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The effects of the kappa opioid agonist U-50,488 on locomotor activity of the preweanling rat following bilateral microinjection into the substantia nigra pars reticulata and striatum

Robert Linwood Collins

THE EFFECTS OF THE KAPPA OPIOID AGONIST U-50,488 ON LOCOMOTOR ACTIVITY OF THE PREWEANLING RAT FOLLOWING BILATERAL MICROINJECTION INTO THE SUBSTANTIA NIGRA PARS RETICULATA AND STRIATUM

A Thesis

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by

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ABSTRACT

The purpose of this thesis was to determine the neuroanatomical site where the kappa opioid agonist U-50,488 induces locomotor activity in the preweanling rat. It was hypothesized that kappa opioid receptors in the substantia nigra pars reticulata mediate the locomotor activating effects of systemically administered U-50,488 in the preweanling rat. Locomotor activity was assessed after 18-day-old rats (N=264) were given systemic injections of U-50,488 and *nor*-BNI (a kappa opioid antagonist), or bilateral microinjections of U-50,488 and *nor*-BNI into the substantia nigra pars reticulata or striatum. In the first experiment, the locomotor activating effects of systemically administered U-50,488 (5 mg/kg) were attenuated following systemic injection of *nor*-BNI (8 and 12 mg/kg). In the second experiment, bilateral microinjection of *nor*-BNI (5, 10, and 20 μg) into the substantia nigra pars reticulata attenuated the locomotor activating effects of systemically administered U-50,488 (5 mg/kg). In contrast, bilateral microinjection of *nor*-BNI into the striatum did not affect the locomotor activating effects of systemically administered U-50,488. In a final experiment, bilateral microinjection of U-50,488 (1.6 and

3.2 µg) into the substantia nigra pars reticulata induced locomotor activity in preweanling rats. Collectively, these results demonstrate that U-50,488 induces locomotor activity in the preweanling rat through actions on kappa opioid receptors in the substantia nigra pars reticulata.

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INTRODUCTION

Ontogenetic Research Strategy

The ontogenetic research strategy is a useful model for assessing the development of underlying neurobiological mechanisms mediating behavior (for reviews see Spear, 1979; Zolman & McDougall, 1983). There are a variety of ontogenetic strategies used to assess development. Anatomical examination of neuronal growth, myelination, and synaptogenesis, for example, allows for comparisons between young and adult animals. While this approach provides a map of anatomical development, it is limited when assessing the functionality of developing neurotransmitter systems. Alternatively, neurotransmitter system functionality can be assessed by using centrally acting drugs. As neurobiological substrates develop in young animals, behavioral repertoires also change. Thus by administering centrally acting drugs during development, ontogenetic alterations in behavior can be used to identify neurotransmitter systems that mediate a particular behavior.

Ontogenetic strategies utilizing a pharmacological approach are most effective when using drugs which have known and specific mechanisms of action on putative

neurotransmitter systems. For example, depressant drugs or minor tranquilizers are of little use in assessing ontogenetic behavioral changes, because mechanisms of action are nonspecific and affect various neurotransmitter systems. However, drugs with known mechanisms of action (e.g., cocaine, apomorphine, U-50,488) can be used to directly determine the relationship between a given neurotransmitter system and a particular behavior. In a sense, a drug with a specific mechanism of action provides a "chemical coding" for an expressed behavior (Spear, 1979). Further, pharmacological manipulation allows for the examination of both quantitative and/or qualitative behavioral changes across development.

Quantitative behavioral changes are more frequently observed than qualitative changes. Animals demonstrating quantitative changes typically show monotonic increases in behavior across age. More specifically, there is an age period when behavioral effects induced by a given drug are negligible, but over time the behavioral effects increase in a linear fashion. For example, rats as young as 4 days of age show increases in locomotor activity when challenged with dopamine receptor agonists (e.g., apomorphine and quinpirole) (Camp & Rudy, 1987; McDougall, Arnold, &

Nonneman, 1990; Moody & Spear, 1992; Shalaby & Spear, 1980). Older rat pups and adults also show increases in locomotor activity when challenged with apomorphine or quinpirole, but locomotor responsiveness increases in a positive linear manner (Moody & Spear, 1992; Shalaby & Spear, 1980). As such, quantitative changes in behavior indicate a relatively simple monotonic increase in the functionality of underlying neurobiological mechanisms governing the behavior.

While quantitative changes in behavior are more frequently observed, qualitative changes are often of greater interest. Behaviors that are unique to the preweanling rat, and not manifest in the adult, are typical of qualitative changes. For example, the dopamine mediated behaviors of young rats are sometimes characterized by the emergence of new behaviors (e.g., grooming), and the occurrence of age specific behaviors (e.g., wall climbing and the short persistence of sensitization), which are only found during a restricted period of development (McDougall, Duke, Bolanos, & Crawford, 1994; Moody & Spear, 1992; Shalaby & Spear, 1980; Tirelli & Ferrara, 1997).

The kappa opioid system also manifests qualitative ontogenetic differences. High doses of the kappa opioid

receptor agonist U-50,488 causes a suppression of motor activity in adult rats and mice (Hayes, Skingle, & Tyers, 1985; Jackson & Cooper, 1988; Leighton, Johnson, Meecham, Hill, & Hughes, 1987; Ukai & Kameyama, 1985; VonVoightlander, Lahti, & Ludens, 1983). In contrast, kappa opioid agonists produce behavioral stimulation in the preweanling rat (Bolanos, Garmsen, Clair, & McDougall, 1995; Collins, Zavala, Ingersoll, Duke, Crawford, & McDougall, 1998; Duke, Meier, Bolanos, Crawford, & McDougall, 1997; Jackson & Kitchen, 1989; McDougall, Garmsen, Meier, & Crawford, 1997). Interestingly, the paradoxical motor activating effects of U-50,488 in the preweanling rat do not appear to be dopamine mediated, since both NPA (an indirect dopamine agonist) and quinpirole (a D_2-like receptor agonist) suppress U-50,488-induced locomotor activity (Duke et al., 1997). As such, qualitative changes in the kappa opioid system are not indicative of a simple quantitative, monotonic increase in kappa receptor functionality. Rather, this qualitative change reflects a more complex ontogenesis of the kappa opioid system, possibly indicating an interaction with other developing neurotransmitter systems.

Opioid System

Opioid Peptides and Ontogeny

Within the opioid system, up to 20 peptides are derived from three opioid precursors: proopiomelanocortin, proenkephalin, and prodynorphin (for reviews see Fowler & Fraser, 1994; Leslie & Loughlin, 1994; McDowell & Kitchen, 1987). A variety of techniques are used to assess the development of these peptides including molecular probes and immunohistochemistry (Fowler & Fraser, 1994; Leslie & Loughlin, 1994; Schafer, Day, Watson, & Akil, 1991). Interestingly, these three peptide families exhibit differential expression throughout development, with peptide levels in the neonate being considerably less than in the adult (Leslie & Loughlin, 1994; McDowell & Kitchen, 1987).

Proopiomelanocortin. Proopiomelanocortin (POMC) is a precursor of the opioid peptide β-endorphin and other non-opioid products (e.g., adrenocorticotrophic hormone)(Shafer et al., 1991). β-endorphin has a high affinity for both delta and mu receptor subtypes (Schoffelmeer et al., 1990). In the adult rat brain, β-endorphin is found in the arcuate nucleus of the hypothalamus and the nucleus tractus

solitarus of the brain stem (Mansour, Khachaturian, Lewis, Akil, & Watson, 1988; Schwartzberg & Nakane, 1982). Processes from the arcuate nucleus extend to forebrain regions, including the medial preoptic area, nucleus accumbens, and septum, as well as midbrain and hindbrain regions. Less is known about POMC containing neurons extending from the nucleus tractus solitarus, however evidence suggests that these processes project to the caudal brain stem (Akil, Young, & Watson, 1988).

Both POMC mRNA and peptide products are detected early in development. POMC mRNA is detectable by embryonic day (E) 10.5 in the mouse (Elkabes, Loh, Nieburgs, & Wray, 1989) and by E 13 in whole rat brain (Bayon, Shoemaker, Bloom, Mauss & Guilleman, 1979; Bloom et al., 1980). β-endorphin is present in both mice and rats at mid-gestation, but the ontogenetic maintenance of β-endorphin levels after birth is unclear (Bayon et al., 1979; Rius, Barg, Bem, Coscia, & Loh, 1991; Rius, Chikuma, & Loh, 1991). While it has been suggested that β-endorphin increases across ontogeny until reaching adult levels, it has also been reported that β-endorphin levels increase rapidly during the preweanling period and then decline to

adult levels (Lugo, Roberts, & Pintar, 1989; McDowell & Kitchen, 1987).

Proenkephalin. Proenkephalin is a precursor for a variety of peptides of varying sizes. Both the smaller pentapeptides ([met] and [leu]enkephalin) and the larger peptides (peptides E and F, metorphamide, MERGL, and MERF) are derived from proenkephalin (Hollt, 1986). The smaller pentapeptides are the endogenous ligands for delta opioid receptors (Lord, Waterfield, Hughes, & Kosterlitz, 1977), whereas larger peptide chains have a greater affinity for mu and kappa opioid receptors (Hollt, 1986). In the adult rat, proenkephalin is found throughout the brain, including the cerebral cortex, basal ganglia, limbic areas, hypothalamus, thalamus, and various caudal cell groups (Fallon & Ciofi, 1990; Harlan, Shivers, Romano, Howells, & Pfaff, 1987; Mansour et al., 1988; Schafer et al., 1991). Enkephalinergic cells are also found in various areas of the telencephalon (i.e., nucleus accumbens, amygdala, caudate putamen, stria terminalis) (Mansour et al., 1988).

In the developing rat fetus, proenkephalin mRNA is found in the basal plate of the brain stem by E 12.5 (Keshet, Polakiewicz, Itin, Ornoy, & Rosen, 1989). Enkephalinergic immunoreactive cells in the fetal rat brain

can be visualized by E 13 and enkephalinergic immunoreactive fibers in the ventral pons and cervical spinal cord can be visualized by E 15 (Dahl, Epstein, Silva, & Lindberg, 1982; Palmer, Miller, Olson, & Sieger, 1982). By E 18, the pattern of immunoreactive cell bodies is similar to adult animals and enkephalinergic terminal fields have mature distributions by postnatal day (PD) 1 (Loughlin, Massimiri, Kornblum, & Leslie, 1985; Palmer et al., 1982; Tecott, Rubenstein, Paxinos, Evans, Eberwine, & Valentino, 1989). Peptide levels in whole brain increase during late gestation and continue to increase in most brain regions across ontogeny (Bayon et al., 1979; Rius, Barg, Bem, Coscia, & Loh, 1991; Rius, Chikuma, & Loh, 1991). Interestingly, striatal [met]enkephalin immunoreactivity peaks around postnatal day 21, while MERGL, MERF, and [leu]enkephalinergic immunoreactive cells remain at fairly constant levels from PD 10 - PD 30 (Baily & Kitchen, 1985). Thus, it appears that proenkephalin products exhibit different maturational rates.

Prodynorphin. Prodynorphin, also known as proenkephalin B, is a precursor for peptides that are structurally related to [leu]enkephalin [e.g., dynorphin A, dynorphin B, dynorphin (1-8)] (Hollt, 1986). Similar to

proenkephalin peptides, long and short prodynorphin peptides have different pharmacological specificities. While dynorphin (1-8) does not appear to be selective for a particular receptor, dynorphin A has a high affinity for the kappa receptor and is its endogenous ligand (Chavkin, James, & Goldstein, 1982).

Dynorphinergic cells are extensively distributed throughout the adult brain (Fallon & Ciofi, 1990; Mansour et al., 1988; Morris, Haarmann, Kempter, Holt, & Herz, 1986; Schafer et al., 1991). Dynorphinergic cells in the hippocampus have their projections in the mossy fiber pathway with terminals in all fields except CA1. In the substantia nigra, the pars reticulata receives dynorphinergic projections from the striatum, whereas enkephalinergic cells are found in the substantia nigra pars compacta (Peppenberg, 1988).

Few prenatal studies have assessed prodynorphin development (Leslie & Loughlin, 1994). Within the striatum, prodynorphin mRNA is detectable by E 15 (Alvarez-Bolado, Fairen, Douglass, & Naranjo, 1990). Striatal prodynorphin mRNA levels increase until PD 8-10, following which there is a 50-fold decline by PD 28 (Rosen & Polakiewicz, 1989). Prodynorphin mRNA levels in the

hypothalamus and medulla have a similar postnatal profile (Rosen & Polakiewicz, 1989); however, prodynorphin develops later in the hippocampal formation, since hippocampal prodynorphin is not detectable until PD 6 (Gall, 1984). Curiously, in other brain structures (e.g. pituitary), prodynorphin products increase progressively from birth to adulthood (Seizinger, Liebish, Grimm, & Herz, 1984).

Opioid Receptors and Ontogeny

Endogenous opioid peptides and synthetic drugs act at three known opioid receptors: mu, kappa, and delta (Kornblum, Hurlbut, & Leslie, 1987; Mansour, Khachaturian, Lewis, Akil, & Watson, 1987; Mansour, Schafer, Newman, & Watson, 1991; Martin, Eades, Thompson, Huppler, & Gilbert, 1976; Zaki, Bilsky, Vanderah, Lai, Evans, & Porreca 1996). All three receptors are widely distributed throughout the brain and show different maturational patterns (Spain, Roth, & Coscia, 1985). Radioligand binding is the primary technique used to assess the ontogeny of receptors (McDowell & Kitchen, 1987). While this technique is generally successful, it is limited by ligands that are not sufficiently selective for targeted receptor populations (Leslie & Loughlin, 1994).

Mu Receptors. Mu receptors are widely distributed throughout the rat brain and have a high affinity for the endogenous opioid peptide β-endorphin and the synthetic drug morphine. Additionally, both the radioligand DAGOL and the peptide β-endorphin have been used to label mu receptors. Receptor binding studies have found both low- and high-affinity binding sites, suggesting the presence of two mu subtypes (Fowler & Fraser, 1994). In the adult rat, a high density of mu binding sites occurs in the cerebral cortex, hippocampus, olfactory bulb, and striatum (Kornblum et al., 1987; Mansour et al., 1991). A high density of labeling also occurs in numerous thalamic nuclei and the medial habenula, while lower densities are found in the hypothalamus (Kornblum et al., 1987).

Mu receptors are first detectable in the fetal rat brain at E 14 and increase until birth (Kent, Pert, & Herkenham, 1982; Kornblum, Loughlin, Fallon, & Leslie, 1989; Spain et al., 1985). After birth, mu receptor densities decline during the first postnatal week, then rapidly increase to peak densities during the following two weeks. Following this proliferation, there is a gradual decline to adult levels (Spain et al., 1985).

Delta Receptors. Delta opioid receptors have an affinity for the pentapeptides [met] and [leu]enkephalin (Fowler & Fraser, 1994; Lord et al., 1977). Although uncertain, subtypes for delta receptors may exist (Fowler & Fraser, 1994; Zaki et al., 1996). In the adult rat, delta receptors are found in forebrain regions, including the olfactory tubercle, striatum, and nucleus accumbens (Mansour et al., 1991). Low levels of binding occur in amygdala and hippocampal nuclei.

Unlike mu receptors, the development of delta receptors occurs primarily during the postnatal period (McDowell & Kitchen, 1986; Spain et al., 1985). Using the radioligand DADL, delta receptors are found in very low levels during the first few postnatal weeks. From the second to fourth postnatal week there is a linear increase in delta receptor density which seems to correlate with the increase in mu receptors (Spain et al., 1985).

Kappa Receptors. Like mu receptors, kappa receptors are widely distributed throughout the brain (Fowler & Fraser, 1994; Spain et al., 1985). Based on pharmacological action, two subtypes of kappa receptors have been distinguished: kappa 1 (κ_1) and kappa 2 (κ_2)

(Fowler & Fraser, 1994). The κ_1 subtype has a high affinity for the endogenous opioid peptide dynorphin A and the synthetic compounds U-50,488 and U-69,593. The κ_2 subtype has a high affinity for the endogenous opioid peptides dynorphin A and β-endorphin and the synthetic compounds ethlyketocyclazocine and bremazocine (Zukin, Eghbali, Olive, Unterwald, & Tempel, 1988). Because the discovery of these kappa receptor subtypes is relatively recent, the ontogeny of κ_1 and κ_2 binding has yet to be thoroughly examined.

In the adult rat, κ_1 receptors are found in only a few brain regions (Fowler & Fraser, 1994; Zukin et al., 1988). Moderate densities can be found in the striatum, nucleus accumbens, olfactory tubercle, and the basolateral and medial nuclei of the amygdala. Within the substantia nigra, κ_1 receptors are found extensively in the pars reticulata (Mansour, Burke, Pavlic, Akil, & Watson, 1996). κ_2 receptors are found in moderate amounts in many forebrain regions including the olfactory tubercles, nucleus accumbens, and striatum (Fowler & Fraser, 1994; Zukin et al., 1988). Within the mesencephalon, κ_2 receptors are distributed in the superior colliculus, central grey,

medial geniculate, substantia nigra pars compacta, and interpeduncular nucleus (Zukin et al., 1988).

In fetal mouse brain homogenates, kappa receptors can be detected at E 14.5 and increase in density until E 18.5 (Spain et al., 1985). At birth, kappa receptors are found in similar densities in both forebrain and hindbrain regions; however, after PD day 1, kappa receptor densities in the hindbrain increase, whereas densities in the forebrain decrease (see Kitchen, Kelly, & Paz Viveros, 1990; Spain et al., 1985). After the second postnatal week the reverse occurs, with the relative density of forebrain kappa receptors increasing and hindbrain receptors decreasing. This reversal is probably due to the maturation of the cerebellum, which contains few kappa sites (Spain et al., 1985).

Dopamine Pathways

The dopamine system is organized topographically based on the length of efferent dopamine fibers. This organization has led to two broad divisions: the mesolimbic-mesocortical pathway and the nigrostriatal pathway (Copper, Bloom, & Roth, 1996; Role & Kelly, 1991).

Mesolimbic-Mesocortical Pathway

Dopamine fibers in the mesolimbic-mesocortical pathway have their cell bodies in the ventral tegmental area (VTA), located between the substantia nigra and the red nucleus (Peppenberg, 1988). The mesocortical component of this pathway connects the VTA with the medial prefrontal, cingulate, and entorhinal cortices. The mesolimbic component of this pathway connects the VTA with other limbic structures, including the amygdala and nucleus accumbens (Cooper et al., 1996).

Recently, it has been shown that mu and kappa opioid neurotransmitter systems modulate dopaminergic activity in the mesolimbic pathway (see Figure 1). Mu agonists increase dopamine levels in the nucleus accumbens, whereas kappa agonists decrease dopamine release in the same region (Di Chiara & Imperato, 1988a; Spanagel, Herz, & Shippenberg, 1990; 1992). The neuroanatomical mechanisms responsible for these dopamine/kappa interactions are known. For example, mu agonists indirectly increase extracellular dopamine levels by releasing the mesolimbic pathway from GABAergic inhibition in the VTA. In contrast, kappa agonists decrease the dopaminergic response by

directly inhibiting dopamine release in the nucleus accumbens (Spanagel et al., 1990; 1992).

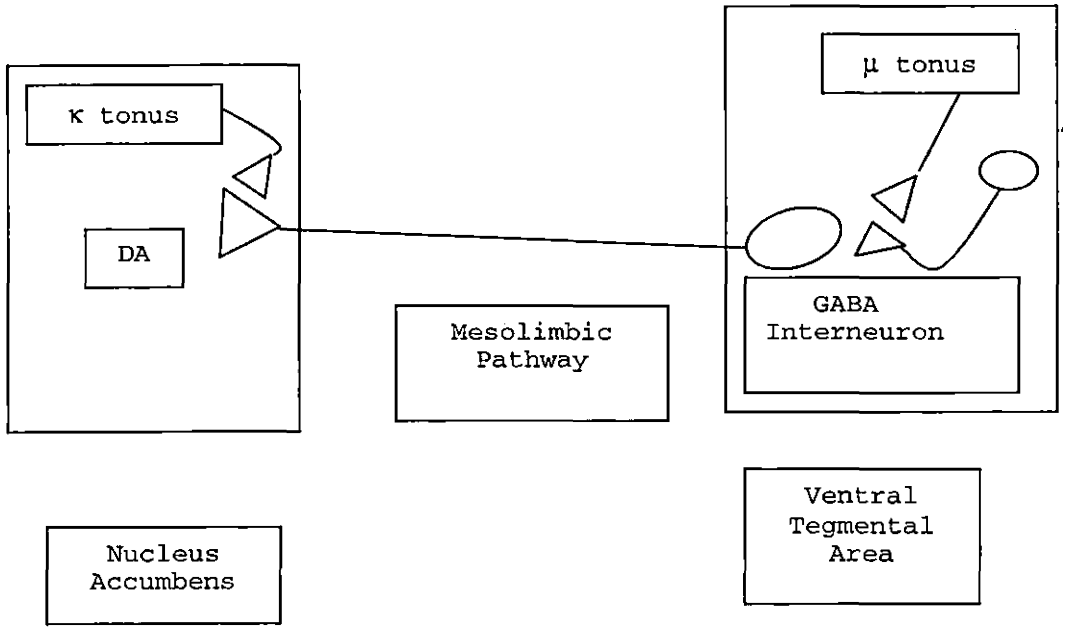

Figure 1. Mu and kappa opioid neurotransmitter systems modulate dopaminergic activity in the mesolimbic pathway of adult rats. Stimulation of mu receptors in the VTA increases dopamine release in the nucleus accumbens by releasing the dopamine pathway from tonic GABAergic inhibition. The behavioral byproduct of stimulating these mu receptors is locomotor activity. Conversely, stimulation of kappa opioid receptors in the nucleus accumbens causes a presynaptic inhibition of dopamine in the same area. The behavioral byproduct of stimulating these kappa receptors is a suppression of locomotor activity.

Therefore, it appears that mu and kappa opioid systems work in concert to modulate dopaminergic functioning in the mesolimbic-mesocortical pathway.

Nigrostriatal Pathway

The nigrostriatal pathway originates in the substantia nigra and projects to the striatum. The substantia nigra is a large nucleus in the tegmentum, superior to the basis pedunculi (Peppenberg, 1988). The substantia nigra is composed of two regions: the pars compacta and pars reticulata. The pars compacta is superior to the pars reticulata and is primarily composed of dopaminergic cell bodies. The pars reticulata is penetrated by pars compacta connections, and contains GABA and opioid (mu and kappa) cell bodies (Matsumoto, Brinsfield, Patrick, & Walker, 1988; Merchenthaler, Maderdrut, Altschuler, & Petrusz, 1986).

The afferent and efferent connections of the substantia nigra are well understood. Afferent GABAergic processes, primarily from the striatum, but also from the subthalamic nucleus, innervate the pars compacta (Mendez, Elisevich, & Flumerfelt, 1993). Dopaminergic fibers from the pars compacta primarily project to the neostriatum, but there are also fibers projecting to the pars reticulata

(Mendez et al., 1993). In addition to dopaminergic input, the pars reticulata receives GABAergic projections from the striatum and the globus pallidus. Within the pars reticulata there appear to be two types of neurons. There are GABAergic neurons that synapse on dopaminergic neurons of the pars compacta and there are GABAergic neurons that project to the thalamus (Mendez et al., 1993).

The kappa opioid system appears to modulate the functioning of dopamine neurons composing the nigrostriatal system. Nondopaminergic pars reticulata cells have a motor activating effect, whereas dopaminergic pars compacta cells have a motor inhibiting effect (Matsumoto et al., 1988). For example, systemic injections of U-50,488 suppress locomotor activity and decrease the firing rate of dopaminergic cells in the substantia nigra (Thompson & Walker, 1990; 1992).

Behavioral Effects of Opioid Receptor Stimulation

Opioid receptors are important for a number of different behaviors, including pain modulation, reward processes, and locomotor activity (Crawford, McDougall, Bolanos, Hall, & Berger, 1995; Di Chiara & Imperato, 1988b; Haney & Miczec, 1995; Mucha & Herz, 1985; Smotherman, Moody, Spear, & Robinson, 1993; Stolerman, 1985). Opioid receptors

have a primarily modulatory role, as opioid agonists inhibit the release of other neurotransmitters. Both mu and delta agonists inhibit the release of cortical noradrenaline and striatal acetylcholine (Wolleman, Benyhe, & Simon, 1993), whereas kappa agonists inhibit dopamine release in striatal and cortical areas (Wolleman et al., 1993).

Behavioral Effects of Opioid Receptor Stimulation in the Adult Rat

Delta and Mu receptor activation. Activation of delta or mu receptors modulates antinociception in the adult rat. A variety of experimental paradigms, including ultrasonic vocalization (a measure of affective distress), tail flick, paw pressure, and hot plate responding are used to assess the analgesic properties of drugs (see McDowell & Kitchen, 1987). Both DPDPE (a delta receptor agonist) and morphine (highly selective for mu receptors) attenuate high frequency vocalizations as well as tail flick responding in the adult rat (Haney & Miczec, 1995). Similarly, [D-Ala2, Glu4]deltorphin (a delta receptor agonist) and the mu agonist dermorphin increase hot plate latencies. Interestingly, delta and mu agonists may interact synergistically in evoking an antinociceptive response, since co-administration of [D-Ala2, Glu4]deltorphin and dermorphin produces a greater analgesic effect than either agonist alone (Negri, Improta, Lattanzi, Potenza, Luchetti, & Melchiorri, 1995; Negri, Noviello & Noviello, 1996).

Delta and mu receptors are also involved in reward processes (see Stolerman, 1985). Experimental paradigms, such as drug self-administration, and place and taste preferences are used to assess the rewarding properties of drugs. In the self-administration paradigm, adult rats maintain high levels of bar press responding when given intracranial injections of either DPDPE (a delta agonist) or DAMGO (a mu agonist) into the VTA (Devine & Wise, 1994; Jenck, Gratton, & Wise, 1987). In addition, adult rats develop conditioned taste and place preferences for morphine. The preference for morphine is often biphasic, since lower doses are preferred and higher doses produce an aversion (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Beach, 1957).

Activation of delta and mu receptors also affects the locomotor activity of adults rats. Systemic and intracranial injections of delta receptor agonists (e.g., DPDPE and [D-Ala2, Glu4]deltorphin) and mu receptor agonists (e.g., DAMGO, morphine, and dermorphin) increase locomotor activity (Klitenick & Wirtshafter, 1995; Meyer & Meyer, 1993; Negri et al., 1995; 1996). These delta and mu receptor-mediated effects are blocked by the delta receptor antagonist naltrindole and the mu receptor antagonist

naloxone, respectively (Negri et al., 1996; Uchihashi, Kuribara, Morita, Kitani, & Sato, 1996). Moreover, delta agonists seem to affect locomotor activity by modulating a dopaminergic mechanism in the striatum, whereas mu agonists appear to affect activity by modulating a dopaminergic mechanism in the nucleus accumbens (Schad, Justice, & Holtzman, 1996; Spanagel et al., 1992).

Kappa Receptor Activation. Similar to delta and mu receptors, kappa receptors have antinociceptive properties (Mucha & Herz, 1985; VonVoightlander et al., 1983). For example, the kappa receptor agonists U-50,488 and enadoline block abdominal licking to visceral pain and formalin-induced nociception in the hindpaw (Craft, Henley, Haaseth, Hruby, & Porreca, 1995; McLaughlin, Tao & Abood, 1995). Importantly, the analgesic response to U-50,488 is blocked by the kappa receptor antagonist *nor*-BNI, indicating that the analgesic response is mediated through actions at the kappa receptor (Craft et al., 1995; McLaughlin et al., 1995).

Unlike delta and mu receptors, activation of kappa receptors have aversive properties when tested in the self-administration paradigm (Di Chiara & Imperato, 1988b; Holtzman & Steinfels, 1994). Not surprisingly, kappa

opioid agonists (U-50,488 and various dynorphin derivatives) induce conditioned place and taste aversions (Mucha & Herz, 1985). The aversive properties of kappa receptor stimulation appear to be mediated centrally, with the aversion resulting from a kappa opioid mediated reduction in extracellular dopamine (Bals-Kubic, Herz, & Shippenberg, 1989; Maisonneuve, Archer & Glick, 1994; Spanagel, Almeida, Bartl, & Shippenberg, 1994; but see Bechara & van der Kooy, 1987).

A variety of kappa opioid agonists (e.g., U-50,488, U-69593, and enadoline) attenuate locomotor activity of adult rats and mice (Duke et al., 1997; Jackson & Cooper, 1988; Leyton & Stewart, 1992; McLaughlin et al., 1995; Ukai & Kameyama, 1985; VonVoigtlander et al., 1983). The attenuation of locomotor activity occurs in a dose-dependent manner. For example, lower doses of U-50,488 (5 and 10 mg/kg) reduce spontaneous activity, while higher doses induce catalepsy (Jackson & Cooper, 1988; Ukai & Kameyama, 1985). Importantly, U-50,488's locomotor suppressing action is blocked by the kappa receptor antagonist *nor*-BNI (Jones & Holtzman, 1992).

It appears that activation of kappa receptors suppresses the locomotor activity of adult rats through

actions on the mesolimbic dopamine pathway. More specifically, dopamine release in the nucleus accumbens is attenuated when U-50,488 is either injected systemically or microinjected directly into the nucleus accumbens (Di Chiara & Imperato, 1988a; Spanagel et al., 1992). Therefore, U-50,488 appears to block the locomotor activity of adult rats by stimulating presynaptic kappa receptors and, thus, inhibiting dopamine release in the nucleus accumbens. Consistent with this model, receptor antagonists (i.e., *nor*-BNI or high doses of naloxone), block U-50,488-induced locomotor suppression in both adult mice and rats (Di Chiara & Imperato, 1988a; Narita, Suzuki, Fanuda, Misawa, & Nagase, 1993).

Importantly, unilateral microinjections of U-50,488 into the substantia nigra pars reticulata induces behavioral activity (contralateral circling) in the adult rat (Matsumoto et al., 1988). This is probably due to kappa receptor stimulation inhibiting GABAergic output projections of the nigotectal and nigrothalamic pathways. Inhibition of these pathways is known to induce behavioral activity (Thompson & Walker, 1992). Thus, stimulation of pars reticulata kappa receptors produces behavioral stimulation, while systemic injections of U-50,488

suppresses locomotor activity. Presumably, U-50,488's ability to inhibit dopamine release in the mesolimbic and nigostriatal pathways masks the locomotor activating effects of kappa receptor stimulation in the pars reticulata.

Behavioral Effects of Opioid Receptor Stimulation in the Preweanling Rat

Delta and Mu Receptor Activation. The selective mu agonist morphine and the selective delta receptor agonists DAMGA and DPDPE have been used to assess antinociception in the young rat. The antinociceptive properties of delta and mu receptors are similar to adult rats and appear to be functional as early as PD 5 (Kehoe & Blass, 1986a; see McDowell & Kitchen, 1987). For example, morphine evokes antinociception after tail immersion in 5-day-old rats and increases hot plate latencies in 10-day-olds (Johannesson & Becker, 1972; Kehoe & Blass, 1986b). Similarly, both DAMGO and DPDPE decrease ultrasonic vocalizations in isolated pups (a sign of affective distress) (Carden, Barr, & Hofer, 1991).

Most of the work assessing the rewarding properties of delta and mu receptor activation has used morphine, and suggests that these receptors are behaviorally functional

early in development. By PD 5, rats treated with morphine develop place preferences to non-preferred odors; this conditioning is blocked by the antagonists naloxone and naltrexone (Kehoe & Blass, 1986a). Morphine's effects on the reward processes of young animals are biphasic. In the 5-day-old rat, low doses of morphine (0.5 mg/kg) induce place preferences, whereas higher doses (2.0 mg/kg) have aversive properties (Bolanos et al., 1996; Randall, Kraemer, Dose, Carbary, & Bardo, 1992). It is possible that higher doses of morphine bind with kappa receptors, either centrally of peripherally, since activation of kappa receptors in both adults and pups is aversive (Bals-Kubic et al., 1989; Bechara & van der Kooy, 1987; Randall et al., 1992).

Interestingly, while delta and mu agonists affect antinociception and reward similarly across ontogeny, these same agonists produce age-dependent effects when locomotor activity is assessed. For example, morphine and DAMGO have cataleptic and sedative effects in the preweanling rat (Bolanos et al., 1996; Caza & Spear, 1980; Jackson & Kitchen. 1989) and the behavioral effects of delta receptor activation are innocuous (Jackson & Kitchen, 1989). In contrast, the adult rat exhibits enhanced locomotor

activity after delta or mu opioid receptor stimulation (Klitenick & Wirtshafter, 1995; Meyer & Meyer, 1993; Negri et al., 1995; 1996).

Kappa Receptor Activation. The antinociceptive properties of kappa receptor activation in the preweanling rat are similar to adults (McLaughlin et al., 1995); however, some quantitative differences are apparent. For example, high doses of the kappa opioid agonist ketocyclazocine (10 mg/kg) produce modest levels of antinociception in 3- to 9-day-old rats, whereas the same dose is more effective at producing antinociception after PD 10 (Barr, Paredes, Erikson & Zukin, 1986).

Similar to adults, kappa opioid receptor stimulation has aversive properties in the fetal and preweanling rat. During late gestation (i.e., E 19-E 21), U-50,488 administered through an artificial nipple is aversive and disrupts appetitive responses towards the nipple (Robinson, Hoeltzel & Smotherman, 1995). By PD 7, rats develop conditioned aversions to U-50,488 (Barr, Wang & Carden, 1994). In addition, U-50,488 enhances ultrasonic vocalizations in 3-, 7-, and 10-day-old rats (Barr et al., 1994; Carden et al., 1991).

Importantly, the locomotor activating effects of kappa receptor activation vary across ontogeny. While U-50,488 suppresses behavioral activity in the adult rat, it produces behavioral stimulation in the preweanling rat (Bolanos et al., 1996; Collins et al., 1998; Duke et al., 1997; Jackson & Kitchen, 1989; Kehoe & Boylan, 1994; McDougall et al., 1997). Furthermore, kappa agonists induce qualitative behavioral differences in fetal and preweanling rats. More specifically, during late gestation (i.e., E 21) U-50,488 (0.1 to 1.0 mg/kg) induces a dose-dependent increase in limb movements (Robinson, Moody, Spear, & Smotherman, 1993; Smotherman et al., 1993). However, in 5- and 10-day-old rats U-50,488 (0.1 and 1.0 mg/kg) enhances locomotor activity and wall climbing, whereas the same doses preferentially increase wall climbing in 20-day-olds (Jackson & Kitchen, 1989). In summary, activation of kappa receptors produces qualitative differences across ontogeny: In the embryonic and young animal, kappa receptor stimulation induces age- and dose-dependent behavioral activation, whereas kappa receptor stimulation in the adult animal suppresses behavioral activity.

Summary and Thesis

In the adult rat, systemic injection of U-50,488 decreases locomotor activity. This is probably due to kappa receptor stimulation causing presynaptic inhibition of dopamine release in the nucleus accumbens (Di Chiara & Imperato, 1988a; Spanagel et al., 1992). Curiously, activation of kappa receptors in the pars reticulata of the substantia nigra increases the motoric activity of adult rats. For example, unilateral injections of prodynorphin peptides [e.g., prodynorphin (1-8) and prodynorphin (1-17)] cause contralateral rotations in adult rats (Friederich, Friederich & Walker, 1987; Herrera-Marschitz, Hokfelt, Ungerstedt & Terenius, 1983; Iwamoto & Way, 1977). Similarly, microinjections of the kappa agonist U-50,488 into the substantia nigra pars reticulata causes motor activity (Matsumoto et al., 1988; Thompson & Walker, 1990; 1992). In summary, systemic injection of U-50,488 decreases locomotor activity in adult rats, while microinjections of U-50,488 into the substantia nigra pars reticulata increases activity. This is most curious, since systemic injections of U-50,488 increase the locomotor activity of preweanling rats.

Although speculative, age-dependent differences in U-50,488 mediated activity may be due to regional differences in a population of kappa receptors. In the adult rat, kappa receptor mediated inhibition of the mesolimbic dopamine pathway masks the locomotor activating effects of the substantia nigra pars reticulata (thus systemic U-50,488 attenuates locomotor activity). In the preweanling rat, however, kappa receptors in the substantia nigra pars reticulata may become functionally mature before kappa receptors in the mesolimbic pathway (or exist in greater numbers). This would explain why systemically administered U-50,488 enhances the locomotor activity of preweanling rats (see Figure 2).

The purpose of this thesis was to determine the neuroanatomical site where U-50,488 induces locomotor activity in the preweanling rat. The first experiment was designed to determine whether the motor activating properties of U-50,488 are attenuated by the kappa opioid antagonist *nor*-BNI. It was predicted that a systemic injection of *nor*-BNI would block U-50,488's motor activating effects in the preweanling rat. The second

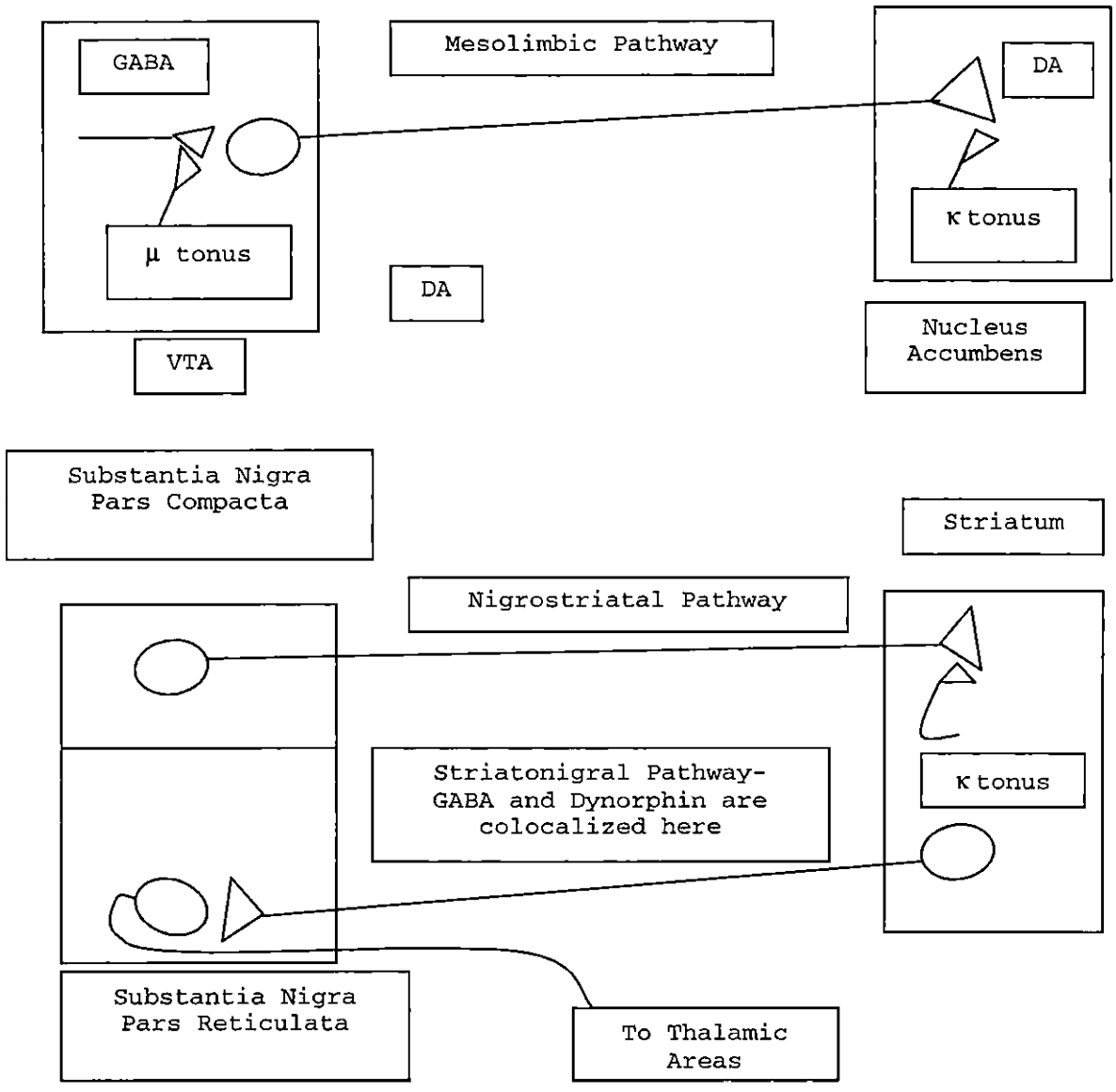

Figure 2. Proposed model for the locomotor activating effects of U-50,488 in the preweanling rat. Kappa opioid neurons in the mesolimbic and nigostriatal pathways, which act to suppress locomotor activity in adult rats when stimulated, may be functionally immature or have low kappa opioid receptor populations. In the substantia nigra pars reticulata, tonically active GABAergic neurons exert an inhibitory influence over target neurons that mediate locomotor activity (e.g. thalamus). Stimulated kappa opioid receptors in the substantia nigra pars reticulata suppress GABAergic activity and induce locomotor activity by releasing target neurons from tonic inhibition.

experiment was designed to determine whether U-50,488 induces motor activity in the preweanling rat through actions in the substantia nigra pars reticulata. It was predicted that bilateral injections of *nor*-BNI into the substantia nigra pars reticulata would block the motor activating effects of a systemic injection of U-50,488. It was also predicted that bilateral injections of *nor*-BNI into the medial striatum would not block the motor activating effects of a systemic injection of U-50,488. These latter experiments would confirm that U-50,488 produces its locomotor activating effects by stimulating kappa receptors in the substantia nigra pars reticulata of the preweanling rat.

GENERAL METHODS

Subjects

Subjects were 264 male and female rats of Sprague-Dawley descent (Harlan). Litters were culled to ten rat pups by three days of age. Pups were kept with the dam until surgery. At 17 days of age, rats were randomly assigned to groups. No more than one rat from each litter was placed into a particular group. The colony room was maintained at 21-23° C and kept on a 12-hr light/dark cycle.

Apparatus

Behavior was assessed in white plywood chambers (35 × 35 × 46 cm). The floors of the test chambers were divided by lines into four equal quadrants.

Drugs

When injected subcutaneously (SC), the kappa opioid agonist (-)-*trans*-(1s,2s)-U-50,588 hydrochloride and the kappa opioid antagonist *nor*-binaltorphamine (Research Biochemicals, Natick, MA) were mixed in double distilled water vehicle at a volume of 5 ml/kg. For intracranial injections (IC), U-50,488 and *nor*-binaltorphimine were mixed in double distilled water vehicle at a volume of 0.25 µl per side.

Surgery

At 17 days of age, rats were bilaterally implanted with stainless steel guide cannulae (26 gauge) according to the general methods described in Cunningham and McKay (1993). At the time of surgery, rats were injected (IP) with 1 ml/kg of a commercially available solution of ketamine hydrochloride and xylazine hydrochloride (Research Biochemicals). Once a surgical plane of anesthesia was achieved, rats were placed in a Cunningham Neonatal Rat Adapter attached to a standard Kopf stereotaxic apparatus (Heller, Hutchens, Kirby, Karapas, & Fernandez, 1979; Tive & Barr, 1992). Stainless steel guide cannulae (Plastics One, Roanoke, VA) were stereotaxically implanted in the substantia nigra pars reticulata (0.0 mm anterior, 2.0 mm lateral) and medial striatum (2.5 mm anterior, 2.9 mm lateral) using coordinates from the developing rat brain atlas of Sherwood and Timiras (1970). The guide cannulae terminated 1 mm above injection sites and were fixed in place using super glue gel (Duro, Cleveland, OH). Stainless steel stylets (Plastics One) were used to seal the cannulae until time of testing.

After surgery, rats were allowed to recover away from the dam in a temperature controlled chamber (30° C). After an individual pup was fully responsive it was returned to the dam. Animals were tested approximately 24 hr after surgery. Immediately prior to testing, inner cannulae (31 gauge), attached to a microsyringe (Hamilton), were inserted into the guide cannulae. Solutions were delivered at a constant rate over a 60 s period. The inner cannulae were left in place for an additional 90 s.

Histology

Following behavioral assessment, rats were given an overdose of Nembutal and their brains were removed and placed in formalin (10%). Following a postfixation period, coronal sections (100 μm) were taken from each brain using a Vibratome 1000 (Ted Pella, Redding, CA). Using a thionin stain, these sections were microscopically assessed for injection site verification (Leyton & Stewart, 1992; Schad et al., 1996). Additional animals were assessed for drug dispersion using both neutral red and crystal violet (Bordi, Carr, & Meller, 1989; Tive & Barr, 1992). These animals received bilateral microinjections of either neutral red or crystal violet dye and their brains were

removed either 5 or 30 min later. Sherwood and Timiras' (1970) Stereotaxic atlas of the developing rat brain was used as a reference source to determine both injection site and dispersion parameters.

Statistical Analyses

Analyses of variance (ANOVA's) for repeated measures (5 min time blocks) were used for statistical analysis of line-crossing data. Line-crosses were defined as the front paws moving forward into an adjacent quadrant. This is a reliable way of assessing locomotor activity and has been validated in many laboratories (Collins et al., 1998; McDougall et al., 1992; Shalaby & Spear, 1980; Tirelli & Ferrara, 1997). Post hoc analyses of simple interactions and simple main effects were made using Tukey's HSD ($p<0.05$).

EXPERIMENT 1

The first experiment was designed to determine whether the motor activating properties of U-50,488 in the preweanling rat can be attenuated by a systemic injection of the kappa opioid antagonist *nor*-BNI. It was predicted that a systemic injection of *nor*-BNI would attenuate U-50,488's motor activating effects.

Method

To examine this, 80 (n=8 per group) 18-day-old rats were systemically injected (SC) with vehicle or 5.0 mg/kg U-50,488 [this dose has been shown to produce robust locomotor activity in preweanling rats (Collins et al., 1998)]. After being injected, rats were placed into the testing chamber for a 5-min habituation period, followed by a 55-min testing session. After the first 15-min of testing, rats were given a systemic injection of *nor*-BNI (0.0, 2.0, 4.0, 8.0, or 12.0 mg/kg) and placed back into the testing chamber. Behavior (line-crosses) was assessed for the entire testing session by an observer blind to treatment conditions. In summary, animals received a systemic injection of U-50,488 or vehicle and then a challenge injection of *nor*-BNI (0.0, 2.0, 4.0, 8.0, or 12.0 mg/kg) 15 min later.

Results

Pretreatment Phase

A summary of the line-cross data for the various groups of rats can be found in Figure 3. Overall, pretreatment with U-50,488 induced robust line-crossing in the young animals (see left portion of Figure 3) [pretreatment main effect, $F(1, 78)=278.5$, $p<0.05$]. Line crosses varied across the pretreatment phase, since there was a progressive increase in line-cross counts during the first 15 min [Pretreatment × Time interaction, $F(2, 156)=40.49$, $p<0.05$]. Animals pretreated with U-50,488 were significantly more active relative to vehicle controls at each of the three time blocks (Tukey HSD, $p<0.05$). Animals pretreated with vehicle remained inactive during the pretreatment phase.

Post-treatment Phase

Line-cross counts during the post-treatment phase can be found in the right portion of Figure 3. Overall, *nor*-BNI (8.0 and 12.0 mg/kg) attenuated the line-crossing of U-50,488 treated rats, and this effect varied across the testing session [Pretreatment × Post-treatment × Time interaction, $F(21, 392)=3.25$, $p<0.05$]. Because only U-

50,488, and not vehicle, stimulated locomotor activity, separate 5 × 8 ANOVAs were done for each pretreatment condition.

Overall, line-crossing of the U-50,488 pretreated rats was attenuated by *nor*-BNI in a dose dependent manner [post-treatment main effect, $F(4, 35)=6.61$, $p<0.05$]. Specifically, both 8 and 12 mg/kg *nor*-BNI diminished the line-crosses of rats given U-50,488. This effect varied across the testing session, since 8 and 12 mg/kg *nor*-BNI attenuated line-crossing, relative to vehicle controls, across the last five time blocks [Post-treatment × Time interaction, $F(44, 385)=3.03$, $p<0.05$]. The two lowest doses of *nor*-BNI (2 and 4 mg/kg) did not suppress line-crosses relative to rats given 0.0 mg/kg *nor*-BNI ($p>0.05$).

The line-crosses of vehicle-pretreated rats can be seen in the bottom portion of Figure 3. *nor*-BNI did not affect the line-crosses of vehicle-pretreated rats during any portion of the testing session ($p>0.05$). These rats showed very little activity across the testing session.

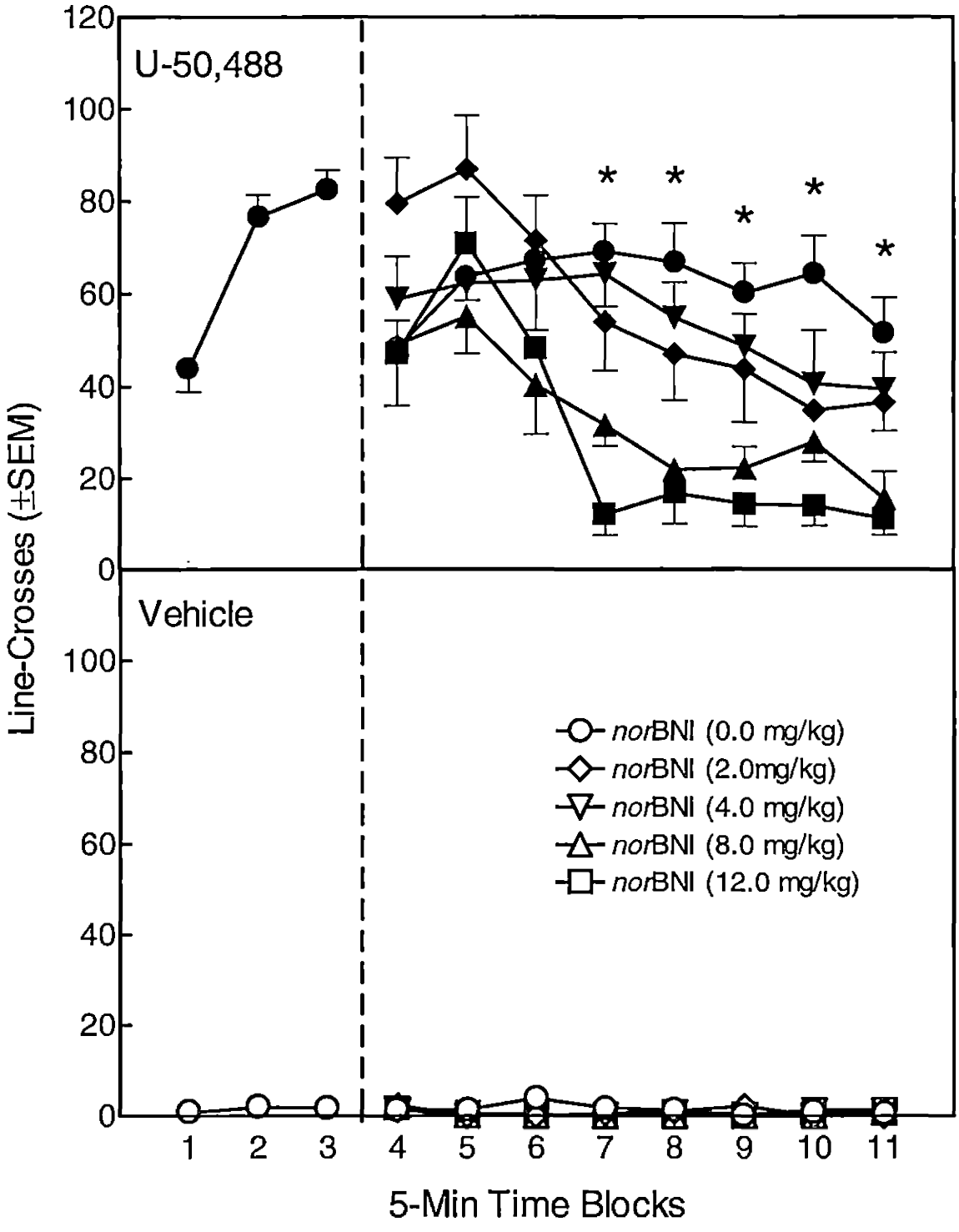

Figure 3. Mean line-cross counts (± SEM) of preweanling rats pretreated with a systemic injection of vehicle or U-50,488 and then systemically challenged with 0.0, 2.0, 4.0, 8.0, or 12.0 mg/kg *nor*-BNI. The pretreatment phase lasted for 15 min, whereas the post-treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session. * Significantly different from groups receiving 8 or 12 mg/kg *nor*-BNI ($p<0.05$).

EXPERIMENT 2

In the first experiment a systemic injection of *nor*-BNI attenuated the motor activating effects of a systemic injection of U-50,488 in the young rat. These results were not surprising, since others have demonstrated that systemically administered *nor*-BNI blocks the motor activating effects of systemic U-50,488 (McLaughlin et al., 1995). The purpose of the second experiment was to determine the neuroanatomical location where U-50,488 produces its locomotor activating effects in the young animal. It was predicted that bilateral microinjections of the kappa opioid antagonist *nor*-BNI into the substantia nigra pars reticulata would block the motor activating effects of a systemic injection of U-50,488. Additionally, it was predicted that bilateral microinjections of *nor*-BNI into the striatum would not block the motor activating effects of a systemic injection of U-50,488.

Method

To examine this, 88 (n=6-8 per group) 17-day-old rats were injected SC with U-50,488 (5 mg/kg) or vehicle and immediately placed into the testing apparatus. Fifteen minutes into the testing session, rats were bilaterally

injected (0.5 µl per side) with 0.0, 5.0, 10.0, or 20.0 µg *nor*-BNI into the substantia nigra pars reticulata or medial striatum (a vehicle pretreatment control was not included for rats receiving microinjections into the striatum). Behavior was assessed as described in Experiment 1.

Results

Substantia Nigra Pars Reticulata

Pretreatment Phase. Line-crosses for rats systemically injected with U-50,488 or vehicle are represented in the left portion of Figure 4. Overall, pretreatment with U-50,488 induced robust line-crossing in the young animals [pretreatment main effect, $F(1, 54)=88.81$, $p<0.05$]. Line-crosses varied across the pretreatment phase, since there was a progressive increase in line-crosses during the first 15 min of the testing session [Pretreatment × Time interaction F, $(2, 108)=18.29$, $p<0.05$]. Animals pretreated with U-50,488 were significantly more active, relative to vehicle controls, at each of the three time blocks (Tukey HSD, $p<0.05$). Animals pretreated with vehicle remained relatively inactive across the pretreatment phase.

Post-Treatment Phase. Line-crosses for rats challenged with intranigral injections of *nor*-BNI are represented in the right half of Figure 4. Overall, *nor*-BNI attenuated the line-crossing of the U-50,488-, but not the vehicle-, treated rats (Pretreatment × Post-treatment interaction, $F(3, 48)=36.24$, $p<0.05$). This effect varied across the testing session [Pretreatment × Post-treatment × Time interaction, $F(21, 392)=8.62$, $p<0.05$]. Because only U-50,488, and not vehicle, enhanced locomotor activity, separate 4 × 8 ANOVAs were done for each condition.

Overall, intranigral injections of *nor*-BNI had a pronounced effect on U-50,588-induced activity (see upper right portion of Figure 4). Specifically, rats pretreated with U-50,488 alone had more line-crosses than U-50,488 pretreated rats given *nor*-BNI (5.0, 10.0, or 20.0 µg) [Post-treatment main effect, $F(3, 28)=50.68$, $p<0.05$]. This effect varied across the testing session, as all groups had relatively few line-crosses during time block 4 [Post-treatment × Time interaction, $F(21, 196)=11.85$, $p<0.05$]. Rats injected with vehicle demonstrated robust line-crossing across the testing session. All three doses of *nor*-BNI (5.0, 10.0, or 20.0 µg) fully attenuated the motor

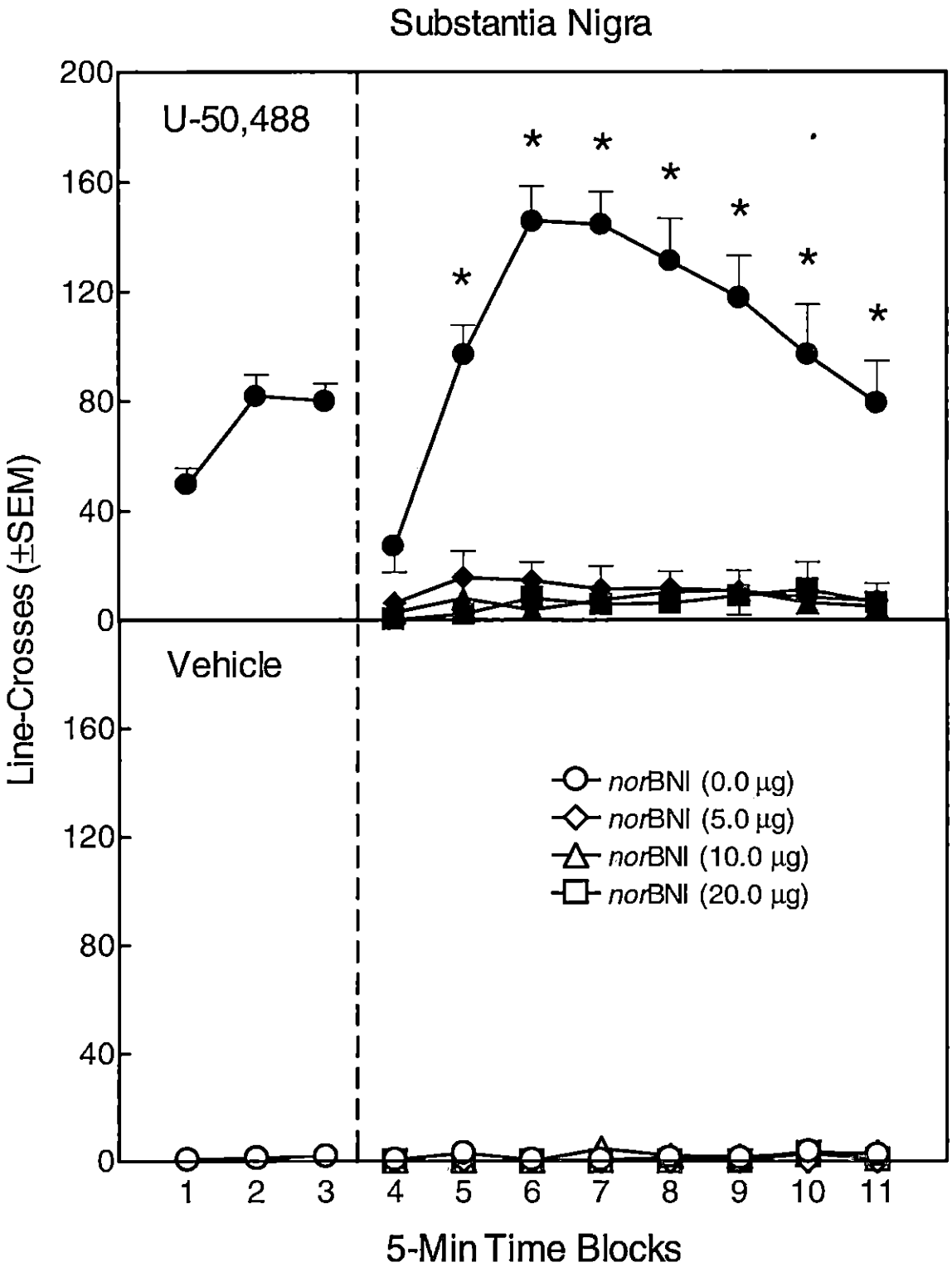

Figure 4. Mean line-cross counts (± SEM) of preweanling rats pretreated with a systemic injection of vehicle or U-50,488 and then challenged with an IC injection of vehicle or 5, 10, or 20 µg *nor*-BNI. The pretreatment phase lasted for 15 min, whereas the post-treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session. * Significantly different from all other groups ($p<0.05$).

activating effects of U-50,488 on time blocks 5-11 (Tukey HSD $p<0.05$).

The line-crosses of the vehicle pretreated rats can be seen in the bottom portion of Figure 4. *nor*-BNI (5.0, 10.0, or 20.0 µg) did not alter the line-crosses of the vehicle-pretreated rats during any portion of the testing session ($p>0.05$). These rats demonstrated little activity across the testing session.

Striatum

Pretreatment Phase. Line-crosses of rats systemically injected with U-50,488 are represented in the left portion of Figure 5 (a vehicle pretreatment group was not included in this experiment). Pretreatment with U-50,488 induced robust line-crossing in the young rats. These rats progressively increased their line-crosses across the pretreatment testing session [time main effect, $F(2,62)=30.63$, $p<0.05$].

Post-Treatment Phase. Striatal microinjections of *nor*-BNI (0.0, 5.0, 10.0, or 20.0 µg) did not significantly affect U-50,488-induced line-crossing (see right portion of Figure 5). Regardless of challenge injection, the U-50,488

pretreated rats maintained a stable level of line-crosses across the testing session.

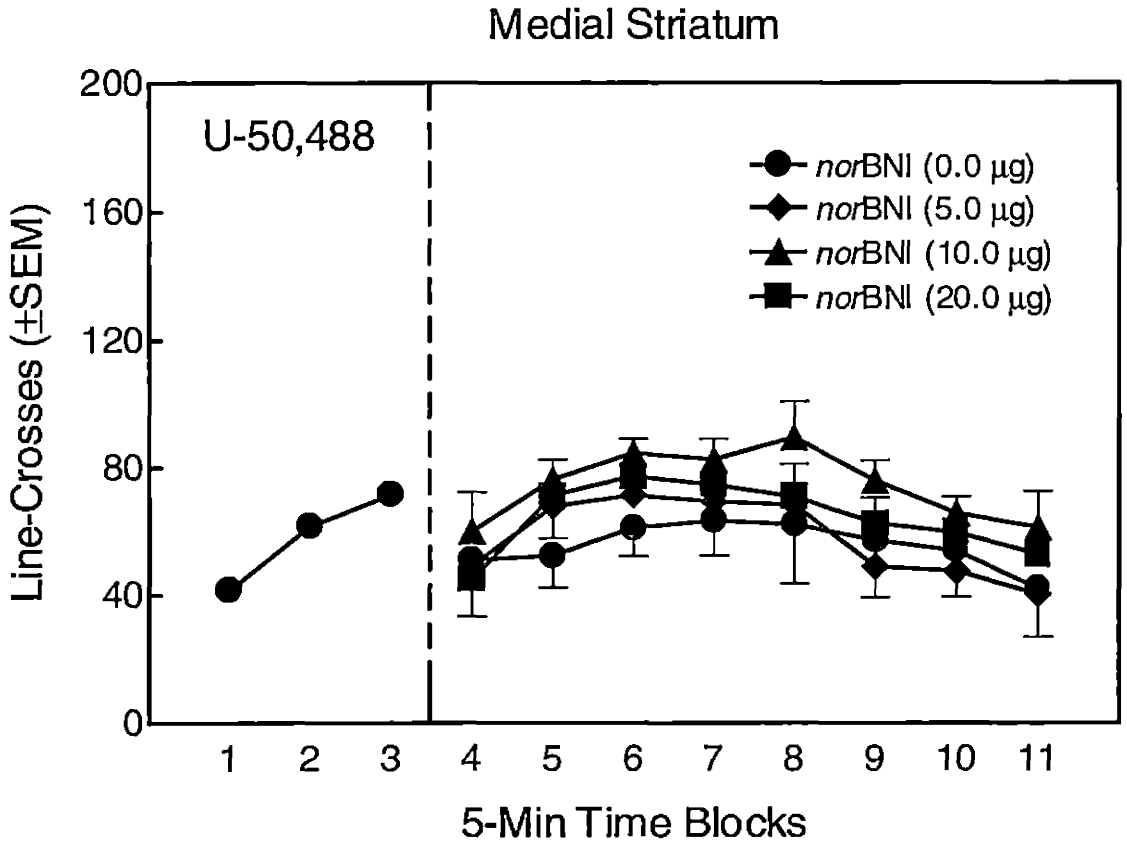

Figure 5. Mean line-cross counts (± SEM) of preweanling rats pretreated with a systemic injection of U-50,488 and then challenged with an IC injection of 0.0, 5.0, 10.0, or 20.0 µg *nor*-BNI. The pretreatment phase lasted for 15 min, whereas the post-treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session.

EXPERIMENT 3

In the second experiment, *nor*-BNI was microinjected at a volume of .5 µl per side. To ensure that this volume did not result in excessive dispersion, an additional experiment was conducted in which *nor*-BNI was administered at .25 µl per side.

Method

A total of 32 (n=8 per group) 17-day-old rats were injected SC with vehicle or U-50,488 (5 mg/kg) and immediately placed into the testing apparatus. Fifteen minutes into the testing session, rats were bilaterally injected (IC) with 0.0 or 10.0 µg *nor*-BNI. Because administering *nor*-BNI into the striatum did not affect line-crossing (see Figure 5), all microinjections were made into the substantia nigra pars reticulata. Behavior was assessed as described in Experiment 1.

Results

Pretreatment Phase

Mean line-crosses can be seen in Figure 6. Overall, animals pretreated with U-50,488 had more line-crosses than vehicle pretreated animals (see left portion of Figure 6) [pretreatment main effect, $F(1, 30)=73.54$, $p<0.05$]. This

effect varied across the pretreatment session, since U-50,488 pretreated rats had progressively more line-crosses across the three time blocks [Pretreatment × Time interaction, $F(2, 60)=15.34$, $p<0.05$]. At each of the three time blocks the U-50,488 pretreated rats were significantly more active than their vehicle controls (Tukey HSD $p<0.05$).

Post-Treatment Phase

Mean line-crosses for the post-treatment phase can be seen on the right side of Figure 6. Following the pretreatment session, animals were given bilateral injections of 0.0 or 10.0 µg *nor*-BNI (.25 µl per side) into the substantia nigra pars reticulata. Rats pretreated with U-50,488 and challenged with 10.0 µg *nor*-BNI had fewer line-crosses than rats given only U-50,488 [Pretreatment × Post-Treatment interaction, $F(1, 28)=11.10$, $p<0.05$]. These drug effects varied across the testing session, since all groups had fewer line-crosses on time block 4 [Pretreatment × Post-Treatment × Time interaction, $F(7, 196)=2.2$, $p<0.05$]. Rats pretreated with U-50,488 and challenged with 10.0 µg *nor*-BNI had fewer line-crosses than the U-50,488 control group on time blocks 5-11 (Tukey HSD $p<0.05$).

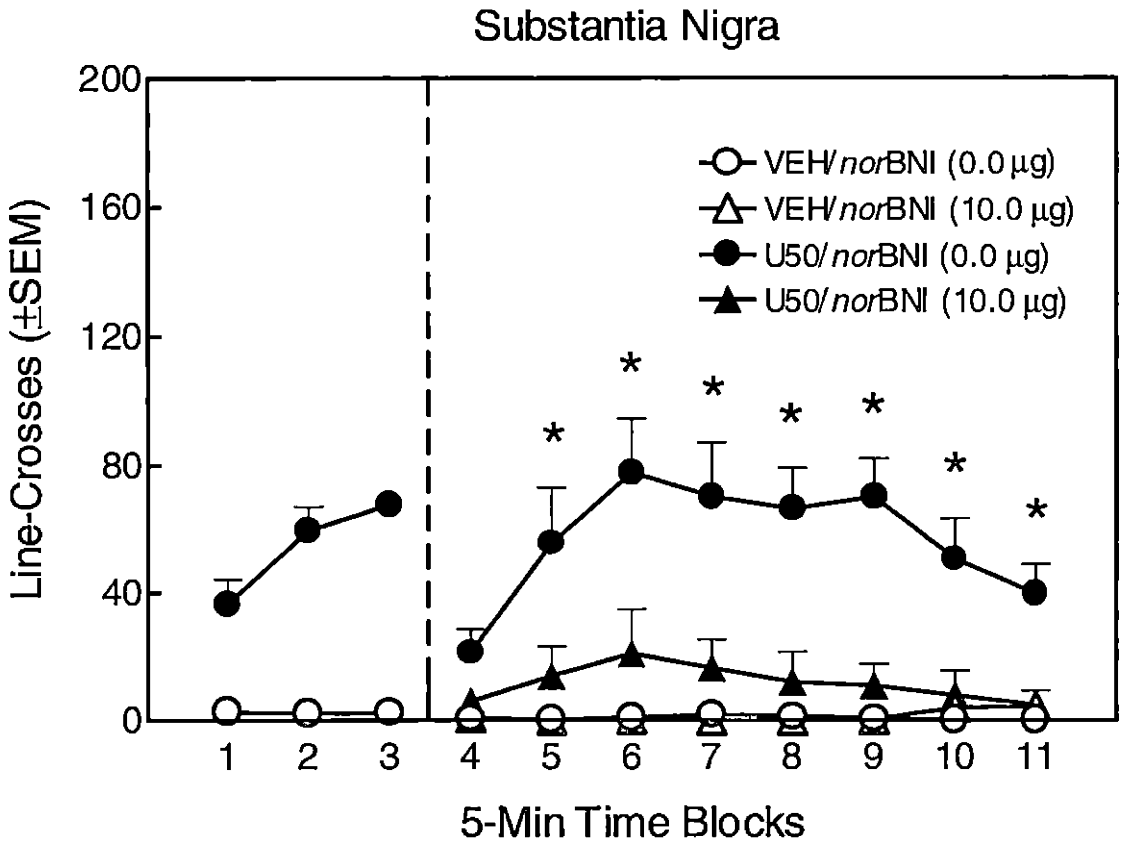

Figure 6. Mean line-cross counts (± SEM) of preweanling rats pretreated with a systemic injection of vehicle or U-50,488 and then challenged with an IC injection of 0.0 or 10 µg *nor*-BNI (0.25 µl per side). The pretreatment phase lasted for 15 min, whereas the post-treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session. * Significantly different from the Vehicle/*nor*-BNI (0.0 µg) control group ($p<0.05$).

EXPERIMENT 4

When injected systemically, U-50,488 induces robust locomotor activity in the young rat, while the same agonist suppresses locomotor activity in the adult rat. In Experiments 2 and 3, antagonizing kappa opioid receptors in the substantia nigra pars reticulata attenuated the U-50,488-induced locomotor activity of preweanling rats. The purpose of the fourth experiment was to determine whether stimulation of kappa opioid receptors in the substantia nigra pars reticulata would enhance the locomotor activity of preweanling rats. It was predicted that a microinjection of the kappa opioid agonist U-50,488 into the substantia nigra pars reticulata would increase line-crosses of preweanling rats.

Method

To examine this, 32 (n=8 per group) 17-day-old rats were bilaterally injected (IC) with 0.0, 0.125, 0.05, or 0.2 μg U-50,488 into the substantia nigra pars reticulata [similar doses of U-50,488 have been administered IC to adult rats (Matsumoto et al., 1988; Thompson & Walker, 1990; 1992)]. After being injected, rats were immediately placed into a testing chamber for 40 min. Behavior was

assessed for the final 30 min of the testing session. An observer blind to treatment conditions assessed the number of line-crosses during the testing session.

Results

A summary of the line-crossing data for rats microinjected with U-50,488 can be seen in Figure 7. U-50,488 (0.0-0.2 μg) did not significantly affect the line-crosses of preweanling rats ($p>0.05$).

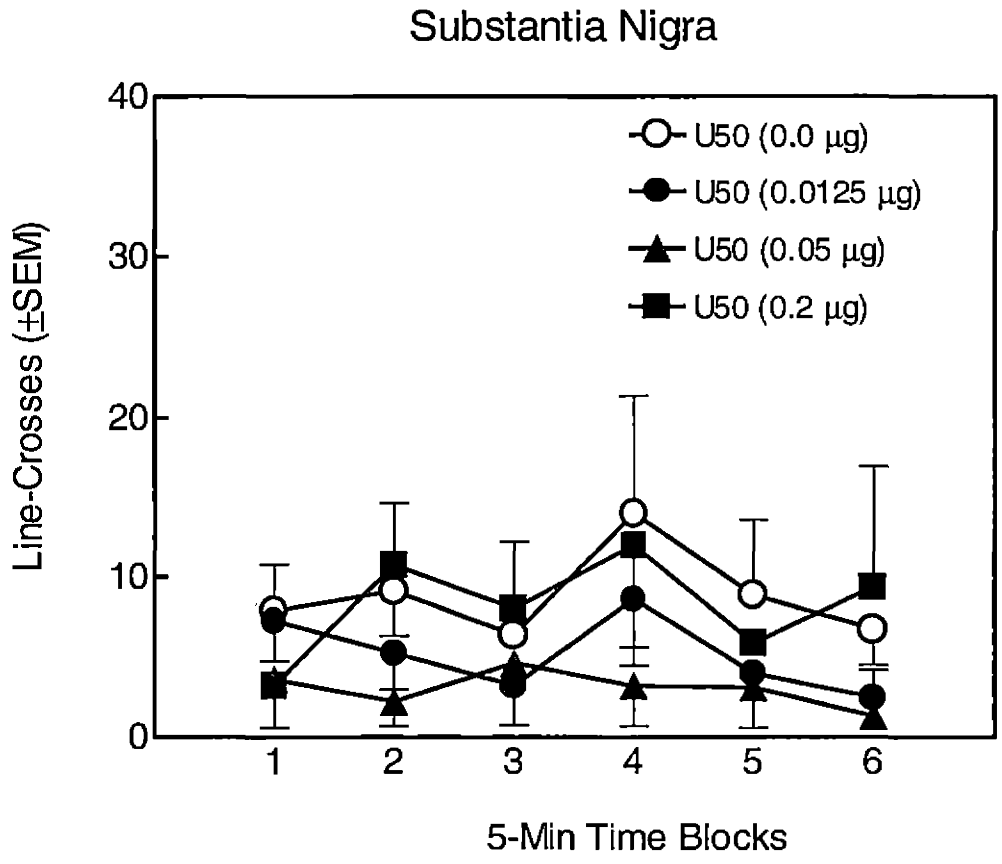

Figure 7. Mean line-cross counts (± SEM) of preweanling rats receiving an intranigral injection of 0.0, 0.125, 0.05, or 0.2 µg U-50,488. The treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session.

EXPERIMENT 5

In Experiment 4, microinjecting U-50,488 (0.0-0.2 µg) into the substantia nigra pars reticulata did not alter the line-crosses of preweanling rats. Although these doses have been shown to induce activity when microinjected into the substantia nigra pars reticulata of adult animals (Matsumoto et al., 1988; Thompson & Walker, 1990; 1992), there may be functionality and/or population differences in kappa opioid receptors in the preweanling rat (see Jackson & Kitchen, 1989; Kitchen, Kelly, & Paz Viveros, 1990; Spain et al., 1985). Thus, the dose range of U-50,488 used in Experiment 4 may not have been adequate to induce line-crossing behavior. In Experiment 5, it was predicted that microinjecting a higher dose of U-50,488 into the substantia nigra pars reticulata would induce line-crossing behavior in the young animal.

Method

To examine this, 32 (n=8 per group) 17-day-old rats were bilaterally injected (IC) with 0.0, 0.8, 1.6, or 3.2 µg U-50,488 into the substantia nigra pars reticulata. After being injected, rats were immediately placed into a testing chamber for 40 min. Behavior was assessed for the final 30

min of the testing session. An observer blind to treatment conditions assessed the number of line-crosses during the testing session.

Results

The line-cross data for preweanling rats microinjected with U-50,488 (0.0, 0.8, 1.6, or 3.2 µg) can be seen in Figure 8. Overall, animals microinjected with 1.6 and 3.2 µg U-50,488 had more line crosses than animals treated with 0.0 µg U-50,488 [drug main effect, $F(3, 28)=7.47$, $p<0.05$]. This effect varied across the testing session, as all groups of rats had relatively few line-crosses on time block 1 [Drug × Time interaction, $F(15, 140)=1.93$, $p<0.05$]. Rats given the highest dose of U-50,488 (3.2 µg) showed an increase in line-crosses from the first to the second time block, at which point line-crosses stabilized. Rats given 3.2 µg U-50,488 had more line-crosses than animals receiving 0.0 or 0.8 µg U-50,488 on time block 2, and more line-crosses than all other groups on time blocks 3-6 (Tukey HSD $p<0.05$). Rats microinjected with 0.0 or 0.8 µg U-50,488 were relatively inactive across the testing session.

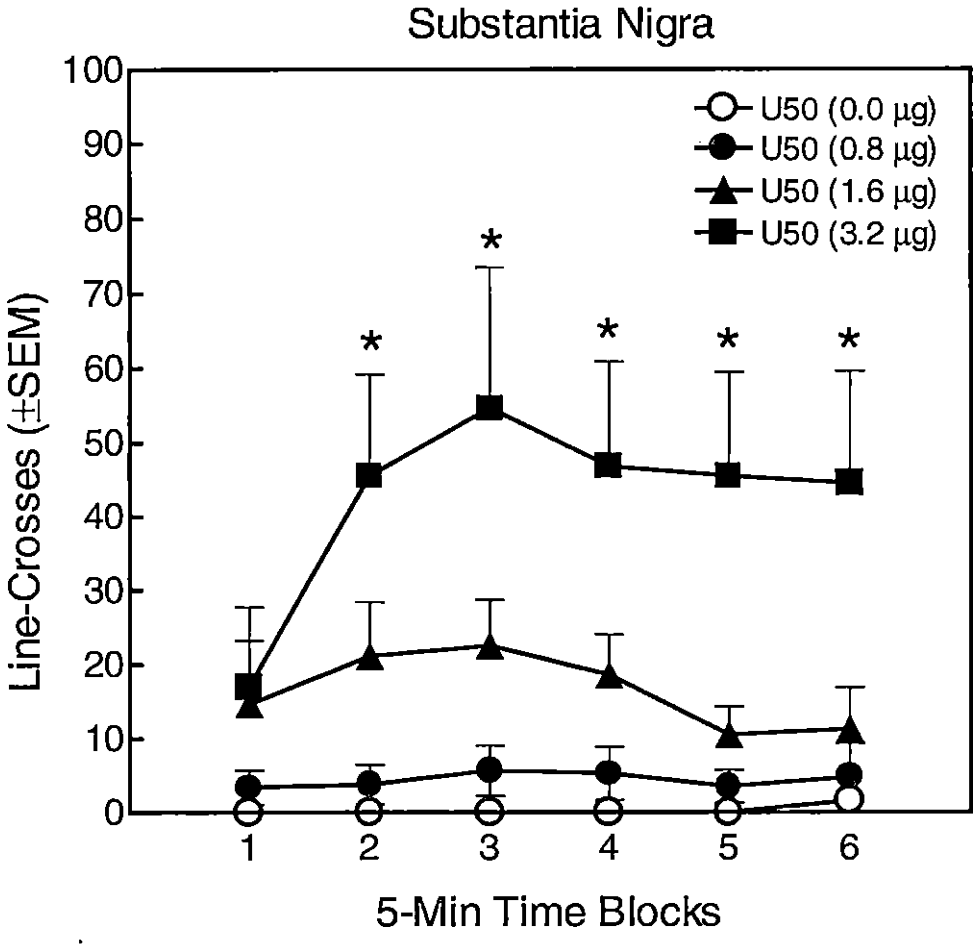

Figure 8. Mean line-cross counts (± SEM) of preweanling rats receiving an intranigral injection of 0.0, 0.8, 1.6, or 3.2 µg U-50,488. The treatment phase lasted for 40 min. Line-cross activity was measured continuously across the 55 min testing session. * Significantly different from the 0.0 µg U-50,488 control group ($p<0.05$).

DISCUSSION

The purpose of the present study was to determine the neuroanatomical site where U-50,488 induces locomotor activity in the preweanling rat. Since directly activating kappa opioid receptors in the substantia nigra pars reticulata of the adult rat induces locomotor activity, it was hypothesized that this same brain region mediates the locomotor activating effects of systemically administered U-50,488 in the preweanling rat.

In the first experiment the motor activating effects of systemically administered U-50,488 were attenuated by systemically administered *nor*-BNI. This was not surprising, since others have shown that a systemic injection of 4.5 mg/kg *nor*-BNI blocks the motor activating effects of systemically administered U-50,488 (0.3 mg/kg) and enadoline at PD 3 (McLaughlin et al., 1995). Moreover, at PD 5 the locomotor activating effects of U-50,488 (10 mg/kg) are blocked by the nonselective opioid antagonist naltrexone, but not by the delta antagonist ICI 174,864 or the opioid antagonist M8008 [an opioid antagonist with a low affinity for kappa receptors] (Jackson & Kitchen, 1989). This strongly suggests that antagonizing kappa

opioid receptors attenuates the motor activating effects of systemically administered U-50,488.

U-50,488 has been shown to dramatically increase the locomotor activity of preweanling rats (Bolanos et al., 1996; Collins et al., 1998; Duke et al., 1997; Jackson & Kitchen, 1989; Kehoe & Boylan, 1994; McDougall et al., 1997). This was also shown in the present study using systemic and intracranial administration: Rats receiving U-50,488 via either subcutaneous (i.e., Experiments 1, 2 and 3) or intranigral (i.e., Experiment 5) injection demonstrated robust line-crossing activity. In the second experiment, however, there was a baseline shift in U-50,488-induced line-crosses. More specifically, rats pretreated with U-50,488 and given an intranigral injection of 0.0 *nor*-BNI (i.e., distilled water) averaged about 150 line-crosses on time blocks 6 and 7 (see Figure 4). In the other experiments, locomotor activity was not as robust, since mean line-crosses ranged from 60-80 for the majority of the testing sessions (see Figures 3, 5, 6, and 8). It is not known why activity was more robust in the second experiment.

Collectively, the results from Experiments 2, 3, and 5 showed that the U-50,488-induced locomotor activity of

preweanling rats is mediated through actions in the substantia nigra pars reticulata. More specifically, microinjecting U-50,488 (1.6 and 3.2 μg) into the substantia nigra pars reticulata of the preweanling rat induced locomotor activity (see Figure 8). Moreover, microinjecting *nor*-BNI into the same brain region attenuated the motor activating effects of systemically administered U-50,488 (see Figures 4 and 6). In contrast, microinjecting *nor*-BNI into the medial striatum did not attenuate the motor activating properties of systemically administered U-50,488 (see Figure 5). That antagonizing kappa receptors in the substantia nigra pars reticulata, and not the medial striatum, attenuated the motor activating effects of systemically administered U-50,488 is central to this thesis, since systemically administered U-50,488 is free to bind with kappa receptors in other brain regions. When these results are considered together, it appears that kappa opioid receptors in the substantia nigra pars reticulata mediate U-50,488-induced activity in the preweanling rat.

There are several alternatives which can be used to explain why systemically administered U-50,488 enhances the

locomotor activity of preweanling rats, but not adults. For example, systemically administered U-50,488 may enhance the locomotor activity of preweanling rats by: (a) directly stimulating a dopaminergic mechanism (Carden, Barr & Hofer, 1991; Kehoe & Boylan, 1994; Duke et al., 1997); (b) producing an aversive state that triggers a locomotor escape response (Barr et al., 1994); or (c), by acting on a population of kappa opioid receptors that are regionally different early in development (Jackson & Cooper, 1988). While the purpose of this thesis was to determine the neuroanatomical location where U-50,488 induces locomotor activity in the preweanling rat, and not to define the precise mechanism, the findings of this study are consistent with all of the proposed models.

As just mentioned, one possibility is that U-50,488 induces locomotor activity in the preweanling rat by directly stimulating a dopaminergic mechanism. Various studies have shown that the dopamine and kappa opioid systems interact, but it appears that the nature of this interaction varies across ontogeny. For example, the D_1 receptor antagonist SCH 23390 fails to inhibit the motor activating effects of U-50,488 in the fetal rat, but in these same animals U-50,488- and SKF 38393-induced activity

are blocked by *nor*-BNI (Smotherman et al., 1993). Thus, during the fetal period the kappa opioid system mediates dopamine's behavioral effects in a unidirectional manner (i.e., kappa systems affect dopamine systems). In the 17-day-old rat, however, the non-selective dopamine receptor agonist NPA and the non-selective dopamine antagonist flupenthixol attenuate U-50,488 induced activity, whereas U-50,488 attenuates NPA induced sniffing (Duke et al., 1997). Thus, during the preweanling period there seems to be a complex reciprocal interaction between the kappa opioid and dopamine systems. Only after the preweanling period does the kappa-dopamine interaction become adult-like, since U-50,488 treatment in adult animals causes a unidirectional inhibition of dopamine stimulated activity (opposite of the fetal period).

In the present study, however, the results indicate that U-50,488 induces locomotor activity in the preweanling rat independent of a direct kappa-dopamine interaction. This becomes clear when two issues are considered. First, microinjecting *nor*-BNI into the substantia nigra pars reticulata, compared to microinjections into the striatum, attenuated the motor activating effects of systemically administered U-50,488. Second, directly microinjecting U-

50,488 into the substantia nigra pars reticulata increased locomotor activity. Only direct manipulation of kappa receptors in the substantia nigra altered locomotor activity in the preweanling rat. It may be possible, though, that the dopaminergic system is activated "downstream" from initial kappa activation in the substantia nigra pars reticulata (e.g., a kappa–GABA-dopaminergic interaction). This was not assessed in the present thesis.

A second possibility is that U-50,488 indirectly induces locomotor activity in the preweanling rat by producing an aversive state that results in an escape response (i.e., an ontogenetic defense reaction) (Barr et al., 1994). Although U-50,488 induces age-dependent differences in locomotor activity, U-50,488 produces an aversive state in both preweanling and adult rats (Bals-Kubic et al., 1989; Barr et al., 1994; Carden et al., 1991; Mucha & Herz, 1985). Thus, increased locomotor activity in the preweanling rat can be explained as a mechanism to escape this aversive state (Barr et al., 1994). Consistent with this idea, preweanling rats (PD 3 and 7) tested in an odor preference/aversion paradigm will avoid a compartment previously paired with U-50,488 and decrease their activity

(Barr et al., 1994). However, assessing activity through an odor preference/aversion paradigm is complex, since preference/aversion is tested in a drug free state. A decrease in activity using a preference/aversion paradigm may indicate: (a) that U-50,488-induced locomotor activity occurs as a response to an aversive state, or (b) it may indicate that U-50,488-induced locomotor activity and U-50,488-induced aversion are mediated through different brain regions. Given the role of the substantia nigra in initiating movement (present study; Hauber, 1998), and the role of the periaqueductal grey in mediating vocalizations and defense reactions (Jürgens, 1994; Lovick, 1993), the second possibility seems more likely. This was not assessed in the present study, however, since only locomotor activity, and not defense reactions, were measured following microinjection of U-50,488 and *nor*-BNI into the substantia nigra and striatum.

Alternatively, the age-dependent differences in U-50,488-induced locomotor activity may be due to regional differences in a population of kappa opioid receptors. In the adult rat, systemically administered U-50,488 presynaptically inhibits the release of dopamine in the nucleus accumbens and there is a corresponding decrease in

locomotor activity (Di Chiara & Imperato, 1998; Spanagel et al., 1992). However, microinjecting U-50,488 into the substantia nigra pars reticulata of adult animals releases thalamic motor areas from GABAergic inhibition and induces motor activity (Hauber, 1998; Matsumoto et al., 1988; Thompson & Walker, 1990; 1992). Presumably, kappa receptor mediated inhibition of the mesolimbic dopamine pathway masks the locomotor activating effects of substantia nigra pars reticulata stimulation in the adult rat. In the preweanling rat, however, kappa receptors in the substantia nigra pars reticulata may become functionally mature before kappa receptors in the mesolimbic pathway (or exist in greater numbers). This may explain why systemically administered U-50,488 induces activity in preweanling rats (present study), but suppresses the activity of adult rats (Collins et al., 1998; Duke et al., 1997; Jackson & Cooper, 1988; Leyton & Stewart, 1992; McLaughlin et al., 1995).

The ontogeny of kappa opioid receptors in the rat has been studied (Georges, Normand, Bloch, & LeMoine, 1998; Kitchen et al., 1990; Kornblum, Hurlbut & Leslie, 1987; Spain et al., 1985). At birth, kappa receptors are found in similar densities in both forebrain and hindbrain regions; however, after PD 1, kappa receptor densities in

the hindbrain increase, whereas densities in the forebrain decrease (see Kitchen et al., 1990; Spain et al., 1985). After the second postnatal week the reverse occurs, with the relative density of forebrain kappa opioid receptors increasing and hindbrain receptors decreasing. This reversal is probably due to the maturation of the cerebellum, which contains few kappa sites (Spain et al., 1985).

It is uncertain whether U-50,488-induced activity in the preweanling rat occurs as a result of regional differences in kappa opioid receptor population, functionality, or a combination of the two. Since κ_1-, and not κ_2-, opioid receptor subtypes are found in the substantia nigra pars reticulata of the adult rat (Mansour et al., 1996; Fowler & Fraser, 1994), and since U-50,488 preferentially binds to κ_1 opioid receptors (Fowler & Fraser, 1994), it is possible that regional differences in κ_1 receptors are responsible for the age-dependent behavioral differences caused by U-50,488. For example, U-50,488-induced locomotor activity in the preweanling rat may occur as a result of kappa opioid receptors in the substantia nigra pars reticulata becoming functionally

mature before kappa opioid receptors in the striatum. Likewise, there may be a transient, regional overabundance of nigral κ_1 opioid receptors early in development which could account for these behavioral differences. Unfortunately, no studies have assessed regional changes in κ_1 opioid receptors across ontogeny, so the relationship between regional population differences and functionality of kappa opioid receptors and U-50,488-induced locomotor activity in the preweanling rat is uncertain.

Conclusion

The present study clearly demonstrates that systemic administration of U-50,488 induces locomotor activity in the preweanling rat through actions in the substantia nigra pars reticulata. This is supported by results showing (a) direct injection of U-50,488 into the substantia nigra pars reticulata induced locomotor activity, and (b) direct injection of *nor*-BNI into the substantia nigra pars reticulata attenuated the locomotor activating effects of systemically administered U-50,488. *nor*-BNI did not attenuate U-50,488 induced locomotor activity when directly injected into the medial striatum. It is likely that the age-dependent motor response in the preweanling rat

following systemic administration of U-50,488 is due to an indirect kappa-dopaminergic interaction, a locomotor response to an aversive state, or κ_1 receptors in the substantia nigra pars reticulata becoming functionally mature, or existing in greater numbers, than κ_1 receptors in the mesolimbic pathway.

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