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The effects of lesions to the superior colliculus and ventromedial thalamus on [kappa]-opioid-mediated locomotor activity in the preweanling rat

Arturo Rubin Zavala

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THE EFFECTS OF LESIONS TO THE SUPERIOR COLLICULUS AND VENTROMEDIAL THALAMUS ON κ-OPIOID-MEDIATED LOCOMOTOR ACTIVITY IN THE PREWEANLING RAT

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree Master of Arts in Psychology

by
Arturo Rubin Zavala

March 2003
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ABSTRACT

The purpose of the present study was to determine the neuronal circuitry responsible for \( \kappa \)-opioid-mediated locomotion in preweanling rats. In two separate experiments, 16-day-old rats were given bilateral electrolytic lesions of the ventromedial thalamus and superior colliculus and, two days later [i.e., postnatal day (PD) 18] locomotor activity was assessed after a systemic injection of the \( \kappa \)-opioid receptor agonist U50,488. To ensure that lesions to the ventromedial thalamus or superior colliculus did not produce a general disruption of non-opioid motor systems, the same rats were retested on PD 19 and given a systemic injection of the dopamine receptor agonist \( R(-) \)-propylnorapomorphine (NPA). It was hypothesized that bilateral lesions of the ventromedial thalamus or superior colliculus would attenuate U50,488-induced locomotor activity, while having no effect on NPA-induced locomotor activity. As predicted, lesions to the ventromedial thalamus and superior colliculus partially blocked U50,488's locomotor activating effects. Importantly, these same lesions failed to disrupt the locomotor activity produced by NPA. A third experiment was
conducted to determine whether lesioning unrelated brain structures would also attenuate U50,488-induced locomotion. For Experiment 3, the nucleus accumbens was lesioned because it was expected that electrolytic lesions of this brain region would not attenuate U50,488-induced locomotor activity, while disrupting NPA-induced locomotion. The results of Experiment 3 were surprising, because: 1) nucleus accumbens lesions attenuated the locomotor activity of U50,488-treated rats; and, 2) NPA-induced locomotor activity was unaffected by nucleus accumbens lesions. It is not clear why lesions to the nucleus accumbens affected κ-opioid-mediated locomotor activity, but one possibility is that the nucleus accumbens is a component of the neural circuitry mediating U50,488-induced locomotion. Therefore, when these experiments are considered as a whole, this is the first study to demonstrate that the nigrothalamic and nigrotectal pathways mediate, at least partially, the U50,488-induced locomotor activity of preweanling rats.
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CHAPTER ONE
BASAL GANGLIA

Relevance.

The basal ganglia is a network of interconnected regions in the brain that form a complex neuronal system mediating a variety of behaviors, including motor movement, cognition, and emotional functioning (Hauber, 1998). Dysfunctions within the basal ganglia have been linked to a number of psychiatric and neurological disorders. For instance, Parkinson’s disease, characterized by difficulty in initiating movements, tremors, and rigidity, is associated with degeneration of the nigrostriatal dopamine pathway (Carlsson, Lindqvist, & Magnusson, 1957; Cotzias, Papavasiliou, & Gellene, 1969; Fahn, 1989; Obeso, Olanow, & Nutt, 2000). Basal ganglia dysfunction has also been implicated in Huntington’s disease (Haddad & Cummings, 1997), obsessive-compulsive disorder (Miguel, Rauch, & Jenike, 1997), tic disorder (Leckman, Peterson, Pauls, & Cohen, 1997), dystonia (Cardoso & Jankovic, 1997), and dyskenisia (Cardoso & Jankovic, 1997).

Despite the fact that the neurological bases for some of these disorders are well known (e.g., Parkinson’s
disease), many disorders resulting from basal ganglia dysfunction are poorly understood. Determining how the basal ganglia functions is a first step towards understanding the etiology of these disorders. Moreover, a basic knowledge of basal ganglia anatomy is important for developing potential pharmacotherapies. The goal of this project is to characterize further how κ-opioid receptors in the basal ganglia modulate motor functioning, particularly during early development.

Basal Ganglia Anatomy

Overview

The basal ganglia consists of a set of interconnected subcortical nuclei (see Figure 1) found in the telencephalon, diencephalon, and midbrain (Albin, Young, & Penney, 1989). The main input structure of the basal ganglia is the striatum (corresponding to the caudate nucleus and putamen in humans), which receives massive glutaminergic (i.e., excitatory) afferent projections from all over the cortex (Ferino, Thierry, Saffroy, & Glowinski, 1987; Parent, 1990; Wilson, 1987). The striatum, in turn, sends projections to the substantia nigra, the main output nuclei of the basal ganglia, via a direct (striatonigral)
pathway and an indirect (striatopallidal) pathway (Gerfen, 1992b; Smith, Bevan, Shink, & Bolam, 1998). The substantia nigra then sends its own output projections to the thalamus, superior colliculus, and pedunculopontine nucleus (Gerfen, 1992a; Gerfen, Staines, Arbuthnott, & Fibiger, 1982; Parent & Hazrati, 1995).

The three target projections of the substantia nigra pars reticulata (i.e., the thalamus, superior colliculus, and pedunculopontine nucleus) send excitatory (i.e., glutaminergic) projections to various brain regions. First, the thalamus sends an excitatory projection back to the cortex and striatum (Mengual, de las Heras, Erro, Lanciego, & Gimenez-Amaya, 1999). Second, the superior colliculus transmits an excitatory projection to the thalamus and cortex (Benevento & Fallon, 1975; Binns, 1999; Harting, Huerta, Frankfurter, Strominger, & Royce, 1980). Third, the pedunculopontine nucleus sends a feedback projection to the substantia nigra pars compacta (Charara, Smith, & Parent, 1996) and the subthalamic nucleus (Bevan & Bolam, 1995; Lavoie & Parent, 1994).

Therefore, it should be clear why the organization of the basal ganglia has been described in terms of parallel loopings, in which distinct circuitries are formed between
Figure 1. Major efferent and afferent pathways of the basal ganglia. The striatum receives input from all over the cortex and sends projections to the substantia nigra, via a direct pathway and an indirect pathway.
the cortex, striatum, substantia nigra, and thalamic output systems (Alexander & Crutcher, 1990; Alexander, DeLong, & Strick, 1986). Specifically, discrete cortical information is cycled through regions of the basal ganglia and sent back, via the thalamus, to discrete cortical areas, thereby forming a circular loop between the cortex and the basal ganglia. Five such parallel circuitries have been identified: 1) motor; 2) oculomotor; 3) dorsolateral prefrontal; 4) lateral orbitofrontal; and 5) anterior cingulate (Alexander & Crutcher, 1990; Alexander et al., 1986). Different functional roles are thought to be associated with each of these circuitries, and they collectively comprise the diversity of behaviors associated with the basal ganglia.

In summary, the basal ganglia is a complex network of interconnected brain regions that receives massive input from all over the cortex. The major input structure is the striatum, which transmits cortical information via a direct and indirect pathway down to the substantia nigra pars reticulata, the main output structure of the basal ganglia. Information is then relayed through the thalamus to several regions of the cortex, thereby completing the cortical loop.
Striatonigral and Striatopallidal Pathways

Excitatory input from the cortex is transmitted to the substantia nigra pars reticulata through a direct (striatonigral) pathway and an indirect (striatopallidal) pathway (Albin et al., 1989; Gerfen, 1992b). In the direct pathway, corticostriatal input is transmitted directly to the substantia nigra pars reticulata (see Figure 1). Neurons which provide this input are GABAergic, but also release two neuropeptides: dynorphin and substance P (Gerfen & Young, 1988; Smith et al., 1998; Steiner & Gerfen, 1998).

In contrast, the indirect pathway sends cortical information to the substantia nigra pars reticulata via an array of interconnections involving the globus pallidus and subthalamic nucleus (see Figure 1) (Smith et al., 1998). GABAergic neurons originating in the striatum, which express and release enkephalin and substance P (Gerfen & Young, 1988; Steiner & Gerfen, 1998), first provide corticostriatal input to the globus pallidus. The globus pallidus then sends an inhibitory projection to the subthalamic nucleus. Two output pathways leave the subthalamic nucleus: an excitatory feedback circuit to the
globus pallidus and an excitatory connection to the substantia nigra pars reticulata.

Different dopamine receptors are associated with the two striatal projections to the substantia nigra pars reticulata (i.e., the direct and indirect pathways). Striatonigral GABAergic neurons generally express dopamine D1 receptors, whereas striatopallidal GABAergic neurons express dopamine D2 receptors (Gerfen, 1992b; Gerfen, Engber, Mahan, Susel, Chase, Monsma, & Sibley, 1990; Steiner & Gerfen, 1998). Only a small number of striatonigral and striatopallidal neurons express both dopamine receptor subtypes (Steiner & Gerfen, 1998).

**Nigrosectal and Nigrothalamic Pathways**

The opioid rich substantia nigra pars reticulata serves as the main output structure for the basal ganglia. There are three prominent output pathways exiting the substantia nigra pars reticulata, and they project to the superior colliculus (nigrosectal pathway), thalamus (nigrothalamic pathway), and pedunculopontine nucleus (nigropedunculopontine pathway) (see Figure 1) (Deniau & Chevalier, 1992; Gerfen, 1992b; Steiner & Gerfen, 1998). Each of these output pathways are inhibitory, because the neurons comprising these connections release GABA. In
fact, the classic view of basal ganglia functioning is that these target regions are under tonic inhibition from the substantia nigra pars reticulata and that the loss of this tonic inhibition results in basal ganglia-mediated behavior (Albin et al., 1989; Chevalier & Deniau, 1990). For instance, unilateral microinjections of GABA into the substantia nigra pars reticulata produces contralateral circling, presumably by inhibiting the inhibitory output pathways of the substantia nigra pars reticulata, resulting in a disinhibition of the superior colliculus and ventromedial thalamus (Kaakkola & Kaariainen, 1980; Kamata, Kameyama, Okuyama, Hashimoto, & Aihara, 1985).

**Nigrostriatal Pathway**

One of the major ascending dopaminergic projections in the brain originates in the substantia nigra pars compacta and terminates in the striatum (Cooper, Bloom, & Roth, 2003; Joel & Weiner, 2000). Interestingly, dopaminergic input via the nigrostriatal tract differentially affects the direct and indirect efferent pathways of the striatum (see Figure 1). Specifically, dopaminergic input excites the striatonigral (i.e., direct) pathway, and inhibits the striatopallidal (i.e., indirect) pathway (Alexander & Crutcher, 1990; Gerfen & Young, 1988). Thus, the role of
dopamine within the striatum may be to strengthen cortical input to the striatum by inhibiting the direct pathway and facilitating the indirect pathway (Alexander & Crutcher, 1990).

The κ-opioid receptor system modulates the activity of dopamine neurons comprising the nigrostriatal pathway. For example, κ-opioid receptor stimulation in the substantia nigra pars compacta decreases the firing rates of dopamine neurons, thus attenuating dopamine release in the striatum (Walker, Thompson, Frascella, & Friederich, 1987). Similarly, at the terminal region of the nigrostriatal pathway (i.e., the striatum), stimulation of presynaptic κ-opioid receptors inhibits the release of dopamine (Di Chiara & Imperato, 1988; Werling, Frattali, Portoghese, Takemori, & Cox, 1988; Zaratin & Clarke, 1994). In summary, dopaminergic activity within the nigrostriatal pathway partially mediates motor functioning, and it is clear that κ-opioid receptors have a significant role in modulating this effect.

**Mesocortical and Mesolimbic Pathways**

The second of the major ascending dopaminergic pathways originates in the ventral tegmental area (Oades &
Halliday, 1987). These cell bodies send prominent projections to cortical and limbic regions (Cooper et al., 2003). The mesocortical pathway is comprised of dopamine fibers projecting to various cortical structures, including the medial prefrontal cortex, cingulate cortex, and entorhinal areas. In contrast, projections to various limbic structures, such as the most ventral part of the striatum (i.e., nucleus accumbens) and amygdala, constitute the mesolimbic pathway.

Dopaminergic activity within the mesolimbic pathway is affected by μ- and κ-opioid receptor activity (see Figure 2). For instance, stimulating μ-opioid receptors in the ventral tegmental area has the effect of inhibiting GABAergic neurons that tonically inhibit dopamine projections to the striatum (Di Chiara & Imperato, 1988; Spanagel, Herz, & Shippenberg, 1990; 1992). In other words, μ-opioid agonists disinhibit neurons of the mesolimbic pathway by inhibiting GABAergic activity within the ventral tegmental area. In contrast, κ-opioid receptor stimulation inhibits dopaminergic activity. Specifically, κ-opioid receptor agonists decrease dopaminergic functioning in the nucleus accumbens by directly inhibiting dopamine
Figure 2. Dopaminergic activity of the mesolimbic pathway is modulated by μ- and κ-opioid receptor stimulation. Increased μ-opioid activity increases dopamine release in the nucleus accumbens by inhibiting GABAergic activity, whereas increased κ-opioid stimulation decreases dopamine release in the nucleus accumbens.

release at the terminal button (Spanagel et al., 1990; 1992). When considered together, it is clear that μ- and κ-opioid receptor activity has opposing actions on the mesolimbic pathway, with μ-opioid receptor stimulation
indirectly enhancing neurotransmission, while κ-opioid receptor stimulation inhibits dopamine release within the nucleus accumbens.
CHAPTER TWO

κ-OPIOID RECEPTOR SYSTEMS

Overview

κ-Opioid receptor systems are involved in regulating motor control within the basal ganglia. Dynorphin peptides, the endogenous ligands that primarily bind to κ-opioid receptors (Chavkin, James, & Goldstein, 1982; Corbett, Paterson, McKnight, Magnan, & Kosterlitz, 1982), are present in high concentrations in the substantia nigra pars reticulata (Zamir, Palkovits, & Brownstein, 1983; 1984). Dynorphin peptides are synthesized in cell bodies located in the striatum and are ultimately released from terminal fibers in the substantia nigra. These peptides serve as neuromodulators, because dynorphin, applied locally into the substantia nigra pars reticulata, modulates the activity of GABAergic cells projecting to the ventromedial thalamus and superior colliculus (Lavin & Garcia-Munoz, 1985; Robertson, Hommer, & Skirboll, 1987). Dynorphin-induced modulation of these GABAergic output pathways produces robust contralateral circling in adult rats (Friederich, Friederich, & Walker, 1987; Matsumoto, Lohof, Patrick, & Walker, 1988b; Morelli & Di Chiara,
1985). Hence, it is clear that κ-opioid receptor systems have an important role in regulating motor control.

κ-Opioid Receptor Classification

Over the last two decades the presence and specificity of κ-opioid receptors have been determined. Specifically, the existence of three κ-opioid receptors have been established: κ1, κ2, and, more recently, κ3 (Cheng, Roques, Gacel, Huang, & Pasternak, 1992; Clark, Liu, Price, Hersh, Edelson, & Pasternak, 1989; Fowler & Fraser, 1994; Leslie & Loughlin, 1994; Mansour, Burke, Pavlic, Akil, & Watson, 1996; Mansour, Fox, Akil, & Watson, 1995). The κ1-opioid receptor subtype has a high affinity for the endogenous peptide dynorphin A and exogenous arylacetamide drugs such as U50,488 and U69,593 (Leslie & Loughlin, 1994; Unterwald, Knapp, & Zukin, 1991). The κ2-opioid receptor has considerable affinity for dynorphin A and other endogenous opioid peptides (e.g., β-endorphin), as well as for exogenous compounds such as diprenorphine, ethylketocyclazocine, and bremazocine (Leslie & Loughlin, 1994). Because the κ3-opioid receptor was discovered recently, less is known about the affinity of endogenous
peptides for this receptor subtype. However, the exogenous compound naloxone benzoylhydrazone has been identified as having a high affinity for \( \kappa_3 \)-opioid receptors (Cheng et al., 1992; Clark et al., 1989).

\textbf{\( \kappa \)-Opioid Receptor Distribution}

\textbf{Adult Brain}

Localization of \( \kappa_1 \)- and \( \kappa_2 \)-opioid receptors in the adult rat brain has been well established, with \( \kappa_2 \)-opioid receptors being more abundant than \( \kappa_1 \)-opioid receptors (Unterwald et al., 1991; Zukin, Eghbali, Olive, Unterwald, & Tempel, 1988). \( \kappa_1 \)-opioid receptors are found in the caudate-putamen (i.e., striatum), nucleus accumbens, medial nucleus of the thalamus, superior colliculus, substantia nigra pars reticulata, ventral tegmental area, and hypothalamus (Mansour et al., 1996; Unterwald et al., 1991). \( \kappa_2 \)-opioid receptors are found throughout the brain, including several regions of the thalamus, inferior colliculus, amygdala, olfactory tubercles, endopiriform nucleus, claustrum, substantia nigra, nucleus accumbens, and striatum (Unterwald et al., 1991; Zukin et al., 1988). Relatively few studies have examined the distribution of \( \kappa_3 \)-
opioid receptors, although binding sites for this receptor have been recognized in the hypothalamus, thalamus, striatum, and midbrain (Cheng et al., 1992).

**Ontogeny**

The ontogenesis of κ-opioid receptors has been examined, although the development of individual κ-opioid receptor subtypes has received minimal empirical study (but see Allerton, Smith, Hunter, Hill, & Hughes, 1989; Kitchen, Kelly, & Viveros, 1990). κ-Opioid receptors begin to emerge in rat brain as early as embryonic day (ED) 14.5 and continue to increase in density until ED 18.5, where they remain stable until birth (Leslie & Loughlin, 1994). After birth, the developmental pattern of κ-opioid receptors is more controversial. For example, one study has reported that adult-like κ-opioid receptor concentrations are attained sometime between postnatal day (PD) 7 and PD 14 (Petrillo, Tavani, Verotta, Robson, & Kosterlitz, 1987). In contrast, Spain and colleagues report a more complex pattern of development, as they found that κ-opioid receptor densities simultaneously increase in hindbrain and decrease in forebrain across the first postnatal week (Spain, Roth, & Coscia, 1985). This effect is reversed during the second
postnatal week, with forebrain κ-opioid receptor densities increasing and hindbrain κ-opioid receptor densities decreasing. Reductions in hindbrain receptor densities are attributed to a lack of κ-opioid receptor binding sites in the cerebellum, a structure which undergoes substantial growth during this time (Spain et al., 1985).

Age-dependent changes in κ-opioid receptor densities have not been ascribed to specific brain regions, because ontogenetic studies have primarily focused on κ-opioid receptor binding in whole brain or spinal cord (see Allerton et al., 1989; Attali, Saya, & Vogel, 1990; Petrillo et al., 1987; Spain et al., 1985). Nevertheless, receptor autoradiography has revealed that by PD 20 κ-opioid receptors are present in the striatum, nucleus accumbens, olfactory tubercle, hypothalamus, amygdala, and thalamic regions (Kornblum, Hurlbut, & Leslie, 1987).
CHAPTER THREE

BEHAVIORAL EFFECTS OF κ-OPIOID RECEPTOR STIMULATION

Adult Studies

Behavioral effects of κ-opioid receptor stimulation in adult rats have been well characterized. Systemic injections with various κ-opioid receptor agonists (e.g., U50,488, PD 117302, and enadoline) decrease the locomotor activity, rearing, and grooming of adult rats and mice (Castellano & Pavone, 1987; Jackson & Cooper, 1988; Leighton, Johnson, Meecham, Hill, & Hughes, 1987; Leyton & Stewart, 1992; Ukai & Kameyama, 1985). Decreased behavioral activity can be reversed by the highly selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) (Portoghese, Lipkowski, & Takemori, 1987; Takemori, Ho, Naeseth, & Portoghese, 1988), indicating that κ-opioid receptors mediate this behavioral effect (Kuzmin, Sandin, Terenius, & Ogren, 2000). The actions of κ-opioid receptor agonists are dose-dependent, because lower doses (0.1-1.0 mg/kg) of U50,488 have no effect on behavioral activity, while higher doses (5.0-10.0 mg/kg) significantly reduce
locomotion, rearing, and grooming (Leighton et al., 1987; Leyton & Stewart, 1992; Ukai & Kameyama, 1985).

Two exceptions to this pattern of results have been reported. First, Kuzmin and colleagues demonstrated that non-habituated mice (i.e., mice that had not been acclimated to the testing chamber) exhibited enhanced locomotion 40 min after being given a low dose (1.25 or 2.5 mg/kg) of U50,488 (Kuzmin et al., 2000). In contrast, mice allowed to explore the testing chamber 30 min before receiving an injection of U50,488, failed to exhibit the same enhanced locomotor response. Importantly, systemic injections of nor-BNI failed to block the increased locomotor activity exhibited by non-habituated mice, indicating that this was not a κ-opioid-mediated effect (Kuzmin et al., 2000).

Second, systemic injections of κ-opioid receptor agonists have different behavioral actions depending on the species being studied. Specifically, adult hamsters show enhanced locomotor activity after a systemic injection of a low dose of U50,488 (1.0 mg/kg) (Schnur & Walker, 1990). A systemic injection of a high dose (10 mg/kg) of U50,488, however, decreased the locomotor activity of hamsters.
Thus, both adult hamsters and adult rats show decreased locomotor activity after systemic administration of a κ-opioid receptor agonist.

It is unlikely that the U50,488-induced locomotor activity of adult hamsters was a result of a non-opioid effect, because the increased locomotion was attenuated by a systemic injection of naloxone (a non-specific opioid receptor antagonist). Rather, Schnur and Walker (1990) suggest that regional differences in the densities of κ-opioid receptors may account for the different behavioral patterns exhibited by U50,488-treated rats and hamsters. More precisely, adult hamsters have comparatively greater densities of κ-opioid receptors in the substantia nigra pars reticulata than adult rats. Importantly, adult rats show increased locomotor activity when the substantia nigra pars reticulata is stimulated by microinjections of κ-opioid receptor agonists (Friederich et al., 1987; Matsumoto et al., 1988b). Thus, it is probable that low doses of U50,488 increase the locomotor activity of adult hamsters by preferentially stimulating κ-opioid receptors in the substantia nigra pars reticulata.
In sum, systemic administration of \(\kappa\)-opioid receptor agonists to adult rats and mice typically results in reduced behavioral activity. Interestingly, systemically administering a low dose of a \(\kappa\)-opioid receptor agonist enhances the locomotor activity of adult hamsters. The latter effect is probably caused by preferential stimulation of \(\kappa\)-opioid receptors in the substantia nigra pars reticulata, because adult hamsters have a greater abundance of \(\kappa\)-opioid receptors in this area compared to adult rats (Schnur & Walker, 1990).

The ability of \(\kappa\)-opioid receptor agonists to suppress the locomotor activity of adult rats is probably due to decreased dopaminergic neurotransmission. Specifically, when U50,488 is given systemically, decreased dopamine release is evident in the nucleus accumbens and striatum (Di Chiara & Imperato, 1988; Maisonneuve, Archer, & Glick, 1994; Zaratin & Clarke, 1994). This decrease in dopaminergic activity may be responsible for U50,488's locomotor inhibiting effects. Consistent with this hypothesis, increased dopamine neurotransmission within the nucleus accumbens and striatum, caused by microinjecting dopamine agonists into these brain regions, enhances the
locomotor activity of adult rats (Essman, McGonigle, & Lucki, 1993; Kelley, Lang, & Gauthier, 1988).

The means by which κ-opioid receptor agonists modulate dopaminergic neurotransmission are well known, as systemic administration of a κ-opioid receptor agonist decreases dopamine activity via two independent mechanisms. One mechanism involves stimulation of presynaptic κ-opioid receptors located on dopamine terminals in the nucleus accumbens and striatum. Local application of U50,488 into these regions directly inhibits the release of dopamine (see Figure 2), an effect that can be blocked by κ-opioid receptor antagonists (Werling et al., 1988).

A second mechanism involves decreasing the neuronal activity of the nigrostriatal pathway. Specifically, the firing rates of dopaminergic nigrostriatal cells are inhibited when U50,488 is either administered systemically or microinjected into the substantia nigra pars reticulata. This reduction in neuronal firing rates causes decreased dopamine release in the striatum (Thompson & Walker, 1990; Walker et al., 1987; but see Lavin & Garcia-Munoz, 1985). Regardless of the mechanism (i.e., stimulating presynaptic κ-opioid receptors or reducing neuronal firing rates), κ-
opioid receptor agonists reduce the locomotion of adult rats by decreasing dopamine transmission in the striatum and/or nucleus accumbens.

In contrast to systemic administration, microinjecting κ-opioid receptor agonists into the substantia nigra pars reticulata of adult rats produces a dramatically different behavioral effect. Specifically, unilateral infusion of U50,488 into the substantia nigra pars reticulata results in robust contralateral circling (Matsumoto, Brinsfield, Patrick, & Walker, 1988a; Thompson & Walker, 1992). Similar effects are observed when various dynorphin peptides (i.e., endogenous ligands for κ-opioid receptors) are administered into the substantia nigra pars reticulata. For instance, unilateral microinjections of dynorphin A_{1-8}, dynorphin A_{1-17}, or dynorphin B into the substantia nigra pars reticulata of adult rats, produces robust contralateral circling (Friederich et al., 1987; Herrera-Marschitz, Christensson-Nylander, Sharp, Staines, Reid, Hokfelt, Terenius, & Ungerstedt, 1986; Herrera-Marschitz, Hokfelt, Ungerstedt, & Terenius, 1983; Herrera-Marschitz, Hokfelt, Ungerstedt, Terenius, & Goldstein, 1984; Matsumoto et al., 1988b; Morelli & Di Chiara, 1985). The finding
that route of administration (i.e., systemic vs. intracranial) produces dramatically different behavioral effects in adult rats, suggests that κ-opioid receptor stimulation has two distinct actions: an inhibitory and a stimulatory effect on locomotor activity.

One possibility is that the locomotor stimulating effects of κ-opioid receptor agonists are caused by increased dopamine neurotransmission in the nigrostriatal and/or mesolimbic pathways. This explanation seems unlikely for three reasons. First, κ-opioid receptor stimulation decreases, rather than increases, dopaminergic neurotransmission by diminishing the firing rate of substantia nigra pars compacta dopamine neurons projecting to the striatum (Walker et al., 1987). Second, lesions of the nigrostriatal pathway fail to inhibit the contralateral circling produced by microinjecting U50,488 into the substantia nigra pars reticulata (1986; Herrera-Marschitz et al., 1984; Morelli & Di Chiara, 1985). Third, lesions of the striatum potentiate, rather than attenuate, κ-opioid-induced circling (1986; Herrera-Marschitz et al., 1984). When considered together, it appears that κ-opioid-induced locomotor activity is not mediated by dopaminergic systems.
Rather, there is evidence suggesting that the enhanced motor movement induced by κ-opioid receptor stimulation of the substantia nigra pars reticulata is a result of disinhibiting GABAergic output neurons originating in the substantia nigra pars reticulata and projecting to the ventromedial thalamus and superior colliculus (Thompson & Walker, 1992). More precisely, under normal conditions the substantia nigra tonically inhibits the motor circuits of the superior colliculus and the ventromedial thalamus via GABAergic output fibers (see Figure 3A). κ-Opioid receptor stimulation in the substantia nigra pars reticulata inhibits these GABAergic output neurons and keeps them from inhibiting the ventromedial thalamus and superior colliculus (see Figure 3B). Thus, through the process of disinhibition, κ-opioid receptor agonists administered into the substantia nigra pars reticulata cause increased motor movement by removing the superior colliculus and ventromedial thalamus from inhibition.

When considered together, it is evident that κ-opioid receptor stimulation has two different, but concurrent, actions in the adult rat. First, κ-opioid receptor agonists inhibit locomotor activity by decreasing dopamine release.
Figure 3. Representation of the normal activity of substantia nigra pars reticulata neurons. During normal conditions, the substantia nigra pars reticulata inhibits the thalamus and superior colliculus (Upper diagram). Dynorphin stimulates \( \kappa \)-opioid receptors located on the GABAergic output projections of the substantia nigra pars reticulata. The dynorphin inhibits these GABA neurons, thus disinhibiting neurons in the ventromedial thalamus and superior colliculus (lower diagram).
in the nucleus accumbens and striatum. Second, κ-opioid receptor agonists stimulate locomotor activity by disinhibiting the GABAergic output neurons of the nigrothalamic and nigrotectal pathways. Apparently, the locomotor inhibiting effects of κ-opioid receptor agonists (mediated by the dopaminergic nigrostriatal and/or mesolimbic pathways), overwhelm the locomotor activating effects of κ-opioid receptor agonists (mediated by GABAergic nigrotectal and nigrothalamic pathways). This conclusion is based on the finding that systemic administration of U50,488 decreases the locomotor activity of adult rats.

**Ontogenetic Studies**

κ-Opioid receptor stimulation produces a dramatically different effect in preweanling rats when compared to adults. Specifically, increased locomotor activity is evident after systemic injections of U50,488 or enadoline in 3-, 5-, 10-, or 17-day-old rats (Bolanos, Garmsen, Clair, & McDougall, 1996; Collins, Zavala, Ingersoll, Duke, Crawford, & McDougall, 1998; Duke, Meier, Bolanos, Crawford, & McDougall, 1997; Jackson & Kitchen, 1989; McDougall, Rodarte-Freeman, & Nazarian, 1999; McLaughlin, Tao, & Abood, 1995). The longevity of this effect is
restricted to the preweanling period, however, because 35-day-old rats, like adult rats, do not exhibit enhanced motor movement after a systemic injection of U50,488 (Bolanos et al., 1996). U50,488's and enadoline's actions are mediated by κ-opioid receptors, since systemic injections of nor-BNI block U50,488- and enadoline-induced locomotor activity in 3- and 18-day-old rats (Collins, Zavala, Nazarian, & McDougall, 2000; McLaughlin et al., 1995).

At present, it is not known why systemic administration of a κ-opioid receptor agonist enhances the locomotor activity of preweanling rats. One possibility is that κ-opioid receptor agonists activate the nigrostriatal or mesolimbic dopamine pathways. This seems unlikely, however, because decreasing dopamine transmission with α-methyl-DL-p-tyrosine (AMPT) fails to attenuate U50,488-induced locomotor activity of preweanling rats (McDougall, Garmsen, Meier, & Crawford, 1997). Importantly, depleting dopamine with AMPT is effective at reversing the locomotor activity induced by amphetamine (an indirect dopamine agonist). Therefore, it appears that the κ-opioid-induced
locomotor activity of preweanling rats is not mediated through dopaminergic mechanisms.

A second possibility is that κ-opioid receptors in the substantia nigra pars reticulata mediate the locomotor activating effects of U50,488. This explanation has received empirical support, because microinjecting U50,488 into the substantia nigra pars reticulata causes a dose-dependent increase in the locomotor activity of 18-day-old rats (Collins et al., 2000). Importantly, this U50,488-induced locomotor activity was blocked by both systemic and intra-nigral injections of nor-BNI, but not by infusions of nor-BNI into the dorsal striatum (Collins et al., 2000). When considered together, it is clear that young rats exhibit robust motor responses after systemic or intra-nigral injections of a κ-opioid receptor agonist. It is also clear that κ-opioid receptors within the substantia nigra pars reticulata mediate this psychopharmacological effect.
CHAPTER FOUR

SUMMARY AND HYPOTHESES

Summary and Purpose

Stimulation of κ-opioid receptors by systemic injections of U50,488 decreases the locomotor activity of adult rats. This effect is probably caused by decreased dopaminergic neurotransmission in the nucleus accumbens and striatum. In contrast, stimulation of κ-opioid receptors in the substantia nigra pars reticulata produces enhanced motor movement in adult rats. For instance, unilateral microinjections of U50,488 or dynorphin peptides (e.g., dynorphin A$_{1-8}$, dynorphin A$_{1-17}$, or dynorphin B) into the substantia nigra pars reticulata causes contralateral circling (Friederich et al., 1987; Herrera-Marschitz et al., 1983; 1984; Matsumoto et al., 1988a; Morelli & Di Chiara, 1985). κ-Opioid-induced circling in adult rats is a result of disinhibiting the nigropectal and nigrothalamic premotor pathways (Thompson & Walker, 1992). In summary, κ-opioid receptor agonists differentially affect the locomotor activity of adult rats, depending on what brain areas are affected: Systemic injections of U50,488 decrease locomotor activity in adult rats, while microinjecting U50,488 into
the substantia nigra pars reticulata enhances motoric movement.

Unlike in the adult rat, systemic administration of a κ-opioid receptor agonist increases the locomotor activity of preweanling rats. For instance, systemic injections of U50,488 enhance the motor responses of 3-, 5-, 10-, or 17-day-old rats (Bolanos et al., 1996; Collins et al., 1998; Duke et al., 1997; Jackson & Kitchen, 1989; McDougall et al., 1999; McLaughlin et al., 1995). The substantia nigra pars reticulata is the neuroanatomical locus for this κ-opioid-mediated effect, since intranigral injections of U50,488 cause a dose-dependent increase in locomotor activity (Collins et al., 2000). Until now, however, no study has determined which nigral output pathway mediates U50,488-induced locomotor activity in preweanling rats.

It is possible that U50,488 enhances the locomotor activity of preweanling rats by disinhibiting GABAergic projections from the substantia nigra pars reticulata to the ventromedial thalamus and superior colliculus. The only support for this hypothesis comes from a non-ontogenetic study which showed that the U50,488-induced contralateral circling of adult rats is attenuated by
lesions to the ventromedial thalamus and superior
colliculus (Thompson & Walker, 1992). If a similar
mechanism mediates the κ-opioid-induced locomotor activity
of preweanling rats, then lesions to the ventromedial
thalamus and superior colliculus should disrupt U50,488’s
locomotor activating effect. Alternatively, if other
nigral output pathways are responsible for κ-opioid-mediated
motor movement, then lesions to the ventromedial thalamus
and superior colliculus should be ineffective at reducing
U50,488-induced locomotor activity.

The purpose of this thesis, therefore, was to
determine the neuronal circuitry mediating U50,488-induced
locomotion in preweanling rats. To this end, preweanling
rats received bilateral electrolytic lesions of the
ventromedial thalamus or superior colliculus and, two days
later, the same rats received a challenge injection of
U50,488. It was predicted that bilateral lesions of the
ventromedial thalamus or superior colliculus would
attenuate the U50,488-induced locomotor activity of 18-day-
old rats.
Experimental Controls

When interpreting the effects of brain lesions on drug-induced changes in locomotor activity it is important to determine whether the lesion caused a generalized disruption of normal motoric functioning. If motor ability was compromised, interpretation of drug-induced behavioral changes would be problematic. Thus, in the present study two measures were taken to ensure that lesions to the ventromedial thalamus and superior colliculus did not produce a general deficit in motor functioning.

First, baseline levels of locomotor activity were assessed prior to U50,488 injections to determine whether the lesions caused a change in basal activity. Second, to ensure that the functioning of non-opioid motor systems were not affected by lesions of the nigrothalamic and nigrotectal pathways, drug-induced changes in locomotor activity were assessed after a systemic injection of the dopamine agonist R(-)-propynorapomorphine (NPA). When given systemically, NPA increases the locomotor activity of rats via dopaminergic mechanisms (Duke et al., 1997; Kafetzopoulos, 1986; Kelly & Roberts, 1983). Thus, NPA-induced increases in locomotor activity were expected to be
largely unaffected by lesions of the ventromedial thalamus and superior colliculus.

For this study, it was also important to determine the specificity of brain lesions. In other words, it was necessary to provide an anatomical control to determine whether lesioning unrelated brain structures also reduces U50,488-induced locomotion. Consequently, an additional experiment was conducted where separate groups of rats were given bilateral electrolytic lesions of the nucleus accumbens and tested with U50,488. The nucleus accumbens is not thought to mediate U50,488-induced locomotor activity, so lesioning this structure was not expected to impact U50,488's behavioral effects. Thus, it was predicted that bilateral lesions of the nucleus accumbens would fail to attenuate the U50,488-induced locomotor activity of 18-day-old rats. Conversely, lesions to the nucleus accumbens were expected to disrupt NPA-induced locomotor activity, since NPA's actions are known to be mediated by the mesolimbic pathway (Kafetzopoulos, 1986; Kelly & Roberts, 1983).
Hypotheses

In summary, three separate experiments were conducted. In the first experiment, rats received bilateral lesions of the ventromedial thalamus and locomotor activity was assessed after systemic injections of U50,488 or NPA. It was hypothesized that: 1) lesions of the ventromedial thalamus would attenuate U50,488-induced locomotor activity; and 2) lesions of the ventromedial thalamus would fail to affect NPA-induced locomotion. In the second experiment, rats received bilateral lesions of the superior colliculus and locomotor activity was assessed after systemic injections of U50,488 or NPA. It was hypothesized that: 1) lesions of the superior colliculus would attenuate U50,488-induced locomotor activity; and 2) lesions of the superior colliculus would have no effect on NPA-induced locomotion. In the third experiment, rats received bilateral lesions of the nucleus accumbens and locomotor activity was assessed after systemic injections of U50,488 or NPA. It was hypothesized that: 1) lesions of the nucleus accumbens would fail to attenuate U50,488-induced locomotor activity; and 2) lesions of the nucleus accumbens would disrupt NPA-induced locomotion.
CHAPTER FIVE
GENERAL METHODS

Subjects
Subjects were 84 male and female rats of Sprague-Dawley descent (Harlan) born and raised at California State University, San Bernardino. Litters were culled to 10 rat pups by PD 3. Pups remained with the dam until time of surgery. At PD 16, rats were randomly assigned to groups. No more than one rat from each litter was placed into a particular group. Effort was made to ensure an equal number of male and female rats in each group. The colony room was maintained at 22°C-24°C and kept on a 24 h light/dark cycle (lights on at 6:00 am).

Apparatus
Behavioral testing was done in commercially available (Coulbourn Instruments, Allentown, PA) activity monitoring chambers (25.5 x 25.5 x 41 cm), consisting of Plexiglas walls, a plastic removable floor, and an open top. Each chamber included an X–Y photobeam array, with 16 photocells and detectors, which were used to determine distance traveled (a measure of horizontal locomotor activity).
Drugs

(±)-trans-U50,488 methanesulfonate (U50,488) and R(-)-
propylnorapomorphine hydrochloride (NPA) were obtained from
Sigma (St. Louis, MO) and dissolved in distilled water.
Both U50,488 and NPA were injected intraperitoneally (ip)
at a volume of 5 ml/kg.

Surgery

On PD 16, rats were anesthetized with a solution of
ketamine and xylazine (Sigma) and placed on a Cunningham
Neonatal Rat Adapter attached to a standard Kopf
stereotaxic apparatus (Cunningham & McKay, 1993). A single
incision was made mid-sagittally along the skull and the
skin was retracted. Separate groups of rats were then
given bilateral electrolytic lesions of the ventromedial
thalmus (Experiment 1), superior colliculus (Experiment
2), or nucleus accumbens (Experiment 3).

Electrolytic lesions were made by passing anodal
constant current (1.0 mA, Grass DC Lesion Maker) through a
Teflon-insulated tungsten electrode (0.2 mm in diameter,
A.M. Systems) that was exposed 1.0 mm at the tip. For the
ventromedial thalamic lesions the electrode was lowered and
left in place for 20 s, with current applied at the
following coordinates: +3.5 mm anteroposterior (AP), ±0.5 mm mediolateral (ML), -4.0 mm dorsoventral (DV). In order to create a complete lesion of the superior colliculus two electrode placements were necessary. For the superior colliculus, the electrode was left in place for 55 s while current was being applied. The coordinates for the superior colliculus lesions were: +0.4 mm AP, ±1.5 mm ML, -7.0 mm DV; and -0.6 mm AP, ±1.5 ML, -7.0 DV. Lesions of the nucleus accumbens required only one electrode placement at -6.2 mm AP, ±2.2 mm ML, -6.8 mm DV. For the nucleus accumbens lesion, current was applied for 30 s. In all cases, coordinates were obtained from the developing rat brain atlas of Sherwood and Timiras (1970). An equal number of rats from each litter were given bilateral sham-lesions using procedures identical to those described above, except that no current was applied. After surgery, rats were allowed to recover in a temperature controlled (30°C) chamber. After becoming fully responsive, rats were placed back with the dam and tested 48 hr and 72 hr later.

Histology

Immediately after behavioral assessment, rats were given an overdose of Nembutal and perfused intracardially
with saline followed by a 4% paraformaldehyde solution. After a 7-day postfixation period, coronal sections were taken from each brain using a Vibratome 1000 sectioning apparatus (Ted Pella, Redding, CA). Sections were stained with thionin, coverslipped, and underwent lesion site verification using a microscope. Rats that had partial or inadequate lesions were removed and replaced.

**Statistical Analyses**

Analysis of Variance (ANOVA) for repeated measures (5 min time blocks) was used for statistical analysis of distance traveled data. The first three time blocks, consisting of baseline locomotor assessment, were analyzed separately from the last nine time blocks. Baseline locomotor activity was not different between sham and lesioned rats in any of the experiments. Body weight data were analyzed using a $3 \times 2$ (day x lesion) repeated measures ANOVA. To control for litter effects, one rat from each litter was placed into a particular group. Litter was then used as the unit of analysis for the statistical analyses. With this statistical model each litter, rather than each rat, was treated as an independent observation (Zorrilla, 1997). Sex differences were assessed using separate
between-subject ANOVAs. No sex differences were found, and thus the data was presented collapsed across male and female rats. Post hoc analyses of simple main effects and interactions were made using Tukey HSD tests \((p < 0.05)\).

For the first and second experiment, in addition to comparing group differences, the correlation between percent of brain area lesioned and U50,488-induced locomotor activity was determined using the Pearson product-moment correlation \((r)\). For these correlations, rats that had improper lesions were included in the analyses. It was predicted that there would be a negative correlation between lesion size and U50,488-induced locomotor activity, with decreased locomotor activity being exhibited by U50,488-treated rats as the percentage of brain area lesioned increased.
CHAPTER SIX

EXPERIMENT ONE

Overview

The first experiment was conducted to determine whether U50,488-induced locomotor activity was attenuated by lesions of the ventromedial thalamus. A second purpose of Experiment 1 was to determine whether lesions of the ventromedial thalamus produced a general decrease in motoric functioning. Consequently, rats were also injected with the dopamine receptor agonist NPA. NPA increases locomotor activity in rats by stimulating dopamine receptors in the nucleus accumbens and striatum (Kafetzopoulos, 1986; Kelly & Roberts, 1983), and not by altering the functioning of nigral output pathways projecting to the ventromedial thalamus. Therefore, it was predicted that bilateral lesions of the ventromedial thalamus would attenuate U50,488's locomotor activating effects, but have no effect on NPA-induced locomotor activity.

Method

A total of 28 rats (n = 7 per group) received bilateral electrolytic lesions or sham lesions of the
ventromedial thalamus on PD 16. On PD 18, these rats were
singly placed in the testing chambers and baseline
locomotor activity was assessed for 15 min. Sham- and
ventromedial thalamus-lesioned rats were then injected with
saline or U50,488 (5.0 mg/kg, ip) and returned to the
testing chambers for an additional 45 min. This dose of
U50,488 was chosen because previous studies have shown that
5.0 mg/kg U50,488 reliably produces robust locomotor
activity in preweanling rats (Collins et al., 1998; 2000).

Following behavioral testing, rats were returned to
their home cage and placed back with the dam. After an
additional 24 hr, rats were retested as described above,
except that half of the rats (counterbalanced for previous
drug treatment) were given a single injection of saline and
the other half an injection of NPA (0.01 mg/kg, ip). This
dose of NPA was chosen because it has been shown that 0.01
mg/kg NPA produces robust locomotor activity in preweanling
rats (Duke et al., 1997).

Results

Histology

A representative lesion of the ventromedial thalamus
is shown in Figure 4. Rats that did not have at least 75%
Figure 4. Image of a thionin-stained section indicating the damage produced by an electrolytic lesion of the ventromedial thalamus on postnatal day 16.

of the ventromedial thalamus destroyed on each hemisphere were removed from the experiment and replaced.

Body Weight

The body weights of sham- and ventromedial thalamus-lesioned rats are shown in Figure 5 (left panel). Rats in Experiment 1 showed an increase in body weight across the
Figure 5. Mean body weight (±SEM) of rats (n = 7-9) from Experiments 1, 2, and 3. Regardless of experiment, no body weight differences were found between sham or lesioned rats (p > 0.05).

four days of the experiment [day main effect, F(2, 52) = 50.49, p < 0.001]. Importantly, body weights of the sham and ventromedial thalamus-lesioned rats did not differ.

Locomotor Activity

Distance traveled (i.e., locomotor activity) data for sham- and ventromedial thalamus-lesioned rats given systemic injections of U50,488 and NPA are presented in
Figure 6. Overall, 18-day-old rats injected with U50,488 (5 mg/kg, ip) exhibited more locomotor activity than saline-treated rats [agonist main effect, $F(1, 6) = 139.79$, $p < 0.001$; agonist $\times$ time interaction, $F(8, 48) = 19.86$, $p < 0.001$]. Bilateral lesions of the ventromedial thalamus attenuated the locomotor activity of 18-day-old rats injected with U50,488 [lesion $\times$ agonist interaction, $F(1, 6) = 41.53$, $p < 0.001$; lesion $\times$ agonist $\times$ time interaction, $F(8, 48) = 2.79$, $p < 0.05$]. Specifically, sham-lesioned rats given U50,488 exhibited more locomotor activity on time blocks 5-12 than ventromedial thalamus-lesioned rats given U50,488 (see upper graph, Figure 6). Bilateral lesions of the ventromedial thalamus did not completely block U50,488-induced locomotor activity, however, since ventromedial thalamus-lesioned rats exhibited significantly more locomotor activity on time blocks 7-12 compared to saline-treated rats. Thus, lesions to the ventromedial thalamus result in a disruption of U50,488-induced locomotor activity. The extent of damage to the ventromedial thalamus was negatively correlated with the amount of locomotor activity exhibited after systemic injections of U50,488 (data not shown) [$r(14) = -0.37$], but
Figure 6. Mean distance traveled (±SEM) of rats that had received sham or electrolytic lesions of the ventromedial thalamus on postnatal day (PD) 16. On PD 18, rats (n = 7) were injected with saline or U50,488 (5 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). On PD 19 the same rats were injected with saline or NPA (0.01 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). (a) Significantly different from the Sham-Saline group (open symbols). (b) Significantly different from the Lesion-U50,488 group (filled square).
this effect did not reach statistical significance (p > 0.05).

On the following day (i.e., PD 19), the same rats were injected with saline or 0.01 mg/kg NPA (see lower graph, Figure 6). Rats given systemic injections of NPA exhibited greater locomotor activity on time blocks 5-10 compared to rats injected with saline [agonist main effect, $F(1, 6) = 37.88, p < 0.001$; agonist $\times$ time interaction, $F(8, 48) = 28.98, p < 0.001$]. Importantly, lesions of the ventromedial thalamus failed to affect NPA-induced locomotor activity, because sham- and ventromedial thalamus-lesioned rats responded similarly to a systemic injection of NPA.
CHAPTER SEVEN

EXPERIMENT TWO

Overview

In the second experiment, the ability of superior colliculus lesions to attenuate U50,488-induced locomotor activity was assessed. As in Experiment 1, rats were retested with NPA to determine whether superior colliculus lesions cause a general decrease in motor functioning. It was predicted that bilateral lesions of the superior colliculus would attenuate U50,488's locomotor activating effects, while having no effect on NPA-induced locomotion.

Method

A total of 28 rats (n = 7 per group) received bilateral electrolytic lesions or sham lesions of the superior colliculus on PD 16. On PD 18, these rats were singly placed in the testing chambers and baseline locomotor activity was assessed for 15 min. Sham- and superior colliculus-lesioned rats were then injected with saline or U50,488 (5.0 mg/kg, ip) and returned to the testing chambers for an additional 45 min.

Following behavioral testing, rats were returned to their home cage and placed back with the dam. After an
additional 24 hr, rats were retested as described above, except that half of the rats (counterbalanced for previous drug treatment) were given a single injection of saline and the other half an injection of NPA (0.01 mg/kg, ip).

Results

Histology

A representative lesion of the superior colliculus is shown in Figure 7. Rats that did not have at least 75% of the superior colliculus destroyed on each hemisphere were removed from the experiment and replaced.

Body Weight

The body weights of sham- and superior colliculus-lesioned rats are shown in Figure 5 (middle panel). An increase in body weight was evident in both sham- and superior colliculus-lesioned rats, with no differences being apparent between the two treatment groups [day main effect, \( F(2, 52) = 123.30, p < 0.001 \)].

Locomotor Activity

Distance traveled (i.e., locomotor activity) data for sham- and superior colliculus-lesioned rats given systemic injections of U50,488 and NPA are presented in Figure 8. Systemic injections of 5.0 mg/kg U50,488 were again found
Figure 7. Image of a thionin-stained section indicating the damage produced by an electrolytic lesion of the superior colliculus on postnatal day 16.

to increase the locomotor activity of 18-day-old rats [agonist main effect, $F(1, 6) = 29.50$, $p < 0.001$; agonist x time interaction, $F(8, 48) = 8.75$, $p < 0.001$]. The U50,488-induced locomotor activity exhibited by these rats was diminished by bilateral lesions of the superior colliculus [lesion x agonist x time interaction, $F(8, 48) = 2.79$, $p < 0.05$]. More precisely, sham-lesioned rats given
Figure 8. Mean distance traveled (±SEM) of rats that had received sham or electrolytic lesions of the superior colliculus on postnatal day (PD) 16. On PD 18, rats ($n = 7$) were injected with saline or U50,488 (5 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). On PD 19 the same rats were injected with saline or NPA (0.01 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). (a) Significantly different from the Sham-Saline group (open symbols). (b) Significantly different from the Lesion-U50,488 group (filled square).
U50,488 had greater locomotor activity on time blocks 5-10 when compared to superior colliculus-lesioned rats given U50,488 (see upper graph, Figure 8). Lesioning the superior colliculus was not sufficient to block the locomotor activity induced by systemic injections of U50,488, since the Lesion-U50,488 group was significantly more active than saline controls (see Figure 8). Therefore, superior colliculus lesions, like lesions of the ventromedial thalamus, attenuated the locomotor activity produced by systemic injections of U50,488.

In order to examine whether non-specific or incomplete brain lesions affect the locomotor activity produced by κ-opioid receptor agonist, a correlational analysis examined the relationship between lesion size and U50,488-induced locomotor activity. A scatterplot representing the relationship between lesion size and the distance traveled scores of all lesioned-rats given systemic injections of U50-488 is shown on Figure 9. As predicted, the amount of locomotor activity exhibited by rats treated with U50,488 was negatively correlated with the amount of damage to the superior colliculus (see Figure 9) \(r(14) = -0.37\). Thus, it is not likely that the reduction in locomotor activity
Figure 9. Scatterplot representing the relationship between lesion size and distance traveled scores of U50-488-treated rats. Distance traveled scores were summated over the last 45 min of the testing session (i.e., the period beginning immediately after U50,488 was injected).

The observation in superior colliculus-lesioned rats is a result of non-specific lesions to surrounding brain areas.

When the same rats were retested with saline or NPA (0.01 mg/kg, ip) on PD 19, both sham- and superior colliculus-lesioned rats exhibited greater locomotor activity on time blocks 5-10 when given NPA [agonist × time interaction, F(8, 24) = 7.18, p < 0.001]. Lesioning the superior colliculus did not disrupt NPA-induced locomotor
activity, because no differences between the Sham-NPA group and the Lesion-NPA group were observed.
CHAPTER EIGHT

EXPERIMENT THREE

Overview.

The first two experiments were conducted to determine whether disruptions of the nigrothalamic and nigroventral pathways attenuate U50,488- and NPA-induced locomotor activity. The purpose of the third experiment was to determine whether lesioning another brain region causes non-specific reductions in U50,488-induced locomotion. Simply stated, Experiment 3 was conducted to provide an anatomical control in which lesions to a particular brain region were not expected to affect U50,488-induced locomotor activity. To this end, rats were given sham or bilateral lesions of the nucleus accumbens and tested with U50,488 and NPA on two consecutive days. It was expected that lesions to the nucleus accumbens would not affect U50,488-induced locomotor activity. However, because NPA-induced locomotor activity is mediated via dopaminergic mechanisms in the striatum and nucleus accumbens (Kafetzopoulos, 1986; Kelly & Roberts, 1983), it was hypothesized that lesions to the nucleus accumbens would attenuate NPA-induced locomotor activity.
Method

A total of 36 rats ($n = 9$ per group) received bilateral electrolytic lesions or sham lesions of the nucleus accumbens on PD 16. On PD 18, these rats were singly placed in the testing chambers and baseline locomotor activity was assessed for 15 min. Sham- and nucleus accumbens-lesioned rats were then injected with saline or U50,488 (5.0 mg/kg, ip) and returned to the testing chambers for an additional 45 min.

Following behavioral testing, rats were returned to their home cage and placed back with the dam. After an additional 24 hr, rats were retested as described above, except that half of the rats (counterbalanced for previous drug treatment) were given a single injection of saline and the other half an injection of NPA (0.01 mg/kg, ip).

Results

Histology

A representative lesion of the nucleus accumbens is shown in Figure 10. Rats that did not have at least 50% of the nucleus accumbens destroyed on each hemisphere were removed from the experiment and replaced.
Figure 10. Image of a thionin-stained section indicating the representative damaged produced by an electrolytic lesion of the nucleus accumbens on postnatal day 16.

Body Weight

The body weights of sham- and nucleus accumbens-lesioned rats are shown in Figure 5 (right panel). All rats showed a significant increase in body weight across the four days of the experiment [day main effect, $F(2, 52) = 142.42, p < 0.001$]. In particular, sham- and nucleus
accumbens-lesioned rats exhibited similar body weight gain during the experiment.

**Locomotor Activity**

Distance traveled (i.e., locomotor activity) data for sham- and nucleus accumbens-lesioned rats given systemic injections of U50,488 and NPA are presented in Figure 11. Systemically administered U50,488 significantly enhanced the locomotor activity of 18-day-old rats compared to saline controls [agonist main effect, $F(1, 8) = 103.37$, $p < 0.001$; agonist $\times$ time interaction, $F(8, 64) = 6.35$, $p < 0.001$]. Contrary to my predictions, bilateral lesions to the nucleus accumbens disrupted the U50,488-induced locomotor activity of 18-day-old rats [lesion $\times$ agonist interaction, $F(1, 8) = 7.31$, $p < 0.05$; lesion $\times$ agonist $\times$ time interaction, $F(8, 64) = 2.31$, $p < 0.05$]. Specifically, the Sham-U50,488 group exhibited greater activity on time blocks 6 and 8-12 than rats in the Lesion-U50,488 group (see upper graph, Figure 11). Lesioning the nucleus accumbens, however, did not completely prevent U50,488 from enhancing the locomotor activity of 18-day-old rats, since the Lesion-U50,488 group was more active during time blocks 5-12 compared to saline-treated rats (see
**Figure 11.** Mean distance traveled (±SEM) of rats that had received sham or electrolytic lesions of the nucleus accumbens on postnatal day (PD) 16. On PD 18, rats \( (n = 7) \) were injected with saline or U50,488 (5 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). On PD 19 the same rats were injected with saline or NPA (0.01 mg/kg, ip) 15 min into the behavioral testing session (indicated by the dashed line). (a) Significantly different from the Sham-Saline group (open symbols). (b) Significantly different from the Lesion-U50,488 group (filled square).
Figure 11). Thus, like lesions of the nucleus accumbens, lesions of the ventromedial thalamus and superior colliculus attenuated the U50,488-induced locomotor activity of 18-day-old rats.

On PD 19, these same rats were injected with saline or NPA (0.01 mg/kg, ip). Systemic injections of NPA produced robust locomotor activity in both sham- and nucleus accumbens-lesioned rats, with these differences reaching statistical significance on time blocks 5-9 [agonist main effect, $F(1, 8) = 112.36, p < 0.001$; agonist x time interaction, $F(8, 64) = 24.08, p < 0.001$]. Unexpectedly, lesions of the nucleus accumbens did not affect the NPA-induced locomotor activity of 19-day-old rats. This result was surprising since the nucleus accumbens has substantial numbers of dopamine receptors (Gelbard, Teicher, Faedda, & Baldessarini, 1989; Rao, Molinoff, & Joyce, 1991) and NPA’s effects are known to be mediated by dopamine receptors.
CHAPTER NINE

DISCUSION

Rationale

The purpose of the present study was to determine the neuronal circuitry responsible for \( \kappa \)-opioid-mediated locomotion in preweanling rats. Convincing evidence now exists that systemic injections of U50,488 produces its locomotor stimulating effects by activating \( \kappa \)-opioid receptors in the substantia nigra pars reticulata (Collins et al., 2000). It is uncertain which nigral output pathway is responsible for mediating the U50,488-induced locomotor activity of preweanling rats, but it is possible that \( \kappa \)-opioid receptor stimulation enhances locomotion by inhibiting GABAergic projections from the substantia nigra pars reticulata to the ventromedial thalamus and superior colliculus.

Nonontogenetic studies have provided the only evidence that the nigrostriatal and nigrotectal pathways may be important for \( \kappa \)-opioid-mediated locomotion. Specifically, although systemic administration of U50,488 does not produce locomotor activity in adult rats (Jackson & Cooper, 1988; Leyton & Stewart, 1992), unilateral microinjections
of U50,488 into the substantia nigra pars reticulata produces robust contralateral circling (Matsumoto et al., 1988a). Importantly, lesions of the ventromedial thalamus or superior colliculus attenuate U50,488-induced circling in adult rats (Thompson & Walker, 1992). Therefore, in the present study, 16-day-old rats were given bilateral lesions of the ventromedial thalamus (Experiment 1) or superior colliculus (Experiment 2) followed, two days later (i.e., on PD 18), by a systemic injection of U50,488. It was predicted that bilateral lesions of the ventromedial thalamus or superior colliculus would attenuate the U50,488-induced locomotor activity of preweanling rats.

Role of the Ventromedial Thalamus and Superior Colliculus in U50,488- and NPA-Induced Locomotion

Systemic administration of U50,488 produced robust locomotor activity in 18-day-old rats (see Figures 6, 8, and 11). That U50,488 caused this behavioral effect was not surprising, because systemic administration of U50,488 reliably increases the locomotor activity of preweanling rats (Bolanos et al., 1996; Collins et al., 1998; Duke et al., 1997; Jackson & Kitchen, 1989; McDougall et al., 1997; McLaughlin et al., 1995). As expected, lesions to the
ventromedial thalamus or superior colliculus significantly decreased the U50,488-induced locomotor activity of 18-day-old rats. More specifically, rats with bilateral lesions of the ventromedial thalamus or superior colliculus exhibited an attenuated response to a systemic injection of U50,488 (see upper graphs, Figures 6 and 8). Importantly, the same lesions did not disrupt the locomotor activity produced by systemic injections of the dopamine agonist NPA (see lower graphs, Figures 6 and 8). Regardless of the brain area lesioned (ventromedial thalamus or superior colliculus), systemic injections of NPA stimulated the locomotor activity of sham and lesioned rats in a similar manner.

When considered together, it is apparent that the nigrothalamic and nigrotectal pathways are part of an integral circuitry responsible for the ability of κ-opioid-mediated locomotor activity of preweanling rats. Moreover, the functioning of non-opioid motor systems (i.e., those systems mediating NPA-induced locomotion) were not affected by lesions of the ventromedial thalamus and superior colliculus. This effect is important because it reveals that ventromedial thalamus and superior colliculus lesions
specifically disrupt the stimulatory actions of \( \kappa \)-opioid receptor agonists.

Lesions of the ventromedial thalamus or superior colliculus alone were not capable of completely blocking U50,488-induced locomotor activity (see Figures 6 and 8). Several explanations may account for this effect. First, it is possible that lesions of the ventromedial thalamus and superior colliculus were incomplete, thus allowing U50,488-induced locomotor activity to be expressed at a reduced level. This explanation seems unlikely, however, since care was taken to ensure that only animals with complete lesions were included in the experiments. Second, it is possible that both the ventromedial thalamus and superior colliculus are necessary for the full expression of \( \kappa \)-opioid-mediated locomotor activity. In other words, when only one brain area was destroyed, the remaining non-lesioned brain area (i.e., the ventromedial thalamus or superior colliculus) may have been sufficient to produce a weakened, although significant, level of U50,488-induced locomotor activity. Third, the inability of ventromedial thalamus and superior colliculus lesions to completely attenuate U50,488-induced locomotor activity, leaves open
the possibility that other nigral output pathways (e.g., pathways projection to the striatum and pedunculopontine nucleus) may mediate some of the behavioral effects induced by κ-opioid receptor agonists. This possibility cannot be eliminated by the present study. In any event, it remains certain that the nigroreticular and nigrothalamic pathways mediate, at least partially, the locomotor activity produced by κ-opioid receptor agonists.

Role of the Nucleus Accumbens in U50,488-Induced Locomotion

For this study, it was important to determine the specificity of lesion-induced effects. Thus, an additional experiment was conducted to determine whether lesioning an unrelated brain structure would reduce U50,488-induced locomotion. The nucleus accumbens was the brain region chosen as the anatomical control for two reasons. First, lesioning the nucleus accumbens was not expected to affect U50,488-induced locomotor activity, since U50,488’s locomotor activating effects are known to be mediated by the substantia nigra pars reticulata (Collins et al., 2000). Second, lesioning the nucleus accumbens was expected to disrupt NPA-induced locomotor activity, since
NPA's actions are known to be mediated by the mesolimbic pathway (Kafetzopoulos, 1986; Kelly & Roberts, 1983). The results from Experiment 3, however, suggest that I did not choose an appropriate anatomical control.

Perhaps the most surprising finding was that the nucleus accumbens lesions attenuated the U50,488-induced locomotor activity of preweanling rats. More precisely, rats with lesions to the nucleus accumbens exhibited a decreased responsiveness to a systemic injection of U50,488 (see upper graph, Figure 11). These findings are problematic because they complicate interpretation of data from the other lesion experiments. Put another way, because lesions of the nucleus accumbens, like lesions of the ventromedial thalamus and superior colliculus, attenuated U50,488-induced locomotor activity, it remains possible that lesioning any brain region may reduce the locomotor activity produced by k-opioid receptor stimulation.

Even so, this explanation, that U50,488-induced locomotor activity is attenuated by non-specific brain lesions, is unlikely for two reasons. First, there was a strong correlation between U50,488-induced locomotor
activity and the amount of damage produced by superior colliculus lesions (see Experiments 2 and Figure 9). Moreover, lesions to the ventromedial thalamus and superior colliculus did not produce a global decrease in motor ability nor did they decrease NPA-induced activity. When considered together, these findings provide strong evidence that the ventromedial thalamus and superior colliculus mediate the stimulatory effects of κ-opioid receptor agonists and do not cause a general disruption of motor functioning.

Second, there is evidence to suggest that lesions of the nucleus accumbens may indirectly affect the locomotor stimulating effects of U50,488 by way of direct projections to the substantia nigra pars reticulata. More precisely, recent anatomical studies have demonstrated that the nucleus accumbens provides input to cell bodies in substantia nigra pars reticulata (Deniau, Menetrey, & Thierry, 1994; Montaron, Deniau, Menetrey, Glowinski, & Thierry, 1996). This is important, because nucleus accumbens lesions may have indirectly affected U50,488-induced locomotor activity by altering the function of the nigrothalamic and nigrocostal pathways. Behavioral
evidence for this explanation is available, since the U50,488-induced locomotor activity of preweanling rats is decreased by dopamine receptor antagonist drugs (Duke et al., 1997; Nazarian, Rodarte-Freeman, & McDougall, 1999). When these results are considered together, it appears that the nucleus accumbens may not have been an appropriate anatomical control for the present study, particularly because the nucleus accumbens may be a component of the circuitry mediating U50,488-induced locomotor activity.

Role of the Nucleus Accumbens in NPA-Induced Locomotion

Although lesions of the nucleus accumbens were effective in disrupting U50,488-induced activity, the same lesions did not disrupt the locomotor activating effects of NPA (see lower graph, Figure 11). This finding was unexpected because the nucleus accumbens has a substantial number of dopamine receptors that are evident as early as the first postnatal week (Gelbard et al., 1989; Rao et al., 1991). Moreover, lesions to the nucleus accumbens have been shown to disrupt the NPA-induced locomotor activity of adult rats (Kafetzopoulos, 1986; Kelly & Roberts, 1983). Importantly, adult and preweanling rats do not demonstrate a qualitatively different behavioral profile after systemic
administration of NPA, as both age groups show marked increases in locomotor activity after NPA treatment (Duke et al., 1997; McDougall, Crawford, & Nonneman, 1992; Mestlin & McDougall, 1993). Consequently, it was reasonable to expect that nucleus accumbens lesions would affect the NPA-induced locomotor activity of preweanling and adult rats similarly.

At least two explanations may account for the inability of nucleus accumbens lesions to disrupt NPA-induced activity. First, it is possible that lesions of the nucleus accumbens were not complete enough to disrupt the locomotor enhancing effects of NPA, since complete lesions of the nucleus accumbens are necessary to disrupt the behavior of adult rats (Kafetzopoulos, 1986; Kelly & Roberts, 1983). In the present study, histological results revealed that many of the rats included in the analyses had only partial lesions (≈50%) of the nucleus accumbens. Thus, the extent of nucleus accumbens damage may have been insufficient to disrupt the NPA-induced locomotor activity of preweanling rats.

Second, it is possible that surviving regions of the nucleus accumbens or striatum may have compensated for the
loss of dopaminergic activity. Consistent with this hypothesis are studies showing that neurochemical and behavioral deficits are only evident when more than 95% of dopamine cell bodies are destroyed (for a review, see Robinson, Castaneda, & Whishaw, 1990). Moreover, extracellular concentrations of dopamine remain unaffected (i.e., are within normal control levels) even when 80% of dopamine neurons in the substantia nigra are lesioned (Castaneda, Whishaw, & Robinson, 1990). These findings may be the result of compensatory changes by the remaining population of dopamine neurons, including, but not limited to, increased dopamine synthesis and release. Similar compensatory changes in dopaminergic activity may account for the lack of behavioral deficits exhibited by 6-hydroxydopamine-treated rats given systemic injections of a dopamine agonist. For instance, amphetamine-induced locomotion and rearing are not disrupted until dopamine depletion reaches 95% (Castaneda et al., 1990; Robinson et al., 1990). In light of these findings, it is plausible that compensatory changes in dopaminergic activity may have allowed for the expression of NPA-induced locomotor activity in preweanling rats.
Conclusion

The present study has demonstrated that the locomotor activity produced by systemic administration of U50,488 is, at least partially, mediated by the ventromedial thalamus and superior colliculus. This conclusion is supported by results showing that: (a) lesions to the ventromedial thalamus and superior colliculus attenuate U50,488-induced locomotor activity, and (b) the same lesions fail to disrupt the locomotor activity produced by systemic injections of NPA. The specificity of these lesion-induced effects is somewhat ambiguous, because nucleus accumbens lesions also attenuated the locomotor activity of U50,488-treated rats. The latter finding is difficult to interpret, since the nucleus accumbens may indirectly affect the activity of the ventromedial thalamus. At present, therefore, the most parsimonious conclusion is that the ventromedial thalamus and superior colliculus are the primary output structures mediating the U50,488-induced locomotor activity of preweanling rats. Future research will be needed to determine whether the mesolimbic dopamine pathway is also important for κ-opioid-mediated locomotion.
REFERENCES


Gerfen, C. R. (1992a). The neostriatal mosaic: multiple levels of compartmental organization. Trends in Neuroscience, 15(4), 133-139.


following recovery from injury induced by 6-OHDA or methamphetamine: a review of evidence from microdialysis studies. Canadian Journal of Psychology, 44(2), 253-275.


