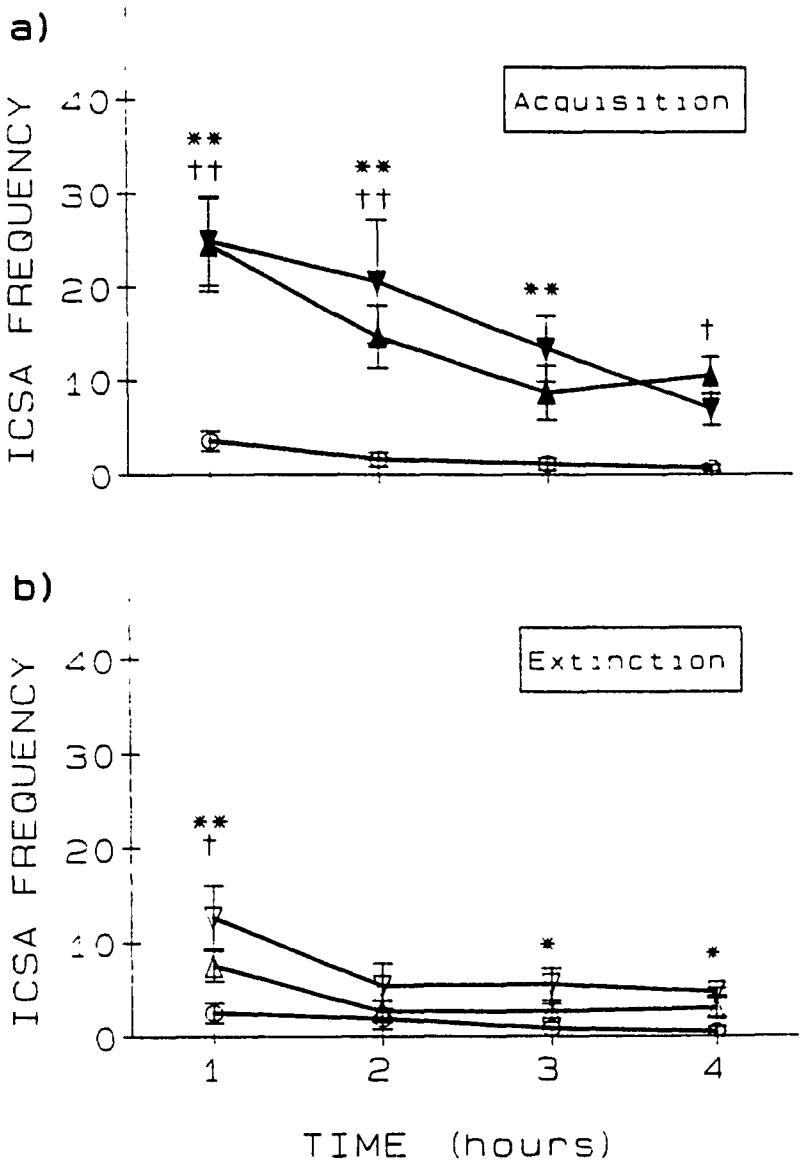

Fig. 2.5 Hourly rates of lever-pressing responses in the rats given access to VTA DAMGO (▼), DPDPE (▲), or aECF vehicle (O). **a)** The rats given access to DAMGO, and the rats given access to DPDPE exhibited significantly higher hourly rates of lever-pressing, averaged across the fourth and fifth days of testing, than did the rats given access to aECF ($F_{(2,15)} = 9.279$, $p < .01$). **b)** The rats given access to DAMGO, and the rats given access to DPDPE exhibited significantly higher hourly rates of lever-pressing during the first hour on the sixth day of testing than did the rats given access to aECF ($F_{(2,15)} = 10.42$, $p < .01$). Values expressed are group means ± SEM. Significant differences in hourly lever-pressing frequencies between the rats in the aECF group with the rats in the DAMGO and DPDPE groups for each hour (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for DAMGO; † $p < .05$, †† $p < .01$ for DPDPE.

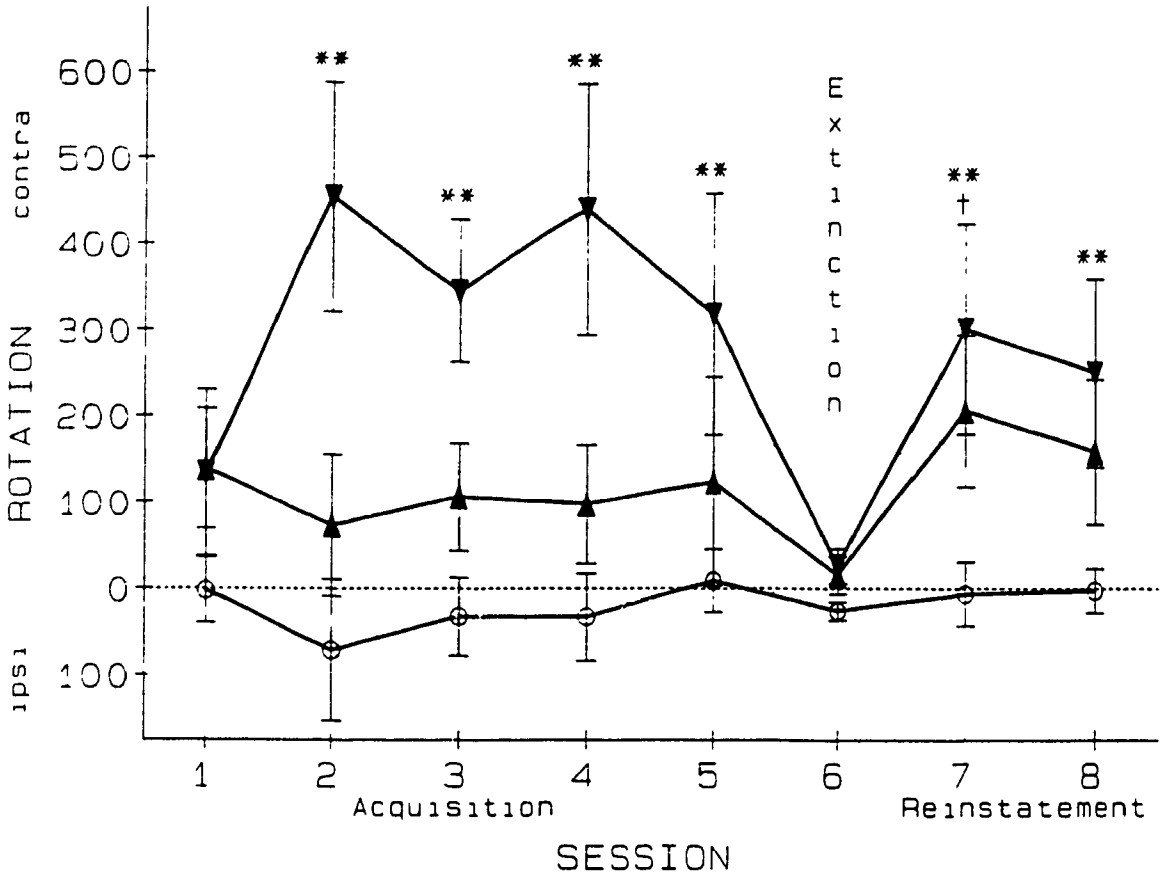

Fig. 2.6 Daily rates of circling in the rats given access to VTA DAMGO (▼), DPDPE (▲), or aECF vehicle (O). There was a significant group x trial interaction, indicating that the rats given access to DAMGO exhibited significantly higher daily rates of contraversive circling on six of the eight testing days than did the rats given access to aECF ($F_{(14,105)} = 2.198$, $p < .05$). The rats given access to DPDPE also appeared to exhibit higher daily rates of contraversive circling than did the rats given access to aECF, but this trend was not statistically significant (Tukey's tests). Values expressed are group means ± SEM. Significant differences in daily circling frequencies between the rats in the aECF group with the rats in the DAMGO and DPDPE groups for each daily session (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for DAMGO; † $p < .05$, †† $p < .01$ for DPDPE.

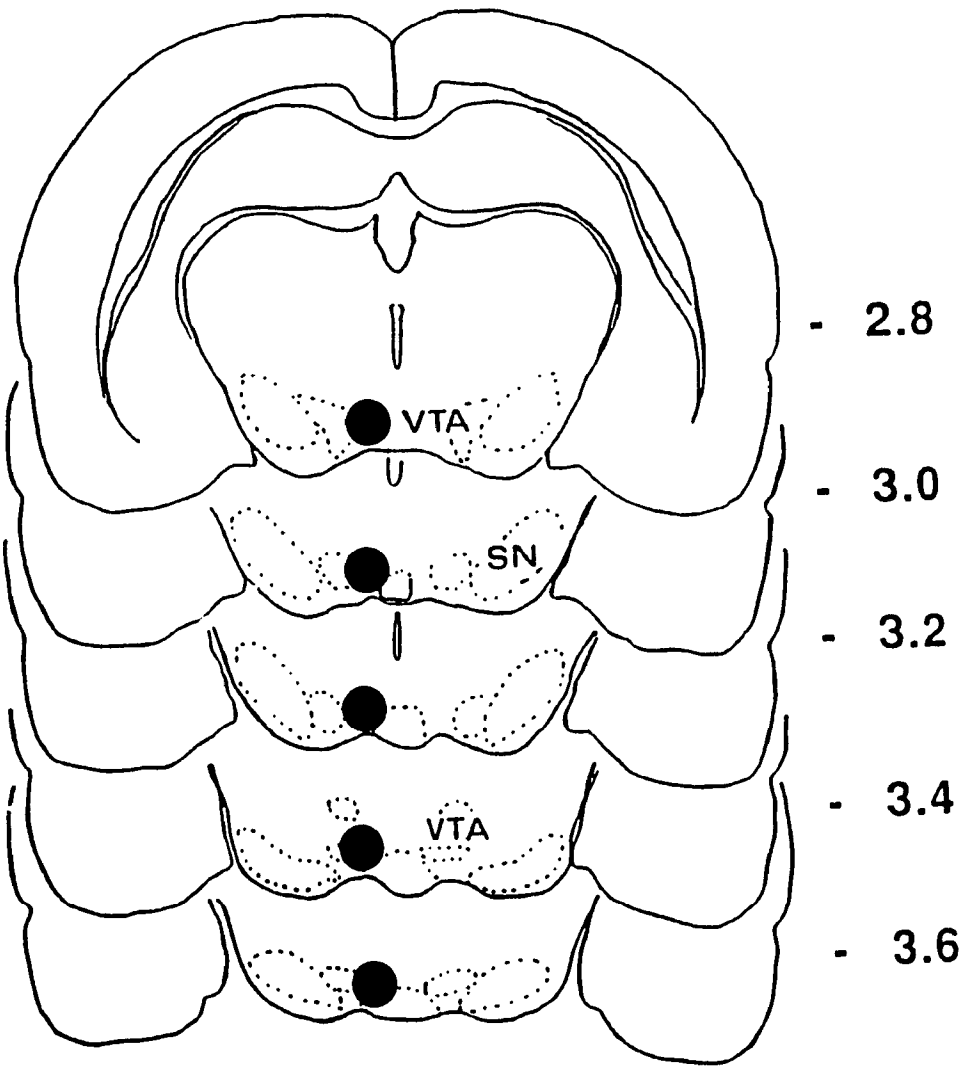

Fig. 2.7 Histological reconstruction of sites where injector tips were identified in the ventral tegmentum. Abbreviations used: SN = substantia nigra; VTA = ventral tegmental area (adapted from Pellegrino *et al.*, 1979).

2.4 Discussion

2.4.1 Intracranial Self-administration of Morphine, DAMGO, and DPDPE

The rats that were given response-contingent microinfusions of morphine, DAMGO, and DPDPE into the VTA each acquired a lever-pressing habit and maintained that habit when drug was accessible. They ceased lever-pressing when vehicle was substituted for the opiates, and reacquired the lever-pressing when the opiates were again available. Thus, VTA microinfusions of DAMGO and of DPDPE (as well as morphine) each appear to be reinforcing.

Within sessions, the rates of lever-pressing for each of the three opioid agonists was highest during the first hour of testing, and dropped off significantly during the following hours. This is consistent with the within-session pattern of responding that has been seen with intravenous codeine (Downs and Woods, 1974) and intracranial morphine self-administration (Bozarth and Wise, 1981a). Thus, as has been proposed previously (Bozarth and Wise, 1981a), the rats that self-administered morphine, DAMGO, and DPDPE each appeared to establish satiating concentrations of the opiates in the VTA and then maintained these concentrations by titrating their frequency of lever-pressing during the ensuing hours of the self-administration sessions.

2.4.2 The Involvement of VTA μ and ∂ Receptors in the Reinforcing Effects of DAMGO and DPDPE

Self-administration of DAMGO and DPDPE raises the possibility that VTA μ and ∂ opioid receptors may each be involved in the rewarding and habit-forming effects of opiates. However, since much higher doses of DPDPE were required to initiate and maintain self-administration, the possibility that DPDPE cross-reacted with μ receptors must be considered. In fact, the minimal effective dose of DPDPE (3.17×10^{-10} moles per infusion) was 100-fold higher than the effective dose of DAMGO (3.17×10^{-10} moles per infusion). Yet, these doses of DAMGO and DPDPE maintained responding at roughly equivalent rates.

However, despite these differences in the effective doses of DAMGO and DPDPE, there are a number of reasons to believe that DPDPE exerted selective actions on ∂ receptors in the dose range that was used. In the first place, the effective dose of morphine (3.17×10^{-10} moles per infusion), like DPDPE, was 100-fold higher than the effective dose of DAMGO. In other words, morphine and DPDPE each initiated and maintained intracranial self-administration at equal doses, while DAMGO worked at a much lower dose. On the basis of this comparison, it appears that the effective dose of DPDPE may not have been inordinately high. Secondly, when comparing the effective doses of DAMGO and DPDPE, one should take into consideration a variety of factors that contribute to drug potency. In the present case, two factors in particular appear to make important contributions to the

potency of DAMGO and DPDPE. The first factor is the number of receptors that these drugs can act upon in the VTA. In general, lower receptor densities require higher concentrations of a given drug to achieve equal magnitudes of response (Clark, 1926). (An exception to this relationship is found in tissues with different *receptor reserves* for a given agonist, wherein different proportions of the receptors must be occupied to achieve a maximal neuronal response; see Stephenson, 1956.) It is known that the density of ∂ receptors in the VTA is lower than the density of μ receptors in this area (see Section 1.5.1). Therefore, it is likely that a relatively high concentration of DPDPE would be required to act upon the sparse ∂ receptor population, while a relatively low concentration of DAMGO would suffice for the abundant μ receptor population. The second determinant of drug potency that may be important for the present comparison is the affinity of the drug for the receptor. Low rates of association and high rates of dissociation between the drug and the receptor are associated with low drug potency (Ariëns, 1954; Paton, 1961). In brain tissue, DPDPE exhibits slow association kinetics relative to other opiates (Akiyama *et al.*, 1985). Thus, DPDPE is expected to have a lower potency than other opiates. When these factors are considered, it is not surprising that the effective dose of DPDPE was higher than the effective dose of DAMGO.

Similar differences in the potency of DAMGO and DPDPE have been reported in studies of conditioned place preference following icv injections (Bals-Kubik *et al.*, 1990a), and locomotor activation and nucleus accumbens (NAcc) DA metabolism following VTA

injections (Latimer *et al.*, 1987). Further, VTA DAMGO and DPDPE each increase ventral striatal DA and DA metabolite concentrations. Roughly equivalent magnitudes of increases occur with doses of DPDPE that are 100-fold higher than the equi-effective doses of DAMGO, and these effects appear to occur through selective actions of DAMGO on VTA μ receptors and through selective actions of DPDPE on VTA ∂ receptors (see Chapter 4), even at doses that are 4-5 times more concentrated than the doses that were self-administered.

2.4.3 Contraversive Circling during Self-administration of DAMGO and DPDPE

The daily rates of self-administration and of contraversive circling were positively correlated in both the DAMGO and DPDPE groups. Thus, it seems that rats will self-administer μ and ∂ opioid agonists into the VTA in amounts that are sufficient to elicit forward locomotion. It is thought that contraversive circling results from locomotor activation (Pycock and Marsden, 1978) that is associated with increased dopaminergic activity on the side of the intracranial microinjection (Anden *et al.*, 1966; Ungerstedt, 1971; Glick *et al.*, 1976; Pycock and Marsden, 1978). Accordingly, the results of the present self-administration study suggest that self-administered microinjections of μ and ∂ agonists into the VTA may have produced increases in dopaminergic activity. The possibility that VTA μ and ∂ opioid receptors are involved in modulation of mesolimbic DA

neurotransmission has been assessed using intracranial microdialysis (see Chapter 4)

2.4.4 Summary and Conclusions

In summary, animals learned to lever-press for ventral tegmental microinjections of μ- and ∂-selective opioids. The effective dose of DPDPE was 100-fold higher than the effective dose of DAMGO, a finding that is commensurate with the densities of μ and ∂ receptors in the VTA, and with the slow rate of association of DPDPE with CNS ∂ receptors. These data suggest that VTA μ and ∂ receptors may each be involved in the rewarding and habit-forming effects of self-administered opiates.

Chapter 3

Surgical Preparation of Ventral Striatal Tissue Facilitates Early Sampling in Microdialysis and Reveals an Index of Neuronal Damage

3.1 Introduction

3.1.1 Orientation

In Chapters 4 through 6 of this thesis, experiments are reported in which the neurochemical concomitants of VTA opiate administration were explored. In these experiments, microdialysis and high pressure liquid chromatography (HPLC) were used to extract and quantify extracellular dopamine (DA) and DA metabolite concentrations from the ventral striatum. Selective opioid agonists and antagonists were injected into the ventral mesencephalon, and alterations in the concentrations of dialysate DA and DA metabolite concentrations were measured.

In order to conduct these investigations, a microdialysis preparation was developed and tested in anesthetized rats. This preparation is now described, before the experiments on neurochemical effects of VTA opioid administration are reported.

3.1.2 The Problem of Damages in Microdialysis Preparations

Microdialysis has gained wide acceptance as a tool for *in vivo* sampling of neurotransmitters in the extracellular compartment of the brain (for reviews see Ungerstedt, 1984, 1986; Westerink *et al.*, 1987a; Benveniste and Huttemeier, 1990; Di Chiara, 1990), and the combination of microdialysis with HPLC has resulted in very sensitive assays for various neurotransmitters. However, insertion of a microdialysis probe into the brain damages neurons in the vicinity of the probe, producing an initial period of neurotransmitter efflux that is unrelated to normal neuronal activity (Westerink and de Vries, 1988). Therefore, when using microdialysis, it is important to ascertain that the neurotransmitter efflux that is sampled does not result from damages that occur during probe insertion.

Exocytotic release of DA in the caudate and NAcc is dependent upon extracellular calcium (Ca^{2+}: Augustine *et al.*, 1987). Accordingly, depletion of the extracellular Ca^{2+} concentration (Westerink and de Vries, 1988; Westerink *et al.*, 1988; Drew *et al.*, 1989; Santiago and Westerink, 1990) and local administration of Ca^{2+} antagonists (Westerink *et al.*, 1988) have been used to evaluate exocytotic release of DA after insertion of a microdialysis probe. Evaluations of the impulse-dependence of DA release have been conducted through local administration of tetrodotoxin (TTX: Westerink *et al.*, 1987b; Westerink and

de Vries, 1988; Drew *et al.*, 1989; Santiago and Westerink, 1990), which blocks voltage-dependent sodium channels to prevent neuronal depolarization (Narahashi, 1974; Strichartz *et al.*, 1987). During dialysis with TTX, residual dialysate DA concentrations indicate efflux of DA that is not dependent upon neuronal action potentials (i.e., damage-induced efflux).

Two critical factors have been identified that affect the amount of damage-induced efflux of DA; the size of the probe, and the post-insertion interval. Currently available microdialysis probes are roughly one thousand times the diameter of synapses in the CNS, and these probes are roughly 10-100 times the diameter of commonly used micropipettes and microelectrodes (Di Chiara, 1990). The amount of damage may be limited by use of a small concentric probe. Santiago and Westerink (1990) compared TTX-sensitivity and Ca^{2+}-dependence of extracellular striatal DA and metabolite release after implantation of four different types of microdialysis probes. Under these conditions, implantation of probes with a concentric design (similar to the probes used in the present study) produced less damage-induced efflux of DA than larger trans-striatal or U-shaped probes. However, implantation of any one of these probes resulted in substantial DA efflux that was insensitive to TTX and independent of extracellular Ca^{2+} concentrations when assayed within the first 24 hours after implantation. Complete elimination of dialysate DA during dialysis with TTX, and a higher degree of Ca^{2+}-dependent exocytosis was achieved by 24 hours after probe implantation in

all cases (Westerink and de Vries, 1988; Santiago and Westerink, 1990).

3.1.3 Methodological Implications

These observations have led to the conclusion that microdialysis experiments should not be conducted until 24 hours after surgical implantation of a microdialysis probe, to allow recovery from damage (Westerink and de Vries, 1988; Santiago and Westerink, 1990). In the present study, the feasibility of performing microdialysis was examined four hours after insertion of a probe into striatal tissue that was surgically prepared to facilitate probe insertion. Five to ten days prior to the microdialysis experiment, an obturator was implanted into the ventral striatum through a guide cannula, preparing a track for subsequent insertion of the microdialysis probe. This track greatly reduced the amount of acute damage produced by insertion of the probe on the morning of test days, resulting in basal release of DA that was Ca^{2+}-dependent and completely TTX-sensitive within four hours after probe insertion. Further, the ratio of DOPAC (3,4-dihydroxyphenylacetic acid) to DA under baseline conditions provides a reliable index of the TTX-sensitivity of extracellular DA concentrations in the ventral striatum.

3.2 Methods

3.2.1 Animals and Surgery

Twenty male Long-Evans rats (Charles River, Boston, MA) weighing 375-400 grams were implanted under sodium pentobarbital anaesthesia (65 mg/kg, ip) with 20 gauge guide cannulae terminating 1.0 mm above the NAcc (AP +3.4, ML +2.6, DV -4.5). Surgery was performed with the incisor bar set 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. (All measurements in the dorsal-ventral plane refer to distances along the cannula track at 10° from the vertical.) Stainless steel obturators (diameter = 356 µm; 28 gauge) were put in place at the time of surgery and removed at the time of testing. Obturators extended 4.2 mm beyond the tips of the guide cannulae (extended obturators) in twelve rats, and they ended flush with the tips of the guide cannulae (short obturators) in the other eight rats.

3.2.2 Drugs

TTX was purchased from Sigma Chemical Co. (St. Louis, MO). D-Amphetamine sulphate was purchased from Smith Kline & French (Montreal, Qué.). Ca^{2+}-free aECF was made by excluding the Ca^{2+} from aECF (see Section 2.2.2) and replacing it with additional Mg^{2+} to maintain the ionic balance (total = 2.2 mM Mg^{2+}). TTX was

dissolved in aECF and administered into the ventral striatum through the microdialysis probes. Ca^{2+}-free aECF was also dialysed through the ventral striatal microdialysis probes. D-Amphetamine was dissolved in physiological saline and injected intraperitoneally (ip).

3.2.3 Microdialysis Probes

Removable microdialysis probes were constructed according to the concentric design of Robinson and Whishaw (1988). Each probe consisted of a polyethylene tubing (PE20) fluid inlet, attached to a 26 gauge stainless steel outer cannula. This cannula was cemented to a 4 mm length of cellulose fibre (Brain Research Institute, i.d. = 215 μm; o.d. = 251 μm; 6,000 Dalton molecular weight cutoff) that was sealed at the end with acrylic cement. This cement covered the bottom 1 mm of cellulose fibre. The fluid outlet consisted of an inner cannula of silicon capillary tubing (Polymicro Technologies, i.d. = 75 mm; o.d. = 143 mm) that began 1 mm from the plugged tip of the dialysis tubing. The silicon tubing exited the polyethylene tubing through a hole that was sealed with epoxy cement, terminating in a collection vial which was located approximately 15 cm from the probe (see Fig. 3.1). On test days, probes were prepared by immersing them in test tubes containing aECF, and perfusing them with aECF at a flow rate of 2 μl/min for at least 20 min immediately prior to insertion.

The *in vitro* recovery of DA through the dialysis membrane was determined by immersing probes in aECF solution at 37°C containing: 325 fmole/µl of DA, 600 fmole/µl of DOPAC, 550 fmole/µl homovanillic acid (HVA), and 525 fmole/µl 5-hydroxyindole acetic acid (5-HIAA). Probes were dialyzed with aECF, and dialysate samples were analyzed by HPLC. Recovery of DA was 12.38 ± 2.016% (mean ± SEM) of the external concentration. Recovery of DOPAC was 9.519 ± 0.6213%. Recovery of HVA was 8.382 ± 0.7483%. Recovery of 5-HIAA was 12.63 ± 0.4556%.

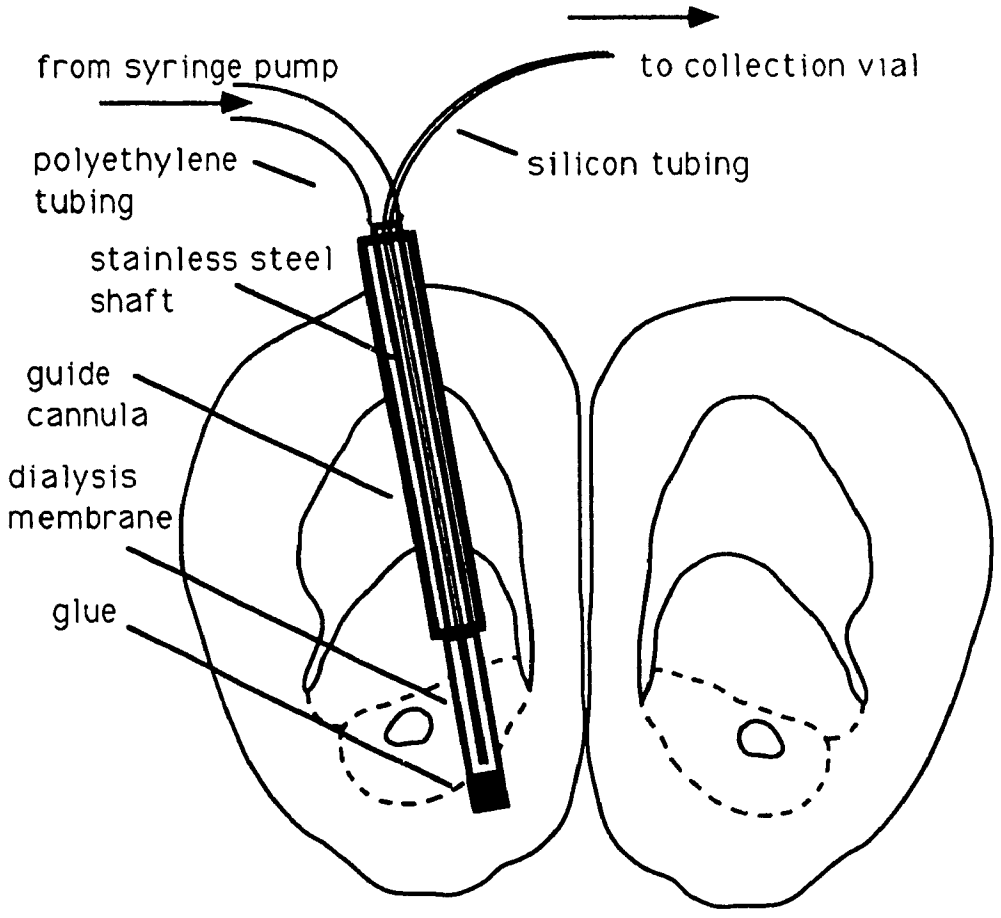

Fig. 3.1 A schematic representation of a microdialysis probe implanted in the ventral striatum.

3.2.4 Microdialysis Procedure

On test days (5-10 days after surgery) the rats were anaesthetized with chloral hydrate (400 mg/kg, ip) and given atropine sulphate (0.5 mg/kg, ip) to reduce salivation and mucous secretions. Supplemental doses (approximately 20 mg) of chloral hydrate were given when signs of arousal were observed (approximately once per hour). A microdialysis probe was inserted into the ventral striatum and dialysed with aECF at a flow rate of 2 µl/min. Dialysate samples were collected at 20 minute intervals until a stable baseline was obtained (four consecutive DA and DOPAC peak areas that varied by less than 10%, approximately four hours after probe insertion). The rats that had been implanted with extended obturators were randomly assigned to drug treatment groups, and treated as follows:

Group 1. When stable baselines were obtained, the perfusates were switched to Ca^{2+}-free aECF (n = 4) and dialysate samples were collected for another 80 minutes.

Group 2. When stable baselines were obtained, the perfusates were switched to aECF containing 1.0×10^{-6} M TTX (n = 4) and dialysate samples were collected for another 80 minutes. The rats were then injected ip with *d*-amphetamine (2.0 mg/kg) while dialysis with aECF containing TTX continued. Dialysate samples were collected for an additional 80 minutes after the *d*-amphetamine injections.

Group 3 (controls). After stable baselines were obtained, the rats were dialysed continuously with aECF (n = 4), and dialysate samples were collected for another 160 minutes.

The rats that had been implanted with short obturators were randomly assigned to the drug treatment groups, and treated as follows:

Group 4. The rats (n = 4) were treated the same as the rats in Group 1.

Group 5. The rats (n = 4) were treated the same as the rats in Group 2.

Finally, each rat was perfused with physiological saline followed by 10% formalin. Each brain was removed immediately, stored in formalin for one or more days, frozen, sliced in 40 µm coronal sections, and stained with thionin for localization of probe tracks.

3.2.5 Analytical Procedure

Dialysate was assayed for DA, DOPAC, 5-HIAA and HVA using isocratic, reverse-phase, HPLC with electrochemical detection. Each 40 µl sample of dialysate was injected onto a 15 cm x 4.6 mm (i.d.) C-18 column (spherisorb ODS/2, 5 µm) via a Rheodyne injection valve with a 100 µl sample loop. The recycled mobile phase (0.06 M NaH_2PO_4, 0.03 M citric acid, 7.98×10^{-5} M sodium dodecyl sulphate, 9.94×10^{-5} M EDTA (ethylenediaminetetra acetic acid) and 25% HPLC grade methanol in nanopure water; adjusted to pH 3.35 with 1 N NaOH) was pumped

at 1.4 ml/min by a Waters pump (model 510). DA, DOPAC, 5-HIAA and HVA (retention times of approximately 6, 2, 3 and 4 min respectively) were quantified on an ESA Coulochem II Detector (model 5200) with a conditioning electrode (Conditioning Cell model 5201; +100 mV) and two analytical electrodes (Analytical Cell model 5011), an oxidizing electrode (+340 mV; 500 nA), and a reducing electrode (-260 mV; 20 nA). Metabolites were quantified on the oxidizing electrode and DA was quantified on the reducing electrode. Areas under the DA, DOPAC, HVA, and 5-HIAA peaks were evaluated using a dual channel Spectra-Physics SP4270 integrator, and Spectra-Physics Winner software on a 386 IBM-compatible computer. The integrator was calibrated (using areas under the peaks) with standards in aECF.

3.2.6 Statistics

Between-groups differences in basal extracellular DA, DOPAC, HVA and 5-HIAA concentrations were evaluated using 5 x 4 repeated-measures analyses of variance (ANOVAs) which were calculated using raw (fmole/µl) scores. Ratios of DOPAC to DA (fmole/µl) were calculated for each sample collected during baseline, to evaluate differences in the DOPAC/DA ratio between groups of rats that had been surgically implanted with long obturators, and rats that had been implanted with short obturators.

Effects of Ca^{2+} depletion

Scores were transformed to percent of baseline means for each group, and 3 x 4 repeated-measures ANOVAs were calculated for each neurochemical measure evaluating differences between Ca^{2+}-free aECF-dialysed rats that had been implanted with short obturators, Ca^{2+}-free aECF-dialysed rats that had been implanted with long obturators, and rats that were dialysed with aECF. Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the aECF-dialysed group with the Ca^{2+}-free aECF-dialysed groups. All significant effects were further analysed with Tukey's tests, comparing values for each sample from the aECF control group with the corresponding samples from the Ca^{2+}-free aECF-dialysed groups. Tukey's tests were also used to evaluate differences in the responses to Ca^{2+}-free aECF between rats that had been implanted with short obturators and rats that had been implanted with long obturators.

Effects of TTX and d-amphetamine

Scores were transformed to percent of baseline means for each group, and 3 x 4 repeated-measures ANOVAs were calculated for each neurochemical measure evaluating differences between TTX-dialysed rats that had been implanted with short obturators, TTX-dialysed rats that had been implanted with long obturators, and aECF-dialysed control rats. Additionally, 3 x 4 repeated-measures ANOVAs were calculated for each neurochemical measure evaluating differences between

TTX-dialysed and amphetamine-treated rats that had been implanted with short obturators, TTX-dialysed and amphetamine-treated rats that had been implanted with long obturators, and rats that were dialysed with aECF.

Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the aECF-dialysed group with the TTX-dialysed groups, or between the aECF-dialysed and TTX- plus amphetamine-treated groups. All significant effects were further analysed with Tukey's tests, comparing values for each sample from the aECF control group with the corresponding samples from the TTX, or TTX plus amphetamine-treated groups. Tukey's tests were also used to evaluate differences in the responses to TTX between rats that had been implanted with short obturators and rats that had been implanted with long obturators, and to evaluate differences in the responses to amphetamine in TTX-dialysed rats that had been implanted with short obturators and rats that had been implanted with long obturators.

A linear regression analysis was calculated with mean baseline DOPAC/DA ratios from each TTX-dialysed rat regressed on DA concentrations (% of mean baseline concentrations) in the fourth sample during TTX dialysis, to evaluate how well the baseline DOPAC/DA ratio predicted the efficacy of TTX to eliminate DA from the dialysate samples.

3.3 Results

Extracellular ventral striatal DA and DOPAC concentrations in the first sample collected immediately after probe insertion were higher in the rats that had been implanted with short obturators than in the rats that had been implanted with extended obturators (Fig. 3.2). During the four hours between probe insertion and drug treatments, DA concentrations decreased (95.66%) and DOPAC concentrations increased (46.98%) in the rats with that had been implanted with short obturators. On the other hand, DA and DOPAC concentrations were relatively stable during the initial four hours in the rats that had been implanted with extended obturators (Fig. 3.2).

Baseline concentrations of extracellular ventral striatal DA differed significantly between groups. Similarly, significant between-groups differences were found in baseline concentrations of DOPAC, HVA, and 5-HIAA. The baseline concentrations of DA, DOPAC, HVA and 5-HIAA, and tests of significance are summarized in Table 3.1.

Dialysis of the ventral striatum with Ca^{2+}-free aECF decreased concentrations of extracellular ventral striatal DA to 7.503% (± 3.145) of baseline concentrations in the rats that had been implanted with extended obturators. Thus, in this group, DA concentrations were reduced to 0.164 fmoles/µl. On the other hand, in the rats that had been implanted with short obturators, dialysis of the ventral striatum with Ca^{2+}-free aECF only

decreased DA concentrations to 0.287 fmoles/µl, or 47.19% (± 9.253%) of baseline concentrations (Fig. 3.3).

Ventral striatal DOPAC and HVA concentrations were also decreased by Ca^{2+}-free aECF dialysis, and these percent

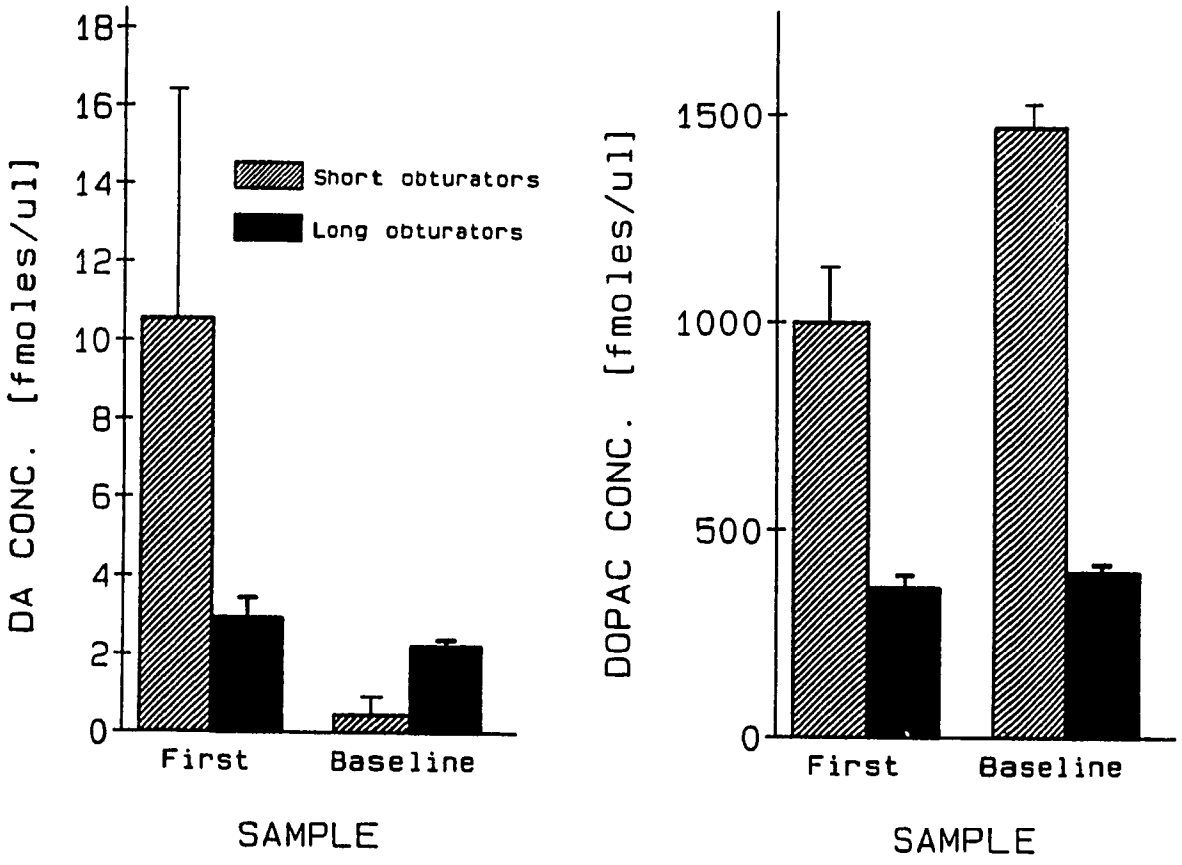

Fig. 3.2 Initial extracellular ventral striatal DA and DOPAC concentrations were substantially higher in the rats that had been implanted with short obturators than concentrations in the rats that had been implanted with extended obturators. DA concentrations decreased and DOPAC concentrations increased during the four hours between the initial sample and the baseline samples in the rats that had been implanted with short obturators. Conversely, DA and DOPAC concentrations were stable in the rats that had been implanted with extended obturators. Values expressed are group means ± SEM. Baseline scores represent means of the four samples collected just prior to drug treatments (Ca^{2+}-free aECF or TTX dialysis).

	Group	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	aECF extended	1.772 ± 0.289			
	-Ca^{2+} extended	2.188 ± 0.091			
	TTX extended	2.672 ± 0.365			
	-Ca^{2+} short	0.609 ± 0.019 **			
	TTX short	0.308 ± 0.024 **			
	overall	1.510 ± 0.138	22.84	4,75	<.01
DOPAC	aECF extended	308.2 ± 8.238			
	-Ca^{2+} extended	333.4 ± 9.757			
	TTX extended	548.2 ± 36.94			
	-Ca^{2+} short	1403.9 ± 101.6 **			
	TTX short	1535.7 ± 52.69 **			
	overall	825.9 ± 64.53	121.9	4,75	<.01
HVA	aECF extended	372.7 ± 16.61			
	-Ca^{2+} extended	526.5 ± 31.90 **			
	TTX extended	631.4 ± 27.69 **			
	-Ca^{2+} short	595.1 ± 27.36 **, ††			
	TTX short	390.4 ± 27.06			
	overall	503.3 ± 16.56	19.47	4,75	<.01
5-HIAA	aECF extended	94.60 ± 6.093			
	-Ca^{2+} extended	87.41 ± 2.443			
	TTX extended	95.07 ± 5.006			
	-Ca^{2+} short	82.64 ± 2.109			
	TTX short	79.77 ± 3.521			
	overall	87.88 ± 1.926	2.797	4,75	<.05

Table 3.1. Baseline means and standard errors for DA and metabolites for 5 treatment groups (aECF perfusate controls, Ca^{2+}-free perfusate with short obturators, Ca^{2+}-free perfusate with extended obturators, TTX perfusate with short obturators, and TTX perfusate with extended obturators), and tests of significance for between-groups differences in baseline means. Significant differences in baseline concentrations between groups (Tukey's T-tests) are depicted as follows: ** $p < .01$ for comparisons between each group and the aECF controls; †† $p < .01$ for comparisons between groups with short obturators treated that were treated with Ca^{2+}-free aECF or TTX. No other between-groups comparisons revealed significant differences.

reductions were equivalent between groups (Fig. 3.4). 5-HIAA concentrations were not reliably altered by dialysis with Ca^{2+}-free aECF (Fig. 3.4).

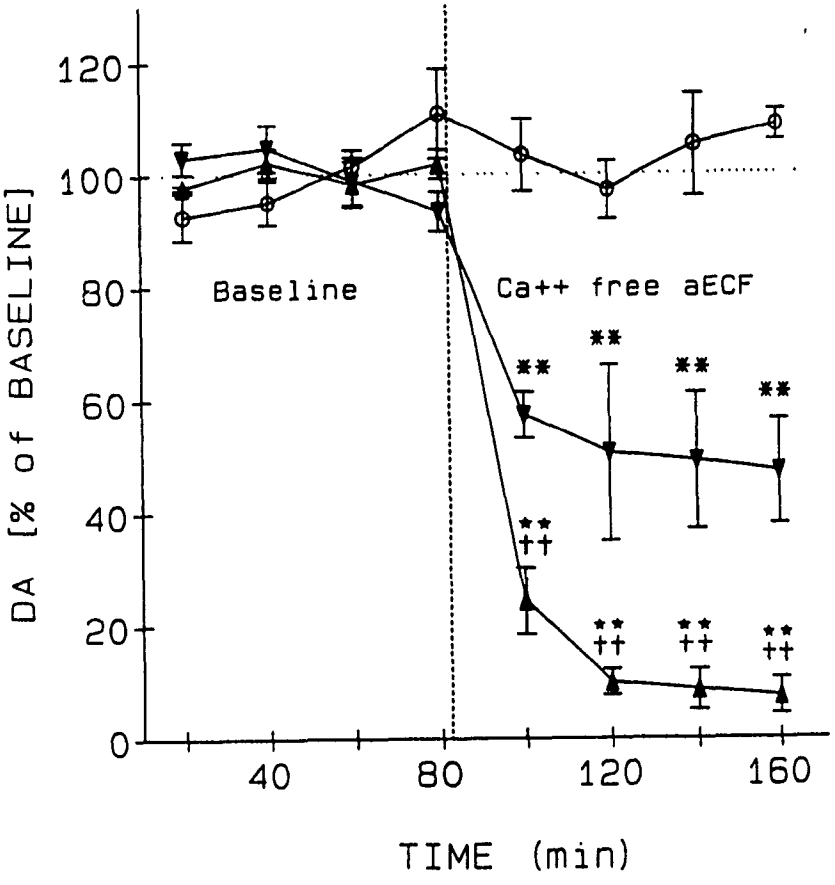

Fig. 3.3. Extracellular ventral striatal DA concentrations were significantly lower in each group of Ca^{2+}-free aECF-dialyzed rats than in the aECF controls (O) ($F_{(2,9)} = 57.78$, $p < .01$). Tukey's tests reveal that DA concentrations were reduced to a smaller fraction of the baselines during dialysis with Ca^{2+}-free aECF in the rats that had been implanted with extended obturators (▲) than in the rats that had been implanted with obturators that did not extend below the tip of the guide cannulae (▼). Values expressed are group means ± SEM. Significant differences in DA concentrations between the drug-treated and control rats at each sample (Tukey's T-tests) are depicted as follows: ** $p < .01$ for short obturators; †† $p < .01$ for extended obturators. Significant differences in DA concentrations between the rats that had been implanted with short obturators and the rats that had been implanted with extended obturators during Ca^{2+}-free aECF dialysis at each sample (Tukey's T-tests) are depicted as follows: ★★ $p < .01$.

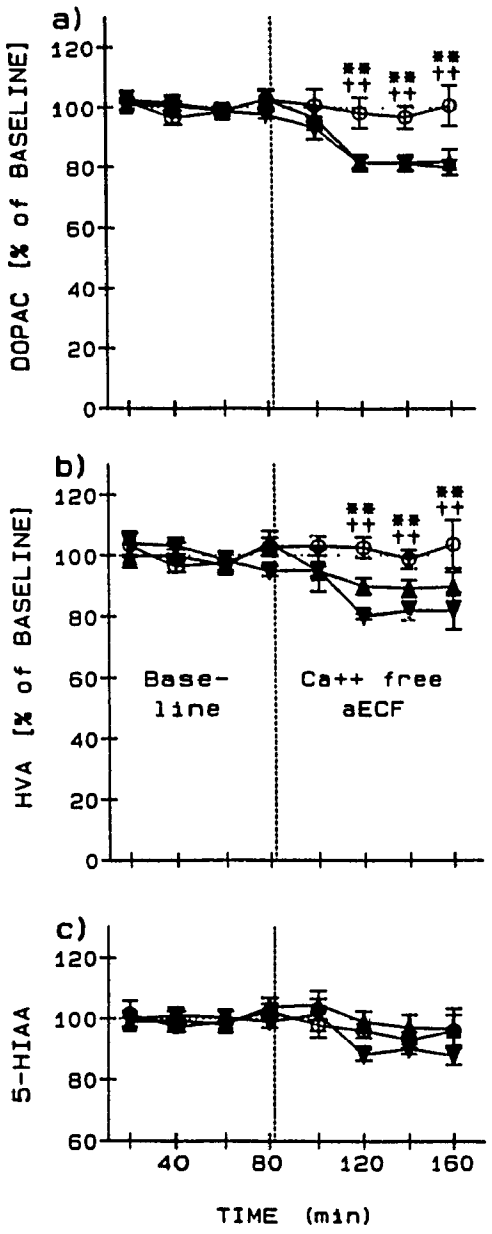

Fig. 3.4. Extracellular ventral striatal DOPAC ($F_{(2,9)} = 9.363$, $p < .01$) and HVA ($F_{(2,9)} = 9.273$, $p < .01$) concentrations were significantly lower in each group of Ca^{2+}-free aECF-dialyzed rats than in the aECF controls (○). Tukey's tests reveal that the DOPAC and HVA concentrations were reduced to equivalent fractions of the baselines during dialysis with Ca^{2+}-free aECF in the rats that had been implanted with short obturators (▼) and in the rats that had been implanted with obturators that extended below the tip of the guide cannulae (▲). Extracellular ventral striatal 5-HIAA concentrations were not significantly altered by dialysis with Ca^{2+}-free aECF ($F_{(2,9)} = 1.713$, $p > .05$). Values expressed are group means ± SEM. Significant differences in concentrations between the drug-treated and control rats at each sample (Tukey's T-tests) are depicted as follows: ** $p < .01$ for short obturators; †† $p < .01$ for extended obturators.

In the rats that had been implanted with extended obturators, dialysis of the ventral striatum with aECF containing TTX decreased DA concentrations to undetectable levels. On the other hand, in the rats that had been implanted with short obturators, TTX dialysis only decreased DA concentrations to 0.091 fmoles/µl, or 29.55% (± 6.695%) of baseline concentrations (Fig. 3.5).

In the TTX-dialysed rats, ip injections of *d*-amphetamine produced reliable increases in ventral striatal DA concentrations, which reached a maximum in the second post-injection sample (Fig. 3.5). DA concentrations were substantially higher in the rats that had been implanted with extended obturators (228.5 ± 22.29 fmoles/µl) than in the rats that had been implanted with short obturators (124.5 ± 5.631 fmoles/µl) after *d*-amphetamine injections. However, the increases that were observed in the rats that had been implanted with short obturators constituted a significantly higher percentage increase from baseline than the increases that were observed in the rats that had been implanted with extended obturators (Fig. 3.5).

Ventral striatal DOPAC and HVA concentrations were decreased to 72.51% (± 5.786%) of baseline by dialysis with TTX, and these reductions were equivalent between groups (Fig. 3.6). 5-HIAA concentrations were not reliably altered by dialysis with TTX.

DOPAC and HVA concentrations were decreased in all rats following ip *d*-amphetamine injections. 5-HIAA concentrations were not reliably altered by *d*-amphetamine (Fig. 3.6).

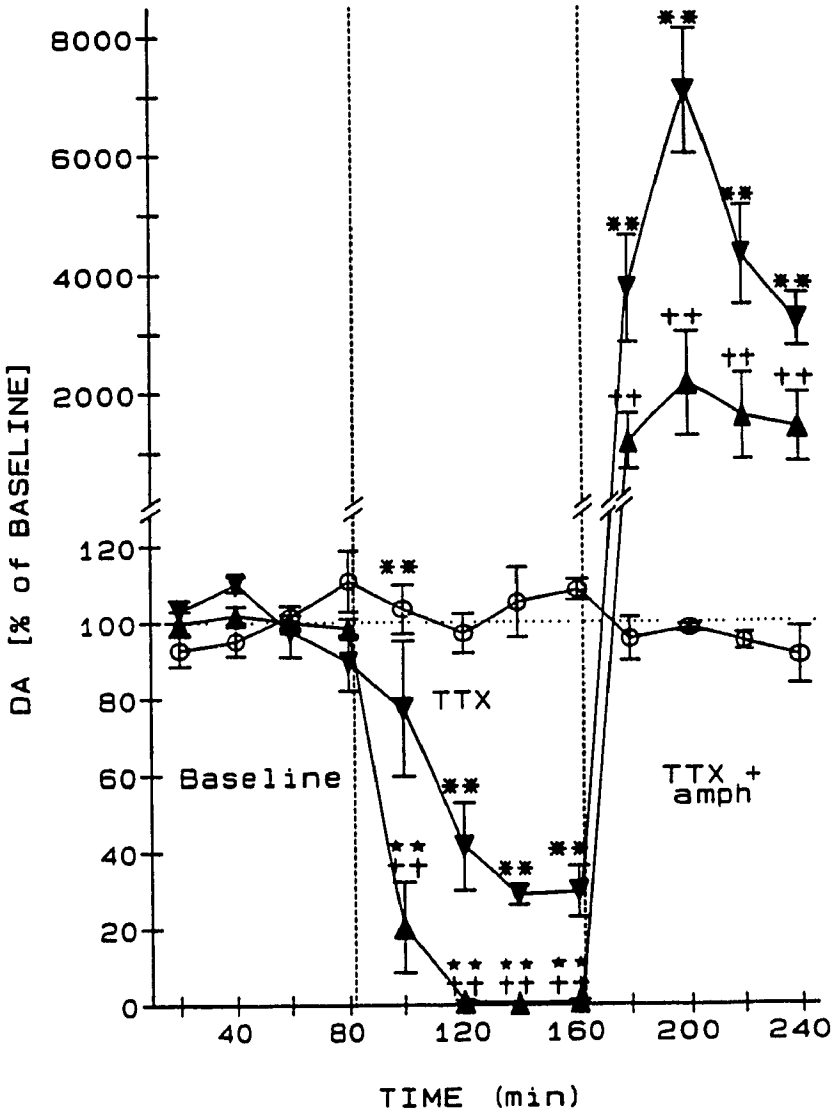

Fig. 3.5. Extracellular ventral striatal DA concentrations were significantly lower in each group of TTX-dialyzed rats than in the aECF controls (O) ($F_{(2,9)} = 118.7$, $p < .01$). T ' ey's tests reveal that DA concentrations were reduced to a smaller fraction of the base..es during dialysis with TTX in the rats that had been implanted with extended obturators (▲) than in the rats that had been implanted with obturators that did not extend below the tip of the guide cannulae (▼). D-amphetamine injections in each group of TTX-dialyzed rats produced DA concentrations that were significantly higher than in the aECF controls ($F_{(6,27)} = 21.27$, $p < .01$). Tukey's tests reveal that DA concentrations were increased to a greater percentage of the baselines during dialysis with TTX in the rats that had been implanted with obturators that did not extend below the tip of the guide cannulae than in the rats that had been implanted with extended obturators. Values expressed are group means ± SEM. Significant differences in concentrations between the drug-treated and control rats at each sample (Tukey's

T-tests) are depicted as follows: ** $p < .01$ for short obturators; †† $p < .01$ for extended obturators. Significant differences in DA concentrations between the rats that had been implanted with short obturators and the rats that had been implanted with extended obturators during TTX-dialysis at each sample (Tukey's T-tests) are depicted as follows: ★ $p < .05$, ★★ $p < .01$.

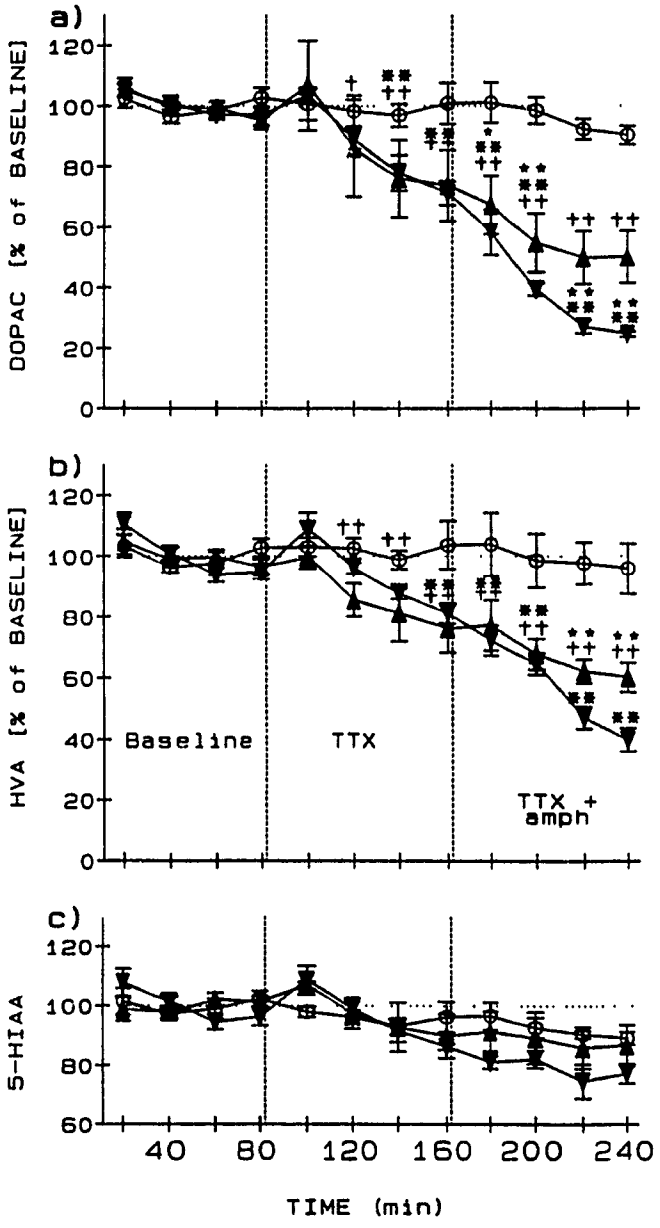

Fig. 3.6. Extracellular ventral striatal DOPAC ($F_{(6,27)} = 5.427$, $p < .01$) and HVA ($F_{(6,27)} = 2.707$, $p < .05$) concentrations were significantly lower in each group of TTX-dialyzed rats than in the aECF controls (O), and these reductions were equivalent between the rats that had been implanted with short obturators (▼) and the rats that had

been implanted with extended obturators (▲). D-amphetamine injections in TTX-dialysed rats produced DOPAC ($F_{(6,27)} = 4.039$, $p < .01$) and HVA ($F_{(6,27)} = 3.957$, $p < .01$) concentrations that were significantly lower than concentrations in aECF controls. Tukey's tests reveal that DOPAC and HVA concentrations were decreased to a lower percentage of the baselines during dialysis with TTX in the rats that had been implanted with obturators that did not extend below the tip of the guide cannulae than in the rats that had been implanted with extended obturators. Extracellular ventral striatal 5-HIAA concentrations were not significantly altered by dialysis with TTX ($F_{(2,9)} = 0.0143$, $p > .05$), or by *d*-amphetamine injections ($F_{(2,9)} = 2.088$, $p > .05$) in the TTX-dialysed rats. Values expressed are group means ± SEM. Significant differences in concentrations between the drug-treated and control rats at each sample (Tukey's T-tests) are depicted as follows: ** $p < .01$ for short obturators; † $p < .05$, †† $p < .01$ for extended obturators. Significant differences in DOPAC and HVA concentrations between the rats that had been implanted with short obturators and the rats that had been implanted with extended obturators during TTX dialysis at each sample (Tukey's T-tests) are depicted as follows: ★ $p < .05$, ★★ $p < .01$.

Linear regression analysis revealed a significant correlation ($r = 0.8202$) between mean baseline DOPAC/DA ratios and dialysate DA concentrations assayed in the fourth sample during TTX dialysis

(Fig. 3.7).

The dialyzing surfaces (approx. 3 mm) of microdialysis probes were localized in the medial NAcc, with a small portion in the ventral aspect of the caudate nucleus. Placements of ventral striatal microdialysis probes are depicted in Fig. 3.8. There were no apparent between-groups differences in the locations of probe tracks.

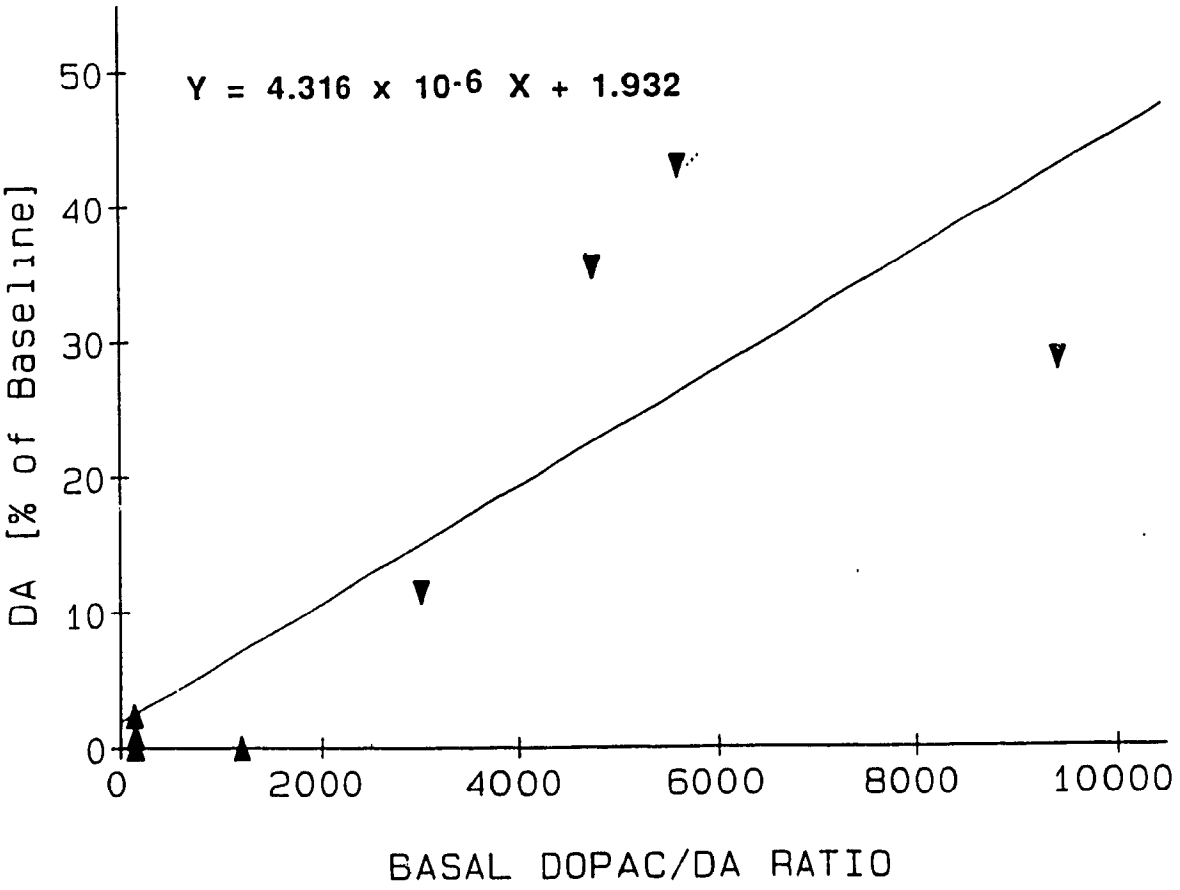

Fig. 3.7. Linear regression analysis reveals a significant relationship between mean baseline DOPAC/DA ratios and levels of DA (% of baseline means) assayed in the fourth sample during TTX dialysis ($F_{(1,6)} = 12.33$, $p < .05$), in the rats that had been implanted with short obturators (▼) and in the rats that had been implanted with obturators that extended below the tip of the guide cannulae (▲). The linear regression is represented by the solid line, while the 95% confidence interval is represented by the area bounded by the two dotted lines.

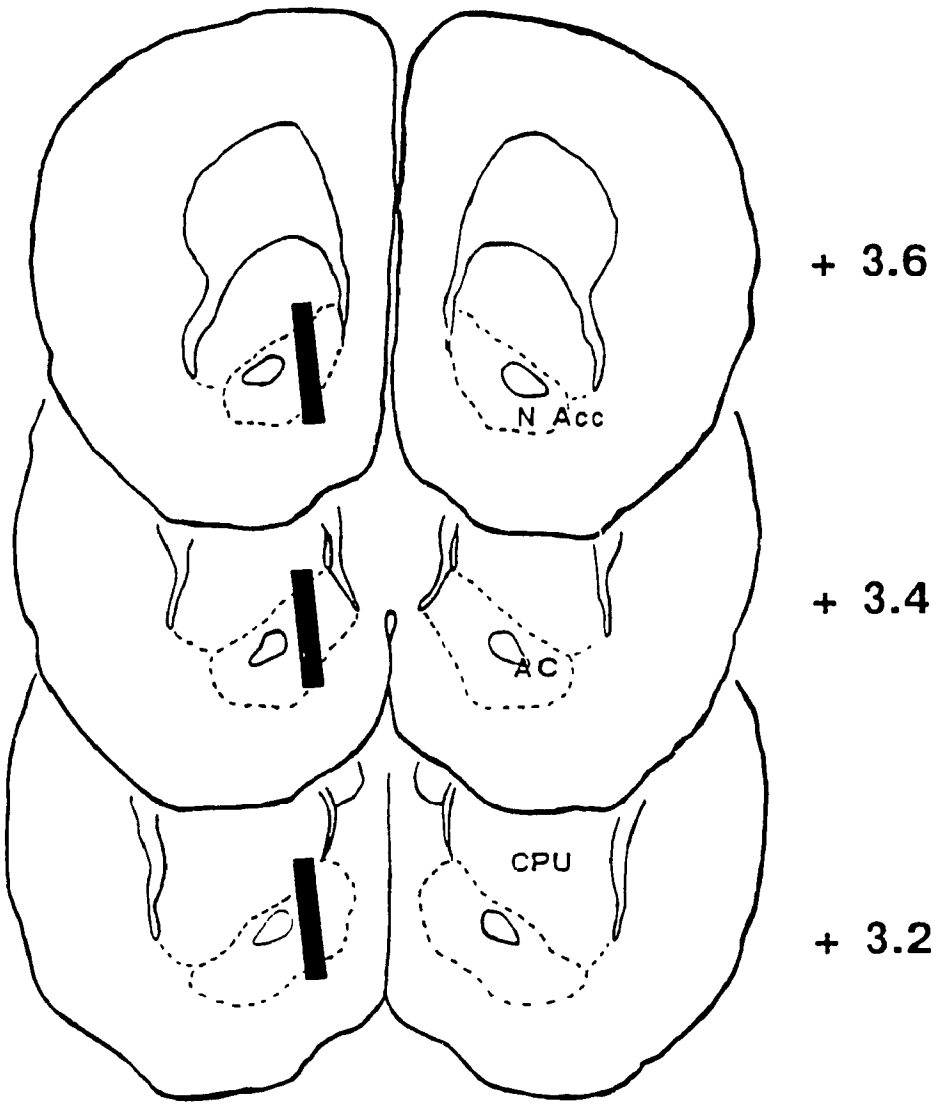

Fig. 3.8. Histological reconstruction of representative microdialysis probe tracks in the ventral striatum. Abbreviations used: CPU = caudate putamen; NAcc = nucleus accumbens; AC = anterior commissure (adapted from Pellegrino *et al.*, 1979).

3.4 Discussion

3.4.1 Between-Groups Differences in Initial DA and DA Metabolite Efflux

The pattern of DA efflux during the initial four hours of microdialysis was quite different between groups. There was a massive efflux of DA during the first sample after probe insertion in the rats that had been implanted with short obturators. This was followed by a rapid decline in dialysate DA concentrations during subsequent samples in these rats. Similar (but smaller) declines in dialysate DA concentrations after acute implantation of a microdialysis probe have been previously reported (Camp and Robinson, 1992). In contrast, dialysate DA concentrations were stable throughout the samples collected during the first four hours in the rats that had been implanted with extended obturators.

The initial efflux of DA in the rats that had been implanted with short obturators may have resulted from acute damage to neuronal membranes. Membrane damage could cause intracellular stores of DA to spill out into the extracellular fluid either by passive diffusion or as a consequence of an exchange of ions across the membrane to produce exocytosis of vesicular DA. In fact, Benveniste *et al.* (1989) have reported that a spontaneously reversible spreading depression of Leao occurs during the first 0.5-3 minutes after the acute implantation of a microdialysis

probe. This depression is characterized by sudden alterations in the extracellular ion concentrations, and a transient hyperpolarization of the local membrane potential.

Differences were also noted in the efflux of DOPAC during the first four hours of microdialysis in these two groups of rats. The rats that had been implanted with short obturators exhibited higher dialysate concentrations of DOPAC in the first sample after probe implantation than did the rats that had been implanted with extended obturators. The DOPAC concentrations in the rats that had been implanted with short obturators increased further during the first four hours after probe insertion. Similar (but smaller) increases in dialysate DOPAC concentrations after acute implantation of a microdialysis probe have been reported previously (Camp and Robinson, 1992). Conversely, stable, relatively low basal DOPAC concentrations were found in the rats that had been implanted with extended obturators. These results provide further evidence that acute tissue trauma was greater in the rats that had been implanted with short obturators, than in the rats that had been implanted with extended obturators. The persistently higher dialysate concentrations of DOPAC in the rats that had been implanted with short obturators may have resulted from damage to neuronal membranes, producing efflux of the intracellular stores of DOPAC, or from the increased synthesis and turnover of DA that occur following lesions of the ascending dopaminergic fibres (Commissiong *et al.*, 1990).

We have previously examined the effects of knife-cut lesions of the ascending DA fibres in the cell body region of these

fibres on extracellular basal striatal DA and DA metabolite concentrations. In these rats, stable baselines were obtained. Then, after knife-cuts, the extracellular DA concentrations fell, and the DOPAC concentrations rose to levels that are comparable to the baseline levels that were observed in the rats that had been implanted with short obturators in the present experiment (unpublished observations). Thus, it appears that the effects of acute implantation of a microdialysis probe resembles the effects of knife cut lesions to the dopaminergic fibres, while these effects do not occur after probe implantation in rats that have been implanted with extended obturators.

Baseline DA concentrations were significantly higher in the rats that had been implanted with extended obturators than in those that had been implanted with short obturators. This may indicate that there is a recovery of functioning of the dopaminergic system that occurs within a few days after damage from penetration of the striatal tissue. De Boer *et al.* (1992) recently reported that tonic endogenous dopaminergic regulation of striatal acetylcholine (ACh) is absent 16-24 hours after probe implantation, but is recovered by 40-96 hours post-implantation. In the present experiment, the initial tissue trauma occurred when the extended obturators were surgically implanted into the striatum, 5-10 days prior to the experiment, and it appears that little additional insult was inflicted by removal of these obturators and insertion of the microdialysis probes into this prepared tissue. Thus, the dopaminergic system may exhibit a recovery of activity in this preparation.

3.4.2 Dialysis with TTX

The linear relationship that was found between baseline DOPAC/DA ratios (measured in fmole/µl) with the efficacy of TTX to reduce dialysate DA concentrations in individual rats suggests that basal DOPAC/DA ratios provide an index of the amount of acute insult to striatal tissue upon insertion of a microdialysis probe. Examination of the regression line (Fig. 3.7) for the correlation of basal DOPAC/DA ratios with TTX efficacy suggests that rats with DOPAC/DA ratios that are greater than 2000 will likely have more than 10% of the extracellular concentration of DA resulting from voltage-independent (i.e., TTX-insensitive) efflux. Thus, basal DOPAC/DA ratios may be used to evaluate the recovery from acute insult following probe implantation in individual rats during experiments when it would be inappropriate to dialyse the animals with TTX (e.g. studies of pharmacological manipulations of dopaminergic function). Investigators may wish to eliminate rats with high basal DOPAC/DA ratios, or wait until levels stabilize before applying pharmacological manipulations in these animals. If percent recoveries for DOPAC and DA differ significantly from those of the probes used in the present experiment (9.5% for DOPAC; 12% for DA), the maximal acceptable DOPAC/DA ratio must be adjusted accordingly.

The between-groups differences in the efficacy of TTX to reduce dialysate DA concentrations demonstrates that there were substantial differences in the injury-induced efflux of DA in these groups four hours after probe insertions. Since TTX eliminated

dialysate DA in the rats that had been implanted with extended obturators, it seems that the extracellular DA concentrations in these animals reflect release of DA that is completely derived from neuronal activity. Conversely, the high residual dialysate DA concentrations in the rats that had been implanted with short obturators indicates that a substantial proportion (about 30%) of the extracellular DA concentrations originated from injury-induced efflux of DA that was not dependent upon neuronal activity. Similar damage-induced efflux during the first 24 hours after probe insertion (without previous penetration of the striatal tissue) has previously been reported (Westerink *et al.*, 1987b; Westerink and de Vries, 1988; Santiago and Westerink, 1990).

While TTX eliminated DA from dialysate samples, the concentrations of DOPAC and HVA (in both groups of rats) were only reduced to about 70% of baseline values during TTX dialysis. These residual metabolite concentrations probably result from differences in the diffusion kinetics of DA and its metabolites. DOPAC and HVA diffuse quite freely in striatal tissue, while the diffusion of DA is extremely limited due to high affinity uptake into neurons (Horn 1979, 1990; Rice *et al.*, 1985; Cass *et al.*, 1992). Thus, the residual metabolite concentrations may reflect diffusion from cell populations that were not affected by local dialysis (Westerink *et al.*, 1987b). Further, it seems that a significant portion of the extracellular concentrations of DOPAC and HVA are produced by DA metabolism that is related to metabolism of newly synthesized DA rather than metabolism of previously released DA (Westerink *et al.*, 1987b), since

treatments that eliminate impulse-dependent DA release (γ-butyrolactone, TTX) fail to reduce extracellular DOPAC and HVA concentrations to less than 30-50% of baseline (Roth *et al.*, 1976; Westerink *et al.*, 1987b; Drew *et al.*, 1989), even when high concentrations of these toxins are administered. Similar reductions in the concentrations of extracellular DOPAC and HVA concentrations during TTX administration have previously been reported (Westerink *et al.*, 1987b).

3.4.3 Dialysis with Ca^{2+}-free aECF

Dialysis with Ca^{2+}-free aECF four hours after probe insertion reduced, but did not eliminate extracellular DA concentrations in both groups of rats. The residual DA concentrations may be accounted for by the fact that dialysis with Ca^{2+}-free aECF does not completely eliminate extracellular Ca^{2+} in the neuronal environment (Benveniste *et al.*, 1989). Between-groups differences in the effects of Ca^{2+} depletion indicate that the severity of acute trauma from probe insertion differed between groups. Extracellular DA concentrations were reduced to lower levels in the rats that had been implanted with extended obturators than they were in the rats that had been implanted with short obturators. Thus, it would seem that a significant portion of the extracellular pool of DA in the rats that had been implanted with short obturators was derived from mechanisms that did not involve Ca^{2+}-dependent exocytosis.

The magnitudes of the decreases in DA concentrations during dialysis with Ca^{2+}-free aECF in the rats with extended obturators were notably greater than those reported in previous studies. This may have resulted from either of two differences between this study and the previous studies. The composition of Ca^{2+}-free aECF in the present study included additional Mg^{2+} to replace the Ca^{2+}, whereas the previous studies maintained the ionic balance of the dialysis fluid by replacing Ca^{2+} with Na^{+} (Santiago and Westerink, 1990; Westerink and de Vries, 1988; Westerink *et al.*, 1988), or Ca^{2+} was simply omitted from the dialysis medium (Drew *et al.*, 1989). Mg^{2+} acts as a Ca^{2+} channel antagonist (Augustine *et al.*, 1987), and dialysis with high concentrations of Mg^{2+} in the striatum produces complete elimination of extracellular DA (Westerink *et al.*, 1988). The concentration of Mg^{2+} that eliminated extracellular DA was much greater than the dose used in the present study, and the threshold dose has not been characterized. It is unclear if the concentration presently used is sufficient to alter dopaminergic activity. Another possible explanation for the pronounced effects of Ca^{2+} depletion in this study is that chloral hydrate-anaesthetized rats were used, and anaesthesia is known to depress neuronal activity (Di Chiara, 1990), possibly through actions upon Ca^{2+}-dependent mechanisms (Mody *et al.*, 1991).

Dialysis with Ca^{2+}-free aECF had very little effect on extracellular DOPAC and HVA concentrations, and no between-groups differences were found in concentrations of these metabolites during dialysis with Ca^{2+}-free aECF. These residual

metabolite concentrations are probably derived from diffusion from distal sites at which the Ca^{2+} concentration was not affected by dialysis with Ca^{2+}-free aECF, and from metabolism of intracellular DA that is unrelated to release mechanisms. The magnitudes of reductions in DOPAC and HVA concentrations that were observed in the present experiment during dialysis with Ca^{2+}-free solutions are similar to those that have previously been reported (Westerink *et al.*, 1988; Santiago and Westerink, 1990).

The fact that extracellular concentrations of 5-HIAA were unaffected by dialysis with Ca^{2+}-free aECF or by dialysis with TTX suggests that serotonergic neurons may not terminate in the vicinity of the microdialysis probes in the present experiment, or that 5-HIAA is not a sensitive enough measure to evaluate serotonergic function under these conditions. (5-HIAA may diffuse in sufficient quantities to mask any effects of Ca^{2+} depletion or low dose TTX infusion on serotonergic function.) Santiago and Westerink (1990) have also reported failures of Ca^{2+} depletion and TTX dialysis to alter extracellular 5-HIAA concentrations in the striatum.

3.4.4 *d*-Amphetamine Injections

Injections of *d*-amphetamine (2.0 mg/kg) in the TTX-dialysed rats produced large increases in the extracellular concentrations of DA, indicating that the dopaminergic nerve terminals were responsive to pharmacological manipulation in both groups of rats. These increases are thought to reflect

interference with carrier-mediated mechanisms (Fischer and Cho, 1979; McMillen *et al.*, 1980), that are Ca^{2+}-independent (Hurd and Ungerstedt, 1989) and TTX-insensitive (i.e., not dependent upon voltage-gated mechanisms; Nomikos *et al.*, 1990). In the present study, DA concentrations were increased to a much higher percentage of baseline concentrations in the rats that had been implanted with short obturators than in the rats that had been implanted with extended obturators. However, it appears that this difference is an artifact of the extremely low baseline DA concentrations in the rats that had been implanted with short obturators. In fact, the dialysate DA concentrations after *d*-amphetamine in the rats that had been implanted with extended obturators reached a peak concentration of about 225 fmoles/µl, while DA concentrations in the rats that had been implanted with short obturators peaked at only 125 fmoles/µl. Thus, the *d*-amphetamine-induced 21.5-fold increase in DA efflux in the rats that had been implanted with extended obturators represents a greater magnitude of response than the 71-fold increase in DA efflux in the rats that had been implanted with short obturators. Hurd and Ungerstedt (1989) have previously reported 20 to 25-fold increases in extracellular striatal DA after subcutaneous injection of *d*-amphetamine at a dose of 1.5 mg/kg.

Amphetamine-induced increases in extracellular DA concentrations are accompanied by substantial decreases in DOPAC and HVA concentrations (Imperato and Di Chiara, 1984; Butcher *et al.*, 1988; Hurd and Ungerstedt, 1989; present results). These effects are thought to occur due to either inhibition of the

activity of monoamine oxidase (MAO: Hurd and Ungerstedt, 1989), or efflux of newly synthesized cytosolic pools of DA, removing the main substrate for intracellular metabolism by MAO (Zetterstrom *et al.*, 1986; Hurd and Ungerstedt, 1989).

3.4.5 Permeability of the Blood-Brain Barrier

An additional concern for early sampling of microdialysis perfusate is the integrity of the blood-brain barrier (BBB) after insertion of a microdialysis probe. However, it seems that coagulation restores this integrity within 1 hour after probe insertion (Tossman and Ungerstedt, 1986; Ungerstedt, 1984; Benveniste *et al.*, 1984). Intravenous injection of the radio-isotope sodium technetate ($Na^{99m}TcO_4$; which does not cross the BBB) immediately after probe insertion produced 1500 scintillation counts per 10 µl of blood, but radioactivity was not found in CNS perfusate. Conversely, iv injection of $[^3H]H_2O$ (which crosses the BBB) produced 2000 counts in 10 µl of blood, and 1200 counts in 10 µl of perfusate (Tossman and Ungerstedt, 1986; Ungerstedt, 1984). Similar results have been reported by Benveniste *et al.* (1984) using γ-amino-isobutyrate (which doesn't cross the BBB). Integrity of the BBB was found within approximately 1 hour after probe implantation. Therefore, it appears that the BBB is restored prior to the time at which baselines were collected in the present experiment.

3.4.6 Pharmacological Responsiveness

In addition to the present evidence for pharmacological responsiveness of dopaminergic terminals shortly after microdialysis probe insertion in the rats that were previously implanted with extended obturators, extracellular DA and DA metabolite concentrations are responsive to pharmacologic manipulations of presynaptic inputs to the dopaminergic neurons when this method is used. VTA microinjections of morphine (Leone *et al.*, 1991) increase extracellular ventral striatal DA concentrations, and VTA microinjections of the μ opioid agonist DAMGO, or the ∂ opioid agonist DPDPE each produce dose-orderly increases in extracellular ventral striatal DA, DOPAC and HVA (see Chapter 4). Westerink *et al.* (1987b) found that morphine-induced increases in DA release are TTX sensitive, so they originate from impulse-dependent activity of the dopaminergic neurons.

3.4.7 Summary and Conclusions

In summary, implantation of a guide cannula and an obturator that extends along the trajectory that will subsequently be occupied by the microdialysis probe allows recovery from acute insult to striatal tissue prior to the day of a microdialysis experiment. Careful insertion of the microdialysis probe into tissue that is prepared in this manner produces little acute insult to the tissue so that stable, Ca^{2+}-dependent, and TTX-sensitive

baselines are established within four hours after probe insertion. This method may have benefits over methods in which a probe is acutely implanted into unprepared tissue. A substantial interval is allowed to pass after initial tissue trauma in the striatum, which may allow a restoration of dopaminergic function. Another potential benefit of the method reported herein is that it provides an alternative to longer term implantation of probes. We have noted decreases in recovery of DA and metabolites *in vitro* after 14-16 hour long *in vivo* experiments (unpublished observations). Finally, the relationship between basal DOPAC/DA ratios and TTX-sensitivity indicates that these ratios may be used as an indirect measure to evaluate the acute tissue trauma after striatal probe insertion in experiments when it is unfeasible or undesirable to evaluate the voltage-dependence of extracellular DA concentrations with TTX.

Chapter 4

Modulation of Ventral Striatal DA and DA Metabolite Concentrations by VTA Microinjections of Selective μ, ∂, and κ Opioid Agonists

4.1 Introduction

4.1.1 Opioid Modulation of Mesolimbic Dopaminergic Activity

The neural mechanisms that mediate the rewarding effects of opiates are being investigated in a number of laboratories worldwide. One notable candiate mechanism is increased mesolimbic DA neurotransmission. In fact, it is well established that a variety of opiates produce increased activity of the ascending dopaminergic fibers. Systemic (Gysling and Wang, 1983; Matthews and German, 1984) or iontophoretic (i.e. VTA) administration of morphine (Yim and Mogenson, 1980a; Gysling and Wang, 1983) increases the firing rates of mesolimbic and nigrostriatal dopaminergic neurons, and the increases in the firing rates of the mesolimbic neurons are greater than the increases in the firing rates of the nigrostriatal neurons after systemic morphine administration (Matthews and German, 1984). Thus, it seems that the mesolimbic system is preferentially activated by

morphine. Further, it has recently been demonstrated that microinjections of morphine into the VTA produce increases in extracellular DA concentrations in the NAcc (Leone *et al.*, 1991).

4.1.2 The Involvement of DA in Opiate Reward

There is a substantial body of behavioural evidence that implicates DA neurotransmission in the rewarding effects of opiates. The fact that rats will self-administer opiates directly into the cell body region of the mesolimbic dopaminergic neurons (Bozarth and Wise, 1981a, 1984; Welzl *et al.*, 1989; present results, Chapter 2) suggests that these neurons may participate in opiate reward. Further, a variety of treatments that interfere with DA transmission block the rewarding effects of opiates. Pretreatment with the DA antagonist haloperidol blocks the place preferences produced by systemically administered heroin (Spyraki *et al.*, 1983) or DALA (Phillips *et al.*, 1983), and pretreatment with the DA antagonist pimozide blocks place preferences produced by systemically administered heroin (Bozarth and Wise, 1981b). Destruction of the mesolimbic dopaminergic neurons with 6-hydroxydopamine (6-OHDA) also blocks the development of place preferences from systemically administered heroin (Spyraki *et al.*, 1983). These data implicate dopaminergic neurotransmission in the rewarding effects of opiate administration.

On the other hand, opiate-conditioned place preferences and intravenous opiate self-administration have been observed under

conditions when DA neurotransmission is disrupted. The DA antagonists α-flupenthixol and haloperidol failed to block place preferences produced by systemic morphine injections (Mackey and Van der Kooy, 1985). Further, 6-OHDA lesions of the ascending DA fibers failed to antagonize intravenous heroin and (Pettit *et al.*, 1984) and morphine (Smith *et al.*, 1985; Dworkin *et al.*, 1988) self-administration. In addition, α-flupenthixol decreased intravenous heroin self-administration, but failed to produce compensatory increases in responding (Ettenberg *et al.*, 1982). Thus, while mesolimbic DA neurotransmission is implicated in the rewarding effects of opiates, the question of whether it is an *essential* component in the mediation of opiate reward is a subject of current debate (see Wise, 1978b, 1989; Wise and Bozarth, 1982; Koob and Bloom, 1988; Koob *et al.*, 1989; Koob, 1992).

4.1.3 The Involvement of μ, ∂, and κ Receptors in Opioid Modulation of Mesolimbic Dopaminergic Activity

While it is known that opiates modulate the functioning of mesolimibic DA neurons, and that DA participates in some manner in the rewarding effects of opiates, the receptor mechanisms that underly these effects have not been completely characterized. Recently, investigations into the involvement of μ, ∂, and κ opioid receptors in opiate-modulation of mesolimbic dopaminergic activity have begun. Intracerebroventricular administration of

β-endorphin (Spanagel *et al.*, 1990b), or of either of the selective opioid agonists DAMGO or DPDPE (Spanagel *et al.*, 1990a) results in significant elevations in extracellular NAcc DA and DA metabolite concentrations. β-Endorphin-induced increases are antagonized by icv pretreatment with either the selective ∂ antagonist ICI 174,864 or the selective μ antagonist CTOP (Spanagel *et al.*, 1990b). DAMGO-induced increases are blocked by pretreatment with icv CTOP, but not by pretreatment with ICI 174,864, whereas DPDPE-induced increases are blocked by icv pretreatment with ICI 174,864, but not by pretreatment with CTOP (Spanagel *et al.*, 1990a). On the other hand, icv administration of the κ agonist E-2078 (a stable dynorphin analog) significantly decreases extracellular ventral striatal DA and DA metabolite concentrations. This effect is blocked by pretreatment with the κ antagonist nor-binaltorphimine (nor-BNI: Spanagel *et al.*, 1990a). Thus, it appears likely that μ and ∂ opioid receptors may each participate in facilitation of mesolimbic DA neurotransmission, while κ receptors appear to exert an inhibitory input to DA transmission. However, the anatomical localization of these receptor-selective effects are not identified by these experiments with icv opiate injections. One experiment has addressed the possibility that μ opioid receptors in the VTA contribute to modulation of mesolimbic dopaminergic activity. Microinjections of DAMGO into the VTA produced dose-orderly increases in extracellular DA and DA metabolite concentrations in the NAcc, and these increases were antagonized by VTA

microinjections of CTOP (Spanagel *et al.*, 1992). The roles of VTA ∂ and κ receptors have not been examined.

The present experiments were designed to evaluate the involvement of VTA μ, ∂, and κ opioid receptors in modulation of mesolimbic DA activity. Microdialysis and HPLC were used to assay extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA. Responses to VTA microinjections of selective opioid agonists, and to combinations of selective opioid agonists and antagonists were evaluated.

4.2 Methods

4.2.1 Animals and Surgery

Ninety male Long-Evans rats (Charles River, Boston, MA) weighing 350-450 grams were implanted under sodium pentobarbital anaesthesia (65 mg/kg, ip) with 20 gauge guide cannulae terminating approximately 1.0 mm above the NAcc (AP +3.4, ML +2.9, DV -4.3) and with 22 gauge guide cannulae terminating 1.0 mm above the ipsilateral VTA (AP -3.2, ML +2.2, DV -7.2). Surgery was performed with the incisor bar set 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. (All measurements in the dorsal-ventral plane refer to distances along the track at 10° from the vertical.) Stainless steel obturators (28 gauge) extending 4.2 mm (NAcc) or 1.1 mm (VTA) beyond the tip of each

guide cannula were put in place at the time of surgery and removed at the time of testing.

4.2.2 Drugs

DAMGO and DPDPE were purchased from Sigma Chemical Co. (St Louis, MO). U-50,488H (trans-(dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclo-hexyl]-benzeneacetamide) methane sulfonate hydrate; a selective κ agonist: Lahti *et al.*, 1982, 1985; von Voightlander *et al.*, 1983; Hruby and Gehrig, 1989) was a gift from The Upjohn Company (Kalamazoo, MI; courtesy of Dr. P. F. Von Voightlander). CTOP (D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; a selective μ antagonist: Pelton *et al.*, 1986; Kramer *et al.*, 1989) was purchased from Peninsula Laboratories Inc. (Belmont, CA), and naltrindole (17-cyclopropylmethyl-6,7-dehydro-4,5-epoxy-3,14-dihydroxy-6,7,2',3'-indolmorphinan hydrochloride; a selective ∂ antagonist: Portoghese *et al.*, 1988; Rogers *et al.*, 1990; Sofuoglu *et al.*, 1990; Portoghese, 1991) was purchased from Research Biochemicals Inc. (Natick, MA). Each drug was dissolved in aECF and injected into the VTA via a 28 gauge stainless steel injector that extended 1 mm beyond the guide cannula. Injections were delivered in a volume of 1.0 μl over 92 seconds using a 1.0 μl glass syringe driven by a syringe pump. Injectors were left in place for 3 minutes after injection to limit drug efflux up the cannula shaft. U-50,488H was dissolved in physiological saline and injected ip in six animals.

4.2.3 Microdialysis Probes

Removable microdialysis probes were constructed as previously described (see Section 3.2.3).

4.2.4 Microdialysis Procedure

On test days (at least 5 days after surgery) the animals were anaesthetized with chloral hydrate (400 mg/kg, ip) and given atropine sulphate (0.5 mg/kg, ip) to reduce salivation and mucous secretions. Supplemental doses (approximately 20 mg) of chloral hydrate were given when signs of arousal were observed (approximately once per hour). A microdialysis probe was inserted into the ventral striatum and was perfused with aECF at a flow rate of 2 µl/min. Dialysate samples were collected at 20 minute intervals until a stable baseline was obtained (4 consecutive DA and DOPAC peak areas which varied by less than 10%, approximately 4-5 hours after probe insertion).

4.2.4.1 Experiment 1

Fifty four rats were randomly assigned to drug treatment groups (6 rats/drug dose) and microinjected in the VTA with DAMGO (1.32×10^{-12} moles, 1.32×10^{-11}, or 1.32×10^{-10} moles), DPDPE (1.32×10^{-10} moles, 1.32×10^{-9} moles, or 1.32×10^{-8} moles), U-50,488H (1.32×10^{-9} moles, or 1.32×10^{-8} moles), or aECF vehicle. Dialysate samples were collected for four hours

after the injections. Six of the rats were administered U-50,488H systemically (10 mg/kg, ip) four hours after their VTA injection of U-50,488H, and dialysate samples were collected for an additional hour. Finally, each rat was perfused with physiological saline followed by 10% formalin. Each brain was removed immediately, stored in formalin for one or more days, frozen and sliced in 40 μm coronal sections, and stained with thionin for localization of cannula and probe tracks.

4.2.4.2 Experiment 2

Thirty six rats were randomly assigned to drug treatment groups and microinjected with either the ∂ opioid receptor antagonist naltrindole (3.0×10^{-9} moles) (n = 18), or the μ receptor antagonist CTOP (3.0×10^{-10} moles) (n = 18) in the VTA. Dialysate samples were collected for an 1 hour after antagonist injections. Then, the rats in each antagonist pretreatment group were given VTA microinjections of DAMGO (1.32×10^{-11} moles), DPDPE (1.32×10^{-9} moles), or aECF vehicle (n = 6/drug combination). Doses of DAMGO and DPDPE were selected from Experiment 1 to yield clearly identifiable increases in extracellular ventral striatal DA and DOPAC concentrations, at minimal effective doses. Dialysate samples were collected for an additional four hours. Finally, the rats were perfused and brains were examined as in the earlier experiment.

4.2.5 Analytical Procedure

Dialysate was assayed for DA, DOPAC, HVA, and 5-HIAA as previously described (see Section 3.2.5).

4.2.6 Statistics

4.2.6.1 Experiment 1

Between-groups differences in basal DA, DOPAC, HVA, and 5-HIAA measures were evaluated using 9 (group) x 4 (sample) repeated-measures analyses of variance (ANOVAs), which were calculated using raw (pg/40 µl) scores. Scores were then transformed to percents of each rat's baseline mean, and 4 (drug dose) x 12 (sample) repeated-measures ANOVAs were calculated to assess the effects of each of the three drugs for each neurochemical measure (DA, DOPAC, HVA, and 5-HIAA). Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the drug-treated and aECF-treated groups. All significant drug effects were further analysed with Tukey's tests, comparing values for each post-injection sample, at each dose of a given agonist, with the corresponding sample for the aECF control group. Finally, effects of ip injections of U-50,488H were evaluated with 2 x 3 repeated-measures ANOVAs, and significant effects were analysed with Tukey's tests, comparing values for each post-ip

injection sample with the final post-VTA injection sample for each neurochemical measure.

4.2.6.2 Experiment 2

Between-groups differences in basal DA, DOPAC, HVA, and 5-HIAA scores were evaluated using 6 (group) x 4 (sample) repeated-measures ANOVAs, which were calculated using raw (pg/40 µl) baseline scores. Scores were then transformed to percents of each rat's baseline mean. The effects of opioid antagonists (naltrindole or CTOP) on each neurochemical measure, and between-groups differences in response to each of the antagonists were evaluated with 3 (group) x 6 (sample) repeated-measures ANOVAs. Finally, 3 (group) x 12 (sample) repeated-measures ANOVAs were calculated for each of the opioid antagonists to determine the effects of selective agonists after antagonist pretreatments. Again, treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the drug treated and aECF treated groups. Effects of agonists after antagonist-pretreatment were further analysed with Tukey's tests, comparing values for each post-injection sample of either agonist with the corresponding sample for the aECF control group.

4.3 Results

4.3.1 Experiment 1

Extracellular ventral striatal DA concentrations were reliably increased in a dose-orderly fashion after VTA microinjections of DAMGO (1.32×10^{-12} to 1.32×10^{-10} moles; Fig. 4.1). Extracellular ventral striatal DOPAC and HVA concentrations were elevated in a dose-orderly fashion after VTA microinjections of the two higher doses of DAMGO (Fig. 4.2a, 4.2b).

VTA microinjections of DPDPE also produced dose-orderly increases in ventral striatal DA concentrations. However, the effective range of doses of DPDPE (1.32×10^{-10} to 1.32×10^{-8} moles) was 100-1000 times higher than the effective range of DAMGO doses (Fig. 4.3). DOPAC and HVA concentrations were elevated in dose-orderly fashion after VTA microinjections of the two higher doses of DPDPE (Fig. 4.4a, 4.4b).

Ventral striatal DA concentrations were not reliably altered by VTA U-50,488H at doses of 1.32×10^{-9} or 1.32×10^{-8} moles (Fig. 4.5) or at any of the lower doses that were tested (data not shown). VTA microinjections of U-50,488H also failed to alter DOPAC and HVA concentrations (Fig. 4.6a, 4.6b). Conversely, subsequent systemic injections of U-50,488H (10 mg/kg) reliably decreased ventral striatal DA (Fig. 4.5), DOPAC and HVA concentrations (Fig. 4.6a, 4.6b).

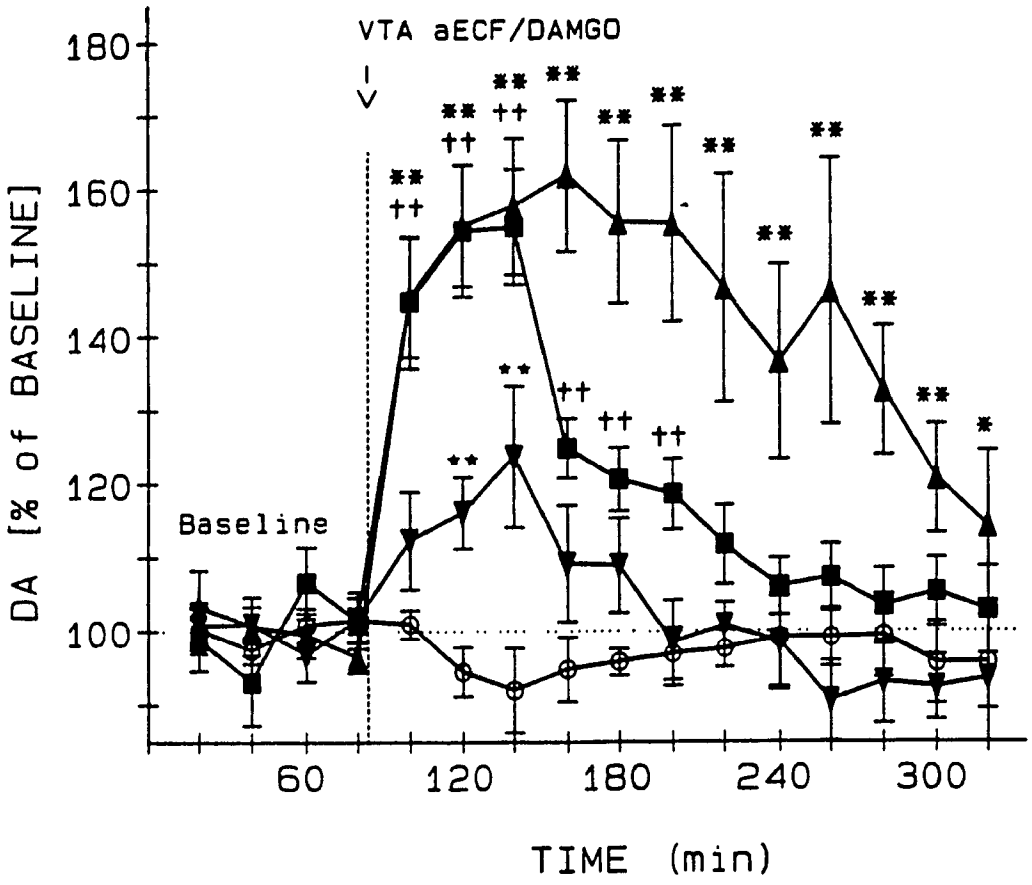

Fig. 4.1. Dose-response curves for DAMGO-induced alterations in ventral striatal DA concentrations. VTA DAMGO produced significant increases in DA concentrations ($F_{(33,220)} = 3.25$, $p < .01$). Values expressed are group means ± SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-12} moles/µl (▼), VTA DAMGO at 1.32×10^{-11} moles/µl (■), VTA DAMGO at 1.32×10^{-10} moles/µl (▲). Significant differences in concentrations between drug treated and control rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for high dose; †† $p < .01$ for medium dose; ★★ $p < .01$ for low dose.

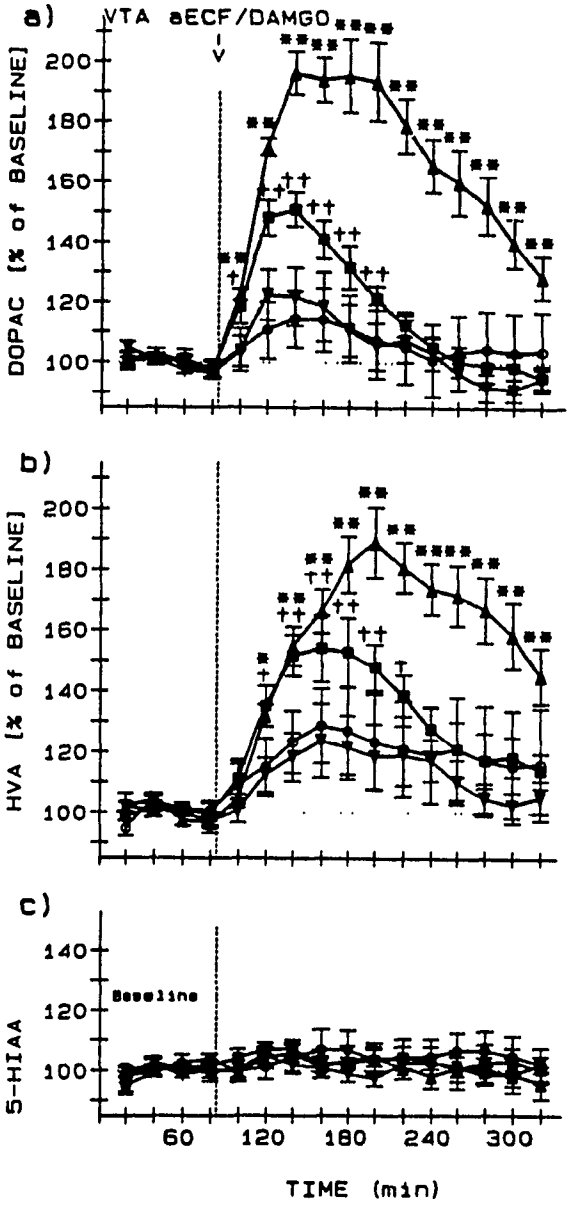

Fig. 4.2. Dose-response curves for DAMGO-induced alterations in ventral striatal **a)** DOPAC, **b)** HVA, and **c)** 5-HIAA concentrations. VTA DAMGO produced significant increases in DOPAC ($F_{(33,220)} = 8.54$, $p < .01$) and HVA ($F_{(33,220)} = 4.61$, $p < .01$) concentrations, but did not alter 5-HIAA concentrations ($F_{(3,20)} = 0.22$, $p > .05$). Values expressed are group means ± SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-12} moles/µl (▼), VTA DAMGO at 1.32×10^{-11} moles/µl (■), VTA DAMGO at 1.32×10^{-10} moles/µl (▲). Significant differences in concentrations between drug treated and control rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for high dose; † $p < .05$, †† $p < .01$ for medium dose.

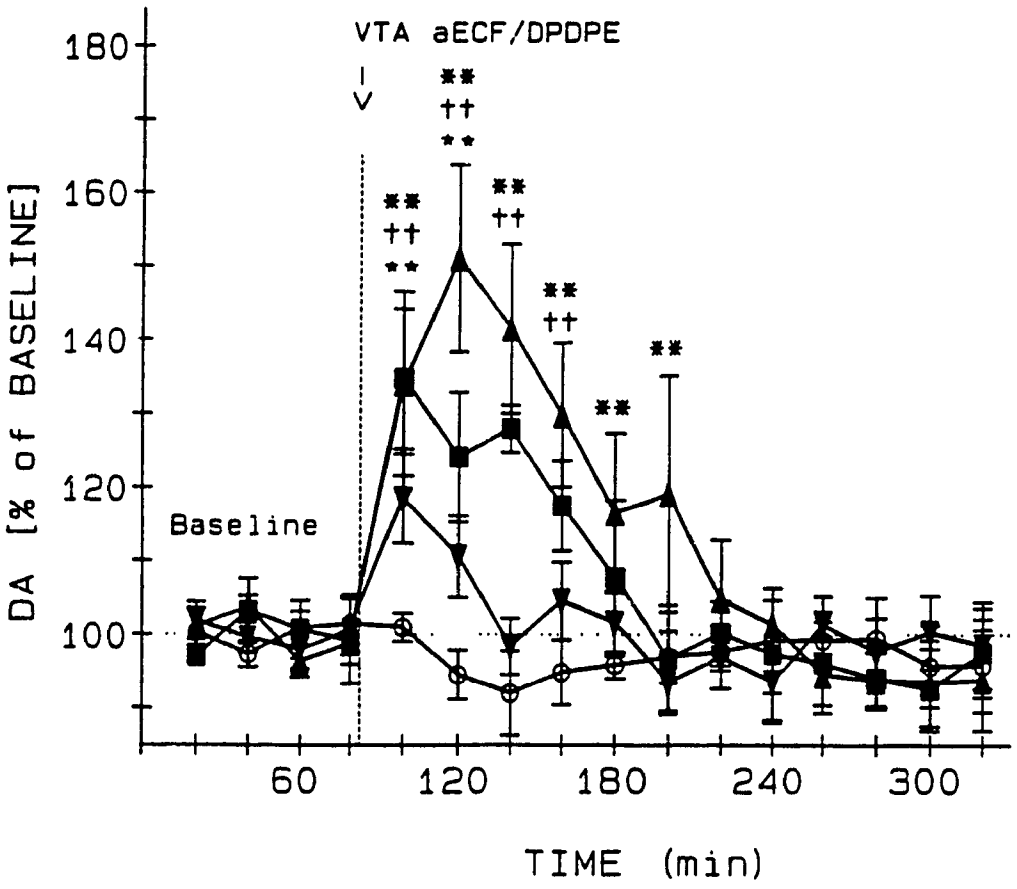

Fig. 4.3. Dose-response curves for DPDPE-induced alterations in ventral striatal DA concentrations. VTA DPDPE produced significant increases in DA concentrations ($F_{(33,220)} = 4.20$, $p < .01$). Values expressed are group means ± SEM; VTA aECF (○), VTA DPDPE at 1.32 x 10^{-10} moles/µl (▼), VTA DPDPE at 1.32 x 10^{-9} moles/µl (■), VTA DPDPE at 1.32 x 10^{-8} moles/µl (▲). Significant differences in concentrations between drug treated and control rats for each sample (Tukey's tests) are depicted as follows: ✱✱ $p < .01$ for high dose; †† $p < .01$ for medium dose; ★★ $p < .01$ for low dose.

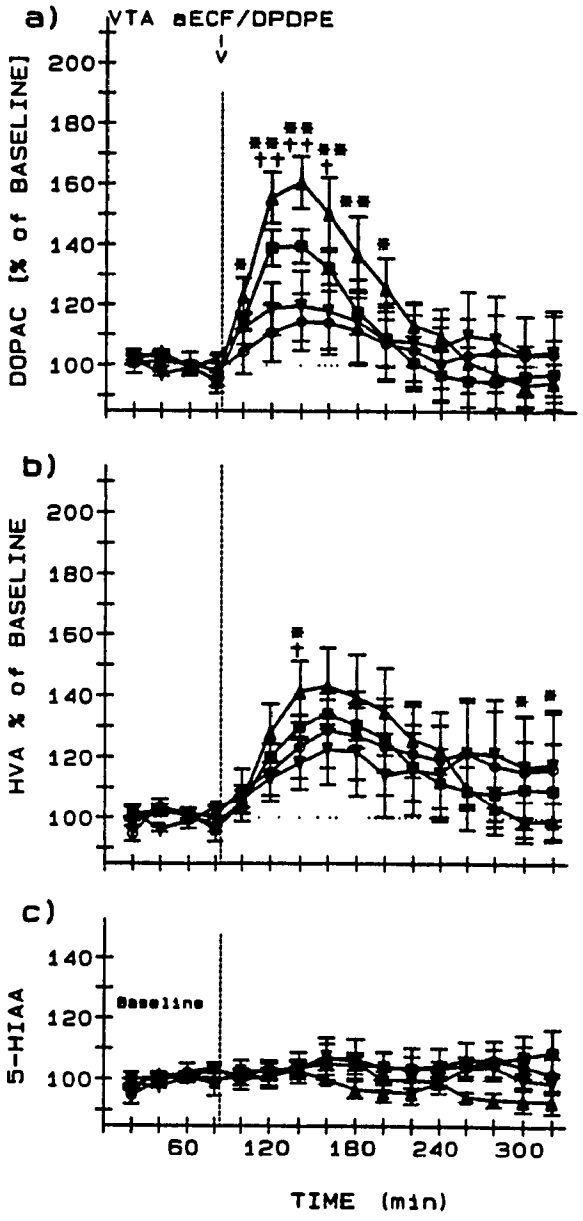

Fig. 4.4. Dose-response curves for DPDPE-induced alterations in ventral striatal **a)** DOPAC, **b)** HVA, and **c)** 5-HIAA concentrations. VTA DPDPE produced significant increases in DOPAC ($F_{(33,220)} = 4.98$, $p < .01$) and HVA ($F_{(33,220)} = 1.61$, $p < .05$) concentrations, but did not significantly alter 5-HIAA concentrations ($F_{(3,20)} = 1.07$, $p > .05$). Values expressed are group means ± SEM; VTA aECF (O), VTA DPDPE at 1.32×10^{-10} moles/µl (▼), VTA DPDPE at 1.32×10^{-9} moles/µl (■), VTA DPDPE at 1.32×10^{-8} moles/µl (▲). Significant differences in concentrations between drug treated and control rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for high dose; † $p < .05$, †† $p < .01$ for medium dose.

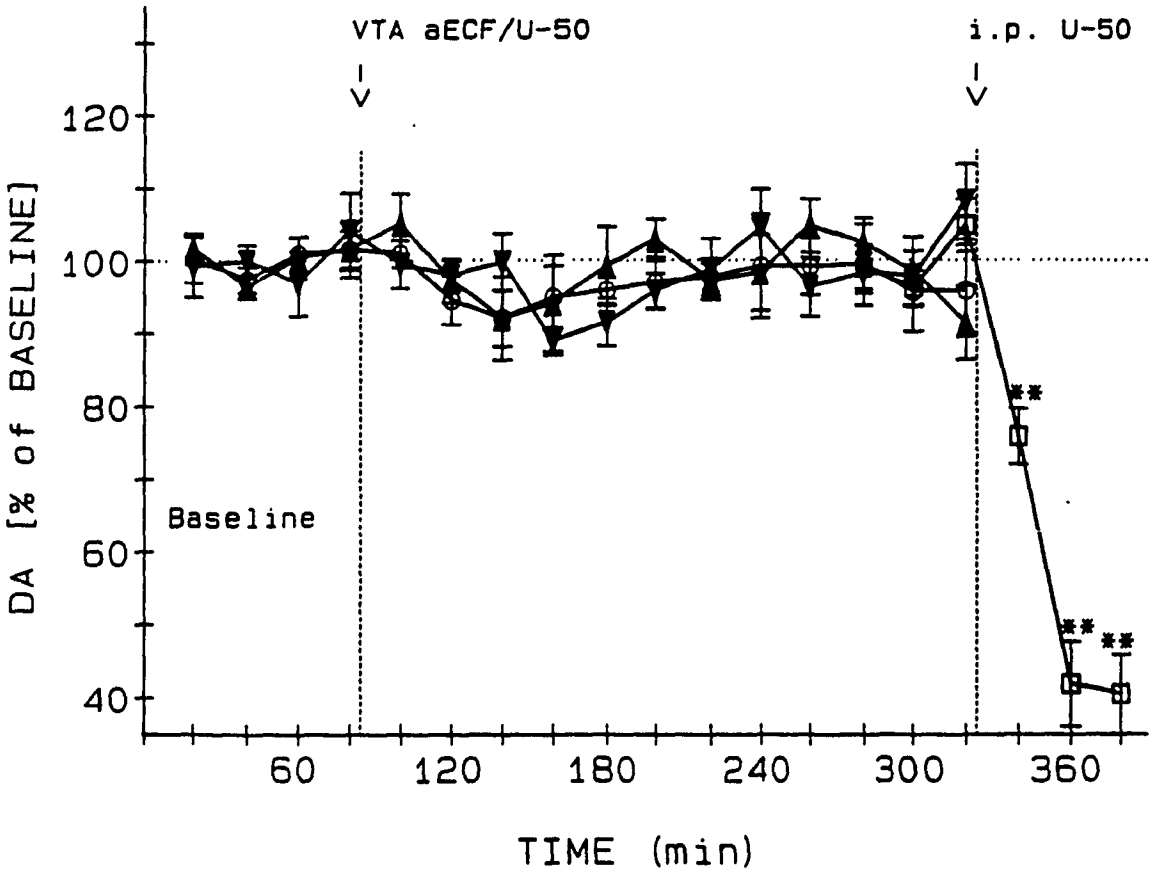

Fig. 4.5. Dose-response curves for U-50,488H-induced alterations in ventral striatal DA concentrations. VTA U-50,488H did not significantly alter DA concentrations ($F_{(2,15)} = 0.11$, $p > .05$). U-50,488H administered ip produced significant decreases in DA concentrations ($F_{(2,20)} = 14.54$, $p < .01$). Values expressed are group means ± SEM; VTA aECF (○), VTA U-50,488H at 1.32×10^{-9} moles/µl (▼), VTA U-50,488H at 1.32×10^{-8} moles/µl (▲), ip U-50,488H at 2.15×10^{-5} moles/kg (□). Significant differences in concentrations from the final sample prior to systemic U-50,488H for each post-systemic injection sample (Tukey's tests) are depicted as follows: ** $p < .01$.

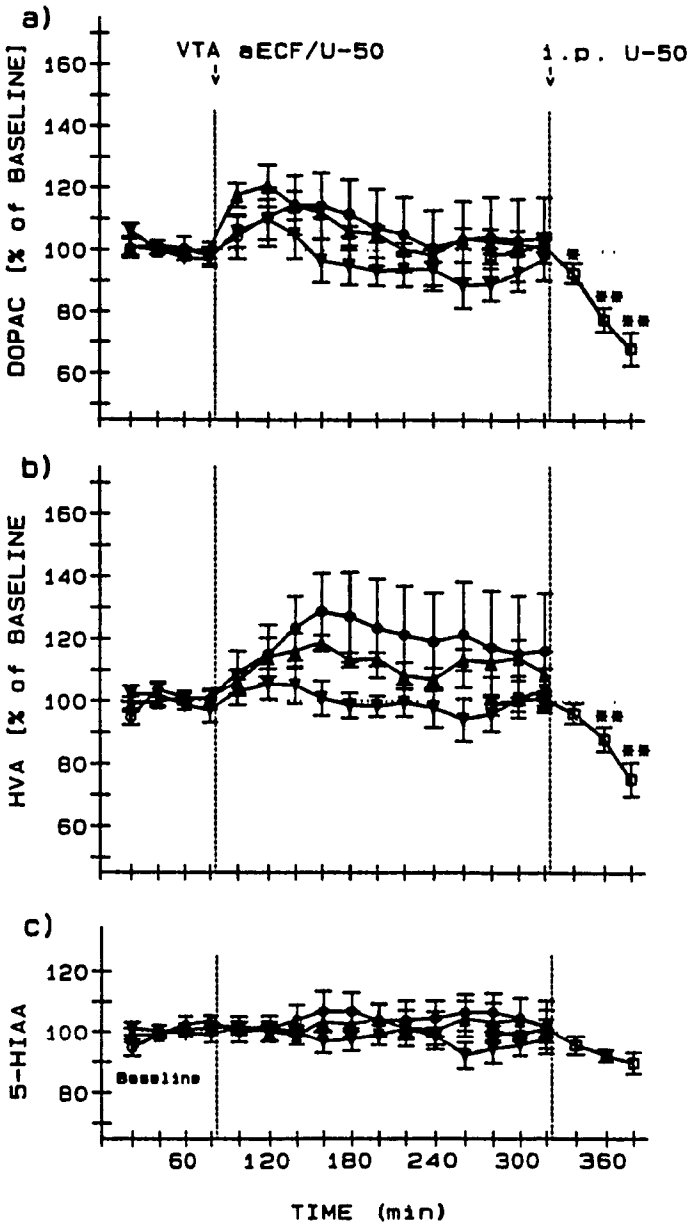

Fig. 4.6. Dose-response curves for U-50,488H-induced alterations in ventral striatal **a)** DOPAC, **b)** HVA, and **c)** 5-HIAA concentrations. VTA U-50,488H did not significantly alter DOPAC ($F_{(2,15)} = 0.69$, $p > .05$), HVA $F_{(2,15)} = 1.39$, $p > .05$), or 5-HIAA concentrations ($F_{(2,15)} = 1.39$, $p > .05$). U-50,488H administered ip produced significant decreases in ventral striatal: DOPAC ($F_{(2,20)} = 11.24$, $p < .01$), and HVA concentrations ($F_{(2,20)} = 6.58$, $p < .01$), but did not significantly alter 5-HIAA concentrations ($F_{(2,20)} = 0.76$, $p > .05$). Values expressed are group means ± SEM; VTA aECF (○), VTA U-50,488H at 1.32×10^{-9} moles/µl (▼), VTA U-50,488H at 1.32×10^{-8} moles/µl (▲), ip U-50,488H at 2.15×10^{-5} moles/kg (□). Significant differences in concentrations from the final sample prior to systemic U-50,488H for each post-systemic injection sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$.

Extracellular ventral striatal 5-HIAA concentrations were not reliably altered by VTA aECF, DAMGO, DPDPE or U-50,488H at any dose tested (Fig. 4.2, 4.4, 4.6). Further, there were no reliable alterations in 5-HIAA concentrations after ip injections of U-50,488H (Fig. 4.6).

Baseline concentrations of extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups (Table 4.1).

	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	3.134 ± 0.107	1.3556	8,45	>.05
DOPAC	406.1 ± 11.36	1.0497	8,45	>.05
HVA	361.7 ± 9.524	1.1739	8,45	>.05
5-HIAA	91.58 ± 2.117	0.8641	8,45	>.05

Table 4.1. Baseline means and standard errors for DA and metabolites for 9 treatment groups combined (3 doses of DAMGO, 3 doses of DPDPE, 2 doses of U-50,488H, and aECF controls), and tests of significance for between-groups differences in baseline means.

4.3.2 Experiment 2

VTA microinjections of naltrindole (3.0×10^{-9} moles) decreased extracellular ventral striatal DA concentrations in the three samples following the drug injection. These decreases were consistent between groups that were subsequently treated with aECF, DAMGO or DPDPE (Fig. 4.7). Surprisingly, these decreases in

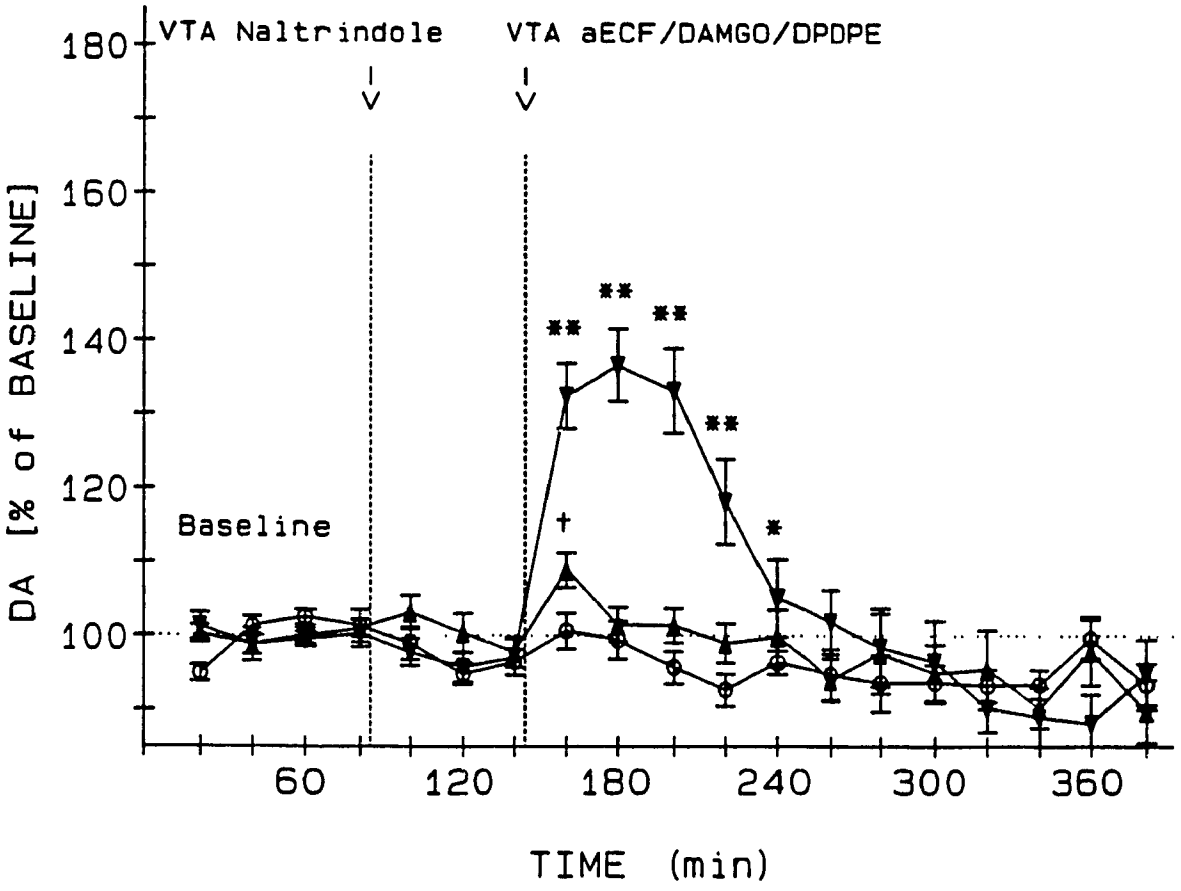

Fig. 4.7. Effects of aECF, DAMGO and DPDPE on ventral striatal DA concentrations after pretreatment with naltrindole. VTA naltrindole produced significant decreases in DA concentrations ($F_{(5,75)} = 2.48$, $p < .05$). One hour after naltrindole, aECF, DAMGO and DPDPE differentially affected DA concentrations ($F_{(22,165)} = 9.28$, $p < .01$). Values expressed are group means ± SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-11} moles/µl (▼), VTA DPDPE at 1.32×10^{-9} moles/µl (▲). Significant differences in concentrations between agonist treated and aECF control rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for DAMGO; † $p < .05$ for DPDPE.

DA concentrations were accompanied by increases in ventral striatal DOPAC and HVA concentrations (Fig. 4.8a, 4.8b), that were equivalent between groups.

In naltrindole pretreated rats, VTA DAMGO reliably increased ventral striatal DA concentrations in the first five samples collected after the VTA microinjections of DAMGO. VTA DPDPE increased DA concentrations during only the first 20 minute sampling interval (Fig. 4.7). In naltrindole pretreated rats, ventral striatal DOPAC and HVA concentrations were also increased after VTA microinjections of DAMGO. DOPAC and HVA concentrations were not reliably altered by VTA DPDPE after naltrindole pretreatment (Fig. 4.8a, 4.8b). Ventral striatal 5-HIAA concentrations were not reliably affected by VTA naltrindole or combinations of naltrindole with DAMGO or DPDPE (Fig. 4.8c).

VTA microinjections of CTOP (3.0×10^{-10} moles) produced a reliable increase in ventral striatal DA concentrations during the three samples following the drug injection. This increase was consistent between groups that were subsequently treated with aECF, DAMGO or DPDPE (Fig. 4.9). DOPAC and HVA concentrations were also increased by VTA microinjections of CTOP (Fig. 4.10), and again the increases were consistent between groups.

In CTOP pretreated rats ventral striatal DA concentrations were reliably increased in the first five samples collected after the VTA microinjections of DPDPE. Conversely, DA concentrations were not reliably altered by VTA DAMGO (Fig. 4.9). In CTOP pretreated rats concentrations of ventral striatal DOPAC and HVA were also elevated following VTA DPDPE, while DOPAC and HVA

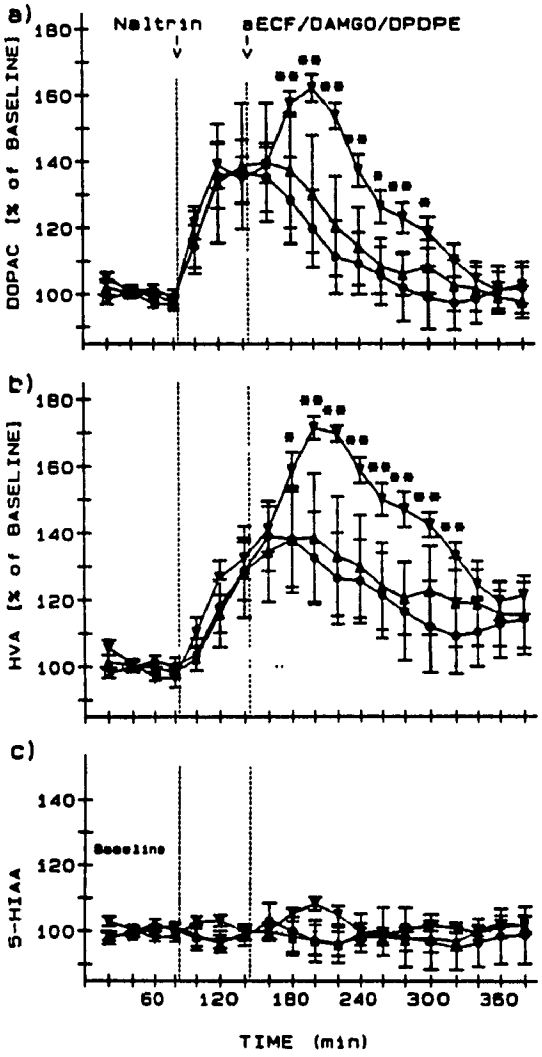

Fig. 4.8. Effects of aECF, DAMGO and DPDPE on ventral striatal **a)** DOPAC, **b)** HVA, and **c)** 5-HIAA concentrations after pretreatment with naltrindole. VTA naltrindole produced significant increases in DOPAC ($F_{(5,75)} = 22.66$, $p < .01$) and HVA ($F_{(5,75)} = 21.81$, $p < .01$) concentrations, but did not significantly alter 5-HIAA concentrations ($F_{(5,75)} = 0.23$, $p > .05$). One hour after naltrindole, aECF, DAMGO and DPDPE differentially affected DOPAC ($F_{(22,165)} = 1.87$, $p < .01$) and HVA concentrations ($F_{(22,165)} = 1.90$, $p < .01$); but did not significantly alter 5-HIAA concentrations ($F_{(22,165)} = 0.84$, $p > .05$). Values expressed are group means ±SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-11} moles/µl (▼), VTA DPDPE at 1.32×10^{-9} moles/µl (▲). Significant differences in concentrations between agonist treated and aECF control rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for DAMGO.

concentrations were unaffected by VTA DAMGO in CTOP pretreated rats (Fig. 4.10a, 4.10b). Ventral striatal 5-HIAA concentrations were not reliably affected by VTA CTOP, or by combinations of CTOP with DAMGO or DPDPE (Fig. 4.10c).

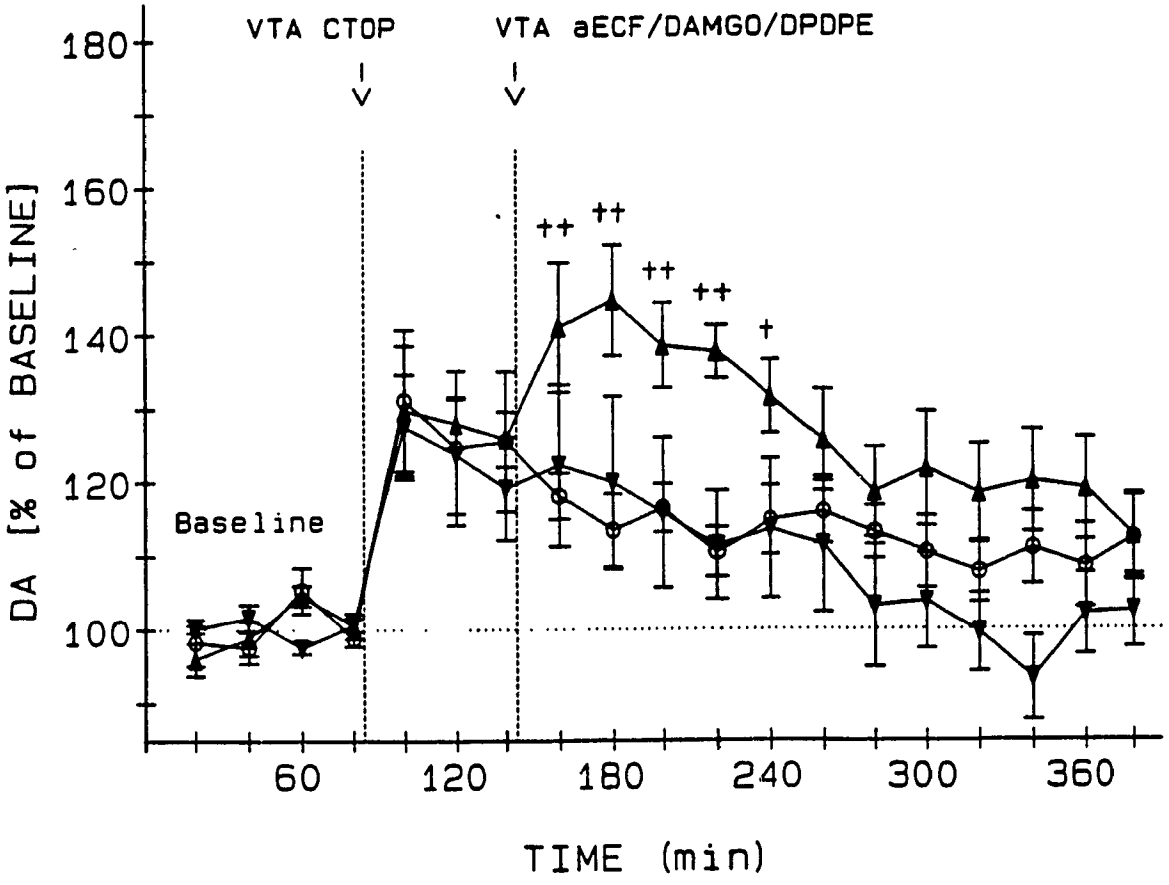

Fig. 4.9. Effects of aECF, DAMGO and DPDPE on ventral striatal DA concentrations after pretreatment with CTOP. VTA CTOP produced significant increases in DA concentrations ($F_{(5,75)} = 26.06$, $p < .01$). One hour after CTOP, aECF, DAMGO and DPDPE differentially affected DA concentrations ($F_{(22,165)} = 1.95$, $p < .01$). Values expressed are group means ± SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-11} moles/µl (▼), VTA DPDPE at 1.32×10^{-9} moles/µl (▲). Significant differences in concentrations between agonist treated and aECF control rats for each sample (Tukey's tests) are depicted as follows: + $p < .05$, ++ $p < .01$ for DPDPE.

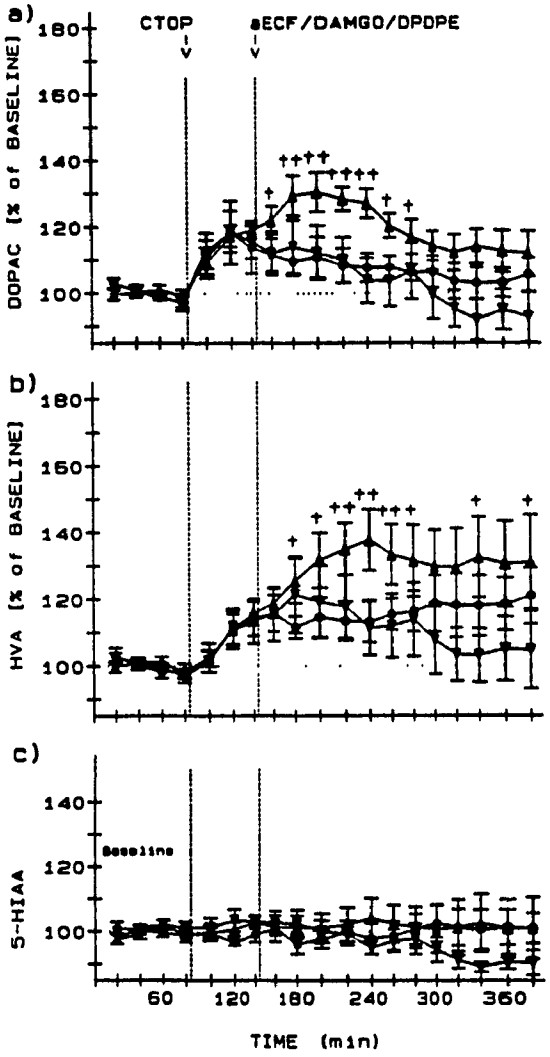

Fig. 4.10. Effects of aECF, DAMGO and DPDPE on ventral striatal **a)** DOPAC, **b)** HVA, and **c)** 5-HIAA concentrations after pretreatment with CTOP. VTA CTOP produced significant increases in DOPAC ($F_{(5,75)} = 18.60$, $p < .01$) and HVA ($F_{(5,75)} = 13.56$, $p < .01$) concentrations, but did not significantly alter 5-HIAA concentrations ($F_{(5,75)} = 0.50$, $p > .05$). One hour after CTOP, aECF, DAMGO and DPDPE differentially affected DOPAC ($F_{(22,165)} = 2.43$, $p < .01$) and HVA concentrations ($F_{(22,165)} = 1.83$, $p < .01$); but did not significantly alter 5-HIAA concentrations ($F_{(22,165)} = 1.2027$, $p > .05$). Values expressed are group means ± SEM; VTA aECF (○), VTA DAMGO at 1.32×10^{-11} moles/µl (▼), VTA DPDPE at 1.32×10^{-9} moles/µl (▲). Significant differences in concentrations between agonist treated and aECF control rats for each sample (Tukey's tests) are depicted as follows: † $p < .05$, †† $p < .01$ for DPDPE.

Baseline concentrations of extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups (Table 4.2).

	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	3.336 ± 0.142	1.0587	5,30	>.05
DOPAC	460.0 ± 14.48	1.5464	5,30	>.05
HVA	464.2 ± 11.30	1.1093	5,30	>.05
5-HIAA	93.20 ± 1.774	1.7261	5,30	>.05

Table 4.2. Baseline means and standard errors for DA and metabolites for 6 treatment groups combined (CTOP + DAMGO, DPDPE, or aECF controls; naltrindole + DAMGO, DPDPE, or aECF controls), and tests of significance for between-groups differences in baseline means.

Injector tips were located in the VTA in each experiment. The dialyzing surface (approx. 3 mm) of microdialysis probes were localized in the medial NAcc, near the anterior commissure, with a small portion in the ventral aspect of the caudate nucleus. Placements of ventral striatal microdialysis probes and VTA injector tips are depicted in Fig. 4.11. There were no apparent

between-groups differences in the locations of ventral striatal probe tracks or in the locations of VTA injector tracks.

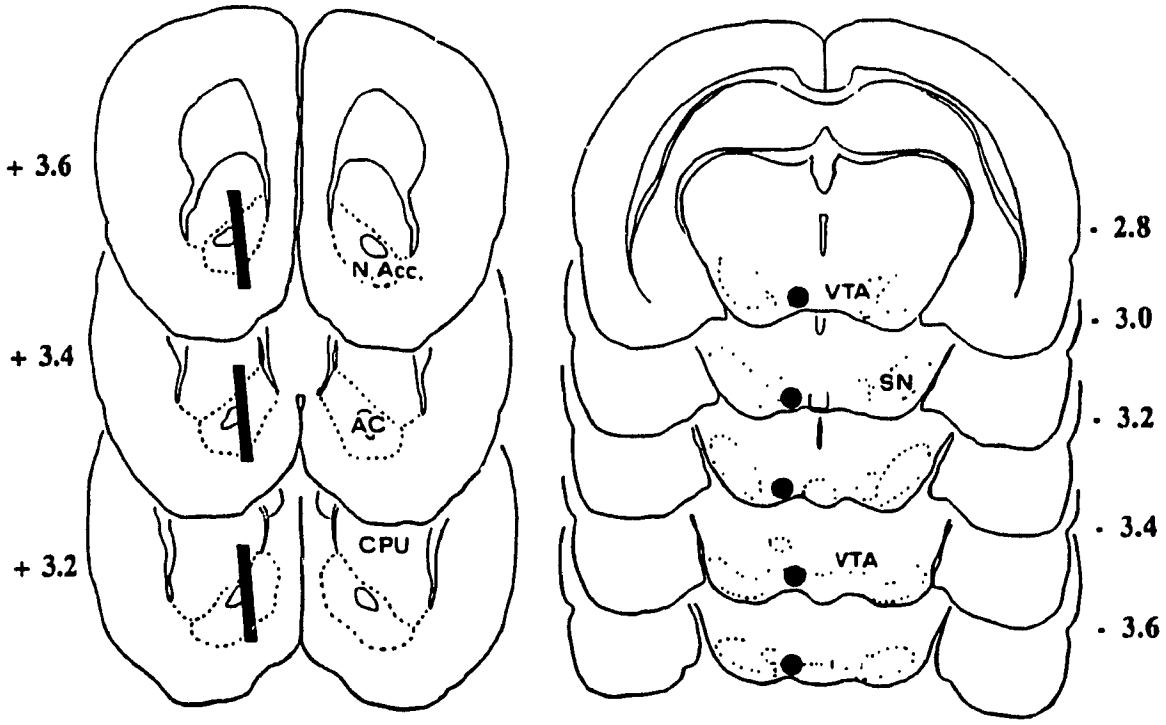

Fig. 4.11. Histological reconstruction of a representative microdialysis probe track in the ventral striatum and the area wherein injector tips were identified in the VTA. Abbreviations used: CPU = caudate putamen; NAcc = nucleus accumbens; AC = anterior commissure; SN = substantia nigra; VTA = ventral tegmental area (adapted from Pellegrino *et al.*, 1979).

4.4 Discussion

4.4.1 The Roles of VTA μ and ∂ Receptors in Modulation of Mesolimbic DA

The fact that VTA microinjections of highly selective μ and ∂ opioid agonists caused dose-orderly increases in extracellular ventral striatal DA, DOPAC, and HVA concentrations suggests that VTA μ and ∂ receptors each play a role in modulation of basal mesolimbic dopaminergic activity. This conclusion is further supported by the finding that these increases were blocked by the appropriate and highly selective μ and ∂ antagonists.

4.4.2 The Mechanism of VTA μ Receptor-Mediated Modulation of Mesolimbic DA

Antagonism by VTA CTOP of VTA DAMGO-mediated increases in ventral striatal DA and DA metabolite concentrations indicates that these consequences of VTA DAMGO administration were exerted through actions on VTA μ receptors. These data concur with the recent report that icv microinjections of CTOP antagonized icv DAMGO-induced increases in extracellular NAcc DA and DA metabolite concentrations (Spanagel *et al.*, 1990a). Further, the failure of VTA microinjections of naltrindole to antagonize DAMGO-mediated increases in ventral striatal DA and DA metabolite concentrations (at a dose that was sufficient to

antagonize DPDPE-mediated increases in DA and DA metabolite concentrations) indicates that DAMGO did not exert its effects through actions on VTA ∂ receptors.

The mechanism by which VTA DAMGO increases ventral striatal DA and DA metabolite concentrations has been well characterized, and evidence has accumulated that the relevant μ receptor population resides on VTA GABAergic neurons in the VTA. Accordingly, there appears to be a dense population of μ receptors in the VTA (see Section 1.4.1), as well as a substantial enkephalinergic innervation (Johnson *et al.*, 1980), which appears to originate (at least in part) in the NAcc (Groenewegen and Russchen, 1984; Troiano and Siegel, 1978a) and ventral pallidum (Troiano and Siegel, 1978a; Groenewegen and Russchen, 1984; Haber *et al.*, 1985; Zahm, 1989). This innervation provides an endogenous source of opioids that may act upon the VTA μ receptors (and probably also on the VTA ∂ receptors).

Kelley *et al.* (1980) proposed that opioid agonist-induced increases in the firing rates of dopaminergic fibers result from an indirect, disinhibitory action in the VTA. In support of this hypothesis, ventral mesencephalic 6-OHDA lesions fail to alter VTA [^{125}I]DAMGO binding, but quinolinic acid lesions of this area substantially decrease this binding (Dilts and Kalivas, 1989). Thus, VTA μ receptors appear to reside on VTA interneurons, rather than the dopaminergic cells themselves. DAMGO leads to a hyperpolarization of non-dopaminergic neurons in VTA slice preparations, and met-enkephalin reduces the γ-aminobutyric acid (GABA) components of VTA evoked synaptic potentials (Johnson

and North, 1992), indicating that these interneurons are GABAergic. In fact, an inhibitory modulation of dopaminergic activity by VTA GABA has been found. Microiontophoretic application of GABA in the VTA increases spike height of dopaminergic neurons while decreasing impulse flow, which is thought to be a consequence of DA membrane hyperpolarization (Matthews and German, 1984). These effects of GABA on dopaminergic neurons appear to be mediated through $GABA_B$ receptors located directly on the dopaminergic cells (Pinnock, 1984; Lacey *et al.*, 1988). On the other hand, $GABA_A$ agonists appear to act presynaptically on GABAergic neurons, producing an inhibition of these neurons, to yield a net disinhibition of dopaminergic neurons (Grace and Bunney, 1979). Recently, Kalivas *et al.* (1990) found that VTA microinjections of the $GABA_B$ agonist baclofen antagonized increases in NAcc DA from VTA microinjections of DAMGO, providing further evidence that VTA DAMGO mediates a disinhibition of mesolimbic dopaminergic activity through a GABA-mediated mechanism.

4.4.3 The Mechanism of VTA ∂ Receptor-Mediated Modulation of Mesolimbic DA

While the mechanism of VTA DAMGO-mediated increases in mesolimbic dopaminergic activity has been well characterized, the mechanism of action of VTA DPDPE is less clear. As discussed in the case of intracranial self-administration (see Section 2.4.2), the differential potencies of VTA DAMGO and DPDPE could possibly

indicate cross-reactivity of DPDPE with VTA μ receptors. In light of this possibility, the ∂ receptor selectivity of VTA DPDPE was evaluated. Antagonism by VTA naltrindole of VTA DPDPE-mediated increases in ventral striatal DA and DA metabolite concentrations demonstrates that these consequences of VTA DPDPE administration were exerted through actions on VTA ∂ receptors. Further, the failure of VTA CTOP to antagonize DPDPE-mediated increases in ventral striatal DA and DA metabolite concentrations (at a dose that was sufficient to antagonize DAMGO-mediated increases in DA and DA metabolite concentrations) indicates that DPDPE did not significantly cross-react with VTA μ receptors. Taken together, these data suggest that VTA microinjections of DPDPE produce increases in ventral striatal DA and DA metabolites through selective actions on VTA ∂ receptors.

However, anatomical and electrophysiological evidence for a population of VTA ∂ receptors capable of mediating the modulation of mesolimbic DA is less clear than it is in the case of μ receptors. Evidence that the VTA ∂ receptor population is sparse has previously been discussed (see Section 1.5). These receptors appear to be localized primarily in the interfascicular and paranigral nuclei of the VTA (Delay-Goyet *et al.*, 1990). Thus, the low density and regional specificity of ∂ receptors in the VTA may contribute to the differential efficacy of VTA μ and ∂ agonists in modulating mesolimbic DA.

Moreover, ∂ receptors appear not to be associated with VTA GABAergic interneurons. Johnson and North (1992) have reported

that DPDPE fails to hyperpolarize non-dopaminergic neurons in VTA slice preparations. Thus, VTA ∂ receptors appear uninvolved in GABA-mediated disinhibition of DA. It would seem that VTA DPDPE modulates the activity of mesolimbic DA neurons through a mechanism quite different from the mechanism activated by DAMGO. VTA μ receptors act on VTA GABAergic interneurons, while VTA ∂ receptors appear to act through another unknown mechanism.

4.4.4 The Actions of μ and ∂ Antagonists after VTA Administration

The increases in ventral striatal DA, DOPAC, and HVA concentrations that were observed after pretreatment with the μ antagonist CTOP in the VTA were quite unexpected, considering that administration of the μ agonist DAMGO in the VTA also produced increases in ventral striatal DA, DOPAC, and HVA concentrations. The reason for these effects of CTOP is unclear. It is possible that CTOP exerts a partial agonist activity in the range of doses that was used. On the other hand, Spanagel *et al.* (1992) found that VTA CTOP produced dose-orderly decreases in NAcc DA and DA metabolite concentrations. The reason for these conflicting findings is not obvious. The effects of VTA CTOP on ventral striatal DA and DA metabolite concentrations are further examined in Chapter 6.

Pretreatment with VTA naltrindole produced slight decreases in ventral striatal DA concentrations, coupled with

substantial increases in ventral striatal DOPAC and HVA concentrations. Again, the reason for these effects is not clear, but they do not appear to have resulted from injection-induced hydraulic pressure or mechanical damage, as vehicle injections did not produce these effects.

4.4.5 The Mechanism of κ Receptor-Mediated Modulation of Mesolimbic DA

The failure of VTA microinjections of U-50,488H to alter extracellular ventral striatal DA and DA metabolite concentrations suggests that VTA κ receptors do not participate in modulation of basal mesolimbic dopaminergic activity. This conclusion is supported by a recent report that VTA microinjections of U-69,593 fail to affect NAcc DA or DA metabolite concentrations (Spanagel *et al.*, 1992). Further, Johnson and North (1992) have reported that U-50,488H fails to hyperpolarize non-dopaminergic neurons in VTA slice preparations.

However, there does seem to be a κ receptor-mediated input to the mesolimbic dopaminergic system. Di Chiara and Imperato (1988b) found that systemic administration of κ-selective compounds (U-50,488H, bremazocine, trifluadom) result in decreased NAcc DA and DA metabolite concentrations, and these findings are confirmed by the present results. The κ receptor-mediated input to mesolimbic DA appears to reside in the terminal fields of mesolimbic dopaminergic neurons.

Perfusion of the NAcc with U-69,593 during microdialysis decreases extracellular DA and DA metabolite concentrations (Spanagel *et al.*, 1992). Further, electrically-evoked release of [^{3}H]DA is inhibited by dynorphin (Mulder *et al.*, 1989), or bremazocine (Schoffelmeer *et al.*, 1988) in superfused striatal slices, and electrically-evoked release of [^{3}H]DA is inhibited by bremazocine or U-50,488H (Heijna *et al.*, 1990) in punches of NAcc tissue. DAMGO and DPDPE are ineffective in either of these preparations (Schoffelmeer *et al.*, 1988; Mulder *et al.*, 1989; Heijna *et al.*, 1990). K^+-evoked release of [^{3}H]DA is also inhibited by U-50,488H, but not by DAMGO or DPDPE (Werling *et al.*, 1988) in striatal tissue slices, and the above effects are completely antagonized by nor-BNI (Werling *et al.*, 1988; Heijna *et al.*, 1990) or naloxone (Werling *et al.*, 1988). Taken together, the data lead to the conclusion that VTA κ receptors do not participate in modulation of basal mesolimbic dopaminergic activity, but a κ-regulation of mesolimbic dopaminergic activity occurs in the terminal field.

This conclusion is also supported by evidence from anatomical analyses. The autoradiographic distribution of CNS κ receptors (which comprise less than 5% of opioid receptors in the rat brain; Lahti *et al.*, 1985) has been assessed with [^{3}H]ethylketocyclazocine (Tempel and Zukin, 1987) or [^{3}H]bremazocine (Mansour *et al.*, 1987, 1988) in the presence of saturating concentrations of DAMGO and DPDPE, or with [^{125}I-Tyr^1,D-Pro^{10}]dynorphin $A_{(1-11)}$ (Jomary *et al.*, 1988). No

significant κ receptor binding has been identified in the VTA, despite the use of an iodinated ligand. Further, little or no dynorphinergic innervation has been found in the VTA (Weber *et al.*, 1982), in contrast to the dense dynorphinergic innervation of the substantia nigra (SN) (Watson *et al.*, 1982a, 1982b; Weber *et al.*, 1982). On the other hand, a moderate (Jomary *et al.*, 1988) to high (Mansour *et al.*, 1987, 1988; Tempel and Zukin, 1987; Jomary *et al.*, 1988) density of κ receptor binding has been observed in the caudate putamen and NAcc, which coincides with a substantial dynorphinergic innervation (Watson *et al.*, 1982a, 1982b; Weber *et al.*, 1982).

4.4.6 Summary and Conclusions

In summary, evidence has been presented for VTA μ and ∂ receptor involvement in modulation of mesolimbic DA. The μ effect appears to involve opioid inhibition of GABAergic interneurons that normally provide a tonic inhibition of dopaminergic cell firing; the mechanism for ∂ effects is currently unknown. Finally, this study and others provide evidence that the inhibition of mesolimbic dopaminergic function induced by systemic or icv injections of κ agonists does not involve opioid actions in the VTA.

Chapter 5

Ventral Mesencephalic ∂ Opioid Receptors are Involved in Modulation of Basal Mesolimbic Dopaminergic Activity: An Anatomical Localization Study

5.1 Introduction

VTA microinjections of DPDPE produced increases in the extracellular ventral striatal DA and DA metabolite concentrations (see Chapter 4). However, the anatomical specificity of these effects were questionable, given the possibility that the DPDPE could have diffused to nearby structures in the ventral mesencephalon. In fact, the density of ∂ opioid receptors in the VTA is quite sparse (see Section 1.4.1), while a high density of ∂ receptors has consistently been identified in the adjacent interpeduncular nucleus (IPN: Mansour *et al.*, 1987, 1988; Tempel and Zukin, 1987; Delay-Goyet *et al.*, 1990; Dilts and Kalivas, 1990). Further, there are neural connections between the IPN and the VTA which could potentially mediate the effects of ∂ opioid receptor agonists on mesolimbic DA transmission. The IPN extends fibres which directly innervate the VTA (Tork *et al.*, 1984). Additionally, a substantial reciprocal

interconnection exists between the IPN and the habenula (Velayos and Reinoso-Suarez, 1982), which in turn, has reciprocal connections with the VTA (Beckstead *et al.*, 1979; Phillipson, 1979; Tork *et al.*, 1984). Thus, it seemed possible that VTA microinjections of DPDPE could diffuse to the IPN, initiating opioid effects that were relayed back to the DA cell body region through either the direct connection with the VTA, or through the polysynaptic loop incorporating the habenula. This possibility could explain the finding that VTA DPDPE appears to be about 100 times less potent than DAMGO in its effects on mesolimbic dopaminergic activity (Latimer *et al.*, 1987; present results, Chapter 4), as well as in behavioural assays (Latimer *et al.*, 1987; Bals-Kubik *et al.*, 1990a; present results, Chapter 2) after VTA administration. Diffusion to the IPN would require a higher concentration of agonist at the distal injection site to compensate for the diffusion gradient.

In the present experiment, the ability of DPDPE to elicit increases in extracellular NAcc DA and DA metabolite concentrations was compared after injections into the VTA or IPN. DPDPE microinjections into the VTA increased NAcc DA and DOPAC concentrations more potently than did microinjections into the IPN. Thus, diffusion to the IPN cannot account for the effects of DPDPE after VTA microinjections. It appears likely that increases in NAcc DA and DA metabolite concentrations after VTA microinjections of DPDPE arise from actions in the VTA.

5.2 Methods

5.2.1 Animals and Surgery

Twenty four male Long-Evans rats (St. Constant, Qué.) weighing 375-410 grams were implanted under sodium pentobarbital anaesthesia (65 mg/kg, ip) with 22 gauge guide cannulae terminating 1.0 mm above either the VTA (3.2 posterior to bregma, 2.2 mm lateral to the midsagittal suture, 7.0 mm ventral to dura) or the IPN (4.4 mm posterior, 2.1 mm lateral, 8.0 mm ventral), and with 20 gauge guide cannulae terminating at the dorsal border of the ipsilateral NAcc (3.4 mm anterior to bregma, 2.3 mm lateral, 5.3 mm ventral). Surgery was performed with the incisor bar set at 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. (All measurements in the dorso-ventral plane refer to distances along the cannula track at 10° from the vertical.) Stainless steel obturators (diameter = 356 µm; 28 gauge) extending 1.1 mm (VTA and IPN) or 3.2 mm (NAcc) beyond the tip of each guide cannula were put in place at the time of surgery and removed at the time of testing.

5.2.2 Drugs

DPDPE was purchased from Sigma Chemical Co. (St Louis, MO). DPDPE was dissolved in aECF and injected into the VTA or

IPN via a 28 gauge stainless steel injector that extended 1 mm beyond the guide cannula. Injections were delivered in a volume of 0.25 µl over 23 seconds. Injectors were left in place for 3 minutes after injections to reduce drug efflux up the cannula shaft.

5.2.3 Microdialysis Probes

Removable microdialysis probes were constructed as previously described (see Section 3.2.3), with the following modification. The cannula of each probe was cemented to a 3 mm length of cellulose fibre. (i.e. These probes were 1 mm shorter than those previously described.)

5.2.4 Microdialysis Procedure

On test days (5-10 days after surgery) the animals were anaesthetized with sodium pentobarbital (65 mg/kg, ip) and given atropine sulphate (0.5 mg/kg, ip) to reduce salivation and mucous secretions. The rats were implanted with an ip cannula, and sodium pentobarbital was continuously administered at a flow rate of 65 µg/min throughout the experiment. A 3 mm concentric microdialysis probe (Robinson and Whishaw, 1988) with a 2 mm dialyzing surface (The ventral 1 mm was covered by glue.) was inserted into the ventral striatum and was perfused with aECF at a flow rate of 2 µl/min. Dialysate samples were collected at 20 minute intervals and assayed for DA, DOPAC, 5-HIAA and HVA

using isocratic, reverse-phase, HPLC with electrochemical detection. VTA or IPN injections were administered after a stable baseline was obtained (4 consecutive DA and DOPAC peak areas which varied by less than 10%, approximately 4-5 hours after probe insertion).

The rats were randomly assigned to drug treatment groups, and treated as follows:

Group 1 (VTA DPDPE; n = 6). After stable baselines were obtained, the rats were injected in the VTA with 0.25 µl aECF containing 2.5×10^{-11} moles DPDPE, and dialysate samples were collected for another 80 minutes. After 80 minutes, the rats were injected in the VTA with aECF containing 7.5×10^{-11} moles DPDPE, and dialysate was collected for another 80 minutes. This procedure was then repeated with VTA injections of aECF containing 2.5×10^{-10}, and 7.5×10^{-10} moles DPDPE respectively, for a total of four injections.

Group 2 (IPN DPDPE; n = 6). The rats were treated the same as Group 1 except that DPDPE injections were delivered in the IPN.

Group 3 (VTA controls; n = 6). After stable baselines were obtained, the rats were injected in the VTA with 0.25 µl aECF, and dialysate samples were collected for another 80 minutes. After 80 minutes, the rats were again injected with aECF in the VTA. This procedure was repeated for a total of four injections.

Group 4 (IPN controls; n = 6). The rats were treated the same as Group 3 except that aECF injections were delivered in the IPN.

Finally, each rat was perfused with physiological saline followed by 10% formalin. Each brain was removed immediately,

stored in formalin for one or more days, frozen and sliced in 40 µm coronal sections, and stained with thionin for localization of cannula and probe tracks.

5.2.5 Analytical Procedure

Dialysate was assayed for DA, DOPAC, HVA, and 5-HIAA as previously described (see Section 3.2.5).

5.2.6 Statistics

Between-groups differences in basal DA, DOPAC, HVA and 5-HIAA measures were each evaluated using 4 x 4 repeated-measures analyses of variance (ANOVAs) which were calculated using raw (fmole/µl) scores. Scores were then transformed to percent of baseline means for each group, and 4 x 16 repeated-measures ANOVAs were calculated for each neurochemical measure to evaluate the effects of the four doses of DPDPE. All significant effects were further analysed with Tukey's tests, comparing values for each post-injection sample at each dose of VTA DPDPE with the corresponding sample from the VTA aECF control group, and comparing values for each post-injection sample at each dose of IPN DPDPE with the corresponding sample from the IPN aECF control group. Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the

DPDPE-treated groups and the appropriate (VTA or IPN) aECF-treated groups. Tukey's tests were also used to compare values for each post-injection sample at each dose of VTA DPDPE with the corresponding sample from the IPN DPDPE group.

5.3 Results

Extracellular NAcc DA (Fig. 5.1) and DOPAC (Fig. 5.2) concentrations were reliably increased after both VTA and IPN microinjections of DPDPE (2.5×10^{-11} to 7.5×10^{-10} moles). Extracellular NAcc HVA (Fig. 5.3) and 5-HIAA (Fig. 5.4) concentrations were not reliably altered by VTA or IPN DPDPE.

The significant increases in DA concentrations occurred after VTA injections of DPDPE at all doses tested (2.5×10^{-11} to 7.5×10^{-10} moles; Fig. 5.1a). On the other hand, increases in DA concentrations occurred after IPN injections at only the two higher doses of DPDPE (2.5×10^{-10} to 7.5×10^{-10} moles; Fig. 5.1b), and these increases were less pronounced than the increases after VTA injections (Tukey's tests; Fig. 5.1a). DPDPE injections produced increases in DOPAC concentrations at the two higher doses (2.5×10^{-10} to 7.5×10^{-10} moles) after injections into either the VTA or IPN, but the increases after VTA injections were greater than they were after IPN injections (Tukey's tests; Fig. 5.2a).

Baseline concentrations of extracellular NAcc DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups (Table 5.1). Injector tips were located in the VTA (Groups 1 and

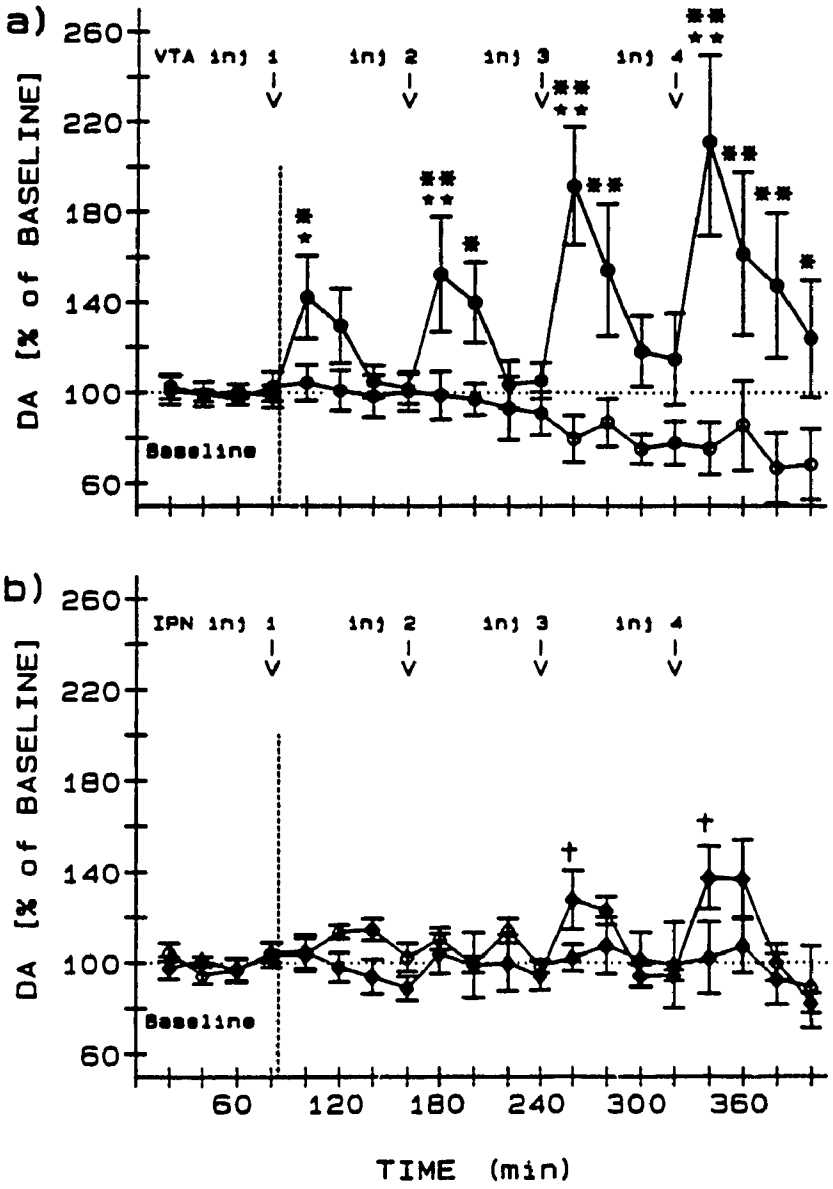

Fig. 5.1. Extracellular NAcc DA concentrations are significantly higher after **a)** VTA (●) microinjections of DPDPE (2.5 x 10^{-11} to 7.5 x 10^{-10} moles) than they are after VTA (○) aECF. NAcc DA concentrations are significantly higher after **b)** IPN (◆) microinjections of DPDPE at the two higher concentrations (2.5 x 10^{-10} to 7.5 x 10^{-10} moles) than they are after IPN (◇) aECF ($F_{(45,300)} = 2.840$, $p < .01$). Values expressed are group means ± SEM. Significant differences in concentrations between DPDPE-treated and aECF-treated rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for VTA DPDPE (Fig. 5.1a); † $p < .05$ for IPN DPDPE (Fig. 5.1b). Significant differences in concentrations between rats treated with DPDPE in the VTA, and rats treated with DPDPE in the IPN (Tukey's tests) are depicted as follows: ★ $p < .05$, ★★ $p < .01$ (Fig. 5.1a).

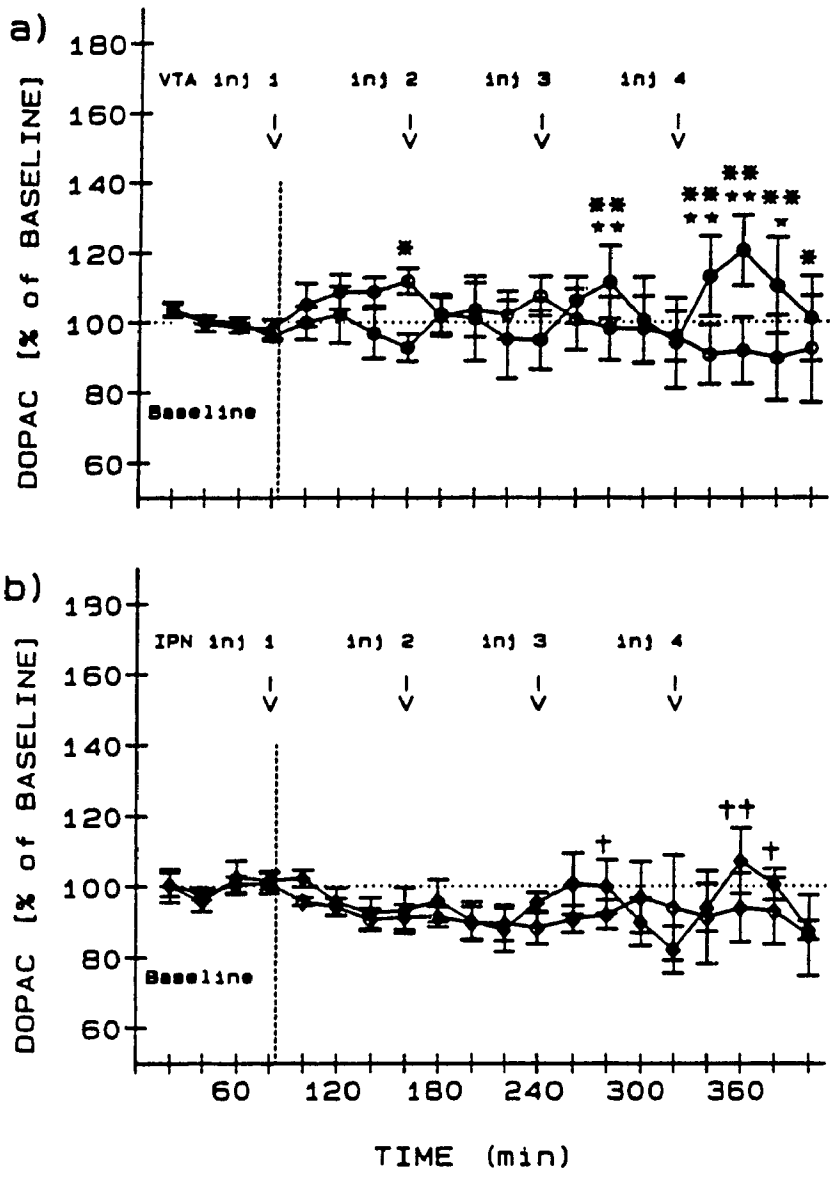

Fig. 5.2. Extracellular NAcc DOPAC concentrations are significantly higher after **a)** VTA (●) microinjections of DPDPE at the two higher concentrations (2.5×10^{-10} to 7.5×10^{-10} moles) than they are after VTA (○) aECF. NAcc DOPAC concentrations are significantly higher after **b)** IPN (◆) microinjections of DPDPE at the two higher concentrations (2.5×10^{-10} to 7.5×10^{-10} moles) than they are after IPN (◇) aECF ($F_{(45,300)} = 2.286$, $p < .01$). Values expressed are group means ± SEM. Significant differences in concentrations between DPDPE-treated and aECF-treated rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for VTA DPDPE (Fig. 5.2a); † $p < .05$, †† $p < .01$ for IPN DPDPE (Fig. 5.2b). Significant differences in concentrations between rats treated with DPDPE in the VTA, and rats treated with DPDPE in the IPN (Tukey's tests) are depicted as follows: ★ $p < .05$, ★★ $p < .01$ (Fig. 5.2a).

2) or IPN (Groups 3 and 4). The dialyzing surfaces (approx. 2 mm) of microdialysis probes were located in the medial NAcc (all Groups). Placements of NAcc microdialysis probes and VTA injector tips are depicted in Fig. 5.6. There were no apparent

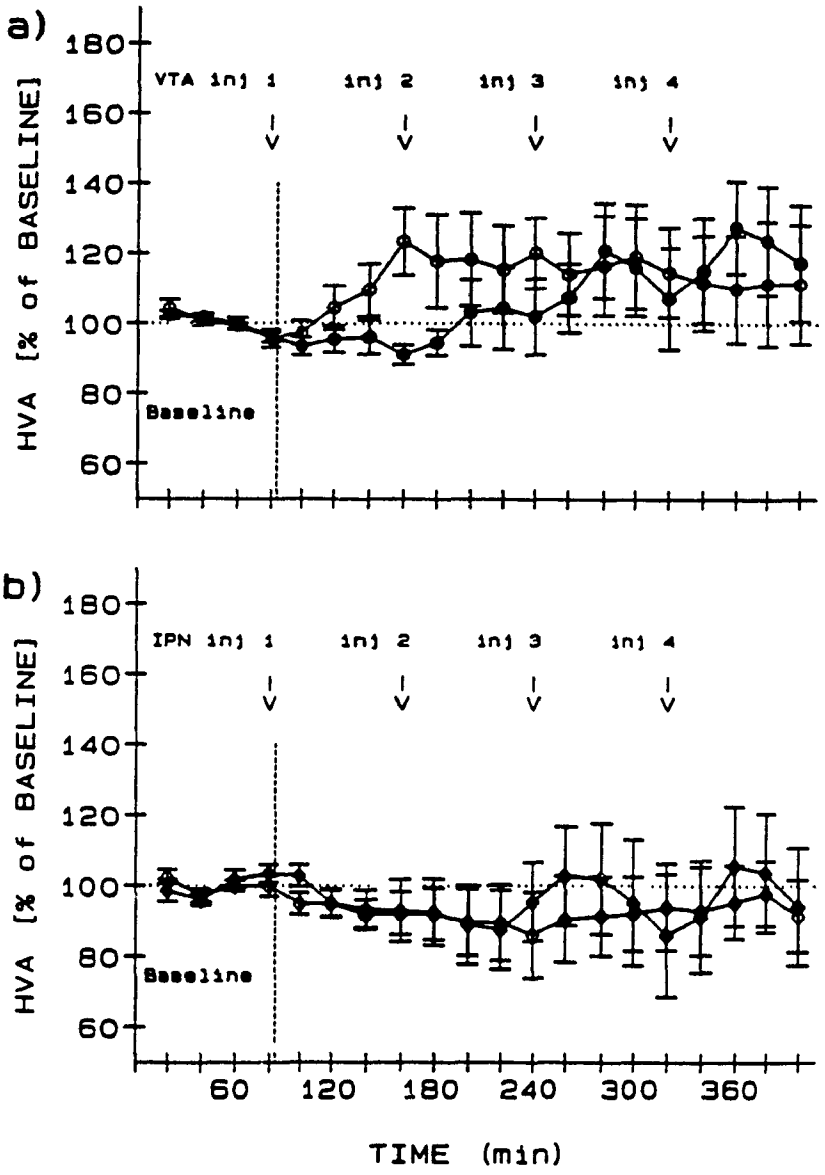

Fig. 5.3. Extracellular NAcc HVA concentrations are not significantly altered by **a)** VTA (●) or **b)** IPN (◆) microinjections of DPDPE at any dose tested, when compared with concentrations after **a)** VTA (○) or **b)** IPN (◇) aECF ($F_{(3,15)} = 0.250$, $p > .05$). Values expressed are group means ± SEM.

between-groups differences in the locations of VTA (Groups 1 and 2) or IPN (Groups 3 and 4) injector tracks or in the locations of NAcc probe tracks (all Groups).

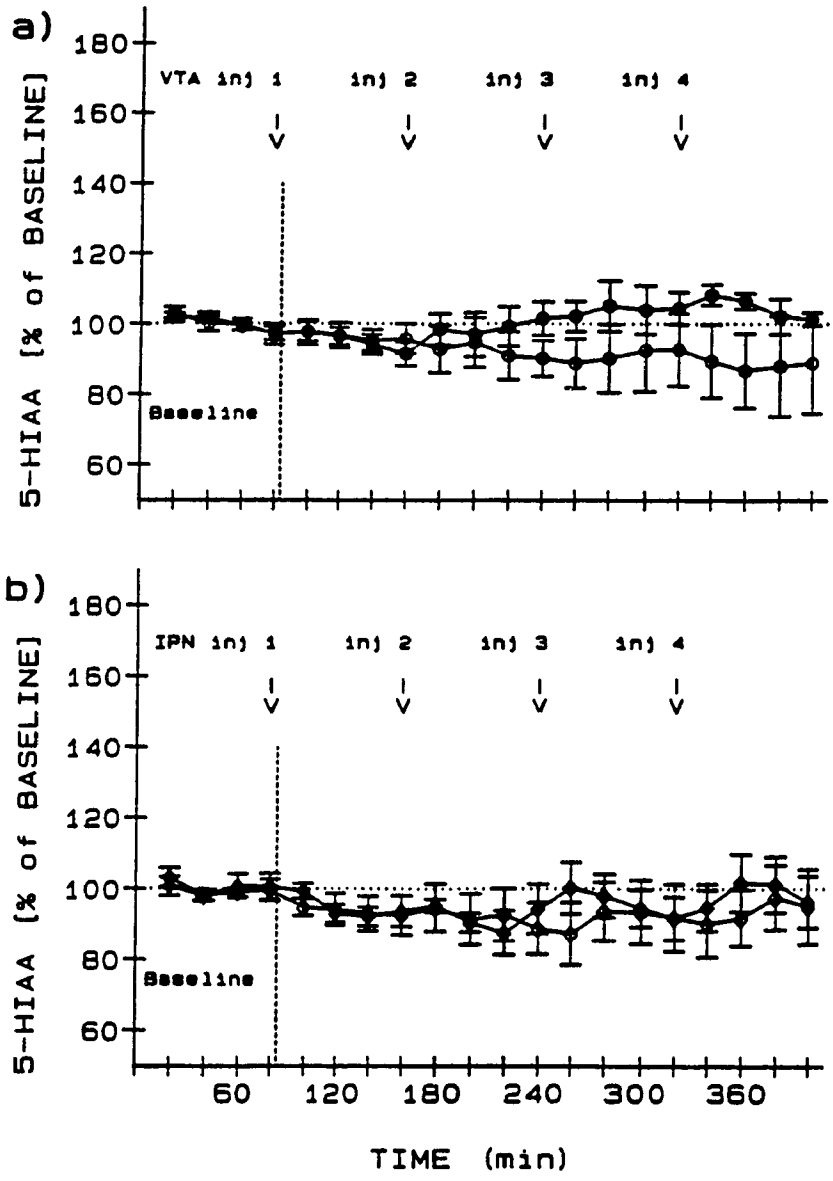

Fig. 5.4. Extracellular NAcc 5-HIAA concentrations are not significantly altered by **a)** VTA (●) or **b)** IPN (◆) microinjections of DPDPE at any dose tested, when compared with concentrations after **a)** VTA (○) or **b)** IPN (◇) aECF ($F_{(3,15)} = 0.1094$, $p > .05$). Values expressed are group means ± SEM.

	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	1.200 ± 0.093	2.120	3,20	>.05
DOPAC	286.4 ± 14.06	1.943	3,20	>.05
HVA	184.1 ± 10.45	2.032	3,20	>.05
5-HIAA	32.97 ± 0.967	1.563	3,20	>.05

Table 5.1. Baseline means and standard errors for DA and metabolites for 4 groups combined (VTA aECF, VTA DPDPE, IPN aECF, IPN DPDPE), and tests of significance for between-groups differences in baseline means.

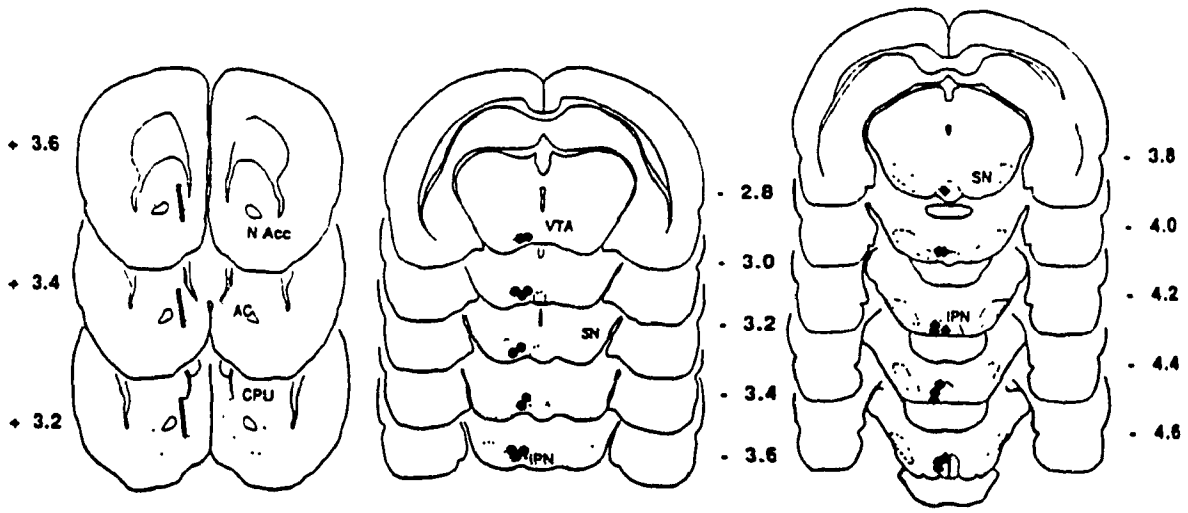

Fig. 5.5. Histological reconstruction of representative microdialysis probe tracks in the NAcc, and the sites where injector tips were identified in the VTA (●) and IPN (◆). Abbreviations used: CPU = caudate putamen; NAcc = nucleus accumbens; AC = anterior commissure; SN = substantia nigra; VTA = ventral tegmental area; IPN = interpeduncular nucleus (adapted from Pellegrino *et al.*, 1979).

5.4 Discussion

5.4.1 Effects of VTA and IPN Microinjections of DPDPE

The potency of DPDPE to increase extracellular NAcc DA and DOPAC concentrations was greater after VTA microinjections than after IPN microinjections (i.e. threshold dose, magnitude and duration of effect). Thus, it seems that diffusion to the IPN cannot account for the increases in NAcc DA and DA metabolite concentrations after VTA microinjections of DPDPE. It is possible that ∂ receptors in the IPN may contribute to opioid modulation of mesolimbic DA, but it seems more likely that the observed effects after IPN microinjections resulted from diffusion of the drug to the VTA. The lack of effect of VTA DPDPE injections on NAcc HVA in the present study does not concur with our previous finding that VTA DPDPE increases NAcc HVA concentrations (see Chapter 4). This is probably a result of the low doses used in the present study. In fact, NAcc HVA concentrations appeared to increase after the two highest doses of DPDPE administered into the VTA, but these trends were not statistically significant. The lack of effect of VTA and IPN DPDPE on NAcc 5-HIAA concentrations is consistent with our previous finding that mesencephalic opioid injections do not alter ventral striatal 5-HIAA concentrations (see Chapter 4).

5.4.2 Microanatomy of ∂ Receptor Distribution in the VTA

In the immediate region of the VTA injection site, the highest density of ∂ opioid receptors has been found in the interfascicular and paranigral nuclei of the VTA (see Fig. 5.6). These two nuclei are moderately labelled with [^{3}H]DSTBULET (a selective ∂ ligand), whereas the SN and the surrounding nuclei of the VTA (i.e. rostral linear and parabrachial pigmented nuclei) are very poorly labelled (Delay-Goyet *et al.*, 1990). Thus, it seems that VTA injections of DPDPE may elicit alterations in mesolimbic DA functioning through actions on ∂ receptors located in the interfascicular and paranigral nuclei of the VTA.

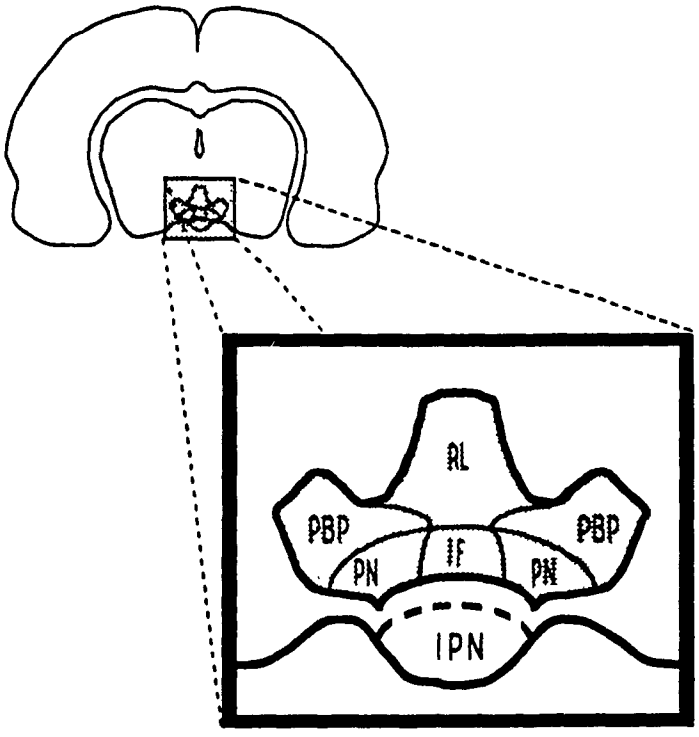

Fig. 5.6 The anatomy of the VTA. Abbreviations used: RL = Rostral Linear nucleus; PBP = Parabrachial Pigmented nucleus; PN = Paranigral nucleus; IF = Interfascicular nucleus; IPN = interpeduncular nucleus (adapted from Halliday and Tork, 1988).

Chapter 6

Modulation of Basal Mesolimbic Dopaminergic Activity By Administration of μ-Opioid Receptor Antagonists into the Ventral Tegmental Area

6.1 Introduction

The CTOP-induced increases in extracellular ventral striatal DA and DA metabolite concentrations (see Chapter 4) were unexpected, as these effects resembled the effects of μ agonists. Further, these μ antagonist-induced increases in DA and DA metabolite concentrations conflict with another recent report. Spanagel *et al.* (1992), reported that microinjections of CTOP into the VTA produced decreases in extracellular DA, DOPAC, and HVA concentrations in the NAcc. It is unclear why increases were seen in one study, while decreases were seen in the other. One possibility arises from differences in the drug vehicle and microdialysis perfusate solutions that were used in the two studies. In the study where decreased DA and DA metabolite concentrations were found (Spanagel *et al.*, 1992), the NAcc was dialysed with Ringers solution containing high concentrations of calcium and potassium, and CTOP was dissolved in sterile water for VTA microinjections. In the study where increased DA and DA

metabolite concentrations were found (Chapter 4), aECF that contained physiological concentrations of these ions (Moghaddam and Bunney, 1989) was used for the dialysate and drug vehicle solutions. It has recently been demonstrated that local alterations in ion concentrations can produce alterations in the responses of neurons to pharmacological manipulations (Imperato and Di Chiara, 1984; Westerink *et al.*, 1988; Drew *et al.*, 1989; Moghaddam and Bunney, 1989; de Boer *et al.*, 1990; Westerink *et al.*, 1990).

In the present experiments, the possibility is examined that the previously observed increases in ventral striatal DA and metabolite concentrations may have resulted from a partial opioid agonist activity of CTOP, or from non-selective actions of CTOP at receptors other than μ opioid receptors. The responses of mesolimbic dopaminergic neurons to VTA administration of CTOP are assessed over a range of CTOP doses, and the effects of CTOP are compared with the effects of VTA microinjections of a structurally dissimilar μ antagonist, β-funaltrexamine (β-FNA).

6.2 Methods

6.2.1 Animals and Surgery

6.2.1.1 Experiment 1

Twenty four male Long-Evans rats (St. Constant, Qué., Canada) weighing 360-400 grams were implanted under sodium

pentobarbital anaesthesia (65 mg/kg, ip) with 20 gauge guide cannulae terminating 1.0 mm above the NAcc (3.2 mm anterior to bregma, 2.8 mm lateral to the midsagittal suture, 4.5 mm ventral to dura), and with 22 gauge guide cannulae terminating 1.0 mm above the ipsilateral VTA (3.6 mm posterior to bregma, 2.2 mm lateral, 7.1 ventral). Surgery was performed with the incisor bar set at 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. (All measurements in the dorso-ventral plane refer to distances along the cannula track at 10° from the vertical.) Stainless steel obturators (diameter = 356 µm; 28 gauge) extending 4.2 mm (NAcc) or 1.1 mm (VTA) beyond the tip of each guide cannula were put in place at the time of surgery and removed at the time of testing.

6.2.1.2 Experiment 2

Eighteen male Long-Evans rats (Charles River, Boston, MA) weighing 375-400 grams were implanted under sodium pentobarbital anaesthesia (65 mg/kg, ip) with 20 gauge guide cannulae terminating at the dorsal boundary of the medial NAcc (3.6 mm anterior to bregma, 2.3 mm lateral to the midsagittal suture, 5.3 mm ventral to dura), and with 22 gauge guide cannula terminating 1.0 mm above the ipsilateral VTA (3.4 mm posterior, 2.2 mm lateral, 7.0 mm ventral). Surgery was performed with the incisor bar set at 5 mm above the interaural line, and cannulae were angled toward the midline at 10° from the vertical. Stainless steel obturators (356 µm; 28 gauge) extending 3.2 mm

(NAcc) or 1.1 mm (VTA) beyond the tip of each guide cannulae were put in place at the time of surgery and removed at the time of testing.

6.2.2 Drugs

CTOP was purchased from Peninsula Laboratories Inc. (Belmont, CA). β-FNA ({E)-4[[(5a,6β)-17-cyclopropylmethyl) 4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic acid methyl ester hydrochloride, a selective μ antagonist: Portoghese *et al.*, 1980; Takemori *et al.*, 1981; Ward *et al.*, 1982a; Ward *et al.*, 1982b; Jiang *et al.*, 1990; Rothman *et al.*, 1991) was purchased from Research Biochemicals Inc. (Natick, MA). CTOP and β-FNA were each dissolved in aECF and injected into the VTA via a 28 gauge stainless steel injector that extended 1 mm beyond the guide cannula. Injections were delivered in a volume of 1.0 μl over 92 seconds (Experiment 1), or 0.5 μl over 46 seconds (Experiment 2) using a 1.0 μl glass syringe driven by a syringe pump. Injectors were left in place for 3 minutes after injections to limit drug efflux up the cannula shaft.

6.2.3 Microdialysis Probes

Removable microdialysis probes were constructed as previously described (see Section 3.2.3), with the following modification. The probes used in Experiment 1 were constructed

with a 4 mm length of cellulose fibre (as previously described), while the probes used in Experiment 2 were constructed with a 3 mm length of cellulose fibre.

6.2.4 Microdialysis Procedure

On test days (at least 5 days after surgery) the animals were anaesthetized with chloral hydrate (400 mg/kg, ip) and given atropine sulphate (0.5 mg/kg, ip) to reduce salivation and mucous secretions. Supplemental doses (approximately 20 mg) of chloral hydrate were given when signs of arousal were observed (approximately once per hour). A microdialysis probe was inserted into the ventral striatum and was perfused with aECF at a flow rate of 2 µl/min. Dialysate samples were collected at 20 minute intervals until a stable baseline was obtained (4 consecutive DA and DOPAC peak areas that varied by less than 10%, approximately 4-5 hours after probe insertion).

6.2.4.1 Experiment 1

The rats were randomly assigned to drug treatment groups (6 rats/drug dose), and microinjected in the VTA with 1.0 µl aECF vehicle, or with 1.0 µl aECF containing CTOP (3.0×10^{-11}, 3.0×10^{-10}, or 3.0×10^{-9} moles). Dialysate samples were collected for one hour after the injections.

Finally, each rat was perfused with physiological saline followed by 10% formalin. Each brain was removed immediately,

stored in formalin for one or more days, frozen and sliced in 40 μm coronal sections, and stained with thionin for localization of cannula and probe tracks.

6.2.4.2 Experiment 2

The rats were randomly assigned to drug treatment groups (6 rats/drug), and microinjected in the VTA with 0.5 μl aECF vehicle, or with 0.5 μl aECF containing CTOP (5.0×10^{-10} moles), or β-FNA (5.0×10^{-10} moles). Dialysate samples were collected for four hours after the injections. Finally, the rats were perfused, and the brains were examined as in the earlier experiment.

6.2.5 Analytical Procedure

Dialysate was assayed for DA, DOPAC, HVA, and 5-HIAA as previously described (see Section 3.2.5).

6.2.6 Statistics

6.2.6.1 Experiment 1

Between-groups differences in basal DA, DOPAC, HVA and 5-HIAA measures were evaluated using 4 x 4 repeated-measures analyses of variance (ANOVAs), which were calculated using raw (fmole/μl) scores. Scores were then transformed to percent of

baseline means for each group, and 4 x 3 repeated-measures ANOVAs were calculated for each neurochemical measure to evaluate the effects of the three doses of CTOP. Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the CTOP-treated and aECF-treated groups. All significant effects were further analysed with Tukey's tests, comparing values for each post-injection sample at each dose of CTOP with the corresponding sample from the aECF control group.

6.2.6.2 Experiment 2

Between-groups differences in basal DA, DOPAC, HVA and 5-HIAA measures were evaluated using 3 x 4 repeated-measures analyses of variance (ANOVAs), which were calculated using raw (fmole/μl) scores. Scores were then transformed to percent of baseline means for each group, and 3 x 12 repeated-measures ANOVAs were calculated for each neurochemical measure to evaluate the effects of the two μ opioid antagonists. Treatment-associated increases or decreases in the neurochemical measures were treated as statistically reliable when there were significant differences between the CTOP- or β-FNA-treated and aECF-treated groups. All significant effects were further analysed with Tukey's tests, comparing values for each post-injection sample after VTA CTOP or β-FNA with the corresponding sample from the aECF control group.

6.3 Results

6.3.1 Experiment 1

Extracellular ventral striatal DA (Fig. 6.1), DOPAC, and HVA (Fig. 6.2) concentrations were reliably increased in a dose-orderly fashion after VTA microinjections of CTOP (3.0×10^{-11} to 3.0×10^{-9} moles). On the other hand, extracellular ventral striatal 5-HIAA concentrations were not reliably altered by VTA CTOP at any dose tested (Fig. 6.2).

Baseline concentrations of extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups (Table 6.1). Injector tips were located in the parabrachial pigmented or paranigral nuclei of the VTA. The dialyzing surface (approx. 3 mm) of microdialysis probes were localized in the medial NAcc, near the anterior commissure, with a small portion in the ventral aspect of the caudate nucleus. Placements of ventral striatal microdialysis probes and VTA injector tips are depicted in Fig. 6.3. There were no apparent between-groups differences in the locations of ventral striatal probe tracks or in the locations of VTA injector tracks.

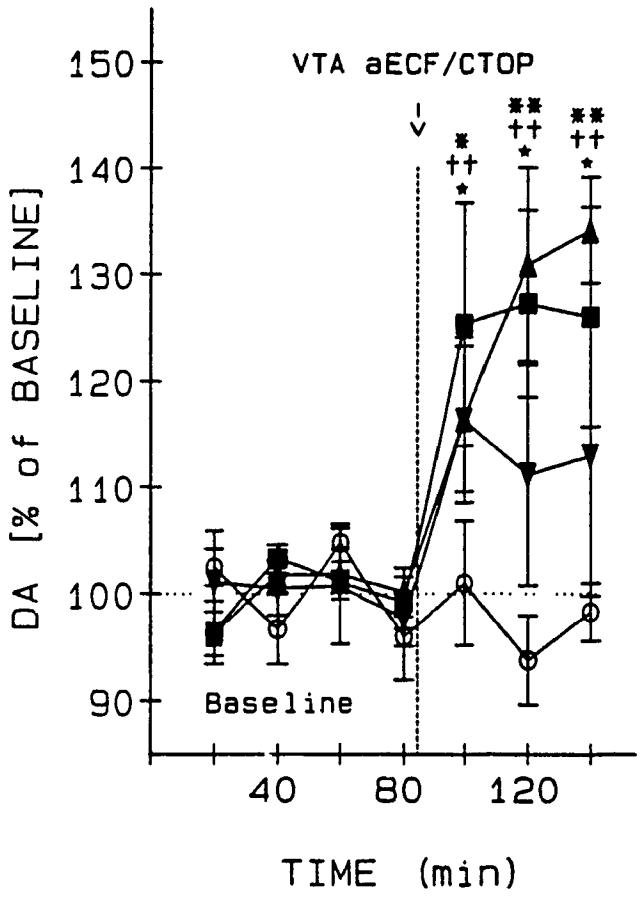

Fig. 6.1. Dose-response curves illustrate significant between-groups differences in extracellular ventral striatal DA concentrations after VTA aECF (○), or VTA CTOP at 3.0×10^{-11} (▼), 3.0×10^{-10} (■), or 3.0×10^{-9} (▲) moles/µl ($F_{(3,20)} = 3.4184$, $p < .05$). Values expressed are group means ± SEM. Significant differences in concentrations between drug-treated and vehicle-treated rats for each sample (Tukey's tests) are depicted as follows: ★ $p < .05$, for low dose; †† $p < .01$ for medium dose; * $p < .05$, ** $p < .01$ for high dose.

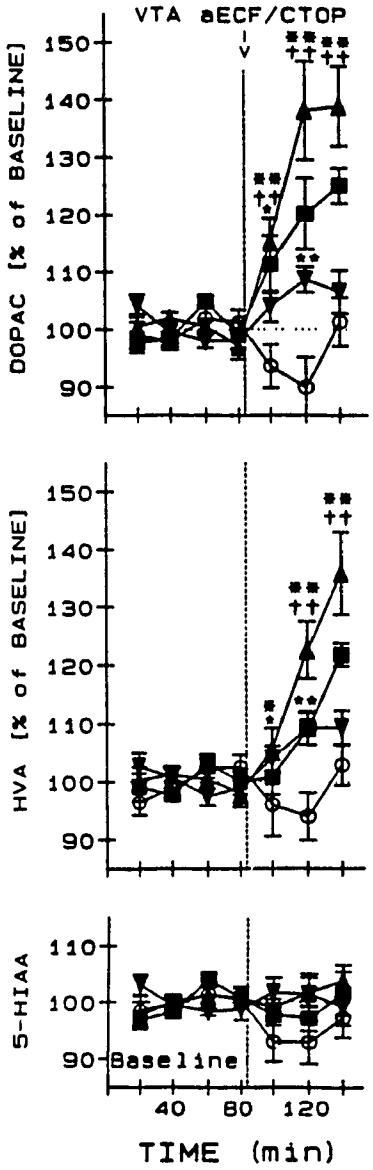

Fig. 6.2. Dose-response curves illustrate significant between-groups differences in extracellular ventral striatal DOPAC ($F_{(6,40)} = 3.9113$, $p < .01$) and HVA ($F_{(6,40)} = 6.0290$, $p < .01$) concentrations after VTA aECF (○), or VTA CTOP at 3.0×10^{-11} (▼), 3.0×10^{-10} (■), or 3.0×10^{-9} (▲) moles/µl. There were no significant between-groups differences in 5-HIAA concentrations after VTA CTOP or aECF ($F_{(3,20)} = 1.6196$, $p > .05$). Values expressed are group means ± SEM. Significant differences in concentrations between drug-treated and vehicle-treated rats for each sample (Tukey's tests) are depicted as follows: ★ $p < .05$, ★★ $p < .01$ for low dose; †† $p < .01$ for medium dose; * $p < .05$, ** $p < .01$ for high dose.

	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	2.870 ± 0.147	2.825	3,20	>.05
DOPAC	438.1 ± 22.53	0.103	3,20	>.05
HVA	425.6 ± 26.75	0.070	3,20	>.05
5-HIAA	86.60 ± 2.585	0.227	3,20	>.05

Table 6.1. Baseline means and standard errors for DA and metabolites for 4 treatment groups combined from Experiment 1 (3 doses of CTOP, and aECF controls; with 4 mm probes). Baseline concentrations of extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups.

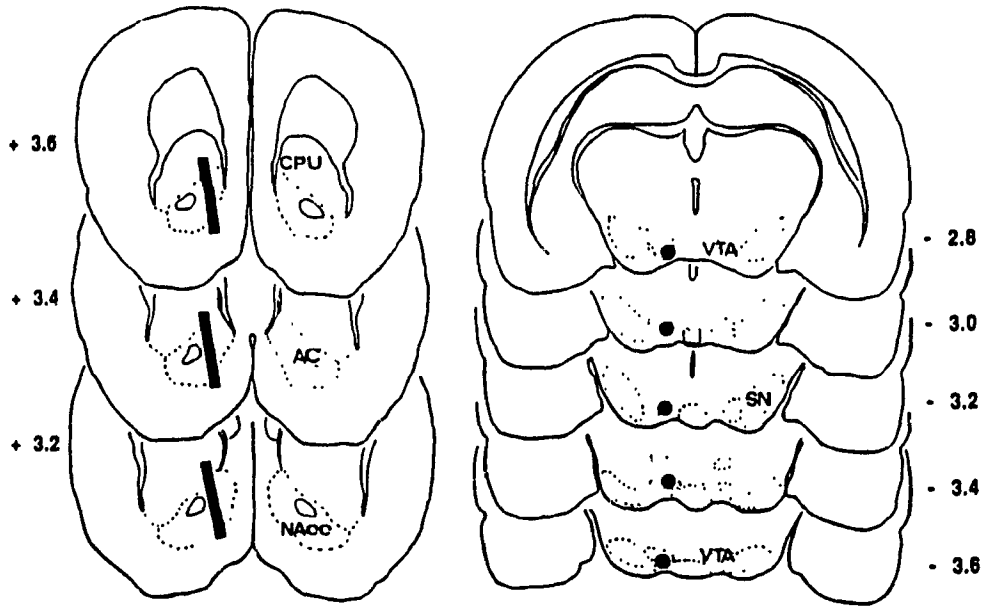

Fig. 6.3. Histological reconstruction of representative microdialysis probe tracks in the ventral striatum, and the area in which injector tips were identified in the ventral tegmentum from Experiment 1. Abbreviations used: CPU = caudate putamen; NAcc = nucleus accumbens; AC = anterior commissure; SN = substantia nigra; VTA = ventral tegmental area (adapted from Pellegrino *et al.*, 1979).

6.3.2 Experiment 2

Extracellular NAcc DA concentrations were reliably increased after VTA microinjections of either CTOP or β-FNA (5.0×10^{-10} moles), and these increases persisted in all samples collected during four hours after the VTA microinjections (Fig. 6.4). Extracellular DOPAC and HVA concentrations were also elevated after VTA microinjections of the μ antagonists (Fig. 6.5). Extracellular 5-HIAA concentrations were elevated transiently after VTA CTOP and β-FNA (Fig. 6.5).

Baseline concentrations of extracellular NAcc DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups (Table 6.2). Injector tips were located in the parabrachial pigmented or paranigral nuclei of the VTA, and the dialyzing surface (approx. 2 mm) of microdialysis probes were localized entirely within the medial NAcc (Fig. 6.6). There were no apparent between-groups differences in the locations of NAcc probe tracks or in the locations of VTA injector tracks.

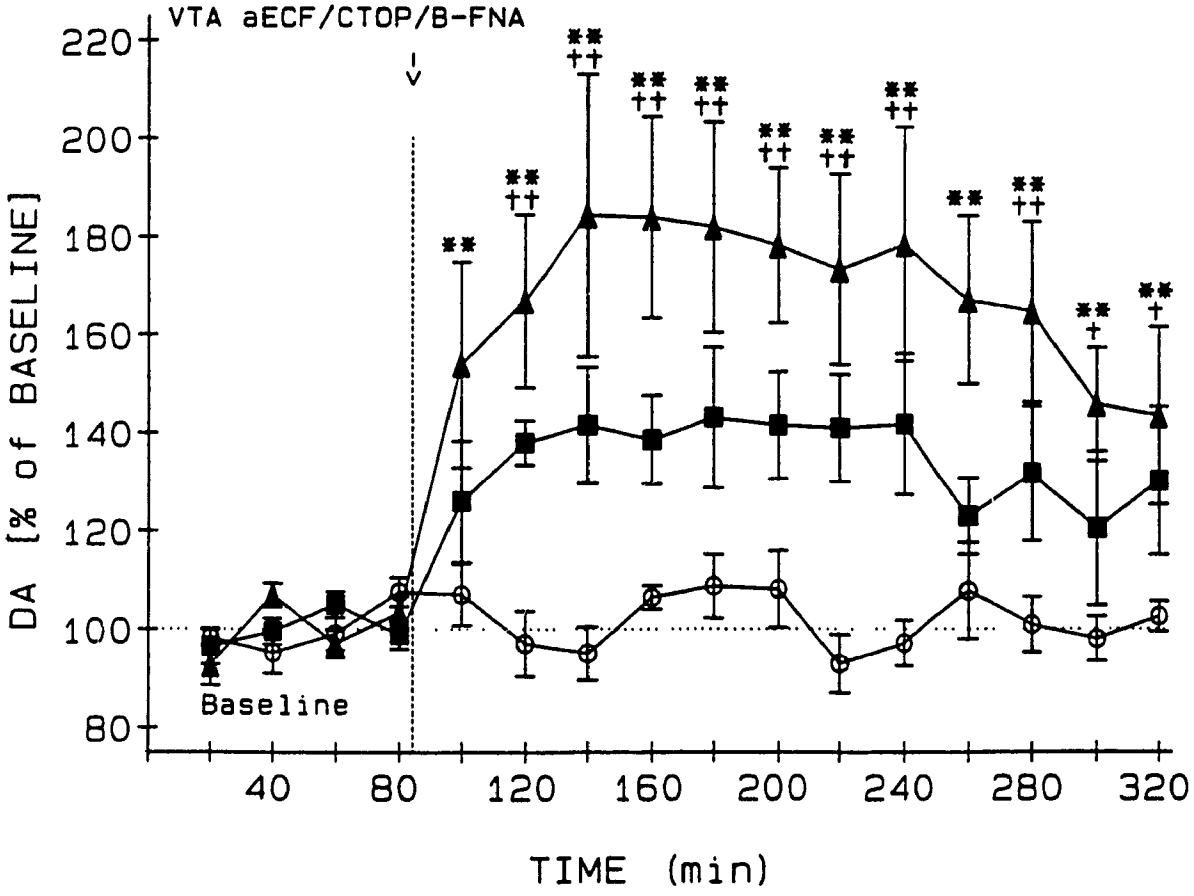

Fig. 6.4. Extracellular NAcc DA concentrations are significantly higher after VTA microinjections of either CTOP (5.0×10^{-10} moles) (▲) or β-FNA (5.0×10^{-10} moles) (■) than they are after VTA microinjections of aECF (○) ($F_{(2,15)} = 7.8998$, $p < .01$). Values expressed are group means ± SEM. Significant differences in concentrations between drug-treated and vehicle-treated rats for each sample (Tukey's tests) are depicted as follows: * $p < .01$ for CTOP; † $p < .05$, †† $p < .01$ for β-FNA.

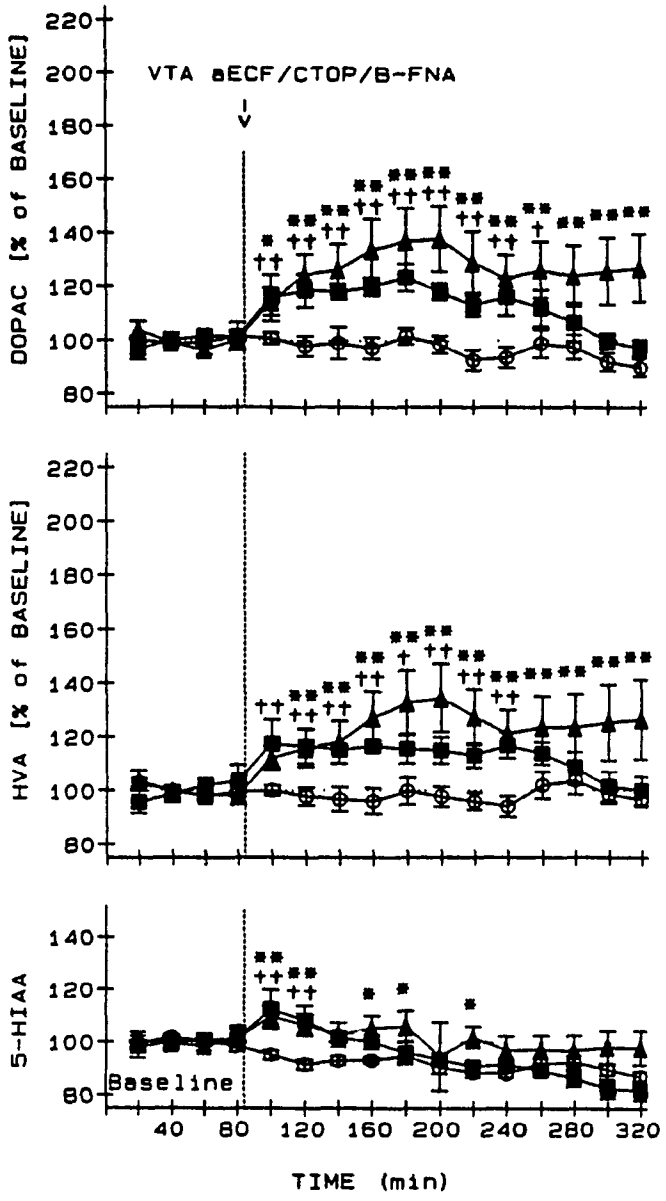

Fig. 6.5. Extracellular NAcc DOPAC ($F_{(22,165)} = 1.6929$, $p < .05$) and HVA ($F_{(22,165)} = 1.7039$, $p < .05$) concentrations are higher, and 5-HIAA concentrations are transiently higher ($F_{(2,15)} = 4.0240$, $p > .05$) after VTA microinjections of either CTOP (▲) or β-FNA (■) than they are after VTA microinjections of aECF (○). Values expressed are group means ± SEM. Significant differences in concentrations between drug-treated and vehicle-treated rats for each sample (Tukey's tests) are depicted as follows: * $p < .05$, ** $p < .01$ for CTOP; † $p < .05$, †† $p < .01$ for β-FNA.

	Baseline mean ± SEM (fmoles / 1.0 µl)	F-ratio	df	p
DA	0.859 ± 0.068	0.486	2,15	>.05
DOPAC	206.1 ± 10.52	0.516	2,15	>.05
HVA	172.6 ± 7094	1.294	2,15	>.05
5-HIAA	56.67 ± 1.224	1.115	2,15	>.05

Table 6.2. Baseline means and standard errors for DA and metabolites for 4 treatment groups combined from Experiment 2 (CTOP, β-FNA, and aECF controls; with 3 mm probes), and tests of significance for between-groups differences in baseline means. Baseline concentrations of extracellular ventral striatal DA, DOPAC, HVA, and 5-HIAA did not differ significantly between groups.

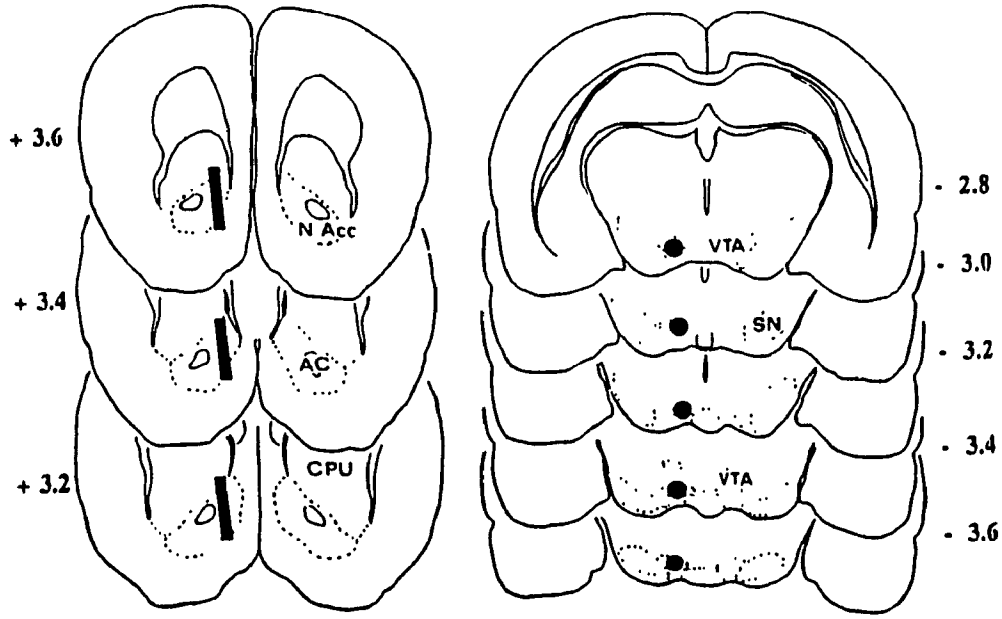

Fig. 6.6. Histological reconstruction of representative microdialysis probe tracks in the nucleus accumbens, and the area in which injector tips were identified in the VTA from Experiment 2. Abbreviations used: CPU = caudate putamen; NAcc = nucleus accumbens; AC = anterior commissure; SN = substantia nigra; VTA = ventral tegmental area (adapted from Pellegrino *et al.*, 1979).

6.4 Discussion

6.4.1 Effects of VTA Microinjections of CTOP and β-FNA

The effects of CTOP in the present study replicate our previous findings (see Chapter 4) that VTA microinjections of CTOP increase extracellular ventral striatal DA, DOPAC and HVA concentrations when physiological vehicle and dialyzing solutions are used. Further, the CTOP-induced increases in DA and metabolite concentrations occur in a dose-orderly fashion, and the structurally dissimilar μ antagonist β-FNA produces similar increases in these neurotransmitter and metabolite levels. These findings are further supported by the observation that VTA microinjections of CTOP produce hyperlocomotion in rats (Badiani, personal communication), and locomotor activation has been associated with increases in dopaminergic activity (Stinus *et al.*, 1980; Kalivas and Richardson-Carlson, 1986; Latimer *et al.*, 1987; Wise and Bozarth, 1987).

Nevertheless, the VTA CTOP- and β-FNA-induced increases in extracellular ventral striatal DA, DOPAC and HVA concentrations are perplexing, considering the fact that VTA microinjections of opioid receptor agonists also produce increases in mesolimbic dopaminergic activity (Gysling and Wang, 1983; Kalivas *et al.*, 1983; Matthews and German, 1984; Latimer *et al.*, 1987; Cador *et al.*, 1989; Leone *et al.*, 1991; Spanagel *et al.*,

1992; present results, Chapter 4). One possibility is that increases in DA and DA metabolite concentrations arose from partial opioid agonist activities of the two opioid antagonists. Activation of VTA μ (Spanagel *et al.*, 1992; present results, Chapter 4) and ∂ (present results, Chapter 4) opioid receptors each produce increases in ventral striatal DA and DA metabolite concentrations. Therefore, if CTOP and β-FNA were to exhibit agonist activity at either of these two receptor types, this could account for the observed increases.

6.4.2 Pharmacological Profile of CTOP

The possibility of μ opioid agonist activity of CTOP has been examined in several preparations. CTOP (10 μM) fails to inhibit electrically-evoked contraction of the guinea pig ileum *in vitro*, but it potently antagonizes the effects of the μ agonist PL017 in this preparation (Kramer *et al.*, 1989). In mice, CTOP (1.0 μg, icv) fails to induce hot plate analgesia or to inhibit gastrointestinal transit (1.0 μg, intrathecally) *in vivo* but it antagonizes the effects of PL017 with a potency about 10 times higher than naloxone in both preparations (Kramer *et al.*, 1989). CTOP antagonizes DAMGO-mediated inhibition of electrically-evoked release of [^{3}H]noradrenaline from rat cortical slices in a dose-orderly fashion, but CTOP administered alone (1.0 μM) does not exert any effects on spontaneous or electrically-evoked efflux of [^{3}H]noradrenaline (Mulder *et al.*, 1991). Thus, CTOP exhibits

potent μ antagonist activity in all of these preparations, without any apparent μ agonist properties.

Analyses of the activity of CTOP at ∂ receptors has yielded less clear results. CTOP exhibits a significant inhibition of electrically-evoked contraction in the mouse and rabbit vas deferens *in vitro*, at concentrations as low as 300 nM. These effects are marginally decreased by induction of somatostatin tolerance, or by administration of naloxone or the ∂ antagonist ICI 174,864 (Kramer *et al.*, 1989). Thus, it is possible that CTOP exerts agonist activity at somatostatin or ∂ opioid receptors in these preparations. However, it appears unlikely that CTOP exerts somatostatin or ∂ opioid agonist activity in the rat brain. CTOP is a substituted somatostatin analogue that exhibits approximately 8,100-11,000 fold higher affinity for μ opioid receptors than somatostatin receptors in the rat brain (Gulya *et al.*, 1986; Pelton *et al.*, 1986; Kazmierski *et al.*, 1988). Further, CTOP binding in the rat brain has approximately 1,300-4,800 fold higher affinity for μ receptors than for ∂ receptors (Gulya *et al.*, 1986; Pelton *et al.*, 1986; Kazmierski *et al.*, 1988), and activity at ∂ receptors has not been found in the rat brain. CTOP (1 μM) fails to antagonize DSTBULET-mediated inhibition of electrically-evoked release of [^{3}H]DA from rat striatal slices, and CTOP administered alone fails to alter spontaneous or electrically-evoked efflux of [^{3}H]DA from these slices, indicating a lack of ∂ agonist or antagonist effects in this preparation (Mulder *et al.*, 1991). Further, CTOP (0.1 μM) fails to alter inhibition of electrically evoked release of [^{3}H]DA

from striatal slices by the κ agonist U-69,593, indicating a lack of κ activity in the rat brain (Mulder *et al.*, 1991).

6.4.3 Pharmacological Profile of β-FNA

In the case of β-FNA, extensive pharmacological characterization has demonstrated a lack of agonist activity at μ or ∂ opioid receptors. β-FNA is a derivative of naltrexone that acts as an irreversible μ receptor antagonist, and as a reversible κ receptor agonist in the guinea pig ileum (Portoghese *et al.*, 1980; Takemori *et al.*, 1981) and mouse vas deferens (Ward *et al.*, 1982a) *in vitro*. Similar μ antagonist and κ agonist activities have been found *in vivo* in acetic acid writhing tests of antinociception in mice (Ward *et al.*, 1982b; Jiang *et al.*, 1990). Further, β-FNA fails to exhibit antinociceptive effects in the radiant heat tail-flick test, indicating a lack of μ agonist activity (Ward *et al.*, 1982b), and ∂ agonist activity has not been found in guinea pig ilea, mouse vas deferens, or antinociception tests (Portoghese *et al.*, 1980; Takemori *et al.*, 1981; Ward *et al.*, 1982a, 1982b; Jiang *et al.*, 1990).

The reversible κ agonist effects of β-FNA cannot account for the increases in NAcc DA and metabolite concentrations after VTA microinjections. VTA microinjections of the κ agonists U-69,593 (Spanagel *et al.*, 1992) and U-50,488H (present results, Chapter 4) fail to affect ventral striatal DA and metabolite concentrations.

Thus, VTA κ receptors are uninvolved in modulation of basal mesolimbic dopaminergic activity.

6.4.4 Doses of CTOP and β-FNA

It should be noted that the concentrations of CTOP and β-FNA administered into the VTA were higher than the concentrations that were used in the *in vitro* characterizations of the receptor selectivities of these compounds, but the total number of moles of CTOP and β-FNA that were injected into the VTA in the present study was very low (3.0×10^{-11} to 3.0×10^{-9} moles). Further, the doses of CTOP that were selected for study in the present experiment were chosen from doses that were previously tested for antagonism of the effects of a μ agonist in the VTA. In the previous investigation, 3.0×10^{-10} moles/μl was the threshold dose of CTOP that was required to antagonize increases in ventral striatal DA, DOPAC and HVA produced by a moderate dose of VTA DAMGO (see Chapter 4). This dose of CTOP was chosen as the middle dose in the present dose-response analysis. Equimolar doses of β-FNA and CTOP were then examined (5.0×10^{-10} moles/0.5 μl). In the study that reported possible δ opioid and somatostatin effects of CTOP (Kramer *et al.*, 1989), the doses that were required to produce these non-selective actions greatly exceeded the doses that were required to antagonize selective μ agonists. Thus, it seems

unlikely that CTOP or β-FNA exerted either non-selective or mixed agonist-antagonist actions in the present study.

6.4.5 An Explanation of Agonist-Like Effects of CTOP and β-FNA, Based upon VTA Neuroanatomy

An alternative explanation for the CTOP- and β-FNA-induced increases in DA, DOPAC and HVA concentrations is based upon evidence that the relevant μ receptors are on GABAergic neurons and that GABAergic afferents and GABAergic interneurons interact in a complex manner in the SN and in the VTA to modulate midbrain dopaminergic activity. GABAergic afferents (Feltz, 1971; Precht and Yoshida, 1971; McGeer *et al.*, 1974; Hattori *et al.*, 1975; Nagy *et al.*, 1978; Fisher, 1989) and interneurons (Francois *et al.*, 1979; Nitsch and Riesenberg, 1988) have been identified in the SN, and each of these populations of GABAergic neurons is thought to contribute to modulation of dopaminergic neurons. Kondo and Iwatsubo (1978) found that morphine inhibits the release of preloaded [^{3}H]GABA from the SN evoked by electrical stimulation of the caudate. Further, iontophoretic application of GABA in the substantia nigra pars reticulata (SNR) produces a decrease in firing of non-dopaminergic (putative GABAergic) neurons, and a concomitant increase in firing of dopaminergic neurons (Grace and Bunney, 1979). Iontophoretic application of GABA into the substantia nigra pars compacta (SNC) inhibits the firing of dopaminergic neurons, but the

non-dopaminergic neurons of the SNR are 20 times more sensitive to the inhibitory effects of iontophoretically applied GABA than are the dopaminergic neurons of the SNC (Grace and Bunney, 1979). Further, Grace and Bunney (1985) found that differing intensities of striatal stimulation produce opposing effects on nigrostriatal dopaminergic activity. High current intensity (500uA or more) striatal trains of stimulation inhibit DA cell firing, while lower levels of stimulation (20-50uA) increase firing of DA neurons. Non-dopaminergic SNR neurons are consistently inhibited by striatal stimulation. Thus, inhibition of nigrostriatal dopaminergic neurons appears to be mediated directly through GABAergic striatonigral input, while activation of nigrostriatal dopaminergic neurons is mediated through disinhibition from an SNR interneuron. SN interneurons are more sensitive to the inhibitory effects of GABA than the dopaminergic cells are, so the predominant effect of GABA release from striatonigral afferents is disinhibition of the dopaminergic neurons.

Analogous GABA interactions may occur in the VTA. Afferent fibres project from the NAcc and ventral pallidum to the VTA (Troiano and Siegel, 1978a, 1978b; Phillipson, 1979; Zahm, 1989). These afferents seem to include a GABAergic input (Olpe *et al.*, 1977; Waddington and Cross, 1978; Wolf *et al.*, 1978; Walaas and Fonnum, 1980; Yim and Mogenson, 1980a, 1980b), and GABAergic interneurons have also been identified in the VTA (Fonnum *et al.*, 1977; Nagai *et al.*, 1983). Iontophoretic application of GABA reduces the firing rates of dopaminergic and non-dopaminergic neurons in the VTA (Wolf *et al.*, 1978; Yim and

Mogenson, 1980a), but superfusion of VTA slices with GABA or the GABA agonist muscimol increases spontaneous release of [^{3}H]DA (Beart and McDonald, 1980). Further, electrical stimulation of the NAcc produces patterns of excitation and inhibition similar to the effects of striatal stimulation on SN neurons (Wolf *et al.*, 1978; Yim and Mogenson, 1980b). The inhibitory effects are potentiated by iontophoretic application of the GABA uptake inhibitor nipecotic acid (Yim and Mogenson, 1980b), and reversed by iontophoretic application of the GABA antagonists bicuculline (Wolf *et al.*, 1978) or picrotoxin (Yim and Mogenson, 1980b). VTA microinjections of muscimol also produce increases in extracellular concentrations of DA, DOPAC and HVA in the NAcc, and these increases are antagonized by pretreatment with baclofen (Kalivas *et al.*, 1990).

The effects of μ-selective opiate agonists on mesolimbic dopaminergic activity have been attributed to actions on μ receptors located on GABAergic interneurons (Dilts and Kalivas, 1989; Johnson and North, 1992). However, it is possible that μ opioid receptors may also be located on terminals of GABAergic afferents to the VTA. Quinolinic acid lesions of the VTA only produce a 50% reduction in [^{125}I]DAMGO binding in the VTA (Dilts and Kalivas, 1989), raising the possibility that some VTA μ receptors are located on neurons with perikarya that are not contained within the VTA. Thus, μ opioid agonists and antagonists may act upon both populations of μ receptors, producing complex interactions between opioids, GABA, and DA in the VTA.

Local adminstration of a μ agonist would increase opioid inhibition of VTA GABAergic afferents and interneurons. This would reduce the inhibition of dopaminergic activity from GABAergic afferents that synapse directly on the dopaminergic neurons. Inhibitory input from GABAergic afferents onto VTA GABAergic interneurons would also be reduced, but these interneurons would be directly inhibited by agonist actions at μ receptors located on the interneurons. Thus, dopaminergic activity would be disinhibited through both sets of GABAergic inputs (i.e. direct inputs from GABAergic afferents, and polysynaptic input involving GABAergic interneurons).

VTA administration of a μ antagonist would block opioid inhibition of GABAergic afferents and interneurons in the VTA. This would increase the direct GABA-mediated inhibition of dopaminergic neurons from GABAergic afferents. However, GABA-mediated inhibition of GABAergic interneurons would also be increased. This inhibition would compensate for antagonism of opioid inhibition of the interneurons themselves, and produce a disinhibition of mesolimbic dopaminergic neurons. Further, given that the sensitivity of GABAergic interneurons to the inhibitory effects of GABA is 20 times higher than the sensitivity of dopaminergic neurons (Grace and Bunney, 1979), the effects of disinhibition of dopaminergic neurons through actions of the GABAergic interneurons would be expected to outweigh the effects of direct GABA-mediated inhibition of DA resulting from antagonism of μ receptors on the GABAergic afferents that synapse directly on dopaminergic neurons. A schematic

representation of this proposed VTA circuitry is presented in Fig. 6.7.

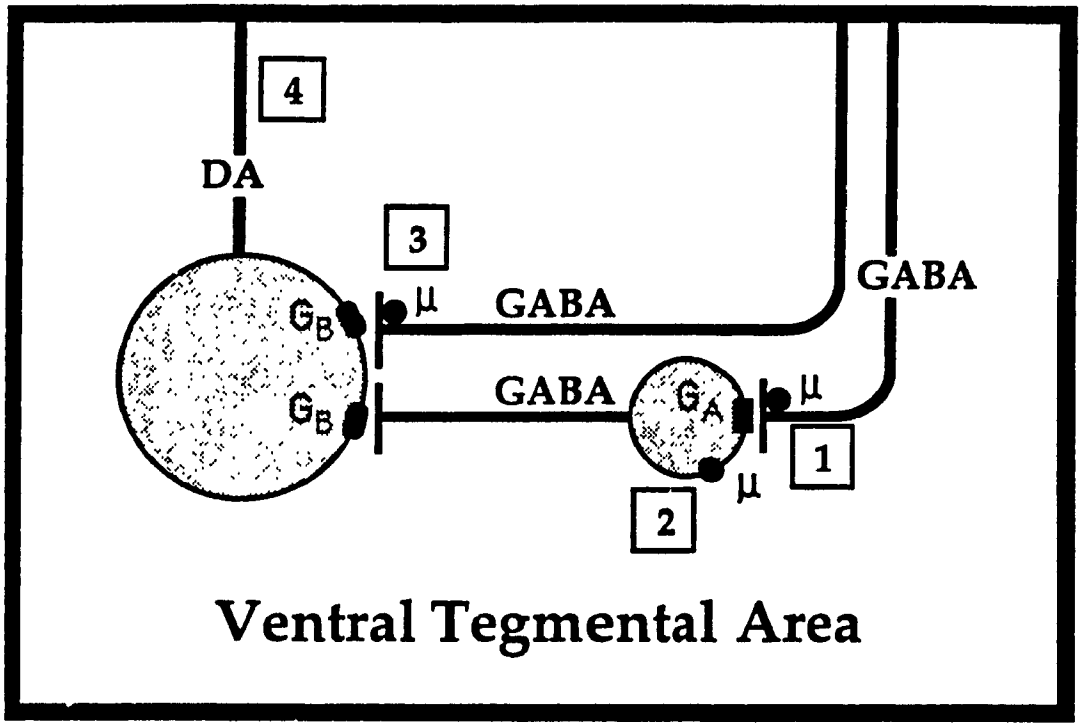

Fig. 6.7. A schematic representation of possible GABA interactions in the VTA. **μ AGONISTS:** Agonists acting at μ receptors on GABAergic terminals presynaptic to GABAergic interneurons (1) decrease GABA-mediated inhibition of the GABAergic interneurons - but μ agonists acting on GABAergic interneurons (2) and on terminals of long axon GABAergic afferents presynaptic to the dopaminergic neurons (3) inhibit these neurons, resulting in a net disinhibition of mesolimbic dopaminergic neurons (4). **μ ANTAGONISTS:** Antagonists acting at μ receptors on GABAergic terminals presynaptic to GABAergic interneurons (1) increase GABA-mediated inhibition of the GABAergic interneurons. Therefore, antagonism of tonic opioid inhibition of GABAergic interneurons (2) is without effect, and the net activity of GABAergic interneurons is reduced due to increased GABA-mediated inhibition. μ Antagonists acting on terminals of long axon GABAergic afferents presynaptic to the dopaminergic neurons (3) reduce opioid inhibition of these neurons, to increase inhibitory GABA input from these neurons onto the dopaminergic neurons. However, the removal of inhibitory input from GABAergic interneurons (2) outweighs the increased GABA-mediated input from long axon GABAergic afferents, so again the result is a net disinhibition of mesolimbic dopaminergic neurons (4).

6.4.6 Effects of VTA Microinjections of CTOP and β-FNA on 5-HIAA Concentrations

In the second experiment reported herein, extracellular NAcc 5-HIAA concentrations were significantly higher after VTA microinjections of CTOP and β-FNA than they were after VTA microinjections of aECF for a short period (2 samples) after VTA CTOP or β-FNA injections. However, this finding is not consistent with the results of the first experiment, and with the results of our previous experiment (see Chapter 4), when 5-HIAA concentrations were not altered significantly by any dose of CTOP. The between groups differences in 5-HIAA concentrations found in the second experiment are believed to reflect slight decreases in 5-HIAA concentrations that were observed after vehicle injections in the control group. The reason for these decreases is unclear, but significant decreases have not been observed in control rats in previous experiments.

6.4.7 Summary and Conclusions

In summary, VTA microinjections of selective μ opioid antagonists produce significant increases in ventral striatal DA, DOPAC and HVA concentrations that mimic the effects seen after VTA microinjections of selective μ opioid agonists. While the possibility of non-selective or partial agonist actions of CTOP and β-FNA cannot be ruled out in the present study, it appears likely that the effects occur as a consequence of antagonism of complex

interactions between opioid actions on GABAergic afferents to the VTA and GABAergic interneurons within the VTA. This possibility may be better addressed by electrophysiological analyses of the effects of local administration of GABA and selective opioids.

Chapter 7

General Discussion

7.1 A Comparison between the Results of Intracranial Self-Administration and Microdialysis Studies

It is well established that opiate actions in the VTA produce activation of mesolimbic DA neurotransmission, and it is commonly believed that these actions of opiates play a role in the rewarding effects of these drugs (for review see Section 4.1). The present studies implicate VTA μ and ∂ opioid receptors in both the DA-modulating and the rewarding effects of opiates. Moreover, a comparison of the effective doses of DAMGO and DPDPE in the behavioural and neurochemical assays reveals some interesting correlations between the rewarding and DA-modulating efficacies of these drugs. These drugs were differentially rewarding, and their potencies to produce self-administration occurred in close proportion to their potencies to activate the mesolimbic DA system.

The minimum doses of DAMGO and DPDPE that rats self-administered were comparable to the minimum doses that produced elevations in extracellular ventral striatal DA concentrations. Each self-administered microinfusion of DAMGO contained 3.17×10^{-12} moles of the opiate. This

self-administered dose of DAMGO was similar in magnitude to the dose of DAMGO (1.32×10^{-12} moles) that produced small (i.e. just detectable) increases in ventral striatal DA concentrations (see Fig. 4.1) after microinjections into the VTA. In the case of DPDPE, each self-administered microinfusion contained 3.17×10^{-10} moles of the opiate. As in the case of DAMGO, the self-administered dose of DPDPE was similar to the dose of DPDPE (1.32×10^{-10} moles) that produced small (just detectable) increases in DA concentrations (see Fig. 4.3). Thus, the threshold doses of DAMGO and DPDPE that initiated and maintained self-administration of each of these drugs were approximately equal to the threshold doses that produced measurable increases in extracellular ventral striatal DA and DA metabolite concentrations.

Further, DAMGO was more potent than DPDPE in both experiments, and the difference in the potencies of DAMGO and DPDPE was similar in the self-administration and microdialysis experiments. While it required 100 times more DPDPE than DAMGO to reinforce lever-pressing, the doses of DPDPE that elevated DA concentrations were 100 to 1,000 times higher than the doses of DAMGO that produced roughly equivalent increases in these concentrations. These similarities in the potencies of selective μ and ∂ opioid agonists to initiate and maintain self-administration, and to elevate extracellular ventral striatal DA concentrations provide further support for the idea that mesolimbic DA neurotransmission plays an important role in the rewarding effects of opiates after administration into the VTA

(see Wise and Bozarth, 1982, 1987; Bozarth and Wise, 1983, 1985; Bozarth, 1986, 1987b; Di Chiara and Imperato, 1988a; Wise and Rompré, 1989).

7.2 The Relative Involvements of VTA μ and ∂ Opioid Receptors in Mediation of the Actions of Endogenous Opioid Peptides

The differential potencies of DAMGO and DPDPE after VTA microinjections suggest that VTA μ and ∂ receptors may differentially participate in the rewarding and DA-modulating actions of exogenous opiates. The high potency of DAMGO after VTA microinjections (Latimer *et al.*, 1987; Bals-Kubik *et al.*, 1990a; present results, Chapters 2 and 4), coupled with the high density of μ opioid receptors in the VTA, suggests that VTA μ receptors play an important role in mediating the rewarding and DA-modulating effects of opiates. On the other hand, the low potency of DPDPE actions after VTA microinjections, and the low density of ∂ receptors in the VTA lead to the conclusion that VTA ∂ receptors play a minor role in the rewarding and DA-modulating effects that result from opiate admininstration.

However, when considering the roles of VTA opioid receptors in mediation of the actions of endogenous opioid peptides, their relative involvements may differ from their involvements in the actions of exogenous opiates. The VTA is richly innervated by neurons that release methionine- and leucine-enkephalin (Johnson *et al.*, 1990), endogenous opioid

neurotransmitters that are moderately selective for ∂ opioid receptors (Lord *et al.*, 1977). Further, it appears that enkephalin actions in the VTA are involved in the neurobiology of opioid-reward and in opioid-modulation of dopaminergic activity. Conditioned place preferences (Glimcher *et al.*, 1984) and increased locomotion (Kalivas and Richardson-Carlson, 1986) have been produced by inhibition of enkephalinase activity in the VTA.

While the contribution of VTA ∂ receptors to mediation of the effects of exogenous opiates may be small, there is reason to believe that these receptors play a substantial role in mediation of the rewarding and DA-modulating actions of endogenous opioid peptides. The receptor mechanisms by which enkephalin actions are expressed in the VTA are influenced by at least two factors (as discussed previously in the case of exogenous opiates, see Section 2.4.2): the relative densities of μ and ∂ receptors in the VTA, and the affinity of the the enkephalins for these two receptor types. In the case of μ receptors, there is a high receptor density, and a low to moderate receptor affinity. In the case of ∂ receptors, there is a low receptor density, and a high receptor affinity. Thus, it appears that the enkephalins have the potential to act upon both μ and ∂ opioid receptors in the VTA. Accordingly, the role of ∂ receptors may be quite important in mediating the rewarding and DA-modulating actions of enkephalin in the VTA.

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Appendix 1

List of Abbreviations Used

aECF,	artificial extracellular fluid
β-FNA,	{E}-4[[(5a,6β)-17-cyclopropylmethyl)4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic acid methyl ester hydrochloride
BBB,	blood-brain barrier
CNS,	central nervous system
CTOP,	D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2
DA,	dopamine
DALA,	D-Ala^2-methionine enkephalin
DAMGO,	[D-Ala^2,N-Me-Phe^4-Gly^5-ol]-enkephalin
df,	degrees of freedom
DOPAC,	3,4-dihydroxyphenyl acetic acid
DPDPE,	[D-Pen^2,D-Pen^5]-enkephalin
EDTA,	ethylenediaminetetra acetic acid
GABA,	γ-aminobutyric acid
HPLC,	high pressure liquid chromatography
HVA,	homovanillic acid
icv,	intracerebroventricular
ip,	intraperitoneal
LH,	lateral hypothalamus
MFB,	medial forebrain bundle
$Na^{99m}TcO_4$,	sodium technetate

naltrindole	17-cyclopropylmethyl-6,7-dehydro-4,5-epoxy-3,14-dihydroxy-6,7,2',3'-indolmorphinan hydrochloride
nor-BNI,	nor-binaltorphimine
NAcc,	nucleus accumbens
p	probability
SNC,	substantia nigra pars compacta
SNR ,	substantia nigra pars reticulata
SEM,	Standard error of the mean
TH,	tyrosine hydroxylase
U-50,488H	(trans-(dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide) methane sulfonate hydrate
VDI,	variable dose per infusion
VTA,	ventral tegmental area
5-HIAA,	5-hydroxyindole acetic acid
6-OHDA,	6-hydroxydopamine