PREFRONTAL CORTEX LTP AND BEHAVIOURAL SENSITIZATION

DOPAMINERGIC MODULATION OF GLUTAMATE-BASED LONG-TERM POTENTIATION IN THE MEDIAL PREFRONTAL CORTEX AND THE MAINTENANCE OF BEHAVIOURAL SENSITIZATION, *IN VIVO*

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ABSTRACT

Drug addiction and behavioural sensitization are associated with reorganization of mesolimbocortical circuitry, which we have attempted to model with glutamatergic-based long-term potentiation (LTP) in the medial prefrontal cortex (mPFC). The objective of the experiments was to examine the effects of dopamine-1 (D_1) and dopamine-2 (D_2) receptor family specific agonists and antagonists on LTP in the mPFC in the chronic *in vivo* preparation using fully-awake, freely-moving rats. Male Long-Evans rats were surgically implanted with stimulating electrodes into the corpus callosum and recording electrodes into the mPFC. Subjects were systemically administered a drug together with high frequency stimulation for the induction of LTP. The rats treated with the D_1 receptor agonist A68930 (0.4mg/kg/ml) showed LTP levels equal to those in the saline LTP group. The D_1 receptor antagonist SKF83566 (0.15mg/kg/ml) blocked the expression of LTP, and instead induced significant long-term depression. The D_2 receptor antagonist sulpiride (3, 6, and 12mg/kg/ml) significantly decreased LTP, compared to the control group, in a dose-dependent fashion. The D_2 receptor agonist quinpirole (0.125, 0.25, and 0.5mg/kg/ml) significantly increased LTP, compared to the control group, in a dose-dependent fashion. The D_2 receptor agonist also induced behavioural sensitization, the intensity and frequency of which was positively correlated with the LTP effect. This is the first

work to show that glutamatergic-based LTP in the mPFC is positively modulated by D_2 receptors in the chronic *in vivo* preparation, and behavioural sensitization is, in turn, modulated by LTP induction. As D_2 receptor-rich neurons are located largely in mesencephalic nuclei that, in turn, project to the mPFC, the D_2 effects may be indirect. This plasticity modulation needs to be more deeply explored to determine its relationship to disorders, such as psychostimulant addiction and schizophrenia that are known to be due to dysregulated dopamine and glutamate function in the mesencephalon and mPFC.

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"HE WHO ENDURES, CONQUERS"
- Persius

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INTRODUCTION

The *Diagnostic and Statistical Manual of Mental Disorders* (2002) refers to drug addiction as a 'substance-related disorder', the essential characteristic of which is a chronic and compulsive pattern of drug-seeking and drug-taking behaviour that continues despite adverse consequences and a loss of control over voluntary acts. Researchers in varied fields have long accepted the biological basis of drug addiction, though the exact mechanisms of initiation, development, and maintenance of the behaviour is yet to be definitively elucidated. It has been determined, however, that drugs of abuse saturate the reward circuitry of the brain. In particular, functioning of the neuromodulator dopamine is known to be usurped in mesolimbocortical circuitry, the phylogenetically ancient brain system innately involved in rewarding behaviours such as feeding, sex, and exercise. Indeed, brain reward systems serve to direct the organism's behaviour toward goals that are normally beneficial and promote survival of the individual (e.g., food) or the species (e.g., reproductive behaviour). Similar to the consequence of these positive behaviours, drugs of abuse create a feeling of euphoria due to the exacerbated release of the 'feel good' neurotransmitter dopamine. Yet, in addition to this state of well-being, chronic drug use leads to the expression of a phenomenon known as behavioural sensitization. Behavioural sensitization refers to the augmentation of behavioural responses, such as locomotion, and is often associated with stereotypy

(repetitive patterns of behaviour). This phenomenon is reliably expressed by chronic psychostimulant users after a period of drug abstinence. This temporal pattern suggests that the neurobiological alterations that underlie behavioural sensitization outlast the immediate alterations that take place in the dopamine systems after and during drug use.

Considerable literature suggests that long-term potentiation, the electrophysiological phenomenon hypothesized to represent learning and memory, may be the substrate for the maintenance and expression of behavioural sensitization during periods of drug abstinence. The objective of the following dissertation was to examine the relationship between mesolimbocortical long-term potentiation and behavioural sensitization. This objective was satisfied with three main areas of inquiry: (1) can long-term potentiation be induced in the prefrontal cortex *in vivo*?; (2) if so, can this long-term potentiation be modulated by dopamine?; (3) does long-term potentiation in the prefrontal cortex affect the development, expression, or maintenance of psychostimulant-induced behavioural sensitization? Our hypothesis was that a psychostimulant analogue would positively modulate long-term potentiation in the prefrontal cortex, and that this would be associated with the expression of behavioural sensitization during a period of drug abstinence.

CHAPTER 1

ANATOMY AND RECEPTOR ORGANIZATION OF THE MESOLIMBOCORTICAL SYSTEM

Ascending dopaminergic pathways in the mammalian central nervous system can be divided into two major systems. One is the *nigrostriatal system* that originates in the substantia nigra and innervates predominantly the dorsal striatum (caudate and putamen) (Sesack, Snyder, & Lewis, 1995). This system is most commonly known to be involved in the control of voluntary movement (Sesack et al., 1995). The *mesolimbocortical dopamine (DA) system* arises from the ventral tegmental area (VTA) and projects to the prefrontal cortex (PFC), hippocampus, amygdala, and ventral striatum/nucleus accumbens (N.Acc.) (Sesack et al., 1995). The mesolimbocortical system can be further divided into two subsystems, which are determined by the localization of cell bodies within the VTA and their projection areas. Dopaminergic cells of the *mesolimbic (mesoaccumbens) division* project to the N.Acc. and are associated with reward and locomotor activity (Schultz, 1998). Those of the *mesocortical division* project to cortical structures, and are involved in the modulation of cognitive functions (Williams & Goldman-Rakic, 1998). See Figure 1.1. Different physiological and pathological conditions such as arousal (Berridge & Stalnaker, 2002), stress (Del Arco & Mora, 2001) drug addiction (Moghaddam & Bunney, 1989), and neuropsychiatric disorders (Carlson, Waters, Holm-Waters et al., 2001; Davis,

Kahn, Ko et al., 1991; Goldstein & Deutch, 1992; Mattay, Tessitore, Callicott et al., 2002; Tzchentke , 2001) have been associated with changes in the release of the neuromodulator dopamine (DA) in the mesolimbocortical system.

1.1 Prefrontal Cortex

The PFC mediates higher-order cognition. The modulation of neural function in the PFC by mesolimbocortical DA is essential for these processes, which include reasoning and decision-making (Birrell & Brown, 2000; Floresco, Seamans, & Phillips, 1997; Kinberg & Farrah, 1993; Phillips, Ahn, & Floresco, 2004), temporally ordering events (Floresco et al., 1987; Phillips et al., 2004) working memory (Kinberg & Farrah, 1993), attentional control (Birrell & Brown, 2000), and organization and planning (Goldman-Rakic, 1995). For example, in a typical delayed-response task used to test short-term working memory in animals, a distinct increase in firing of medial PFC (mPFC) neurons is observed during the brief delay period (Fuster, 1995; Goldman-Rakic, 1995). This is the period when the animal has to temporarily 'hold' specific pieces of information that are to be used to guide and execute forthcoming correct behavioural responses (Fuster, 1995; Goldman-Rakic, 1995). The sustained enhancement of firing during the delay period, and the successful performance of these tasks are highly dependent on intact mesocortical dopaminergic input (Goldman-Rakic,

1992; Sawaguchi & Goldman-Rakic, 1994; Seamans, Floresco, & Phillips, 1998;) and optimal levels of DA (Williams & Goldman-Rakic, 1995).

Compromised PFC function has been implicated in the cognitive deficits that accompany various psychophathologies, including schizophrenia (Laruelle, Kegeles, & Abi-Dargham, 2003; Manoach, Gollub, Benson et al., 2000; Pantelis, Barnes, Nelson et al., 1997; Pantelis, Stuart, Nelson et al., 2001). There is evidence of PFC aberrations in the human schizophrenic brain, such as PFC thinning, reduction in the number of glutamate (Glu) neurons that exhibit dendritic spines (Lewis, Glantz, Pierri et al., 2003; Selemon & Goldman-Rakic, 1999), and reduced PFC regional cerebral blood flow correlated with impaired performance on working memory tasks (Manoach et al., 2000; Meyer-Lindenberg, Miletich, Kohn et al., 2002; Pantelis et al., 1997, 2001).

The PFC is a structurally and functionally heterogeneous group of cortical areas located anterior to the motor and premotor regions of the frontal lobes (Tamminga, 2004). In rat, the PFC is a 2-3 millimeter thick ribbon of gray matter that is divided into six distinct cytoarchitectonic layers (I-VIb), with no layer IV (Ding, Gabbot, Totterdell, 2001). Distributed across these six layers are different types of excitatory glutamatergic pyramidal neurons which represent approximately 85% of cortical neurons (Gabbot, Dickie, Vaid et al., 1997). Inhibitory γ-amino-butyric acid (GABA) interneurons represent the remaining 15%

of PFC neurons (Gabbot et al., 1997). Pyramidal neurons typically have triangularly-shaped cell bodies, a single apical dendrite directed toward the pial surface, and an array of basilar dendrites (Gabbot et al., 1997). Depending on their location, pyramidal neurons provide excitatory projections to different brain regions. Those which innervate the contralateral PFC are distributed in superficial layers II-III and V (Ferino, Thierry, Saffroy et al., 1987). Those in deep layers V-VI integrate excitatory inputs from cortical and subcortical areas, and are the output neurons that transfer information processed within the PFC to distant cortical areas and sub-cortical structures (Fuster, 1997).

The medial PFC (mPFC) is a region located rostrally in the cerebral hemispheres, and is subdivided into the infralimbic cortex, prelimbic cortex, and anterior cingulate cortex (Ding et al., 2001). The anterior cingulate mPFC receives afferents from the caudal mediodorsal thalamus and the neocortex, and sends efferents to the caudal mediodorsal thalamus, motor-somatosensory-visual cortices, dorsolateral striatum, superior colliculus, deep mesencephalic nucleus, pontine and medullary reticular formation, paraoculomotor central gray, and mesencephalic trigeminal nucleus (Berendse, Galis-De Graaf, & Groenewegen 1992; Groenewegen, Berendse, Wolters et al., 1990; Sesack, Deutch, Roth, et al., 1989). The infralimbic mPFC receives afferents from the hypothalamus, amygdala, hippocampus, and autonomic brainstem nuclei (Berendse et al., 1992; Groenewegen et al., 1990), and sends efferents to many regions including the caudate putamen, shell regions of the N.Acc., VTA

(Berendse et al., 1992; McGeorge & Faull, 1989), hypothalamus, amygdala, bed nucleus of the stria terminalis, periaqueductal gray, dorsal motor vagal nucleus, nucleus of the solitary tract, and the parabrachial nucleus (Hurley, Hernert, Moga, et al., 1991; Sesack et al., 1989). The prelimbic mPFC receives afferents from the rostral mediodorsal thalamus, hippocampus, and the VTA, and sends efferents to the N.Acc., (Gorelova & Yang, 1997) rostromediodorsal thalamus, VTA, basolateral amygdala, forebrain (Berendse et al., 1992; Groenewegen et al., 1990), dorsal regions of the dorsomedial striatum (Berendse et al., 1992; McGeorge & Faull, 1989), cingulate-perirhinal cortices, dorsomedial and ventral striatum, lateral hypothalamus, periacqueductal gray region, ventral midbrain tegmentum, laterodorsal tegmental nucleus, raphe nuclei, anterior olfactory nucleus, piriform cortex, and pedunculopontine tegmental-cuneiform region (Sesack et al., 1989).

Prefrontal cortex Glu and DA receptors are located on pyramidal neurons as well as the inhibitory neurons that impinge on the pyramidal neurons (Fuster, 1997). Pyramidal neurons express both the ionotropic Glu receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPAR) and *N*-methyl-$_D$-aspartate (NMDAR), as well as metabotropic GluRs (mGluRs) (Fuster, 1997). Of the dopamine-1 receptor (D_1R) family, which includes the D-1 receptor (D_1R) and D-5 receptor (D_5R), the D_1R subtype predominates in neocortex and striatum, while the D_5R subtype is found in the hippocampus and entorhinal cortex (Fuster,

1997). Of the dopamine-2 receptor (D_2R) family, which includes the D-2 receptor (D_2R), D-3 receptor (D_3R), and D-4 receptor (D_4R), the D_2R subtype predominates in the striatum, limbic sites, and the thalamus, the D_3R predominates in the N.Acc., and the D_4R is found in the hippocampus and PFC (Fuster, 1997). Note that while D_2Rs are primarily located subcortically, the D_4R subtype of the D_2R family is also found in the cortex, albeit at low levels.

Medial Prefrontal Cortex to Nucleus Accumbens

The mPFC excitatory glutamatergic (Groenewegen & Berendse, 1994) and aspartergic (Christie, Summers, Stephenson et al., 1990) projections to the N.Acc. are part of a multisynaptic processing loop that involves the ventral pallidum and mediodorsal thalamus. Cholecystokinin (CCK) is co-localized in this pathway (Morino, Herrera-Marschitz, Castel et al., 1994a; Morino, Mascagni, McDonald et al., 1994b), where pyramidal-shaped CCK-containing neurons of the mPFC project bilaterally to the N.Acc. (Morino et al., 1994b). This circuit plays a role in motivated and cognitive behaviours, and has been implicated in the etiology of drug addiction, schizophrenia and other neuropsychiatric disorders. The projections from areas of the mPFC are topographically arranged. The detailed arrangements with respect to specific compartments within the 'shell' and 'core' areas of the N.Acc. appear to depend upon the layer of origin of the mPFC projections. Deep layers V and VI throughout the mPFC

innervate both the core and shell regions (Brog, Salyapongse, Deutch, et al., 1993; Descarries, Lemay, Doucet et al., 1987; Ferino et al., 1987; Pinto & Sesack, 1998). Specifically, the infralimbic subarea of the mPFC projects to a peripheral, band-like region in the medioventral parts of the shell and medial part of the core (Groenewegen, Wright, Beijer, et al.,1999). Deep layer V of the prelimbic subarea of the mPFC sends fibres to the dorsomedial part of the shell and throughout the core (Groenewegen et al., 1999). Alternatively, the superficial section of mPFC layer V, and mPFC layers II and III preferentially project throughout the core region (Ding et al., 2001; Groenewegen & Uylings, 2000). There is evidence, however, that in the shell region of the N.Acc. the mPFC areal origin of the fibres, rather than their laminar origin, may be more important in determining area of synapse (Berendse et al.,1992). Specifically, throughout the shell region of the N.Acc., fibres from the ventral part of the prelimbic mPFC concentrate inside areas of high cell density and weak enkephalin reactivity (Berendse et al., 1992). Anatomically, the data support a laminar-areal specificity for mPFC to N.Acc. projections, and suggest different roles for these areal-laminae sections in their interactions with the N.Acc. shell, core, and their efferents. However, the functional significance of these anatomical differences is unclear.

The mPFC terminals in the N.Acc. synapse onto dendritic spines of inhibitory medium spiny GABA neurons which form at least 90% of neurons in the ventral striatum (Carr & Sesack, 2000a; Sesack & Pickel, 1992). For each

glutamatergic cortical synapse in the N.Acc., there is convergence with dopaminergic innervation from the VTA (Sesack & Pickel, 1992) so that glutamatergic and dopaminergic terminals dually synapse in the N.Acc. The excitatory input to these GABA projection neurons could provide a mechanism through which the mPFC can inhibit the activity of neurons immediately downstream of the N.Acc. Similarly, mPFC afferents to GABA neurons in the VTA might exert a feed forward inhibitory influence on the N.Acc. output cells, both of which would modulate subcortical DA activity.

Medial Prefrontal Cortex to Ventral Tegmental Area

The majority of excitatory glutamatergic pyramidal neurons that project directly from the mPFC to the VTA reside in deep lamina V and VI (Au-Young & Yang, 1999; Carr & Sesack, 2000; Lu, Churchill, & Kalivas, 1997; Sesack & Pickel, 1992). Both dopaminergic and GABAergic neurons in the VTA receive monosynaptic input from these mPFC afferents, where they synapse onto small and large dendrites (Sesack & Pickel, 1992). Medial PFC terminals selectively synapse onto the VTA DA cells that monosynaptically project back to the mPFC (Carr & Sesack, 2000a). Functional evidence for this circuitry is provided by the finding that mPFC stimulation releases Glu in the VTA (Rossetti, Marcangione, & Wise, 1998) and induces burst firing of VTA dopaminergic neurons (Chergui, Suaud-Chagny, & Gonon, 1994), followed by an increased release of DA in the

N.Acc and mPFC (Taber & Fibiger, 1995). The glutamatergic control of dopaminergic activity in the VTA can be modulated locally, but it is also modulated by processing within the mPFC, which includes the integration of inputs from multiple transmitter systems. Together, these systems determine the output activity of mPFC glutamatergic projections, thereby modulating the release of Glu in the VTA (Adell & Artigas, 2004). Through these connections, the mPFC can mediate a selective influence on the activity of ascending DA and GABA projections. Such a synaptic organization has important implications for models of schizophrenia pathophysiology which hypothesize loss of inhibitory control of mesostriatal DA neurons as a result of mPFC functional deficits (Laruelle, Abi-Dargham, Van Dyck, et al., 1996). This is hypothesized because an abnormal reduction in mPFC glutamatergic receptors (hypoglutamatergia), as in the schizophrenic brain, would lead to deficient stimulation of VTA GABA neurons which synapse onto co-localized VTA DA neurons. The net result would be a disinhibition of DA cell firing in the VTA, allowing DA to be over-released. Mesencephalic hyperdopaminergia is a key characteristic in schizophrenia which is associated with symptoms of paranoia, delusions (persistent erroneous beliefs despite the presentation of truth), hallucinations (unreal auditory and/or visual perceptions), and behavioural sensitization (augmented behavioural responses such as hyperlocomotion) (American Psychiatric Association, 2002).

Excitatory mPFC terminals also monosynaptically synapse onto VTA GABA cells that monosynaptically project to the N.Acc. (Carr & Sesack, 2000a)

and VTA DA neurons (Tong, Overton, & Clark 1996). These PFC synaptic inputs to VTA GABA neurons may regulate mesoaccumbens DA cells through these local connections, which is consistent with the short-latency inhibition typically evoked in VTA DA neurons by PFC stimulation (Tong et al., 1996).

1.2 Ventral Striatum: Nucleus Accumbens

The term ventral striatum denotes a nuclear mass in the rostroventral neostriatum known as the N.Acc., which is bordered medially by the septum and ventrally by the olfactory tubercule (Lovinger, Partridge, & Tang, 2003). The N.Acc. was originally suggested to play a role in the pathophysiology of schizophrenia (Stevens, 1973), and was proposed to form a limbic-motor interface (Heimer & Wilson, 1975). Since then, disordered dopaminergic signaling in the N.Acc. has been thought to be critical in several other common neuropsychiatric disorders, including Tourette's syndrome and drug addiction. The N.Acc. has also been defined as integral to limbic-motor interactions, and important in reward, motivation, learning and memory.

The N.Acc. receives inputs from cortical-limbic areas including the mPFC, hippocampus, entorhinal cortex, amygdala, thalamus, ventral pallidum, VTA, retrorubral area, raphe nuclei, and nucleus of the solitary tract (Berendse et al., 1992; Brog et al.,1993; Groenewegen, Vermeulen-Van Der Zee, Te Kortschot, et al, 1987; Lovinger et al., 2003; Wright, Beijer, & Groenewegen, 1996). These inputs innervate the neurons on the necks of the spines covering the outer two-

thirds of the dendrites (Kincaid, Zheng, & Wilson, 1998; Smith, Bennet, Bolam et al., 1994), as well as the aspiny dendrites of N.Acc. interneurons (Hidaka & Totterdell, 2001). Outputs from the N.Acc. reach the ventral pallidum, thalamus, hypothalamus, VTA, caudal areas of the mesencephalon, substantia nigra, and globus pallidus (Groenewegen et al., 1999). The existence of a pathway from the N.Acc. to the ventral pallidum, and from the ventral pallidum to the mediodorsal thalamic nucleus, is consistent with the participation of the N.Acc. in a prefrontal channel which parallels the sensorimotor circuits of the dorsal striatum (Groenewegen, Berendse, & Haber, 1993). There is now evidence that the substantia nigra pars reticulata also provides a substantial innervation to thalamic areas projecting to the mPFC (Deniau & Chevalier, 1992) adding another link between the N.Acc. and the mPFC circuitry as well as with the basal ganglia motor system.

The N.Acc. is considered to consist of three main subdivisions. These include the central 'core' which ensheaths the anterior limb of the anterior commissure and gradually merges into the ventral part of the dorsal striatum; a 'shell' which compromises medial, ventral, and lateral divisions that surround the core; and finally a 'rostral pole' where core and shell regions cannot easily be differentiated in the rat (Ding et al., 2001; Groenewegen et al., 1999). Many different markers, including acetylcholinesterase, immunoreactivity for enkephalin, substance P, CCK, and calbindin D28k (CaDP), and the distribution of DA receptors, are distributed across the shell and the core. (Groenewegen,

Berendse, Meredith, et al., 1991). These compartments are not homogeneous, and further cythoarchitectonic and chemical subdivisions can be made. For example, in the medial parts of the shell, dense concentration of substance P, dynorphin, and tyrosine hydroxylase overlap enkephalin or CaDP poor zones, whereas laterally, moderate immunoreactivity for substance P and CaDP overlap with less dense dynorphin and enkephalin (Groenewegen et al., 1991). In the core, small rostral and caudal zones are enkephalin and opioid receptor rich, yet CaDP poor (Groenewegen et al., 1991). Overall, afferents to the N.Acc. from different sources innervate specific areas within the central core and peripheral shell.

Approximately 90% of the N.Acc. is comprised of medium spiny stellate projection neurons, which are inhibitory GABAergic neurons (Chang & Kitai, 1985; Lovinger et al., 2003). As such, they function to inhibit downstream nuclei. They are referred to as spiny stellate neurons because they have dendrites that are spine-free at proximal segments, up to the first branch point, but are densely covered with spines more distally, and each cell has an axon that leaves the soma to arborize locally before projecting out of the N.Acc. (Meredith, 1999). The projection neurons contain GABA as the primary neurotransmitter, but they also immunoreact for CaDP and a number of neuropeptides including neurotensin, enkephalin, dynorphin, substance P, and neurokinin B (Meredith, Pennartz, & Groenewegen, 1993; Pickel, Joh, & Chan, 1988). Local circuit neurons make up approximately 10% of all N.Acc. neurons and manufacture

either acetylcholine, GABA (Hussain, Johnson, & Totterdell, 1995) or nitric oxide synthase (Lovinger & McCool, 1995), as well various peptides: vasoactive intestinal peptide, CCK, neurotensin, and CaDP (Hussain, Johnson, & Totterdell, 1994).

Various receptor types can be found on N.Acc. medium spiny neurons, as the variety of neurotransmitters and neuropeptides that are manufactured and interact with these neurons suggests. Overall, DA receptors in the neostriatum are expressed predominantly on postsynaptic membranes of medium spiny neurons (Gerdeman, Partridge, Lupica et al., 2003). Both the D_1R and D_2R families are highly expressed in the striatum (Lovinger et al., 2003). It has been traditionally thought that D_1Rs and D_2Rs do not colocalize, but D_3R expression can be found in subsets of D_1R- and D_2R -expressing neurons (Le Moine & Bloch, 1996). The D_1R subtype is present on the cell bodies, dendrites, and spines of local circuit neurons (Smiley, Levey, Ciliax et al., 1994), and is correlated with the expression of substance P (Lu, Ghasemzadeh, & Kalivas, 1998; Schwartz, Diaz, Bordet et al., 1998). Further, D_1R family mRNA is expressed in local dynorphinergic neurons and the majority of cholinergic reactive cells (Le Moine & Bloch, 1995). The D_2R subtype is present on the cell bodies, dendrites, and spines of local circuit neurons (Smiley et al., 1994). D_2R family mRNA has been localized to local enkephalinergic and cholinergic reactive cells (Le Moine & Bloch, 1995; Lu et al., 1998). Ventral tegmental area-derived D_2R subtype autoreceptors also exist on dopaminergic afferents innervating the

striatum (Gerdeman et al., 2003). The D_3R and D_5R subtypes are expressed at lower levels (Lovinger et al., 2003) and are correlated with the expression of substance P (Lu et al., 1998; Schwartz et al., 1998). Overall, D_4R and D_5R subtype expression is very low or undetectable (Sibley, 1995), yet, D_5Rs may be expressed in cholinergic neurons (Nicola, Surmeier, & Malenka, 2000).

Both ionotropic and metabotropic GluRs are also present on N.Acc. medium spiny neurons (Rouse, Marino, Bradley et al., 2000; Tarazi & Baldessarini, 1999). The medium spiny neurons contain AMPA and NMDA ionotropic receptors, as well as group I mGluRs (Lovinger et al., 2003). Group II mGluRs reside mostly on glutamatergic cortical afferents, where they appear to act as autoreceptors (Lovinger & McCool, 1995). Glutamatergic transmission in the N.Acc. is primarily mediated by the AMPA/kainate receptors, although NMDARs also contribute to excitatory post-synaptic potentials (Pennartz, Groenewegen, & Lopes Da Silva, 1994). There may be regional differences in the contributions of these receptor types to excitatory transmission, since NMDARs are activated in the shell but not the core during reduced GABAergic inhibition (Pennartz et al., 1994). There are also a large number of inhibitory receptors. $GABA_B$ receptors exist mainly on the cortical afferents, while many $GABA_A$ receptors are expressed on different striatal neurons (Lovinger et al., 2003). The striatum also receives serotonergic input from the raphe nuclei, and cholinergic innervation from the pedunculopontine nucleus and lateral dorsal tegmental nucleus, so there are a variety of serotonin and cholinergic receptors

(Genova, Berke, & Hyman, 1997; Lovinger et al., 2003). Several subunits that form the nicotinic and muscarinic subtypes of the acetylcholine receptor are also present (Hersch, Gutekunst, Rees et al., 1994). In addition, delta and mu opiate receptors are in abundance (Hohmann & Herkenham, 2000), CB-1 type cannabinoid receptors (Hohmann & Herkenham, 2000), CCKa, and CCKb receptors (Genova et al.,1997), and somatostatin receptors (Lovinger et al., 2003).

1.3 Ventral Tegmental Area

The VTA is a small subcortical structure, and in conjunction with the substantia nigra, is the point of origin of all DA in the mesolimbocortical system. Mesocortical DA neurons originate in the VTA, and 10% innervate the PFC (Westerink, Enrico, Feimann et al., 1998). Ascending DA afferents from the VTA are known to play an important role in the mediation of cognitive and affective functions, including attention, motivation, reward, learning, and memory (Blackburn, Pfaus, & Phillips, 1992; Fibiger & Phillips, 1986; Le Moal & Simon, 1991; Sachs & Meisel, 1988). The VTA of the rat contains about 30,000 neurons on each side (Halliday & Tork, 1986). Eighty to 85% of the total neuronal population in the VTA are DA-containing cells while 15-20% are GABA-containing cells (Kalivas, 1993). The GABA content comes from both intrinsic and extrinsic sources. The former includes VTA GABAergic neurons that project to the N.Acc. and mPFC (Carr & Sesack, 2000; Thierry, Deniau, Herve et al.,

1980; Van Bockstaele & Pickel, 1995). GABA and DA are not co-localized in the VTA, but rather GABAergic terminals form synapses with dopaminergic cells (Kosaka, Kosaka, Hataguchi et al., 1987). A background extracellular pool of GABA exists in the VTA (Frantz, Harte, Ungerstedt et al., 2002) and exerts its influence over dopaminergic neurons via both GABAa and GABAb receptors on VTA neurons (Charara, Heilman, Levey et al., 2000). The extrinsic sources of GABA originate from N.Acc., ventral pallidum, and the pedunculopontine nucleus (Smith, Charara, & Parent, 1996, 1996a).

Both DA- and GABA-containing mesoprefrontal terminals form synaptic contacts onto distal dendrites and dendritic spines of projection and local circuit neurons (Carr & Sesack, 2000). Excitatory glutamatergic pyramidal neurons of layer V receive innervation from the VTA (Lewis & O'Donnell, 2000) which play a modulatory role on pyramidal cell activity (Groenewegen & Uylings, 2000). Approximately 60% of VTA projections to the PFC are GABAergic, and form synapses on the distal dendrites of the pyramidal and GABA-containing local circuit neurons (Carr & Sesack, 2000; Carr & Sesack, 2000a). Thus, GABA afferents from the VTA may mediate a disinhibitory influence within the PFC through inhibition of inhibitory local circuit neurons. Approximately 33% of VTA projections to the PFC are dopaminergic (German & Manaye, 1993; Swanson, 1982). These VTA DA terminals converge with excitatory terminals from intrinsic collaterals of neighbouring mPFC cells rather than from extrinsic sources (Carr & Sesack, 1996, 1998; Pinto & Sesack, 1998; Sesack & Miner, 1997). The large

number of GABAergic cells in the VTA primarily project to the same terminal fields as the dopaminergic cells (Swanson, 1982).

Glutamatergic innervation of the VTA originates mainly from the mPFC (Lu et al., 1997) and synapses with both dopaminergic and GABAergic cells (Bonci & Malenka, 1999; Sesack & Pickel, 1992; Smith et al., 1996a). Specifically, this mPFC glutamatergic input has been shown to synapse on dopaminergic cells that project back to the mPFC and synapse onto GABAergic and pyramidal cells in deep layers V and VI throughout the prelimbic and infralimbic mPFC (Descarries et al., 1987; Goldman-Rakic, Leranth, Williams, et al., 1989). These areas/layers contain the highest concentration of DA terminals in the rat PFC (Descarries et al., 1987; Goldman-Rakic et al., 1989). In turn, mPFC neurons monosynaptically project to the N.Acc. and VTA (Beckstead, 1979; Carr & Sesack, 2000; Sesack et al., 1989). Further evidence for this circuitry is provided by electrophysiological studies which show that mPFC pyramidal cells projecting to the N.Acc. respond to applied DA or stimulation of the VTA (Yang & Seamans, 1996). Although earlier studies reported extensive collateralization of PFC pyramidal cell axons to multiple targets (Thierry, Chevalier, Ferron et al., 1983), more recent work has reported only limited collateralization of accumbens-projecting mPFC neurons to the contralateral PFC, amygdala, or VTA (Pinto and Sesack. 1998). Thus, the modulatory influence mediated by DA on this population of pyramidal neurons is likely to be heavily weighted to its

monosynaptic projection to the N.Acc. As such, these synaptic contacts of VTA DA terminals onto the dendritic tree of cortico-accumbens neurons is likely to play a role in regulating the functional output of neurons in the N.Acc.

Both DA and GABA neurons in the VTA house a relatively small set of receptor types: GABARs, GluRs, DARs (Bonci & Malenka, 1999; Westerink et al., 1998), and AchRs (Westerink et al., 1998). D_1R mRNA is found in both GABAergic and glutamatergic inputs to the VTA from the mPFC (Lu et al., 1997), and D_1R perfusion into the VTA triggers both Glu and GABA release (Kalivas & Duffy, 1995), which can control resident DA neurons. Both the D_1 and D_5 subtype of the D_1R family are present on DA cells (Ciliax, Nash, Heilman et al., 2000) and GABAergic afferents (Cameron & Williams, 1993). The D_2R family is highly expressed in the VTA of rodents (Sesack, Aoki, & Pickel, 1994). The highest density corresponds to the D_2 subtype; the D_3 subtype is expressed moderately, followed by the D_4 subtype (Diaz, Pilon, Le Foll et al., 2000). The majority of all D_2R subtypes are expressed on DA neurons (Chen, Qin, Szele et al., 1991), including pre-synaptic D_2 autoreceptors. In addition to their role as autoreceptors, D_2Rs can also function as 'heteroreceptors' in non-dopaminergic neurons (Pickel, Chan, & Nirenberg, 1996). This is supported by electrophysiological evidence that dendritic release of DA may regulate the firing rate of VTA dopaminergic neurons through D_2Rs located on glutamatergic terminals (Koga & Momiyama, 2000). Infusion of a D_2R antagonist also increases extracellular DA in the ipsilateral PFC (Westerink et al., 1998),

supporting the notion that D_2 autoreceptors at somatodendritic sites of mesocortical neurons participate in tonic inhibition. Infusion of a D_2R antagonist also reveals that the three types of DA neurons all contain D_2R autoreceptors; mesocortical DA neurons display a higher sensitivity for D_2R stimulation than do mesolimbic and nigrostriatal neurons, however (Westerink et al., 1998).

Both ionotropic and metabotropic GluRs are also present in the VTA (Bonci & Malenka, 1999; Paquet, Tremblay, Soghomonian et al., 1997). All three subtypes of the ionotropic receptor – AMPA, kainate, and NMDA – are present on the dendrites of DA and GABA mesocortical neurons (Bonci & Malenka, 1999; Paquet et al., 1997; Wang & French, 1995; Westerink et al., 1998; Zhang, Chiodo, & Freeman, 1994). This is consistent with electrophysiological studies that have provided evidence for the presence of NMDARs and non-NMDARs on DA cell bodies in the VTA (Johnson & North, 1992; Wang & French, 1995; Zhang et al., 1994).

Both inhibitory GABAR subtypes, 'a' and 'b', are also present in the VTA. Infusion of GABAaR and GABAbR agonists into the VTA decreases extracellular DA in the ipsilateral mPFC (Westerink et al., 1998), indicating the presence of both GABAR subtypes on somatodendritic sites of mesocortical DA neurons. The localization of these receptor types on the cell bodies of VTA DA neurons is also supported by anatomical (Bayer & Pickel, 1991) and *in vitro* electrophysiological studies (Johnson & North, 1992; Jiang, Pessia, & North, 1993; Seutin, Johnson, & North, 1994). Nonetheless, only GABAaRs tonically

inhibit mesocortical DA neurons (Westerink et al., 1998), indicating that mesocortical neurons are less sensitive to GABAb stimulation than are mesolimbic or nigrostriatal DA neurons.

Electrophysiological studies on brain slices also indicate the presence of both muscarinic and nicotinic subtypes of the acetylcholine receptor (mAchR & nAchR, respectively) on DA cell bodies in the VTA (Calabresi, Lacey, & North, 1989; Lacey, Calabresi, & North, 1990). Infusion of a general AchR antagonist into the VTA results in a marked increase in extracellular DA in the PFC (Westerink et al., 1998) illustrating the ability of cholinergic afferents to stimulate mesocortical DA cells at the level of the VTA. This is consistent with electrophysiological data that DA neurons in the VTA are excited by AchR agonists (Seutin, Verbanck, Massotte et al., 1990). Infusion of a general AchR antagonist also reveals that the three types of DA neurons all receive cholinergic input, although the mesocortical DA neurons display a higher sensitivity for AchR stimulation than the mesolimbic and nigrostriatal neurons (Westerink et al., 1998). Intra-tegmental infusions of mAchR- and nAchR-specific agonists are, however, without effect on the extracellular levels of DA in the PFC (Westerink et al., 1998). This supports the notion that the mesolimbic DA system is phasically rather than tonically regulated by cholinergic receptor activation within the VTA.

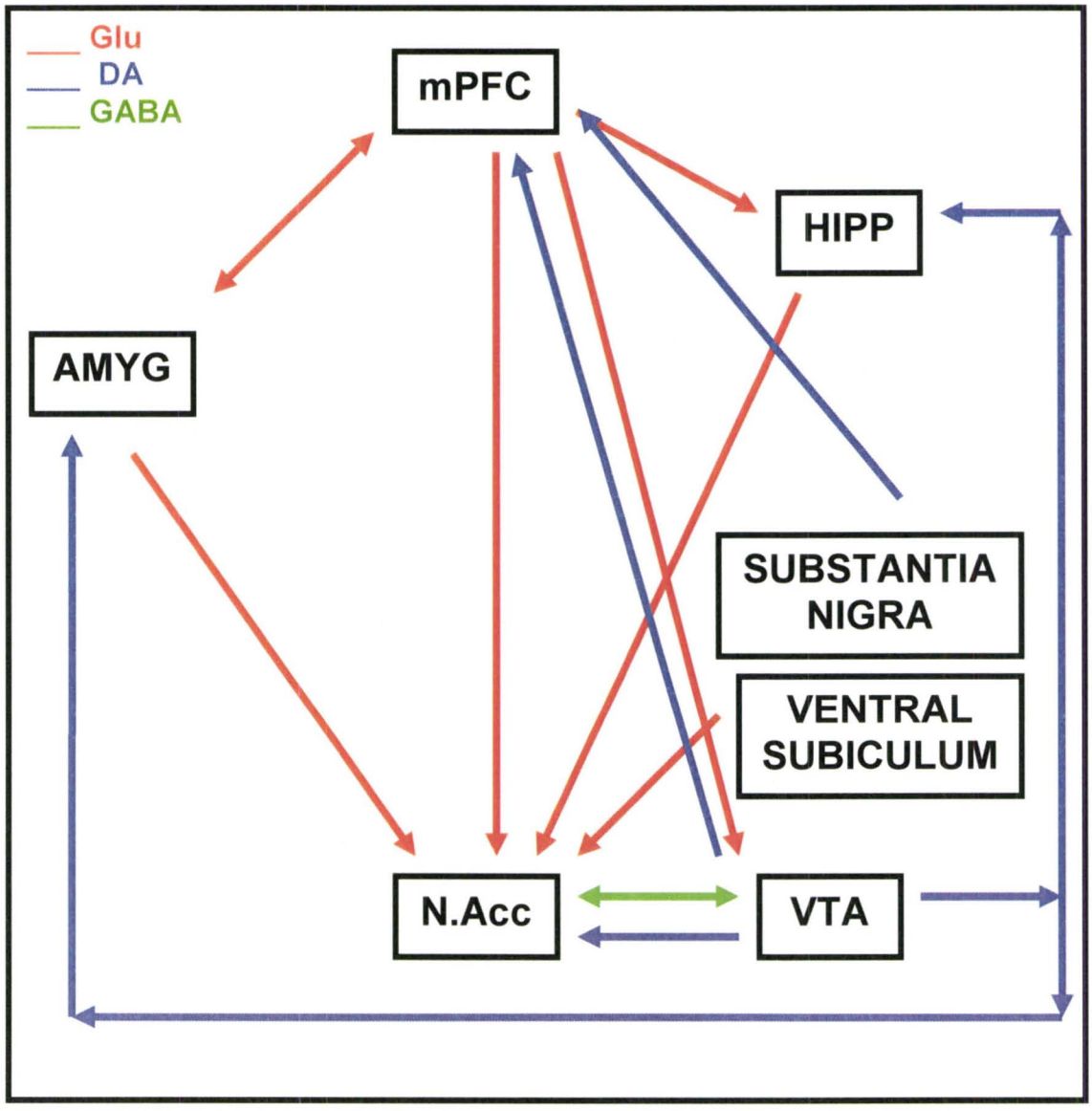
1.4 Ventral Tegmental Area to Nucleus Accumbens, and Back Again

Only dopaminergic afferents from the VTA are generally reported to reach all regions of the N.Acc. (Voorn, Jorritsma-Byham, Van-Dijk et al., 1986). There are two types of projections that run between the VTA and N.Acc.: dopaminergic and GABAergic. Approximately 85% of VTA projections to the N.Acc. are dopaminergic (German & Manaye, 1993; Swanson, 1982), and these neurons colocalize CCK (Maidment & Villafranca, 1997). These VTA dopaminergic cells synapse predominantly on the medial and ventral parts of the shell, with a lesser amount terminating in the medial core region (Groenewegen et al., 1999). The remaining 15% of mesoaccumbens projections are GABAergic (Groenewegen et al., 1999). The majority of the N.Acc.-VTA projections originate in the medial (Meredith, 1999) and rostrolateral parts of the shell, and are entirely GABAergic (Groenewegen et al., 1999). In the rostrolateral part of the N.Acc. shell, fibres coming from the deep layers of the prelimbic area of the mPFC distribute in an inhomogeneous way over a moderately enkephalin reactive compartment (Berendse, Groenewegen, & Lohman, 1992). Since the neurons that project from the N.Acc. to the VTA are located in this compartment (Berendse et al., 1992; Groenewegen, Meredith, Berendse et al., 1989), only the afferents from the deep layers of the prelimbic mPFC area may be selectively related to the VTA neurons receiving N.Acc. input. Overall, there are reciprocal GABAergic projections to and from the VTA and N.Acc. which play a strong role in

dampening or maintaining DA levels originating in the VTA that extend to both the PFC and N.Acc. (Groenewegen et al., 1989).

1.5 Figures

Figure 1.1



1.6 Figure Captions

Figure 1.1 Mesolimbocortical System

This figure represents the basic anatomical connections of the mesolimbocortical system which arises from the ventral tegmental area (VTA) and sends dopaminergic (DA) (*blue*) efferents to the medial prefrontal cortex (mPFC), hippocampus (HIPP), amygdala (AMYG), and nucleus accumbens (N.Acc.). The mesolimbocortical system can be divided into two subsystems: DA cells of the mesolimbic (mesoaccumbens) division project to the N.Acc., and those of the mesocortical division project to cortical structures. Glutamatergic (Glu) (*red*) efferents originate from pyramidal cells in the mPFC which synapse onto GABAergic cells in the N.Acc. and VTA. Bilateral GABAergic (GABA) (*green*) efferents project between GABAergic cells in the N.Acc. and GABAergic and DA cells in the VTA.

CHAPTER 2

CELL FIRING AND DOPAMINE RELEASE

2.1 Dopamine Cell Firing and Neurotransmission

Dopamine cells in the intact animal fire in two distinct modes: tonic irregular single spikes firing at rates of 2-8 spikes per second, or bursts of 3-8 spikes occurring at high intra-burst frequencies of approximately 10-20 Hz with pauses between bursts (Grace & Bunney, 1984). Evidence indicates that both patterns of DA cell firing are regulated by glutamatergic neurons in the PFC (Grace, 1991, 2000). There also appear to be phasic and tonic forms of DA transmission.

Phasic DA transmission occurs with a high-amplitude transient signal, in which periterminal DA concentrations are estimated to reach into the low millimolar range (e.g., ~1.6 mM in N.Acc.) (Garris, Ciolkowski, Pastore et al., 1994). Phasic DA transmission results from brief (~300ms) DA cell-burst firing (Floresco, West, Ash et al., 2003; West, Floresco, Charara et al., 2003) with the released DA acting on DARs (West et al., 2003). Periods of DA cell burst-firing have been correlated with conditioned stimuli that predict the delivery of reward (Schultz, 1998), so this phasic form of DA transmission may mediate rapid behaviourally relevant activation of the DA system. This transmission is regulated by glutamatergic and cholinergic inputs (Gronier & Rasmussen, 1998; Kitai, Shepard, Callaway et al., 1999) and terminated rapidly via reuptake

mechanisms that eliminate DA from the synaptic cleft by the high-affinity pre-synaptic DA transporter (DAT) which plays an important role in limiting diffusion of DA from the synaptic space (West et al., 2003).

It has been shown that manipulations that selectively increase burst firing of DA neurons beyond baseline levels, however, produce no discernable increase in extra-cellular DA levels in the N.Acc. (West et al., 2003). This is surprising considering that bursting is the primary mechanism by which behaviourally relevant DA release is facilitated (Garris et al., 1994; Grace & Bunney, 1984). It has, thus, been posited that the lack of effect of enhanced bursting of DA neurons on DA efflux may be due to the actions of the DAT (West et al., 2003). This theory is supported by studies which show that blocking the DAT results in an approximate nine-fold increase in extra-cellular DA levels, and that this is further increased by ~300% when burst firing of DA neurons is activated (West et al., 2003). This indicates that a selective increase in burst firing of DA neurons induces a massive increase in DA release at the terminal level.

In contrast to phasic transmission, tonic DA transmission accounts for the extra-synaptic pool of DA that is present at steady-state concentrations within the extra-cellular space (West et al., 2003). The tonic DA pool is tightly regulated and maintained within a narrow concentration range (e.g., ~ 4-20 nM in N.Acc.) (Parsons & Justice, 1992), and alterations in tonic levels of DA efflux occur on a

much slower time scale than changes in phasic levels (Schultz, 1988; West et al., 2003). These conditions hold true even when the DA system is compromised (West et al., 2003). The slower timescale of DA level changes may enable a variety of motor, cognitive, and motivational states. Tonic levels of DA are maintained primarily by presynaptic glutamatergic signaling pathways and the overflow of DA released from the terminals during tonic firing activity (Grace, 1991; Howland, Taepavararpruk, & Phillips, 2002; West et al., 2003). Presumably, tonic DA levels are affected by the total number of DA neurons active within the VTA (i.e. population activity) (Grace, 1991). Tonic extra-cellular DA levels act to down-regulate the responsivity of the DA system to bursts of action potentials thought to be generated during behavioural activation (Grace, 1991). Tonic extracellular levels of DA are not altered dramatically by increases in DA cell burst firing. However, the disinhibition of non-firing DA cells can increase the overall population activity of midbrain DA neurons and result in an elevation of tonic DA transmission in the N.Acc. (West et al., 2003). It has also been proposed that tonic extracellular DA levels modulate the phasic component of DA signaling through the activation of synthesis- and release-regulating autoreceptors. These autoreceptors are thought to be located extra-synaptically on the DA terminal, and play an important role in suppressing synaptic DA levels (West et al., 2003).

The data discussed in the previous paragraphs provide support for the notion that there are at least two distinct components of DA neurotransmission at the terminal level. The phasic component, driven primarily by bursting events at the level of the DA cell body, is highly compartmentalized and spatially limited by the DAT. Phasic DA transmission likely serves as an important teaching signal by modulating the changes in synaptic strengths of selected inputs to particular ensembles of medium spiny neurons in the striatum (O'Donnell, 2003; Schultz, 1998). In contrast, slower changes in tonic levels of DA are not influenced by bursting of DA neurons. Rather these levels are regulated by the overall levels of activity of the entire population of DA neurons, as well as by Glu-mediated mechanisms in the N.Acc. (West et al., 2003). Tonic DA communication, acting via volume transmission, would have an influence over a larger number of distributed medium spiny neurons in the N.Acc. This tonic release presumably serves a distinctly different function, both at the cellular and behavioural level, to that of intra-synaptic phasic DA transmission (West et al., 2003).

As noted above, both types of DA cell firing and neurotransmission are primarily regulated by glutamatergic neurons in the PFC (Grace, 1991, 2000). Stimulation of the PFC by local infusion of Glu agonists is known to increase DA levels within the PFC itself, while local infusion of Glu antagonists decreases DA levels within the PFC (Jedema & Moghaddam, 1996; Takahata & Moghaddam,

1998). Descending glutamatergic projections from the PFC are also known to modulate dopaminergic transmission in both the N.Acc. and VTA, where PFC stimulation and inactivation increases and decreases DA levels, respectively (Chergui, Charlety, Akaoka et al., 1993; Murase, Grenhoff, Chouvet et al., 1993; Overton & Clark, 1992; Sesack & Pickel, 1992; Tong et al.,1996). The PFC may regulate DA release in the N.Acc. via a direct cortex-accumbens pathway, or via an indirect cortex-VTA pathway. Support for the former mechanism is provided by studies showing that local application of GluR agonists to the N.Acc. increases DA release in this area (Imperato, Honoré, & Jensen, 1990; Youngren, Daly, & Moghaddam, 1993). In contrast, support for the latter mechanism is provided by evidence that the increase in DA release in the N.Acc. produced by electrical stimulation of the PFC occurs independently of GluRs located in the N.Acc. (Taber & Fibiger, 1995). Thus, GluRs in the N.Acc. do not appear to be involved in the regulation of subcortical DA release. These results provide support for the notion that PFC control of N.Acc. DA release occurs primarily through glutamatergic projections to the VTA and that these projections stimulate the release of accumbal DA. This mechanism is further supported by evidence that PFC stimulation-induced DA release in the N.Acc. is attenuated by application of GluR antagonists to the VTA (Murase et al., 1993; Taber, Das, & Fibiger, 1995), and increased by stimulation of GluRs of VTA dopaminergic neurons (Karreman, Westerink, & Moghaddam, 1996).

It must be considered, however, that the excitatory effect of PFC stimulation on mesoaccumbens DA cell activity may be produced not by a direct projection from the PFC to the VTA, but rather by an indirect projection in which the PFC activates another area, which in turn, projects to the VTA. Such an indirect mechanism is consistent with findings that PFC stimulation produces excitatory responses in VTA cells with a latency inconsistent with a monosynaptic projection (Tong et al., 1996). Thus, alternative sources of Glu that project to the VTA are a likely source. The PFC projects to the neurons of the pedunculopontine tegmentum (Oakman, Faris, Kerr et al., 1995; Sesack et al., 1989; Smith et al., 1996), habenular nucleus (Beckstead, 1979) and subthalamic nucleus (Kita & Kitai, 1987), each of which projects to the VTA and synapses onto DA neurons in primates (Oakman et al., 1995; Sesack et al., 1989; Smith et al., 1996).

Activation of mPFC neurons induces release of CCK, a Glu co-peptide, in the N.Acc. (You, Tzschentke, Brodin et al., 1998). While alternative extrinsic sources of CCK via the amygdala and nucleus tractus solitarus may contribute (Kresse, Reyes, Micevych et al., 1995), the mPFC is the largest source of CCK innervation to the N.Acc. (Kresse et al., 1995). Although there are glutamatergic projections from mPFC to DA CCK neurons in the VTA, which project to the N.Acc., mPFC stimulation does not elevate N.Acc. CCK by trans-synaptically

activating the mesoaccumbens pathway. Medial PFC stimulation is sufficient to cause Glu release in the N.Acc. and VTA, and the Glu release in the VTA is sufficient to cause DA release in the N.Acc., but not CCK release in the N.Acc. (You et al., 1998). This implies that the direct Glu-CCK pathway from the mPFC to the N.Acc. is the major source of the stimulation-induced CCK release in the N.Acc.

It has also been shown that GABA participates in the PFC regulation of subcortical levels of DA. The infusion of GABA antagonists into the PFC stimulates the release of DA in the N.Acc., an effect blocked by the application of GluR antagonists into the VTA (Karreman & Moghaddam, 1996). Furthermore, the blockade of GABAergic interneurons within the N.Acc. itself has been shown to increase striatal DA release (Karreman & Moghaddam, 1996). In addition, subcortical afferents other than Glu modulate DA levels within subcortical areas. For example, the ventral pallidum receives GABAergic inputs from the N.Acc. and in turn provides inhibitory GABAergic input to the VTA (Wu, Hrycyshyn, & Brudzynski, 1996; Zahm & Heimer, 1990), selectively regulating population activity of VTA DA neurons (Floresco et al., 2003). The pedunculopontine tegmental nucleus in the hindbrain sends glutamatergic and cholinergic projections to DA cell bodies (Oakman et al., 1995), selectively regulating burst firing of VTA DA neurons (Floresco et al., 2003). These manipulations cause

corresponding changes to DA release in both the N.Acc. and PFC (Clement & Grant, 1990; Floresco et al., 2003; Oakman et al., 1995). Infusions of GABAa/b or AchR antagonists, or NMDA/kainateR agonists, into the VTA produce an increase in extracellular levels of DA in the ipsilateral mPFC, while NMDA/kainateR antagonists into the VTA led to a decrease in extracellular levels of DA in the ipsilateral PFC (Westerink et al., 1998). These results indicate that mesocortical receptors at the level of the VTA contribute to both cortical and subcortical DA release.

Prefrontal cortical Glu- and GABA-based modulation of subcortical DA cell firing and release have been proposed to be an integral part of the pathophysiology in both psychiatric and physiological disorders, including schizophrenia, Parkinson's disease, and drug addiction.

2.2 Membrane Potential and Cell Firing

Like several other G-protein-coupled receptors (Hille, 1994), DARs can modulate neuronal excitability by altering the properties of voltage-dependent ion channels (Nicola et al., 2000). Because of this mode of action, deciphering the role of DA in regulating cellular activity has been more difficult than deciphering the role of conventional fast neurotransmitters that can be normally thought of as excitatory or inhibitory. The following summarizes some membrane physiology for neurons in the PFC, VTA, and N.Acc., as well as the role that DA and DARs

play in modulating fluctuations between states of hyperpolarization and depolarization in the mesolimbocortical system.

Prefrontal Cortex

Two main classes of pyramidal neurons have been described *in vitro*: regular spiking and intrinsic bursting cells (Kang & Kayano, 1994). *In vivo* studies revealed regular spiking cells that were either fast-adapting or slow-adapting (Dégenètais, Thierry, Glowinski, et al. 2002; Nuñez, Amzica, & Steriade, 1993) and intrinsic bursting cells that tended to be non-inactivating (Nuñez et al., 1993). *In vivo*, PFC pyramidal neurons exhibit a bi-stable membrane potential. A very negative resting membrane potential ('down' state) is interrupted by plateau depolarizations ('up' states) (Branchereau, van Bockstaele, Chan et al., 1996; Lewis & O'Donnell, 2000). During the 'down' state, no action potential firing occurs, but the 'up' state brings the membrane potential close to firing threshold (Lewis & O'Donnell, 2000). Thus, 'up' states can be interpreted as enabled periods in PFC neurons. Although the firing pattern of pyramidal neurons depends on their intrinsic membrane properties, data suggests that the ongoing spontaneous synaptic activity also participates in shaping their neuronal output activity (Dégenètais et al., 2002). Descending glutamatergic projections from the PFC are known to modulate GABA and DA neuron burst firing in the N.Acc. and VTA, respectively (Chergui et al., 1993;

Murase et al., 1993; Overton & Clark, 1992; Sesack & Pickel, 1992; Tong et al., 1996). The role of PFC Glu is supported by work showing that the application of Glu increases burst firing in VTA DA neurons, while application of NMDAR antagonists reduces the level of bursting (Cherugi et al., 1993; Overton & Clark, 1992). Furthermore, cooling the PFC (Svensson & Tung, 1989), or injecting the area with a local anesthetic (Murase et al., 1993), has been reported to reduce burst activity of VTA DA neurons.

Ventral Tegmental Area

Ventral tegmental area DA neurons of the anaesthetized rat are known to discharge action potentials in a pattern which consists of irregular single spikes or bursts of spikes with short inter-spike intervals, decreasing spike amplitude, and increasing spike duration (Au-Young & Yang, 1999; Overton & Clark, 1992). The stimulation of GluRs on VTA dopaminergic neurons has been shown to increase cell firing in the VTA (Wang & French, 1993). Furthermore, the train stimulation of Glu afferents on VTA dopaminergic neurons with pulses mimicking DA cell burst firing evokes cell firing and DA release in the forebrain (Gonon, 1988; Westerink et al., 1998), and a prolonged depolarization in mPFC pyramidal neurons resembling the 'up' state (Goto & O'Donnell, 2001, 2001a; Lewis & O'Donnell, 2000). The involvement of DA in this prolonged depolarization is supported by *in vitro* studies showing that DA can maintain depolarization during

tetanic stimulation of Glu afferents in rat PFC slices (Otani, Blond, Desce et al., 1998). These effects are reduced with application of a selective D_1R antagonist (Goto & O'Donnell, 2001a; Lewis & O'Donnell, 2000; Westerink et al., 1998). This is significant for theories of cortical learning and memory given that burst firing in the VTA seems to be correlated with reward-related cues (Romo & Schultz, 1990; Schultz, & Romo, 1990) and attention and motivational mechanisms (Schultz, 1998). Further, D_1Rs in the PFC are necessary for accurate performance in working memory tasks (Sawaguchi & Goldman-Rakic, 1994). The involvement of DA in these functions may be achieved by its promotion of 'up' events in PFC neurons. Supporting this is work which illustrates that it is easier to elicit increases in EPSPs in the PFC by hippocampal stimulation following a train of stimuli to the VTA (Jay, Burette, & Laroche, 1996), even though PFC neurons decrease their firing rate following VTA stimulation (Lewis & O'Donnell, 2000). A long-lasting VTA-evoked 'up' state may provide PFC neuron depolarization that is sufficient to facilitate NMDA-dependent synaptic plasticity.

It is also possible that activation of VTA GABA projection cells may also contribute to PFC membrane potential and bistability. Ventral tegmental area and PFC GABAergic projection neurons mediate burst firing of midbrain DA neurons via disinhibition by striatal and tegmental GABARs. While originally attributed to both GABAaRs and GABAbRs (Tepper, Martin, & Anderson, 1995), this is currently attributed to the functioning of GABAaRs (Westerink et al., 1998).

Glutamate of VTA origin may also be involved, as DA cells have been shown to have the capacity to release Glu *in vitro* in adolescent tissue (Sulzer, Joyce, Lin et al., 1998). Lastly, natural burst patterns of stimulation in the VTA have also been found to be effective in the release of co-localized peptide neurotransmitters in DA neurons. While the principal substance released from dopaminergic neurons is DA, they also contain and release neuropeptides such as neurotensin and CCK (Oéth & Lewis, 1992). Interactions of neuropeptides with DA do occur at the terminal fields of DA neurons which can alter DA release patterns, as well as postsynaptic responses of mPFC neurons (Bean & Roth, 1991).

Nucleus Accumbens

Medium spiny neurons of the N.Acc. have low to moderate spontaneous firing rates (Pennartz et al.,1994) and exhibit a bi-stable membrane potential very similar to that exhibited by PFC neurons (O'Donnell & Grace, 1995, 1998). N.Acc neurons undergo characteristic state transitions (~1 Hz) between a hyperpolarized and depolarized state (O'Donnell & Grace, 1995; Plenz & Kitai, 1998; Wilson, 1993; Wilson & Kawaguchi, 1996), and these shifts are highly correlated across cells (Stern, Jaeger, and Wilson, 1998). The 'down' state in N.Acc. medium spiny neurons occurs at -85 to -90mV, which characterizes physiologically 'silent' cells which do not generate action potentials (Groenewegen et al., 1999; O'Donnell & Grace, 1995). In order to activate the

cells, it is necessary to provide sufficient synchrony of input to achieve a depolarization that can remove resident inhibition in these GABAergic cells (Wilson, 1993). Occasionally, N.Acc. medium spiny neurons reach an 'up' state with a relatively depolarized membrane potential of approximately -55mV which approaches spike threshold (Groenewegen et al., 1999). However, the excitability of medium spiny neurons is also highly dependent on the relative timing of different active synaptic inputs. *In vivo* electrophysiological studies have shown that N.Acc. neurons require relatively large numbers of excitatory afferents to be simultaneously active in order to reach spike threshold (O'Donnell & Grace, 1995; Pennartz et al., 1994; Wilson, 1993). Thus, the additive effect of temporally correlated synaptic inputs is thought to induce a short-term increase in the intrinsic excitability of medium spiny neurons recorded in the N.Acc. (Goto & O'Donnell, 2002). This implies that spatial and temporal synaptic integration at N.Acc. neurons may be required to reach spike threshold large enough to compensate for resident GABAergic shunting. The naturally low firing rate (high spike threshold) for N.Acc. neurons highlights the important role of convergence in evoking accumbal spike firing. Nitric oxide-mediated synchronization of striatal neuron clusters may also be important for promoting this transition of bi-stable neurons to the depolarized 'up' state (O'Donnell & Grace, 1997). Weaker, less temporally coherent synaptic input typically fails to trigger an 'up' state transition, and the neuron falls back to the resting or 'down' state. Once a transition occurs, neurons can stay in the 'up' state for a variable period of time, sometimes

extending for a second or more (Wilson, 1993). In this state, spikes can be generated in response to further depolarization.

The 'up' state in N.Acc. neurons is also dependent upon population activity in hippocampal input. For example, the ventral hippocampus has been shown to induce N.Acc. neurons to switch to the depolarized state (Goto & O'Donnell, 2001; O'Donnell & Grace, 1995). In contrast, activation of the hippocampus does not elicit transitions to the 'up' state in PFC neurons (Lewis & O'Donnell, 2000), and hippocampal lesions fail to alter the frequency or duration of 'up' events in the PFC (O'Donnell, Lewis, Lerman, Weinberger et al., 1999). It is thought that the hippocampus' effect on N.Acc. neuronal membrane potential may occur via a high degree of topography of monosynaptic convergent inputs from the entorhinal cortex, subiculum, and CA1 areas of the hippocampus, and the infralimbic, and prelimbic subareas of the PFC, to individual medium spiny neurons of the N.Acc core and shell (Finch, 1996; French & Totterdell, 2000). It is only when the cells are in this depolarized state that stimulation of the afferents from the PFC evoke spike firing in medium spiny neurons, suggesting that hippocampal afferents to the N.Acc. gate the input from the PFC (O'Donnell & Grace, 1995), a function which may be amplified or attenuated by DA (Floresco, Todd, & Grace, 2001; Goto & O'Donnell, 2001).

Convergence and interactions of basolateral amygdala and hippocampal inputs are also found in the medial shell/core regions of the N.Acc. (Mulder,

Hodenpijl, & Lopes da Silva, 1998). Both hippocampal and amygdalar terminals in the N.Acc. synapse primarily onto dendritic spines (Meredith et al., 1993). Within this region of convergence, activation of a single pathway before the other results in a modulation of the response to the latter, either an enhancement in the response to activation of the hippocampus-N.Acc. pathway when stimulation is applied to the amygdala, or a reduction in the response to activation of the amygdala-N.Acc. pathway when stimulation is applied to the hippocampus (Mulder et al., 1998). Enhanced EPSPS in the hippocampus are accompanied by reduced EPSPs in N.Acc. neurons evoked by stimulation of the non-tetanized amygdala-to-N.Acc. pathway. These interactions were recorded in regions of the N.Acc. where hippocampal-amygdalar convergence is encountered (Mulder et al., 1998). It is noteworthy that these amygdalar-hippocampal areas of convergence closely correspond to the N.Acc. targets of VTA output (Wright et al., 1996).

Retrograde tracing studies indicate regions of overlap such that discrete regions of the N.Acc. may receive direct input from all of the areas mentioned in the above paragraph (Brog et al., 1993). This convergence is supported by evidence that each of these input regions can elicit responses in the same regions of the N.Acc. (Finch, 1996). However, stimulation of the PFC is more effective than other sites in producing an elevated excitatory response in otherwise silent N.Acc. cells (Finch, 1996) and 'up-down' oscillations in N.Acc. medium spiny neurons are correlated with frontal cortical electroencephalograms

(Mahon, Deniau, & Charpier, 2001). The pattern of inputs indicates that the N.Acc. likely receives information relevant to attention and cognition (PFC), emotion (amygdala), and explicit memory (hippocampus), with the heaviest weighting given to PFC inputs. The N.Acc. may play a role beyond the mechanisms of motor production. In particular, disruption of accumbal processing may contribute to cognitive, affective, and behavioural pathology.

It has been reported that DA can either excite or inhibit N.Acc. neurons (Nicola et al., 2000). This may be partly due to the differences in time course and spatial profile of synaptically released DA compared to that achieved with bath application, ionotophoresis, electrical stimulation, or chemical stimulation. In any case, *in vitro* studies of dopaminergic effects on medium spiny neuron function have produced mixed results. Some studies from N.Acc. slices found no DA-related changes in post-synaptic properties (Nicola, Kombian, & Malenka, 1996; Nicola & Malenka, 1997; Pennartz, Dolleman-Van der Weel, Kitai et al., 1992), whereas others observed significant, yet inconsistent, DA-dependent changes in resting membrane potential (O'Donnell & Grace, 1996; Uchimura, Higashi, & Nishi, 1986; Uchimura & North, 1990). The latter results are supported by findings that augmentation of endogenous DA transmission following electrical or chemical stimulation of the VTA contributes to shifts in membrane potential in medium spiny neurons and depolarizes their membrane potential (Goto & O'Donnell, 2002). Also, increases in endogenous DA in the N.Acc. that accompany tetanic stimulation of the hippocampus or amygdala

potentiates firing activity in N.Acc. evoked by the hippocampal or amygdalar inputs, respectively. This effect is blocked by D_1R or NMDAR antagonism (Floresco, Blaha, Yang, et al., 2001, 2001a, 2001b). However, *in vivo* anaesthetized studies have reported that VTA stimulation, DA ionotophoresis, or dopaminergic drug administration reduces stimulus-evoked excitatory responses from either hippocampal (DeFrance, Sikes, & Chronister, 1985; Yang & Mogenson, 1984) or amygdaloid afferents (Floresco et al., 2001; Pennartz, et al., 1994; Yang & Mogenson, 1984; Yim & Mogenson, 1982).

Recent experiments in which the activity of cells in the N.Acc. were recorded in the awake, behaving animal have produced results that are largely consistent with the picture emerging from the *in vitro* literature. The first such studies combined single-unit recording with ionotophoresis of DA in monkeys (Rolls, Thorpe, Boytim et al., 1984) and in rats (Kiyatkin & Rebec, 1996). The net effect of DA was to enhance evoked firing relative to spontaneous 'noise' firing (Kiyatkin & Rebec, 1996; Rolls et al., 1984). The notion that DA increases the signal-to-noise ratio for evoked activity is consistent with several other observations. For instance, ionotophoresis of DA onto striatal neurons of awake rats can be either excitatory or inhibitory. Cells that responded during motor behaviour tended to be excited by DA, whereas cells that were quiescent during the animal's movements were usually inhibited (Pierce & Rebec, 1995). These results can be interpreted within the context of the known effects of signals arriving over the various input pathways on the intrinsic properties of medium

spiny neurons. That is, the response to strongly convergent excitatory input in the motor-related neurons was enhanced, whereas the response to weak or temporally dispersed excitatory input ('noise') in the neurons, unrelated to the movement, was suppressed. Similar observations have been made in studies of N.Acc. neurons during cocaine self-administration in rats. Lever-pressing to receive doses of psychostimulants tends to be rapid at the beginning of a self-administration session, and then slow as the level of DA in the N.Acc. plateaus (Koob, 1992; Wise, Newton, Leeb et al., 1995). The elevated level of DA achieved during the plateau phase, which generally suppresses activity unrelated to the operant task, provides a background against which the increased N.Acc. activity specifically related to lever-press behaviour stands out (Peoples, Uzwiak, Guyette et al., 1998).

There is agreement between the *in vitro* and *in vivo* literature that once the 'up'-state transition in the N.Acc. has been achieved, D_1R stimulation enhances evoked activity. Evidence has accumulated that the activation of D_1Rs by endogenous DA appears to facilitate membrane depolarization in medium spiny neurons, and enhances spontaneous and evoked activity when the cell is strongly depolarized (Harvey & Lacey, 1996, 1997; Higashi, Inanaga, Nishi, et al, 1989; Nicola & Malenka, 1997, 1998; O'Donnell & Grace, 1994; Pennartz et al., 1992; West et al., 2003). For example, *in vivo* chemical stimulation of the VTA or burst firing of DA neurons induces increased activity in N.Acc. neurons by activating D_1Rs (Gonon, 1997; Gonon & Sundstrom, 1996). This facilitatory D_1R-

mediated effect is particularly relevant when the neuron is in the depolarized 'up' state, as it has been shown that intra-striatal infusion of a D_1R antagonist depresses the amplitude of naturally occurring 'up' states and decreases the maximal depolarized membrane potential measured in the 'up' state (Gonon & Sundstrom, 1996; West et al., 2003; West & Grace, 2002). Dopamine also has been found to depress inhibitory synaptic transmission in the N.Acc., likely via a presynaptic D_1R (Nicola & Malenka, 1997). Other studies, however, have shown that D_1R activation depresses the EPSPs of N.Acc. neurons recorded during stimulation of hippocampal and amygdalar afferents, yet facilitates the hippocampus-evoked response in a small subset of N.Acc. medium spiny neurons (West et al., 2003). Dopamine D_1R activation also reduces GABAaR currents in medium spiny neurons (Flores-Hernandez, 1997) and facilitates NMDAR-mediated responses (Cepeda & Levine, 1998). Taken together, these findings suggest that D_1R activation suppresses the response to weak excitatory synaptic input, making it more difficult to make the transition from 'down'- to 'up'-states. However, once the 'up'-state transition has been achieved, D_1R stimulation appears to enhance evoked activity. In conclusion, the net effect of DA acting on D_1Rs may be to enhance the activity of cells that receive highly convergent excitatory input, while the activity in cells receiving less temporally coherent inputs is depressed. This would result in focusing activity in the specific ensembles of medium spiny neurons that are receiving the greatest, and presumably, most important, inputs from other brain regions (Nicola et al., 2000).

A major limitation of this hypothesis is that it does not take into account the effects of DA on other subtypes of DAR or on other cell types within the striatum and N.Acc.

Endogenous release of DA can also modulate medium spiny neurons via a D_2R-mediated inhibition (Liang, Wu, Yim et al., 1991; Yang & Mogenson, 1984; Yim & Mogenson, 1982, 1986, 1988). Initial studies using intracellular recording in medium spiny neurons found that DA or cocaine (a D_2R agonist) causes a D_1R-mediated hyperpolarization and a D_2R-mediated depolarization, respectively (Higashi et al., 1989; O'Donnell & Grace, 1994; Uchimura et al., 1986; Uchimura & North, 1990). This is supported by later work showing that D_2R stimulation does not attenuate N.Acc. neuron EPSPs evoked following the stimulation of either amygdalar or hippocampal afferent systems (West et al., 2003). Conversely, in the intact animal D_2R blockade depolarizes both the 'up' and 'down' state membrane potentials of medium spiny neurons, and increases medium spiny neuron responsiveness to depolarizing current and electrical stimulation of the PFC (West & Grace, 2002).

Together, these observations provide evidence, albeit inconsistent, for DAR subtype-specific modulation of glutamatergic inputs to the N.Acc. Perhaps the inconsistency is due to D_1 and D_2 receptors acting synergistically in the N.Acc. in either excitatory or inhibitory modes depending upon the source of stimulation. Also, *in vitro* enhancement of medium spiny neuron firing in the

N.Acc. shell has been shown to require co-activation of D_1Rs and D_2Rs, because neither agonist alone modifies spike firing, and the effect of DA is inhibited by either a D_1R or a D_2R antagonist (Hopf, Cascini, Gordon et al., 2003). Further, the duration of VTA-evoked 'up' states in the N.Acc. is reduced by combined administration of D_1R and D_2R antagonists (Goto & O'Donnell, 2001a). These results suggest that while DA may modulate 'up' events in the PFC via D_1Rs, a similar effect of DA in the N.Acc. requires co-activation of D_1Rs and D_2Rs. Conversely, studies using brain slice preparations of the N.Acc. have shown that DA and psychostimulants depress excitatory synaptic transmission via D_1R- and D_2R-mediated mechanisms (Harvey & Lacey, 1996; Hu & White, 1994; Nicola et al., 1996; Wachtel, Hu, Galloway et al., 1989; Zhang, Cooper, & White, 2002).

These results may provide a cellular mechanism to explain observations from behavioural studies suggesting a cooperative activation of D_1Rs and D_2Rs in the N.Acc. For example, rats will self-administer D_1R and D_2R agonists into the N.Acc. in combination, but will not self-administer either alone (Ikemoto, Glazier, Murphy et al., 1997). Furthermore, synergistic and additive effects of D_1R and D_2R activation in the N.Acc. have been reported by studies of conditioned reinforcement (Chu & Kelley, 1992; Wolterink, Phillips, Cador et al., 1993), amphetamine self-administration (Phillips, Robbins, & Everrit, 1994), and evaluating the relative cost of obtaining a reward (Koch, Schmidt, & Schnitzler, 2000; Nowend, Arizzi, Carlson, et al., 2001).

CHAPTER 3

SYNAPTIC PLASTICITY

Long-term potentiation (LTP) and long-term depression (LTD) are activity-dependent persistent increases and decreases in synaptic strength, respectively. Together, these phenomena serve as the prevailing model of information storage, but there is still considerable debate concerning their exact role in learning and memory formation. The occurrence of these phenomena is correlated with performance on various cognitive tasks, supporting the hypothesis that LTP and LTD represent the neural form of learning and memory. This two-way phenomenon illustrates that there is, as predicted by connectionist modelers, bidirectional control of synaptic strength. This allows for some degree of protection in the storage capacity of neuronal circuits. These phenomena also appear to be involved in the modification of neural circuitry that occurs during development.

3.1 Long-Term Potentiation and Long-Term Depression

In 1973, Timothy Bliss and Terje Lømo demonstrated that a high-frequency train of electrical stimulation (tetanus) applied to the perforant pathway between the entorhinal cortex and hippocampus produces a long-lasting increase in the amplitude of the population EPSP evoked in the dentate gyrus. This response enhancement was given the name LTP. The induction of LTP leaves neurons

increasingly responsive to afferent input over the activated lines, so that a constant level of test stimulation produces a stronger response. Only the activated lines are affected, illustrating that the mechanism is synaptic, and the increased synaptic efficacy lasts from days to months. *Long-term depression* is produced by the application of a repetitive low-frequency train of electrical stimulation to afferent fibres, resulting in a reduction in the amplitude of the EPSP. These stimuli appear to be particularly effective in returning potentiated synapses toward their previous level of connection strength prior to LTP induction. Thus, neural networks are shaped, in part, by the pattern of LTP and LTD induction in the circuitry.

An important requirement for the induction of LTP is that the pre- and postsynaptic neurons be coactive. Thus, LTP appears to be a physiological instantiation of the *Hebb Rule*: "When an axon of cell A...excite[s] cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased" (Hebb, 1949). To date, LTP and LTD have been documented many times in *in vitro* and *in vivo* preparations, primarily in cortical systems such as the hippocampus and neocortex (Chapman, Trepel, Ivanco et al., 1998; Doyère, Errington, Laroche et al., 1996; Froc, Chapman, Trepel et al., 2000; Froc & Racine, 2005; Huang, Rowan, & Anwyl, 1997; Ivanco & Racine,

2000; Ivanco, Racine, & Kolb, 2000; Kulla & Manahan-Vaughan, 2000; Kulla, Reymann, & Manahan-Vaughan, 1999; Rick & Milgram, 1996; Trepel & Racine, 1992, 1998, 2000).

Long-term potentiation has been divided into an induction phase and a maintenance phase. The induction phase refers to the first steps which characterize the triggering mechanisms for these phenomena. During this phase, the activated axons release the excitatory neurotransmitter Glu, which binds predominantly to a variety of GluRs. The AMPAR is fast and accounts for a large proportion of the depolarization in most brain neurons. The receptor that is most responsible for the modifiability of the glutamatergic synapses is the NMDAR. NMDARs are typically located on the spines of dendrites which are specialized to receive excitatory synaptic input (Ballard, Pauly-Evers, Higgins et al., 2002). The NMDAR channel is normally blocked by the presence of magnesium (Mg^{+}) ions, but becomes unblocked when the postsynaptic cell is depolarized via presynaptic neuronal input. The release from the Mg^{+} block allows the receptor to become activated (Ballard et al., 2002). Thus, the NMDAR represents a doubly gated channel: it is gated both by activation of the NMDAR by Glu binding (the presynaptic contribution), and by membrane depolarization (the postsynaptic contribution) which removes the Mg^{+} blockade. This double gating provides the synapse with its Hebbian properties. Once the NMDA channel is unblocked and activated, diffusion of calcium (Ca^{2+}) and

(Na^+) across the plasma cell membrane occurs (Ballard et al., 2002). The Ca^{2+} is particularly important in triggering the downstream pathways that lead to an enduring change in connection strength.

The maintenance phase of LTP requires kinase activation and *de novo* protein synthesis, and possibly new synapse formation, a process which sustains this phenomenon for days to months. Glutamate activates adenylyl cyclase which converts adenosine 3′, 5′ monophosphate (AMP) to cyclic AMP (cAMP) (Nicoll, Kauer, & Malenka, 1988). Cyclic AMP activates cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), and Ca^{2+}-calmodulin-dependent kinase II (CaMK II) (Nicoll et al., 1988). The enzyme CaMKII phosphorylates the AMPAR (Nicoll et al., 1988). Phosphorylation makes the AMPAR more permeable to Na^+ ions, thus, lowering the resting membrane potential of the cell and making it more sensitive to incoming impulses (Nicoll et al., 1988). The phosphorylation of AMPARs also leads to its externalization and insertion into the postsynaptic membrane (Malinow & Malenka, 2002; Wolf, Sun, Mangiavacchi et al, 2004). Thus, insertion of the Glu1R subunit of the AMPAR into the postsynaptic membrane is often used as a 'marker' for LTP (Wolf et al., 2004). Calcium influx also activates a second messenger that causes the release of a retrograde plasticity factor, possibly nitric oxide, from the dendritic spines of the active postsynaptic neuron (Harvey & Lacey, 1997). This retrograde messenger diffuses into the presynaptic terminals

to activate additional second messengers that further maintain LTP by way of additional neurotransmitter release (Harvey & Lacey, 1997).

3.2 Dopaminergic Modulation of Hippocampal Synaptic Plasticity

While LTP and LTD in the hippocampus have been demonstrated mainly at glutamatergic synapses, DA has been shown to modulate these phenomena. The CA1-subiculum region of the hippocampus receives dopaminergic input from the substantia nigra and VTA (Gasbarri, Packard, Campana et al., 1994). The persistence of CA1 LTP (Frey, Matthies, Reymann et al., 1991; Gurden, Takita, & Jay, 2000; Huang & Kandel, 1995; Li, Cullen, Anwyl et al., 2003; Otmakhova & Lisman, 1996; Swanson-Park, Coussens, Mason-Parker et al., 1999) and dentate gyrus LTP (Kusuki, Imahori, Ueda et al., 1997) has been prevented with D_1R-like antagonists and 6-hydroxydopamine lesions of dopaminergic inputs to the hippocampus (Yang, Lin, Yen et al.,2002). Further, the reduction in CA1 LTP has been reversed with the administration of D_1R-like agonists (Li et al., 2003; Swanson-Park et al., 1999). The involvement of D_1Rs in LTP of the Schaffer collateral-commissural pathway of CA1 neurons has been highlighted using D_1R-deficient mice. These mice show reduced LTP compared to wild type controls, demonstrating the necessity of D_1R activation for LTP maintenance (Matthies, Becker, Schroeder et al., 1997). These findings support the notion that DA modulates hippocampal LTP at D_1Rs. Stimulation in the presence of D_2R

agonists, which are negatively coupled to adenylate cyclase, have been reported to have no effect on hippocampal LTP (Huang & Kandel, 1995; Manahan-Vaughan & Kulla, 2003). Conversely, however, the administration of D_2R-like agonists dose-dependently inhibits basal synaptic transmission and LTD in the dentate gyrus *in vivo*, effects which are prevented by D_2R-like antagonists (Manahan-Vaughan & Kulla, 2003). These results suggest a specific role for DA receptors in the regulation of LTD and LTP *in vivo*.

3.3 LTP and LTD in the Mesolimbocortical System

Both LTP and LTD can be elicited in the N.Acc., VTA, and PFC, but little work on their underlying mechanisms has been done, compared to that in the hippocampus. The majority of LTP and LTD research in the mesolimbocortical system has been done in the *in vitro* slice preparation: cortico-accumbal slices for the N.Acc., midbrain slices for the VTA., and neocortical slices for the PFC. A few *in vivo* studies in the acute anaesthetized preparation have been reported, but no work has been published on LTD or LTP in chronic *in vivo* fully awake preparations. The NMDAR-dependency requirements in these systems is not entirely certain, but generally, the story is consistent with more thoroughly tested systems such as the hippocampus. A number of studies have considered the role of modulatory inputs (Jay, 2003). So far, the strongest evidence for such modulation comes from work on the DA systems.

Prefrontal Cortex

The effect of DA upon the function of glutamatergic PFC neurons has been diverse, ranging from reductions to increases in glutamatergic efficacy (Blond, Crepel, & Otani, 2002; Gao, Krimer, & Goldman-Rakic, 2001; Gurden et al., 2000; Gurden, Tassin, & Jay, 1999; Hirsch & Crepel, 1991; Jay et al., 1995; Law-Tho, Hirsch, & Crepel, 1994; Otani, Auclair, Desce et al., 1999; Otani et al., 1998; Pantelis et al., 1997). The bi-directionality of these results is presumably a reflection of the underlying diversity of the pharmacological and biochemical actions of DA itself. As a neuromodulator, DA acts to increase or decrease the activity of targeted neurons.

As in most other cortical areas, synaptic plasticity in the PFC is mediated by the NMDAR. Among neuromodulators, the role of the D_1R in PFC synaptic plasticity has gained most of the attention, presumably because this is the predominant DA receptor type in the cortex. The hippocampal-PFC pathway expresses LTP and LTD (Izaki, Takita, & Akema, 2003), the production of which is thought to be dependent upon intact mesocortical dopaminergic input. *In vitro* animal research has shown that DA inhibits NMDAR-dependent LTP and facilitates LTD in the PFC via D_1R s (Hirsch & Crepel, 1991; Jay et al., 1995; Law-Tho et al., 1994, 1995; Otani et al., 1998; Otani et al., 1999; Otani, Daniel, Roison et al., 2003; Tseng & O'Donnell, 2003). However, alternative evidence suggests that DA facilitates LTP in the slice preparation. In this case, DARs must be pre-stimulated ('primed') before the application of high-frequency

stimulation in the presence of DA (Matsuda, Marzo, & Otani, 2006; Otani et al., 1999). However, acute *in vivo* anesthetized preparations show that increases in endogenous DA in the PFC, via stimulation of the VTA and ventral hippocampus, produces increases in hippocampal-PFC LTP without priming (Gurden et al., 1999, 2000; Huang & Kandel, 1995; Mulder, Arts, & Lopes da Silva, 1997; Otani et al., 1999). This *in vivo* effect is mimicked by infusion of the adenylate cyclase activator forskolin (Gurden et al., 2000) and other cAMP agonists (Huang & Kandel, 1995), while blocked by the administration of protein synthesis inhibitors (Huang & Kandel, 1995) and D_1R antagonists (Gurden et al., 2000; Huang & Kandel, 1995). Further, in the acute *in vivo* anaesthetized preparation, extracellular levels of PFC DA significantly increase during and after hippocampal LTP (Gurden et al., 2000), suggesting that DA may modulate the storage of information previously processed by the hippocampus in the PFC.

NMDARs in the PFC share a close spatial arrangement with D_1Rs, both of which have been shown to regulate working memory and choice behaviour (Sawaguchi & Goldman-Rakic, 1994; Seamans et al., 1998; Williams & Goldman-Rakic, 1995). Since LTP in the PFC requires NMDARs (Hirsch & Crepel, 1991; Jay et al., 1995) and is modulated via D_1Rs (Gurden et al., 1999, 2000), activation of both receptor types is likely necessary for normal cognition regulated by the PFC. The requirement of coincident activation of D_1R s and NMDARs in the PFC for the acquisition of appetitive operant conditioning in the rat (Baldwin, Sadeghin, & Kelly, 2002) suggests that both receptor types are

required for learning and memory. It has also been shown that stress blocks LTP in the mPFC following *in vivo* tetanization of the basolateral amygdala (Maroun & Richter-Levin, 2003). This highlights the interaction between the emotional state of an animal and its ability to learn and remember. This has particular relevance for psychiatric conditions where PFC aberrations are thought to underlie learning and memory impairment. For example, recent evidence indicates that abnormal NMDAR-mediated neurotransmission in the PFC may be etiologically relevant to schizophrenia. According to this NMDAR-hypofunction model, NMDARs operate at a deficient level in the PFC, thereby contributing to cognitive impairment (Laruelle et al., 2003; Lewis et al., 2003; Meyer-Lindenberg et al., 2002; Moghaddam & Jackson, 2003). This model is supported by evidence that NMDAR antagonists produce schizophrenic symptomatology in non-schizophrenic healthy individuals and drug addicts (Krystal, Karper, Seibyl et al., 1994) and exacerbates symptoms in schizophrenic individuals (Lahti, Koffel, LaPorte et al., 1995; Seeman, Weinshenker, Quiron et al., 2005). Furthermore, administration of NMDAR agonists ameliorates NMDAR antagonist-induced cognitive impairment, such as disruptions in sensorimotor gating and associative learning (Coyle, Tsai, & Goff, 2003; Lipina, Labrie, Weiner et al., 2005; Zajaczkowski, Czyrak, & Wedzony, 2003). The neuregulin-1 (NRG1) model of schizophrenia posits that NRG1 may be a susceptibility gene for schizophrenia (Stefansson, Sigurdsson, Steinhorshdottir et al., 2002). Neuregulin-1 regulates the expression of NMDARs by activating a tyrosine kinase that is colocalized with

the NMDAR (Ozaki, Sasner, Yano et al., 1997). Mutant mice heterozygous for NRG1 show reduced levels of NMDAR expression and a behavioural phenotype that overlaps with the NMDAR-hypofunction model (Stefansson et al., 2002).

Nucleus Accumbens

Evidence of both LTP and LTD in the N.Acc. demonstrates that there is also bidirectional control of synaptic strength at these connections. In fact, in a single N.Acc. cell the same change in calcium concentration can have opposite effects: LTP of non-NMDAR-mediated responses, and LTD of NMDAR-mediated responses (Jones, Kornblum, & Kauer, 2000; Kombian & Malenka, 1994). This finding implies that *in vitro* bi-directional plasticity in the N.Acc. is dependent upon, and perhaps modulated by, different receptor groups. In both *in vivo* (Peasey-Truger & Bruggencate, 1994) and *in vitro* (Pennartz, Ameerun, Groenewegen et al., 1993) preparations, high-frequency stimulation of cortical inputs to the N.Acc. elicits LTP in the N.Acc. which is attenuated with the administration of NMDAR antagonists. A similar debate over the role of the NMDAR in accumbal LTD exists. In some instances LTD in the N.Acc. is unaffected by an NMDAR blocker (Pennartz et al., 1993), while in others it seems to require NMDAR activation (Thomas, Malenka, & Bonci, 2000).

The role of DA, in particular D_1Rs, in accumbal LTP and LTD is also controversial. It has been shown that neither N.Acc. LTP (Pennartz et al., 1993) nor LTD (Thomas et al., 2000) is affected by the application of DA, and at least in

some cases, activation of D_1Rs does not seem to be required for the generation of either LTP or LTD (Pennartz et al., 1993; Thomas & Malenka, 1999; Thomas et al., 2000). Actually, the activation of D_1Rs has been shown to reduce NMDAR-mediated responses in the N.Acc. (Nicola et al., 1996). Yet, DA application in other instances has been shown to enhance NMDAR-mediated responses via D_1Rs (Cepeda, Colwell, Itri, et al., 1998) which have been shown to be necessary for accumbal LTP and LTD induction (Calabresi, Gubellini, Centoneze et al., 2000).

As for the role that D_2Rs play in the direction of accumbal synaptic plasticity, a few studies have tested the effects of psychostimulants (D_2R agonists). In one of these, N.Acc. slices were prepared from rats sensitized to cocaine *in vivo* (Thomas, Beurrier, Bonci et al., 2001). The ratio of AMPAR- to NMDAR-mediated excitatory post synaptic currents (EPSCs) was decreased at synapses made by PFC afferents onto medium spiny neurons in the N.Acc. shell. The amplitude of EPSCs at these synapses was also decreased, as was the magnitude of LTD. These data suggest that chronic *in vivo* administration of cocaine elicits a depression of excitatory synaptic transmission in the N.Acc. D_2R agonism may increase glutamatergic input to the N.Acc., leading to an activation of local N.Acc. GABAergic neurons as well as their efferent GABAergic target neurons in the VTA. Ultimately, activation of this GABAergic loop would disinhibit VTA DA neurons. Thus, psychostimulants may remove the normal inhibition of the glutamatergic drive onto DA neurons, and the depression of

excitatory synaptic transmission in the N.Acc. may be integral to how psychostimulants usurp the normal functioning of the mesolimbocortical reward pathway and lead to addiction and psychosis.

Ventral Tegmental Area

The first report of LTP in a VTA slice preparation came from Bonci and Malenka (1999) who used midbrain slices to test the responses of both local dopaminergic and GABAergic neurons to various patterns of stimulation. Paired pulse and medium frequency trains were shown to produce transient depression in DA neurons and facilitation in GABA neurons. When stimulation was paired with postsynaptic depolarization, small LTP effects were induced in DA neurons, but not GABA neurons, implying that cell type is important for tegmental plasticity. This is consistent with previous work in the hippocampus showing little or no LTP in excitatory synapses on GABA interneurons (Maccaferri & McBain, 1996). Excitatory synapses on VTA DA neurons in slices have also been shown to express LTD that was independent of both NMDARs (Jones et al., 2000) and D_2Rs (Thomas et al., 2000), but dependent on both AMPARs and PKA. These results are consistent with a D_1R-dependent mechanism.

The role of the D_2R in N.Acc. synaptic plasticity seems a little more clear than that of the D_1R. A single *in vivo* exposure to the D_2R agonist cocaine induces LTP of AMPAR-mediated currents at excitatory synapses onto DA cells, sustainable for 5 days following the termination of cocaine exposure (Ungless,

Whistler, Malenka et al., 2001). This effect is blocked when an NMDAR antagonist is administered with cocaine (Ungless et al., 2001). These results show that a prominent form of synaptic plasticity can be elicited by a single *in vivo* exposure to a D_2R agonist, and therefore, may be involved in the early stages of the development of drug addiction. This work supports previous work showing that VTA DA neurons exhibit enhanced responsiveness to AMPA Glu stimulation after chronic psychostimulant exposure (Nestler & Aghajanian, 1997). It is thought that this is mediated by increased VTA and N.Acc. AMPAR expression following chronic psychostimulant administration.

The D_2R also plays a role in VTA LTD. *In vitro*, VTA DA neurons exhibit NMDAR-independent LTD in response to low-frequency stimulation and modest depolarization (Jones et al., 2000). Further, VTA LTD at glutamatergic synapses is blocked by brief exposure to the general D_2R agonist amphetamine (Jones et al., 2000). This work is significant because it shows that by inhibiting LTD, DA agonization may contribute to the removal of the normal brake on the glutamatergic drive to DA neurons. This may be an important mechanism by which normal function of the brain reward system may be impaired in neuropsychiatric conditions such as drug addiction and schizophrenia. However, it has also been shown that LTD at excitatory synapses onto DA cells is enhanced by *in vivo* cocaine exposure (Ungless et al., 2001). Again, the DAR story is not consistent.

CHAPTER 4

PSYCHOSTIMULANTS

4.1 Reward System and Psychostimulants

Our current understanding of the brain circuitry through which various rewards gain control over behaviour has developed from studies of brain stimulation which revealed that the brain has specialized 'centres' for reward functions (Olds & Milner, 1954). Due to its ubiquitous involvement in the regulation of reward-related behaviour, the mesolimbocortical system has been characterized as the substrate of reward. Specifically, the dopaminergic projections from the VTA to the N.Acc. (Koob, 1992; Spanagel & Weise, 1999) and PFC (Bardo, 1998; Spanagel & Weiss, 1999) are considered to represent a crucial part of the reward system, and it is thought that virtually all addictive drugs enhance dopaminergic neurotransmission in these pathways. The mesolimbocortical system utilizes endogenous DA that is agonized or mimicked by drugs of abuse.

Psychostimulants, such as cocaine and amphetamine, induce many effects on the mesolimbocortical system, including increased synthesis of DA, increased release of DA (Calabresi, Maj, Pisani et al., 1992; Hope, Kosofsly, Hyman et al., 1992), and high-affinity binding to the presynaptic DAT (Hope et al., 1992). For example, cocaine blocks the DAT, a member of the family that

also includes the norepinephrine and serotonin transporters (Genova et al., 1997; Heikkila, Orlansky, & Cohen, 1975a), and amphetamine causes reverse transport of DA through the DAT (Heikkila, Orlansky, Mytilneou et al., 1975b). The rewarding effects of these agents are attenuated by selective DA antagonists only, and not by selective noradrenergic (Yokel & Wise, 1975) or serotonergic (Lyness & Moore, 1983) antagonists. The net effect of blocking the DAT is an elevation of the extracellular DA concentrations in the terminal region of midbrain dopaminergic neurons (Koob, 1992).

Psychoactive drugs elicit intense euphoria, increased energy, and sympathetic arousal in those who use them (Genova et al., 1997). However, the increases in DA during psychostimulant use cannot explain the process of addiction since drugs of abuse increase DA both in addicted and non-addicted subjects. Moreover, the magnitude of the drug-induced DA increases is smaller in the addicted than in non-addicted subjects (Volkow, Wang, Fowler et al., 1997). There is also no evidence that the pleasurable response to drugs is the important variable, since it is not necessarily more intense in addicted than in non-addicted subjects (Volkow et al., 1997). Yet, because drug addiction requires chronic drug administration, it is possible that addiction results from the repeated perturbation of the DA system – marked increases followed by marked decreases – and the consequential disruption of the circuits that it regulates.

4.2 Behavioural Sensitization

Pharmacologists use the term sensitization to refer to an increase in a drug effect with repeated drug administration. In other words, the change in drug effect is in the opposite direction as seen with the development of tolerance (a decrease in a drug effect with repeated administration). Repeated administration of psychostimulants produces a more pronounced (sensitized) increase in DA levels than the increase seen after acute administration (Kalivas & Stewart, 1991). This sensitized increase in DA levels is accompanied by a change in behavioral response, called behavioural sensitization. This phenomenon refers to the intensification of behaviour upon repeated exposure to a stimulus, and in the context of addiction research, this stimulus is the repeated administration of a drug. Behavioural sensitization is seen in the addicted state, and is symptomatically represented in particular psychopathologies in the drug-free state (Deutsch, Rosse, Billingslea, et al., 2002). Thus, behavioural sensitization is a useful animal model of the neuroadaptations underlying the addictive and psychotogenic effects of psychostimulant drugs involved in the initial stages of human psychostimulant addiction and psychostimulant-induced (schizophreniform) psychosis (Hiroi, Brown, Haile, et al., 1997).

In humans and animals, behavioural sensitization is evident as an increase in arousal, attention, and motor behaviour, producing heightened locomotion, exploration, and approach (Szumlinski, Goodwill, & Szechtman,

2000). In humans and animals receiving higher doses, psychomotor effects can also include intense repetitive stereotyped movements (Wise & Bozarth, 1987) as well as delusions and hallucinations in humans (Szumlinski et al., 2000). The relevance of animal models of behavioural sensitization for psychopathological conditions is evident, as many express sensitization symptoms in the drug-free state. For example, there is evidence of heightened locomotion and stereotypy in schizophrenia, mania, and obsessive compulsive disorder; autistic disorder, and Tourette's Syndrome (Deutsch et al., 2002).

The study of behavioural sensitization has provided a great deal of information about factors that influence its induction, development, and expression. These include genetic, hormonal, and experiential determinants of individual differences in susceptibility to behavioural sensitization, the roles of pharmacological factors such as drug dose, and of psychological factors such as learning and stress (Robinson & Becker, 1986; Stewart & Baldini, 1993). For example, it has been shown that behavioural sensitization is produced by various drugs of abuse, including amphetamines, cocaine, opiates, methylphenidate, ethanol, phencyclidine, and nicotine. It has also been shown that sensitization is strongest when high or escalating doses are given, especially when the drug is administered rapidly (Samaha, Li, & Robinson, 2002) and intermittently (Robinson & Berridge, 2003). One of the most important features of behavioural sensitization is its remarkable persistence. In animals, behavioural sensitization, especially motoric activation, can persist for months to years after drug treatment

is discontinued (Paulson, Camp, & Robinson, 1991). Motoric sensitization has also been described in humans which can last most of a lifetime (Strakowski & Sax, 1998; Strakowski, Sax, Setters et al., 1996).

Whether behavioural sensitization is linked to enhanced rewarding efficacy has been considered, but is still not certain. Alternative hypotheses postulate that repeated drug use leads to a progressive and persistent hypersensitivity of neural systems that mediate 'incentive salience' – the properties that make them 'wanted' - which results in excessive craving (De Vries, Schoffelmeer, Binnekade, et al.,1998; Robinson & Berridge, 2003). The central idea is that addictive drugs persistently alter N.Acc.-related brain systems that mediate a basic incentive-motivational function, the attribution of incentive salience. As a consequence, these neural circuits may become persistently hypersensitive (or 'sensitized') to specific drug effects, such as DA agonization, and to drug-associated stimuli. This brain change, called neural sensitization, is proposed to lead to excessive attribution of incentive salience to drugs and drug-cues (ie., the environment where drugs are taken, the time of day drugs are taken, or the paraphernalia people take drugs with). This neural sensitization is proposed to cause psychopathological 'wanting' to take drugs. By way of sensitization, drug-associated stimuli are endowed with increasing incentive salience up to the point at which the urge to take the drug becomes so powerful that it gains control over and suppresses voluntary behaviour (Robinson &

Berridge, 2003). Thus, an amphetamine pre-exposed rat will work harder to obtain a drug than a drug naïve rat. Further, animals remain sensitized for months to years (Wolf, 2003), reminiscent of the persistence of vulnerability to relapse in recovering addicts. Regardless of which theory one adheres to regarding the role of sensitization, the view that it is associated with addiction is supported by the following findings: (1) sensitization includes functional alterations which are very stable. In the rat, sensitized locomotor activity can persist for several months after the end of drug administration (Coppa-Hopman, 2006; Robinson & Becker, 1986). (2) The degree of sensitization determines the vulnerability for recidivism; that is, strongly sensitized animals will take drugs after a drug-free period more easily than weakly sensitized animals (De Vries et al., 1998). (3) Sensitization facilitates subsequent drug-taking. For example, amphetamine or cocaine sensitization facilitates the acquisition of cocaine self-administration (Schenk & Partridge, 2000). (4) There is considerable inter-individual variation in the degree of sensitization that develops under a given drug treatment schedule, which is correlated with large inter-individual differences in the vulnerability to develop addiction (Piazza & Le Moal, 1996).

4.3 Behavioural Sensitization and Long-Term Potentiation

For many years, drug-induced alterations of DA-mediated neurotransmission have been the focus of the majority of research into the actions of drugs of abuse in the brain. Based on the clinical and preclinical

evidence demonstrating an important role for DA in mediating the actions of addictive drugs, this emphasis on the role of DA in drug addiction has been well-placed. One normal function of DA may be to help consolidate stimulus-response learning, so individuals acquire the habit of pursuing those stimuli that are both rewarding and necessary. But why do cocaine and amphetamine lead to addiction, while food and sex usually do not? The answer might be that these drugs increase DA release in a more prolonged and unregulated manner than natural stimuli (Wolf, 2002).

However, it is now generally thought that the transient adaptations that occur in the dopaminergic system during the development of behavioural sensitization are followed by critical secondary changes in the glutamatergic system. Furthermore, there is good evidence that a major part of the role of Glu in addiction is related to the modification of the activity of the dopaminergic system. Thus, a sequence of neuroadaptations produced by repeated psychostimulants might first involve adaptations in signaling pathways related directly to DA transmission that become more permanently manifested by changes in Glu transmission, which themselves alter dopaminergic functioning.

An important conceptual advance in the past decade has been the understanding that the process of drug addiction shares striking commonalities with memory and synaptic plasticity phenomena. Dopamine and Glu appear to play an integrative role in motivation, learning, and memory, thus, modulating adaptive behaviour. Experience-dependent or drug-dependent reorganization of

neural circuitry can occur via several mechanisms. Changes in synaptic strength might result from a change in neurotransmitter release, neurotransmitter receptors, or receptor-mediated signaling. Drugs and LTP induction activate common signal transduction pathways (Hyman and Malenka, 2001; Nestler, 2001). Also, both drugs and LTP produce similar morphological changes, including increased dendritic length, density, and arborization (Robinson & Kolb, 1997, 1999a, b). It is possible that these sensitization-related alterations in dendritic structure reflect changes in patterns of synaptic connectivity, and thus, may alter information processing in N.Acc- and PFC-related circuitry.

The best candidate mechanisms for synaptic plasticity are LTP and LTD, as evidence is accumulating that they play a major role in experience-dependent plasticity such as learning and memory. Perhaps psychostimulant-induced alterations in Glu sensitivity strengthens the neuronal pathways that link memories of drug-taking experiences with high reward, thereby feeding the desire to seek the drug. It has long been suggested that long-term memories are associated with the formation of new synaptic connections that lead to altered circuit function. Further, LTP and LTD can also occur in brain areas outside the hippocampus and neocortex, suggesting that they may represent general mechanisms for altering synaptic strength in response to changes in synaptic use, regardless of whether the inducing stimulus is healthy or not. The abnormal engagement of LTP and LTD may be the first step in the cascade leading to changes underlying addiction. As such, drug addiction is often described as a

form of maladaptive learning, as drugs of abuse usurp the neuronal circuitry involved in motivation and reward (Wolf, 2002). Abused drugs modulate LTP and LTD in these neuronal circuits, suggesting a way for the behavioural consequences of drug-taking to become reinforced by learning mechanisms.

A wealth of indirect evidence supports the notion that psychostimulants can alter Glu function. The persistence of behavioural sensitization and paranoia, and the propensity to relapse after a period of abstinence, indicates there must be neural substrates underlying long-term neuroplastic changes. For example, in the human, even if drug-abstinence is achieved, psychostimulant addicts remain vulnerable for years to episodes of craving and relapse triggered by stimuli previously associated with drugs (Gawin & Kleber, 1986). The role for Glu in this process receives support from imaging studies of the brains of human cocaine addicts: stimuli previously associated with drug use (e.g., drug paraphernalia) trigger intense drug craving, and at the same time, activate Glu-rich neuronal circuits implicated in learning and memory (Bonson, Grant, Contoreggi et al., 2002). Animal studies also support the altered role of Glu following psychostimulant administration. For example, repeated cocaine or amphetamine administration enhances the responsiveness to ionotophoretic glutamatergic stimulation of mesolimbic DA neurons and reduces the responsiveness to ionotophoretic glutamatergic stimulation of N.Acc. neurons (White, Hu, Zhang, et al., 1995; Zhang, Hu, White, et al., 1997). Psychostimulants also alter the expression of AMPAR GluR subunits in the

mesolimbic system (Churchill, Swanson, Urbina et al., 1999; Fitzgerald, Ortiz, Hamedani, et al., 1996; Ghasemzadeh, Nelson, Lu, et al., 1999). This is significant since cocaine induces a transient increase in GluR1 which is linked to more enduring cellular changes in the N.Acc. and the maintenance of behavioural sensitization (Carlezon & Nestler, 2002), and results in increased Glu release in the N.Acc. (Pierce, Bell, Duffy, et al., 1996; Reid & Berger, 1996).

While many actions of Glu derive their importance from a stimulatory interaction with the DA system, there are some Glu mechanisms that contribute to addiction independently of DA systems. There is compelling evidence that mGluRs regulate the stimulatory effects of drugs of abuse on locomotor activity, and are involved in the progressive sensitization of behavioural effects. For example, mice lacking the fifth subtype of metabotropic GluR (mGluR5), which is highly expressed in the N.Acc. (Ghasemzadeh et al., 1999), do not express behavioural sensitization or reward responses following cocaine administration (Chiamulera, Epping-Jordan, Zocchi et al., 2001). This deficit occurs despite apparently normal DA function in the mGluR5 null mutants, suggesting that the deficits in the mutant mice may not have been solely due to changes in the dopaminergic system, but perhaps also affected by changes in Glu signaling. The interaction between DA and Glu in the N.Acc. in this process may take place at the level of the medium spiny neuron since these cells express both DA and mGlu5 receptors (Robinson & Becker, 1986; Tallaksen-Greene, Kaatz, Romano et al., 1998).

As outlined previously, there is good evidence that LTP- and LTD-like changes occur in mesolimbocortical circuitry as a consequence of drug administration, and that these changes are important in the development of addiction. There is also more direct evidence that psychostimulant-induced behavioural sensitization involves N.Acc. and VTA NMDAR-dependent synaptic plasticity (Bonci & Malenka, 1999; Overton, Richards, Berry, et al., 1999). Systemic injections of an NMDAR antagonist has been shown to block both cocaine and amphetamine behavioural sensitization, implicating Glu activation of NMDARs in the sensitization that psychostimulants induce (Karler, Calder, Chaudry, et al., 1989). Of course, the NMDAR-dependent form of LTP is also blocked by NMDAR antagonists. Consequently, it has been suggested that the synaptic plasticity at glutamatergic synapses in the N.Acc. and VTA are integral to the development of behavioural sensitization to rewarding stimuli.

So, behavioural sensitization, like LTP, requires Glu transmission (Karler et al., 1989), and the VTA was later pinpointed as the necessary site for Glu transmission, leading to the suggestion that the excitatory synapses in the VTA were responsible for the initiation of behavioural sensitization (Wolf, 1998). This would also account for the increase in dopaminergic cell activity that is critical for the early stages in behavioural sensitization. Behavioural sensitization became directly linked with the induction of LTP in VTA DA neurons when the excitatory transmission was found to be enhanced in midbrain slices from mice injected the day before with a single injection of cocaine (Ungless et al., 2001) or

amphetamine (Saal, Dong, Bonci et al., 2003). Slices from cocaine or amphetamine-treated mice showed an increase in the portion of the AMPAR-mediated excitatory postsynaptic currents relative to those mediated by NMDARs. An increase in AMPAR/NMDAR ratio follows the insertion of AMPARs into postsynaptic sites and is typically seen following LTP induction (Malinow & Malenka, 2002). The cocaine/amphetamine-induced LTP was correlated with cocaine-induced behavioural sensitization, as both were prevented if cocaine was co-administered with an NMDAR antagonist. Indirect evidence for this came from electrophysiological and microdialysis studies which showed that during withdrawal from cocaine or amphetamine, dopaminergic neurons are more sensitive to the excitatory effects of AMPA (Giorgetti, Hotsenpiller, Ward, et al., 2001; Zhang et al., 1997). This is consistent with the idea that psychostimulants produce NMDAR-dependent LTP. Furthermore, LTP could no longer be induced in DA neurons from sensitized rats, which is consistent with the idea that LTP was already maximally induced by cocaine. The demonstration of synaptic plasticity at excitatory synapses in mesolimbic dopaminergic structures support a role for synaptic plasticity in the development of addiction.

Long-term depression-like processes may also underlie sensitization, as the phenomenon has been correlated with decreased synaptic efficacy in the N.Acc. following cocaine administration (Gerdeman et al., 2003). Amphetamine has been shown to block LTD at Glu synapses on VTA DA neurons in acute slices (Jones et al., 2000) perhaps via inhibition of calcium channels (Calabresi

et al., 2000; Paladini, Fiorillo, Morikawa et al., 2001), which may ultimately increase neuronal excitability and thus promote LTP. Also, a behaviourally sensitizing treatment regimen of cocaine produces LTD at excitatory synapses between afferents from the PFC and medium spiny neurons in the N.Acc. shell, and this LTD lasts at least two weeks (Thomas et al., 2001). These results have led to the hypothesis that LTD may serve to limit the synaptic drive onto N.Acc. and VTA cells in order to prevent the pathological over-expression that may contribute to drug addiction via N.Acc. and VTA LTP (Thomas et al., 2000). Furthermore, the blockade of LTD by DA may facilitate LTP which may generate the synaptic reorganization leading to drug addiction and behavioural sensitization (Thomas et al., 2000).

While significant, LTP was demonstrated in these studies after only acute exposure to a drug, which cannot be equated with the commonly studied behavioural sensitization produced by repeated drug administration. Also, these animals showed a sensitized response only when tested in the same environment in which the drug was first administered. The mechanisms of synaptic plasticity have begun to be identified in these reduced preparations, but it is important to examine their role in intact, behaving animals. The crucial question remains as to whether drugs of abuse actually facilitate changes in synaptic strength *in vivo* in a chronic preparation. Thus, a critical next step, fulfilled by the dissertation research reported here, is to evaluate the relationship

between LTP and behavioural sensitization following repeated drug administration in a chronic *in vivo* preparation.

4.4 The Development of Behavioural Sensitization

There is good evidence that the development of behavioural sensitization and the expression of established behavioural sensitization are mediated by different neurochemical mechanisms and brain structures. The development of behavioural sensitization refers to the incremental increase of the behavioural response with repeated drug administration, and it is generally thought that transient alterations that take place in the VTA are most critical to this phase. The development of sensitization requires a transient increase in the activity of Glu transmission between PFC and VTA DA neurons, leading to an activation of DA cell firing. Somehow, this sensitization is transferred to the N.Acc. where the effects become long-lasting, accounting for expression and maintenance.

Various studies support the role of the VTA in the development of behavioural sensitization. Direct injection of amphetamine into the VTA has no acute effect on locomotor activity, but repeated intra-VTA administration results in a sensitized locomotor response to systemic injections of amphetamine, cocaine, or morphine (Wolf, 2002). Glutamatergic input to the VTA also increases the activity of dopaminergic cells and enhances DA release in the N.Acc. (Tzschentke, 2001; Tzschentke & Schmidt, 2000). Further, the development of behavioural sensitization is blocked by intra-VTA administration

of GluR antagonists or by lesions of the PFC (Everitt & Wolf, 2002; Nestler, 2001; Vanderschuren & Kalivas, 2000), as well as by intra-VTA administration of DA antagonists (Pierce et al., 1996). Destruction of the PFC-VTA glutamatergic pathway also weakens drug reward (Tzschentke & Schmidt, 1999). Lastly, the firing rate of VTA DA neurons is increased for several days after discontinuing repeated administration of cocaine or amphetamine, which normalize after about one week (Wolf, 1998, 2002).

Repeated drug administration may result in LTP at synapses between Glu nerve terminals and VTA DA neurons, leading to a transient increase in the firing rate of DA neurons (Vanderschuren & Kalivas, 2000). This, in turn, would increase DA release in the N.Acc., PFC, amygdala, and hippocampus. A transient increase in excitatory drive to VTA DA neurons occurs shortly after discontinuing stimulant administration (Wolf, 1998), suggesting that plasticity at synapses between glutamatergic afferents and VTA DA neurons is responsible for driving downstream changes related to sensitization. This idea is directly supported by evidence that cocaine sensitization is accompanied by LTP in VTA DA neurons (Ungless et al, 2001). Although it was originally imagined that such plasticity occurred between Glu terminals originating in PFC and midbrain DA neurons, anatomical studies have revealed that PFC afferents to the VTA synapse on mesoaccumbens GABA, rather than DA, neurons (Carr & Sesack, 2000a). So the route of communication between PFC and VTA DA neurons may be indirect, perhaps involving PFC projections to the laterodorsal tegmentum and

mesopontine nuclei (Forster & Blaha, 2000) or bed nucleus of the stria terminalis (Georges & Aston-Jones, 2001), which send excitatory glutamatergic and cholinergic projections to VTA DA neurons.

Once it has developed, LTP is expressed as an enhancement of postsynaptic AMPAR transmission. Thus, if the initiation of behavioural sensitization is associated with LTP at Glu synapses on VTA DA neurons, then these DA neurons in sensitized rats should exhibit increased responsiveness to AMPA. This has been confirmed. First, VTA DA neurons recorded from chronic psychostimulant-pretreated rats are more responsive to the excitatory effects of AMPA (Giorgetti et al., 2001). Second, intra-VTA infusion of AMPA results in a larger increase in extracellular levels of DA and Glu in the VTA and N.Acc. in psychostimulant-pretreated rats compared to saline-pretreated rats (Giorgetti et al., 2001; Ungless et al., 2001). Conversely, intra-N.Acc. infusion of NMDA produces a similar increase in extracellular DA in the VTA and N.Acc., but does not affect extracellular levels of Glu. Third, plasticity at these synapses, as measured by the increase in the AMPAR/NMDAR ratio, has been correlated with behavioural sensitization and reported after a single dose of cocaine (Ungless et al., 2001) and amphetamine (Faleiro, Jones, & Kauer, 2003). These results indicate that an early step in the cascade leading to the establishment of drug-induced alterations involves increased Glu signaling at AMPARs in the VTA. There is also specific evidence that it is the GluR1 subunit of the AMPAR that is externalized during the development of sensitization (Carlezon & Nestler, 2000).

Elevated levels of the GluR1 subunit favours the formation of Ca^{2+}-permeable AMAPR formation, which would favour LTP.

There is also indirect evidence of altered AMPAR functioning in the PFC. The administration of NMDAR antagonists into the PFC produces reward and behavioural arousal (Feenstra, Botterblom, & van Uum, 2002), which is accompanied by an increase in the levels of DA. Yet, this behavioural activation and reward is not blocked by co-infusion of DA antagonists, but rather by co-infusion of AMPAR antagonists (Feenstra et al., 2002). This suggests that the behavioural activation and reward is not causally related to the increase in DA, but rather to increased glutamatergic transmission at AMPARs within the PFC. The role of the increased DA may, therefore, be related to the modulation of plasticity in the system.

4.5 The Expression of Behavioural Sensitization

The circuitry involved in the expression of sensitization is less well established; however, the N.Acc. is thought to be a critical site. This notion is supported by evidence that direct injection of amphetamine into the N.Acc. is sufficient to elicit a behavioural sensitization response in rats that received repeated systemic or intra-VTA injections (Nestler, 2001; Vanderschuren & Kalivas, 2000). It has been shown that the N.Acc. shell is more clearly associated with DA-dependent reward, while the core is linked to the enduring cellular changes elicited by repeated use of addictive drugs (Kalivas and

McFarland, 2003). Some studies indicate that expression of sensitization requires activation of glutamatergic projections from the PFC to the N.Acc (Pierce, Reader, Hicks, et al., 1998). Others have shown that PFC lesions do not alter expression, although they do prevent the development of sensitization (Li, Hu, Berney et al., 1999). Yet, there is growing concensus that despite the increase in mesencephalic DA following drug intake, the maintenance of sensitization is associated with the loss of inhibitory DA tone (ie., movement towards a depolarized 'up' state) in the PFC (Karler, Calder, Thai, et al., 1998; Prasad, Hochstatter, Sorg, 1999). This would lead to an increase in PFC glutamatergic excitation of the N.Acc.

As discussed previously, DA is known to modulate Glu signals in the N.Acc. originating in the PFC, amygdala, and hippocampus in a manner consistent with a gating mechanism (Floresco et al., 2001, 2001a, 2001b). Glutamatergic pyramidal projection neurons in the PFC (Yang, Seamans, & Gorelova, 1996) and GABAergic medium spiny projection neurons in the N.Acc. show membrane potential fluctuations between hyperpolarized 'down' states and depolarized 'up' states, and action potentials can be more easily triggered by excitatory input when the cells are in the 'up' state. Although DA released from the mesocortical and mesoaccumbal projections do not directly induce firing in these projection neurons, it does promote the 'up' state in these cells, thus, increasing the probability that these cells fire action potentials (Lewis & O'Donnell, 2000). This mechanism might be enhanced after repeated

psychostimulant treatment, since repeated psychostimulant administration results in increased Glu released in the N.Acc. (Pierce et al., 1996; Reid & Berger, 1996), as well as in increased DA released in both the N.Acc. and PFC. Stimulation of the PFC usually suppresses the activity of N.Acc. projection neurons via inhibitory interneurons. Yet, increased DA release via psychostimulants may 'overstimulate' PFC D_1Rs, sending PFC neurons into a depolarized 'up' state and, thus, increase excitatory transmission (Lewis & O'Donnell, 2000; Nestler, 2001). Increased Glu transmission to the N.Acc. would result in increased stimulation of resident D_1Rs. Indirect support for this is evidence that D_1Rs, not D_2Rs, on N.Acc. neurons become hypersensitive after sensitization (White & Kalivas, 1998) and increase in responsiveness during chronic cocaine administration (Hope et al., 1992),. The consequences of D_1R stimulation, namely the induction of cAMP-dependent PKA and subsequent changes in protein function and gene expression in the N.Acc., have been shown to be critical to establishing sensitization (Nestler, 2001).

The notion that bistability (ie., transition between 'up' and 'down' states) perturbation underlies behavioural sensitization is supported by i*n vivo* intracellular recording of PFC pyramidal cells projecting to the N.Acc. or VTA which reveal a loss in membrane bistability following repeated psychostimulant administration (Trantham, Szumlinski, McFarland, et al., 2002). Given that the fluctuation in membrane potential is regulated by both dopaminergic and glutamatergic afferents, the loss of membrane bistability following chronic

psychostimulant use reflects changes within the pyramidal cells or changes in dopaminergic and/or glutamatergic afferents, as well as possible changes in related GABAergic interneurons. If, in the sensitized state, there is loss of inhibitory regulation of N.Acc. neurons, this would impair PFC-induced inhibitory regulation of N.Acc. neurons, and the enhancing effects of DA release in N.Acc. outflow would be unopposed. The same could occur at cortical inputs to inhibitory interneurons of the basolateral amygdala, which project to the N.Acc. (Everitt & Wolf, 2002). The net enhancement of excitatory drive to the N.Acc. could contribute to Glu-dependent mechanisms implicated in behavioural sensitization. Furthermore, the loss of membrane bistability is consistent with an emerging view that the PFC may fluctuate between hyperactive and hypoactive states, the hypoactive resembling that in schizophrenics (Goldstein & Volkow, 2002; Kalivas, McFarland, Bowers et al., 2003). Prefrontal cortex functioning is assessed in traditional neuropsychological tests such as the Wisconsin Card Sorting Task, which evaluates working memory, attentional set-shifting, organization, planning, and inhibition (Phillips et al., 1994). Evidence of a hypoactive PFC in addicts is indicative of decreased cognitive ability to regulate drug-seeking behaviours (ie., reduced inhibition, faulty organization and planning) (Goldstein & Volkow, 2002; Kalivas et al., 2003).

Nucleus accumbens neurons are normally quiescent, their activation requiring synchronous activation of multiple excitatory inputs (O'Donnell & Grace, 1995). Thus, LTP or LTD in excitatory pathways impinging on these neurons

would have profound effects on their output, because these processes influence synchronized activation. Psychostimulants may therefore influence LTP and LTD in the N.Acc. by acting at a systems level to alter neuronal activity in excitatory pathways underlying motivation and reward, leading to abnormal LTP or LTD. For example, acute cocaine administration fails to alter Glu release in the N.Acc. of drug-naïve animals, but produces marked Glu release in the N.Acc. of animals previously treated with repeated cocaine (McFarland, Lapish & Kalivas, 2003). The enhanced release of Glu occurs against a background of reduced basal levels of Glu in the extracellular space and inside presynaptic terminals (Baker, McFarland, Lake, et al., 2003). It has been hypothesized that the reduced Glu background may serve to accentuate the synaptic signal delivered by Glu released in the projection from the PFC to the N.Acc. (Baker et al., 2003; McFarland et al., 2003). Behavioural sensitization and LTP in the N.Acc. are further linked by evidence that amphetamine-induced sensitization increases syntaxin-1, a protein that has been strongly implicated in synaptic plasticity and LTP, in the N.Acc. but not the VTA, (Stroemer, Kent, & Husebosch, 1998; Subramaniam, Marcotte, & Srivasta, 2001).

CHAPTER 5

METHODS

5.1 Research Questions and Methods

The objective of this research was to examine the relationship between LTP in the PFC and behavioural sensitization, *in vivo*. This objective was addressed via three main areas of inquiry. First, we examined whether or not LTP could be induced in the mPFC, *in vivo*. This was done by stimulating the corpus callosum with high-frequency stimulation (HFS) and recording resultant population EPSPs in the mPFC. Secondly, we examined whether or not LTP in the PFC could be modulated by DA. This was done by administering specific DAR agonists and antagonists concomitantly with HFS. Lastly, we examined whether or not LTP in the mPFC affects the development, expression, or maintenance of psychostimulant-induced behavioural sensitization. This was done by administering a psychostimulant analogue concomitantly with HFS, and measuring the effect on both LTP and behavioural sensitization. Our hypothesis was that a psychostimulant analogue would positively modulate long-term potentiation in the mPFC, and that this would be associated with the expression of behavioural sensitization during a period of drug abstinence. All experimental methods listed were accepted by the McMaster Animal Care Committee according to the guidelines set forth by the Canadian Council on Animal Care.

5.2 Surgery

One hundred and forty one male Long-Evans rats (325-350gms; Charles River, Quebec) were used as subjects. They were individually housed in Plexiglas shoe-box cages, maintained on an *ad libitum* feeding schedule, and kept on a 12:12hr light:dark cycle. In order to prevent respiratory distress, the rats received a pre-anesthetic injection of atropine (*atropine sulfate salt monohydrate,* SIGMA*)* at 1.0mg/kg, intraperitoneal, i.p). The rats were then anesthetized with the barbiturate sodium pentobarbital (Somnotol) (CEVA) (65mg/kg, i.p.). During surgery, rats received an injection of penicillin (*Derapen)* (Ayerst) (0.05ml, intramuscular, i.m.), an antibiotic (Baytril) (Bayer) (5mg/ml, i.m.), an anti-inflammatory/analgesic (Anafen) (Merial) (5mg/kg, subcutaneous, s.c.), and a topical anaesthetic (Xylocaine) (AstraZeneca) (0.05ml. s.c.). Throughout surgery, Xylocaine and sterile saline or water was administered to the skull and surrounding dura mater and skin. Twisted wire bipolar electrodes were constructed of teflon-coated stainless steel wire (0.005"-0.008" in diameter). A stimulating electrode with a tip separation of 0.5mm was implanted into the forceps minor corpus callosum (fmiCC), 1.6mm anterior to Bregma and 1.8mm lateral to Bregma. This site was chosen as it targets mPFC cells that also receive inputs from the VTA. Mesocortical dopaminergic projections to the rat PFC run adjacent to the N.Acc. between the corpus callosum and anterior commissure (Berger, Thierry, Tassin et al., 1976). Also, callosal terminals target

the spines of pyramidal neurons in the PFC (Carr & Sesack, 1998). Further, maintained activation of NMDARs by synaptically released Glu can produce a sustained enhancement of the EPSPs in the striatum following tetanus of the corpus callosum, *in vitro* (Walsh & Dunia, 1993). Lastly, DA terminals make synaptic contact with the distal dendrites of callosally projecting PFC neurons (Carr & Sesack, 2000b).

A recording electrode with a tip separation of 2.0mm was implanted into the prelimbic mPFC, 3.2mm anterior to Bregma and 1.0mm lateral to Bregma. All electrodes were adjusted during surgery to provide optimal response amplitudes. The optimal depth range for the electrodes was 2.0-2.2mm for the mPFC and 2.8-3.4mm for the fmiCC. The electrodes were connected to gold-plated male pins that were inserted into a nine-pin miniature connector plug. This was mounted onto the skull with dental cement and anchored with stainless steel screws, one of which served as the ground electrode. At surgery completion, rats received an analgesic Buprenorphine (Temgesic) (Schering-Plough) (0.01-0.05 mg/kg, s.c.) and saline (5ml, s.c.). Surgery was followed by a two-week recovery period. During the first week, Jello cubes containing 0.5ml of liquid acetaminophen (Tylenol) were left in each cage for consumption, and all rats received Baytril and Anafen every other day for 3-5 days 6-12 hours apart. During the full two-week recovery period, all rats received penicillin every other day. If there was any evidence of dehydration, rats received 5-10ml of saline.

5.3 Morphological Characterization

In order to resolve the monosynaptic and polysynaptic components of the field responses, the following three tests were administered.

Recovery from Anaesthesia

The differential susceptibility of field components to anaesthesia suggested that monitoring the recovery of components from deep anaesthesia could help resolve monosynaptic and polysynaptic components (Barry & Pentreath, 1976; Chapman et al., 1998). Somnotol (65mg/kg, i.p.) was administered to five rats following input/output (I/O) testing (single pulses of increasing intensity delivered to the corpus callosum at a frequency of 0.1Hz; 10 field responses were evoked at each intensity, amplified, digitized at 10kHz, and averaged at each of four intensities: 160, 400, 500, and 1000μA). Once a level of general anaesthesia was reached (absence of reflexive blinking and the righting reflex), field responses in response to a single test pulse delivered every 30 seconds at the same four intensities were measured until the rats completely recovered from anaesthesia (three and a half hours).

Paired-Pulse Test

Paired-pulse tests were conducted on the last baseline day. Two pulses were delivered at 7 interpulse intervals (50, 70, 100, 150, 200, 300, and 500ms).

Pulse intensity was set to evoke an early component approximately 75% of maximum amplitude. Amplitudes for the response evoked by the second pulse were expressed as a percentage of the amplitudes for the response evoked by the first pulse (Chapman et al., 1998). The results were averaged across animals and plotted as a function of interpulse interval (Chapman et al., 1998).

Frequency of Following

Monosynaptic responses 'follow' (or are capable of being repeatedly evoked by) much higher frequencies of stimulation than polysynaptic responses. Polysynaptic components usually fail at frequencies <40-50Hz, whereas monosynaptic components should follow frequencies near 100Hz (Berry & Pentreath, 1976; Chapman & Racine, 1997). Responses following at 300Hz typically reflect fibre volleys and/or antidromic invasion. Frequency of following tests were conducted on five rats after baseline I/O tests. Trains of 10 pulses were delivered at six frequencies (20, 40, 60, 80, 160, 320Hz). Three trains at each frequency were applied at an intensity of 250μA, and tests were then repeated with 500μA. The intertrain interval was five minutes.

5.4 Pharmacological Characterization

In order to resolve the pharmacology of the field responses, seven receptor antagonists were administered to rats following the administration of a single test pulse delivered 10 times at each of seven intensities (32, 80, 160,

250, 400, 500, and 1000μA). Ten minutes following drug injection, the procedure was repeated. Rats received the D_1R antagonist SKF 83566 Hydrobromide *(8-Bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrobromide)* (TOCRIS) at 0.15mg/kg/ml, i.p. (n=5) (Samini, Fakhrian, Mohagheghi et al., 2000; Serafim & Felicio, 2001); the D_2R antagonist Sulpiride *((RS)-(+/-)-Sulpiride)* (TOCRIS) at 12mg/kg/ml, i.p. (n=5); the NMDAR antagonist Dizocilpine/MK-801 *((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate)* (SIGMA) at 0.25mg/kg/ml, s.c. (n=5) (Dall'Olio, Gandolfi, & Montanaro, 1991; Jeziorski, White, & Wolf, 1994; Verma & Kulkarni, 1991); the AMAPR antagonist GYKI 52466 *([1-(4-aminophenyl-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride])* (SIGMA) at 1.2mg/kg/ml, i.p. (n=5) (Yoshiyama, Roppolo, & de Groat, 1995); the kainate receptor antagonist SYM 2081 *(2S, 4R)-4-methylglutamic acid)* (SIGMA) at 0.15mg/kg/ml, i.p.; the GABAaR antagonist Picrotoxin *(Picrotoxic Acid/Cocculin)* (SIGMA) at 1mg/kg/ml, i.p. (n=5) (Koryntora, Kubova, Tutka et al., 2002); and the GABAbR antagonist Phaclofen *(3-Amino-2-(4-Chlorophenyl) propanephosphoric acid)* (SIGMA) at 0.75mg/kg/ml, i.p. (n=5) (Humeniuk, White, & Ong, 1993). Drug preparation followed guidelines provided by the drug company with strict adherence to accompanying Material and Safety Data Sheet instructions. Drugs were dissolved in glacial acetic acid when required, and 1-2ml of distilled water, then brought up to working volume with 0.9% physiological saline (pH [7.0-7.4]).

All drugs and saline were syringe-filter (0.2μm) sterilized and stored at 4° Celsius.

5.5 Drug Preparation and Administration

The dose and route of administration for each drug was determined by published literature employing the drug in rats *in vivo.* The dose of the selective D_1R antagonist *SKF 83566* was 0.15mg/kg/ml, and was administered i.p. (Samini et al., 2000; Serafim & Felicio, 2001). The dose of the selective D_1R agonist A68930 Hydrochloride *((4aR-trans)-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride)* (TOCRIS) was 0.4mg/kg/ml, and was administered i.p. (Britton, Kebabian, & Curzon, 1991; Isacson, Kull, Wahlestedt, et al., 2004; Kebabian, DeNinno, Schoenleber et al., 1992). The doses of the selective D_2R antagonist Sulpiride was 3mg/kg/ml, 6mg/kg/ml, and 12mg/kg/ml, and was administered i.p. (Benelli, De Poi, Poggioli et al., 2000; Gioanni, Thierry, Glowinski et al., 1998; Gnegy, Bernabei, & Treisman, 1983; Lacroix, Hows, Shah et al., 2003; Mattingly, Hart, Lim et al., 1994; Mattingly, Rowlett, Graff et al., 1991; Noda, Miyamoto, Mamiya et al., 1998; Oliveira, Messinger, Tannhauser et al., 1999). The dose of the selective D_2R agonist Quinpirole Hydrochloride *((-)-Quinpirole hydrochloride)* (TOCRIS) was 0.15mg/kg/ml, 0.25mg/kg/ml, and 0.5mg/kg/ml, and was administered s.c. (Kurylo, 2004; Szechtman, Eckert, Tse et al., 2001; Szechtman, Talangbayan, Eilam, 1993). Drug preparation followed guidelines provided by the drug company with strict adherence to accompanying

Material and Safety Data Sheet instructions. All drugs were dissolved in glacial acetic acid when required, and 1-2ml of distilled water, then brought up to working volume with 0.9% physiological saline (pH [7.0-7.4]). All drugs and saline were syringe-filter (0.2μm) sterilized and stored at 4° Celsius.

5.6 Electrophysiological and Drug Testing

All animals were run at the same time each day (+/- 30 minutes) in a relaxed, fully-awake state. All testing was carried out in a 30X40cm wooden chamber with Plexiglas front and wire-grid floor. Electrical stimuli were generated with a Grass S88 stimulator and photoelectric stimulus isolation units (Grass SIU6B) were used to deliver 0.1ms biphasic constant current pulses. Both Monopolar and differentially recorded field potentials were filtered (0.3-3kHz), amplified (Grass Model 12) and digitized (10kHz); 12-bit A/D board) for storage on computer hard disk.

Baseline Phase (3 days)

Three sets of field potential measures, spaced at 48 hours, were taken to establish a series of baseline input/output (I/O) curves to monitor the stability of the response in each animal. Single pulses of increasing intensity were delivered at a frequency of 0.1Hz. Ten field responses were evoked at each intensity, amplified, digitized at 10kHz, and averaged at each of seven intensities (32, 80, 160, 250, 400, 500, and 1000μA). The responses were averaged together at

each intensity each day to track the baseline response. Among the 145 rats, response morphologies were divided into matched pairs. These matched pairs were then randomly split into control and test subjects and randomly distributed among the three test groups (D_1R agonist, D_1R antagonist, saline) and three control groups (D_1R agonist, D_1R antagonist, saline). The intensity that evoked the approximate half-maximum response amplitude for each test rat was selected as the LTP-induction intensity.

LTP Induction Phase (15 days)

Test Groups: Drug was administered 10 minutes prior to the delivery of high-frequency trains (8 pulses, 3.3ms interpulse interval) each day using stimulation parameters previously shown to be effective in neocortical LTP induction (Chapman et al., 1998). There were nine test groups: 0.9% physiological saline (n=11) (Table 5.6.1); D_1R agonist A68930 at 0.4mg/kg (n=7) and antagonist SKF 83566 at 0.15mg/kg (n=6) (Table 5.6.2); D_2R agonist Quinpirole low dose at 0.125mg/kg (n=7), middle dose at 0.25mg/kg (n=9), and high dose at 0.5mg/kg (n=10) (Table 5.6.3); D_2R antagonist Sulpiride low dose at 3mg/kg (n=6), middle dose at 6mg/kg (n=11), and high dose at 12mg/kg (n=10) (Table 5.6.3). The baseline paradigm was administered every other day (days 1, 3, 5, 7, 9, 11, 13, 15), prior to HFS, to track the development of LTP.

Control Groups: Drug was administered each day 10 minutes prior to running the baseline paradigm; no HFS was administered. There were nine

control groups: D_1R agonist A68930 at 0.4mg/kg (n=7); D_1R antagonist SKF 83566 at 0.15mg/kg (n=7); D_2R agonist Quinpirole low dose at 0.125mg/kg (n=7); D_2R agonist Quinpirole middle dose at 0.25mg/kg (n=7); D_2R agonist Quinpirole high dose at 0.5mg/kg (n=9); D_2R antagonist Sulpiride low dose at 3mg/kg (n=6); D_2R antagonist Sulpiride middle dose at 6mg/kg (n=9); D_2R antagonist Sulpiride high dose at 12mg/kg (n=10); and 0.9% physiological saline (n=11).

Decay Phase (17 days)

Following the completion of the testing phase, all subjects in all groups were run through the original baseline paradigm to assess the maintenance of any changes in response amplitude beyond the HFS and drug administration phase. Four decay tests were administered: 24 hours following the last HFS day, 48 hours following the first decay day, one week following the second decay day, and one week following the third decay day. The responses were averaged together at each intensity each day to track the decay of LTP.

5.7 Behavioural Testing During Stimulation

All rats were scored on a Sensitization Observation Scale (SOS) during all phases of electrophysiological testing every day of the experiment: baseline, induction, and decay (total 22 days). Each session began 10 minutes following drug/saline injection once animals were in the testing chamber and electrophysiological testing commenced. The duration of behavioural

observation lasted the entirety of the electrophysiological session (15 minutes) for each animal.

The SOS was composed of 10 sensitization behaviours that are known to be expressed in rodents following administration of D_2R agonists, and that are well represented in the literature. The behaviours chosen represent a scale of sensitization behaviours beginning with milder forms that are expressed with low doses of D_2R agonists soon after administration (sniffing, locomotion, rearing), medium forms that are expressed with middle doses of D_2R agonists a few days after chronic administration (circling, paw/tail licking), and intense forms that are expressed with high doses of D_2R agonists several days after chronic administration (gnawing, jumping, biting, vocalization, stereotypy – repetitive sensitization behaviours). Rats were given a score of one for each of the 10 types of behaviours expressed each day.

The intensity (frequency per 15 minute trial) of expressed sensitization behaviours was also recorded. Rats in the mild category expressed behaviours during one third of the 15 minute trial (1-5 minutes), and were given an intensity score of one. Rats in the medium category expressed behaviours during two thirds of the 15 minute trial (6-10 minutes), and were given an intensity score of two. Rats in the high category expressed behaviours during the full trial (11-15 minutes), and were given an intensity score of three.

The total behavioural expression score (maximum 10) per day was added to the intensity score (maximum 3) per day for each rat, for an overall possible

total score of 13 per day. The average total score across animals between groups and doses was calculated per day.

5.8 Histology

The deepest positions of depth recordings was marked by DC lesions. All rats were deeply anaesthetized with urethane, and transcardially perfused with 0.9% neutral-buffered formol-saline and 37% formaldehyde. Brains were removed and stored in 30% sucrose + formalin solution. Following at least two weeks, the brains were cut into 50μm sections and mounted onto microscope slides. Sections with DC lesions were stained with Prussian Blue (5% KFeCN in 1% HCl) and Neutral Red, all others with Cresyl Violet. Light photomicrographs of electrode tracks were obtained with a Zeiss Axioskop microscope. All histological sites of interest were compared with those from the sections of the rat brain stereotaxic atlas (Paxinos & Watson, 1997) via light microscopy.

5.9 Statistical Analyses

Repeated measure ANOVAs, with a critical value of $p=0.05$, were performed at a common stimulation intensity for all LTP control and test groups. Chi-square tests, with a critical value of $p=0.05$, were performed for all behavioural data. All figures represent the standardized mean difference from baseline across days.

5.10 Tables

Table 5.6.1 Saline Experimental Groups

GROUP	DRUG	N = 22
Control	0.9% Physiological Saline	11
Test	0.9% Physiological Saline	11

Table 5.6.2 D_1R Experimental Groups

GROUP	DRUG	N = 28
Control	D_1R Agonist A68930 hydrochloride (0.4mg/kg)	7
Test	D_1R Agonist A68930 hydrochloride (0.4mg/kg)	7
Control	D_1R Antagonist SKF83566 hydrobromide (0.15mg/kg)	7
Test	D_1R Antagonist SKF83566 hydrobromide (0.15mg/kg)	6

Table 5.6.3 D_2R Experimental Groups

GROUP	DRUG	N = 101
Control	D_2R Agonist Quinpirole (0.125mg/kg)	7
Test	D_2R Agonist Quinpirole (0.125mg/kg)	7
Control	D_2R Agonist Quinpirole (0.25mg/kg)	7
Test	D_2R Agonist Quinpirole (0.25mg/kg)	9
Control	D_2R Agonist Quinpirole (0.5mg/kg)	9
Test	D_2R Agonist Quinpirole (0.5mg/kg)	10
Control	D_2R Antagonist Sulpiride (3mg/kg)	6
Test	D_2R Antagonist Sulpiride (3mg/kg)	6
Control	D_2R Antagonist Sulpiride (6mg/kg)	9
Test	D_2R Antagonist Sulpiride (6mg/kg)	11
Control	D_2R Antagonist Sulpiride (12mg/kg)	10
Test	D_2R Antagonist Sulpiride (12mg/kg)	10

5.11 TABLE CAPTIONS

Table 5.6.1 Saline Experimental Groups

This table summarizes the baseline (saline) control and test groups.

Table 5.6.2 D_1R Experimental Groups

This table summarizes the D_1R agonist and antagonist control and test groups.

Table 5.6.3 D_2R Experimental Groups

This table summarizes the D_2R agonist and antagonist control and test groups.

CHAPTER 6

RESPONSE CHARACTERIZATION

6.1 Morphological Characterization

Response Morphology

All baseline field potentials showed an early spike, an early population excitatory post synaptic potential (EPSP), and a longer latency polysynaptic component (Figure 6.1.1). All components increased in amplitude with stimulation intensity (Figure 6.1.2). The polysynaptic components were variable across animals and, sometimes, across tests in the same animal. Otherwise, response amplitudes were reasonably stable across baseline tests.

Recovery from Anaesthesia

The differential susceptibility of field components to anaesthesia and the differential recovery from anaesthesia can be used to resolve monosynaptic and polysynaptic components (Barry & Pentreath, 1976; Chapman et al., 1998). In all animals, the evoked mPFC potentials recorded in the fully awake state demonstrate clear monosynaptic and polysynaptic components which increase in amplitude with increasing intensity. Under deep anaesthesia, these components show large amplitude reductions in all animals (Figure 6.1.3). On the first post-injection recording (20 minutes following anaesthesia) the peak amplitude of the early component (approximately 13ms to peak) was reduced by 0.77mV (51% of

baseline amplitude), and the peak amplitude of the late component (approximately 28ms to peak amplitude) was reduced by 1.34mV (94% of baseline amplitude). This was followed by graded increases in peak amplitude in both components over time until anaesthesia expired and baseline amplitudes were restored. One hour post-injection, the monosynaptic component was reduced 0.20mV (13% of baseline amplitude). The polysynaptic component was still reduced 0.99mV (70% of baseline amplitude). One and a half hours post-injection, the monosynaptic component had nearly recovered and was now reduced by only 0.02mV (1.3% of baseline amplitude), and the polysynaptic component was reduced 0.52mV (37% of baseline amplitude). Both components then gradually recovered toward baseline levels over the next two hours. At two hours post-injection, the monosynaptic component was reduced 0.08mV (5% of baseline amplitude), and the polysynaptic component was reduced 0.26mV (18% of baseline amplitude). At two and a half hours post-injection, the monosynaptic component was reduced 0.13mV (9% of baseline amplitude), and the polysynaptic component was reduced 0.01mV (7% of baseline amplitude). At three hours post-injection, the monosynaptic component was reduced 0.06mV (4% of baseline amplitude), and the polysynaptic component was reduced 0.02mV (1.4% of baseline amplitude). Lastly, at three and a half hours post-injection, the monosynaptic component was reduced 0.01mV (0.66% of baseline amplitude), and the polysynaptic component was reduced 0.01mV (0.07% of

baseline amplitude). Both the monosynaptic and polysynaptic components were considered to be fully recovered by over three and a half hours.

Paired Pulse Stimulation

The results were averaged across animals and plotted as a function of interpulse interval (IPI) (Figures 6.1.4-6.1.10). Over all animals there was a 21-73% reduction in the monosynaptic component of the response to the second pulse relative to the responses evoked by the first pulse across 50-500ms IPIs (50% at 50 and 70ms; 43% at 100ms; 21% at 150ms; 73% at 200ms; 62% at 300ms; 50% at 500ms).

With respect to the polysynaptic component, all animals showed a facilitation in the polysynaptic component of the response to the second pulse relative to the responses evoked by the first pulse. The only exception to this occurred at an IPI of 50ms where there was a 49% reduction in the response. However, a 16-54% facilitation of the response occurs at IPIs of 70-500ms (50% at 70ms; 54% at 100ms; 45% at 150ms; 52% at 200ms; 50% at 300ms; 16% at 500ms).

Frequency of Following

The frequency of following results were the same for both pulse intensities administered: 250 and 500uA (Figure 6.1.11-6.1.16). A portion of the initial spike component followed trains of 10 pulses at frequencies up to 320Hz ,

suggesting this component reflected contribution of fibre volley and/or antidromic activation.

Monosynaptic responses 'follow' (are capable of being repeatedly evoked by) much higher frequencies of stimulation than polysynaptic responses, often following frequencies near 100Hz (Berry & Pentreath, 1976; Chapman & Racine, 1997). At 40 and 60Hz stimulation, the monosynaptic response was comparable to that at 20Hz. While attenuated considerably (60%) at frequencies of 80Hz and greater, the initial spike and the subsequent early component still followed stimulation at these higher frequencies, suggesting that monosynaptic inputs also drive a large part of these responses. The early component was, however, eliminated at a frequency of 320Hz.

Polysynaptic components usually fail at frequencies near 40-50Hz (Berry & Pentreath, 1976; Chapman & Racine, 1997). The polysynaptic field potential component was attenuated (53%) by stimulation at only 40Hz, and virtually eliminated (97%) during 60Hz stimulation. Stimulation at all higher frequencies (80, 160, and 320Hz) failed to produce a polysynaptic component. Failure to follow these higher frequencies suggests that the late component is generated polysynaptically.

6.2 Pharmacological Characterization

The administration of receptor antagonists revealed the receptor-dependency of the mPFC field potential components. The greatest attenuation

of both monosynaptic and polysynaptic components was induced by the NMDAR antagonist MK-801 (0.25mg/kg), indicating that a large proportion of this response is NMDAR-dependent. On average, administration of the NMDAR antagonist attenuated the monosynaptic component by 0.67mV (51% of baseline amplitude), and the polysynaptic component by 1.13mV (72% of baseline amplitude) (Figure 6.2.1). On average, smaller amplitude reductions were produced in both the monosynaptic and polysynaptic components by the remaining ionotropic glutamate receptor antagonists. The AMPAR antagonist GYKI 52466 (1.2mg/kg) reduced the monosynaptic component by 0.52mV (40% of baseline amplitude) and by 0.89mV (57% of baseline amplitude). The kainate receptor antagonist SYM 2081 (0.15mg/kg) attenuated the monosynaptic component by 0.69mV (47% of baseline amplitude) and the polysynaptic component by 0.63mV (57% of baseline amplitude) (Figure 6.2.1). The D_1R antagonist SKF 83566 (0.15mg/kg) reduced the monosynaptic component by 0.52mV (34% of baseline amplitude), and the polysynaptic component by 0.87mV (55% of baseline amplitude). The D_2R antagonist Sulpiride (12mg/kg) also reduced the monosynaptic component by 0.74mV (56% of baseline amplitude), and the polysynaptic component by 1mV (64% of baseline amplitude) (Figure 6.2.2). The smallest amplitude changes followed the administration of the GABAR antagonists. While the GABAaR antagonist Picrotoxin (0.1mg/kg) marginally increased the amplitude of the monosynaptic component by 0.6mV (5% of baseline amplitude), it attenuated the polysynaptic

component by 0.19mV (12% of baseline amplitude). The GABAbR antagonist Phaclofen (0.075mg/kg) attenuated the monosynaptic component by 0.25mV (19% of baseline amplitude), and reduced the polysynaptic component by 0.41mV (26% of baseline amplitude) (Figure 6.2.3).

6.3 Histology

All stimulating electrodes were localized to the forceps minor corpus callosum (Figure 6.3.1 and 6.3.2). All recording electrodes were localized to the prelimbic mPFC (Figure 6.3.3 and 6.3.4). All electrodes were within a 1-4mm range of one another, correlating well with the 1-3mm within subjects variance of the location of Bregma.

6.4 Figures

Figure 6.1.1

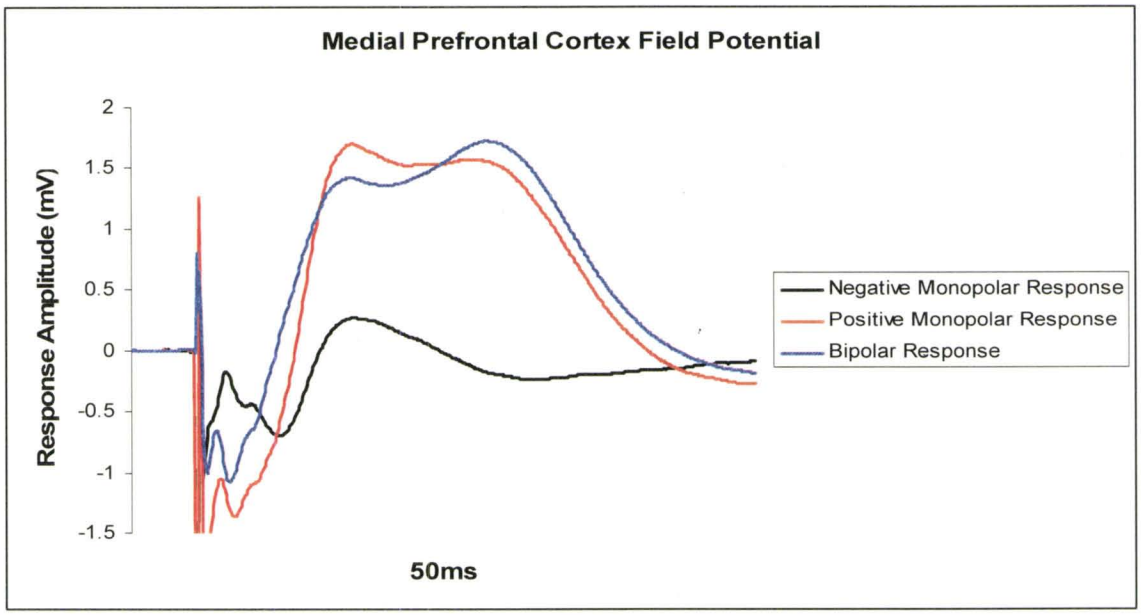


Figure 6.1.2

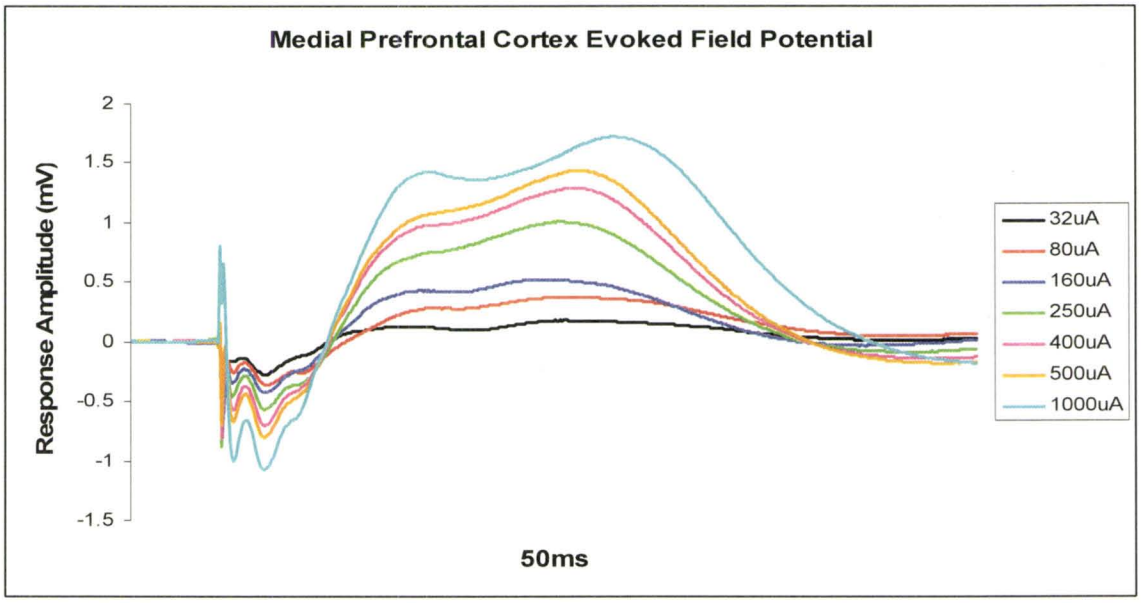


Figure 6.1.3

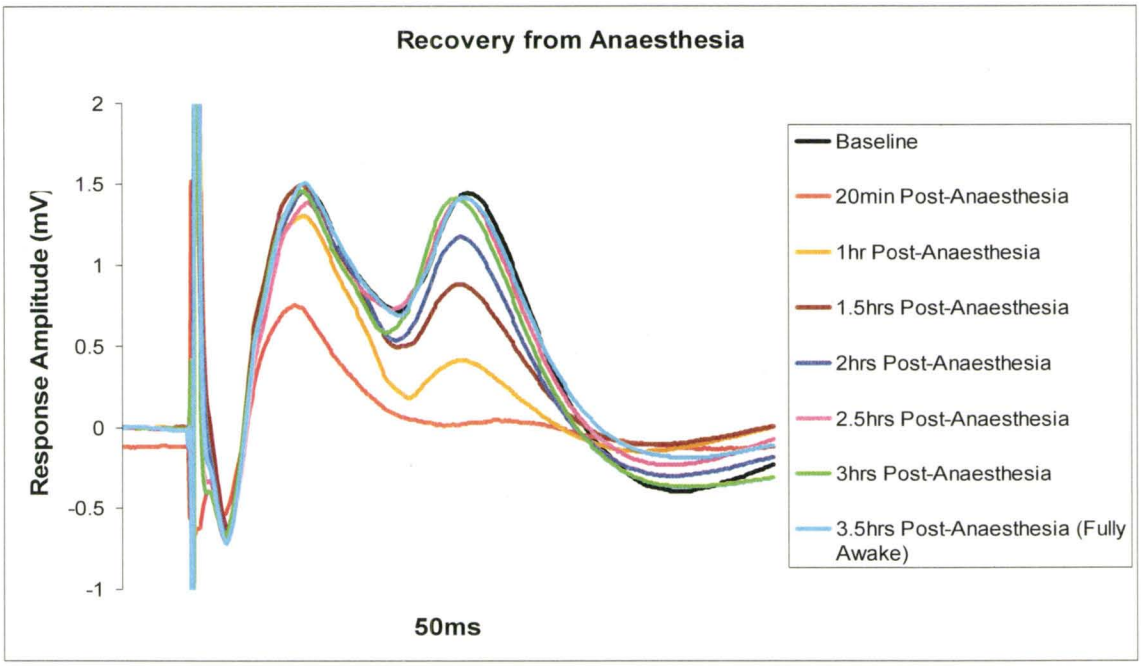

Recovery from Anaesthesia
Response Amplitude (mV)
2
1.5
1
0.5
0
-0.5
-1
50ms
Baseline
20min Post-Anaesthesia
1hr Post-Anaesthesia
1.5hrs Post-Anaesthesia
2hrs Post-Anaesthesia
2.5hrs Post-Anaesthesia
3hrs Post-Anaesthesia
3.5hrs Post-Anaesthesia (Fully Awake)

Figure 6.1.4

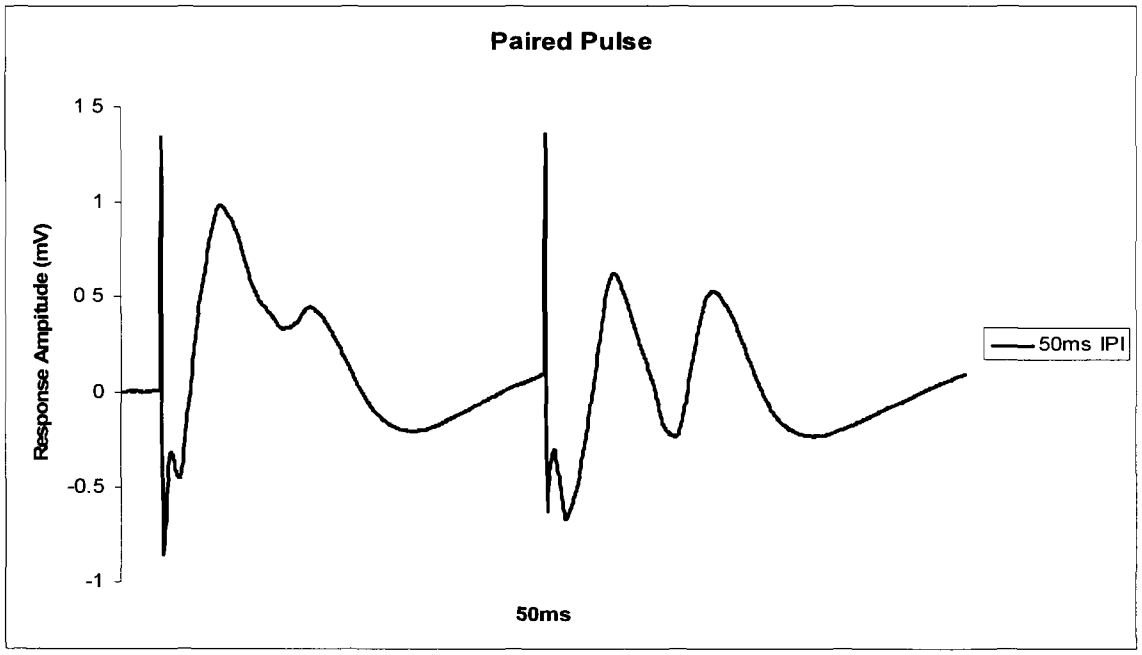


Figure 6.1.5

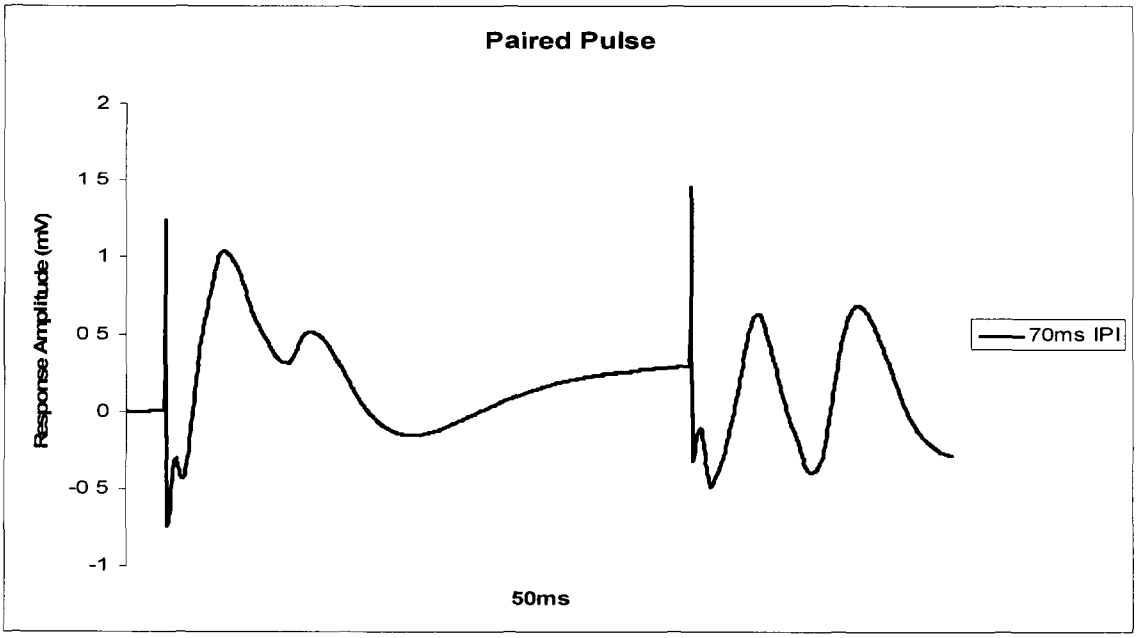


Figure 6.1.6

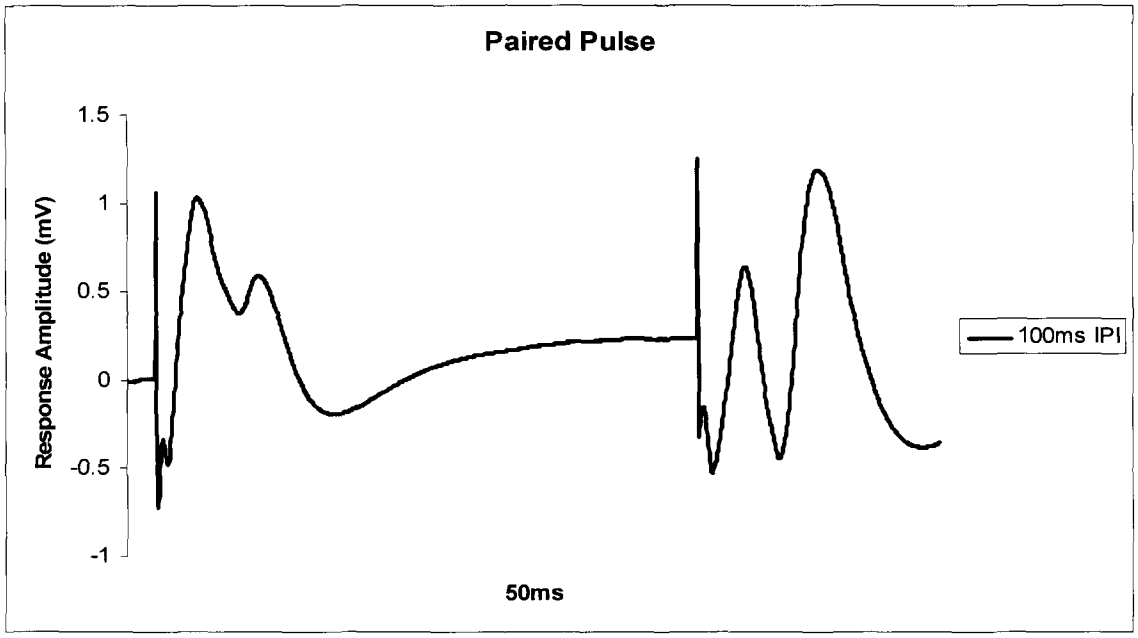


Figure 6.1.7

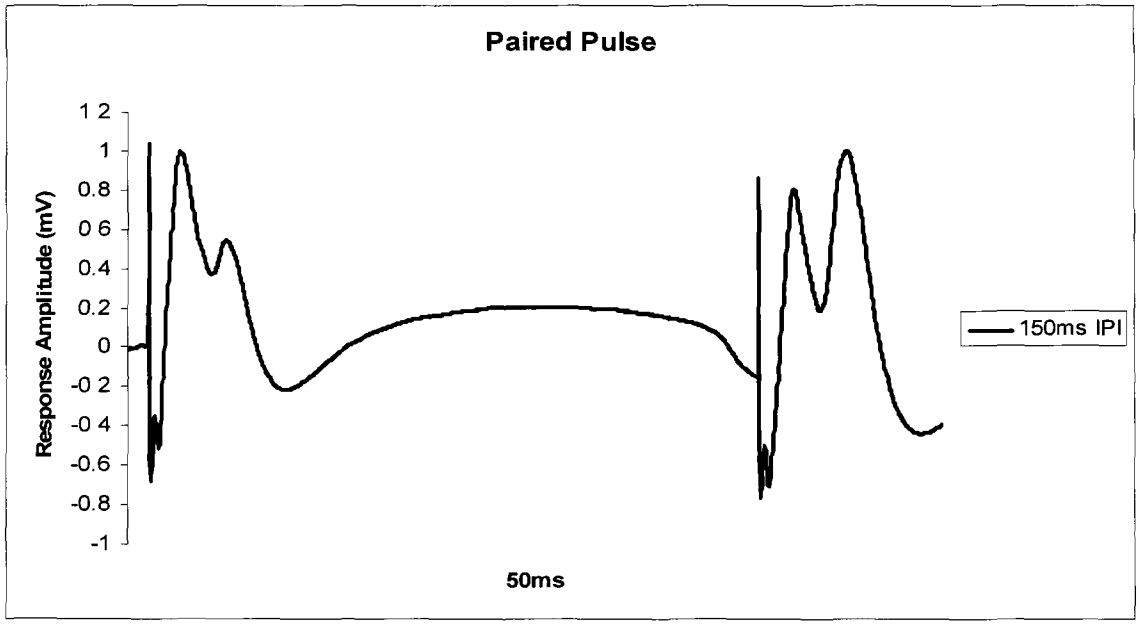


Figure 6.1.8

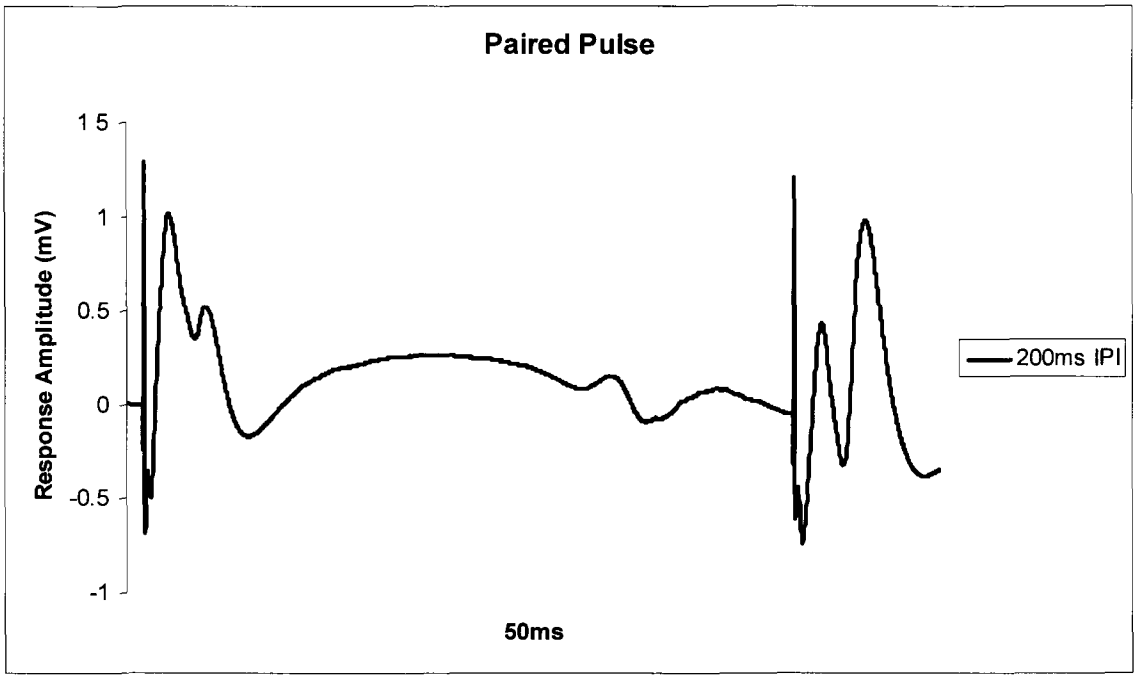


Figure 6.1.9

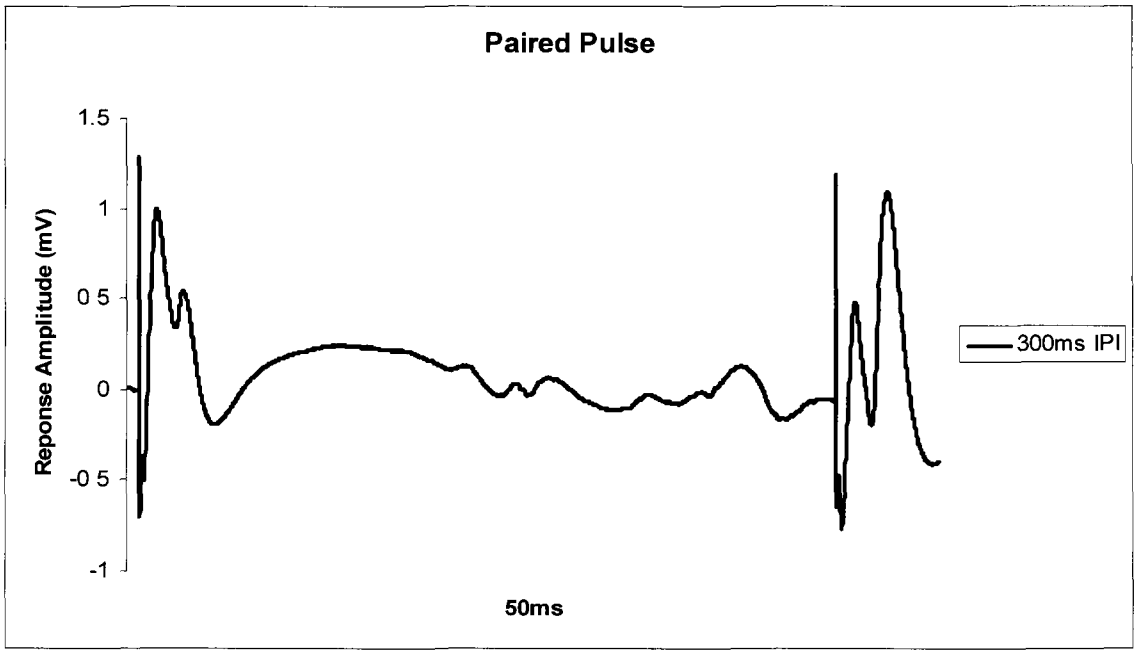


Figure 6.1.10

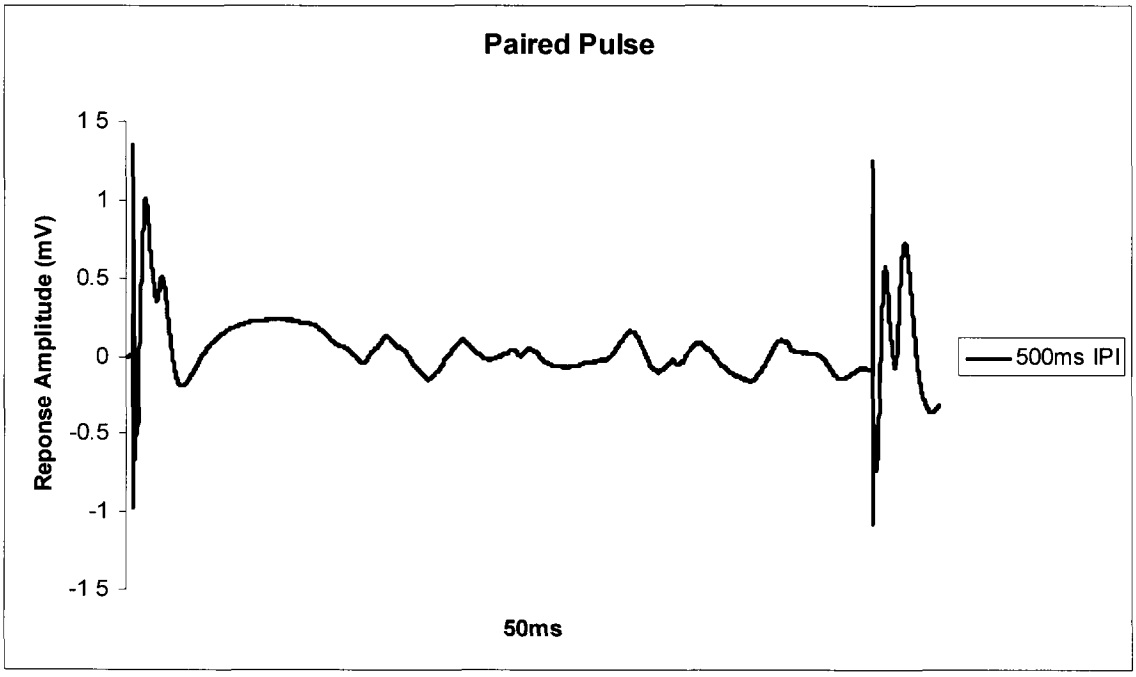

Paired Pulse
Reponse Amplitude (mV)
1 5
1
0.5
0
-0.5
-1
-1 5
50ms
500ms IPI

Figure 6.1.11

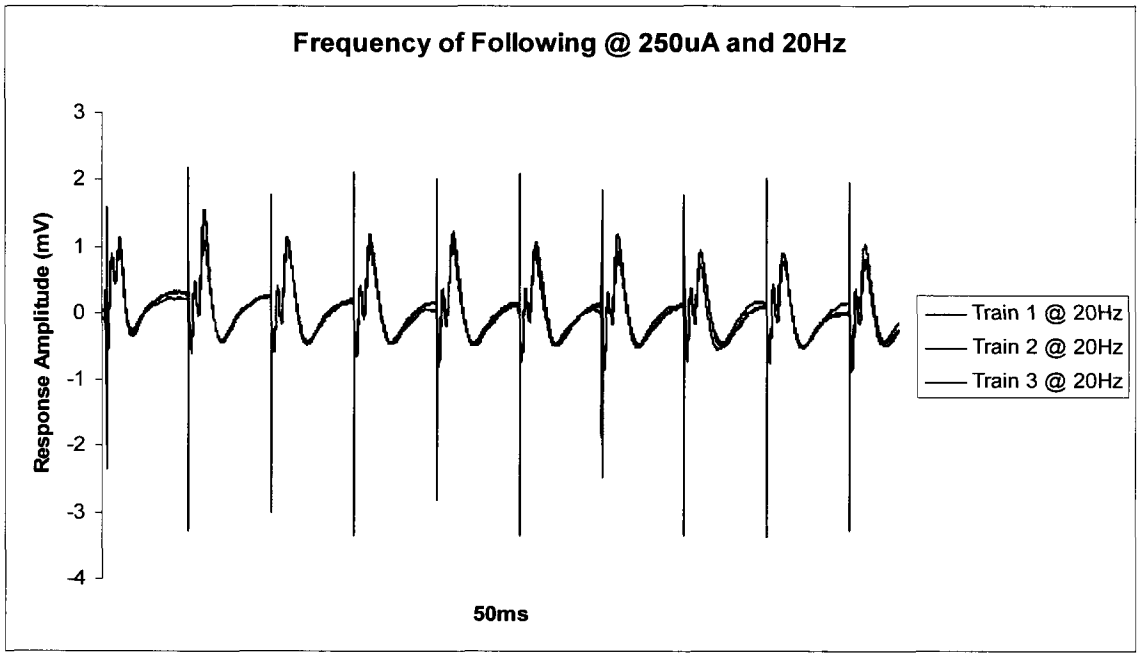


Figure 6.1.12

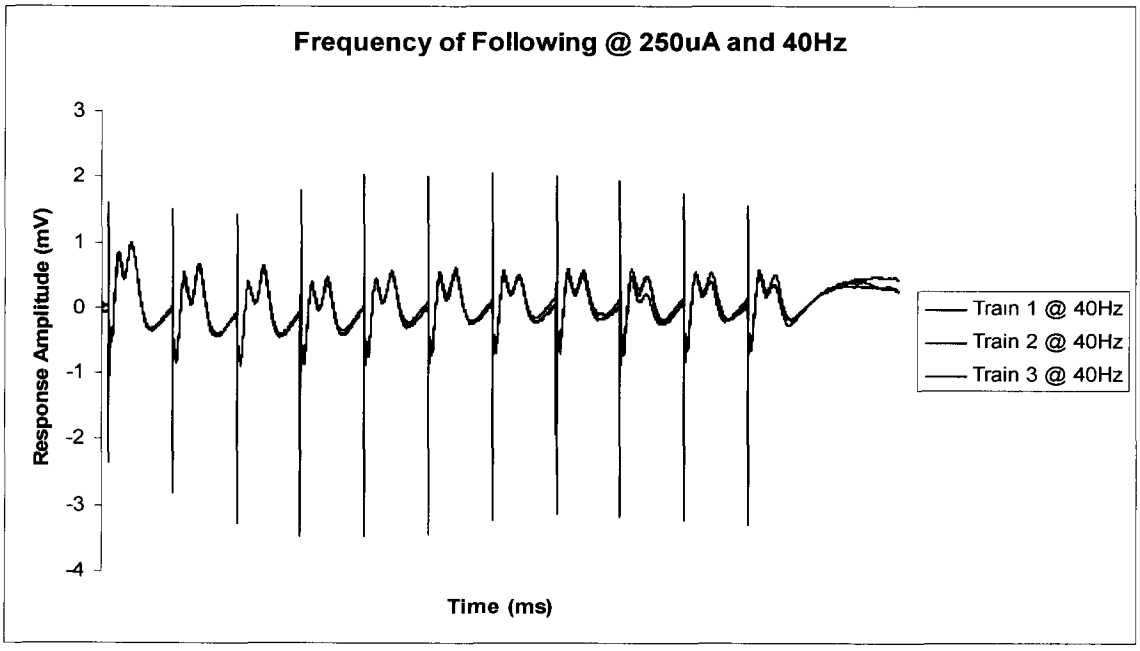


Figure 6.1.13

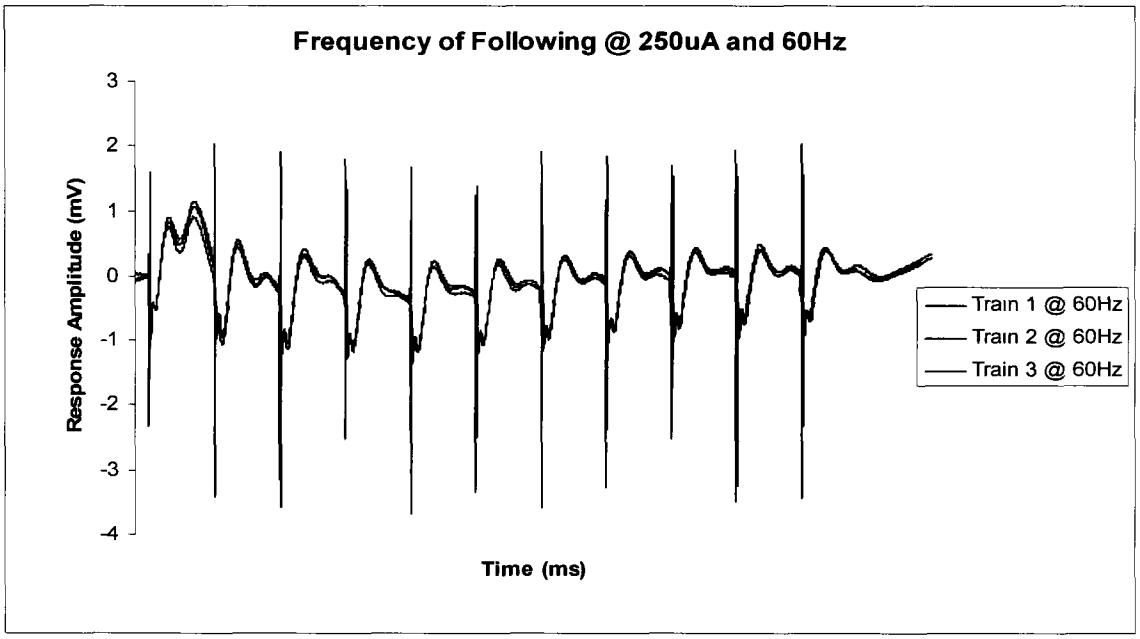


Figure 6.1.14

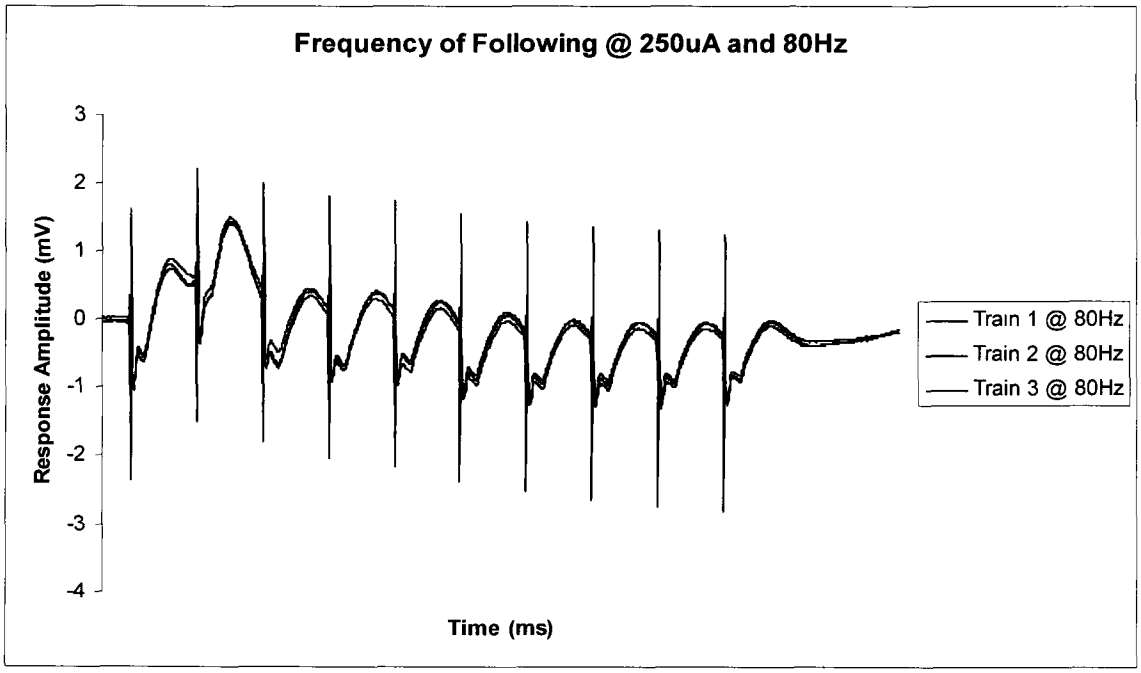


Figure 6.1.15

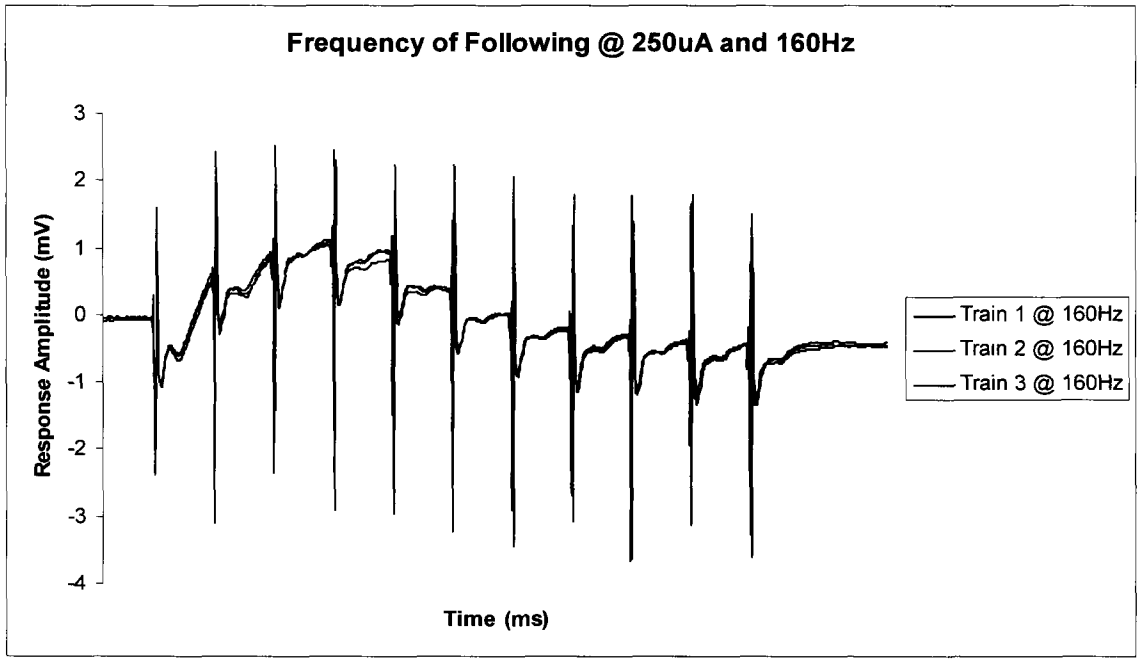


Figure 6.1.16

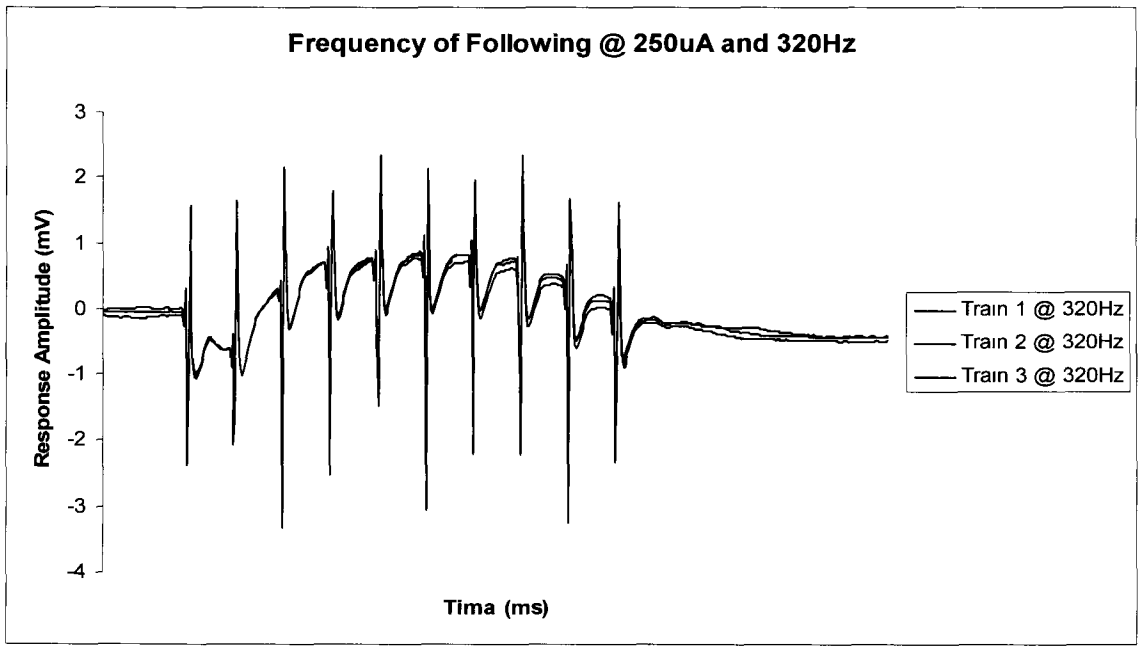


Figure 6.2.1

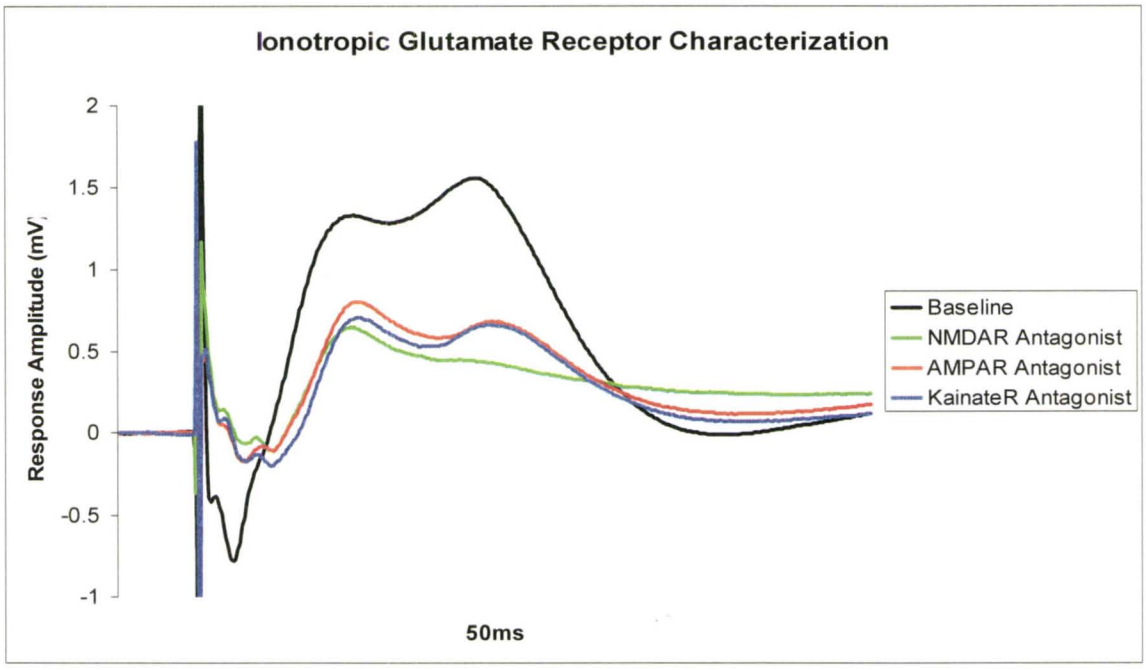


Figure 6.2.2

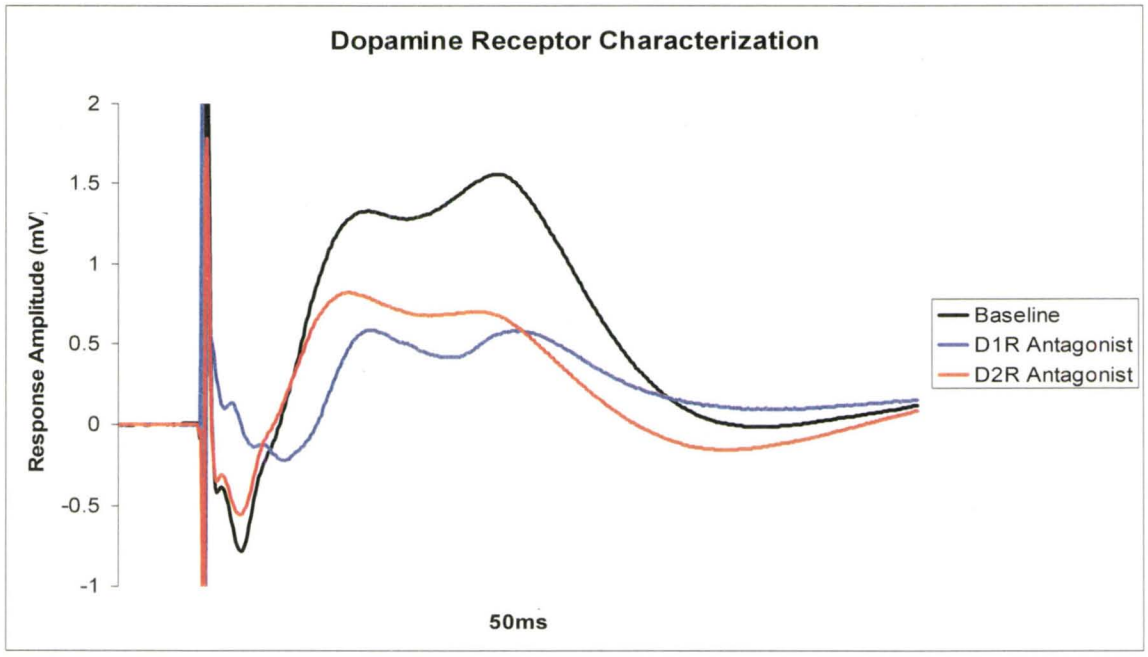


Figure 6.2.3

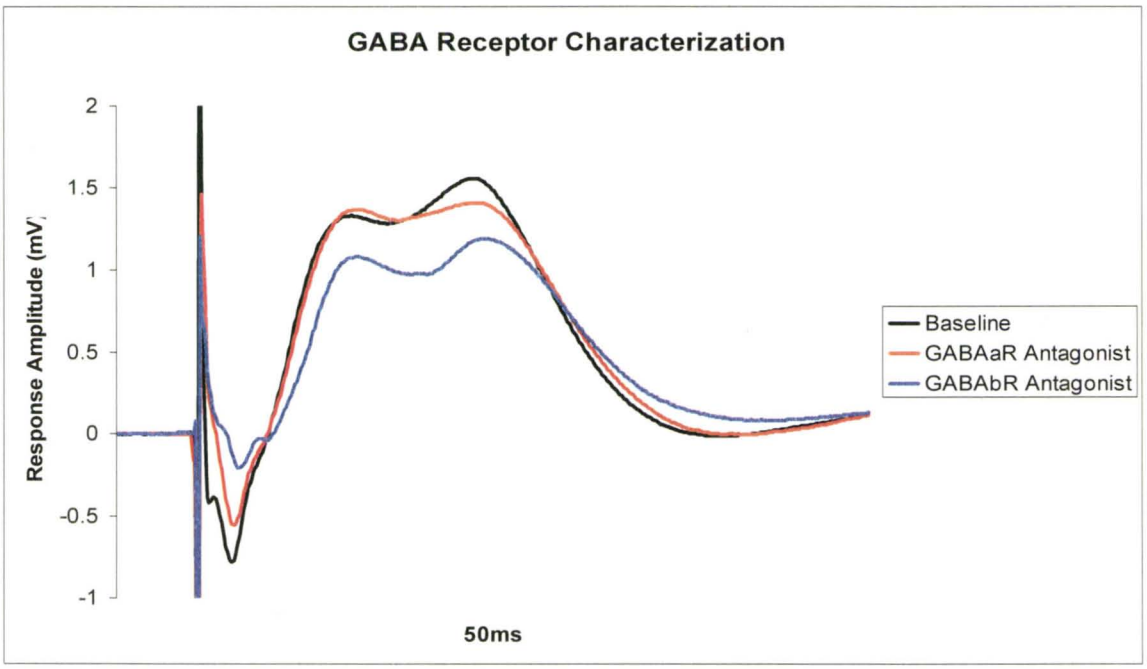

GABA Receptor Characterization
Response Amplitude (mV)
2
1.5
1
0.5
0
-0.5
-1
50ms
Baseline
GABAaR Antagonist
GABAbR Antagonist

Figure 6.3.1

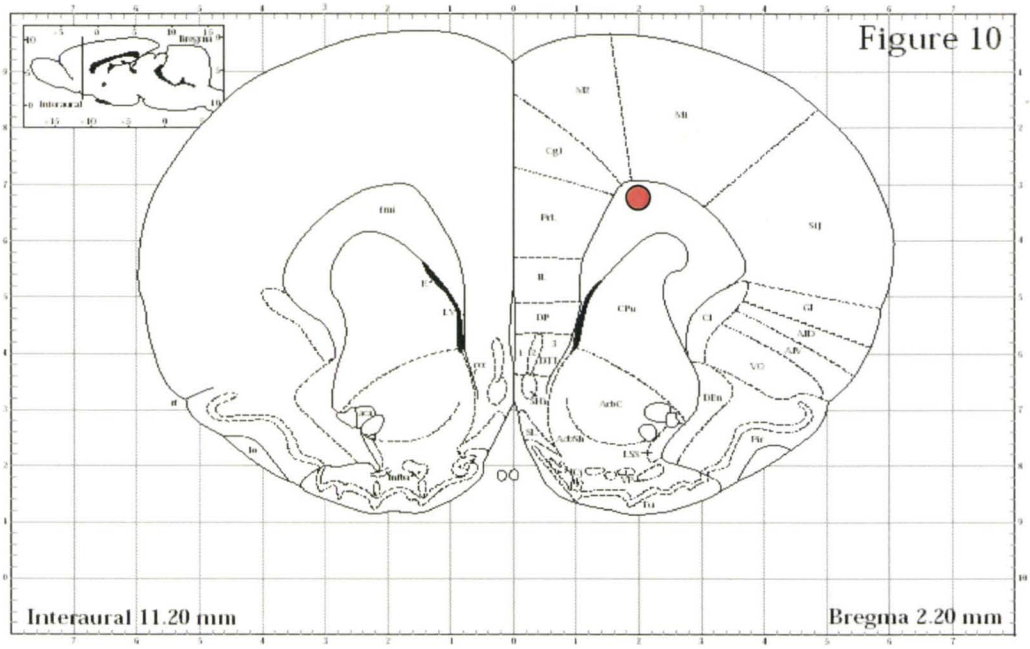


Figure 6.3.2

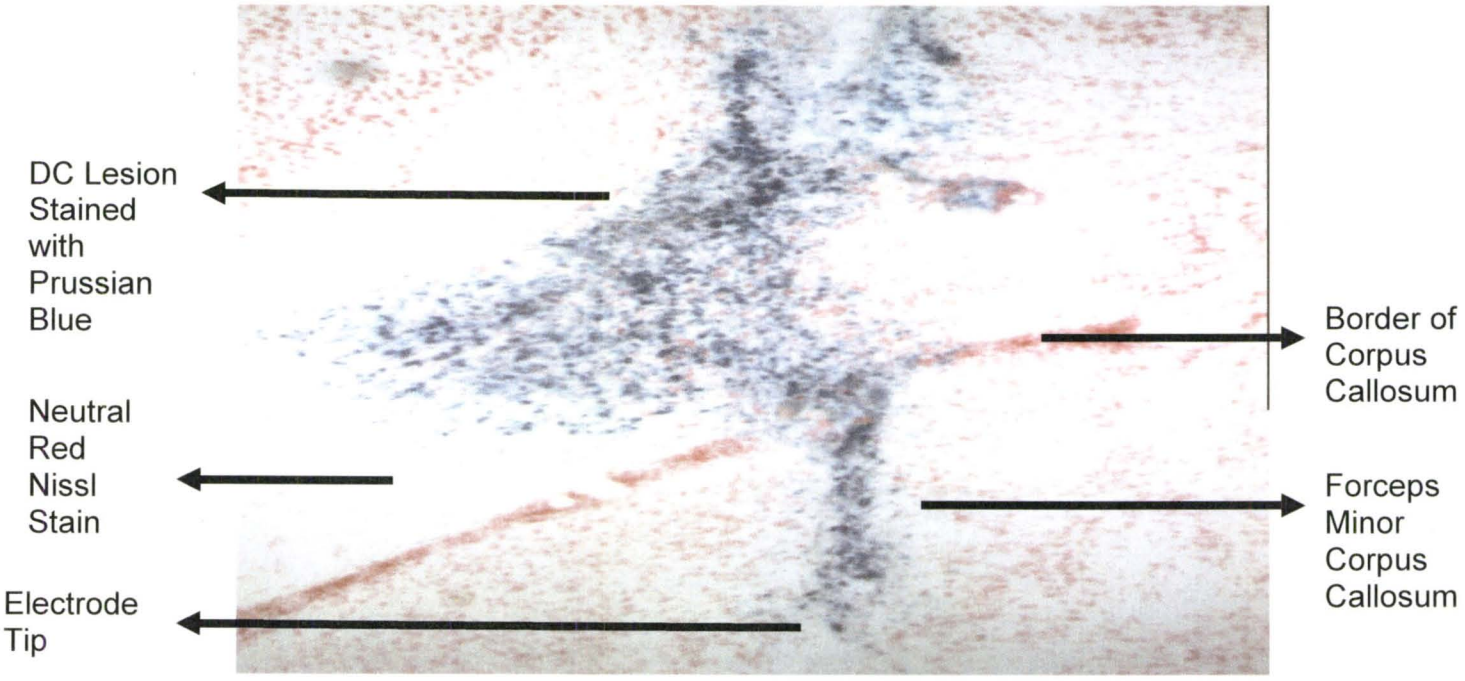


Figure 6.3.3

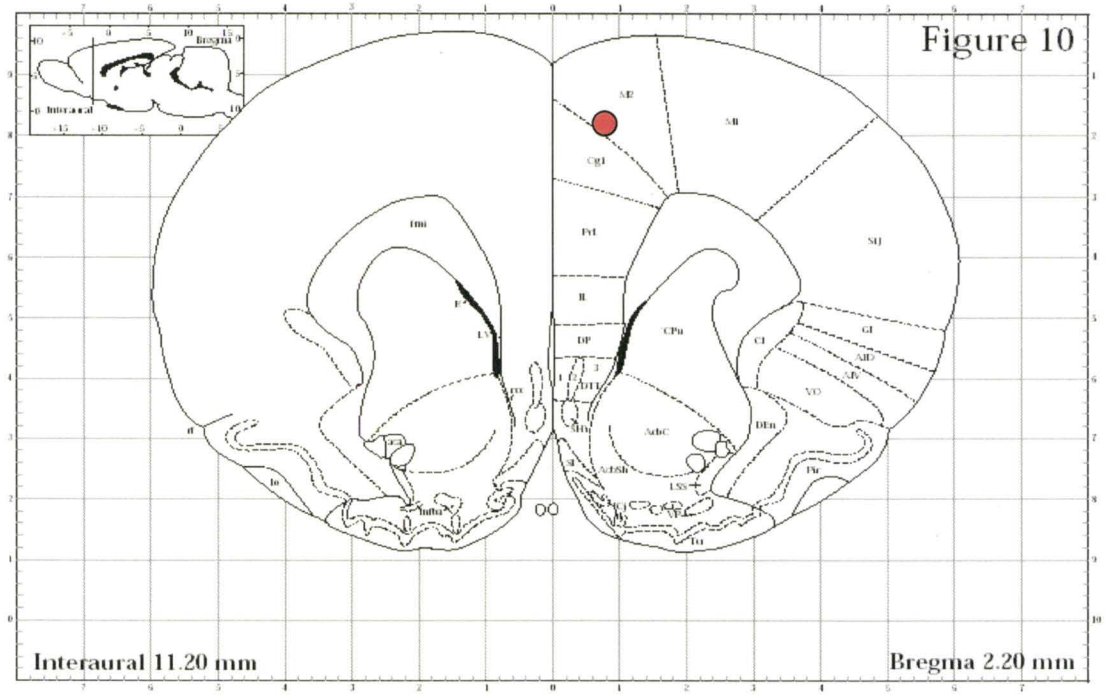
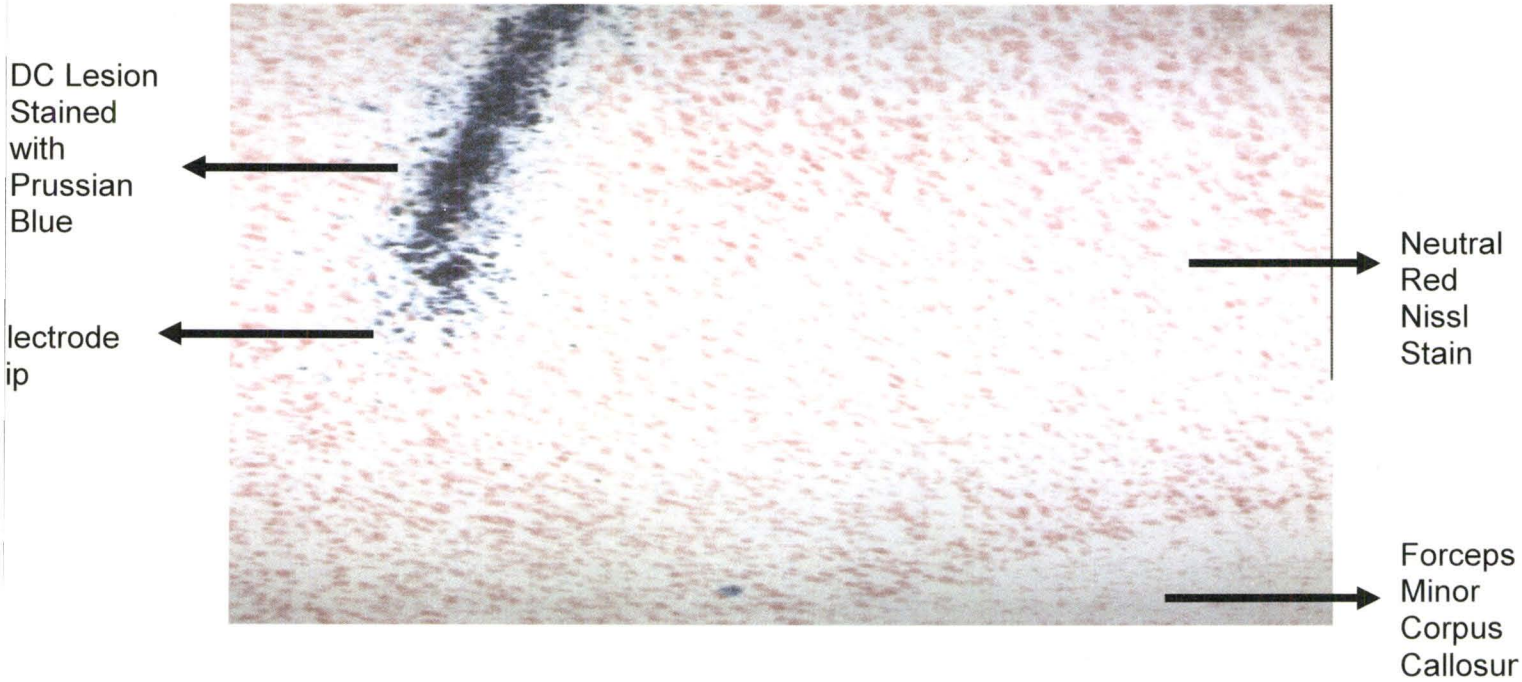


Figure 6.3.4



6.5 Figure Captions

Figure 6.1.1 Medial Prefrontal Cortex Monopolar & Bipolar Field Potential

This figure depicts the negative monopolar mPFC recording (*black*), positive mPFC recording (*red*), and bipolar mPFC recording (*blue*).

Figure 6.1.2 Medial Prefrontal Cortex Evoked Field Potential

This figure illustrates a representative averaged evoked field potential (response amplitude in mV) across 50ms in the mPFC. Single representative animal. Single pulses of increasing intensity were delivered at a frequency of 0.1Hz, and 10 field responses were evoked at each intensity, digitized at 10kHz, and averaged at each of seven intensities (32, 80, 160, 250, 400, 500, and 1000uA).

Figure 6.1.3 Morphological Characterization: Recovery from Anaesthesia

Sodium pentobarbital (65mg/kg, i.p.) was administered to five rats following input/output testing (single pulses of increasing intensity delivered to the corpus callosum at a frequency of 0.1Hz; 10 field responses (mV) were evoked at each intensity, amplified, digitized at 10kHz, and averaged at each of four intensities (160, 400, 500, and 1000μA). Once a level of general anaesthesia was reached, field responses in the mPFC in response to a single test pulse delivered every 30 seconds and the same four intensities were

measured until the rats completely recovered from anaesthesia. The peak amplitude was reduced by 0.77mV (51% of baseline amplitude) and 1.34mV (94% of baseline amplitude) on the first post-anaesthetic recording (20 minutes following anaesthesia) in the monosynaptic and polysynaptic components, respectively. One hour post-anaesthesia, the monosynaptic component was reduced 0.20mV (13% of baseline amplitude), and the polysynaptic component was reduced 0.99mV (70% of baseline amplitude). One and a half hours post-anaesthesia, the monosynaptic component was reduced 0,02mV (1.3% of baseline amplitude), and the polysynaptic component was reduced 0.52mV (37% of baseline amplitude). Two hours post-anaesthesia, the monosynaptic component was reduced 0.08mV (5% of baseline amplitude), and the polysynaptic component was reduced 0.26mV (18% of baseline amplitude). Two and a half hours post-anaesthesia, the monosynaptic component was reduced 0.13mV (9% of baseline amplitude), and the polysynaptic component was reduced 0.01mV (70% of baseline amplitude). Three hours post-anaesthesia, the monosynaptic component was reduced 0.06mV (4% of baseline amplitude), and the polysynaptic component was reduced 0.02mV (1.4% of baseline amplitude). Lastly, three and a half hours post-anaesthesia, the monosynaptic component was reduced 0.01mV (0.66% of baseline amplitude), and the polysynaptic component was reduced 0.01mV (0.07% of baseline amplitude).

Figure 6.1.4-6.1.10 Morphological Characterization: Paired Pulse Tests

Two pulses were delivered at seven interpulse intervals: 50ms (Figure 6.4), 70ms (Figure 6.5), 100ms (Figure 6.6), 150ms (Figure 6.7), 200ms (Figure 6.8), 300 (Figure 6.9), and 500ms (Figure 6.10). In all animals there is a 21-73% reduction in the monosynaptic component of the response to the second pulse relative to the responses evoked by the first pulse across 50-500ms IPIs (50% at 50 and 70ms; 43% at 100ms; 21% at 150ms to 21%; 73% at 200ms; 62% at 300ms; 50% at 500ms). With respect to the polysynaptic component, in all animals there is a facilitation in the polysynaptic component of the response to the second pulse relative to the responses evoked by the first pulse (16-54% facilitation of the response occurs at IPIs of 70-500ms (50% at 70ms; 54% at 100ms; 45% at 150ms; 52% at 200ms; 50% at 300ms; 16% at 500ms), except at an IPI of 50ms (49% reduction).

Figure 6.1.11-6.1.16 Morphological Characterization: Frequency of Following Tests

Trains of 10 pulses were delivered at six frequencies: 20Hz (Figure 6.11), 40Hz (Figure 6.12), 60Hz (Figure 6.13), 80Hz (Figure 6.14), 160 (Figure 6.15), 320Hz (Figure 6.16). The intertrain interval was five minutes. At 40 and 60Hz stimulation, the monosynaptic response was comparable to that at 20Hz. While attenuated considerably (60%) at frequencies of 80Hz and greater, the initial spike and the subsequent early component still followed stimulation at higher frequencies. The early component was, however, eliminated at a frequency of

320Hz. The polysynaptic field potential component was attenuated (53%) by stimulation at only 40Hz, and virtually eliminated (97%) during 60Hz stimulation. Stimulation at all higher frequencies (80, 160, and 320Hz) failed to produce a polysynaptic component.

Figure 6.2.1 Pharmacological Characterization: Ionotropic Glutamate Receptor

Twenty minutes following drug injection a single test pulse delivered 10 times at each of seven intensities (32, 80, 160, 250, 400, 500, and 1000μA), glutamate receptor antagonists were administered. The NMDAR antagonist MK-801 (0.25mg/kg) attenuated the monosynaptic component by 0.67mV (51% of baseline amplitude), and the polysynaptic component by 1.13mV (72% of baseline (*black*) amplitude) (*green*). The AMPAR antagonist GYKI 52466 (1.2mg/kg) attenuated the monosynaptic component by 0.52mV (40% of baseline amplitude) and the polysynaptic component by 0.89mV (57% of baseline amplitude) (*red*). The kainate receptor antagonist SYM 2081 (0.15mg/kg) attenuated the monosynaptic component by 0.62mV (47% of baseline amplitude) and the polysynaptic component by 0.94mV (60% of baseline amplitude) (*blue*).

Figure 6.2.2 Pharmacological Characterization: Dopamine Receptor

Twenty minutes following drug injection a single test pulse delivered 10 times at each of seven intensities (32, 80, 160, 250, 400, 500, and 1000μA),

dopamine receptor antagonists were administered. The D_1R antagonist *SKF83566* (0.15mg/kg) attenuated the monosynaptic component by 0.52mV (34% of baseline (*black*) amplitude), and the polysynaptic component by 0.87mV (55% of baseline amplitude) (*blue*). The D_2R antagonist Sulpiride (12mg/kg) attenuated the monosynaptic component by 0.74mV (56% of baseline amplitude) and the polysynaptic component by 1mV (64% of baseline amplitude) (*red*).

Figure 6.2.3 Pharmacological Characterization: GABA Receptor

Twenty minutes following drug injection a single test pulse delivered 10 times at each of seven intensities (32, 80, 160, 250, 400, 500, and 1000μA), GABAR antagonists were administered. The GABAaR antagonist Picrotoxin (1mg/kg) actually facilitated the monosynaptic component by 0.06mV (5% of baseline (*black*) amplitude) (*red*), but reduced the polysynaptic component by 0.19mV (12% of baseline amplitude). The GABAbR antagonist Phaclofen (0.75mg/kg) reduced the monosynaptic component by 0.25mV (19% of baseline amplitude), and the polysynaptic component by 0.41mV (26% of baseline amplitude) (*blue*).

Figure 6.3.1 Cerebral Coronal Section: Corpus Callosum

This section (Paxinos & Watson, 1997) depicts the stimulation site (forceps minor corpus callosum) coordinates anterior (1.5mm) and lateral (1.8mm) to bregma (*red dot*).

Figure 6.3.2 Histology: Corpus Callosum Coronal Section

The deepest positions of depth stimulation was marked by DC lesions. The brains were cut into 50μm sections and those with DC lesions were stained with Prussian Blue and Neutral Red.

Figure 6.3.3 Cerebral Coronal Section: Medial Prefrontal Cortex

This section (Paxinos & Watson, 1997) depicts the recording site (mPFC) coordinates anterior (3.2mm) and lateral (1.0mm) to bregma (*red dot*).

Figure 6.3.4 Histology: Medial Prefrontal Cortex Coronal Section

The deepest positions of depth stimulation was marked by DC lesions. The brains were cut into 50μm sections and those with DC lesions were stained with Prussian Blue and Neutral Red.

CHAPTER 7

PREFRONTAL CORTEX LTP INDUCTION RESULTS

7.1 Baseline (Saline) Prefrontal Cortex LTP Induction

Again, all baseline field potentials showed an early spike, an early population excitatory postsynaptic potential (EPSP), and a longer latency polysynaptic component. All components increased in amplitude with stimulation intensity, and the polysynaptic components were variable across animals and, sometimes, across tests in the same animal. Otherwise, response amplitudes were reasonably stable across baseline tests.

During LTP induction, the saline plus HFS test group showed a small 0.23mV decrease in the monosynaptic component, representing 15% of baseline amplitude (Figure 7.1.1, 7.1.2). This attenuation in peak amplitude approached significance, indicating that the stimulation may have led to decreased amplitudes across sessions. A further decrease in amplitude occurred during the decay phase (0.42mV; 27%), which was maintained. The saline-alone control group, however, remained reasonably stable over the same time period. In the motor cortex, similar decreases are due to an increase in the population spike components, which generate field currents in opposition to the EPSP components. However, the motor cortex response also shows a change in morphology of the early component response indicative of population spikes (Chapman et al., 1998). No such changes were observed in the PFC response,

at least not clearly, so these decreased amplitudes may represent a depression effect. Single unit recording will be required to resolve this issue.

The saline plus HFS test group showed a significant 0.46mV increase in the amplitude of the polysynaptic component compared to the saline-only control group, representing 19% of baseline amplitude ($F[10, 200]=4.183$, $p=0.005$) (Figure 7.1.1, 7.1.3). This effect was significantly maintained (0.21mv; 9%) during the decay phase ($F[1, 20]=4.827$, $p=0.05$). The polysynaptic component of the saline control group remained stable over the test period. Note that all saline main effects can be found in Table 7.1.1.

7.2 Modulation of Prefrontal Cortex LTP Induction: D_1R Effects

In general, administration of the D_1R agonist increased response amplitude while administration of the D_1R antagonist decreased response amplitude. Behaviourally, rats showed less movement and activity, and ate less, following the administration of the D_1R agonist. There were no obvious changes in behaviour during the administration of the D_1R antagonist. Note that all D_1R drug main effects can be found in Table 7.2.1.

D_1R Agonist (A 68930 Hydrochloride, 0.4mg/kg/ml)

The D_1R agonist plus HFS test group showed a significant decrease in the monosynaptic component (0.34mV; 20% of baseline amplitude) compared to the saline plus HFS group (day by group interaction: $F[10, 120]=3.033$, $p=0.002$)

(Figure 7.2.1, 7.2.2). The decrease was larger than that seen versus the saline control group, which did not reach significance. The decrease in the D_1R agonist plus HFS group was significantly maintained during the decay phase (0.62mV; 37% of baseline amplitude) in comparison to both the saline-only group (day by group interaction: $F[3,48]=5.070$, $p=0.004$) and the saline plus HFS group (day by group interaction: $F[3, 48]=5.916$, $p=0.002$). The shift toward baseline appeared to be slow, and much longer decay periods would be required to determine the longevity of these changes.

The increase in amplitude of the polysynaptic component following HFS plus administration of the D_1R agonist did not exceed the increased response amplitude induced with HFS plus saline. The increases were also similarly maintained during the decay phase in both groups. As expected, the increase in polysynaptic component amplitude (1mV; 67% of baseline amplitude) was significant when compared to the drug control group response (day by group interaction: $F[10, 120]=3.086$, $p=0.002$) (Figure 7.2.1, 7.2.3). Also as expected, the increase in polysynaptic component amplitude over days was significant when compared to the saline control group response (day by group interaction: $F[10,160]=4.555$, $p=0.000$), but this increase was not maintained during the decay phase of the experiment (1.3mV; 87% of baseline amplitude). Both the drug- and saline-alone control groups remained relatively stable throughout the experiment.

D_1R Antagonist (SKF 83566 Hydrobromide, 0.15mg/kg/ml)

The D_1R antagonist did not alter response amplitude in the monosynaptic component in comparison to the drug and saline control groups (Figure 7.2.4, 7.2.5). The administration of the D_1R antagonist during HFS did, however, nearly significantly block the induction of LTP in the polysynaptic component compared to the saline test group (day by group interaction: $F[10, 120]=3.583$, $p=0.07$) (Figure 7.2.4, 7.2.6). In fact, the LTP was replaced by an LTD (0.15mV reduction representing 16% of baseline amplitude) in the drug test group compared to the drug control group (day by group interaction: $F[10, 110]=3.821$, $p=0.000$), indicating that the effect was not due to the drug alone. There was also a significant block of LTP compared to the saline control group (day by group interaction: $F[10,150)=6.904$, $p=0.000$). During the decay phase, this LTD effect was significantly maintained (0.78mV reduction representing 85% of baseline amplitude) in comparison to the saline-alone control group (day by group interaction: $F[3, 45]=3.00$, $p=0.04$) and nearly so in comparison to the drug-only control group (day by group interaction: $F[3,33]=2.596$, $p=0.06$).

7.3 Modulation of Prefrontal Cortex LTP Induction: D_2R Effects

In general, administration of the D_2R agonist, Quinpirole, dose-dependently increased response amplitude, while administration of the D_2R antagonist, Sulpiride, dose-dependently decreased response amplitude (Figure 7.3.1). Note that all D_2R drug main effects can be found in Table 7.3.1.

7.3.1 D_2R Agonist (Quinpirole)

Three doses of the D_2R agonist Quinpirole were tested for their effect on LTP induction in the mPFC following the application HFS to the corpus callosum. In general, the D_2R agonist produced an overall significant decrease in the monosynaptic component across doses (day by group interaction: F[7, 315]=2.125, p=0.041), and an overall significant increase in the polysynaptic component across doses (day by group interaction: F[7, 308]=7.049, p=0.000; day by dose interaction: F[14, 308]=1.962, p=0.020). The day by group interaction effect was significantly maintained during the decay phase (F[3, 120]=3.675, p=0.014). The agonist also induced a behavioural sensitization effect (see Chapter 8).

D_2R Agonist (Quinpirole) Low Dose (0.125mg/kg/ml)

Combining the low dose of the agonist with HFS had no significant effect on the HFS-induced early component depression (0.24mV; 17% of baseline amplitude); it appeared to be comparable to that induced in the saline plus HFS group (Figure 7.3.1.1, 7.3.1.2). This was nearly significant in comparison to the saline control group (F[10, 190]=1.795, p=0.064). This depression effect was maintained during the decay phase (0.5mV; 35% of baseline amplitude). Both drug and saline control groups remained stable throughout.

Similarly, the polysynaptic component amplitude shift (0.13mV; 10% of baseline amplitude) was comparable between the drug plus HFS test group and

the saline plus HFS group, yet nearly reaching significance when compared to the saline control group (day by group interaction: $F[5, 98]=2.144$, $p=0.064$) (Figure 7.3.1.1, 7.3.1.3). The saline test and drug test groups were not significantly different, showing that administration of 0.125mg/kg of quinpirole did not alter baseline levels of LTP in the polysynaptic component. The saline and drug control groups remained reasonably stable throughout, and comparable to one another. Though it did not quite reach significance, the amplitude change in the drug plus HFS test group appeared to decay slowly toward baseline once the HFS ceased (0.35mV; 26% of baseline amplitude).

D_2R Agonist (Quinpirole) Middle Dose (0.25mg/kg/ml)

The middle dose D_2R agonist plus HFS test group showed a significantly larger decrease (0.125mV; 15% of baseline amplitude) in the monosynaptic component following HFS when compared to the saline plus HFS group ($F[10, 140]=1.927$, $p=0.046$) (Figure 7.3.1.4, 7.3.1.5). This decrease was nonsignificantly maintained (0.15mV; 17% of baseline amplitude) during the decay phase. Both the saline plus HFS group and drug plus HFS group remained relatively stable throughout, and comparable to one another, while the saline and drug control groups remained reasonably stable throughout.

While the amplitude of the polysynaptic component in the drug plus HFS group was significantly increased over days (0.016mV; 3% of baseline amplitude) compared to the drug and saline control groups (day by group interaction: F[10,

140]=3.531, p=0.000) and F[10,180]=5.327, p=0.000, respectively), and was larger than that seen in the saline plus HFS group, it was not significantly different from the latter group (Figure 7.3.1.4, 7.3.1.6). The enhanced responses in both HFS groups were maintained (0.27mV; 46% of baseline amplitude) during the decay phase, significantly so in comparison to the saline control group (F[3, 54)=4.568, p=0.006). The control group responses remained relatively stable throughout the experiment.

D_2R Agonist (Quinpirole) High Dose (0.5mg/kg/ml)

Once again, the drug plus HFS group expressed a greater decrease in response amplitude (0.39mV; 32% of baseline amplitude) compared to the saline plus HFS group in the monosynaptic component (Figure 7.3.1.7, 7.3.1.8), but the difference was not significant. This decrease was still present at the end of the decay phase (0.23mV; 19% of baseline amplitude), during which the drug test group response was significantly lower than the drug control group response (F[10, 120]=1.915, p=0.049). Both the saline and drug control groups remained reasonably stable throughout, although there was a tendency for some upward drift in the drug control group.

The amplitude of the polysynaptic component in the D_2R agonist plus HFS group was significantly increased over days (1.16mV; 75% of baseline amplitude) compared to the saline plus HFS group (day by group interaction: F[10, 160]=3.556, p=0.000) and, of course, highly significant compared to both the

drug control group response, indicating that the effect was not due to the drug alone (day by group interaction: F[10, 120]=9.799, p=0.000), and to the saline control group response (day by group interaction: F[10, 160]=11.064, p=0.000) (Figure 7.3.1.7, 7.3.1.9). The increased amplitudes were maintained (0.75mV; 48% of baseline amplitude) during the decay phase in both HFS groups, significantly so in comparison to the saline control group (F[3, 48]=4.420, p=0.008). The saline and drug control groups remained relatively stable throughout the experiment, with the drug group, as usual, showing slightly more variability.

D_2R Agonist (Quinpirole) Dose Comparisons (0.125, 0.25, 0.5mg/kg/ml)

There was a more rapid decrease in the monosynaptic component with increased doses of the D_2R agonist, significantly so for the 0.125mg/kg versus the 0.25mg/kg test groups (F[10, 170)=2.108, p=0.026) (Figure 7.3.1.10). There was also a greater increase in the polysynaptic component with increased doses of the D_2R agonist (Figure 7.3.1.11). This reached significance in the 0.5mg/kg test group versus 0.125mg/kg test group (F[10, 150]=4.002, p=0.000), and nearly so between the 0.125mg/kg and 0.25mg/kg test groups (F[10, 170)=1.709, p=0.082). For both monosynaptic and polysynaptic components, each of the drug control groups at each dose remained reasonably stable and comparable to one another and to the saline control group.

7.3.2 D_2R Antagonist (Sulpiride)

Three doses of the D_2R antagonist Sulpiride were tested for their effect on LTP induction in the prefrontal cortex following the application HFS to the corpus callosum. In general, the D_2R antagonist produced an overall significant decrease in the polysynaptic component across doses (day by group interaction: $F[7, 336]=12.074$, $p=0.000$; day by dose interaction: $F[14, 336]=1.904$, $p=0.025$). The day by group interaction effect was significantly maintained during the decay phase ($F[3, 138]=3.517$, $p=0.017$). Administration of the antagonist also induced some lethargy and a decrease in appetite, resulting in a block in weight gain, with weight maintained at levels measured during the baseline phase of the experiment (mean baseline weight of 343gms with a range of 330-347gms).

D_2R Antagonist (Sulpiride) Low Dose (3mg/kg/ml)

The drug plus HFS group showed a decrease (0.08mV; 9% of baseline amplitude) in response amplitude of the monosynaptic component that was slightly, but not significantly, greater than the decrease expressed by the saline plus HFS group (Figure 7.3.2.1, 7.3.2.2). However, both the drug control and drug plus HFS groups also show a small increase in response amplitude at the beginning of the injection series. Otherwise, both the saline and drug control groups remained reasonably stable and comparable to one another.

Although there was a significant increase (0.4mV; 33% of baseline amplitude) in the response amplitude of the polysynaptic component in the drug plus HFS group compared to the saline control group (day by group interaction: F[10, 150]=6.344, p=0.000), and a nearly significant increase compared to the drug control group (day by group interaction: F[10, 130]=1.773, p=0.072) (Figure 7.3.2.1, 7.3.2.3), there were no significant differences between the drug plus HFS and the saline plus HFS groups. The difference between the HFS groups and the control groups were maintained (0.34mV; 28% of baseline amplitude) during the decay phase. For example, the drug plus HFS group response was significantly elevated compared to both the saline (day by group interaction: F[3, 45]=5.923, p=0.002) and the drug (day by group interaction: F[3, 39]=4.526, p=0.008) control groups. Both the drug and saline control group responses were reasonably stable throughout the experiment.

D_2R Antagonist (Sulpiride) Middle Dose (6mg/kg/ml)

The decrease in the monosynaptic component (0.21mV; 20% of baseline amplitude) in the drug plus HFS group was slightly larger than for the lower dose group, but still not significantly different from the drug plus HFS group (Figure 7.3.2.4, 7.3.2.5). With the slightly higher variability in these groups, the differences were also not significant between HFS groups and control groups. Although not significant, the decreased response amplitude in the drug plus HFS

group did appear to be maintained during the decay phase (0.24mV; 23% of baseline amplitude).

Although the increase in the response amplitude of the polysynaptic component of the drug plus HFS group (0.17mV; 13% of baseline amplitude) was comparable to that of the saline plus HFS group, the drug plus HFS group response changed rapidly following cessation of stimulation (Figure 7.3.2.4, 7.3.2.6). This drug plus HFS decay effect was nearly significantly different than that seen in the saline-only (day by group interaction: $F[1,18]=3.406$, $p=0.081$) and the drug-only (day by group interaction: $F[1,18]=4.294$, $p=0.053$) groups, which remained stable throughout.

D_2R Antagonist (Sulpiride) High Dose (12mg/kg/ml)

There was a significantly larger reduction (0.12mV; 25% of baseline amplitude) in response amplitude of the monosynaptic component in the high dose of Sulpiride plus HFS group compared to the saline plus HFS group (day by group interaction: $F[10, 190)=2.045$, $p=0.031$), drug control group ($F[10, 180]=2.281$, $p=0.015$), and the saline control group ($F[10, 190]=1.940$, $p=0.042$) (Figure 7.3.2.7, 7.3.2.8). This enhanced decrease in response was maintained during the decay phase of the experiment (0.1mV; 20% of baseline amplitude). Again, the control groups were both relatively stable and comparable to one another.

LTP induction in the drug plus HFS group appeared to track LTP in the saline plus HFS group fairly closely (0.3mV; 37% of baseline amplitude) (Figure 7.3.2.7, 7.3.2.9). The LTP effect was significantly increased in comparison to both the drug control group ($F[10, 180]=2.658$, $p=0.005$), and the saline control group (day by group interaction: $F[10, 190]=2.309$, $p=0.014$). However, when HFS ceased the LTP decayed very rapidly to baseline in the drug plus HFS group. The drug plus HFS group, however, did remain nearly significantly different than the drug control group ($F[3, 54)=2.289$, $p=0.089$). All drug control groups remained reasonably stable, and comparable to one another.

D_2R Antagonist (Sulpiride) Dose Comparisons (3, 6, 12mg/kg/ml)

In the monosynaptic component, increasing doses of the drug plus HFS led to greater, yet nonsignificant, decreases in the response amplitude during the latter half of LTP-induction (i.e., days 12-15) and the decay phase of the experiment (Figure 7.3.2.10). All drug control groups remained relatively stable. Sulpiride appeared to produce a small, yet significant, dose-dependent reduction of LTP in the polysynaptic component, particularly in the latter half of the induction phase (Figure 7.3.2.11). The 6mg/kg dose plus HFS group showed a significant reduction in the peak response amplitude compared to the 3mg/kg dose (day by group interaction: $F[10, 150]=2.902$, $p=0.002$), an effect which was significantly maintained during the decay phase (day by group interaction: $F[3, 45]=2.912$, $p=0.045$). Also, the 12mg/kg dose plus HFS group showed a

significant reduction in response amplitude compared to the 3mg/kg dose (day by group interaction: $F[10, 140]=2.595$, $p=0.006$), and no significant maintenance during the decay phase. It was in the long-term maintenance of the LTP effect that the clearest effect was seen. There was a clear, dose-dependent increase in the LTP decay rates in the drug plus HFS groups. All drug control groups remained reasonably stable, and comparable to one another.

7.3.3 Figures

Figure 7.1.1

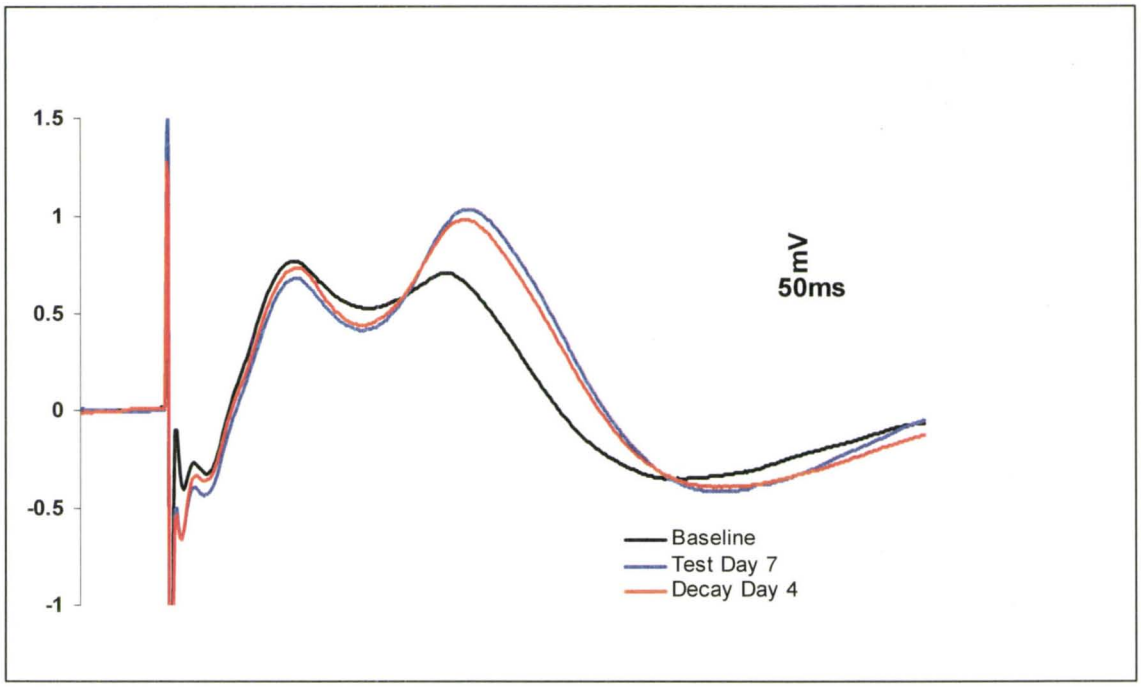
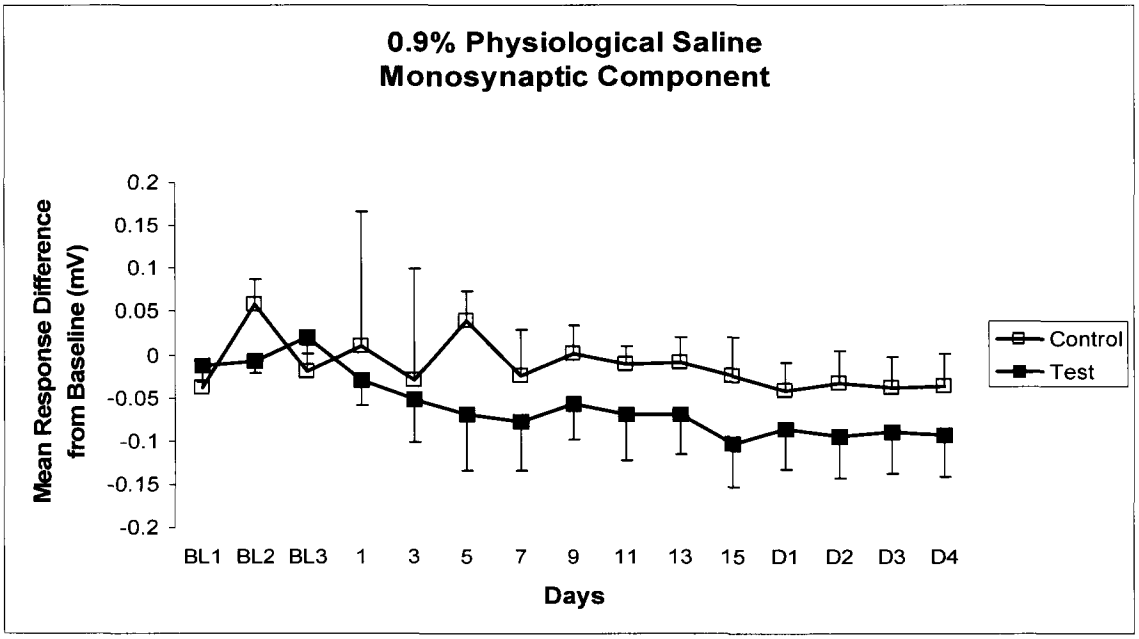


Figure 7.1.2



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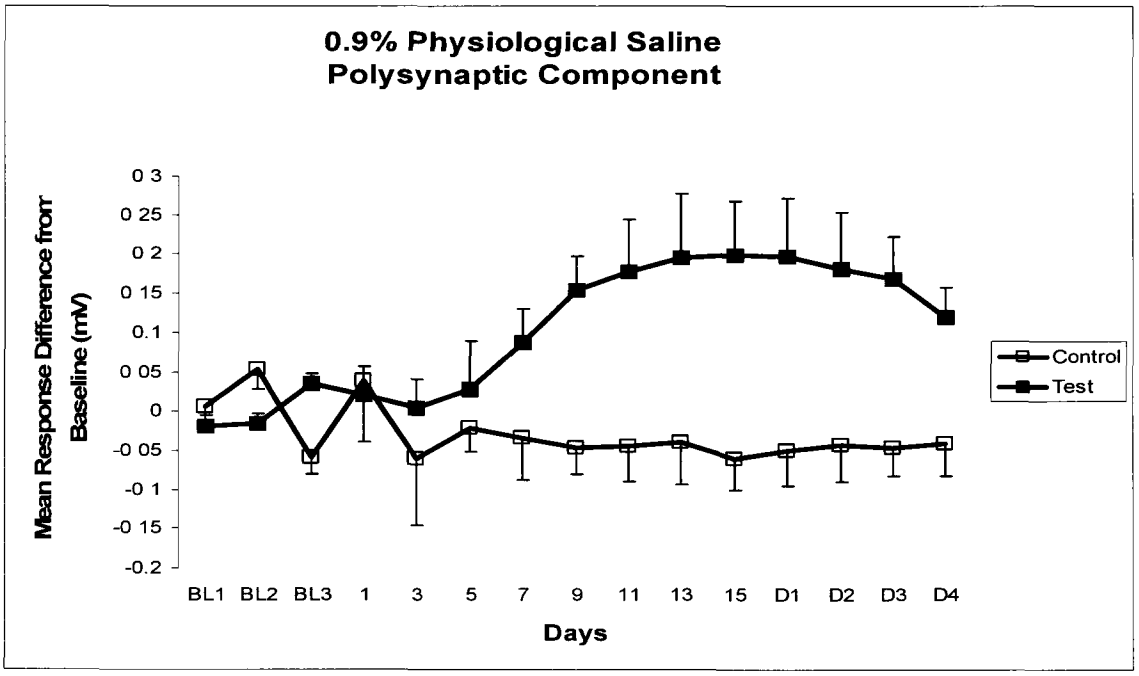

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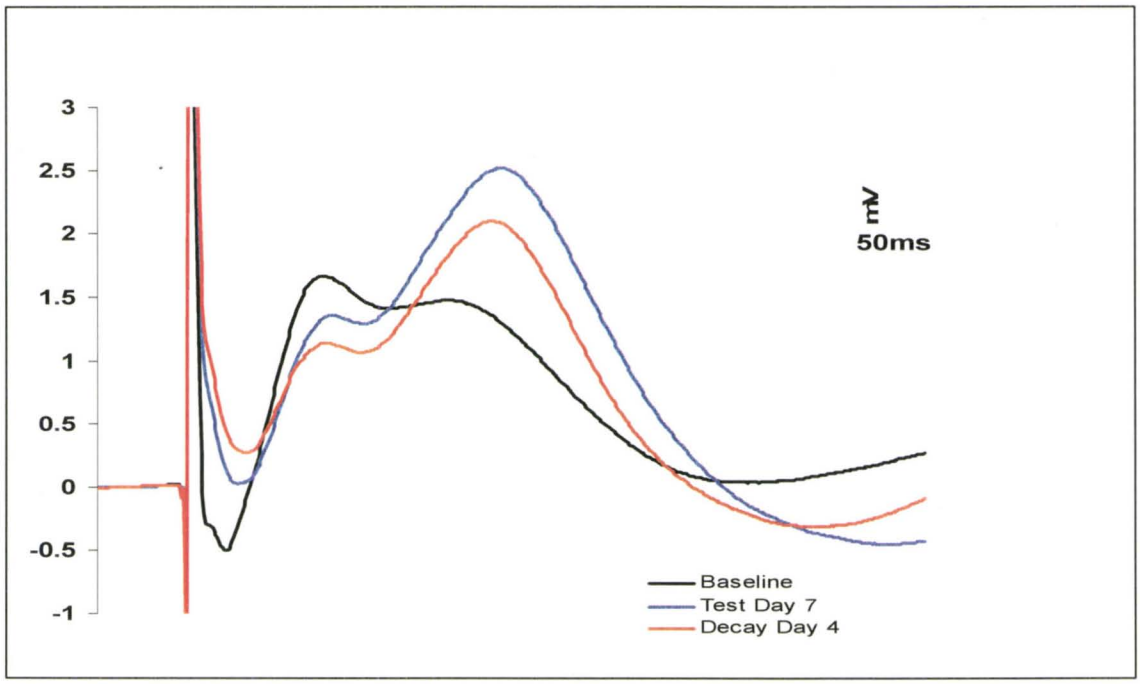

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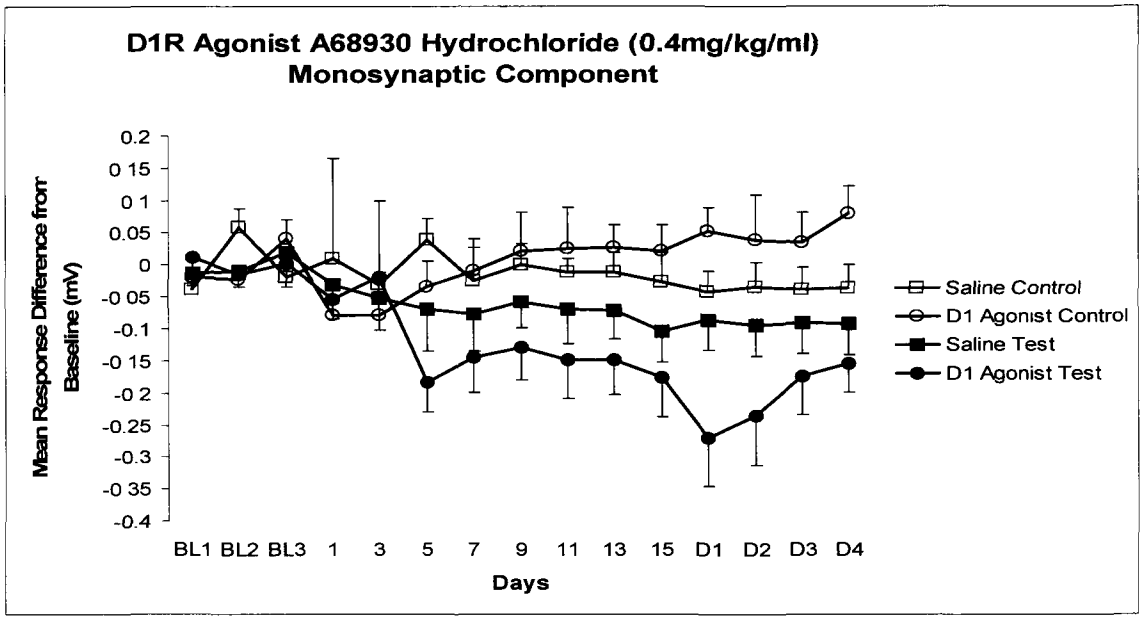

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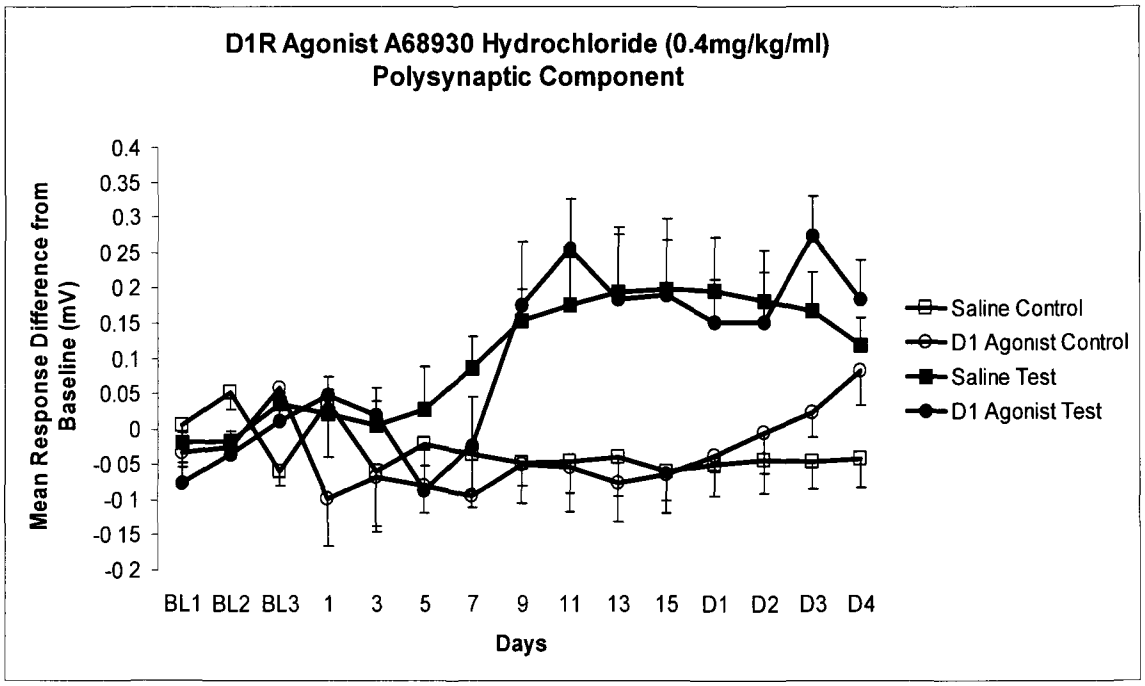

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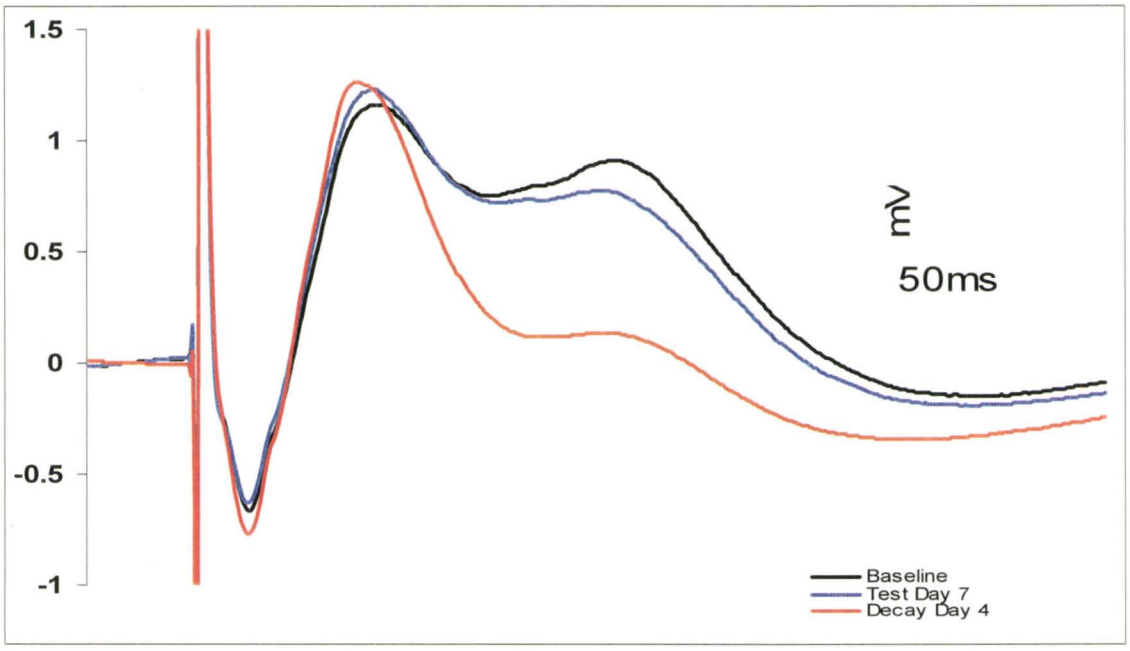

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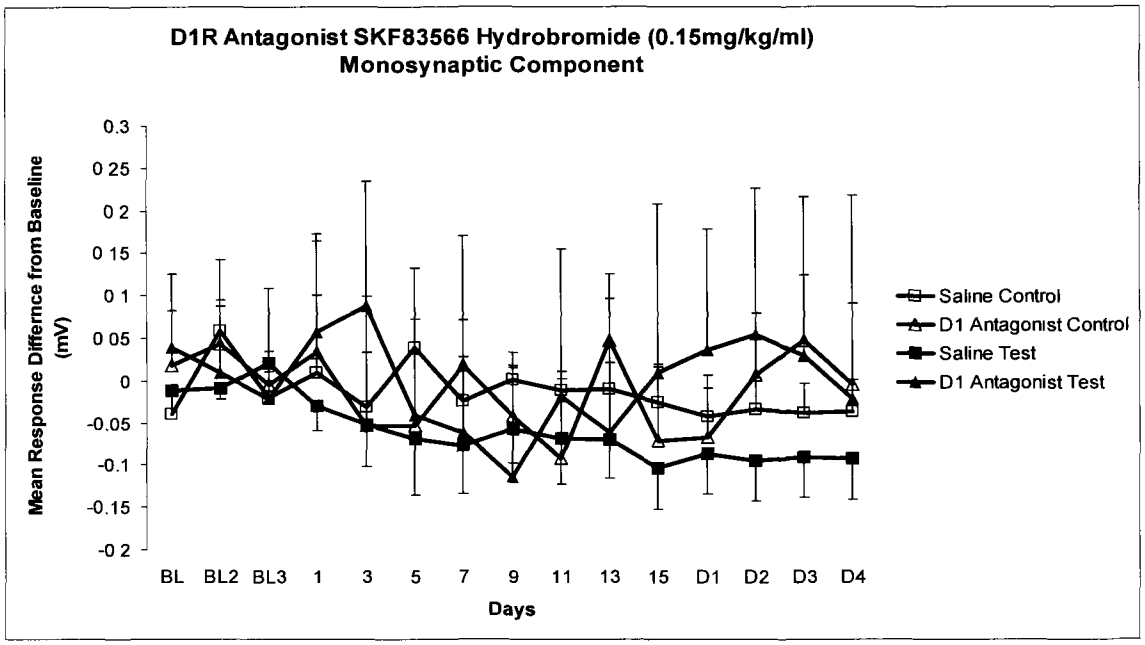

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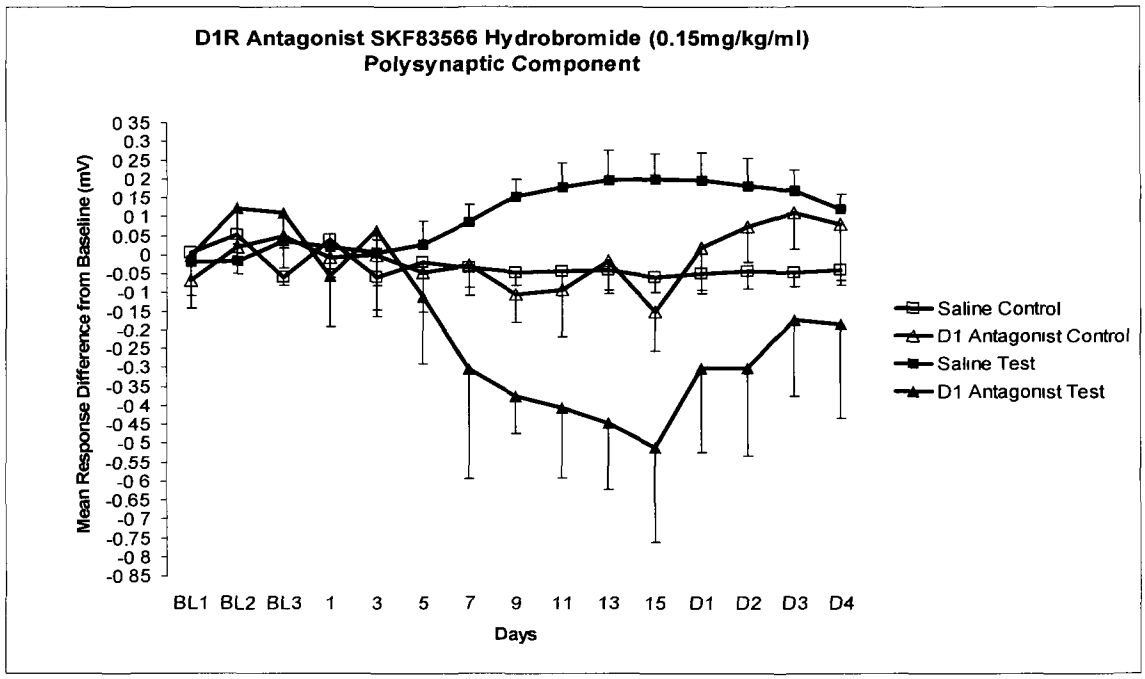

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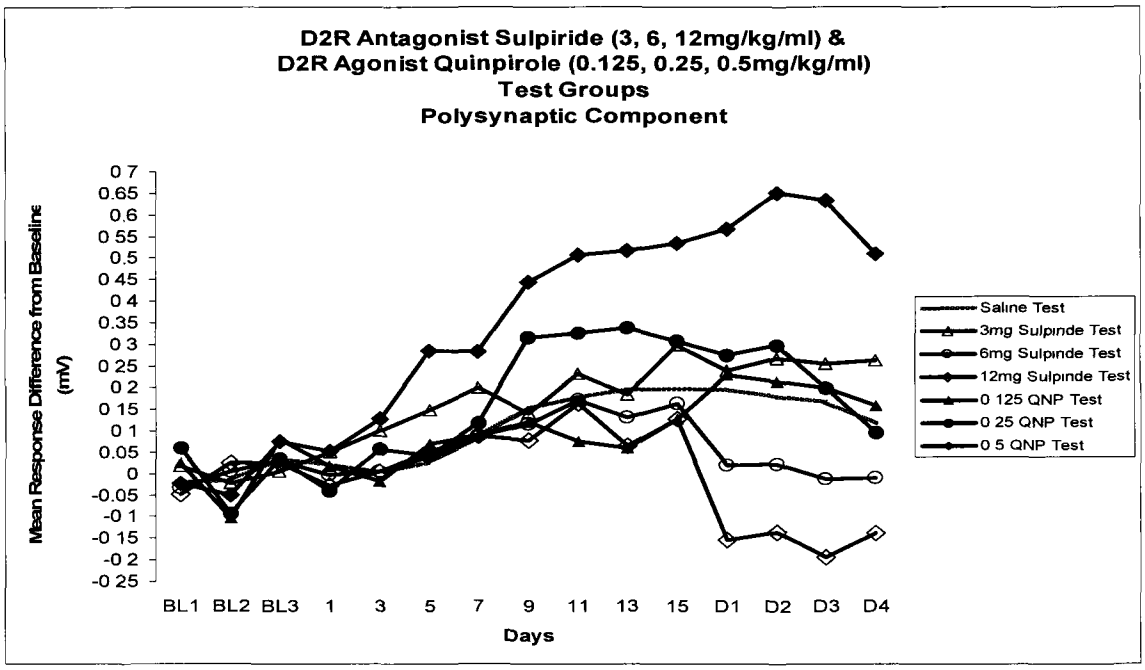
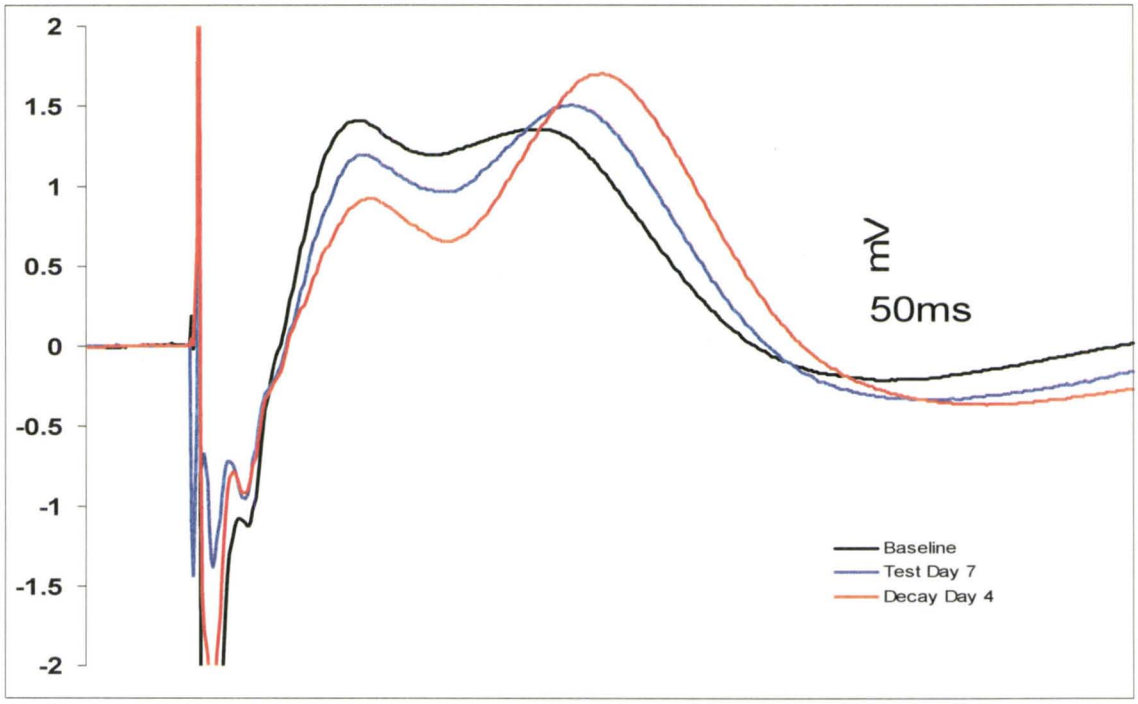

D2R Antagonist Sulpiride (3, 6, 12mg/kg/ml) &
D2R Agonist Quinpirole (0.125, 0.25, 0.5mg/kg/ml)
Test Groups
Polysynaptic Component
Mean Response Difference from Baseline (mV)
0 7
0.65
0.6
0 55
0.5
0 45
0 4
0.35
0 3
0 25
0 2
0 15
0 1
0.05
0
-0.05
-0 1
-0 15
-0 2
-0 25
BL1 BL2 BL3 1 3 5 7 9 11 13 15 D1 D2 D3 D4
Days
Saline Test
3mg Sulpinde Test
6mg Sulpinde Test
12mg Sulpinde Test
0 125 QNP Test
0 25 QNP Test
0 5 QNP Test

Figure 7.3.1.1



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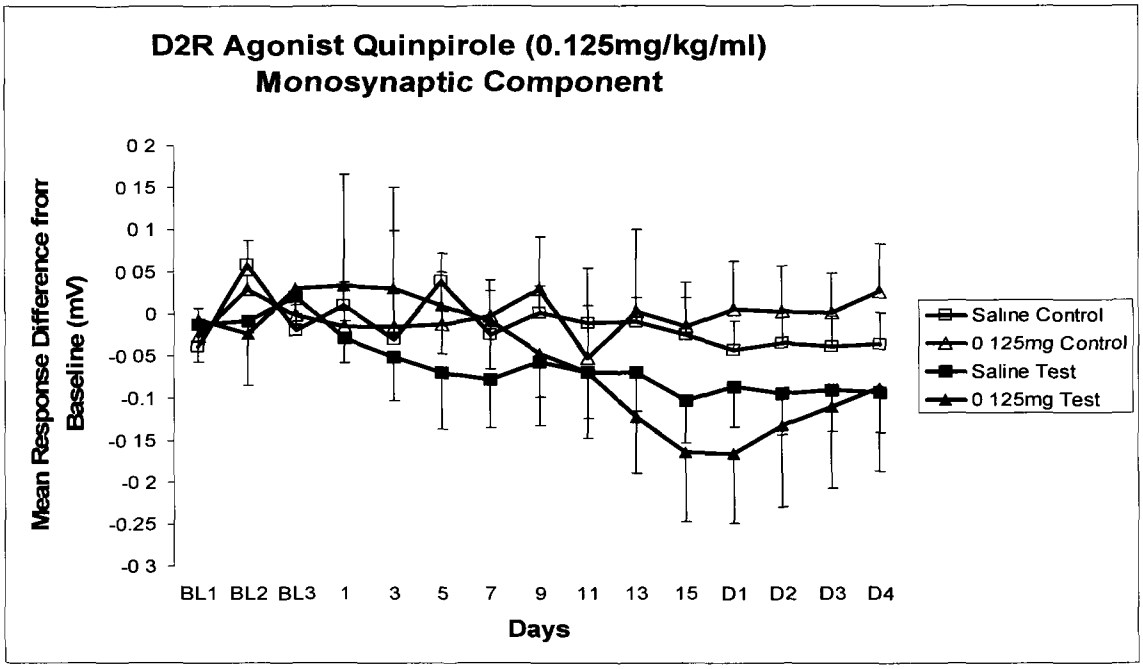

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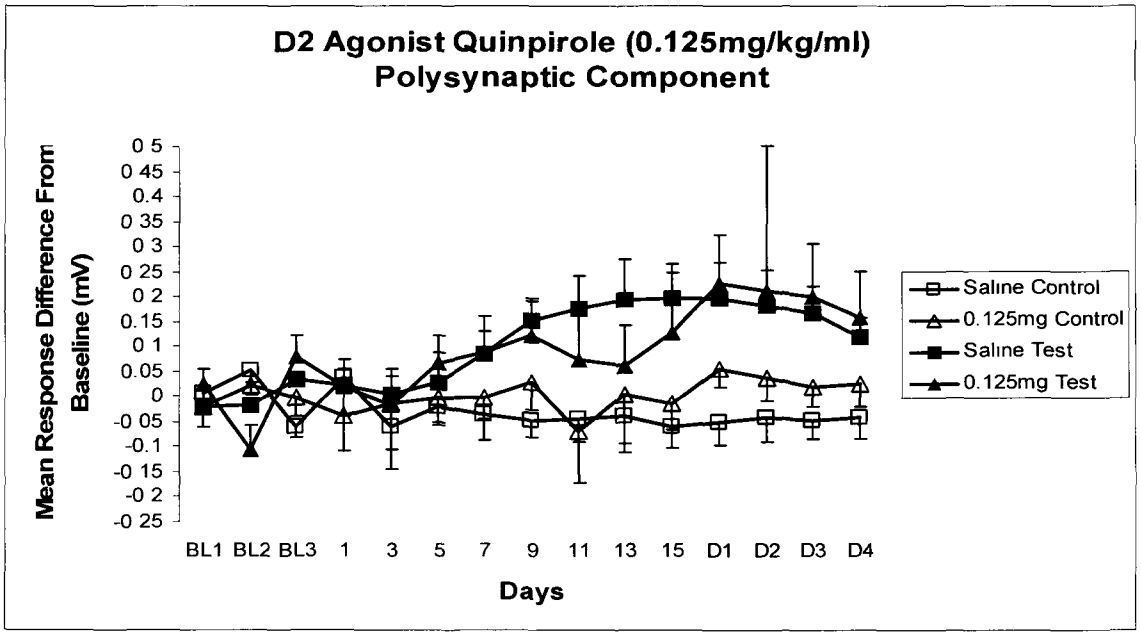

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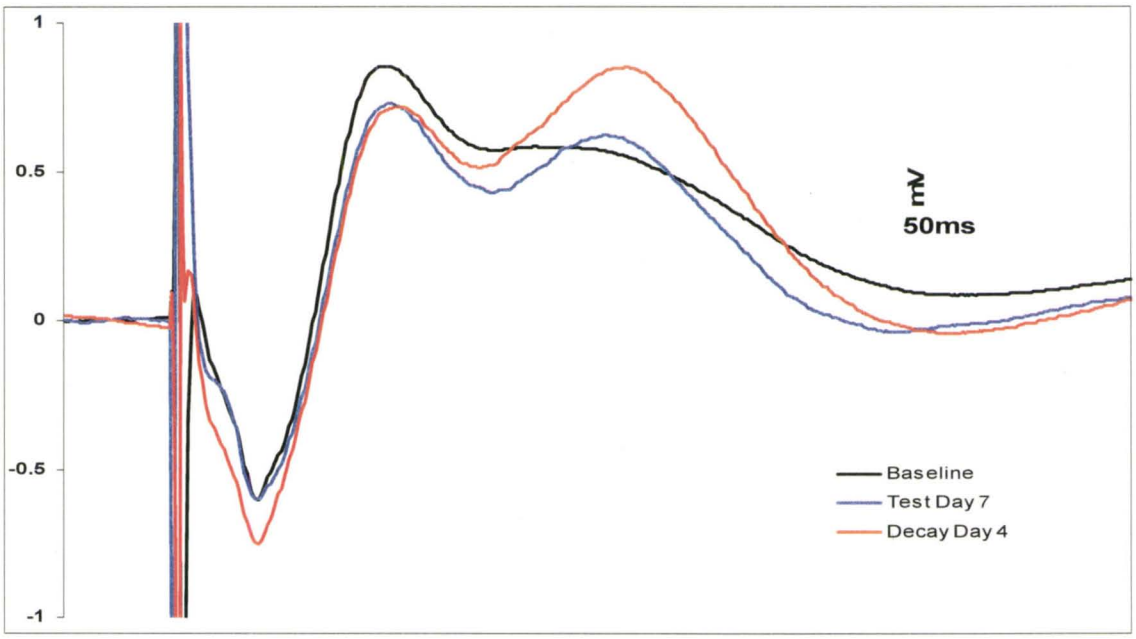

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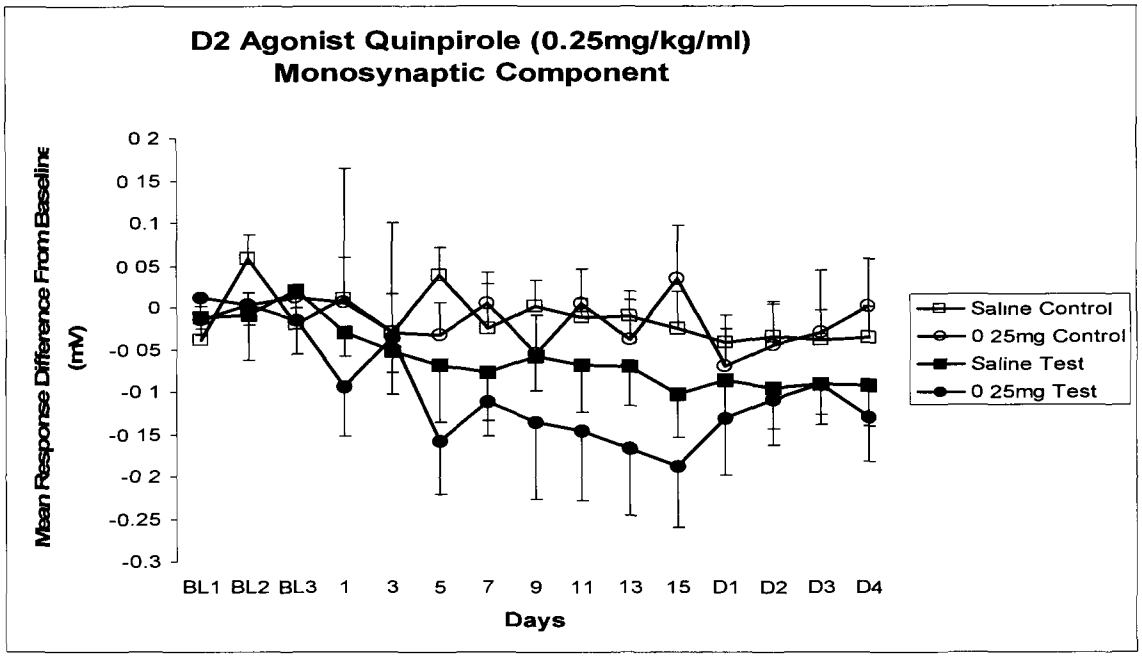

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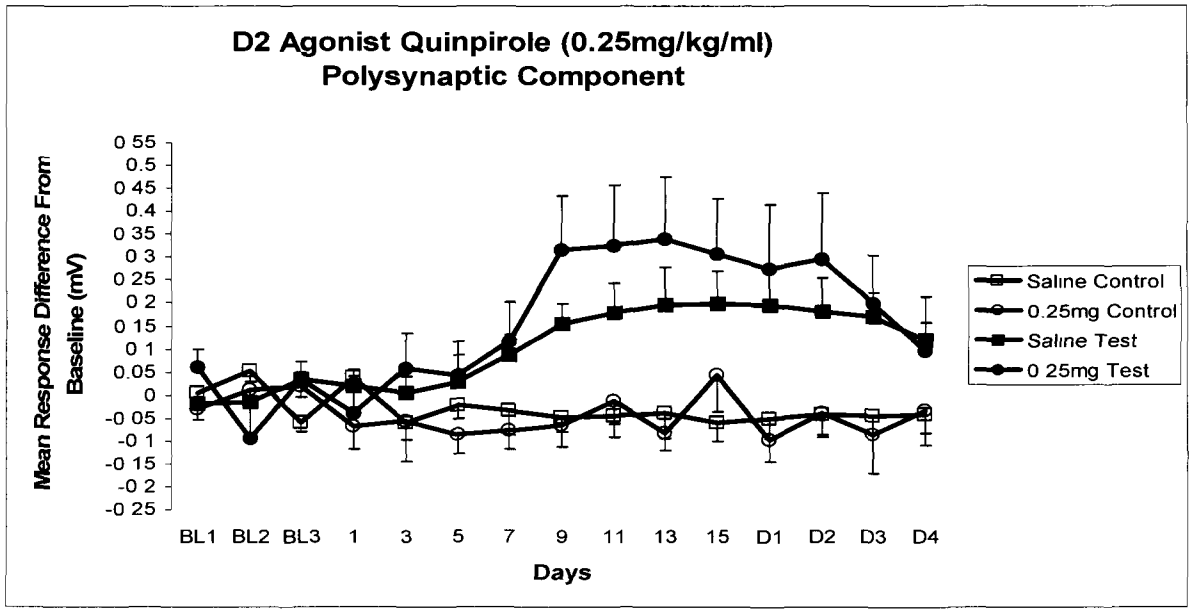

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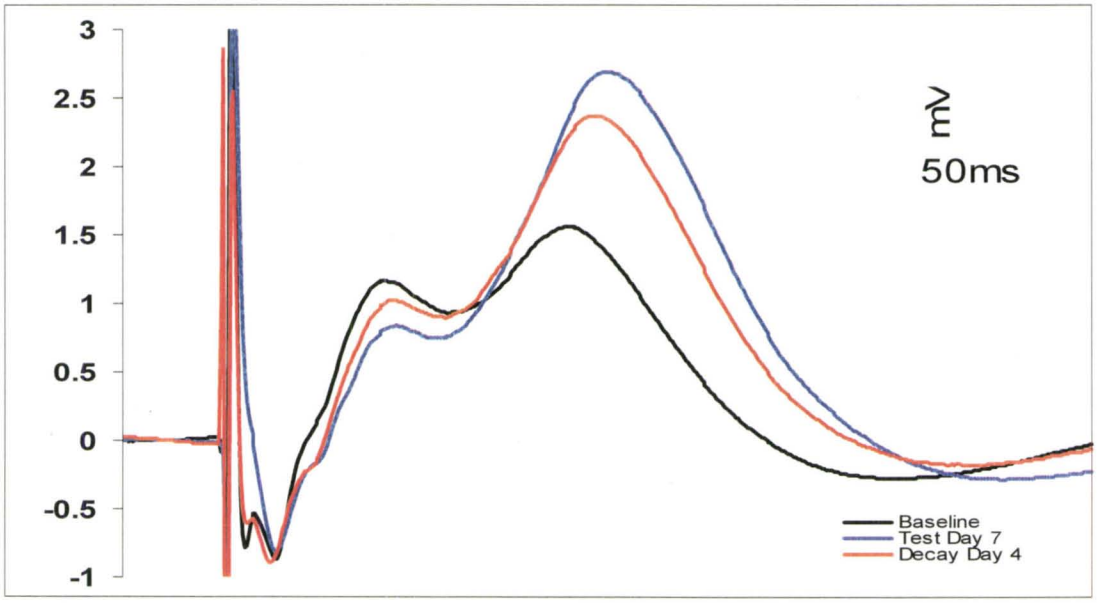

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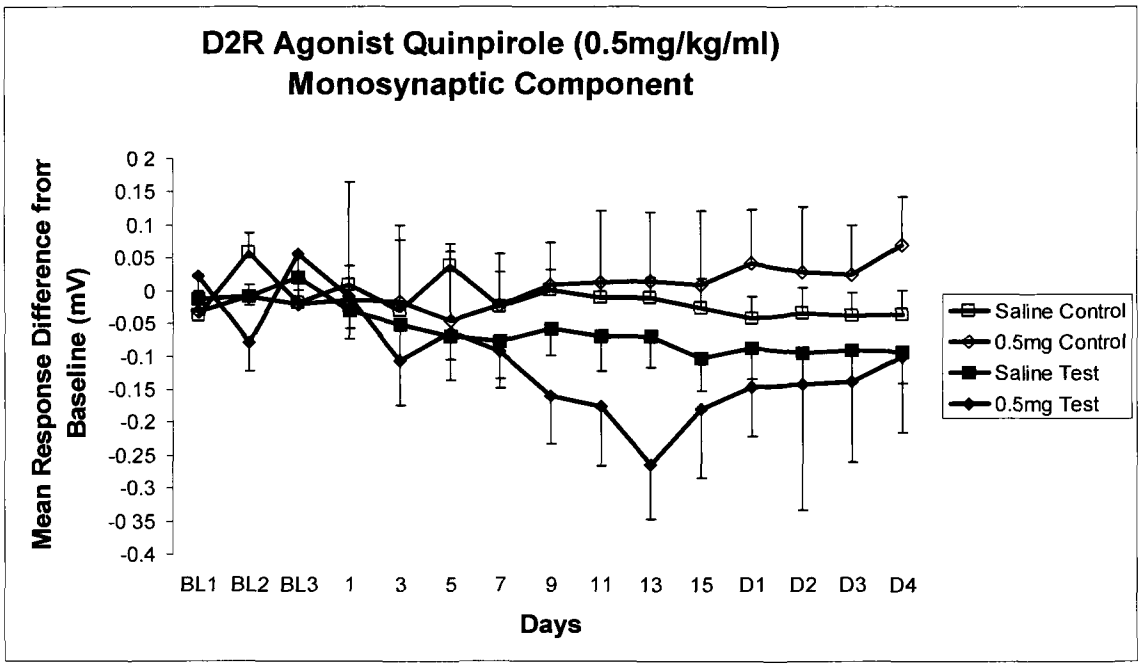

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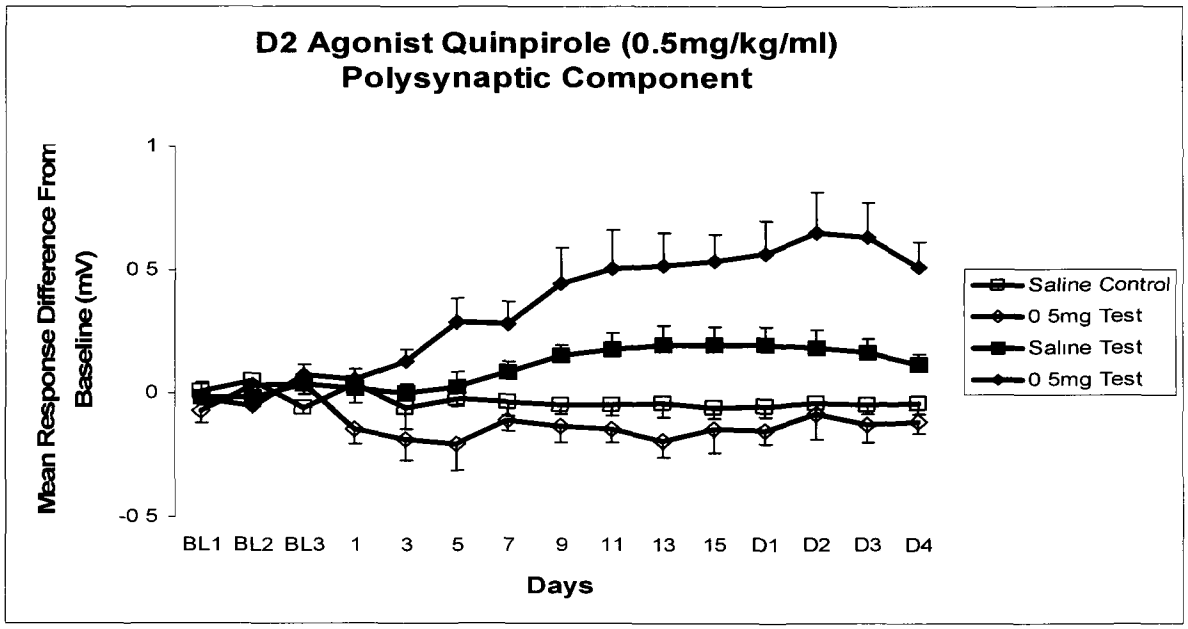

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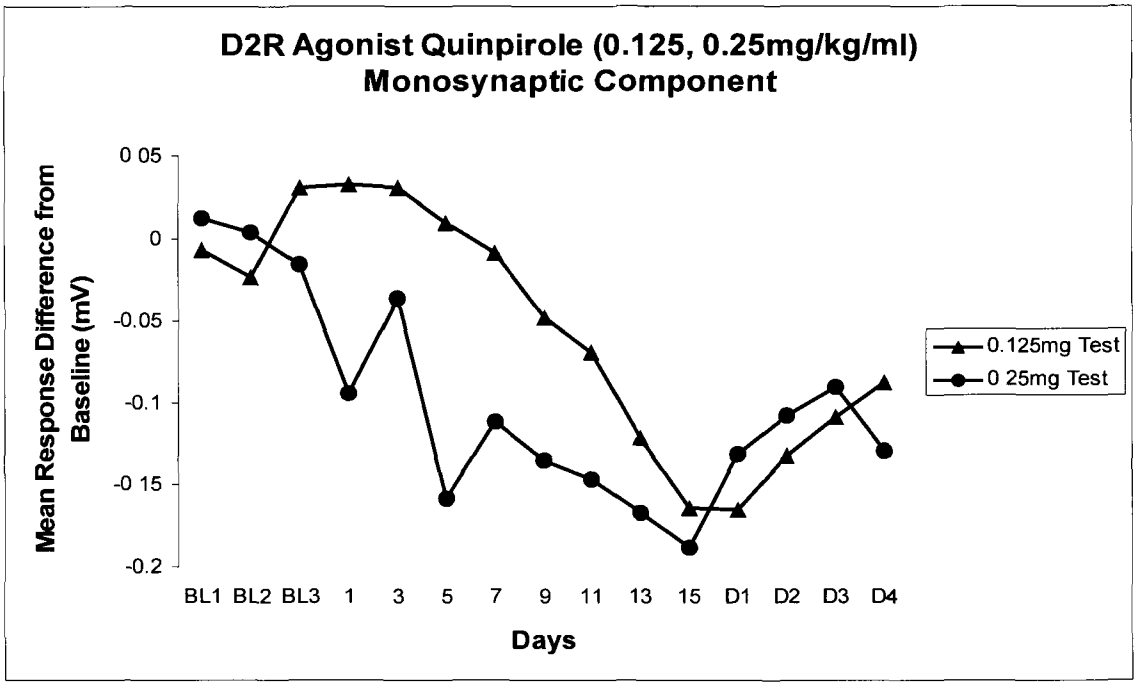

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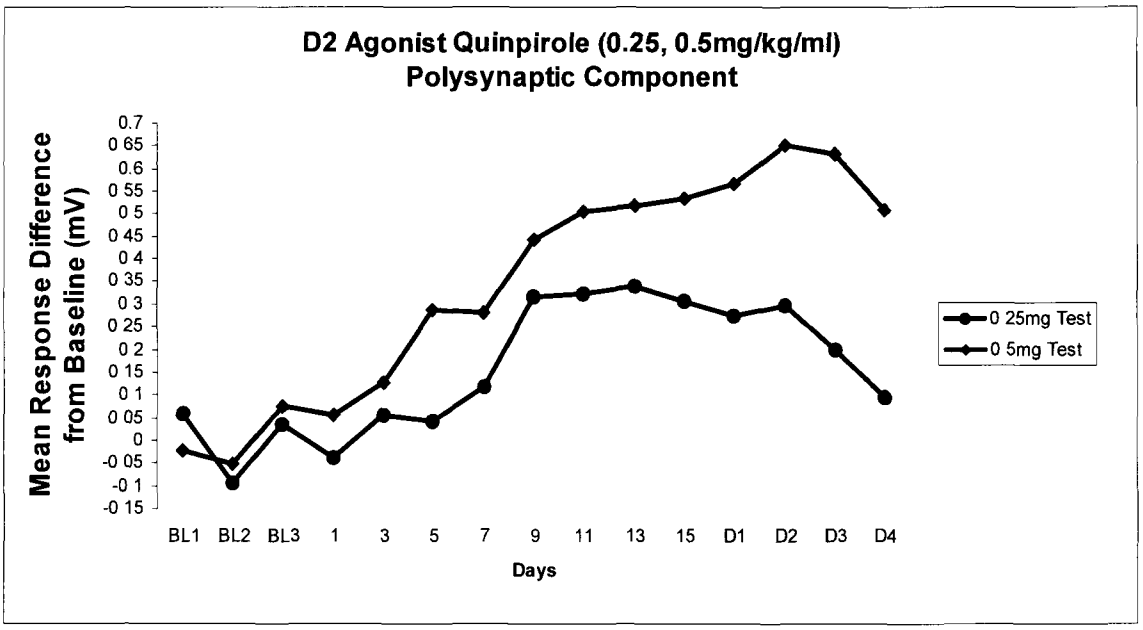

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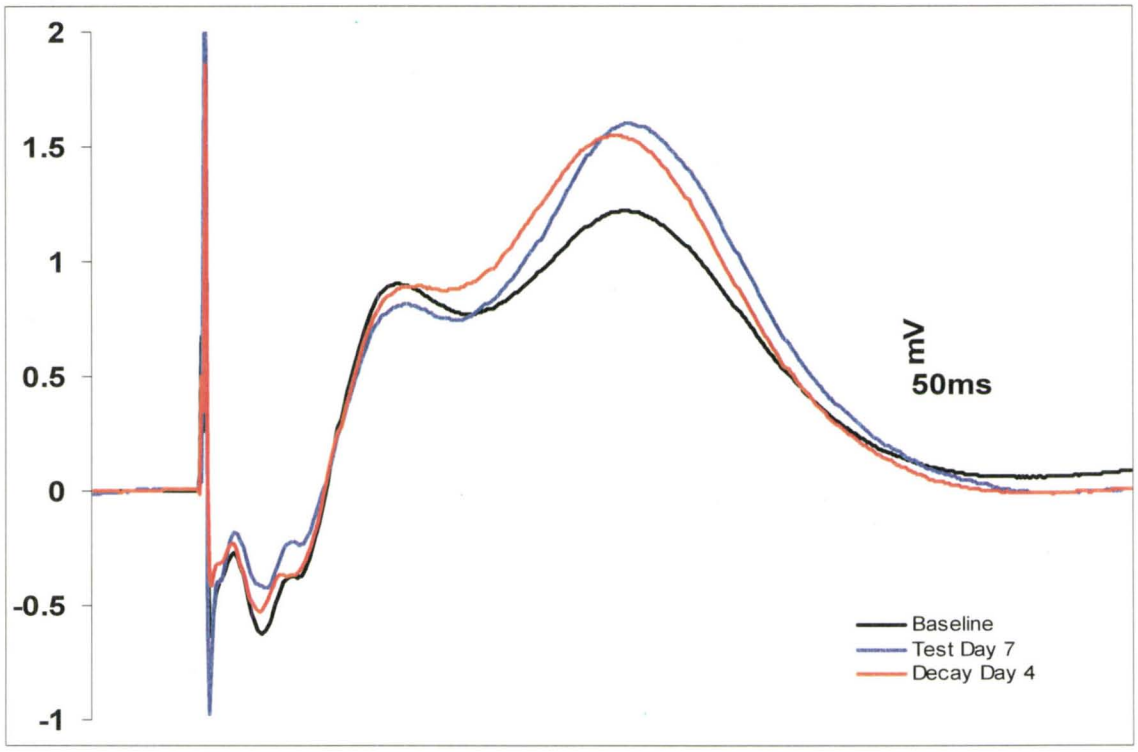

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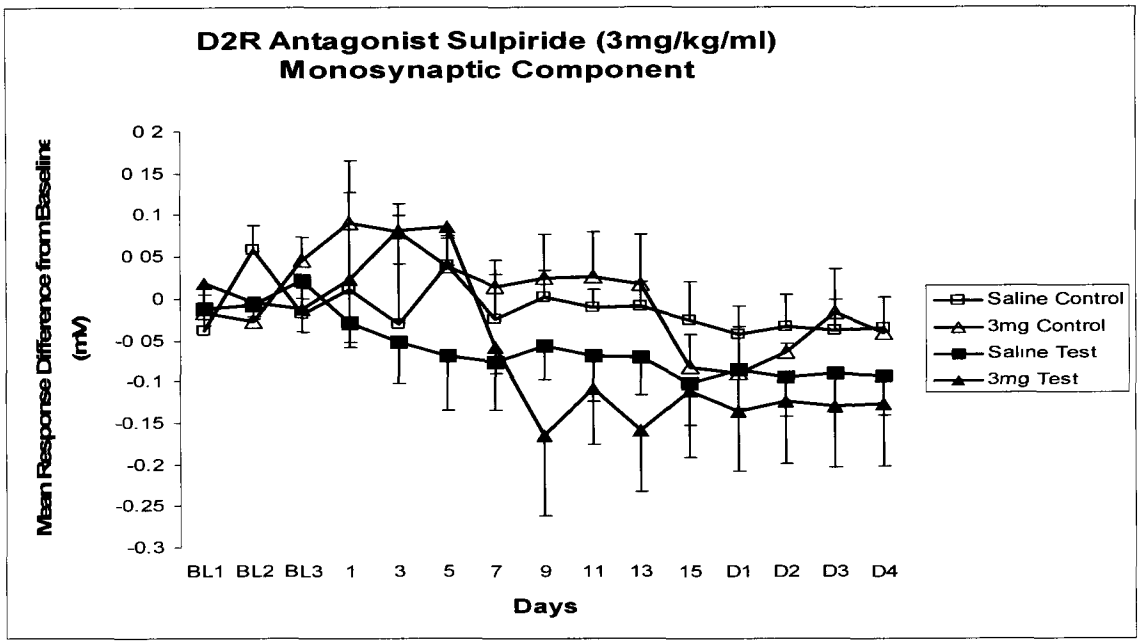

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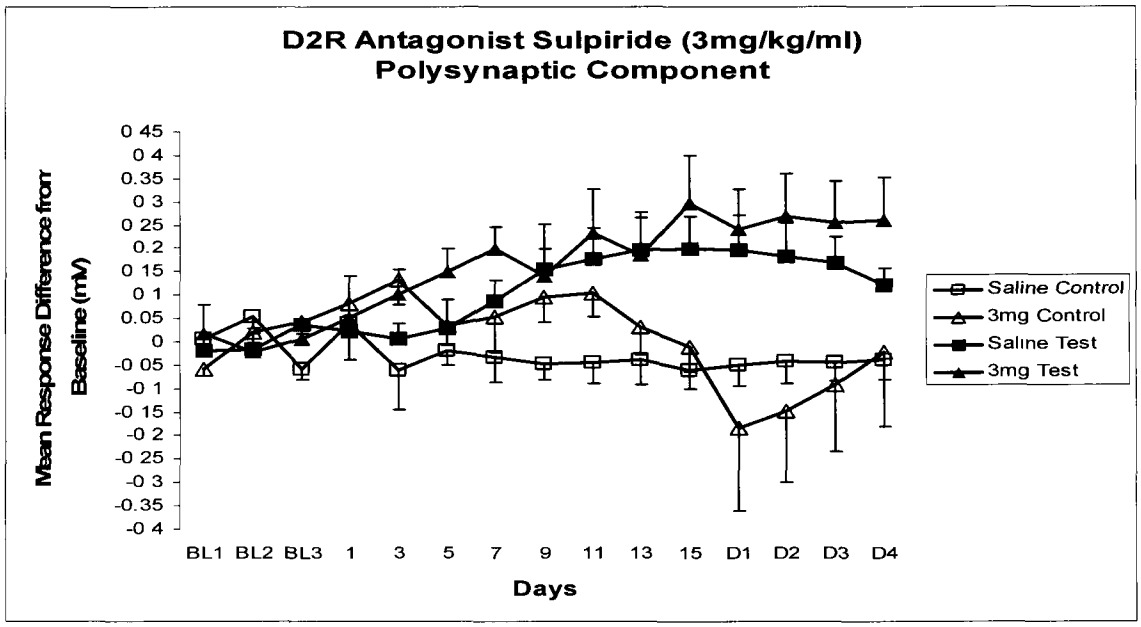

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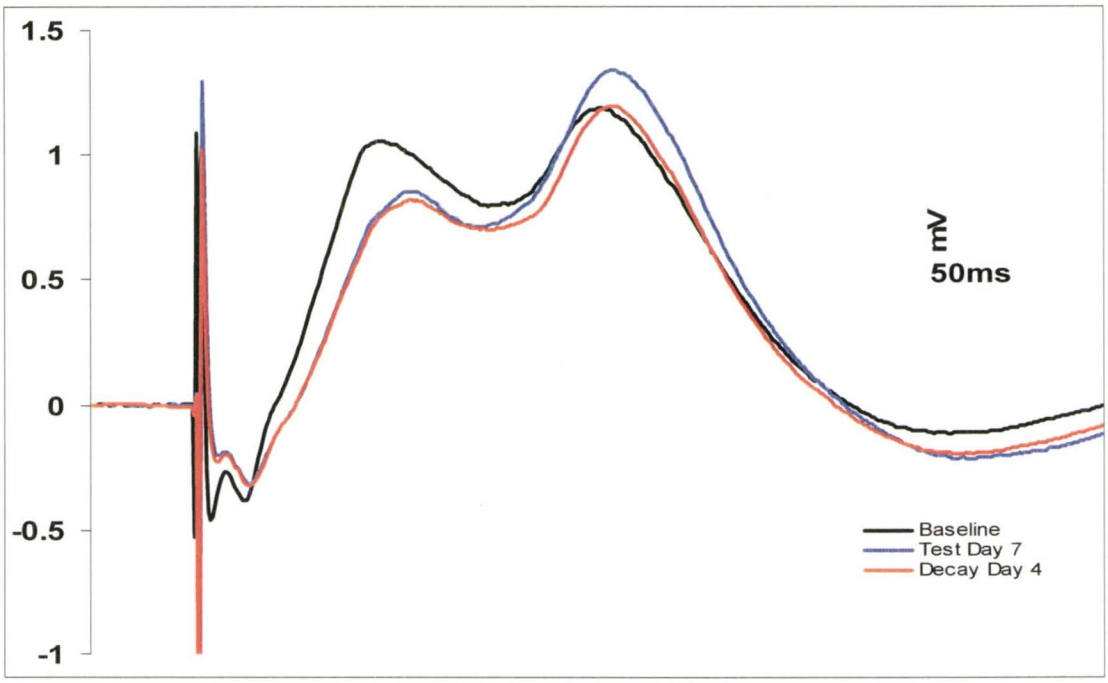

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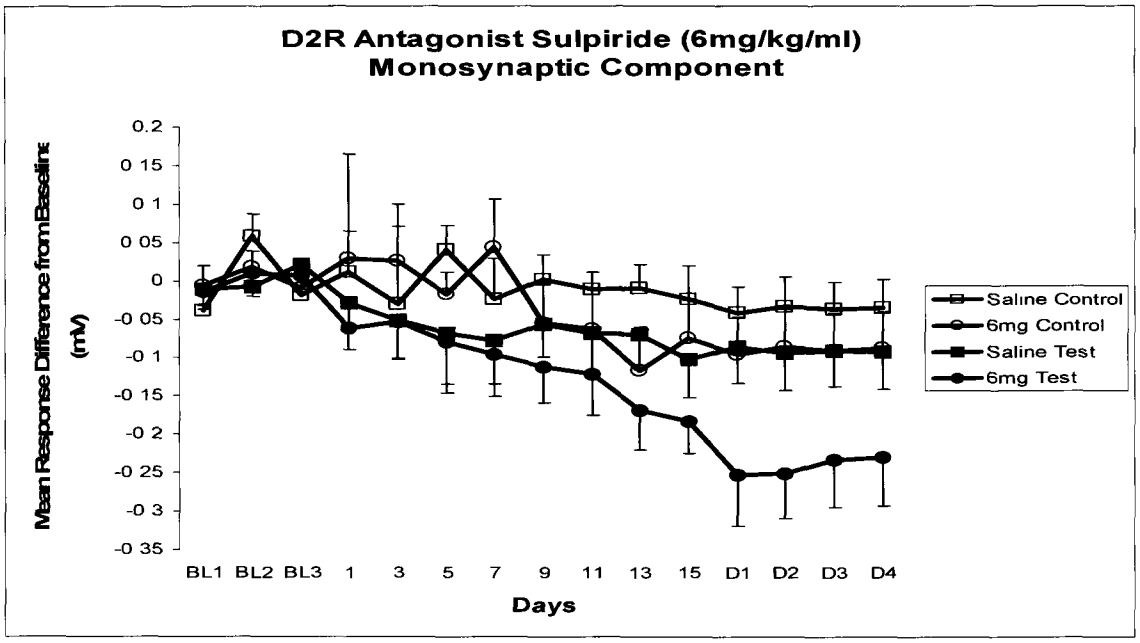

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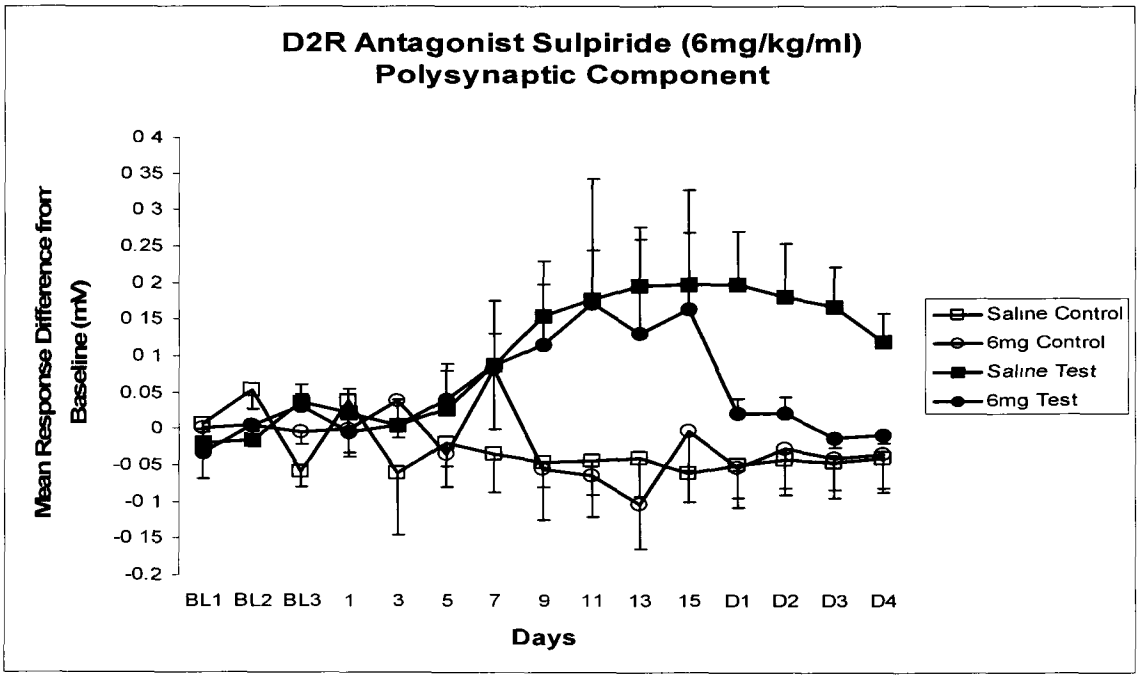

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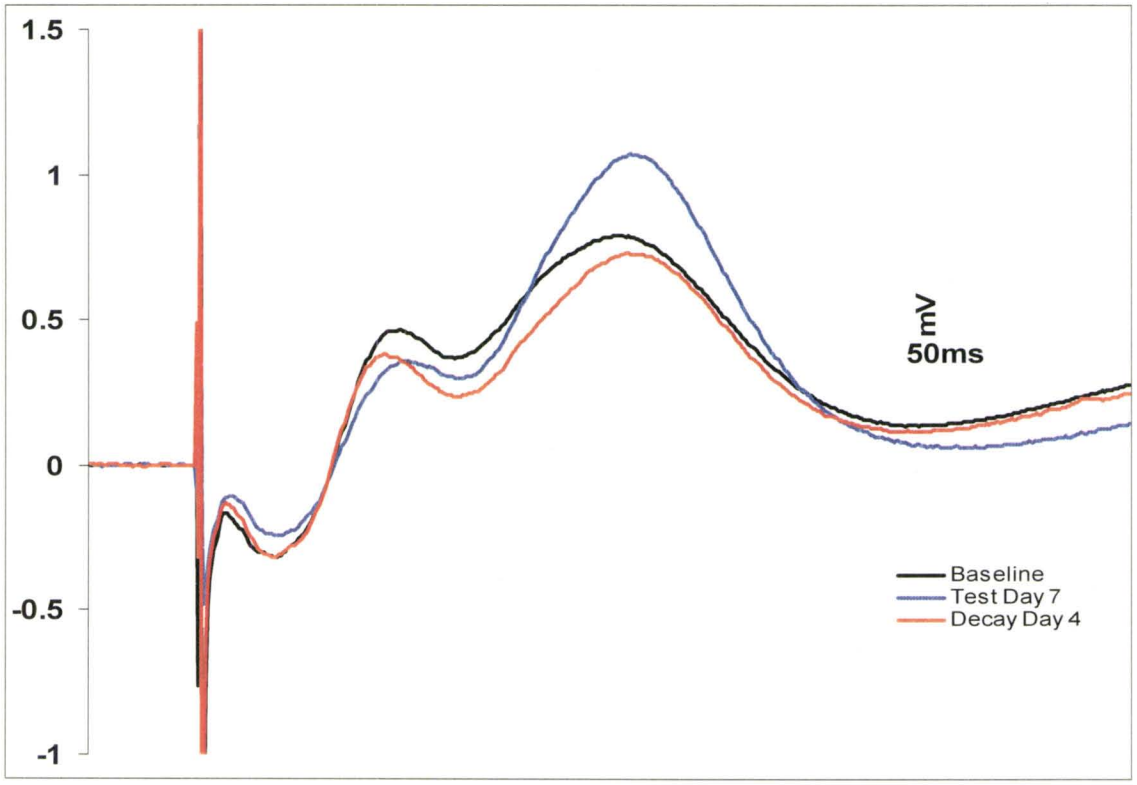

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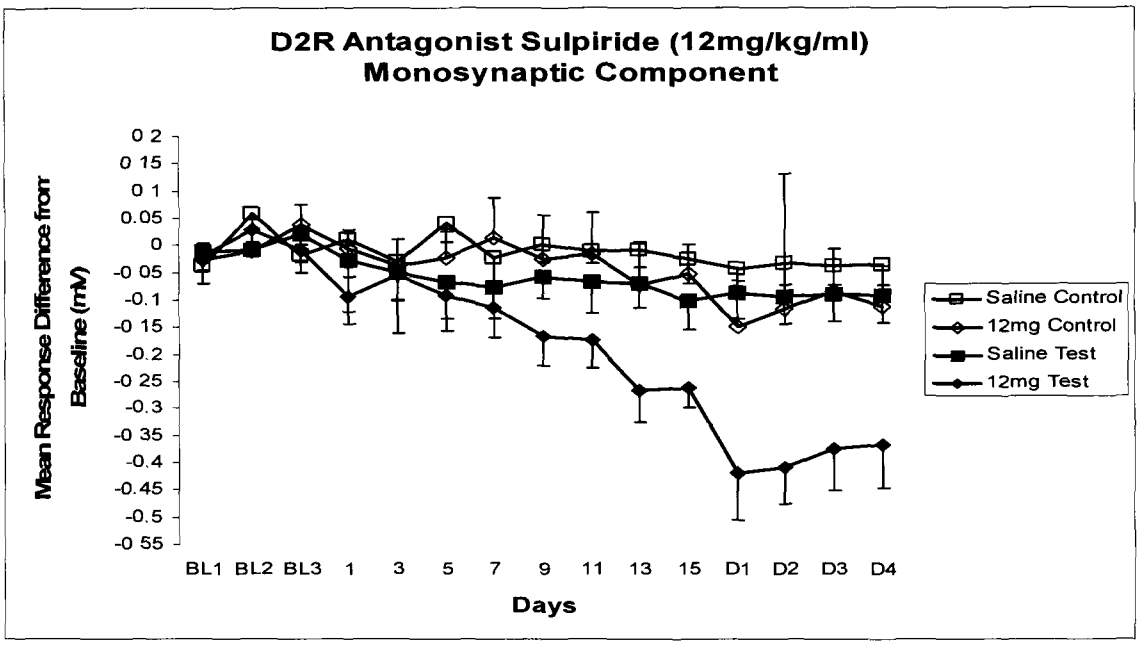

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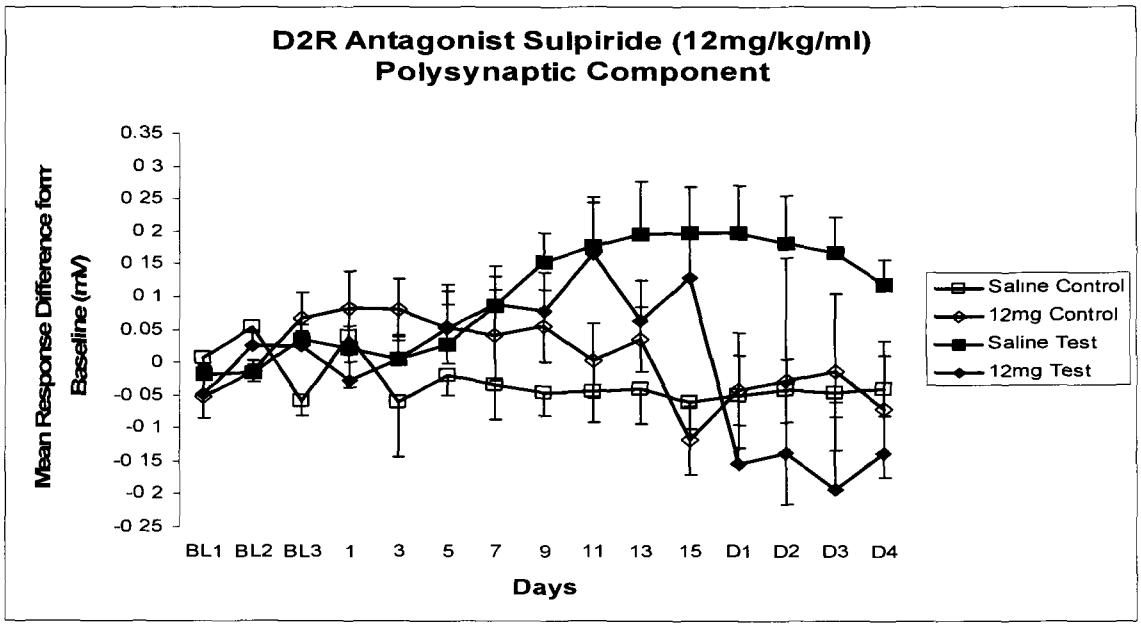

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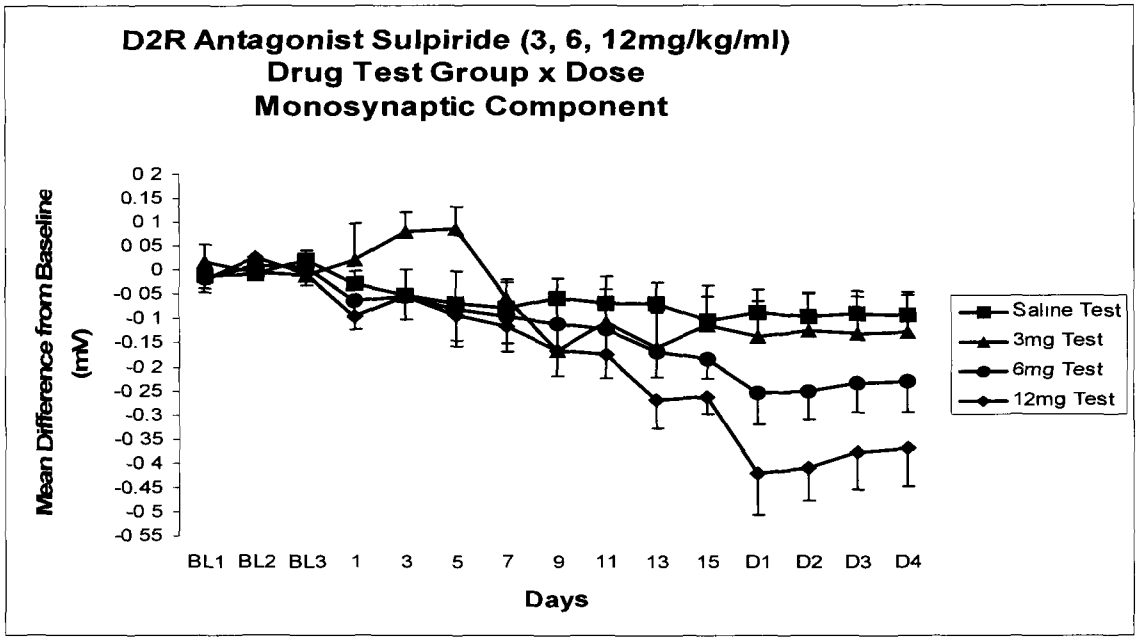

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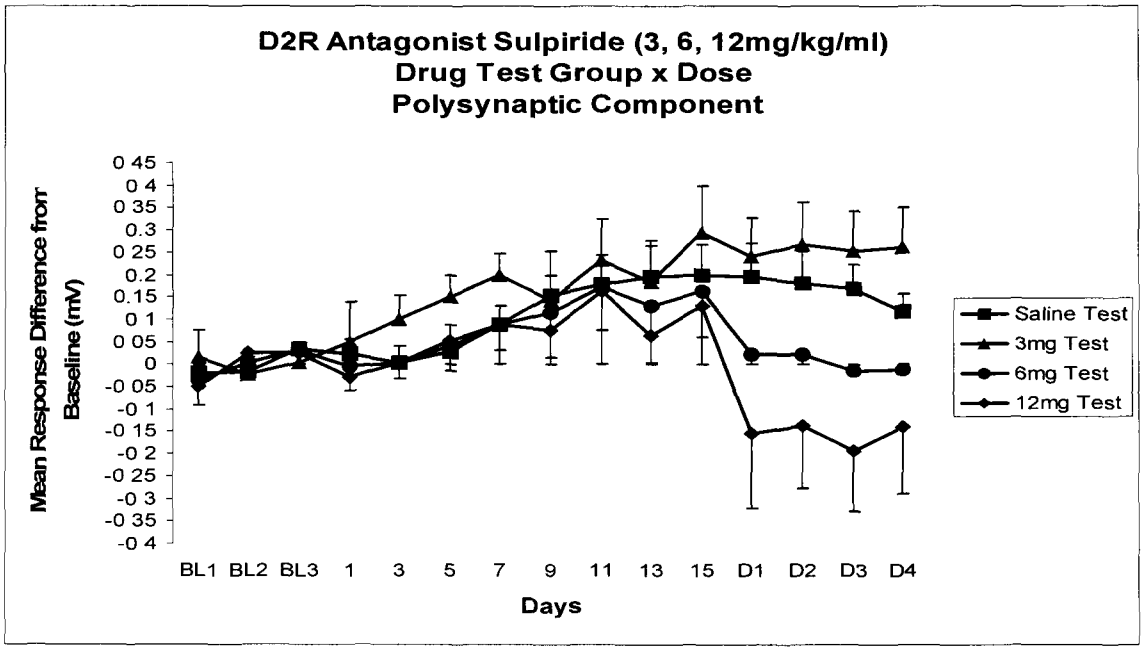


7.3.4 Figure Captions

Figure 7.1.1 Medial Prefrontal Cortex Baseline (Saline) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the saline plus HFS group. Included are sweeps at baseline (*black*), test (saline plus HFS) day 7 (*blue*), and decay (no saline or HFS) day 4 (*red*).

Figure 7.1.2 Medial Prefrontal Cortex Baseline (Saline) LTP Induction: Monosynaptic Component

During LTP-induction (days 1-15), the saline plus HFS test group showed a decrease in response amplitude across days (0.23mV representing 15% of baseline amplitude) in the early component in comparison to the saline-alone control group. This small effect was not significant, and appeared to remain relatively stable during the decay phase (D1-D4) (0.42mV representing 27% of baseline amplitude). BL=baseline; D=decay.

Figure 7.1.3 Medial Prefrontal Cortex Baseline (Saline) LTP Induction: Polysynaptic Component

During LTP-induction (days 1-15), the saline plus HFS test group showed a significant increase in response amplitude (0.46mV representing 19% of baseline amplitude) across days in the polysynaptic component which was not

expressed in the saline-alone control group. This effect was maintained in the decay phase (D1-D4) (0.21mV representing 9% of baseline amplitude). BL=baseline; D=decay.

Figure 7.2.1 Medial Prefrontal Cortex D_1R Agonist A 68930 (0.4mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_1R agonist plus HFS group. Included are sweeps at baseline (*black*), test (D_1R agonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.2.2 Medial Prefrontal Cortex D_1R Agonist A 68930 (0.4mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_1R agonist plus HFS test group showed a significant decrease in response amplitude across days (0.34mV representing 20% of baseline amplitude) in the early component, compared to either the D_1R agonist control group or saline control group. However, there was no effect of the agonist on the response depression when compared to the saline plus HFS test group. The depression in both groups was maintained in the decay phase (D1-D4) (0.62mV representing 37% of baseline amplitude). BL=baseline; D=decay.

Figure 7.2.3 Medial Prefrontal Cortex D_1R Agonist A 68930 (0.4mg/kg) LTP Induction: Polysynaptic Component

During LTP induction, the D_1R agonist plus HFS test group showed a significant increase in response amplitude across days (1mV representing 67% of baseline amplitude) in the polysynaptic component, compared to either the D_1R agonist control group or saline control group. However, there was no effect of the drug on the level of LTP induction. This effect was maintained in the decay phase (D1-D4) (1.3mV representing 87% of baseline amplitude). BL=baseline; D=decay.

Figure 7.2.4 Medial Prefrontal Cortex D_1R Antagonist SKF 83566 (0.15mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_1R antagonist plus HFS group. Included are sweeps at baseline, test (D_1R antagonist plus HFS) day 7, and decay (no drug or HFS) day 4.

Figure 7.2.5 Medial Prefrontal Cortex D_1R Antagonist SKF 83566 (0.15mg/kg) LTP Induction: Monosynaptic Component

The D_1R antagonist (test) did not alter response amplitude in the early component in comparison to the D_1R antagonist and saline control groups. BL=baseline; D=decay.

Figure 7.2.6 Medial Prefrontal Cortex D_1R Antagonist SKF 83566 (0.15mg /kg) LTP Induction: Polysynaptic Component

The administration of the D_1R antagonist during HFS (test) significantly blocked the induction of baseline levels of LTP in the polysynaptic components. The LTP was replaced with a significant LTD (0.15mV reduction representing 16% of baseline amplitude) in comparison to both the saline and D_1R antagonist control groups, both of which remained stable across sessions. This effect was maintained during the decay phase (D1-D4) (0.78mV representing 85% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1 D_2R Antagonist Sulpiride (3, 6, 12mg/kg) and D_2R Agonist Quinpirole (0.125, 0.25, 0.5mg/kg) Dose Response Effects

This figure depicts the dose-response trend for the D_2R agonist (increasing LTP effect with increasing dose) and the D_2R antagonist (decreasing LTP effect with increasing dose). BL=baseline; D=decay.

Figure 7.3.1.1 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.125mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R agonist plus HFS group. Included are sweeps at baseline (*black*), test (D_2R agonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.3.1.2 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.125mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R agonist plus HFS test group showed a nonsignificant decrease in response amplitude across days (0.24mV representing 17% of baseline amplitude) in the early component, comparable to that induced in the saline plus HFS test group. This depression effect was maintained during the decay phase (D1-D4) (0.5mV representing 35% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.3 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.125mg/kg) LTP Induction: Polysynaptic Component

The administration of the D_2R agonist during HFS (test) induced a nonsignificant increase in response amplitude (0.13mV representing 10% of baseline amplitude) across days in the polysynaptic component which was comparable to that in the saline plus HFS group. This effect decayed slowly (D1-D4) (0.35mV representing 26% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.4 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.25mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R agonist plus HFS group. Included are sweeps at baseline (*black*), test (D_2R agonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.3.1.5 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.25mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R agonist plus HFS test group showed a significant decrease in response amplitude across days (0.125mV representing 15% of baseline amplitude) in the early component, in comparison to the saline plus HFS test group. This depression effect was maintained during the decay phase (D1-D4) (0.15mV representing 17% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.6 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.25mg /kg) LTP Induction: Polysynaptic Component

The administration of the D_2R agonist during HFS (test) induced a significant increase in response amplitude (0.016mV representing 3% of baseline amplitude) across days in the polysynaptic component compared to the drug and saline control groups. This effect was maintained during the decay phase (D1-D4) (0.27mV representing 46% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.7 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.5mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R agonist plus HFS group. Included are sweeps at baseline (*black*), test (D_2R agonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.3.1.8 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.5mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R agonist plus HFS test group showed a nonsignificant decrease in response amplitude across days (0.39mV representing 32% of baseline amplitude) in the early component, compared to the saline plus HFS test group. This depression effect was maintained during the decay phase (D1-D4) (0.23mV representing 19% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.9 Medial Prefrontal Cortex D_2R Agonist Quinpirole (0.5mg /kg) LTP Induction: Polysynaptic Component

The administration of the D_2R agonist during HFS (test) induced a significant increase in response amplitude (1.16mV representing 75% of baseline amplitude) across days in the polysynaptic component compared to the saline plus HFS test group and both the drug and saline control groups. This effect was maintained during the decay phase (D1-D4) (0.75mV representing 48% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.1.10 D_2R Agonist Quinpirole (0.125, 0.25mg/kg)-Induced Dose-Dependent Reductions in the Monosynaptic Component

This figure depicts the dose-dependent decrease in the monosynaptic component of the mPFC field potential during drug plus HFS administration. BL=baseline; D=decay.

Figure 7.3.1.11 D_2R Agonist Quinpirole (0.25, 0.5mg/kg)-Induced Dose-Dependent Facilitation in the Polysynaptic Component

This figure depicts the dose-dependent facilitation in the polysynaptic component of the mPFC field potential during drug plus HFS administration. This effect was maintained during the decay phase (D1-D4). BL=baseline; D=decay.

Figure 7.3.2.1 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (3mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R antagonist plus HFS group. Included are sweeps at baseline (*black*), test (D_2R antagonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.3.2.2 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (3mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R antagonist plus HFS test group showed a nonsignificant decrease in response amplitude across days (0.08mV representing 9% of baseline amplitude) in the early component, greater than that expressed in the saline plus HFS test group. BL=baseline; D=decay.

Figure 7.3.2.3 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (3mg /kg) LTP Induction: Polysynaptic Component

The administration of the D_2R antagonist during HFS (test) induced a significant increase in response amplitude (0.4mV representing 33% of baseline

amplitude) across days in the polysynaptic component compared to the drug and saline control groups. This effect was maintained during the decay phase (D1-D4) (0.34mV representing 28% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.2.4 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (6mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R antagonist plus HFS group. Included are sweeps at baseline, test (D_2R antagonist plus HFS) day 7, and decay (no drug or HFS) day 4.

Figure 7.3.2.5 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (6mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R antagonist plus HFS test group showed a nonsignificant decrease in response amplitude across days (0.21mV representing 20% of baseline amplitude) in the early component. This effect was maintained during the decay phase of (D1-D4) (0.24mV representing 23% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.2.6 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (6mg /kg) LTP Induction: Polysynaptic Component

The administration of the D_2R antagonist during HFS (test) induced a significant, but transient, increase in response amplitude (0.17mV representing 13% of baseline amplitude) across days in the polysynaptic component

compared to the saline-only and drug-only control groups. BL=baseline; D=decay.

Figure 7.3.2.7 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (12mg/kg) LTP Induction Field Potential: Monosynaptic and Polysynaptic Component

Representative field potential evoked in the mPFC by pulse stimulation of the corpus callosum of the D_2R antagonist plus HFS group. Included are sweeps at baseline (*black*), test (D_2R antagonist plus HFS) day 7 (*blue*), and decay (no drug or HFS) day 4 (*red*).

Figure 7.3.2.8 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (12mg/kg) LTP Induction: Monosynaptic Component

During LTP induction, the D_2R antagonist plus HFS test group showed a significant decrease in response amplitude across days (0.12mV representing 25% of baseline amplitude) in the early component, compared to the saline plus HFS test group. This effect was maintained during the decay phase of (D1-D4) (0.1mV representing 20% of baseline amplitude). BL=baseline; D=decay.

Figure 7.3.2.9 Medial Prefrontal Cortex D_2R Antagonist Sulpiride (12mg/kg) LTP Induction: Polysynaptic Component

The administration of the D_2R antagonist during HFS (test) induced a significant, but transient, increase in response amplitude (0.3mV representing 37% of baseline amplitude) across days in the polysynaptic component

compared to the saline-only and drug-only control groups. BL=baseline; D=decay.

Figure 7.3.2.10 D_2R Antagonist Sulpiride (3, 6, 12mg/kg)-Induced Dose-Dependent Reductions in the Monosynaptic Component

This figure depicts the dose-dependent decrease in the monosynaptic component of the medial prefrontal cortex field potential during drug plus HFS administration during the latter half of LTP induction. This effect was maintained during the decay phase (D1-D4). BL=baseline; D=decay.

Figure 7.3.2.11 D_2R Antagonist Sulpiride (3, 6, 12mg/kg)-Induced Dose-Dependent Facilitation in the Polysynaptic Component

This figure depicts the significant dose-dependent reduction of LTP in the polysynaptic component, particularly in the latter half of the induction phase. This effect was maintained during the decay phase (D1-D4). BL=baseline; D=decay.

7.3.5 Tables

Table 7.1.1

GROUP	MAIN EFFECT OF DAY
Saline control group vs. saline test group: polysynaptic component	F[10,200]=1.642, p=0.097

Table 7.2.1

GROUP	MAIN EFFECT OF DAY
D_1R Agonist (A68930) test group vs. Saline control group: monosynaptic component	F[1,16]=5.715, p=0.029
D_1R Antagonist (SKF83566) test group vs. Saline control group: polysynaptic component	F[1,15]=8.368, p=0.011

Table 7.3.1

GROUP	MAIN EFFECT OF DAY
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. Saline test group: monosynaptic component	F[4,70]=3.570, p=0.012
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. Saline test group: polysynaptic component	F[1,19)=4.324, p=0.051
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. Saline test group: monosynaptic component	F[1,18)=3.334, p=0.084
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.25mg/kg) control group: polysynaptic component	F[4,59]=2.958, p=0.025
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. Saline control group: polysynaptic component	F[5,85]=2.775, p=0.025
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. Saline test group: polysynaptic component	F[1,18]=8.840, p=0.008
D_2R Agonist (Quinpirole, 0.25mg/kg) test group decay vs. Saline control group decay: polysynaptic component	F[1,18]=4.143, p=0.057
D_2R Agonist (Quinpirole, 0.25mg/kg) test group decay vs. Saline test group decay: polysynaptic component	F[1,18]=6.379, p=0.021
D_2R Agonist (Quinpirole, 0.5mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) control group: monosynaptic component	F[5,62]=2.684, p=0.028
D_2R Agonist (Quinpirole, 0.5mg/kg) test group vs. Saline test group: monosynaptic component	F[6,100]=2.382, p=0.032
D_2R Agonist (Quinpirole, 0.5mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) control group: polysynaptic component	F[1,12]=4.403, p=0.058
D_2R Agonist (Quinpirole, 0.5mg/kg) test group vs. Saline control group: polysynaptic component	F[1,16]=7.065, p=0.017

D_2R Agonist (Quinpirole, 0.5mg/kg) test group vs. Saline test group: polysynaptic component	F[1,16]=16.262, p=0.001
D_2R Agonist (Quinpirole, 0.5mg/kg) test group decay vs. Saline control group decay: polysynaptic component	F[1,16]=4.246, p=0.056
D_2R Agonist (Quinpirole, 0.5mg/kg) test group decay vs. Saline test group decay: polysynaptic component	F[1,16]=5.059, p=0.039
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.25mg/kg) test group: monosynaptic component	F[4,71]=3.340, p=0.014
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) test group: monosynaptic component	F[4,59]=3.559, p=0.012
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) test group: monosynaptic component	F[4,59]=2.634, p=0.040
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.25mg/kg) test group: polysynaptic component	F[1,17]=4.994, p=0.039
D_2R Agonist (Quinpirole, 0.125mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) test group: polysynaptic component	F[1,15]=9.303, p=0.008
D_2R Agonist (Quinpirole, 0.25mg/kg) test group vs. D_2R Agonist (Quinpirole, 0.5mg/kg) test group: polysynaptic component	F[1,14]=12.516, p=0.003
D_2R Agonist (Quinpirole, 0.25mg/kg) test group decay vs. D_2R Agonist (Quinpirole, 0.5mg/kg) test group decay: polysynaptic component	F[1,14]=5.879, p=0.029
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 3mg/kg) control group: monosynaptic component	F[5,70]=2.455, p=0.038
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride,	F[5,60]=2.025, p=0.093

3mg/kg) control group: polysynaptic component	
D_2R Antagonist (Sulpiride, 3mg/kg) test group decay vs. D_2R Antagonist (Sulpiride, 3mg/kg) control group decay: polysynaptic component	F[1,13]=6.083, p=0.028
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Saline control group: polysynaptic component	F[1,15]=4.409, p=0.053
D_2R Antagonist (Sulpiride, 3mg/kg) test group decay vs. Saline control group decay : polysynaptic component	F[1,15]=5.182, p=0.038
D_2R Antagonist (Sulpiride, 3mg/kg) test group decay vs. D_2R Saline test group decay: polysynaptic component	F[1,15]=7.048, p=0.018
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. Saline test group: monosynaptic component	F[1,15]=4.269, p=0.057
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. Saline test group: polysynaptic component	F[1,15]=10.006, p=0.006
D_2R Antagonist (Sulpiride, 6mg/kg) test group vs. D_2R Antagonist (Sulpiride, 6mg/kg) control group: monosynaptic component	F[1,18]=5.830, p=0.027
D_2R Antagonist (Sulpiride, 6mg/kg) test group vs. Saline test group: monosynaptic component	F[1,20]=4.582, p=0.045
D_2R Antagonist (Sulpiride, 6mg/kg) test group vs. Saline test group: polysynaptic component	F[1,20]=9.956, p=0.005
D_2R Antagonist (Sulpiride, 12mg/kg) test group vs. D_2R Antagonist (Sulpiride, 12mg/kg) control group: monosynaptic component	F[1,18]=4.766, p=0.043
D_2R Antagonist (Sulpiride, 12mg/kg) test group vs. Saline control group: monosynaptic component	F[3,58]=2.750, p=0.049
D_2R Antagonist (Sulpiride, 12mg/kg) test group vs. Saline test group: monosynaptic component	F[1,19]=6.368, p=0.021
D_2R Antagonist (Sulpiride, 12mg/kg) test group vs. Saline test group:	F[1,19]=5.552, p=0.029

polysynaptic component	
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 6mg/kg) test group: monosynaptic component	F[1,15]=9.027, p=0.009
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 12mg/kg) test group: monosynaptic component	F[1,14]=9.282, p=0.009
D_2R Antagonist (Sulpiride, 3mg/kg) test group decay vs. D_2R Antagonist (Sulpiride, 6mg/kg) test group decay: polysynaptic component	F[1,15]=4.983, p=0.041
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 6mg/kg) test group: polysynaptic component	F[1,15]=10.779, p=0.005
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 12mg/kg) test group: polysynaptic component	F[1,14]=7.087, p=0.019
D_2R Antagonist (Sulpiride, 6mg/kg) test group vs. D_2R Antagonist (Sulpiride, 12mg/kg) test group: monosynaptic component	F[1,19]=12.875, p=0.002
D_2R Antagonist (Sulpiride, 3mg/kg) test group vs. D_2R Antagonist (Sulpiride, 6mg/kg) test group: polysynaptic component	F[1,19]=6.055, p=0.024

7.3.6 Table Captions

Table 7.1.1 Saline Main Effects

This table summarizes the significant main effects in the saline experimental groups.

Table 7.2.1 D_1R Main Effects

This table summarizes the significant main effects in the D_1R experimental groups.

Table 7.3.1 D_2R Main Effects

This table summarizes the significant main effects in the D_2R experimental groups.

CHAPTER 8

BEHAVIOURAL SENSITIZATION AND INTERACTIONS WITH PREFRONTAL CORTEX LTP

All rats, in both control and test groups, which received the D_2R agonist quinpirole, expressed behavioural sensitization. This was expressed as an increase in the number, type, and intensity of the behaviours expressed with the number of injections. The behaviours included sniffing, rearing, locomotion, circling, paw/tail licking, gnawing, jumping, biting, vocalization, stereotypy (repetitive rearing, circling, paw/tail licking, gnawing, jumping, or biting). While increases were seen across days in both the drug and control groups at each dose, those expressed by the drug control group were significantly greater than the drug test group at 0.125mg/kg ($X^2_{0.05,18} = 15.60$) (Figure 8.1), yet significantly less at 0.25mg/kg ($X^2_{0.05,24} = 5.0$) (Figure 8.2), and 0.5mg/kg ($X^2_{0\ 05,23} = 5.20$) (Figure 8.3).

Further, the increase in behaviours observed across days of quinpirole administration was dose-dependent in both the drug control and drug test groups. The 0.125mg/kg control group expressed significantly less sensitization than the 0.25mg/kg control group ($X^2_{0.05,108} = 120$) and 0.5mg/kg control group ($X^2_{0.05,99} = 120$), and the 0.25mg/kg control group expressed significantly less sensitization than the 0.5mg/kg control group ($X^2_{0.05,132} = 207$) (Figure 8.4).

The same can be said for the test groups, respectively ($X^2_{0.05,120} = 138.75$; $X^2_{0.05,120} = 127.50$; $X^2_{0.05,144} = 209$) (Figure 8.5).

Behaviour onset latency decreased for both drug control and drug plus HFS test groups across doses, meaning that behavioural sensitization was expressed earlier with increased dosage of quinpirole: rats in the 0.125mg/kg group began to express sensitization behaviours on day 4-5, those in the 0.25mg/kg group on day 2-4, and those in the 0.5mg/kg group on day 1-2 (Figure 8.4 and 8.5). There was also an increase in the development of the response with increasing dose, whereby behaviours in both the drug control and test groups increased more quickly, once started, as dose increased. The behaviours at the initial stages included sniffing, locomotion, rearing, and some circling, all at mild intensities except for the high dose (0.5mgm/kg), where some moderate intensities were recorded.

Overall, there was an increase in the amplitude (mean score) of the behaviours expressed in both control and test groups at each dose, meaning that with increased doses of quinpirole more numerous and intense levels of behavioural sensitization were expressed. This increase was significantly different between drug control and drug test groups during days 6-15 at 0.125mg/kg (0.7 reduction in score representing 13.2% of baseline; $X^2_{0\,05,3} = 2.4$) (Figure 8.1), 0.25mg/kg (0.43 increase in score representing 8% of baseline; $X^2_{0\,05,14} = 4.0$) (Figure 8.2), and 0.5mg/kg (0.25 increase in score representing 4%

of baseline; $X^2_{0.05,13} = 3.8$) (Figure 8.3). In addition to sniffing, locomotion, rearing, and some circling as expressed during the initial stages, the rats also expressed paw/tail licking, gnawing, jumping, biting, vocalization, and stereotypy (e.g., repetitive rearing, paw movements, circling). There was also a change in the type of behaviour expressed in the high dose group (0.5mg/kg) as the number of injections increased. Rats in both the drug control and drug plus HFS test groups began to express more ego-focused and stereotypical behaviours (e.g., vocalization, paw licking, tail licking, self-gnawing, self-biting, repetitive jumping) versus the environment-focused behaviours which predominated in the middle (0.25mg/kg) and low dose (0.125mg/kg) groups and during the early stages of drug administration in the high dose (0.5mg/kg) group (i.e., sniffing, rearing, circling, locomotion).

The distinguishing feature between the drug control group and drug plus HFS test group at each dose was that the behavioural sensitization responses were maintained only in the drug plus HFS test groups during the decay phase (D1-D4) of the experiments (Figures 8.1-8.5). In other words, only those rats that expressed LTP maintained the expression of behavioural sensitization following the end of the induction phase. The amplitude of the response showed very little decay, and was maintained until the final decay day (D4) which occurred three weeks following the final day of drug administration plus HFS. This maintenance effect was significant in comparison to all dose-matched control groups:

0.125mg/kg (0.66 increase in score representing 15% of baseline; $X^2_{0.05,7}$ = 8.00), 0.25mg/kg (0.18 increase in score representing 4% of baseline; $X^2_{0.05,5}$ = 2.5), and 0.5mg/kg (1.89 increase in score representing 38% of baseline; $X^2_{0.05,6}$ = 8.00).

In contrast, the drug only groups continued to express sensitization behaviours robustly only on decay day one (D1), which was the first day without drug administration following the last day of drug administration (Day 15). The response was still evident, but greatly reduced, on decay days two (D2) and three (D3), occurring one week and two weeks following decay day one, respectively. By decay day four (D4), which was the fourth and final day without drug administration and occurring three weeks following decay day one (D1), there was little or no evidence of behavioural sensitization.

8.1 Figures

Figure 8.1

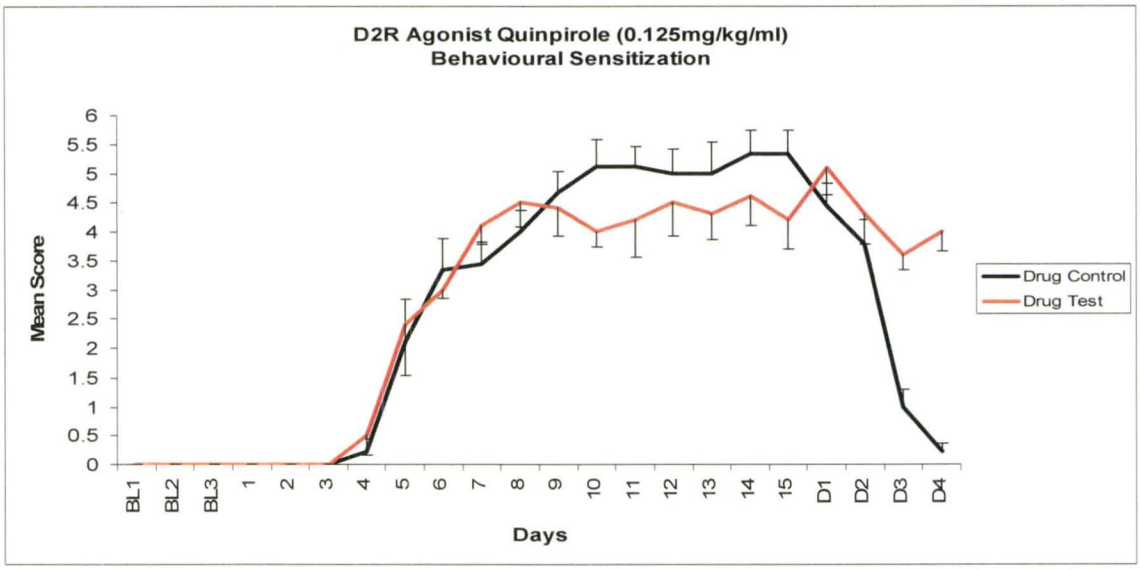


Figure 8.2

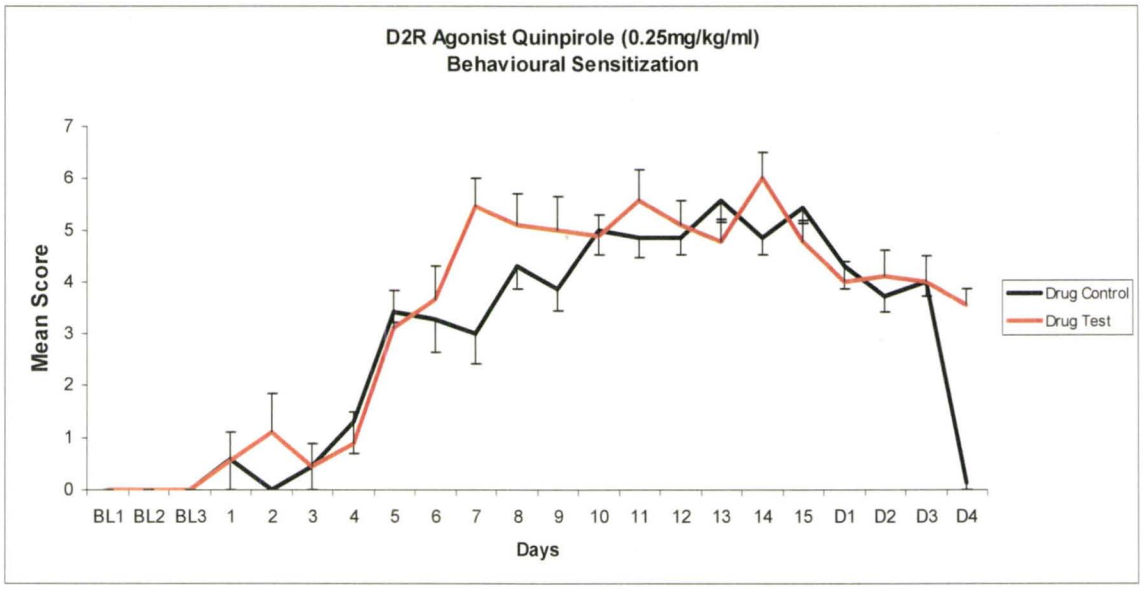


Figure 8.3

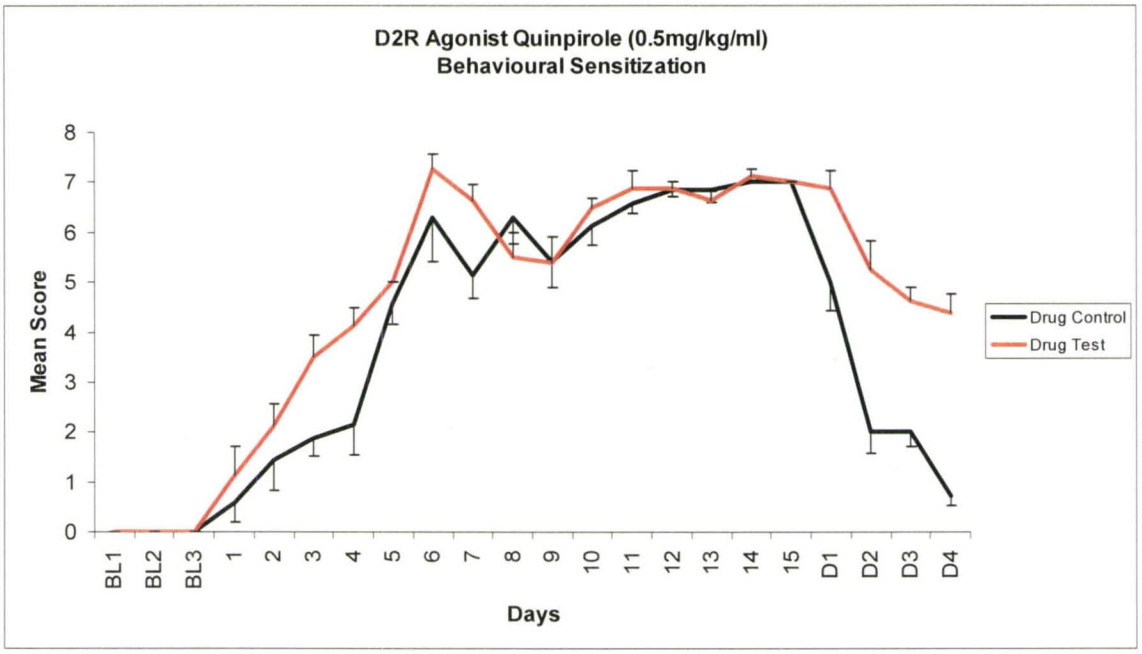

D2R Agonist Quinpirole (0.5mg/kg/ml)
Behavioural Sensitization
Mean Score
0
1
2
3
4
5
6
7
8
BL1
BL2
BL3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
D1
D2
D3
D4
Days
Drug Control
Drug Test

Figure 8.4

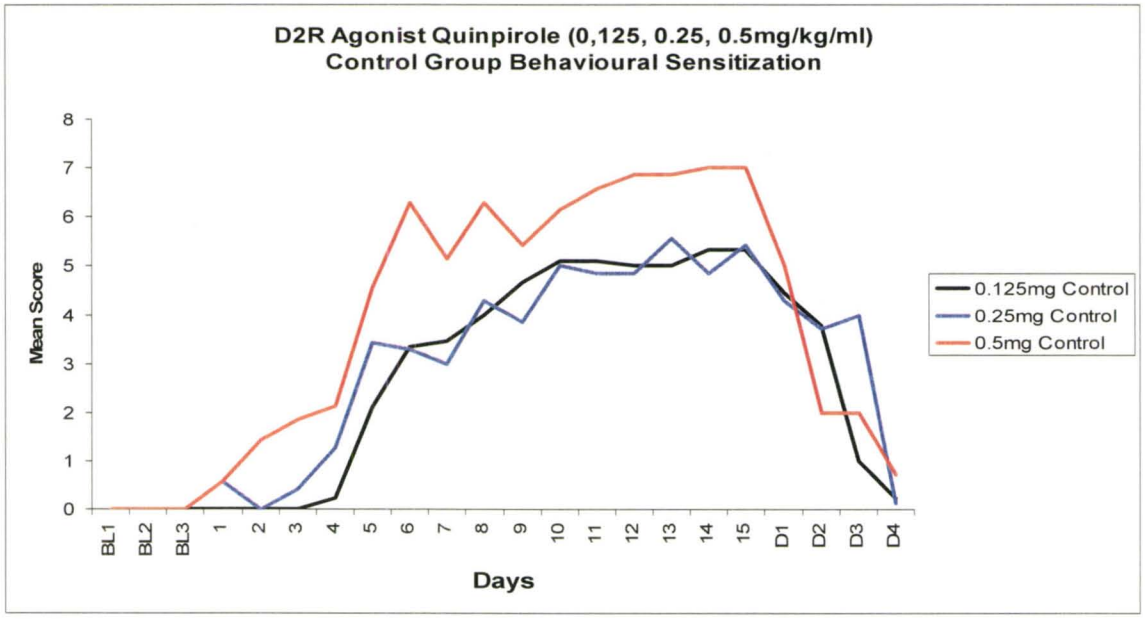
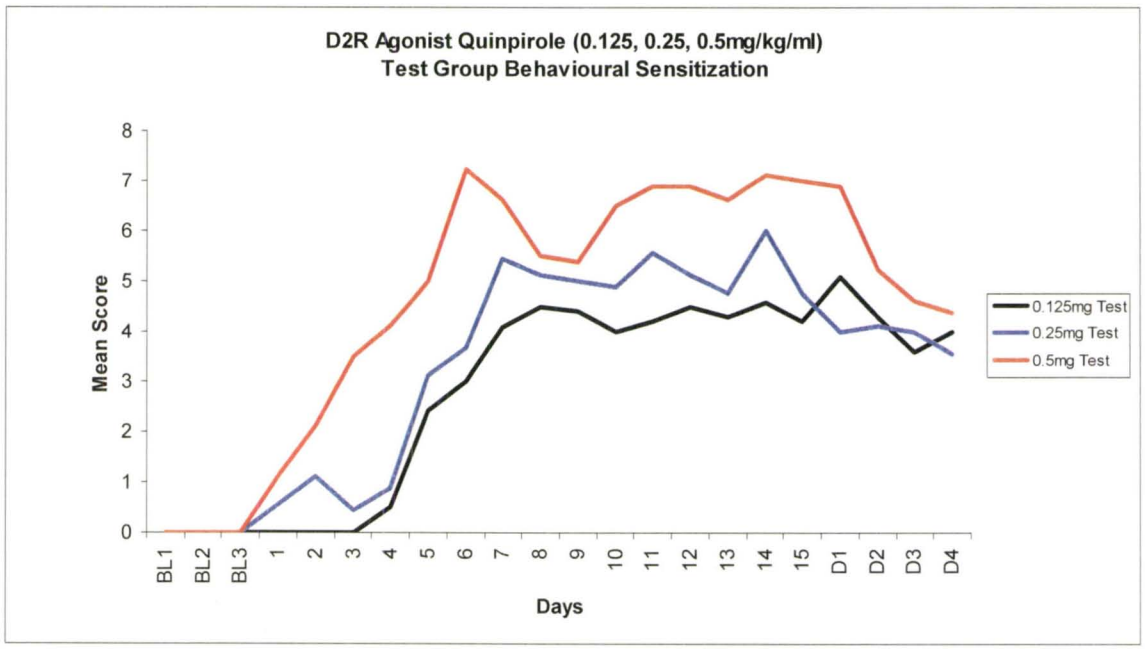


Figure 8.5



8.2 Figure Captions

Figure 8.1 D_2R Agonist Quinpirole (0.125mg/kg)-Induced Behavioural Sensitization

This figure represents mean observed behavioural sensitization during administration of the D_2R agonist quinpirole at 0.125mg/kg across days in the drug control (*black*) and drug test (*red*) groups (0.7 reduction in score representing 13.2% of baseline).

Figure 8.2 D_2R Agonist Quinpirole (0.25mg/kg)-Induced Behavioural Sensitization

This figure represents mean observed behavioural sensitization during administration of the D_2R agonist quinpirole at 0.25mg/kg across days in the drug control (*black*) and drug test (*red*) groups (0.43 increase in score representing 8% of baseline).

Figure 8.3 D_2R Agonist Quinpirole (0.5mg/kg)-Induced Behavioural Sensitization

This figure represents mean observed behavioural sensitization during administration of the D_2R agonist quinpirole at 0.5mg/kg across days in the drug control (*black*) and drug test (*red*) groups (0.25 increase in score representing 4% of baseline).

Figure 8.4 D_2R Agonist Quinpirole (0.125, 0.25, 0.5mg/kg)-Induced Behavioural Sensitization in Drug Control Groups

This figure represents mean observed behavioural sensitization during administration of the D_2R agonist quinpirole in the 0.125mg/kg drug control group (*black*), 0.25mg/kg drug control group (*blue*), and 0.5mg/kg drug control group (*red*).

Figure 8.5 D_2R Agonist Quinpirole (0.125, 0.25, 0.5mg/kg)-Induced Behavioural Sensitization in Drug Plus HFS Test Groups

This figure represents mean observed behavioural sensitization during administration of the D_2R agonist quinpirole in the 0.125mg/kg drug plus HFS test group (*black*), 0.25mg/kg drug plus HFS test group (*blue*), and 0.5mg/kg drug plus HFS test group (*red*).

CHAPTER 9

DISCUSSION

This is the first study of the effect of chronic *in vivo* administration of psychostimulants on LTP induction in the PFC and of the interaction between LTP induction and behavioural sensitization. The effect of administering DAR family specific agonists and antagonists on each of these phenomena was examined. This work is important for characterizing plasticity phenomena that may underlie the long-term expression of drug-induced sensitization as well as the sensitization-like symptomatology in particular psychiatric disorders such as addiction and schizophrenia. The studies indicate that DA modulates Glu-based corticocortical plasticity in the PFC, and that this synaptic plasticity might underlie behavioural sensitization. As the first set of results to look at LTP and behavioural sensitization *in vivo* and chronically, this data is pivotal. After pilot experiments showed little or no evidence for LTP induction in the mesocortical DA pathway, we focused on dopaminergic modulation of the corticocortical response in PFC. Our chronic *in vivo* pilot studies using the general DA agonist *d*-amphetamine showed that increased DA transmission facilitates LTP in the PFC (Coppa-Hopman, 2002). Thus, the following experiments sought to determine the role of specific DAR families in PFC LTP, as well as in DA-induced behavioural sensitization.

9.1 D_1R Modulation of LTP

A variety of *in vitro* studies have shown that DA modulates Glu-based LTP in the PFC via D_1Rs. The effect of DA upon the function of glutamatergic PFC neurons has been diverse, ranging from reductions to increases in glutamatergic efficacy (Blond et al., 2002; Gao et al., 2001; Gurden et al., 1999, 2000; Hirsch & Crepel, 1991; Jay et al., 1995; Law-Tho et al., 1994; Otani et al., 1998, 1999; Pantelis et al., 1997). The bi-directionality of these results is presumably a reflection of the underlying diversity of the pharmacological and biochemical actions of DA itself. As a neuromodulator, DA acts to increase or decrease the activity of targeted neurons. A critical difference between the *in vitro* preparation and the *in vivo* preparation is the lack of intact modulatory pathways, including the DA pathways, in the truncated *in vitro* system. Although these pathways are intact in the acute anesthetized *in vivo* preparation (Gurden et al., 1999, 2000), the anesthesia often has a strong effect on evoked responses. The anesthetized recordings are smaller in amplitude and do not readily express polysynaptic components. The chronic *in vivo* preparation, using fully-awake, freely-moving subjects, does a better job of modeling the normal physiological conditions within which DA receptors and NMDARs interact to produce LTP. We have often found that chronic preparations yield quite different response patterns compared to the *in vitro* and *in vivo* anesthetized chronic preparations. Thus, it was not surprising to find that the direction of dopaminergic modulation in the PFC of chronic

preparations was quite different from that found in the *in vitro* and acute *in vivo* anesthetized preparations.

The group administered the D_1R agonist showed LTP levels comparable to those in the saline group. This indicates that the systemic release of DA during HFS alone must have been adequate for LTP expression. This is in contrast to the *in vitro* and anaesthetized *in vivo* preparations where application of a D_1R agonist decreased LTP in the PFC (Gurden et al., 1999, 2000; Hirsch & Crepel, 1991; Jay et al., 1995; Law-Tho et al., 1994, 1995; Otani et al., 1998, 1999). However, our results are more similar to *in vitro* studies that tried to compensate for the probable inactivation of DA due to fibre truncation. This was done by first exposing PFC slices to a DA bath which induced inhibition. Following recovery from inhibition and a second DA bath that was coupled to stimulation, LTP induction was successful (Blond et al., 2002). An alternate explanation for these results may be that an enhanced induction or occlusion of LTP may only occur at an unknown optimal concentration of the D_1R agonist. Gurden et al. (2000) found that following 1mM administration of a D_1R agonist, the enhancement of NMDAR-dependent LTP at hippocampal-PFC synapses *in vivo* was strong and persistent, however, following a 5mM administration the LTP showed normal induction and maintenance when compared with controls. This suggests that overstimulation of D_1Rs disrupts the facilitatory effects of DA on synaptic

plasticity in the PFC. Future studies should examine the effects of different concentrations of D_1R agonists on LTP induction in the corpus callosum-mPFC synapses *in vivo* in order to better interpret these results.

In our experiments, the D_1R antagonist had no clear effect on the monosynaptic component, but blocked LTP and resulted in an HFS-induced LTD in the polysynaptic component. The fact that the D_1R family is positively linked to adenylate cyclase, one of the key components responsible for the initiation of the molecular cascade underlying LTP, is consistent with this result. The fact that LTP expression was completely blocked, leaving an HFS-induced LTD is strong evidence that D_1Rs do not simply play a facilitator role, but are necessary for the induction of Glu-triggered LTP. These results are in contrast to those from *in vitro* and acute *in vivo* preparations, where application of a D_1R antagonist was found to increase LTP in the PFC.

Our results are potentially relevant for the study of PFC-dependent cognitive processes, as well as for the study of disorders where D_1Rs and NMDARs in the PFC are known to be compromised. Both reduced and increased D_1R activation can disrupt performance on working memory tasks, so dose-response testing is probably essential for working out the effects of agonists and antagonists. Further work is required to determine whether the polysynaptic LTP effects are induced in the horizontal corticocortical connections within the PFC (Chapman et al., 1998) or elsewhere.

Given that D_1R_s predominate in the PFC, it is likely that modulation of these receptors affects PFC Glu transmission to both the N.Acc. and VTA. Prefrontal cortex D_1R agonism increases Glu transmission to the N.Acc. which increases GABA transmission to the VTA, thereby inhibiting local VTA GABA inhibition. Disinhibition of VTA circuitry would lead to an increase in DA transmission to the PFC by VTA fibres that join the fmiCC. Similarly, PFC D_1R agonism increases Glu transmission to the VTA which also increases DA transmission to the PFC by fibres that join the fmiCC.

9.2 D_2R Modulation of LTP

Examination of the role that D_2Rs play in synaptic plasticity has focused primarily on *in vitro* preparations of the N.Acc. and VTA, using psychostimulants. In the N.Acc., administration of psychostimulants elicits a depression of excitatory synaptic transmission. Nucleus accumbens slices prepared from rats sensitized to cocaine *in vivo* show a decrease in the ratio of AMPAR- to NMDAR-mediated excitatory post synaptic currents (EPSCs) at synapses made by PFC afferents onto medium spiny neurons in the shell, as well as a reduction in the amplitude of EPSCs at these synapses (Thomas et al., 2001).

The D_2R also plays a role in VTA synaptic plasticity. The work by Ungless and colleagues (2001) shows that only a single *in vivo* exposure to the D_2R agonist cocaine is required to induce LTP of AMPAR-mediated currents at

excitatory synapses onto DA cells, sustainable for 5 days following the termination of cocaine exposure. This effect is blocked when an NMDAR antagonist is administered with cocaine (Ungless et al., 2001). This work supports previous work showing that VTA DA neurons exhibit enhanced responsiveness to AMPA Glu stimulation after chronic psychostimulant exposure (Nestler & Aghajanian, 1997). *In vitro*, VTA DA neurons exhibit NMDAR-independent LTD in response to low-frequency stimulation and modest depolarization, which is blocked by brief exposure to a D_2R agonist (Jones et al., 2000). This work is significant because it shows that by inhibiting LTD, D_2R agonists remove the normal brake on the glutamatergic drive to DA neurons.

This work indirectly supports the work presented here, namely that chronic pairing of a D_2R antagonist with HFS dose-dependently decreases induced LTP levels, while chronic administration of a D_2R agonist dose-dependently increases levels of LTP induced in the PFC by callosal stimulation. The D_2R antagonist sulpiride did not appear to affect the onset of the induction of the incremental LTP, but it did dose-dependently reduce the maximum amplitude achieved during the latter half of administration. This reduced LTP also declined to LTD levels during the decay phase. The dose-dependent reduction in LTP in the PFC following HFS and D_2R antagonism was characterized by a large reduction of LTP in the polysynaptic component. This indicates that the most prominent effect on potentiation occurs at a latency that is characteristic of multiple synaptic

junctions. Conversely, administration of the D_2R agonist quinpirole to rats during HFS induced LTP at levels above those in saline controls. This was a dose-dependent effect, and the increase in LTP was greater with increases in drug dose. At all doses, the LTP effect was reliably maintained during the decay phase of the experiment, after the cessation of administration of drug and HFS. The dose-dependent effects on HFS-induced response alterations were characterized by a significant reduction in the monosynaptic component and a significant increase in the polysynaptic component, indicating that changes occur at latencies that are characteristic of both direct and indirect pathways, respectively. Overall, these results indicate that the D_2R is integral to establishing full levels of LTP (and perhaps LTD) in the PFC.

D_2R-mediated alterations in PFC plasticity may occur by modulating D_2Rs directly located in the cortex. However, this direct modulation is unlikely for several reasons. Firstly, the predominant DARs found in the PFC are of the D_1R family (Fuster, 1997; Sedvall, 1997). Further, only low levels of one subtype of the D_2R family, the D_4R, exists in the cortex (Fuster, 1997; Sedvall, 1997). Lastly, our chosen D_2R antagonist, sulpiride, has low affinity for the D_4R subtype (Sedvall, 1997), and our D_2R agonist, quinpirole, has no affinity for the D_4R subtype (Kurylo, 2004). Thus, we can say with confidence that our drugs do not significantly affect PFC DARs. This is supported by the demonstration that microinjections of sulpiride into the PFC have no effect on hippocampal-PFC LTP

(Jay et al., 1996), while systemic sulpiride administration in our studies produced large effects in the monosynaptic component of callosal-PFC LTP. Our results therefore suggest that D_2R modulation may indirectly result in alterations in PFC plasticity via modulation of mesolimbic circuitry.

The D_2 family of receptors in mesolimbocortical circuitry is comprised of $D_{2/3}$ autoreceptors present on the dendrites of neurons projecting from the VTA to the N.Acc. and PFC (Diaz et al., 2000). With respect to our D_2R antagonist experiments, blockade of these receptors may effectively antagonize their ability to provide negative feedback to the neuron in the presence of DA, resulting in more DA transmission to the target structures. In fact, sulpiride administration leads to an increase in DA within the N.Acc. and PFC (Ichikawa & Meltzer, 1999; Westerink et al., 1998). This effect may seem contrary to the action of sulpiride as a functional antagonist, however, blockade of D_2Rs found post-synaptically on N.Acc. and PFC neurons prevents target neuron activation (Carr et al., 1999; Morgenstern & Fink, 1985). Further, sulpiride mediates inhibition of drug-induced reinforcement and hypermobility by antagonizing these postsynaptic receptors (Goeders & Smith, 1986; Morgenstern & Fink, 1985). These effects of sulpiride may account for the saline-levels of LTP reached by the D_2R antagonist test groups prior to a reduction in response amplitude.

Chronic D_2R blockade may also lead to changes in receptor density. It has been demonstrated that a significant up-regulation of D_2Rs (Memo, Pizzi,

Nisoli et al., 1987) and D_3Rs (Wang, Hahn, Bishop et al., 1996) occurs in the N.Acc., but not in the frontal cortex, following chronic systemic sulpiride administration. In addition, D_1R expression is not altered by the administration of D_2R drugs (Samini et al., 2000; Serafim & Felicio, 2001). Assuming that up-regulation of D_2Rs in the N.Acc. following chronic sulpiride administration has taken place in the current study, a few arguments may be made. A larger number of D_2Rs in the N.Acc. or VTA following sulpiride administration may mean that it is necessary for this receptor population to be antagonized at a higher dose or for a longer period of time in order to block them at a functional level. This argument would account for the maintenance of saline plus HFS-induced levels of LTP during the initial stages of LTP induction in the D_2R antagonist plus HFS test group. This argument would also account for the delayed reduction in LTP in this group which occurred during the latter half of LTP induction.

In our experiments, chronic D_2R agonization with quinpirole may also have led to changes in receptor density. This is probable as it has been shown that a significant down-regulation of D_2Rs occurs in the mesencephalon following chronic systemic quinpirole administration (Licata, Bari, & Pierce, 2001; Licata, Freeman, Pierce-Bancroft, et al., 2000; Licata & Pierce, 2002). Thus, it is possible that down-regulation of D_2Rs following chronic quinpirole administration may have led to increased sensitivity of D_2Rs. This presumed increase in

sensitivity would result in an augmentation of their post-synaptic effects. Again, D_1R expression is not altered by the administration of D_2R drugs (Samini et al., 2000; Serafim & Felicio, 2001). Assuming that down-regulation of D_2Rs in the N.Acc. following chronic quinpirole administration has taken place in the current study, a few arguments may be made. A lesser number of D_2Rs in the N.Acc. or VTA following quinpirole administration may mean that it is sufficient for this receptor population to be agonized more weakly in order to stimulate them at a functional level. This argument would account for the earlier induction of LTP in the D_2R agonist group in comparison to the D_2R antagonist group. This may also account for the induction of LTP to levels beyond that of the saline plus HFS group. This argument would also account for the long-term maintenance of the effect beyond the administration of drug and HFS.

9.3 Behavioural Sensitization

The central aim of this part of the study was to explore the relationship between LTP and behavioural sensitization. Behavioural sensitization has received considerable attention as an experimental model for the development of behavioural pathology including schizophrenia, mania, post-traumatic stress, panic, obsessive compulsive disorder, autism, and addiction. The reason for this is that these psychopathological categories express symptomatic versions of behavioural sensitization, such as hyperlocomotion, increased speed of motion, and stereotypy.

Evidence has accumulated to support the hypothesis that the adaptations leading to addiction involve the same Glu-dependent cellular mechanisms that enable learning and memory. There are four main areas of indirect support: 1) drugs and LTP mechanisms activate common signal transduction pathways (Hyman & Malenka, 2001; Nestler, 2001); 2) drugs and LTP produce similar changes in dendritic morphology (Robinson & Berridge, 2003); 3) imaging reveals that drug-related stimuli (ie., paraphernalia, environment) activate neuronal circuits implicated in LTP (Wilson, Sayette, & Fiez, 2004); and 4) drugs of abuse influence neuronal plasticity in brain regions related to motivation and reward (Wolf, 2002). Consequently, addiction is often viewed as a form of neuronal plasticity.

The D_2R agonist groups receiving chronic administration of quinpirole expressed dose-dependent behavioural sensitization. At the lowest dose, the onset of mild behavioural sensitization (locomotion, sniffing) occurred on day four of quinpirole administration. At the middle dose, the onset of a moderate form of behavioural sensitization, now including locomotion, sniffing, circling, and rearing occurred on day one of quinpirole administration. The intensity (frequency per trial) of each of these behaviours also increased. At the highest dose, the onset of a more intense behavioural sensitization was evident on day two of quinpirole administration. Again, the range of behaviours expressed was increased (ie., locomotion, sniffing, circling, rearing, licking, gnawing, stereotypy), as well as the intensity of each. There was also a dose-dependent increase in the amount of

the behavioural sensitization response (increased amplitude). In other words, rats at the low and medium dose performed fewer behaviours and at a lower intensity than those in the high dose group.

Quinpirole appeared to have similar effects on behavioural and LTP measures, as both were increased in a dose-dependent fashion. This suggests that there may be a close relationship between LTP and behavioural sensitization phenomena. Behavioural sensitization was expressed somewhat differently between the drug control and drug test groups. At the high dose, the group receiving drug and HFS showed a more rapid induction of behavioural sensitization. Secondly, the expression of behavioural sensitization was markedly different between the drug control and drug plus HFS groups during the decay phase. This difference applied at all doses. Once drug administration stopped, the drug control groups showed a decline in the behavioural measures over days, while the drug test groups continued to express behavioural sensitization throughout the decay phase when quinpirole and HFS were no longer administered. This result suggests that the long-term maintenance of behavioural sensitization is dependent upon the same type of change in corticocortical connectivity that is seen with LTP induction. Normally, such synaptic reorganization may be responsible for encoding memories about drug experience and context and producing the long-term habitual behaviours expressed during addiction. These results provide the first known chronic *in vivo*

support for a link between drug-induced behavioural plasticity and mesolimbocortical synaptic plasticity.

The next major question is how and where does this happen in the brain? At this point, we are left with the task of mapping our results onto those already published regarding the induction, maintenance, and expression of behavioural sensitization, and how it is hypothesized to relate to LTP. Our overall hypothesis is that elevations in subcortical DA transmission lead to more permanent alterations in Glu transmission in the projection from the mPFC to the N.Acc., which mediate the long-term maintenance of behavioural sensitization via an LTP-like process.

Quinpirole sensitization develops with sequential repeated drug infusion (Licata et al., 2000, 2001; Licata & Pierce, 2002). Overall, quinpirole sensitization reflects the accumulation of locomotor-enhancing factors produced by each injection (Szechtman, Talangbayan, Canaran, et al., 1994). D_2R agonists act on DA cell stimulation in the VTA leading to enhanced DA transmission to the PFC and N.Acc. (Berridge & Robinson, 1998). This enhanced DA transmission likely alters the responsiveness of PFC and N.Acc. cells to Glu. In our experiments, the modulatory effects of this expected increase in level of DA transmission to the PFC was augmented by concomitant HFS of the corpus callosum.

The PFC pyramidal cell normally fluctuates between depolarized 'up' states and hyperpolarized 'down' states (Branchereau et al., 1996; Lewis & O'Donnell,

2000). This fluctuation is regulated by DA and Glu afferents, and is thought to reflect activity levels in cortical circuitry (Lewis & O'Donnell, 2000). Chronic psychostimulant administration leads to a loss of this PFC membrane bistability, possibly reflecting a change within pyramidal cells and/or DA/Glu afferents (Lewis & O'Donnell, 2000). Dopamine released from the VTA may promote the 'up' state in PFC pyramidal cells, thus increasing the probability that they will fire action potentials (Lewis & O'Donnell, 2000). Further, it has been shown that in the PFC, D_1R stimulation leads to enhancement of pyramidal neuron excitability (Rosenkrantz & Grace, 2001). *High frequency stimulation plus quinpirole* administration presumably stimulates D_1Rs in the PFC to a greater extent than quinpirole administration alone. This higher level of stimulation may have provided the conditions necessary for sustained conversion to the 'up' state in PFC pyramidal neurons, increasing the probability that they will fire action potentials. This increased firing rate in the PFC may lead to enhancement of excitatory drive within the N.Acc.

The N.Acc. is the interface between limbic and motor systems responsible for the conversion of motivation to action, a process which requires activation of Glu projections from the PFC to the N.Acc. (Pierce et al., 1998). The N.Acc. is also involved in the enduring cellular changes elicited by repeated psychostimulant use (Kalivas et al., 2003). This is supported by the fact that PFC lesions block the development, but do not impede the expression, of behavioural sensitization while N.Acc. lesions do the reverse (Li et al., 1999). As such, the maintenance of

behavioural sensitization has been linked to DA binding to D_1Rs in the mPFC which have efferent projections to the N.Acc., and to D_1Rs in the N.Acc. which receives input directly from the VTA (Kalivas & Duffy, 1991). The central role of D_1Rs in the N.Acc. is supported by the findings that a D_1R agonist injected into the N.Acc. enhances the expression of behavioural sensitization, while D_1R antagonists injected into the N.Acc attenuate the expression of behavioural sensitization (De Vries et al., 1998). Our D_1R agonist groups did not mimic this modulation of behavioural sensitization expression as the drug was administered to groups that had not developed sensitization at all (ie., these D_1R groups did not first receive a D_2R agonist). Enhanced D_1R sensitivity in the N.Acc. following sensitization has also been reported (Henry & White, 1991). Further, prolonged quinpirole administration is known to enhance the capacity of D_1R stimulation to release Glu in the N.Acc. (Kalivas & Duffy, 1998). At the N.Acc., Glu also facilitates DA transmission, presumably by presynaptically influencing DA release (Blaha, Yang, Floresco et al., 1997; Floresco, Yang, Phillips, et al, 1998), an effect which may be predominantly regulated by AMPAr rather than NMDAR (Youngren et al., 1993).

Ultimately, D_1R stimulation of cAMP-dependent protein kinases and subsequent changes in proteins and immediate early gene expression in the N.Acc. are critical to establishing sensitization (Nestler, 2001). It is known that D_1R stimulation enhances phosphorylation of the GluR1 subunit of the AMPAR , leading to increased AMPAR surface expression by increasing the rate of GluR1

externalization onto the cell membrane (Wolf, 2003). Furthermore, studies show increased AMPAR/NMDAR ratios at Glu synapses (Faleiro et al., 2003) and increased GluR1 subunits following sensitization (Carlezon & Nestler, 2002). This is important as GluR1 subunits favour the formation of Ca^{2+}-permeable AMPARs, and thereby would promote LTP induction. However, insertion of GluR1 into synaptic sites has not been reported to occur during behavioural sensitization, as it does during LTP. This is a two-step process: insertion into extrasynaptic sites plus lateral movement in the synapse, a process which requires Ca^{2+}/calmodulin kinase II (Chetkovich, Chen, Stocker, et al., 2002; Passafaro, Piech, & Sheng, 2001).

Enhanced D_1R stimulation in the N.Acc. would also affect these cells' excitability. Nucleus accumbens cells are normally quiescent, but do express 'up' and 'down' states similar to that in the PFC (O'Donnell & Grace, 1995). The activation of N.Acc. cells requires synchronous activation of excitatory input (O'Donnell & Grace, 1995). Thus, the induction of LTP in excitatory PFC pathways likely influences the occurrence of synchronized activation in N.Acc. neurons. Furthermore, N.Acc. cells from sensitized animals are biased toward the 'up' state compared to those from the non-sensitized animals (Brady & O'Donnell, 2004). Thus, the chronic administration of quinpirole plus HFS may influence N.Acc. cell output by promoting synchronized activation. Overall, as 'up' states in N.Acc. cells are driven by Glu input, the enhanced Glu input in sensitized animals exacerbated with HFS would provide the conditions

necessary to create a prolonged 'up' state in N.Acc. cells. This 'up' state would presumably lead to enhanced stimulation of VTA DA neurons, resulting in enhanced mesolimbic DA transmission versus that following chronic quinpirole or HFS administration alone.

This theory suggests that a prolonged 'up' state in N.Acc. cells, driven by a prolonged 'up' state in the PFC following D_2R agonism plus HFS, contributes to the enhancement of basal DA levels in sensitized animals following LTP. This notion is supported by evidence that repeated drug exposure enhances sensitization-induced DA release in the N.Acc. (Cadoni & Di Chiara, 2000). Furthermore, syntaxin-1, a protein strongly implicated in synaptic plasticity and LTP (Stroemer et al., 1998), is increased in the N.Acc. following amphetamine sensitization (Subramaniam et al., 2001). Thus, the net impact of DA neurotransmission is likely to be dependent upon whether convergent excitatory inputs are powerful enough to overcome ongoing inhibitory processes, and allow D_1R/NMDAR-mediated interactions in the PFC and N.Acc. to promote LTP strongly enough to maintain the sensitization behaviours which we hypothesize it supports long-term.

Chronic drug users become dependent on the drug, and when intake stops, an adapted state of the brain (ie., neurotransmitter and cell firing operating at altered levels) continues to function in the absence of the substance and stimuli that created it. Evidence suggests that withdrawal from chronic drug administration decreases activation in the mesocortical system leading to

reduced dopaminergic transmission in the limbic system and cortex (Diana, Melis, Muntoni et al., 1998). During the decay phase of our experiments, when drug and HFS was no longer administered, it is therefore assumed that a reduction in DA release and/or increased reuptake from the synaptic cleft occurred in amidst a system which had functionally and morphologically had adapted to chronic D_2R agonism plus HFS. This would result in a withdrawal-like period with less activation of D_1Rs in the PFC and N.Acc.. In groups expressing LTP, this would occur in a system which presumably has an increased number of AMPARs with GluR1 subunits. Nucleus accumbens neurons are known to be subsensitive to Glu after withdrawal (White, 1995). For example, basal levels of Glu are reduced in the synapse and presynaptic terminals in animals previously treated with cocaine (Baker et al., 2003; McFarland et al., 2003). These reduced levels of DA and Glu may alter the synaptic response to Glu released from the PFC and the synaptic response to DA released from the VTA. Perhaps it is these changes that drive drug craving as a means to reacclimatize the brain to the drug-adapted state, thereby subserving relapse into addiction and recidivism.

9.4 Clinical Implications

There are clinical implications for this work. Despite the well-established role of DA in mechanisms underlying addiction, dopaminergic medications have thus far failed to make valuable contributions to the treatment of drug addiction. In principle then, whether in isolation or in combination with DA-based medications,

glutamatergic agents may provide future therapeutic efficacy. These may be efficacious in blocking the expression of compulsive drug-taking behaviours, reduce physical dependence-withdrawal, or prevent relapse. It has already been shown that low-affinity NMDAR channel blockers reduce the severity of physical withdrawal in opiate addicts (Bisaga, Comer, Ward et al., 2001). AMPAR antagonists might also hold therapeutic potential since these drugs appear to be more effective than NMDAR antagonists in animal studies in inhibiting the expression of addiction-related behaviours such as sensitized locomotion, conditioned place preference, and drug-seeking behaviour (Di Ciano & Everitt, 2001; Wolf, 1998). It should be noted, however, that a major limitation in AMPAR antagonist treatment is that AMPAR activation underlies most of the excitatory synaptic events in the brain. Further, the use-limiting psychotomimetic side-effects of Glu antagonists must be kept in mind.

9.5 Conclusions and Future Considerations

While research has shown that psychostimulant administration induces 'markers' for LTP in the N.Acc. and VTA (Carlezon & Nestler, 2002; Faleiro et al., 2003; Wolf, 2003), there is no evidence that LTP-like alterations in synaptic strength *actually* occur. Our experiments illustrate that chronic *in vivo* administration of a psychostimulant analogue, while presumably leading to the induction of these LTP 'markers', induces robust behavioural sensitization but does not show an LTP effect in the relatively crude population measures that we

are taking. The concomitant application of HFS and quinpirole, however, induces behavioural sensitization and LTP in the PFC. These results imply that an HFS-like pattern of cell firing in afferents to the PFC may be required to result in the maintenance of behavioural sensitization. Without such cell firing, behavioural sensitization will occur, but at reduced levels, and only short term during drug administration. Given that DA is thought to push PFC pyramidal cells into an 'up' state, the combination of increased dopaminergic cell firing in the VTA and psychostimulant administration may be necessary to augment and maintain the behavioural sensitization response long after termination of drug administration. Mesolimbic DA neurons are known to increase their firing rates in response to a large category of salient and arousing events, including appetitive, aversive, high intensity, and novel stimuli (Schultz, 1998). Dopamine's role, in part, is to promote the transition to the 'up' state in PFC neurons (O'Donnell, 1999). A high level of synchronized pyramidal cell firing might be expected to occur when the experience of salient events occurs concomitantly with psychostimulant administration. The subsequent involvement of the N.Acc. would then lead to long-term maintenance of behaviours related to the salient events during the drug state. Studies which place rats into rewarding paradigms may therefore show an 'up' state in the PFC which is translated to the N.Acc. In a human drug addict, the drug paraphernalia, the environment within which drugs are taken, the time of day drugs are used, or the people with which drugs are ingested, may serve as the salient stimuli driving increased DA release from the VTA in a

repetitive, synchronous, HFS-like fashion. This, paired with a further increase in DA due to psychostimulant intake, would lead to increased D_1R stimulation in the PFC, and a sustained 'up' state. As discussed above, this 'up' state in the PFC would be associated with increased Glu efflux to the N.Acc., D_1R stimulation in the N.Acc., and the establishment of a long-term 'up' state in the N.Acc. Such a process may underlie the expression of stereotypical sensitized behaviours in human addicts long after they have stopped taking psychostimulants. This is integral to recidivism, and the lack of therapeutic efficacy with drug addicts.

Other work that needs to be done to provide a clearer understanding of the function of this system is a more detailed characterization of the responses in its component pathways, such as the prefrontal corticocortical pathways from cortex to N.Acc. and VTA. In addition, the capacity of the other component pathways to support synaptic modification must be explored. For example, if neurons in these structures undergo some form of potentiation along with the PFC, then this plasticity must be characterized. The effects of corpus callosum stimulation on DA release in the PFC and N.Acc. and on the firing rates of VTA DA neurons also needs to be characterized. Although the relatively crude techniques that we initially apply in LTP research only scratch the surface, they provide a good starting point for the exploration of the behaviour of intact circuitry in unanaesthetized animals. The emphasis that has been placed on hippocampal and cortical LTP in the literature means that most of what we know about these phenomena comes from clearly laminated systems. The VTA and N.Acc., with

their non-laminated nuclear distribution of neurons presents a physically different system that is more difficult to characterize electrophysiologically. Furthermore, although most of the attention is currently directed to the glutamatergic pathways most directly involved in the information flow through the mesolimbic system, particularly the glutamatergic pathways from the PFC, it is likely that a variety of other systems contribute to the relevant encodings.

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