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Fos Induction Within the Ventral Tegmental Area Following Infusions of Phencyclidine, MK-801, and Nomifensine Into the Core and Shell Subterritories of the Nucleus Accumbens Septi

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ABSTRACT

Fos induction within the ventral tegmental area following infusions of phencyclidine, MK-801 and nomifensine into the core and shell subterritories of the nucleus accumbens septi

Caterina Marcangione

Rats learn to lever-press for phencyclidine (PCP; NMDA channel blocker and dopamine uptake inhibitor), MK-801 (NMDA channel blocker), and nomifensine (dopamine uptake inhibitor) infusions directly into the shell but not the core subterritory of the nucleus accumbens septi (NAS). PCP, MK-801 and nomifensine are thought, through different means, to inhibit a final common path: NAS intrinsic GABAergic output neurons projecting to the ventral tegmental area (VTA). Independent groups of animals were tested to determine whether infusions of PCP, MK-801, or nomifensine directly into the core or shell subterritory of the NAS would induce Fos expression within the VTA. Each rat received a total of 26 unilateral infusions (over a 60 min period) of PCP, MK-801, nomifensine (12 nmol, 1.2 nmol, or 1.7 nmol respectively, per 120 nl infusion) or vehicle into either the NAS core or shell; each animal's infusions were "yoked" to the rate and pattern of administration of a rat that had previously learned to lever-press for PCP infusions. The rats were sacrificed and their brains were processed for histological analysis of Fos-immunoreactivity 75 min after the first infusion of drug. Each drug induced significantly more Fos-positive cells within the lateral and medial VTA. MK-801 and PCP infused into the NAS shell, each induced more Fos within the medial VTA. Overall, Fos induction within the VTA was differentially expressed within the rostral to caudal levels of the VTA. In sum, the present thesis provides preliminary evidence linking suppression of intrinsic NAS GABAergic neurons, *via* both NMDA receptor and dopamine reuptake blockade, to the activation of neurons within the VTA.

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Attempts to identify the neural basis of the rewarding[1] actions of drugs of abuse have been encouraged by a growing consensus that drugs of abuse act on brain circuitry involved in the rewarding actions of natural incentives. Support for this idea has emerged from a wealth of evidence linking the pharmacological actions of drugs of abuse to neural activity within brain circuitry involved in the mediation of rewarding effects of food (Wise *et al.*, 1978; Wise, 1978, 1982), sexual contact (Everitt, 1990; Pfaus and Phillips, 1991), and electrical brain stimulation (Olds and Olds, 1965; Wise, 1980; Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Wise and Rompré, 1989; Gardner, 1992). Each of these rewarding events has been found in numerous studies to affect common neural substrates within the mesocorticolimbic dopamine system and its afferent and efferent connections (Wise and Bozarth, 1987; Di Chiara and Imperato, 1988)—albeit, through a variety of actions at different "trigger zones" (Wise and Hoffman, 1992).

Research on the neural mechanisms of drugs of abuse has focused on understanding the mechanisms of actions of opiates and psychomotor stimulants. Recent developments, however, have stimulated interest in phencyclinoids, a lesser known class of drugs of abuse that includes phencyclidine [1-(phenylcyclohexyl)-piperidine HCl; PCP], dizocilpine (MK-801), and ketamine (Domino, 1964; Marquis and Moreton, 1987). PCP, whose illicit use is not as widespread as that of the opiates and stimulants, has nonetheless been found to have in common with such drugs, the ability to stimulate strong psychoactive effects in both animals and humans (Johnson and Jones, 1990). Moreover, although PCP has been shown to have a variety of neurochemical actions, there is growing evidence that its abuse potential may arise from its effects on glutamatergic and dopaminergic

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[1] The terms "reward" and "reinforcement" are frequently used interchangeably, however, it should be noted that they are not synonymous (Wise, 1989). A "rewarding" effect implies the combined "reinforcing" (both Pavlovian and operant) actions and "priming" (incentive motivational) actions. Given that these two actions cannot be differentiated with the techniques employed in the present study, the more general terms "reward" and "reward-relevant" will be used throughout the thesis to designate collectively drug reinforcement and independent priming effects.

neurotransmission particularly within the mesocorticolimbic dopamine system (Johnson and Jones, 1990).

Like cocaine, amphetamine, and the more specifically acting drug, nomifensine, PCP is a dopamine reuptake inhibitor. PCP is also an antagonist of the N-methyl-D-aspartate (NMDA) receptor (one of the several subtypes of receptors for the excitatory amino acid transmitter glutamate), as are ketamine and MK-801. Both of these pharmacological actions have been shown to lead to elevated synaptic levels of dopamine in the terminal regions of dopamine neurons (e.g., in the nucleus accumbens septi, NAS; Hernandez *et al.*, 1988a, 1988b; Carboni *et al.*, 1989; McCullough and Salamone, 1992), an effect that has been shown to be associated with the rewarding actions of many drugs of abuse (Wise and Bozarth, 1987; Di Chiara and Imperato, 1988). Recently, PCP has been found to have rewarding actions when infused directly into the NAS (Carlezon and Wise, 1996), where it has the potential to block reuptake of dopamine (mimicking the actions of nomifensine) and to antagonize the actions of glutamate at the NMDA receptor (mimicking the actions of MK-801). It was subsequently shown, however, that it was the action of PCP at NMDA receptor and not its effects on dopamine reuptake that appeared to underlie its rewarding actions at this site (Carlezon and Wise, 1996).

The present experiment was carried out to compare further the actions of PCP, MK-801, and nomifensine within the NAS and to extend the analysis of the mechanisms whereby they might have their common effects.

Rewarding Properties of Drugs of Abuse

Drugs of abuse have actions that establish and maintain habitual behaviors in both animals and humans. However, such rewarding actions cannot be directly observed, but rather are inferred on the basis of behavioral observations. Rewarding properties of a drug can best be characterized by the study of its effects on behavior in several paradigms.

In the self-administration paradigm, the rewarding properties of a drug are inferred by the extent to which response-contingent drug injections increase the probability of specific responses, such as lever-pressing (the phenomenon of operant reinforcement; Skinner, 1938; Weeks, 1962; Deneau *et al.*, 1969). The rewarding properties of a drug can be further inferred with the reinstatement paradigm which assesses the ability of a drug to trigger renewed responding in an animal that has extinguished its response habit as a result of termination of operant reinforcement (priming phenomenon; Gerber and Stretch, 1975; Stewart and de Wit, 1987). Yet another property of rewarding drugs, assessed with the conditioned place preference paradigm, is inferred from the ability of a drug to alter an animal's attraction to drug-associated stimuli in the environment. Finally, various reward-potentiation paradigms can be used to determine other rewarding properties of drugs, namely the ability of a response-independent drug injection to potentiate responding or to augment the effects of another rewarding agent (Stewart and Grupp, 1981; Gallistel and Karras, 1984; Gallistel and Freyd, 1987).

Among these behavioral paradigms, the self-administration paradigm has figured prominently in the characterization of rewarding actions of several classes of drugs of abuse including PCP. Studies have shown that a variety of drugs abused by humans, including the opiates morphine and heroin, and the psychomotor stimulants amphetamine and cocaine, reliably establish and maintain intravenous self-administration responding in rats (Weeks, 1962; Weeks and Collins, 1964; Pickens, 1968; Pickens and Harris, 1968; Collins *et al.*, 1984). Some drugs abused by humans, however, do not reliably establish and maintain self-administration responding in rats. For example, PCP which is self-administered by humans (Done, 1976; Burns and Lerner, 1976; Herskowitz and Oppenheimer, 1977), is not readily intravenously self-administered by rats (Yanagita *et al.*, 1977; Cox *et al.*, 1984; Collins *et al.*, 1984). A plausible explanation as to why certain drugs are not readily intravenously self-administered by rats lies in possible aversive effects of the systemically administered drugs. Most drugs, including drugs of abuse, usually

have multiple concurrent actions, that can mask one another. Examining specific drug effects following systemic drug administrations has been problematic due to secondary effects of the drugs that have been difficult to control with this mode of administration.

One way to minimize unwanted side effects of drugs is through the use of direct infusions that limit drug dispersion in the brain (Wise and Hoffman, 1992). Adapting the self-administration paradigm to enable the central or intracranial administration of drugs via response-contingent microinfusions of drugs delivered directly to particular brain structures, has revealed that rats will not only learn to lever-press for intracranial infusions of opiates (Bozarth and Wise, 1981; Devine and Wise, 1994), amphetamine (Hoebel *et al.*, 1983), and cocaine (Goeders and Smith, 1993), but also certain drugs that are not readily intravenously self-administered by rats, including PCP (Carlezon and Wise, 1996). Hence, the use of direct infusions and intracranial self-administration paradigms represent useful tools in identifying and localizing anatomical substrates where drugs have rewarding actions (Wise and Hoffman, 1992).

Sites of Action of Rewarding Drugs Within the Mesocorticolimbic Dopamine System

The VTA and its terminal regions represent central components of the mesocorticolimbic dopamine system. The VTA innervates as well as receives input from a number of cortical and limbic regions, including the NAS and the prefrontal cortex (PFC; Dahlstrom and Fuxe, 1964; Thierry *et al.*, 1973; Heimer and Wilson, 1975; Carter and Fibiger, 1977; Bjorklund and Lindvall, 1978; Fallon and Moore, 1978; Lindvall and Bjorklund, 1978; Nauta *et al.*, 1978; Beckstead *et al.*, 1979; Fallon, 1981; Swanson, 1982; Oades and Halliday, 1987; Zham, 1989; Van Bockstaele and Pickel, 1995). It has been hypothesized that the rewarding properties of several classes of drugs are attributable to each drug's ability to modulate neural activity and neurotransmission within the mesocorticolimbic dopamine system and its efferents and afferents (Wise and Bozarth,

1987), *via* a variety of pharmacological actions at different trigger zones (Wise and Hoffman, 1992). Such that in some cases, the drug's trigger-zone is at or near dopamine cell bodies (e.g., in the VTA), whereas, in other cases it is at or near dopamine terminal regions (e.g., in the NAS or PFC).

Rewarding drugs are thought to affect both neural activity and neurotransmission within particular anatomical substrates of the mesocorticolimbic dopamine system by mimicking or enhancing the actions of endogenous chemical messengers in the brain. For example, opiates mimic the actions of endogenous neurotransmitters at opiate receptors within the brain (Hughes, 1975). Psychomotor stimulants such as cocaine, nomifensine, and amphetamine, promote the synaptic actions of dopamine by increasing its release, by blocking its reuptake, or *via* both actions (Axelrod, 1970; Carlsson, 1970; Heikkila *et al.*, 1975a, 1975b; Fisher and Cho, 1979; Ritz *et al.*, 1987; Ritz and Kuhar, 1989). The glutamatergic NMDA receptor antagonist MK-801, acts as an open ion channel blocker that non-competitively antagonizes glutamate activation of channel opening (Reid *et al.*, 1990). As mentioned previously, PCP has more complex mechanisms of action; it not only promotes the synaptic actions of dopamine by blocking its reuptake (Gerhardt *et al.*, 1987; Carboni *et al.*, 1989; Hondo *et al.*, 1994), but is also a non-competitive NMDA receptor antagonist (Fagg, 1987). The latter mechanism of action is probably responsible for the majority of PCP's rewarding actions, and is better understood at the present time.

The results of numerous electrophysiological and neurochemical studies, have implicated the direct activation of anatomical substrates within the mesocorticolimbic dopamine system in the rewarding actions of several classes of drugs. Some classes of drugs have been found to have rewarding actions within the VTA, whereas, others have been found to have rewarding actions within the NAS. The sites of actions of rewarding drugs within the VTA and the NAS—the strongest terminal region of the VTA dopamine neurons—will be described in the following sections.

Sites Within the VTA

The rewarding actions of opiates within the VTA have been well documented. Rats learn to lever-press for morphine (Bozarth and Wise, 1981; Devine and Wise, 1994), mu and delta opioid agonists DAMGO ([D-Ala^2,N-Me-Phe^4-Gly^5-Ol]-enkephalin) and DPDPE ([D-Pen^2,D-Pen^5]-enkephalin; Devine and Wise, 1994) infusions directly into the VTA. The rewarding effects of opiates have been attributed to their ability to increase dopamine neurotransmission within the NAS, by acting, *via* opiate receptors, directly on neurons within the VTA (Smith and Lane, 1983; Di Chiara and Imperato, 1988; Johnson and North, 1992). Mu opioids disinhibit the firing of dopamine neurons within the VTA region by inhibiting local GABA interneurons that normally suppress the activity of their dopaminergic neighbours (Johnson and North, 1992). This disinhibition is believed to lead to excitation of dopamine neurons within the VTA (Gysling and Wang, 1983) and increased dopamine release in the NAS (Leone *et al.*, 1991; Devine *et al.*, 1993). Yet another drug of abuse, nicotine, also appears to have its rewarding effects through its actions in the VTA. However, its rewarding actions are thought to be triggered *via* nicotinic receptors located on VTA dopamine neurons (Clarke and Pert, 1985); nicotine directly stimulates dopamine neurons, which in turn leads to an increase in extracellular dopamine levels within the NAS (Imperato *et al.*, 1986). Furthermore, disruption of dopamine neurotransmission in the NAS appears to affect the rewarding properties of opiates and nicotine within the VTA. Such that DA antagonists reduce both intravenous heroin (Bozarth and Wise, 1986) and nicotine (Corrigal and Coen, 1991) self-administration, and that selective dopamine depletion by 6-hydroxydopamine lesion of the VTA blocks the acquisition of intravenous opiate (Bozarth and Wise, 1986) and nicotine (Corrigal and Coen, 1991; Corrigall *et al.*, 1994) self-administration.

Sites Within the NAS

Several classes of drugs have been found to have rewarding effects when infused directly into the NAS. Amphetamine and cocaine, as well as other more selective dopamine uptake inhibitors such as nomifensine all have the ability to block dopamine uptake at terminal sites of mesocorticolimbic dopamine neurons (Axelrod, 1970; Carlsson, 1970; Heikkila *et al.*, 1975a, 1975b; Nomikoş *et al.*, 1990). This dopamine uptake blockade results in an increase in dopamine concentration within the synaptic cleft and enhanced dopaminergic neurotransmission within these terminal sites. Paradoxically, although rats learn to self-administer amphetamine (Hoebel *et al.*, 1983; Phillips *et al.*, 1994) and nomifensine (Carlezon *et al.*, 1995) infusions directly into the NAS, rats will not readily self-administer cocaine directly into the NAS (Carlezon *et al.*, 1995; Goeders and Smith, 1983). These results can possibly be explained by cocaine's local anesthetic actions, lacked by amphetamine and nomifensine. As such, the failure of cocaine to have rewarding actions when infused into the NAS may be due to side effects specific to its pharmacological actions. Nevertheless, the circuitry mediating the rewarding effects of these drugs within the NAS, appears to involve dopaminergic synapses on GABAergic medium spiny neurons (Pickel and Chan, 1990).

Recently, phencyclinoids have been reported to have rewarding actions within the NAS. Rats will readily learn to self-administer infusions of PCP and MK-801 directly into the NAS (Carlezon and Wise, 1996). The circuitry mediating the rewarding effects of these drugs at this site, appears to involve both dopaminergic projections from the VTA and glutamatergic projections from the PFC terminating on local NAS GABAergic medium spiny neurons (Sesack and Pickel, 1992). Whereas dopaminergic neurons make symmetric (inhibitory-type) synaptic contacts with the GABAergic medium spiny neurons of the NAS, glutamatergic neurons make asymmetric (excitatory-type) synapses on these neurons (Sesack and Pickel, 1992). Thus, the GABA medium spiny neurons of the NAS

appear to be tonically excited by the glutamatergic input from the PFC, resulting in tonic inhibition of dopamine neurons within the VTA.

Systemic administrations of PCP and MK-801, both have the ability to increase the firing rates of dopamine neurons within the VTA–through their ability to block NMDA receptors in dopamine terminal regions—(Freeman and Bunny, 1984; French, 1986; French and Ceci, 1990; Pawlowski *et al.*, 1990; Zhang *et al.*, 1992; French *et al.*, 1993; Murase *et al.*, 1993) and to enhance dopamine release in the NAS (Carboni *et al.*, 1989; Rao *et al.*, 1990; Maj *et al.*, 1991; Steinpreis and Salamone, 1993; Wang *et al.*, 1994). Similarly, infusions of these drugs directly into the NAS are thought to increase the firing rates of dopamine neurons within the VTA, as they have been found to lead to increases in extracellular concentrations of dopamine within the NAS (Hernandez *et al.*, 1988a, 1988b; McCullough and Salamone, 1992).

Taken together, these findings suggest that NMDA antagonists, by binding to NMDA receptors, block the excitatory effects of glutamate and suppress the activity of GABAergic medium spiny neurons within the NAS, thus leading to both a suppression of inhibitory input to the neurons of the VTA and an increase in firing rate of these neurons. This increase in firing rate of VTA dopaminergic neurons is presumed to lead in turn, to an increase in extracellular dopamine levels within the NAS. Hence, inasmuch as glutamatergic inputs from the PFC, are thought to modulate the functions of VTA dopaminergic neurons via actions within both the VTA and the NAS (Kornhurber and Kornhurber, 1986; Carlsson and Carlsson, 1990; Karremann and Moghaddam, 1996), the most likely explanation for the excitatory effects of NMDA antagonists (e.g., MK-801 and PCP) is that they modulate an inhibitory influence on dopaminergic neurons within the VTA (Imperato *et al.*, 1990; Zhang *et al.*, 1992, 1993; French *et al.*, 1993).

In summary, the GABAergic medium spiny neurons of the NAS, upon which VTA dopaminergic neurons and PFC glutamatergic neurons synapse (Sesack and Pickel, 1992), would appear to be similarly affected by local infusions of dopamine reuptake blockers

(such as amphetamine, cocaine and nomifensine), distal infusions of substances that activate dopaminergic input to the NAS (such as morphine and nicotine), and by local infusions of NMDA antagonists (such as PCP and MK-801). In each case, the GABAergic output neurons of the NAS appear to be inhibited as a result of the self-administered drug (Carlezon and Wise, 1996).

The Present Experiment

There is substantial evidence linking activity within the VTA and NAS to rewarding actions of PCP and related drugs. The effects of these drugs on neural activity have, for the most part, been measured *via* electrophysiological studies and neuroanatomical mapping studies using the metabolic marker [^{14}C]2-deoxyglucose (2DG).

Numerous electrophysiological studies have revealed that whereas systemic administration of PCP and MK-801 increase the firing rates of dopamine neurons within the VTA with a potency that is positively correlated with their ability to block the NMDA receptor (French and Ceci, 1990), systemic administration of the dopamine reuptake blocker, nomifensine, decreases the firing rate of dopamine neurons (Gerhardt *et al.,* 1987; Einhorn *et al.,* 1988). Furthermore, MK-801 and PCP have each been found to decrease the firing rates of non-dopaminergic, presumably GABAergic neurons of the VTA (Zhang *et al.*, 1993). The effects of nomifensine on these neurons, however, have yet to be fully characterized. Likewise, neuroanatomical mapping studies using 2DG, as an indirect indicator of neural activity, have reported activation of specific substrates of the mesocorticolimbic dopamine system following systemic injections of PCP and MK-801 (Piercey and Ray, 1988; Piercey *et al.,* 1988; Piercey and Hoffmann, 1989).

Both electrophysiological and metabolic markers of neural activity allow the identification of brain regions that are active following various forms of stimulation. These methods, however, do not allow one to specify which cell types are activated. Electrophysiological methods enable the study of small portions of the brain (e.g., single

neurons) at one time, and therefore renders the identification of simultaneously activated populations of cells (i.e., nuclei or brain structures) difficult or impossible. In contrast, the 2DG mapping technique, infers neural activity based on the assumption that active neurons take up more glucose than inactive neurons. Nevertheless, the 2DG method has poor spatial resolution, in that it cannot distinguish between altered glucose metabolism in neurons intrinsic to a brain region from that of terminals of afferent fibers, and therefore, it has been used to map functional activity primarily in neuropil (Sokoloff, 1977; Sharp *et al.*, 1993). Because it provides a measure of glucose utilization throughout the cell, the 2DG method may be more sensitive to changes in functional activity within the neuropil rather than within cell bodies (Sharp, 1976; Sagar *et al.*, 1988; Sharp *et al.*, 1993).

Recently, activation of the immediate-early gene c-*fos* has been proposed as a cellular marker of neural activation (Dragunow and Faull, 1989; Morgan and Curran, 1991; Sharp *et al.*, 1993). Because c-*fos* and its protein product Fos mark the nuclei of activated cells, their detection allow the identification of specific cell bodies. Therefore, the detection of c-*fos* mRNA *via in situ* hybridization and the immunocytochemical detection of Fos, have both been used as tools to map neural activity in the brain (Dragunow and Faull, 1989; Morgan and Curran, 1991; Sharp *et al.*, 1993).

In quiescent cells, c-*fos* and Fos, are expressed in low levels, but following a variety of stimulus events, are rapidly and transiently induced *via* multiple second messenger systems (e.g., calcium entry into neurons or by receptor-coupled second messengers such as cAMP; Gallin and Greenberg, 1995; Hill and Treisman, 1995). Furthermore, depolarizing agents such as potassium chloride and sodium channel activators, have been found to induce Fos expression, whereas, the sodium channel blocker tetrodotoxin as well as specific calcium channel inhibitors appear to antagonize this effect (Morgan and Curran, 1986, 1988; Morgan *et al.*, 1987; Sagar et al., 1988; Sheng and Greenberg, 1990; Robertson, 1992). Moreover, *in vivo*, c-*fos* mRNA and its protein product Fos can be observed in discrete regions of the rat brain following various forms of

stimulation, including physiological, pharmacological, and behavioural stimulation (Doucet *et al.*, 1990; Morgan and Curran, 1991).

Because this activation occurs selectively in areas responsible for processing the stimulation received, it has been suggested that the immunocytochemical detection of Fos can be used as a marker for neurons which respond to particular stimuli (Dragunow and Faull, 1989; Sagar *et al.*, 1988). However, not all activated neurons have been found to express the c-*fos* gene (Sagar and Sharp, 1993) or Fos protein (Bullitt, 1990). In spite of this, immunocytochemical localization and quantification of Fos induction have been used to trace functional neural pathways, pertinent to the present experiment, following various forms of stimulation including, electrical brain stimulation (Dragunow and Robertson, 1987; Wisden *et al.*, 1990; Arvanitogiannis *et al.*, 1996, 1997; Flores *et al.*, 1997; Rossetti *et al.*, 1998), and drug administration of psychomotor stimulants (Graybiel, *et al.*, 1990; Graybiel, 1993; Young *et al.*, 1991), opiates (Nestler, 1993; Bontempi *et al.*, 1997), and phencyclinoids (Marcangione and Wise, 1997; Gao *et al.*, 1998). It appears to be the case that both rewarding brain stimulation and pharmacological stimulation with drugs of abuse, activate neurons of specific structures within the mesocorticolimbic dopamine system.

PCP, MK-801, and nomifensine are thought to share a common action, inhibition of the intrinsic GABAergic output neurons of the NAS. Hence, if indeed neurons within the VTA are tonically inhibited by activation of NAS GABAergic medium spiny neurons, interference with the activity of these NAS GABAergic medium spiny neurons, by either blocking the excitatory effects of glutamate or by blocking the reuptake of dopamine, should lead to a disinhibition of neural activity within the VTA. Inasmuch as Fos immunoreactivity can be used as a means of visualizing neural activity within the brain, the increase in VTA neural activity resulting from the inhibition of NAS GABAergic neurons, is expected to manifest itself as increased expression of Fos immunoreactivity within this structure. In addition, although, PCP, MK-801, and nomifensine are each thought to lead to disinhibition of cells within the VTA—albeit, via different mechanisms of action—a

possible difference in the pattern of Fos expression induced by the different drugs, reflecting their different pharmacological actions, can also be expected.

Furthermore, it should be noted that the neurons within the NAS form a heterogeneous collection of distinct and functionally relevant subterritories delineated on the basis of the distribution of neurochemical markers, as well as by the organization of efferent and afferent projections (Groenewegen and Russchen, 1984; Heimer *et al.,* 1991; Berendse *et al.,* 1992; Zahm and Brog, 1992; Brog *et al.,* 1993; Jongen-Rêlo, *et al.,* 1994); such that the NAS can be partitioned into two distinct subterritories, a central region and peripheral region, designated as the "core" and "shell" respectively. Both the core and shell subterritories of the NAS have direct projections to the VTA and the neighbouring substantia nigra. However, the shell projects heavily to the VTA, whereas, the core projects to the substantia nigra (Heimer *et al.,* 1991; Berendse *et al.,* 1992). In addition, the core and shell subterritories of the NAS are differentially innervated by the VTA. The core receives a minor innervation predominantly from the lateral aspect of the VTA, whereas the shell receives a dense inneravtion preferentially from the medial VTA (Beckstead *et al.,* 1979; Swanson, 1982; Phillipson and Griffiths, 1985; Voorn *et al.,* 1986; Voorn *et al.,* 1989; Zahm and Johnson, 1989; Zahm, 1992; Meredith *et al.,* 1993).

These regional differences, with respect to both efferent and afferent connections of the NAS subterritories, raise the possibility that various drugs may affect or have actions on the different output populations differentially (Alexander and Crutcher, 1990). Recent findings suggests that this may indeed be the case; the effective reward sites for the NMDA receptor antagonists PCP and MK-801 (Carlezon and Wise, 1996), and of the dopamine uptake inhibitor nomifensine (Carlezon *et al.,* 1995), appear to be in the shell subterritory of the NAS and not in the more dorsal and lateral core subterritory. Differences between Fos induction within the VTA following microinfusions of drugs in a non-reward-relevant site (core) vs a reward-relevant site (shell) are expected to reflect the heterogeneousness of the two subterritories of the NAS.

Hence, the purpose of the present experiment is threefold. First, to investigate whether intra-NAS infusions of PCP, MK-801 and nomifensine—at doses reported to have been self-administered into the NAS by rats—induce Fos within the lateral and medial aspects of the VTA. Second, if indeed PCP, MK-801, and nomifensine infusions directly into the NAS induce Fos within the VTA, to examine the occurrence of any differences in Fos expression within the rostral to caudal levels of the VTA. Third, to determine whether Fos induction within the rostral to caudal levels of both the lateral and medial aspects of the VTA is differentially affected by infusions of drugs within either the core or shell subterritory of the NAS.

Method

Subjects: Forty-two Long-Evans hooded male rats (Harlen Sprague Dawley, U.S.A.) weighing between 350-400 g at the start of the experiment were used as subjects. All animals were individually housed in shoebox Plexiglas cages (36 x 26 x 19 cm) and maintained at a constant temperature of 21 °C, in a reverse light-dark cycle with lights off at 09h00. Food and water were available *ad libitum*.

Surgery: Prior to the left hemispheric implantation of the guide cannula, each of 40 rats was anesthetized with sodium pentobarbitol (65 mg/kg, ip). Each rat was then placed in a stereotaxic frame and injected both with atropine sulfate (0.25 mg/kg, sc) to minimize bronchial secretions and with the antibiotic amycilin (0.3 ml, im) as a precaution against post-surgical infection. The incisor bar was elevated to 5 mm above the interaural line, and the guide cannula was angled toward the midline at 10° from the vertical. Twenty rats (designated as the shell group) were unilaterally implanted with a 22 gauge guide cannula (2 cm in length) that terminated 1.1 mm above the NAS (3.5 mm anterior to bregma, 2.4 mm lateral to the midline suture, and 5.9 mm below dura). The other 20 rats (designated as the core group) were unilaterally implanted with a 22 gauge guide cannula (2 cm in length) that terminated 2.1 mm above the NAS (3.5 mm anterior to bregma, 2.4 mm lateral to the midline suture, and 4.9 mm below dura). Following implantation, the guide cannula was secured to the skull with stainless steel jeweler's screws and dental cement. A 28 gauge stylet, extending 1.1 mm beyond the tip of the guide cannula, was inserted and removed only during handling sessions and testing. At the end of the surgical procedure, the wound was covered with an antibacterial agent and each animal was placed under a heating lamp until post-surgical locomotion was observed.

Drugs and vehicle solutions: The drugs used were phencyclidine (PCP) hydrochloride (National Institute of Drug Abuse, U.S.A.), MK-801 (Merck Research Laboratories, Rahway, NJ) and nomifensine maleate (Research Biochemicals, Natick, MA). Each drug was dissolved in an artificial cerebrospinal fluid vehicle consisting of a 2.0 mM Sorenson's phosphate buffer (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; Moghaddam and Bunney, 1989). The nomifensine solution was placed in a sealed 10 ml glass bottle and vibrated in an ultrasonic cleaning bath until the drug dissolved.

Apparatus: Microinfusions of drugs or vehicle solutions were conducted in operant chambers (26 x 26 x 26 cm) equipped with a single lever mounted on the rear of the wall, 6 cm above a wire-mesh floor. To prevent the animals from getting their headgear or wire lead entangled with the lever, a wooden block was placed below the lever and secured to the wire-mesh floor. The operant chambers were individually contained in a sound-attenuating cubicle illuminated with a dim (15 W) red light.

An electrolytic microinfusion transducer (EMIT) drug-delivery system (Criswell, 1977; Bozarth and Wise, 1980) was used to deliver the drug or vehicle solutions. Each EMIT unit consisted of two platinum electrodes placed in a drug or vehicle solution-filled reservoir equipped with a 28 gauge injector cannula that extended 1.1 mm beyond the tip of the guide cannula. Each EMIT unit was attached to the guide cannula by a threaded collar, and connected to a constant current generator (Mundl, 1981) *via* a flexible spring-protected wire lead, a swivel and a mercury commutator. The constant current generator was in turn connected to a personal computer that enabled the experimenter to control the delivery of infusate. Each infusate delivery triggered the passage of a 200 μA current across the electrodes for 5 sec; the passage of the current for this period of time generated hydrogen gas from the aqueous solution in the reservoir, which in turn displaced an approximate (120 nl) volume of solution out the tip of the injector. A 10 μA quiescent current prevented

the gas from redissolving into the aqueous solution, but was not sufficient to displace more solution. A yoke delivery program (Cabilio, 1995) designed to control the delivery of infusate was used.

Procedure: Following surgery, the animals were allowed to recover for at least 10 days, during which they were handled on alternating days to minimize the amount of Fos-immunoreactivity induced by stress on the day of the experiment (Dragunow and Faull, 1989). Each handling session took place during the dark phase of the animals' dark-light cycle and lasted approximately 20 min. The animals were removed from their home cages and placed on a cart with a cage and lid, where they were allowed to interact with the other rats and freely explore their immediate environment. During the handling sessions, the experimenter also simulated some of the handling that the animals would receive prior to the microinfusion session, by picking them up by the nape of their necks and both removing and replacing the stylet.

Twenty animals with shell NAS placements were randomly divided into four groups of five animals each; each group was randomly assigned to one of the four drug conditions, namely PCP, MK-801, nomifensine and vehicle. Similarly, the remaining 20 animals with core NAS placements were randomly divided into four groups of five animals each and each group was subsequently randomly assigned to one of the four drug conditions.

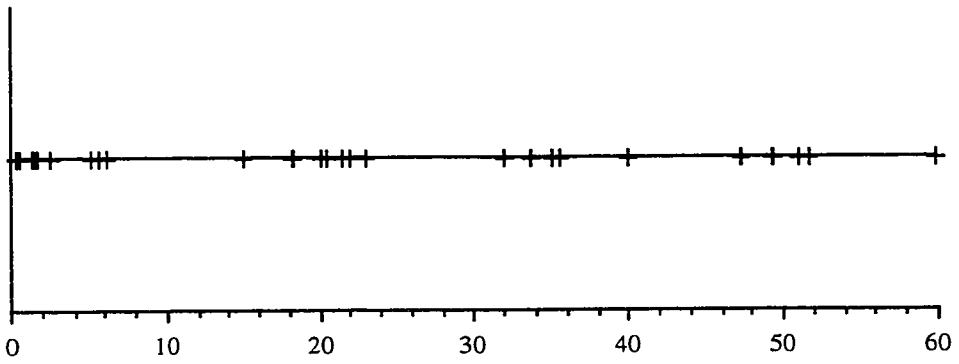
Yoke delivery of drug and vehicle solutions: Before each microinfusion session, the reservoir of each EMIT unit was filled with the appropriate drug or vehicle solution. Furthermore, prior to removing the stylet, inserting the injector cannula through the rat's guide cannula and screwing it into place, each EMIT unit was systematically checked to assure the flow of infusate. Each animal was then placed in an operant chamber and the experimenter engaged the start of the microinfusion session. Each rat received a total of 26

microinfusions of the PCP (12 nmol/120 nl infusion), MK-801 (1.2 nmol/120 nl infusion), nomifensine (1.7 nmol/120 nl infusion) or vehicle solution, spread out during a one hour session; each animal's infusions were yoked to the same rate and pattern of administration as that of a rat that had previously learned to lever-press for PCP infusions (schedule: Figure 1; Carlezon and Wise, 1996). Microinfusions of drug and vehicle solutions were conducted during the dark phase of the animals' dark-light cycle.

Perfusions: Fifteen minutes after the end of the one hour infusion session, the animals were deeply anesthetized with chloral hydrate (400 mg/l, ip) and perfused transcardially with 300 ml of ice-cold heparinized 0.1 M phosphate buffered saline solution followed by 300 ml of ice-cold 4% paraformaldehyde dissolved in 0.1 M phosphate buffer, pH 7.2. Both solutions were perfused using a peristaltic pump. Brains were removed from the skull, post-fixed for 2 to 4 h in 4% paraformaldehyde, blocked and kept in a 30% sucrose solution at 4°C overnight prior to sectioning. Each brain was frozen on the stage of a sliding microtome and marked with a 26 3/8 gauge needle, on the side contralateral to the guide cannula. Consecutive frozen coronal brain sections (30 μm) from the NAS (corresponding to plate 9 to 16 of the Paxinos and Watson, 1986 sterotaxic atlas) and VTA (corresponding to plate 34 to 43 of the Paxinos and Watson, 1986 sterotaxic atlas) regions were cut from each brain on a sliding microtome and collected in cold 50 mM Tris-buffered saline (TBS; pH 7.6).

Fos-immunocytochemistry: All sections were stained for Fos-immunoreactive cells as follows. Sections were washed (3 x 5 min) in cold TBS; and incubated for 48 h at 4°C with an affinity-purified mouse monoclonal antibody raised against the N-terminal sequence of Fos corresponding to the N-terminal residues 4-17 of Fos protein (NCL/BCB Repository, Quality Biotech, Camden, NJ), diluted 1:8000 with a solution of 0.05% Triton X-100 (Fisher Scientific, Pittsburg, PA) in TBS with 1% normal horse serum (Vector

Figure 1. Distribution of the 26 intra-NAS infusions given during a one-hour period. This infusion schedule was adapted from that of a rat self-administering PCP directly into the NAS shell; Carlezon and Wise, 1995).


0
10
20
30
40
50
60
Time of infusion (min)

Laboratories, Burlingame, CA). Following the primary antibody incubation period, sections were rinsed (3 x 5 min) in cold TBS and incubated for 1 h at 4°C with a rat-adsorbed biotinylated anti-mouse IgG made in horse (Vector Laboratories, Burlingame, CA), diluted 1:33 with 0.05% Triton X-100 in TBS with 1% normal horse serum. Following the secondary antibody incubation period, sections were rinsed (3 x 5 min) with cold TBS and incubated for 2 h at 4°C with an avidin-biotin-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA). Following the ABC reagents incubation period, sections were rinsed (3 x 5 min) with cold TBS, and rinsed again for 10 min with cold 50 mM Tris (pH 7.6). Sections were then incubated on an orbital shaker for 10 min with 0.05% 3,3'-diaminobenzidine (DAB) in 50 mM Tris-Hyrochloric acid (HCl) and then again for 10 min in DAB/Tris-HCl with 0.01% hydrogen peroxide and 8% nickel chloride to visualize the protein. After this final incubation, the sections were rinsed with cold TBS, wet-mounted onto gel-coated slides and air dried. The slides were then washed for 60 s in distilled water to remove salt crystals, dehydrated through graded alcohols (70%, 95%, and 100% ethanol) for 10 min each, cleared in xylenes for 2 h and coverslipped with Permount (Fisher Scientific, Pittsburg, PA) for light microscopic examination.

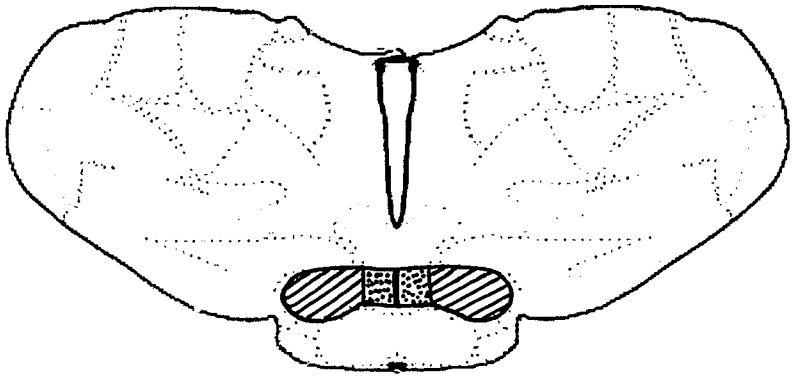
Tyrosine hydroxylase and γ-amino-butyric acid immunocytochemistry: The two rats that did not receive surgical treatment, were perfused and their brains sliced in the same manner as described above. The brain sections of the first of the two rats were stained for tyrosine hydroxylase (TH) immunoreactivity, whereas those of the second rat were stained for γ-amino-butyric acid (GABA) immunoreactivity. All sections were washed (3 x 5 min) in cold TBS and incubated for 20 min at 4 °C in a quenching solution made up of 0.3% hydrogen peroxide in TBS. The sections were then washed (3 x 5 min) in cold TBS before being transferred to a pre-blocking solution consisting of 0.05% Triton X-100 in TBS with 1% normal goat serum (Vector Laboratories, Burlingame, CA) and incubated for 90 min at

4 °C. Following the pre-blocking incubation period, the sections of the first rat were incubated for 48 h at 4 °C with an affinity purified polyclonal rabbit antibody raised against the catecholamine-synthesizing enzyme tyrosine hydroxylase (Eugenetech, NJ), diluted 1:2000 with a solution of 0.05% Triton X-100 in TBS with 1% normal goat serum whereas, the sections of the second rat were incubated for 48 h at 4 °C with an affinity purified polyclonal rabbit antibody raised against γ-amino-butyric acid (Arnel, NY), diluted 1:5000 with a solution of 0.05% Triton X-100 in TBS with 1% normal goat serum. Following the primary antibody incubation period, all sections were rinsed (3 x 5 min) in cold TBS and incubated for 1 h at 4 °C with a biotinylated anti-rabbit IgG made in goat (Vector Laboratories, Burlingame, CA), diluted 1:200 with 0.05% Triton X-100 in TBS with 1% normal goat serum. Following the secondary antibody incubation period, all sections were processed with an avidin-biotin-peroxidase complex and the DAB chromagen in the same manner as described above for the Fos-immunocytochemistry.

Histological and data analyses: The placement coordinates of the guide cannulae tips were identified through the localization of proximate landmarks and structures according to the Swanson (1992) sterotaxic atlas.

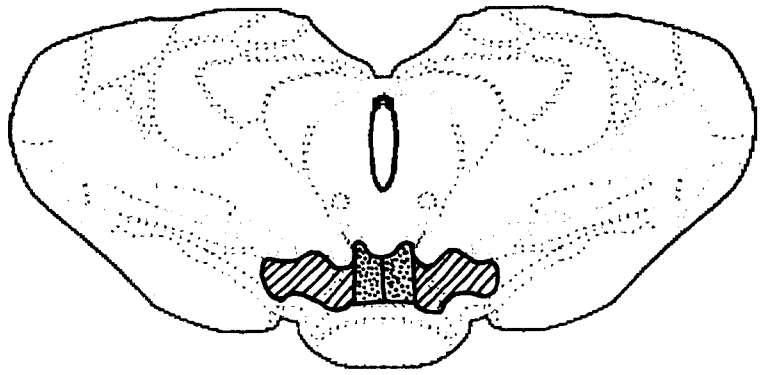
The distribution of Fos-positive cells within the VTA was examined with a Leica microscope (Leitz BMRB), using a 10X objective. Quantitative analysis was conducted using a computerized image analysis system (NIH Image 1.57). TH- and GABA-immunoreactively labeled sections—spanning the entire VTA—served as templates in defining boundaries of where counting of Fos-positive immunoreactive cells was to be restricted. Hence, counting of Fos-positive immunoreactive cell nuclei was restricted to five sections spanning the rostral to caudal VTA (-4.80, -5.00, -5.20, -5.40 and -5.60 mm from to bregma; Figures 2-6, respectively; Paxinos and Watson, 1986), within which borders of the VTA, at the different coordinates, were defined manually to correspond to structure boundaries in the Paxinos and Watson (1986) atlas, Oades and Halliday (1987)

Figure 2. Schematic diagram (top) illustrating the regions of the lateral (hatching) and medial (stippling) VTA that were sampled for analysis of Fos-positive immunoreactive cells. Photomicrographs of TH (center) and GABA (bottom) -immunoreactively labeled cells and processes. Both the diagram (modified from Swanson,1992-4) and the photomicrographs are shown at the level -4.80 mm from bregma. Scale bar = 100 μm.

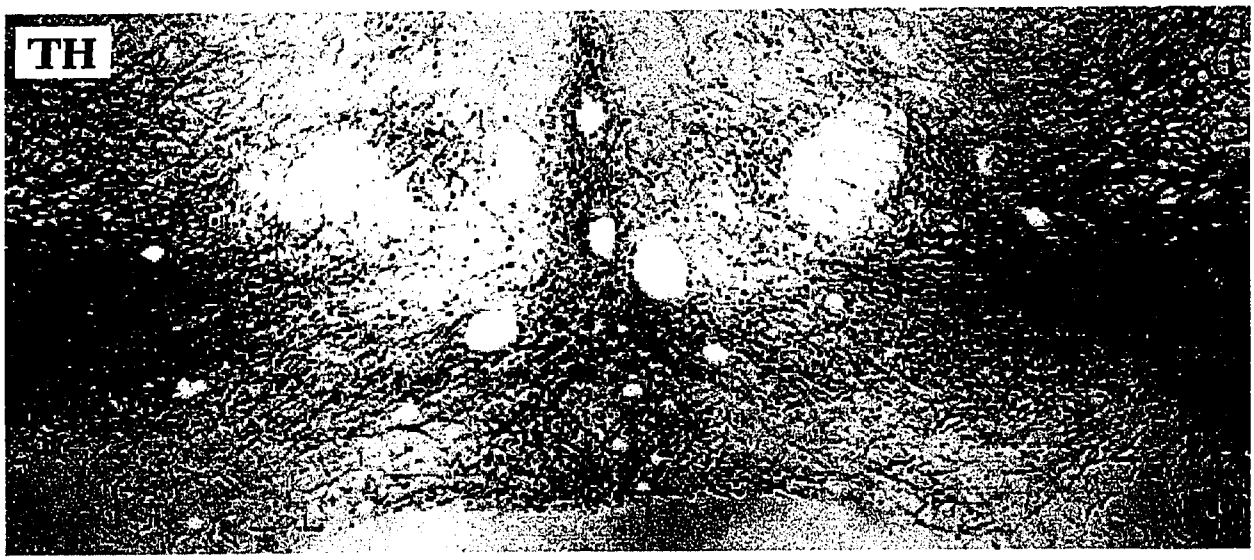


-4.80 mm

Figure 3. Schematic diagram (top) illustrating the regions of the lateral (hatching) and medial (stippling) VTA that were sampled for analysis of Fos-positive immunoreactive cells. Photomicrographs of TH (center) and GABA (bottom) -immunoreactively labeled cells and processes. Both the diagram (modified from Swanson,1992-4) and the photomicrographs are shown at the level -5.00 mm from bregma. Scale bar = 100 μm.



-5.00 mm



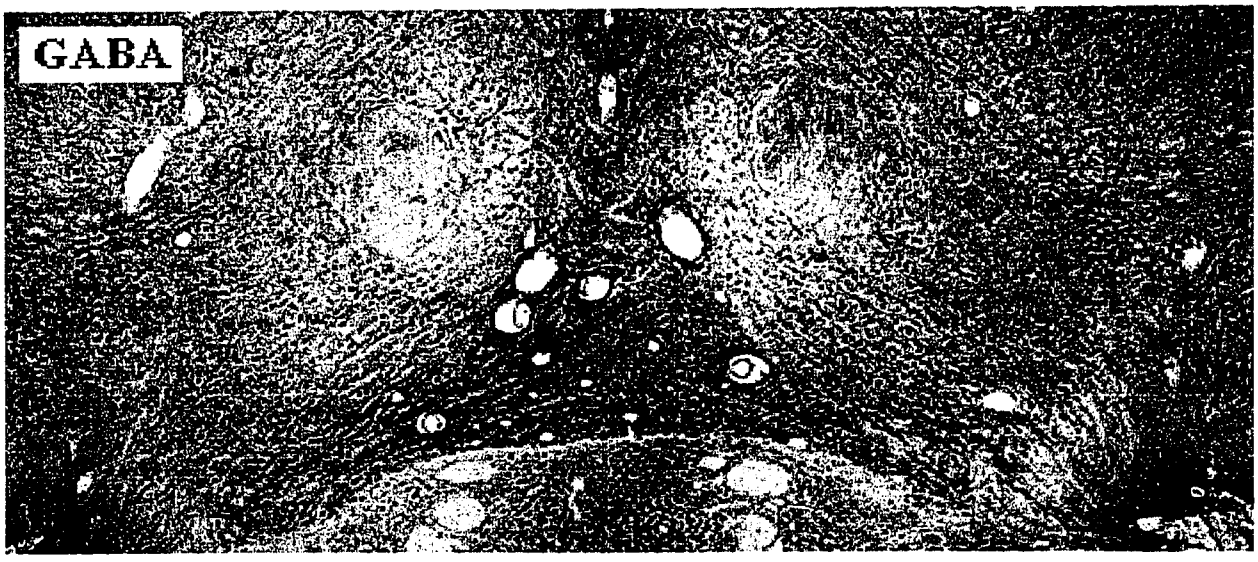
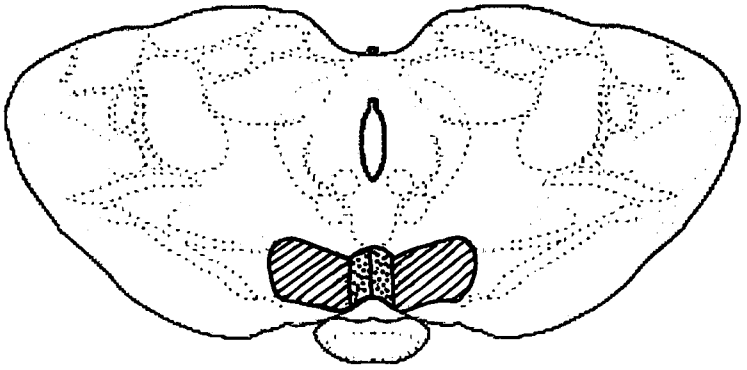
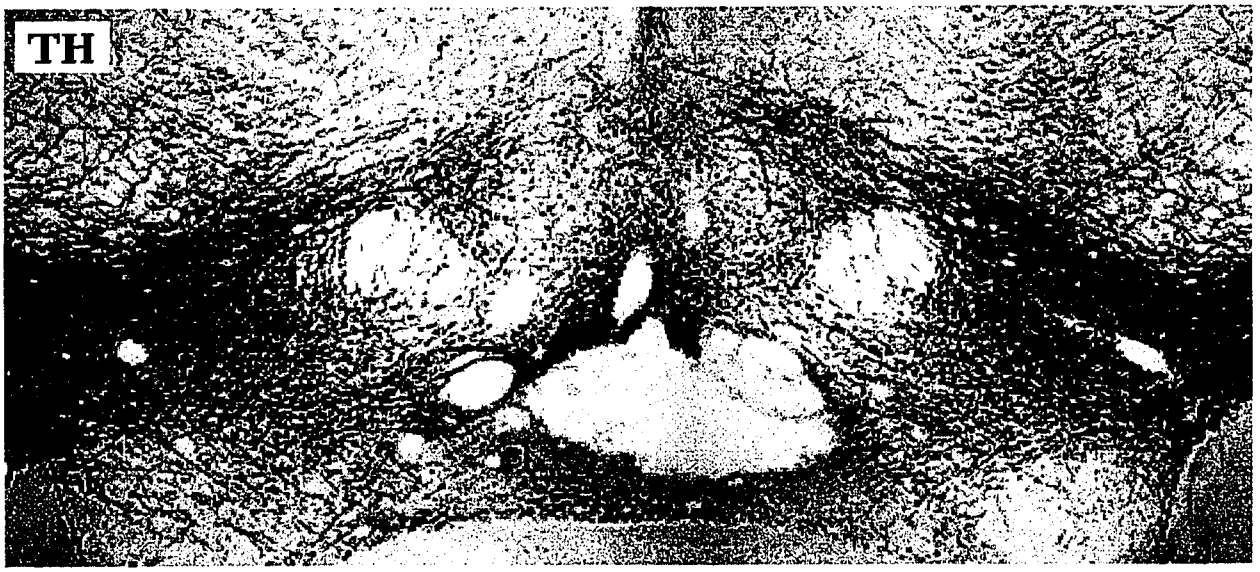


Figure 4. Schematic diagram (top) illustrating the regions of the lateral (hatching) and medial (stippling) VTA that were sampled for analysis of Fos-positive immunoreactive cells. Photomicrographs of TH (center) and GABA (bottom) -immunoreactively labeled cells and processes. Both the diagram (modified from Swanson,1992-4) and the photomicrographs are shown at the level -5.20 mm from bregma. Scale bar = 100 μm.



-5.20 mm



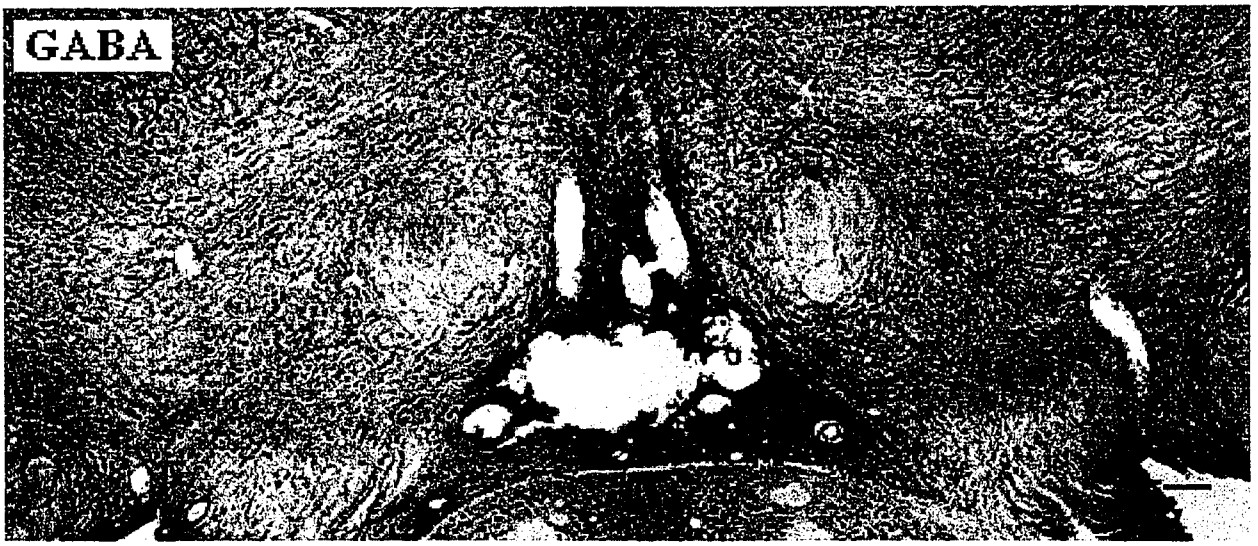
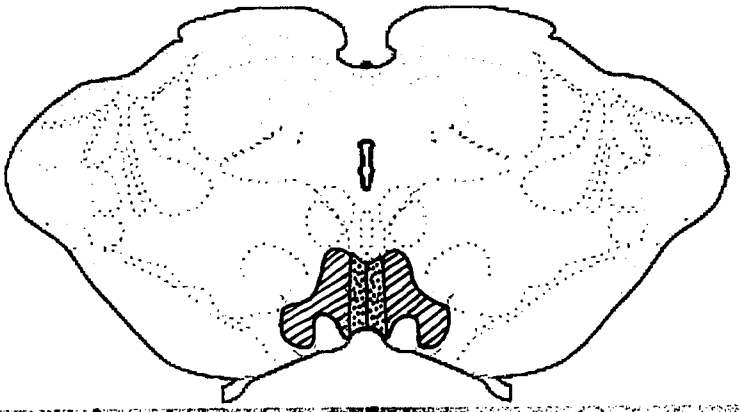


Figure 5. Schematic diagram (top) illustrating the regions of the lateral (hatching) and medial (stippling) VTA that were sampled for analysis of Fos-positive immunoreactive cells. Photomicrographs of TH (center) and GABA (bottom) -immunoreactively labeled cells and processes. Both the diagram (modified from Swanson,1992-4) and the photomicrographs are shown at the level -5.40 mm from bregma. Scale bar = 100 μm.



-5.40 mm

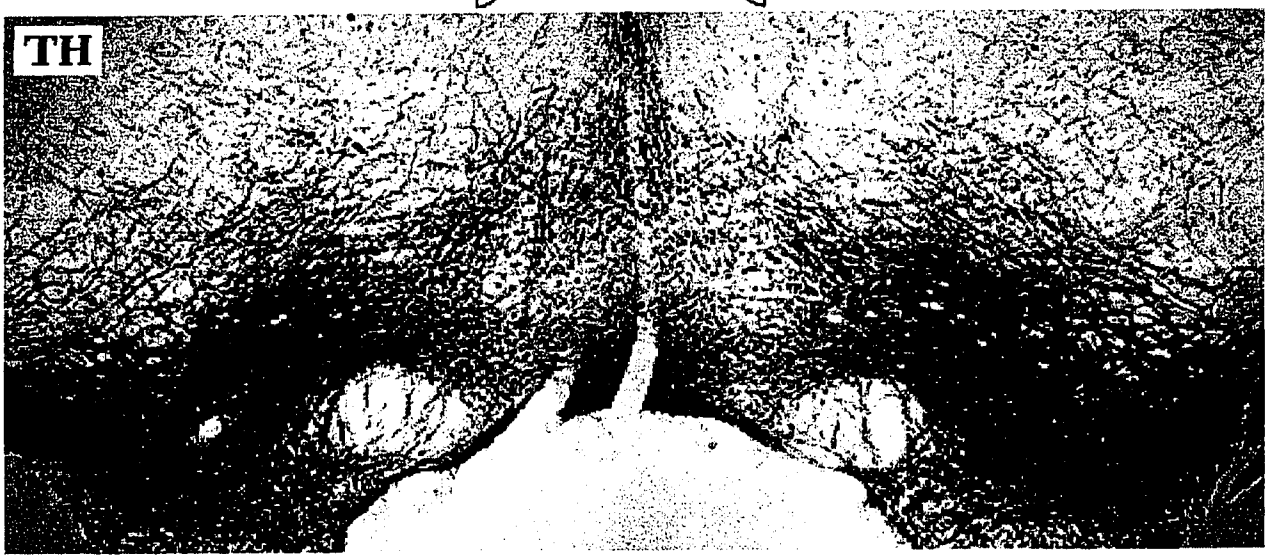
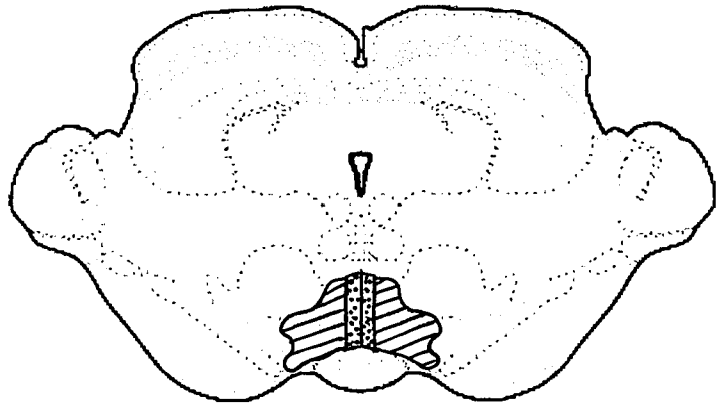
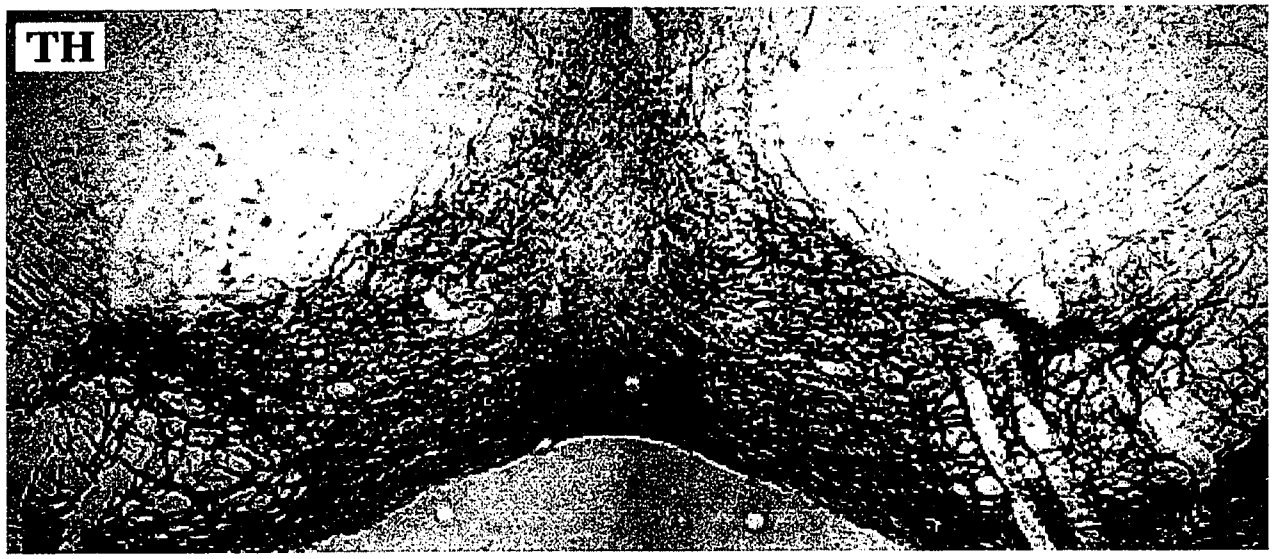
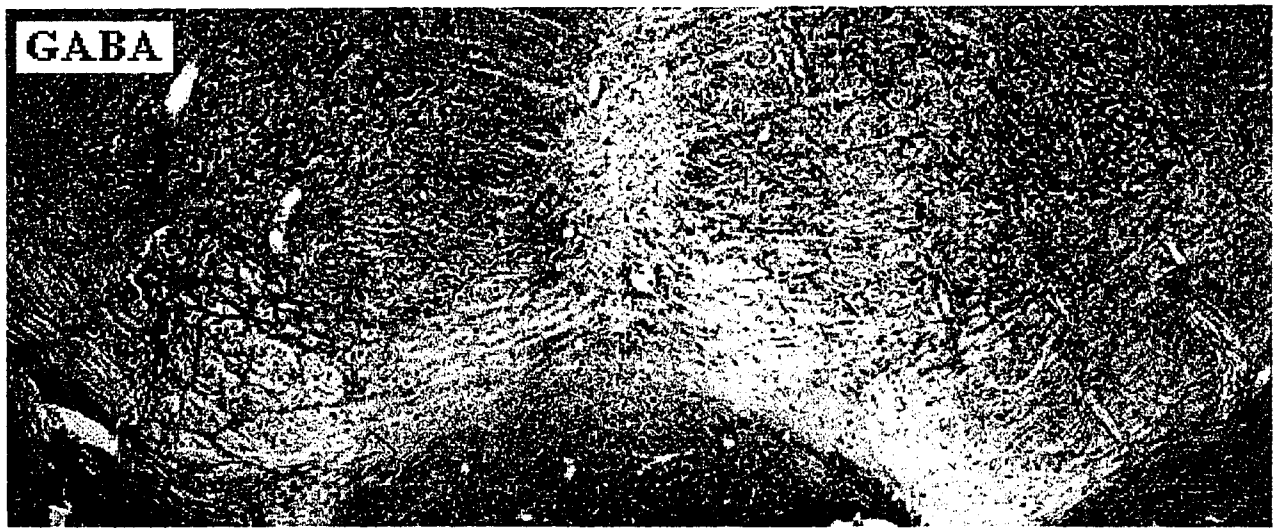


Figure 6. Schematic diagram (top) illustrating the regions of the lateral (hatching) and medial (stippling) VTA that were sampled for analysis of Fos-positive immunoreactive cells. Photomicrographs of TH (center) and GABA (bottom) -immunoreactively labeled cells and processes. Both the diagram (modified from Swanson,1992-4) and the photomicrographs are shown at the level -5.60 mm from bregma. Scale bar = 100 μm.



-5.60 mm





review paper and actual TH- and GABA-immunoreactively labeled sections mentioned above.

Each of the five VTA levels was further divided into four aspects: lateral and medial aspects of both the ipsilateral and contralateral hemispheres. The lateral aspect of the VTA was defined as the area encompassing the nucleus parabrachial pigmentosus and the nucleus paranigralis, whereas the medial aspect of the VTA was defined as the area containing the nucleus interfasicularis, as well as the rostral and caudal nucleus linearis (Oades and Halliday, 1987; Phillipson, 1979; Swanson, 1982; Figures 2-6).

All Fos-positive cell counts were measured as well as the sampling area within the rostral to caudal levels of the VTA—the lateral and medial aspects of both ipsilateral and contralateral hemispheres, corresponding to the NAS infusion site—were counted as primary data.

Statistical analysis: The raw data consisted of the density measurements obtained by dividing the Fos-positive cell counts by the sampling area (mm^2). The mean density and standard error were computed for each of the five VTA levels—ipsilateral and contralateral hemispheres—for each drug, and for each of the two NAS drug infusion sites. The data from the lateral VTA and medial VTA were analyzed separately, each with a 2 X 4 X 2 X 5 mixed-factorial design (SuperANOVA, 1991), with NAS drug infusion site (two levels: core and shell) and drug treatment (four levels: PCP, MK-801, nomifensine, vehicle) as the between-subjects variables and hemisphere (two levels: ipsilateral and contralateral) and VTA level (five levels: -4.80, -5.00, -5.20, -5.40, -5.60 mm from bregma) as the within-subjects variables (repeated measures). No overall effect of hemisphere was found for the lateral VTA. However, a small but significant effect was observed for the medial VTA, revealing more Fos induction within the ipsilateral compared to the contralateral hemisphere (see Appendix A for summary tables). Hence, further

analyses were performed on the data from the ipsilateral hemisphere of the lateral and medial aspects of the VTA.

The data from the ipsilateral lateral VTA and medial VTA were analyzed separately, each with a 2 X 4 X 5 mixed-factorial design (Datasim version 1.1; Bradley, 1988), with NAS drug infusion site (two levels: core and shell) and drug treatment (four levels: PCP, MK-801, nomifensine, vehicle) as the between subjects variables and VTA level (five levels: -4.80, -5.00, -5.20, -5.40, -5.60 mm from bregma) as the within subjects variable (repeated measure). Subsequent tests of simple main effects and post-hoc pairwise comparisons, using the Bonferroni correction test for alpha value, were applied to identify independent variable effects accounting for significant F-values.

Results

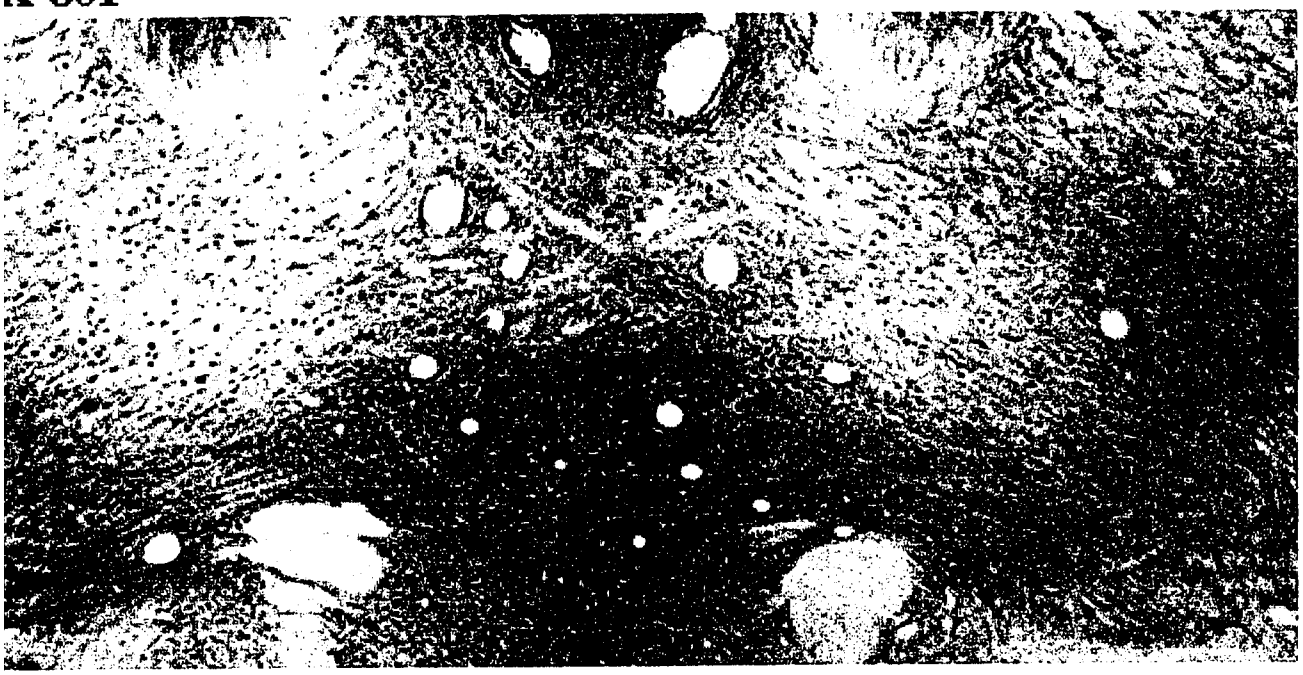
In general infusions of PCP, MK-801, and nomifensine directly into the core and shell NAS, each induced more Fos-positive cells within both the lateral and medial aspects of the VTA, than did infusions of vehicle alone. Differential Fos expression, following infusions of the drugs into either core or shell of the NAS was observed only within the medial VTA, with MK-801 and PCP each inducing more Fos when infused within the shell. In addition, more Fos was seen in rostral aspects of the VTA regardless of the drug infused or drug infusion site. Examples of Fos induction within the lateral and medial aspects of the (a) rostal and (b) caudal VTA, following intra-NAS infusions of MK-801, nomifensine, PCP, and vehicle are represented in Figures 7-10, respectively. Moreover, representative Fos expression following infusions of MK-801, nomifensine, PCP, and vehicle into the (a) core and (b) shell subterritory of the NAS are depicted in Figures 11-14, respectively.

Lateral VTA

The three-way mixed factorial analysis of variance detected significant differences across drug conditions $F(3,32)=12.33$; $p<0.01$, and VTA levels $F(4,128)=277.73$; $p<0.01$, but not for NAS drug infusion site (see Appendix B for summary table). Figure 15 illustrates the overall differential Fos induction across drug conditions. Further analysis using pairwise comparisons showed that intra-NAS infusions of each of the drugs induced significantly more Fos expression than vehicle (see Appendix C for summary table). Intra-NAS infusions of MK-801 were found to induce more Fos than similar infusions of PCP, however, no significant differences were noted between nomifensine and PCP-treated rats. In addition, significantly more Fos-positive cells were observed within the rostral (-4.80 mm and -5.00 mm from bregma) compared to the caudal (-5.20, -5.40, -5.60 mm from bregma) extent of the VTA, regardless of drug treatment or NAS drug infusion site (Figure

Figure 7. Representative photomicrographs showing Fos induction within the lateral and medial aspects of the VTA at the (A) rostral (-4.80 mm from bregma) and (B) caudal (-5.60 mm from bregma) levels of the VTA following MK-801 infusions within the shell subterritory of the NAS. Scale bar = 100 μm.

K-801



K-801

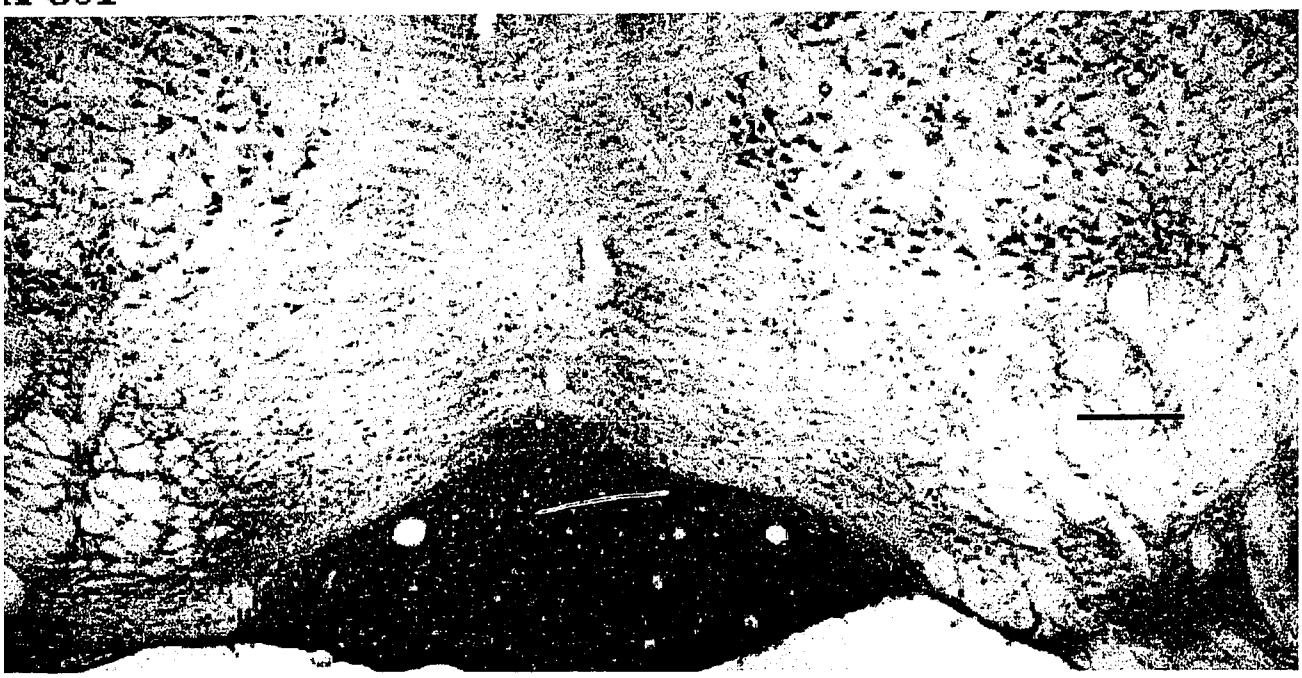
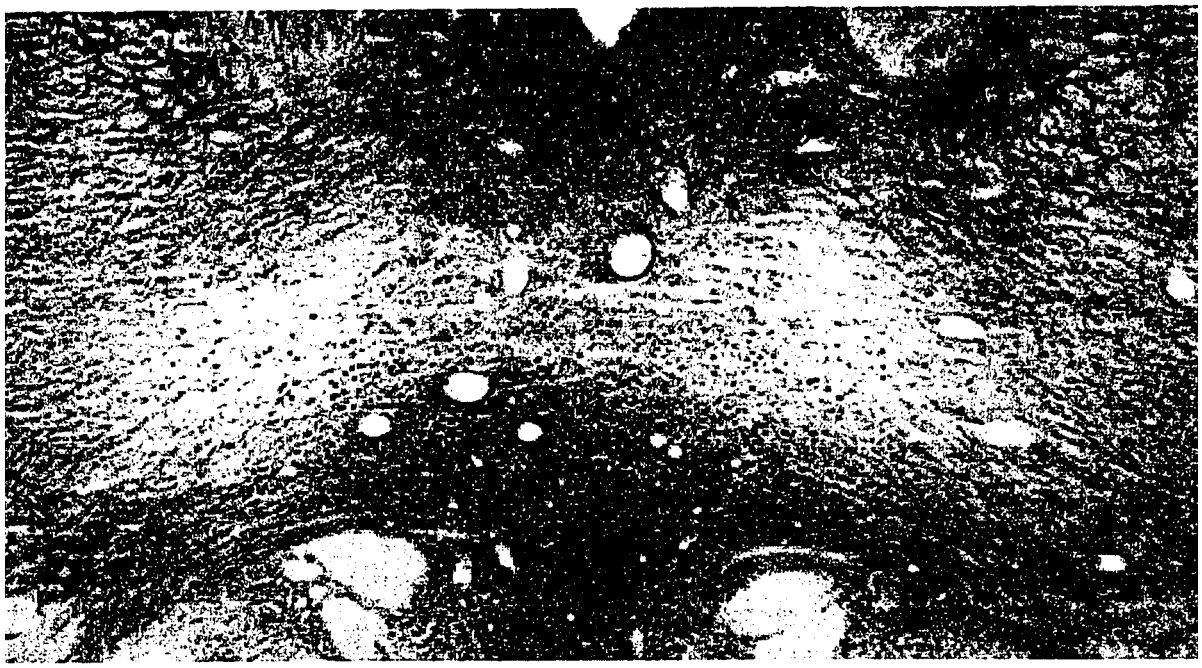


Figure 8. Representative photomicrographs showing Fos induction within the lateral and medial aspects of the VTA at the (A) rostral (-4.80 mm from bregma) and (B) caudal (-5.60 mm from bregma) levels of the VTA following nomifensine infusions within the shell subterritory of the NAS. Scale bar = 100 μm.

mifensine



mifensine

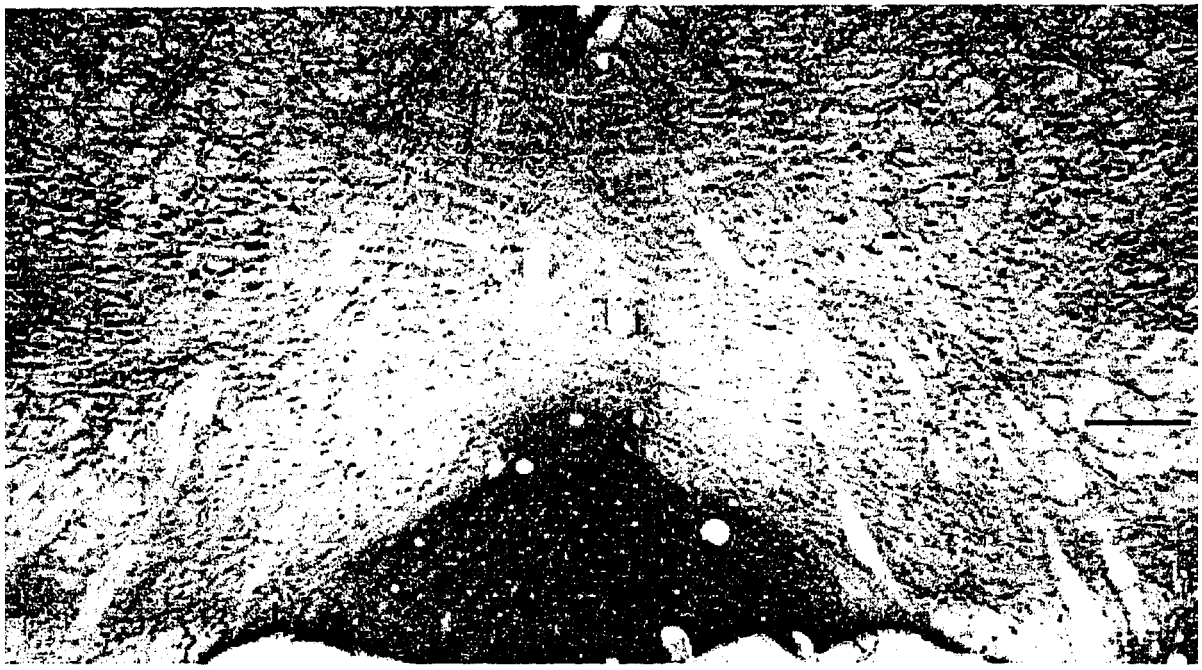
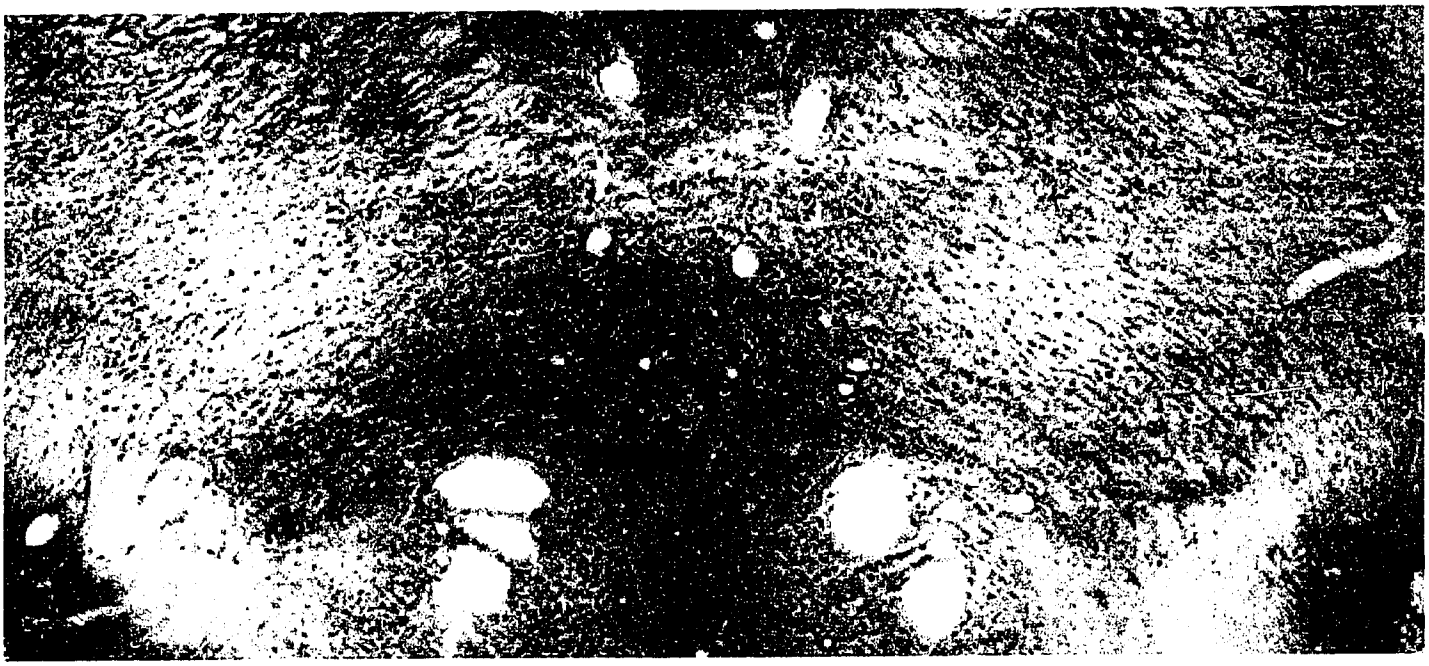


Figure 9. Representative photomicrographs showing Fos induction within the lateral and medial aspects of the VTA at the (A) rostral (-4.80 mm from bregma) and (B) caudal (-5.60 mm from bregma) levels of the VTA following PCP infusions within the shell subterritory of the NAS. Scale bar = 100 μm.

A PCP



B PCP

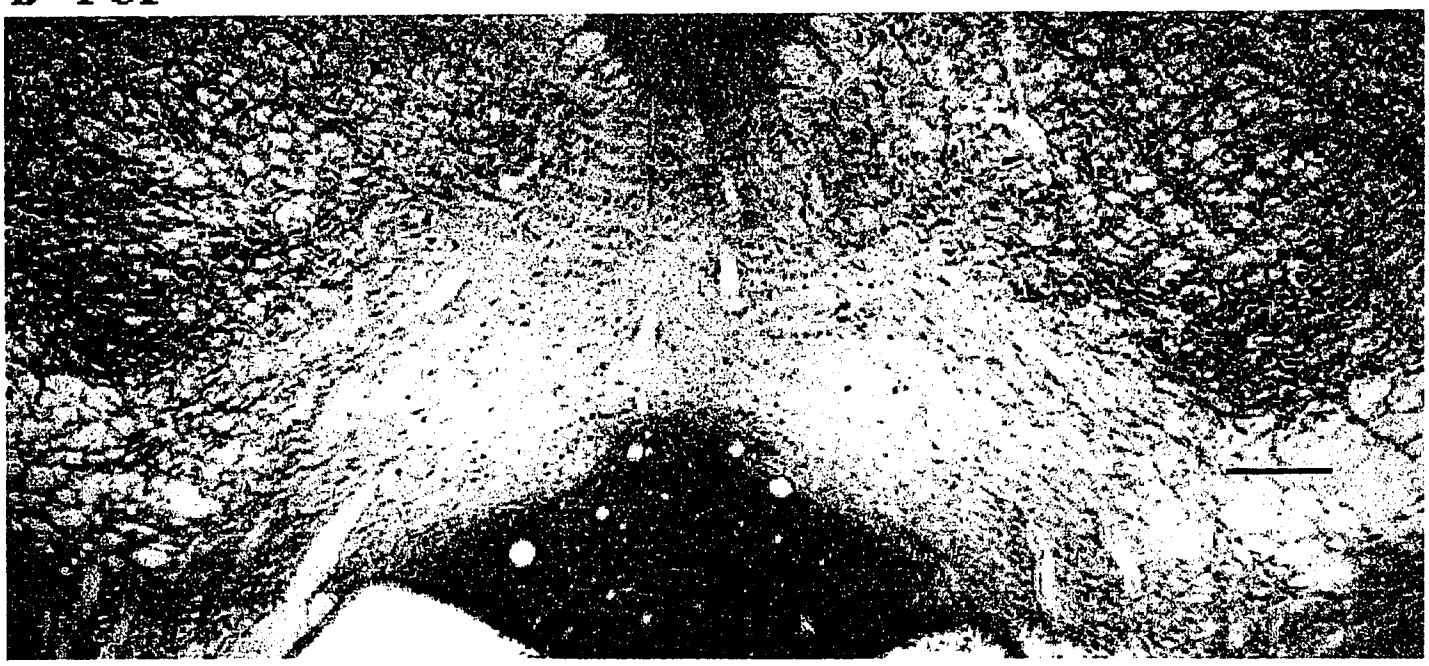
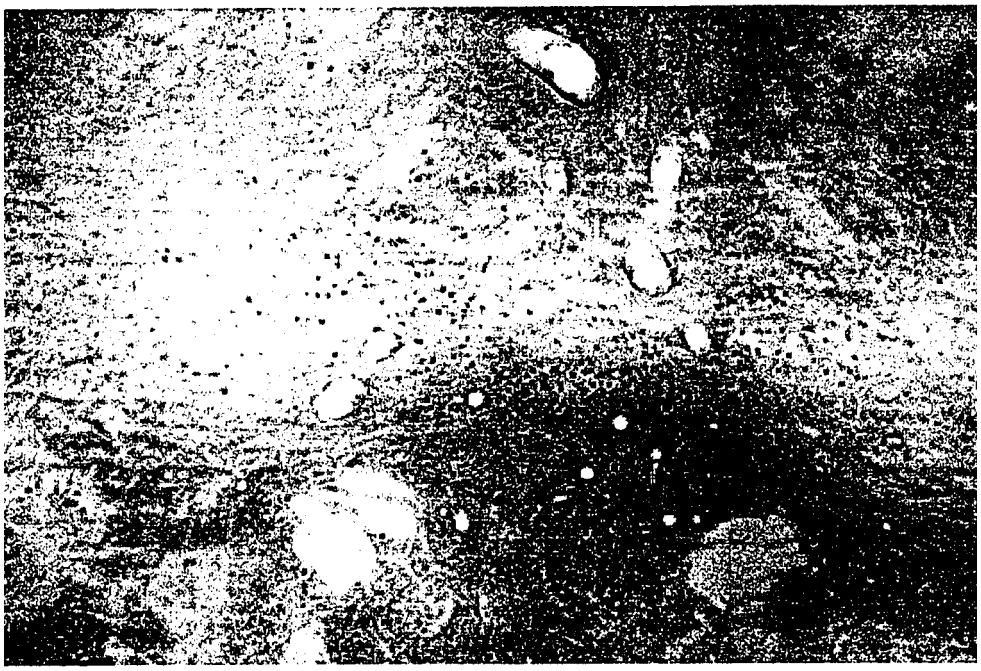


Figure 10. Representative photomicrographs showing Fos induction within the lateral and medial aspects of the VTA at the (A) rostral (-4.80 mm from bregma) and (b) caudal (-5.60 mm from bregma) levels of the VTA following vehicle infusions within the shell subterritory of the NAS. Scale bar = 100 μm.

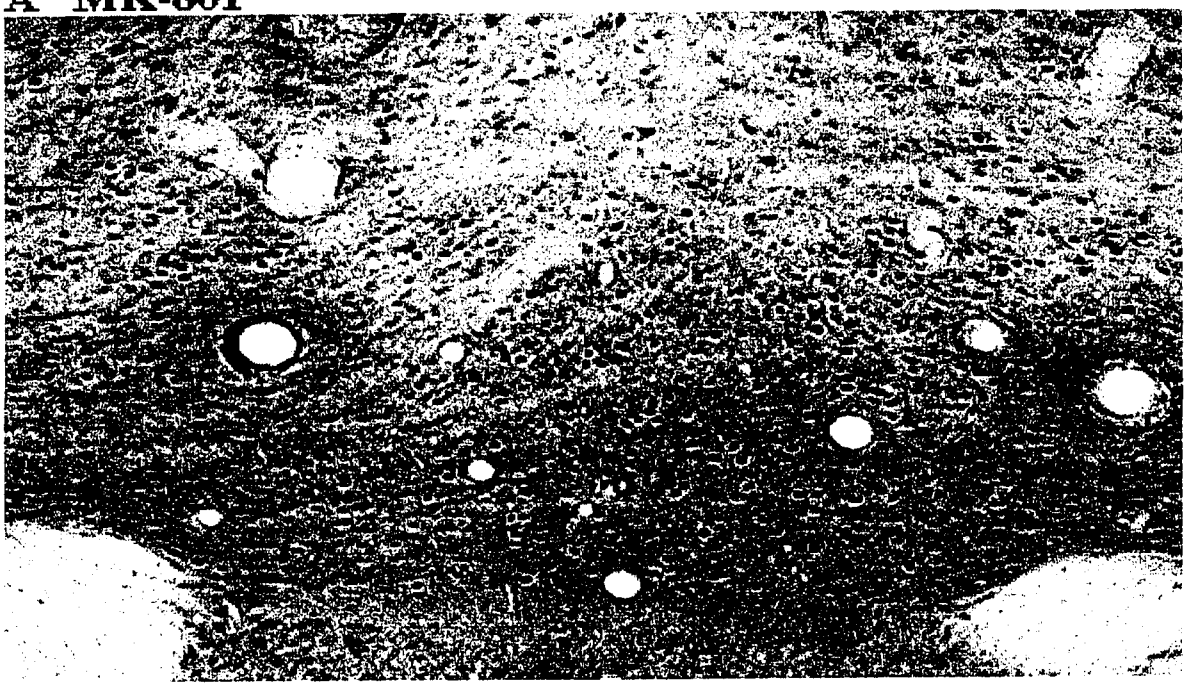
hicle



hicle

Figure 11. Representative photomicrographs showing Fos induction within the medial aspect of the rostral VTA following infusions of MK-801 within the (A) core, and (B) shell subterritory of the NAS. Scale bar = 200 μm.

A MK-801



B MK-801

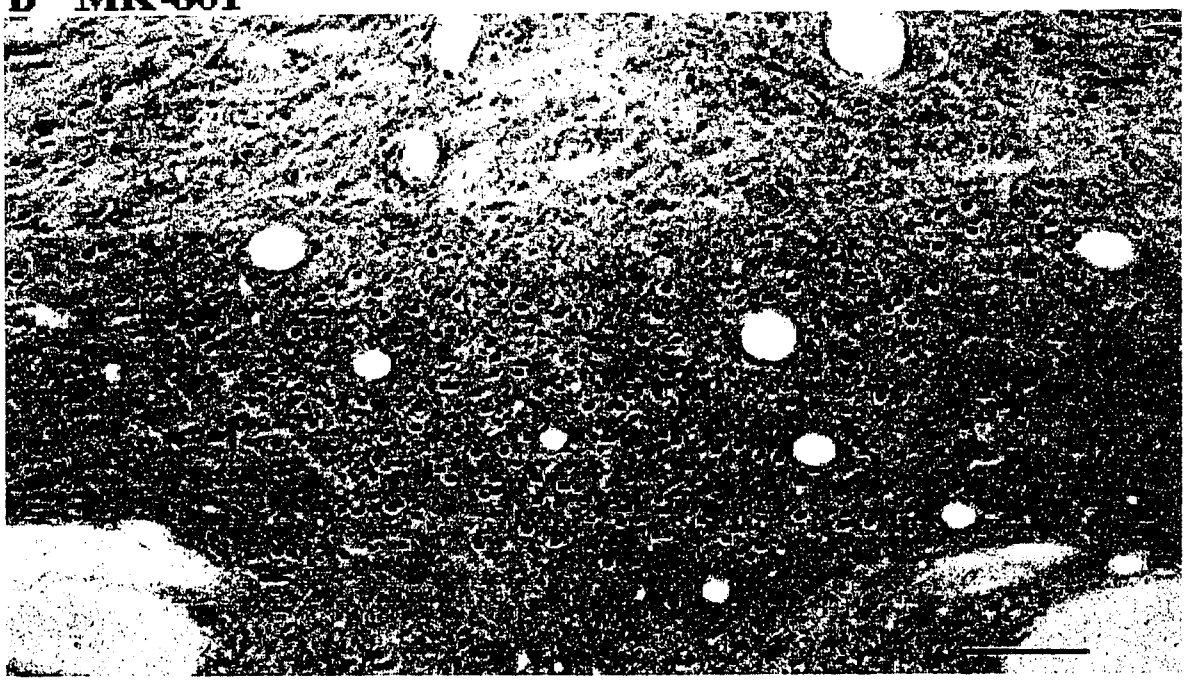
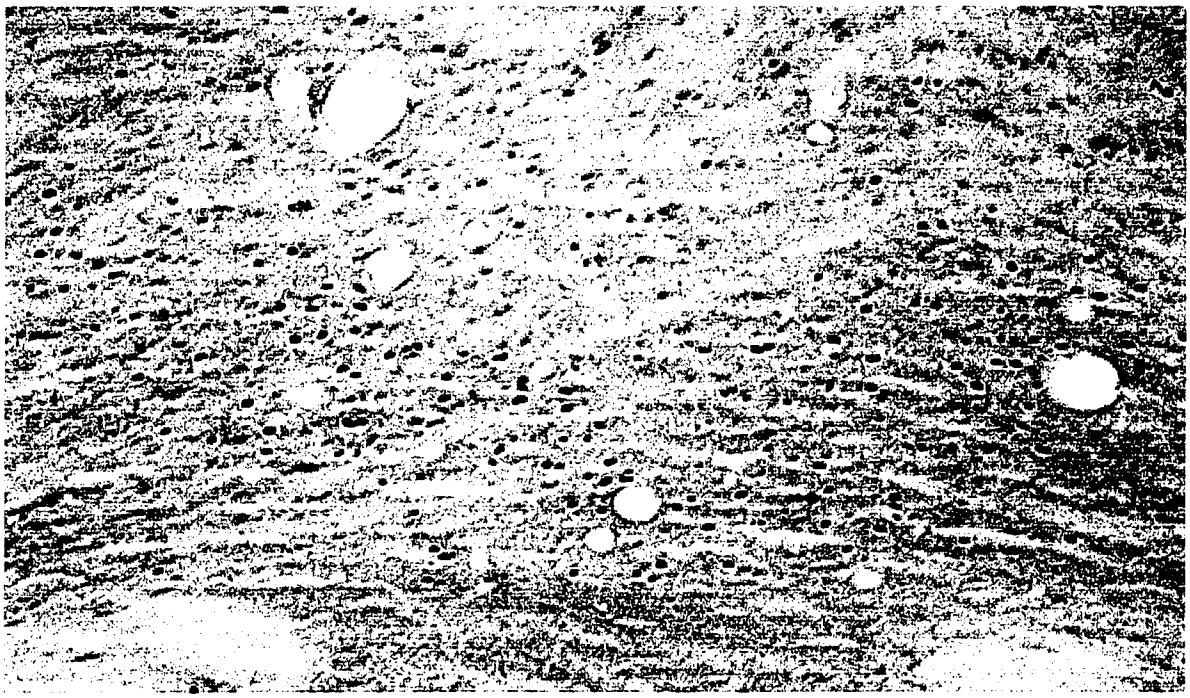


Figure 12. Representative photomicrographs showing Fos induction within the medial aspect of the rostral VTA following infusions of nomifensine within the (A) core, and (B) shell subterritory of the NAS. Scale bar = 200 μm.

A nomifensine



B nomifensine

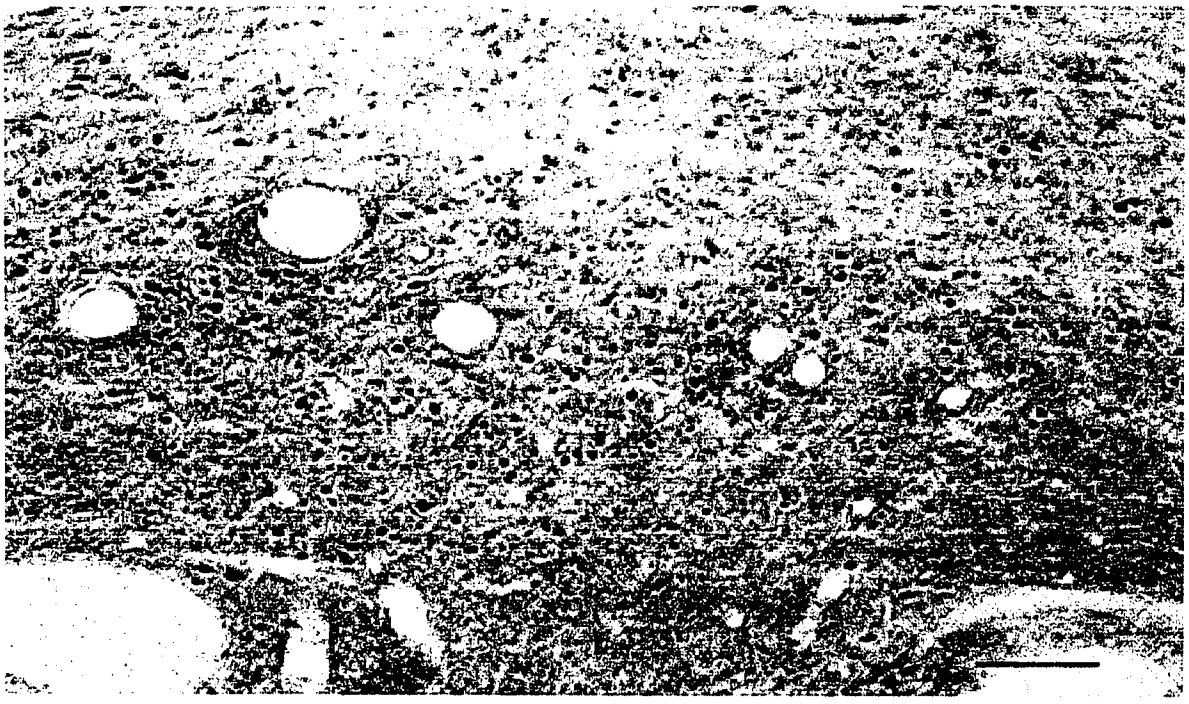
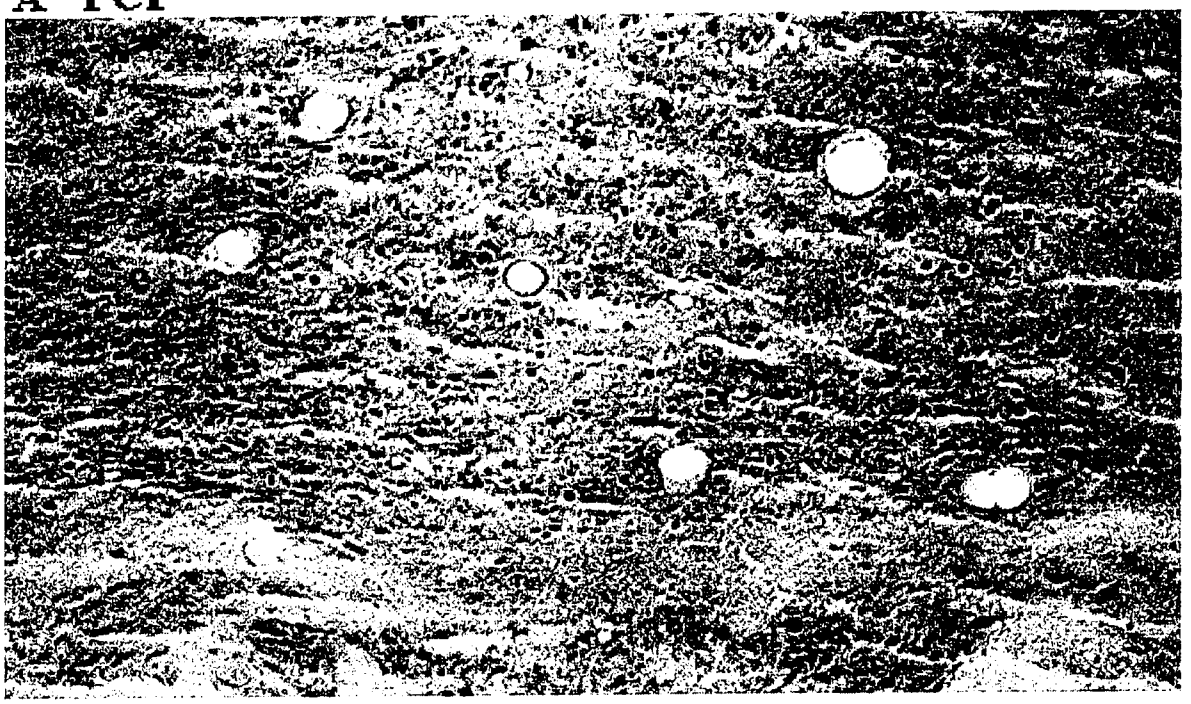


Figure 13. Representative photomicrographs showing Fos induction within the medial aspect of the rostral VTA following infusions of PCP within the (A) core, and (B) shell subterritory of the NAS. Scale bar = 200 μm.

A PCP



B PCP

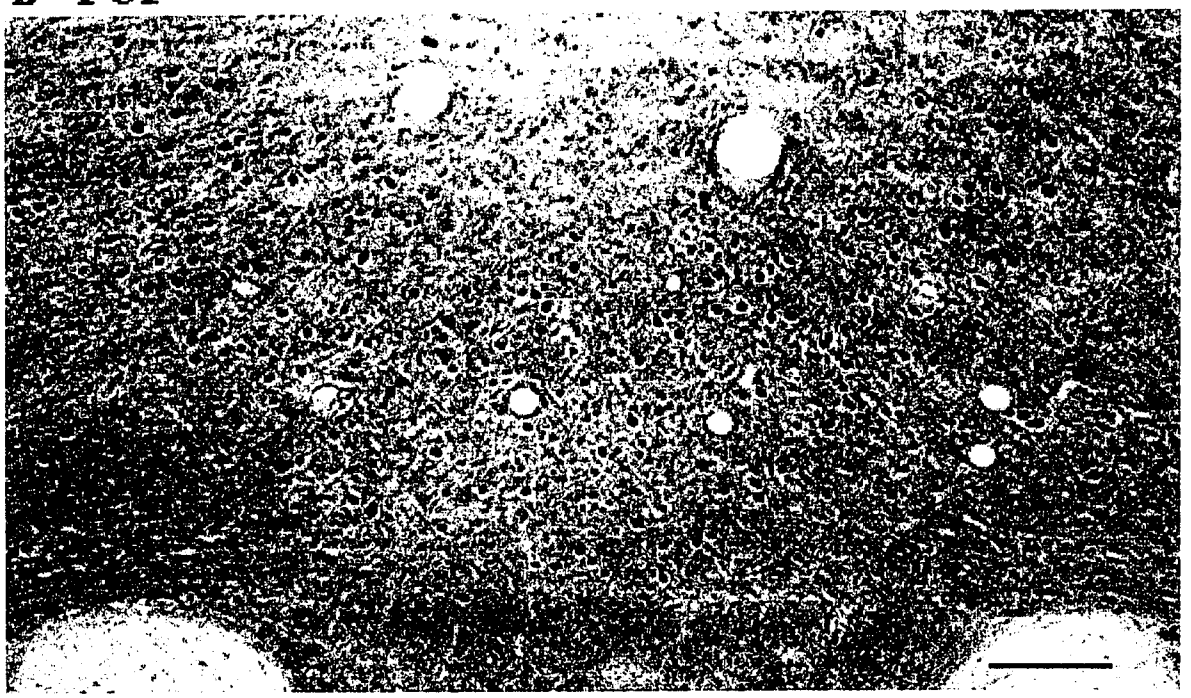
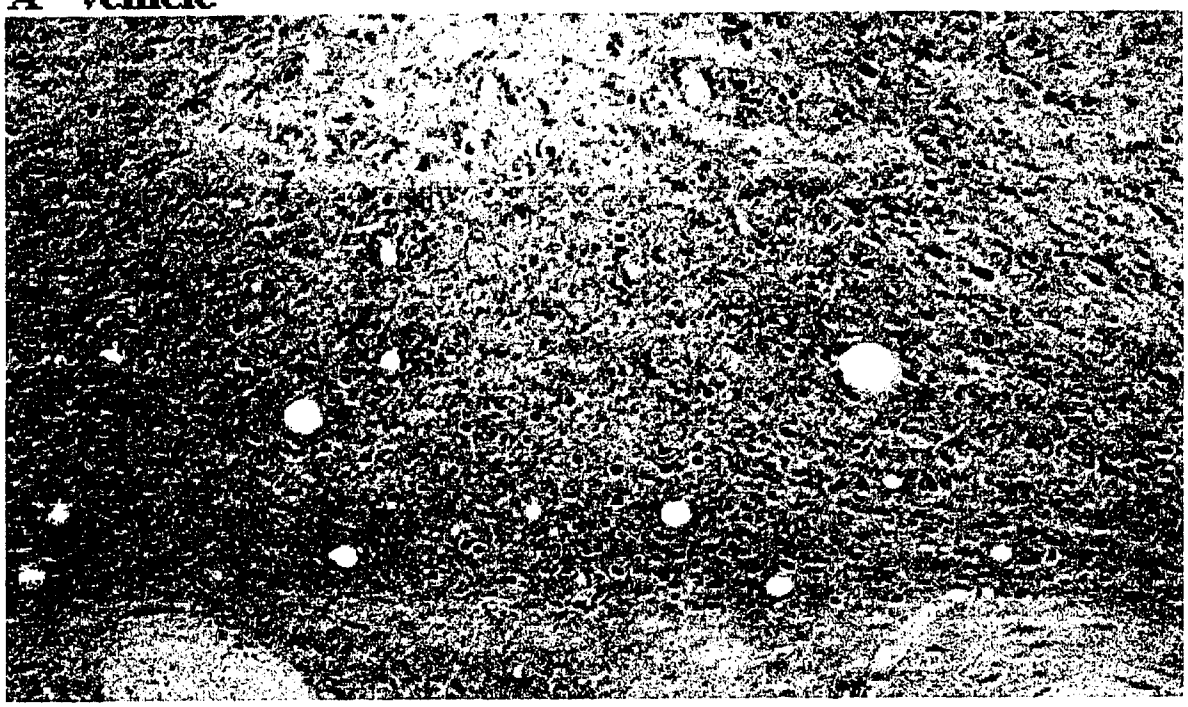


Figure 14. Representative photomicrographs showing Fos induction within the medial aspect of the rostral VTA following infusions of vehicle within the (A) core, and (B) shell subterritory of the NAS. Scale bar = 200 μm.

A vehicle



B vehicle

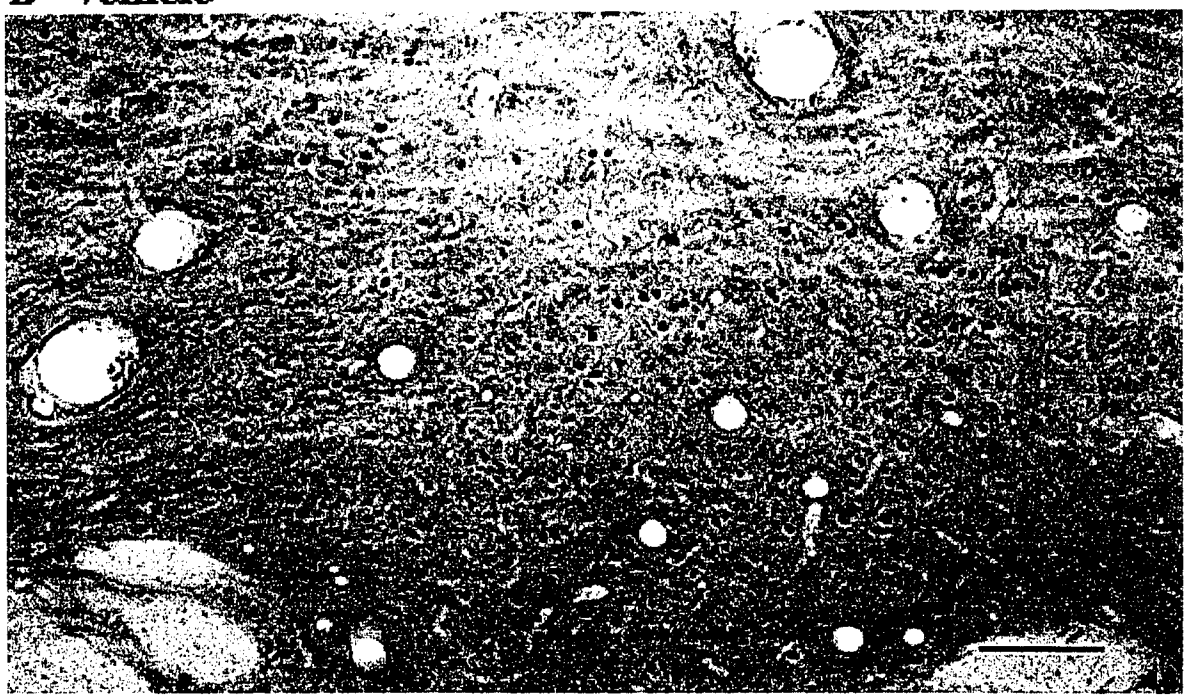
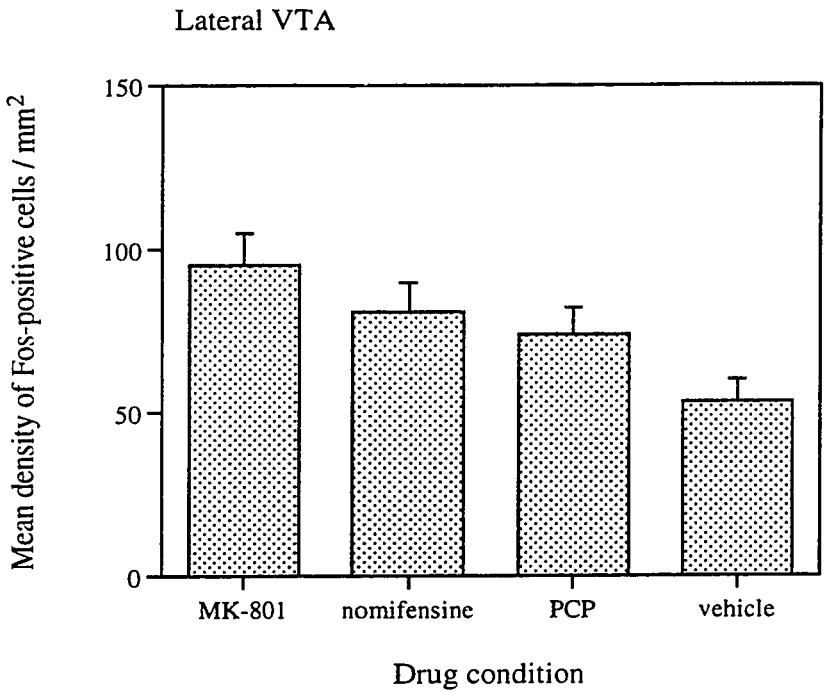


Figure 15. Mean density of Fos-positive cells per mm^2 (± S.E.M.) induced within the lateral aspect of the VTA (all levels and both drug infusion sites combined), following intra-NAS infusions of MK-801, nomifensine, PCP, or vehicle solutions. (Main effect of Drug).


Lateral VTA
Mean density of Fos-positive cells / mm2
150
100
50
0
MK-801
nomifensine
PCP
vehicle
Drug condition

16). Further, more Fos was seen at -4.80 mm than at -5.00 mm (see Appendix C for summary table of pairwise comparisons).

Medial VTA

The three-way mixed factorial analysis of variance yielded significant main effects for NAS drug injection site $F(1,32)=6.34$; $p<0.05$, drug condition $F(3,32)=31.01$; $p<0.01$ and VTA level $F(4,128)=239.22$; $p<0.01$ (see Appendix D for summary table). Subsequent analyses *via* pairwise comparisons revealed that, significantly more Fos-positive cells were seen following infusions within the shell rather than within the core region of the NAS. In addition, intra-NAS infusions of each drug were found to induce significantly more Fos-positive cells than vehicle. However, intra-NAS infusions of MK-801 were found to induce more Fos than infusions of either nomifensine or PCP (see Appendix E for summary tables of pairwise comparisons). Overall, significantly more Fos-positive cells were also found in the two most rostral levels of the VTA compared to rest of the VTA levels examined (see Appendix E for summary tables of pairwise comparisons).

Of greater importance, however, were the significant interactions. The three-way analysis of variance revealed significant two-way interactions between NAS drug infusion site x drug condition $F(3,32)=2.97$; $p<0.05$ and drug condition x VTA level $F(12,128)=4.05$; $p<0.01$, illustrated in Figures 17 and 18, respectively. These results reflect the fact that Fos induction within the medial VTA was not only found to vary as a function of NAS drug infusion site and drug condition, but also as a function of drug condition and VTA level.

The NAS drug infusion site x drug condition interaction was analyzed further using tests of simple effects and post-hoc pairwise comparisons (see Appendix F for summary tables). When infused within the core, MK-801 and nomifensine each induced more Fos than either PCP or vehicle, which did not differ from each other. Whereas, when infused

Figure 16. Mean density of Fos-positive cells per mm^2 (± S.E.M.) induced by infusions into the NAS, regardless of drug or infusion site at each level of the lateral VTA. (Main effect of VTA Level).

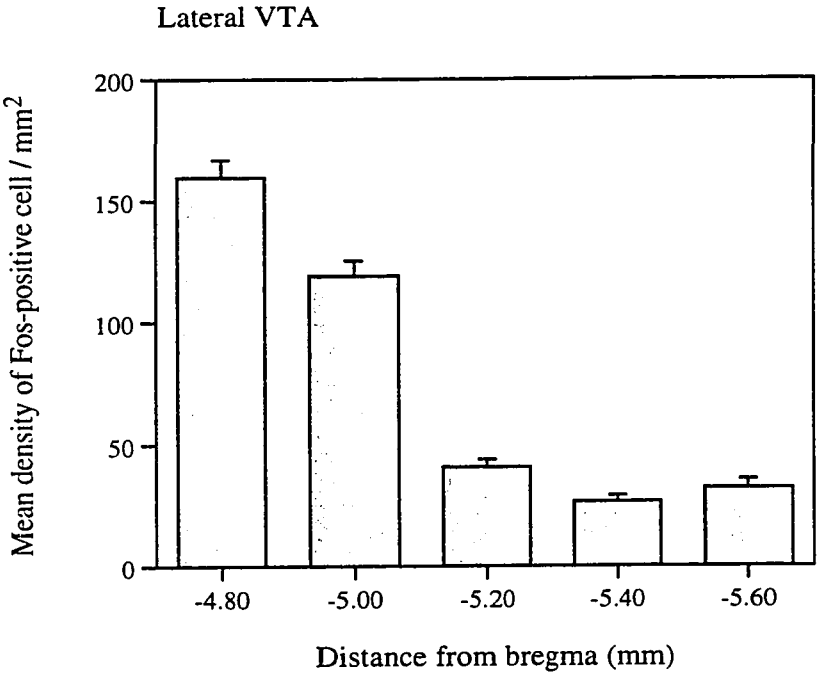

Lateral VTA
Mean density of Fos-positive cell / mm2
200
150
100
50
0
-4.80
-5.00
-5.20
-5.40
-5.60
Distance from bregma (mm)

Figure 17. Mean density of Fos-positive cells per mm^2 (± S.E.M.) within the medial aspect of the VTA following MK-801, nomifensine, PCP, or vehicle infusions into the core or shell subterritory of the NAS. (Interaction between Drug x Infusion Site).

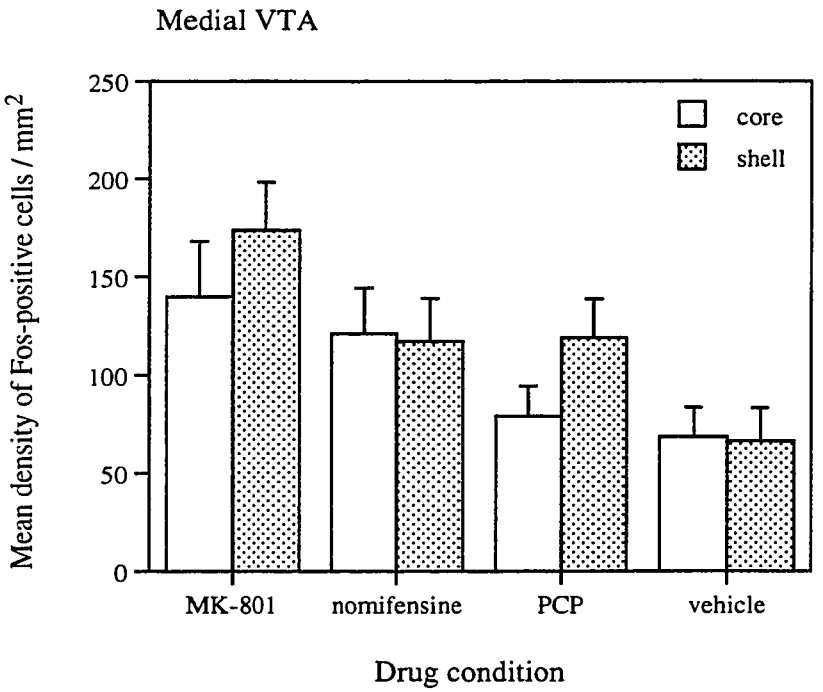
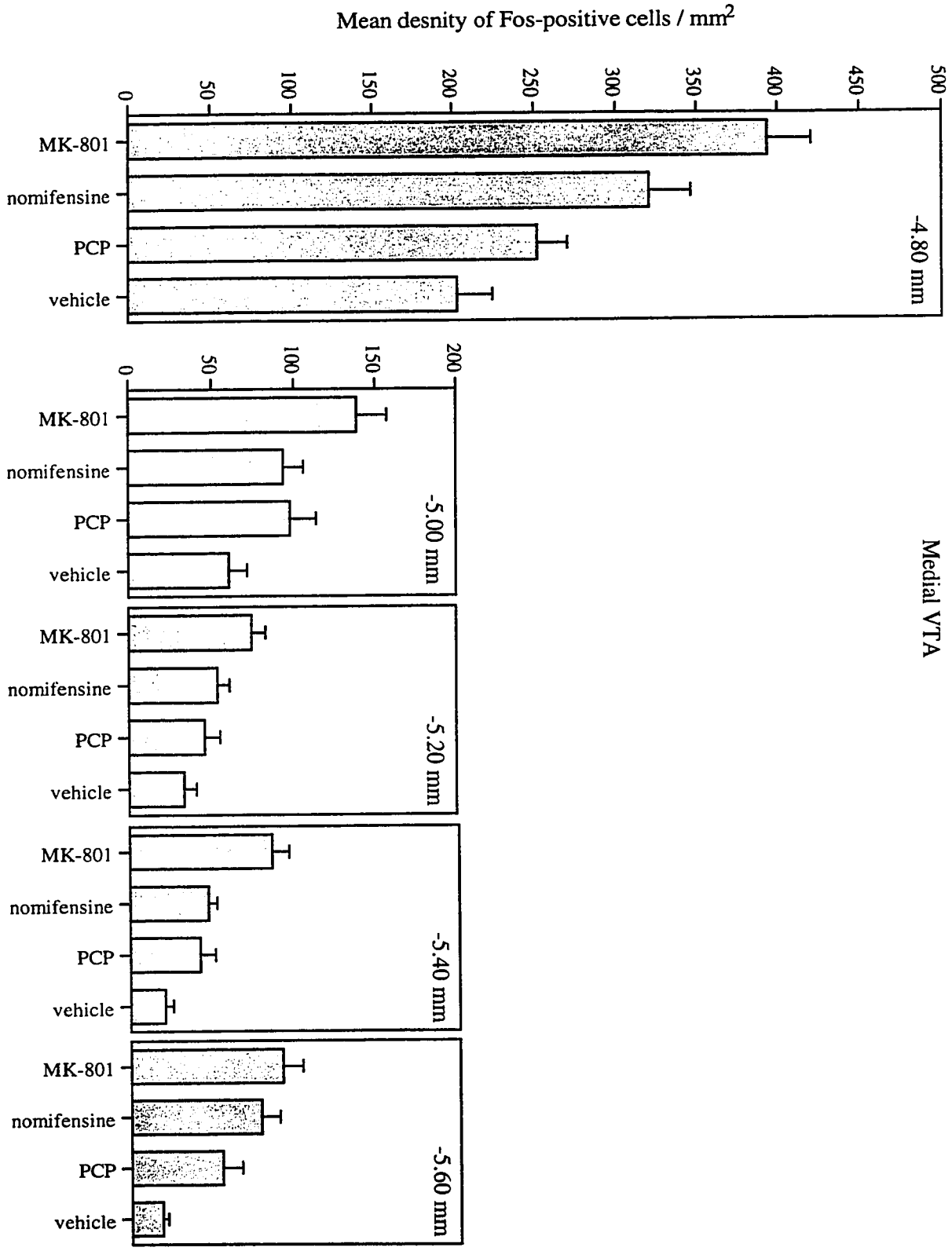

Medial VTA
Mean density of Fos-positive cells / mm2
250
200
150
100
50
0
core
shell
MK-801
nomifensine
PCP
vehicle
Drug condition

Figure 18. Mean density of Fos-positive cells per mm^2 (± S.E.M.) within the -4.80 mm, -5.00 mm, -5.20 mm, -5.40 mm, and -5.60 mm from bregma levels of the medial aspect of the VTA, following intra-NAS infusions of MK-801, nomifensine, PCP, or vehicle solutions. (Interaction of Drug x VTA Level).


Mean desnity of Fos-positive cells / mm2
0
50
100
150
200
250
300
350
400
450
500
MK-801
nomifensine
PCP
vehicle
-4.80 mm
-5.00 mm
-5.20 mm
-5.40 mm
-5.60 mm
Medial VTA

within the shell, MK-801 induced more Fos than either PCP, nomifensine or vehicle, and both nomifensine and PCP each induced more Fos than vehicle. Only MK-801 and PCP-treated groups showed more Fos-positive cells following infusions within the shell than seen following infusions within the core subterritory of the NAS.

The drug condition x VTA level interaction was also explored further using simple effects tests and pairwise comparisons (see Appendix G for summary tables). Each of the drugs was found to induce significantly more Fos-positive cells within the most rostral of the VTA levels examined (i.e., -4.80 mm from to bregma). Furthermore, intra-NAS infusions of MK-801 were found to induce more Fos-positive cells within the -5.00 mm from bregma level compared to the more caudal -5.20 mm from bregma level, whereas similar infusions of PCP were found to induce more Fos within the -5.00 mm from bregma level compared to the most caudal -5.40 mm from bregma level. In addition, the relative efficacy of each drug in inducing Fos varied at each level of the VTA. Within the -4.80 mm from bregma level, intra-NAS infusions of MK-801 and nomifensine, each induced more Fos-positive cells than either PCP or vehicle, with MK-801 inducing more Fos than nomifensine. Within the -5.00 and -5.40 mm from bregma level, only MK-801 was found to induce more Fos than vehicle. Finally, within the -5.60 mm from bregma VTA level, both MK-801 and nomifensine induced more Fos than the vehicle-infused rats.

Guide cannula placements for the core and shell groups are shown in Figure 19 and Figure 20 respectively. Histological reconstructions were made onto tracings of coronal plates from the Swanson atlas (1992-4). *Post mortem* histological examination did not reveal any degree of gross neuronal loss at the sites of infusion and only moderate gliosis along the tracks made by the infusion cannulae.

Figure 19. Guide cannula placements for the core subterritory of the NAS (n = 5 per drug condition). All injector tips are represented as asterisks and were reconstructed, for each drug condition, onto the same coronal hemisection at the level of +1.70 mm from bregma; actual placements varied between +2.00 and +1.00 mm from bregma (adapted from Swanson, 1992-4).

NAS core

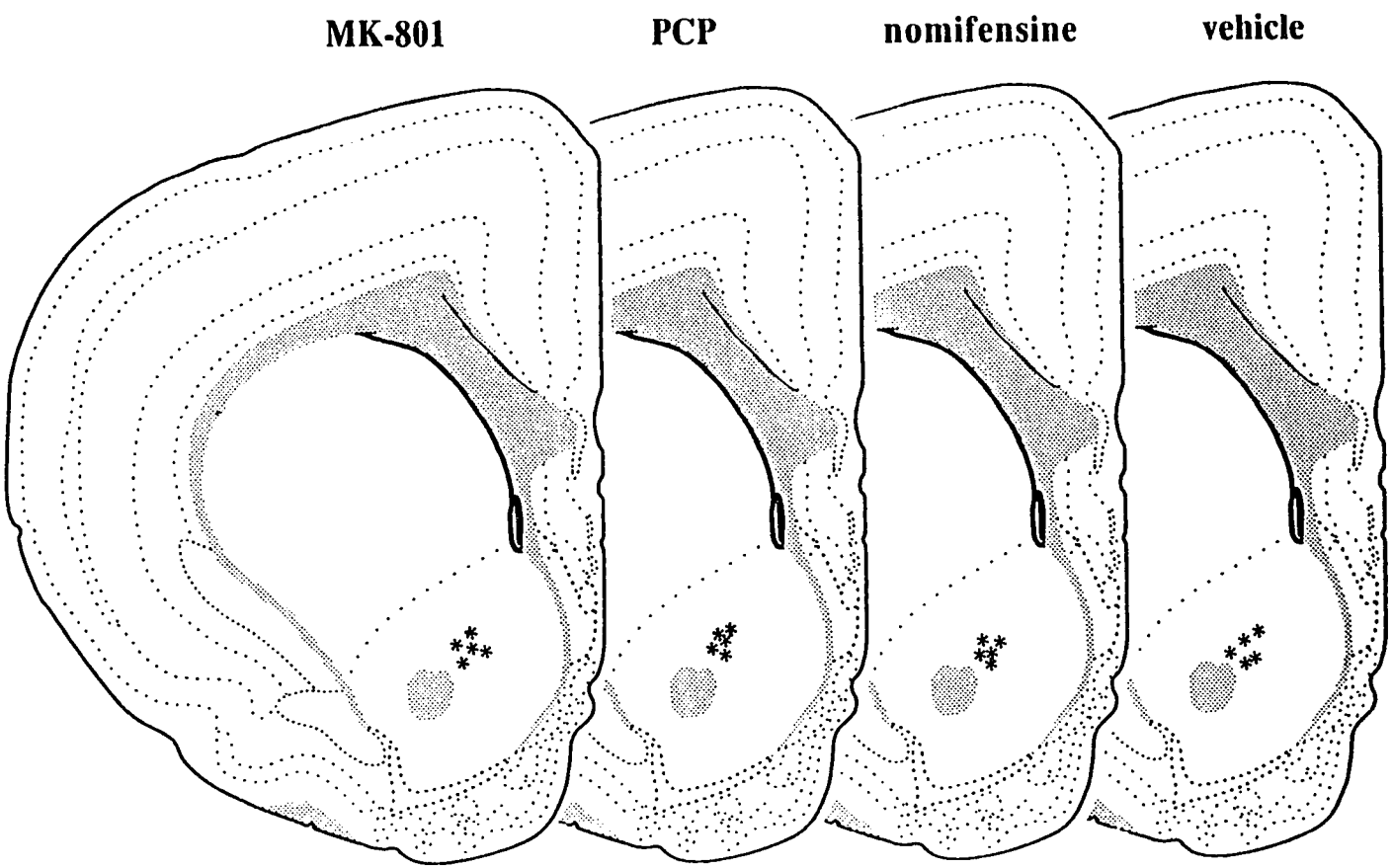
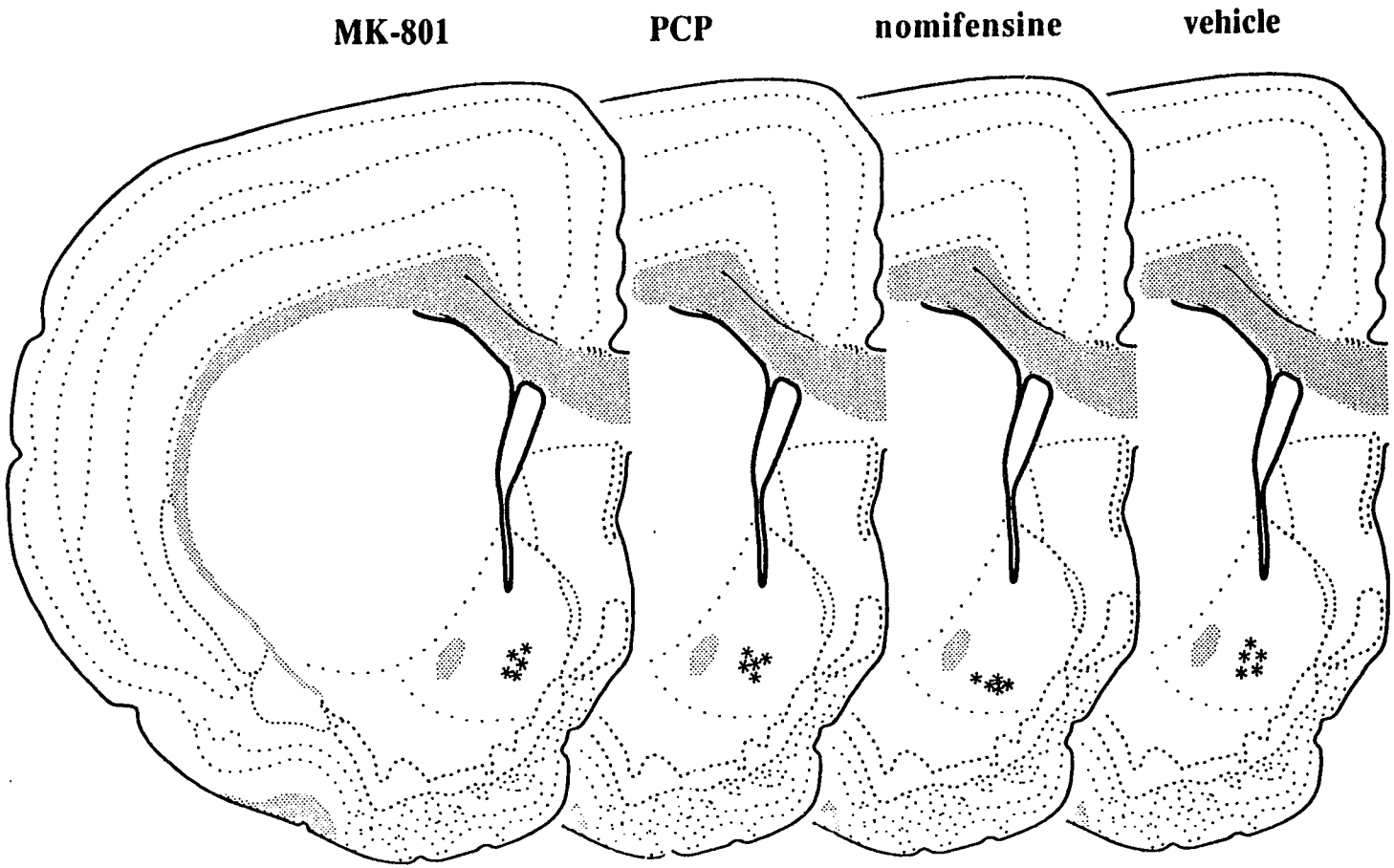


Figure 20. Guide cannula placements for the shell subterritory of the NAS (n = 5 per drug condition). All injector tips are represented as asterisks and were reconstructed, for each drug condition, onto the same coronal hemisection at the level of +1.00 mm from bregma; actual placements varied between +1.60 and +0.60 mm from bregma (adapted from Swanson, 1992-4).

NAS shell



Discussion

Intra-NAS infusions of MK-801, nomifensine, and PCP each resulted in the induction of Fos-positive cells within the VTA. These results suggest that NMDA receptor antagonism and dopamine reuptake blockade at the level of the NAS, both of which serve to suppress the activity of intrinsic GABAergic medium spiny neurons, resulted in an increase in Fos expression within the VTA. Hence, these data add to previous evidence suggesting that NMDA receptor antagonists (such as the phencyclinoids, MK-801 and PCP) and dopamine uptake inhibitors (such as the psychomotor stimulants, amphetamine, cocaine, and nomifensine) have similar yet independent actions in the NAS (Carlsson and Carlsson, 1990; Carlezon *et al.*, 1995; Carlezon and Wise, 1996).

The circuitry mediating the pharmacological actions of these drugs within the NAS, appears to involve both excitatory glutamatergic projections from the PFC and inhibitory dopaminergic projections from the VTA synapsing on local NAS GABAergic medium spiny neurons (Sesack and Pickel, 1992). Both classes of drugs suppress the activity of GABAergic output neurons of the NAS. However, NMDA antagonists, do so by binding to NMDA receptors located on GABA neurons, thereby blocking the excitatory effects of glutamate, whereas, dopamine uptake inhibitors, do so by binding presynaptically on dopamine terminals, which prevents the reuptake of the neurotransmitter and increases the extracellular concentration of dopamine, which in turn binds to dopamine receptors on GABA neurons and inhibits their activity. Finally, suppressing the activity of NAS GABAergic neurons, leads to both a suppression of inhibitory input to the neurons of the VTA and an increase in activity of these neurons.

The present study also found differences between the intra-NAS drug-induced Fos expression observed within the lateral and medial aspects of the VTA. Within the lateral VTA, the number of Fos-positive cells induced following intra-NAS infusions of either MK-801, nomifensine, or PCP were not markedly different from each other. Within the

medial VTA, however, intra-NAS infusions of MK-801 induced significantly more Fos-positive cells than either nomifensine or PCP. Moreover, only within the medial aspect of the VTA, was the Fos induction differentially affected by infusions of drugs into either the core or shell subterritory of the NAS, and only for the MK-801 and PCP-treated rats. More Fos was expressed when MK-801 was infused into the shell than when infused into the core. Likewise, although infusions of PCP into the NAS core failed to induce significant Fos expression, similar infusions into the NAS shell did.

These data are consistent with known pharmacological characteristics of MK-801 and PCP, as well as with both distinct neurochemical composition and topographical organization of efferent and afferent projections of the NAS subterritories. As previously mentioned, MK-801 can block the excitatory actions of glutamate at NMDA receptors, but is not known to block dopamine uptake. In contrast, although PCP has the ability to block both NMDA receptors and dopamine reuptake, it has been found, to bind to NMDA receptors at concentrations lower than those that are needed to block dopamine uptake (Chaudieu *et al.*, 1989; Ohmori *et al.*, 1992). Moreover, MK-801 has been reported to have high affinity and selectivity for the NMDA receptor ion channel complex (Wong *et al.*, 1986), and greater than that of PCP. This receptor is found throughout the NAS, yet it is more abundant in the shell subterritory (Gracy and Pickel, 1996). Additionally, MK-801 and PCP have each been found to increase the firing rates of dopamine neurons within the VTA with a potency that is positively correlated to their ability to block the NMDA receptor (French and Ceci, 1990; Zhang *et al.*, 1992).

Taken together, these observations offer a possible explanation as to why intra-NAS infusions of MK-801 induced more Fos within both the lateral and medial VTA than the other drugs examined. Furthermore, inasmuch as the magnitude of MK-801- and PCP-induced Fos expression appears to reflect each drug's affinity for the NMDA binding site, it seems reasonable to conclude tentatively that MK-801- and PCP-induced Fos expression within the medial VTA, is linked to the drug's actions at the NMDA receptor. This

conclusion is further supported by the following two observations: (1) Fos expression was concentrated in the medial rostral VTA, which is the primary target of ascending efferents of the shell subterritory of the NAS (Heimer *et al.*, 1991; Berendse *et al.*, 1992; Usuda *et al.*, 1998), and (2) both MK-801 and PCP induced more Fos within the medial VTA when infused into a reward-relevant site (shell) than when infused into a non-reward-relevant site (core; Carlezon and Wise, 1996). However it should be noted that, because nomifensine was also found to have rewarding effects when infused into the shell but not the core subterritory of the NAS (Carlezon *et al.*, 1995), and because intra-NAS infusions of nomifensine also resulted in Fos expression within the VTA (present study), the possibility that intra-NAS infusions of PCP, are linked to it's actions as a dopamine reuptake blocker cannot be dismissed.

Finally, unlike MK-801 and PCP, nomifensine induced comparable amounts of Fos within the medial VTA whether infused into either the core or shell subterritory of the NAS. These findings were somewhat unexpected in light of the following considerations. Rats have been shown to readily self-administer infusions of nomifensine directly into the shell, but not the core subterritory of the NAS (Carlezon *et al.*, 1995). In the present study, the dose of each drug used to determine the effects of infusions of these drugs into either the NAS core or shell on inferred neural activity within the VTA was the same as that reported to establish intracranial (NAS shell) self-administration responding in rats (Carlezon *et al.*, 1995; Carlezon and Wise, 1996). The differential behavioral effects of nomifensine given into the core and shell of the NAS are thought to arise from its actions on local, and presumably distinct, populations of neurons with different projections targets. From the present experiment it was not possible to determine whether the Fos expression induced by the infusion at the two sites was found in different cell populations.

It was also found that intra-NAS infusions of each of the drugs induced more Fos-positive cells within the rostral compared to the caudal extent of both the medial and lateral VTA. Within the lateral VTA, the differential rostral-caudal Fos expression was found to

be independent of drug treatment or NAS drug infusion site, whereas within the medial VTA, this effect was found to vary between drug conditions. In particular, within the most rostral levels of the lateral VTA examined, intra-NAS infusions of either MK-801, nomifensine, or PCP, resulted in the induction of more Fos-positive cells than vehicle-treated rats; with MK-801 inducing the greater number of Fos-positive cells, followed by nomifensine and PCP. Finally, rostral-caudal differences in Fos expression within either aspects of the VTA, were not found to be affected by site of drug infusion.

The aforementioned differential rostral-caudal distribution pattern of Fos expression appears to corresponds to the heterogeneous morphological organization of the VTA. Anatomical studies have identified TH-labeled (Swanson, 1982) cells and GABA-labeled (Mugnaini and Oertel, 1985) cells throughout most, but not all, parts of the lateral and medial VTA. However, both the densities of the TH-labeled cells and GABA-labeled cells, decrease progressively from rostral to caudal levels of the VTA (see Figures 2-6). Interestingly, the rostral medial aspect of the VTA not only contains the greatest densities of TH and GABA-labeled cells (see Figure 2), but has been reported to be preferentially innervated by the GABAergic medium spiny neurons of the NAS (Walaas and Fonnum, 1980).

Aside from its topographical organized afferent and efferent projections (see introduction) and the rostral-caudal morphological organization, very little else is known about the heterogeneous nature of the VTA. Support for the idea of distinct rostral-caudal populations of neurons has emerged from studies investigating pharmacological inhibition or stimulation of local VTA neurons. GABA agonists and antagonists were found to have differential effects on locomotor activity following infusions into the rostral and caudal VTA. Infusions of GABA agonists (muscimol and THIP; 4,5,6,7-tetrahydro-isoxazolo-[5,4-c]-pyridin-3-ol) within the rostral VTA induced sedation, whereas local infusions of GABA antagonists (bicuculline and picrotoxin) induce hypermotility (Mogenson *et al.*, 1979; Arnt and Scheel-Krüger, 1979). In contrast, muscimol infusions within the caudal

VTA induced hyperactivity (Arnt and Scheel-Krüger, 1979), similar to effects following lesions of the VTA (Galey and Le Moal, 1976; Galey *et al.*, 1977), whereas infusions of GABA antagonists reduced locomotor activity (Arnt and Scheel-Krüger, 1979). Recently, Yoshida and colleagues (1997), using microdialysis and Fos immunocytochemistry, showed that infusions of muscimol within the NAS led to increases in both extracellular levels of dopamine within the NAS and Fos expression within the rostral aspect of the VTA. Finally, there is evidence to suggest that rats will readily self-administer GABA antagonists picrotoxin and bicuculline directly into the rostral, but not caudal VTA, thereby suggesting that rewarding effects of GABA antagonists are localized within the rostral rather than the caudal aspect of the VTA (Ikemoto *et al.*, 1997).

All things considered, differences in the Fos expression induced by the different drugs appear to not only reflect each drug's different pharmacological actions, but also inherent differences in the neurochemical composition as well as efferent and afferent organization of each of the NAS subterritories in which the drugs were infused.

A somewhat unexpected aspect of the findings presented here was the degree of bilateral activation of Fos within the VTA after unilateral infusions into the NAS. For although there were somewhat more Fos-positive cells within the ipsilateral hemisphere within the medial VTA, no significant difference between the ipsilateral and contralateral hemispheres was observed within the lateral VTA. These results might be explained in part by neuroanatomical studies showing some bilateral innervation of both the lateral and medial aspects of the VTA by the NAS, although the innervation is primarily unilateral (Chronister *et al.*, 1980; Walaas and Fonnum, 1980; Heimer *et al.*, 1991; Berendse *et al.*, 1992; Zahm and Brog, 1992; Zahm and Heimer, 1993; Groenewegen *et al.*, 1996; Usuda *et al.*, 1998). However, the relative amount of Fos expression within the contralateral hemisphere appears to be too high to be solely attributable to fibers crossing over to the contralateral side. Inasmuch as the NAS subterritories have distinct efferent and afferent connections (Heimer and Wilson, 1975; Groenewegen and Becker, 1980; Groenewegen *et*

al., 1987, 1991; Alheid and Heimer, 1988; Zahm, 1989; Zahm and Heimer, 1990; Heimer *et al.*, 1991; Berendse *et al.*, 1992; Zahm and Brog, 1992; Usuda *et al.*, 1998), and that Fos induction can be observed at least two synapses removed from the site of stimulation (Sagar *et al.*, 1988), a more likely explanation for contralateral expression of Fos, may be attributable to the stimulation of some efferents of the NAS which then project bilaterally to the VTA.

However that may be, several factors limit the interpretation of the present findings as well as offer other possible explanation for the bilateral intra-NAS drug-induced Fos expression. First, despite an attempt at minimizing unwanted side effects of MK-801, PCP, and nomifensine through the use of direct infusions that limit drug dispersion in the brain (Wise and Hoffman, 1992), at least two problematic effects of intracranial infusions may have contributed to non-specific activation of Fos within the VTA. The first is associated with possible local physiochemical disturbances that alter brain function by actions unrelated to the normal pharmacological actions of the infused drug (Wise and Hoffman, 1992). Nonetheless, given the fact that all drugs infused into the NAS were found to induce more Fos within the VTA than did vehicle infusions, the observed drug effects do not appear to have resulted simply from damage produced at the drug infusion site, but rather are more likely associated with local pharmacological actions of the drugs.

The second problematic effect of intracranial infusions concerns the fact that, centrally infused drugs do not necessarily act at the site of infusion, but can diffuse from the infusion site, can move by local pressure gradients up the cannula shaft into ventricles, and can cross into the circulation to distal sites of action (Wise and Hoffman, 1992). Inasmuch as activity within the mesocorticolimbic system has also been implicated in arousal (Le Moal and Simon, 1991), and that MK-801, PCP, and nomifensine have all been reported to have strong psychoactive properties (Pijnenburg and van Rossum, 1973; Schmidt *et al.*, 1992; Ali, *et al.*, 1994; Ouagazzal *et al.*, 1994), the possibility that intra-NAS drug-induced Fos expression was due to secondary effects of the drugs cannot be

dismissed. It is worth noting in this context that animals that received intra-NAS infusions of MK-801, nomifensine and PCP, each appeared moderately hyperactive and displayed obvious circling behavior, however this behavior was not systematically measured. Moreover, systemic injections of MK-801, nomifensine, and PCP, have each been found to increase—albeit in varying degrees—both locomotion and Fos expression within the VTA (Marcangione and Wise, 1997).

Finally, coupled to the aforementioned limitations are inherent constraints associated with the Fos immunocytochemical method employed. The Fos immunocytochemical method cannot distinguish between directly stimulated cells and transynaptically-activated cells (Sagar *et al.*, 1988). Consequently, the population of VTA cells expressing Fos may include neurons that are directly-stimulated by NAS GABA medium spiny neurons, neurons that are indirectly linked to the NAS, as well as neurons subserving other functions. This may also, in part, explain the bilateral activation. Moreover, it has been suggested that certain activated cells lack the capacity to express Fos (Bullitt, 1990). Therefore, the possibility that some neurons might not express Fos in response to intra-NAS drug infusions must also be considered.

The constraints on interpretation notwithstanding, the present thesis provides preliminary evidence linking suppression of intrinsic NAS GABAergic neurons, *via* both NMDA receptor and dopamine reuptake blockade, to the activation of neurons within the VTA. Further studies combining Fos immunocytochemistry with additional staining methods (e.g., both TH- and GABA-immunohistochemistry and tracers of axonal transport), would provide information about the neurochemistry, morphology, and location of the activated cells.

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Appendix A

Summary Tables For the Four-Way Mixed Factorial Analysis

of the Lateral and Medial VTA

Table 1

Analysis of variance summary table for lateral VTA

Source of variance	SS	df	MS	F
	Between-subjects			
NAS drug infusion site (placement)	2216.47	1	2216.47	0.99
Drug condition	94299.94	3	31433.31	14.05**
Placement x drug	15099.69	3	5033.23	2.25
Between-subjects error	71595.10	32	2237.35	
	Within-subjects			
VTA level	1247833.26	4	311958.32	450.87**
Placement x level	4198.54	4	1049.64	1.52
Drug x level	29992.28	12	2499.36	3.61**
Placement x drug x level	7505.61	12	625.47	0.90
Within-subjects error	885464.18	128	691.91	
Hemisphere (hemi)	14.21	1	14.21	0.05
Placement x hemi	1157.50	1	1157.50	3.69
Drug x hemi	1544.47	3	514.82	1.64
Placement x drug x hemi	495.10	3	165.03	0.53
Within-subjects error	10029.61	32	313.43	
level x hemi	1985.01	4	496.25	2.09
level x hemi x placement	802.17	4	200.54	0.85
level x hemi x drug	4609.01	12	384.08	1.62
level x hemi x placement x drug	3119.37	12	259.95	1.10
Within-subjects error	30327.50	128	236.93	
Total	2412289.02	399		

Note: ** $p<0.01$

Table 2

Analysis of variance summary table for medial VTA

Source of variance	SS	df	MS	F
	Between-subjects			
NAS drug infusion site (placement)	34579.48	1	34579.48	7.52**
Drug condition	391589.12	3	130529.71	28.39**
Placement x drug	68189.78	3	22729.93	4.94**
Between-subjects error	147141.93	32	4598.19	
	Within-subjects			
VTA level	3356269.27	4	839067.32	272.16**
Placement x level	3161.02	4	790.26	0.26
Drug x level	159430.40	12	13285.87	4.31**
Placement x drug x level	50849.75	12	4237.48	1.37
Within-subjects error	394626.86	128	3083.02	
Hemisphere (hemi)	3816.80	1	3816.80	10.48**
Placement x hemi	275.01	1	275.01	0.76
Drug x hemi	1371.24	3	457.08	1.26
Placement x drug x hemi	3732.52	3	1244.17	3.42*
Within-subjects error	11653.42	32	364.17	
level x hemi	620.54	4	155.13	0.32
level x hemi x placement	682.69	4	170.67	0.35
level x hemi x drug	10761.42	12	896.78	1.86*
level x hemi x placement x drug	6939.90	12	578.32	1.20
Within-subjects error	61676.14	128	481.84	
Total	4707373.29	399		

Note: * $p<0.05$ and ** $p<0.01$

Appendix B

Analysis of Variance Summary Table for Lateral VTA

Table 3

Analysis of variance summary table for lateral VTA

Source of variance	SS	df	MS	F
	Between-subjects			
NAS drug infusion site (placement)	3288.73	1	3288.73	2.63
Drug condition	46335.16	3	15445.05	12.33**
Placement x drug	9591.61	3	3197.20	2.55
Between-subjects error	40087.17	32	1252.72	
	Within-subjects			
VTA level	578439.68	4	144609.92	277.73**
Placement x VTA level	920.24	4	230.06	0.44
Drug x VTA level	9622.18	12	801.85	1.54
Placement x drug x VTA level	5164.47	12	430.37	0.83
Within-subjects error	66647.91	128	520.69	
Total	760097.14	199		

Note: ** $p<0.01$

Appendix C

Summary Tables for Post-hoc Pairwise Comparison of the Drug Condition and VTA levels

Main Effects for the Lateral VTA

Table 4

Pairwise comparisons for main effect of drug condition (lateral VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	11553.23	1	11553.23	9.22*
PCP vs nomifensine	1211.95	1	1211.95	0.97
PCP vs vehicle	10773.07	1	10773.07	8.60*
MK-801 vs nomifensine	5281.35	1	5281.35	4.22
MK-801 vs vehicle	44638.97	1	44638.97	35.63**
nomifensine vs vehicle	19211.75	1	19211.75	15.34**
Between-subjects error	40087.17	32	1252.72	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 5

Pairwise comparisons for main effect of VTA levels (lateral VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	32960.33	1	32960.33	63.30**
-4.80 vs -5.20 mm	282241.79	1	282241.79	542.06**
-4.80 vs -5.40 mm	354506.37	1	354506.37	680.84**
-4.80 vs -5.60 mm	325346.45	1	325346.45	624.84**
-5.00 vs -5.20 mm	122300.26	1	122300.26	234.88**
-5.00 vs -5.40 mm	171275.61	1	171275.61	328.94**
-5.00 vs -5.60 mm	151197.86	1	151197.86	290.38**
-5.20 vs -5.40 mm	4113.94	1	4113.94	7.90*
-5.20 vs -5.60 mm	1530.93	1	1530.93	2.94
-5.40 vs -5.60 mm	625.64	1	625.64	1.20
Within-subjects error	66647.91	128	520.69	

Note: ** $p<0.001$ (Bonferroni correction)

Appendix D

Analysis of Variance Summary Table for Medial VTA

Table 6

Analysis of variance summary table for medial VTA

Source of variance	SS	df	MS	F
	Between-subjects			
NAS drug infusion site (placement)	14343.44	1	14343.44	6.34*
Drug condition	210634.70	3	70211.57	31.01**
Placement x drug	20193.67	3	6731.22	2.97*
Between-subjects error	72444.69	32	2263.90	
	Within-subjects			
VTA level	1720694.43	4	430173.61	239.22**
Placement x VTA level	1449.16	4	362.29	0.20
Drug x VTA level	87366.60	12	7280.55	4.05**
Placement x drug x VTA level	18712.31	12	1559.36	0.87
Within-subjects error	230173.05	128	1798.23	
Total	2376012.05	199		

Note: * $p<0.05$ and ** $p<0.01$

Appendix E

Summary Tables for Post-hoc Pairwise Comparison of the NAS Drug Infusion Site, Drug Condition and VTA level Main Effects for the Medial VTA

Table 7

Pairwise comparisons for main effect of NAS drug infusion site (medial VTA)

Source of variance	SS	df	MS	F
core vs shell	14343.44	1	14343.44	6.34*
Between-subjects error	72444.69	32	2263.90	

Note: * $p<0.05$

Table 8

Pairwise comparisons for main effect of drug condition (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	84510.47	1	84510.47	37.33**
PCP vs nomifensine	10149.48	1	10149.48	4.48
PCP vs vehicle	24467.32	1	24467.32	10.81*
MK-801 vs nomifensine	36085.65	1	36085.65	15.94**
MK-801 vs vehicle	199922.70	1	199922.70	88.31**
nomifensine vs vehicle	66133.81	1	66133.81	29.21**
Between-subjects error	72444.69	32	2263.90	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 9

Pairwise comparisons for main effect of VTA levels (medial VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	758067.21	1	758067.21	421.56**
-4.80 vs -5.20 mm	1159748.61	1	1159748.61	644.94**
-4.80 vs -5.40 mm	1186712.54	1	1186712.54	659.93**
-4.80 vs -5.60 mm	1074733.34	1	1074733.34	597.66**
-5.00 vs -5.20 mm	42537.29	1	42537.29	23.66**
-5.00 vs -5.40 mm	47826.55	1	47826.55	26.60**
-5.00 vs -5.60 mm	27563.61	1	27563.61	15.33**
-5.20 vs -5.40 mm	154.93	1	154.93	0.09
-5.20 vs -5.60 mm	1617.87	1	1617.87	0.90
-5.40 vs -5.60 mm	2774.12	1	2774.12	1.54
Within-subjects error	230173.05	128	1798.23	

Note: ** $p<0.001$ (Bonferroni correction)

Appendix F

Summary Tables of Simple Effects and Post-hoc Pairwise Comparisons of the NAS Drug Infusion Site x Drug Condition Interaction for the Medial VTA

Table 10

Simple effects of NAS drug infusion site across all levels of drug condition for medial VTA

Source of variance	SS	df	MS	F
Placement at PCP	19935.32	1	19935.32	8.81**
Placement at MK-801	14351.48	1	14351.48	6.34*
Placement at nomifensine	193.04	1	193.04	0.09
Placement at vehicle	57.28	1	57.28	0.03
Between-subjects error	72444.69	32	2263.90	

Note: * $p<0.05$ and ** $p<0.01$

Table 11

Pairwise comparisons for NAS infusion site of PCP-infused rats (medial VTA)

Source of variance	SS	df	MS	F
core vs shell	19935.32	1	19935.32	8.81**
Between-subjects error	72444.69	32	2263.90	

Note: ** $p<0.01$

Table 12

Pairwise comparisons for NAS infusion site of MK-801-infused rats (medial VTA)

Source of variance	SS	df	MS	F
core vs shell	14351.48	1	14351.48	6.34**
Between-subjects error	72444.69	32	2263.90	

Note: ** $p<0.01$

Table 13

Simple effects of drug condition across all levels of NAS drug infusion site across all levels of drug condition for medial VTA

Source of variance	SS	df	MS	F
Drugs at core	86372.00	3	28790.67	12.72**
Drugs at shell	144456.37	3	48152.12	21.27**
Between-subjects error	72444.69	32	2263.90	

Note: ** $p<0.01$

Table 14

Pairwise comparisons for drug conditions across core NAS drug infusion site (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	46767.61	1	46767.61	20.66**
PCP vs nomifensine	22135.60	1	22135.60	9.78*
PCP vs vehicle	1312.29	1	1312.29	0.58
MK-801 vs nomifensine	4553.26	1	4553.26	2.01
MK-801 vs vehicle	63748.05	1	63748.05	28.16**
nomifensine vs vehicle	34227.19	1	34227.19	15.12**
Between-subjects error	72444.69	32	2263.90	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 15

Pairwise comparisons for drug conditions across shell NAS drug infusion site (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	37971.73	1	37971.73	16.77**
PCP vs nomifensine	39.77	1	39.77	0.02
PCP vs vehicle	34219.88	1	34219.88	15.12**
MK-801 vs nomifensine	40469.09	1	40469.09	17.88**
MK-801 vs vehicle	144285.66	1	144285.66	63.73**
nomifensine vs vehicle	31926.62	1	31926.62	14.10**
Between-subjects error	72444.69	32	2263.90	

Note: ** $p<0.0017$ (Bonferroni correction)

Appendix G

Summary Tables of Simple Effects and Post-hoc Pairwise Comparisons for the Drug Condition x Medial VTA Level Interaction

Table 16

Simple effects of drug condition across all levels of rostral to caudal levels of the medial VTA

Source of variance	SS	df	MS	F
Drugs at -4.80 mm	205943.21	3	68647.74	36.30**
Drugs at -5.00 mm	30720.32	3	10240.11	5.41**
Drugs at -5.20 mm	8993.00	3	2997.67	1.58
Drugs at -5.40 mm	21750.58	3	7250.19	3.83*
Drugs at -5.60 mm	30594.20	3	10198.07	5.39**
Pooled error	302617.74	160	1891.36	

Note: * $p<0.05$ and ** $p<0.01$

Table 17

Pairwise comparisons for drug conditions across -4.80 mm from bregma VTA levels (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	99767.78	1	99767.78	52.75**
PCP vs nomifensine	23337.52	1	23337.52	12.34**
PCP vs vehicle	12055.71	1	12055.71	6.37
MK-801 vs nomifensine	26599.71	1	26599.71	14.06**
MK-801 vs vehicle	181185.48	1	181185.48	95.80**
nomifensine vs vehicle	68940.23	1	68940.23	35.45**
Pooled error	302617.74	160	1891.36	

Note: ** $p<0.0017$ (Bonferroni correction)

Table 18

Pairwise comparisons for drug conditions across -5.00 mm from bregma VTA levels (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	8345.84	1	8345.84	4.41
PCP vs nomifensine	86.74	1	86.74	0.05
PCP vs vehicle	6927.93	1	6927.93	3.66
MK-801 vs nomifensine	10134.29	1	10134.29	5.36
MK-801 vs vehicle	30481.59	1	30481.59	16.12**
nomifensine vs vehicle	5464.25	1	5464.25	2.89
Pooled error	302617.74	160	1891.36	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 19

Pairwise comparisons for drug conditions across -5.40 mm from bregma VTA levels (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	9462.18	1	9462.18	5.00
PCP vs nomifensine	130.16	1	130.16	0.07
PCP vs vehicle	2227.94	1	2227.94	1.18
MK-801 vs nomifensine	7372.82	1	7372.82	3.90
MK-801 vs vehicle	20872.97	1	20872.97	11.04**
nomifensine vs vehicle	3435.10	1	3435.10	1.82
Pooled	302617.74	160	1891.36	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 20

Pairwise comparisons for drug conditions across -5.60 mm from bregma VTA levels (medial VTA)

Source of variance	SS	df	MS	F
PCP vs MK-801	6674.95	1	6674.95	3.53
PCP vs nomifensine	2750.41	1	2750.41	1.45
PCP vs vehicle	6575.46	1	6575.46	3.48
MK-801 vs nomifensine	855.91	1	855.91	0.45
MK-801 vs vehicle	26500.44	1	26500.44	14.01**
nomifensine vs vehicle	17831.21	1	17831.21	9.43*
Pooled	302617.74	160	1891.36	

Note: * $p<0.0083$ and ** $p<0.0017$ (Bonferroni correction)

Table 21

Simple effects of rostral to caudal levels of the medial VTA across all levels of drug condition

Source of variance	SS	df	MS	F
VTA levels at PCP	315664.37	4	78916.09	43.89**
VTA levels at MK-801	725949.82	4	181487.45	100.93**
VTA levels at nomifensine	524027.10	4	131006.78	72.85**
VTA levels at vehicle	242419.74	4	60604.93	33.70**
Within-subjects error	230173.05	128	1798.23	

Note: ** $p<0.01$

Table 22

Pairwise comparisons for VTA levels of PCP-infused rats (medial VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	119252.93	1	119252.93	66.32**
-4.80 vs -5.20 mm	213531.16	1	213531.16	118.75**
-4.80 vs -5.40 mm	221224.42	1	221224.42	123.02**
-4.80 vs -5.60 mm	195278.78	1	195278.78	108.60**
-5.00 vs -5.20 mm	13633.87	1	13633.87	7.58
-5.00 vs -5.40 mm	15628.71	1	15628.71	8.69*
-5.00 vs -5.60 mm	9326.43	1	9326.43	5.19
-5.20 vs -5.40 mm	68.07	1	68.07	0.04
-5.20 vs -5.60 mm	407.67	1	407.67	0.23
-5.40 vs -5.60 mm	808.91	1	808.91	0.45
Within-subjects error	230173.05	128	1798.23	

Note: * $p<0.005$ and ** $p<0.001$ (Bonferroni correction)

Table 23

Pairwise comparisons for VTA levels of MK-801-infused rats (medial VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	324711.94	1	324711.94	180.57**
-4.80 vs -5.20 mm	509945.24	1	509945.24	283.58**
-4.80 vs -5.40 mm	474626.78	1	474626.78	263.94**
-4.80 vs -5.60 mm	457062.00	1	457062.00	254.17**
-5.00 vs -5.20 mm	20813.69	1	20813.69	11.57**
-5.00 vs -5.40 mm	14184.00	1	14184.00	7.89
-5.00 vs -5.60 mm	11284.52	1	11284.52	6.28
-5.20 vs -5.40 mm	633.67	1	633.67	0.35
-5.20 vs -5.60 mm	1447.11	1	1447.11	0.80
-5.40 vs -5.60 mm	165.59	1	165.59	0.09
Within-subjects error	230173.05	128	1798.23	

Note: ** $p<0.001$ (Bonferroni correction)

Table 24

Pairwise comparisons for VTA levels of nomifensine-infused rats (medial VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	257464.94	1	257464.94	143.18**
-4.80 vs -5.20 mm	356282.84	1	356282.84	198.13**
-4.80 vs -5.40 mm	374180.02	1	374180.02	208.08**
-4.80 vs -5.60 mm	294008.43	1	294008.43	163.50**
-5.00 vs -5.20 mm	8007.44	1	8007.44	4.45
-5.00 vs -5.40 mm	10876.93	1	10876.93	6.05
-5.00 vs -5.60 mm	1212.11	1	1212.11	0.67
-5.20 vs -5.40 mm	219.28	1	219.28	0.12
-5.20 vs -5.60 mm	2988.69	1	2988.69	1.66
-5.40 vs -5.60 mm	4827.07	1	4827.07	2.68
VTA level x S within-group error	230173.05	128	1798.23	

Note: ** $p<0.001$ (Bonferroni correction)

Table 25

Pairwise comparisons for VTA levels of vehicle-infused rats (medial VTA)

Source of variance	SS	df	MS	F
-4.80 vs -5.00 mm	101611.70	1	101611.70	56.51**
-4.80 vs -5.20 mm	144962.45	1	144962.45	80.61**
-4.80 vs -5.40 mm	166258.06	1	166258.06	92.46**
-4.80 vs -5.60 mm	170729.53	1	170729.53	94.94**
-5.00 vs -5.20 mm	3840.71	1	3840.71	2.14
-5.00 vs -5.40 mm	7917.73	1	7917.73	4.40
-5.00 vs -5.60 mm	8916.72	1	8916.72	4.96
-5.20 vs -5.40 mm	729.44	1	729.44	0.41
-5.20 vs -5.60 mm	1053.32	1	1053.32	0.59
-5.40 vs -5.60 mm	29.67	1	29.67	0.02
Within-subjects error	230173.05	128	1798.23	

Note: ** $p<0.001$ (Bonferroni correction)