ANTIDEPRESSANT TREATMENT AND CORTICAL 5-HYDROXYTRYPTAMINE$_{2A}$ RECEPTORS

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Antidepressant Treatment and Cortical 5-Hydroxytryptamine$_{2A}$ Receptors

By

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Abstract

The aim of this research was to functionally assess changes in cortical 5-hydroxytryptamine$_{2A}$ (5-HT $_{2A}$) receptors following exposure to drugs used to treat depression or to electroconvulsive shock. The literature suggests a possible role for 5-HT$_{2A}$ receptors in the onset and treatment of depression but, to date, no extensive functional assessment of cortical 5-HT$_{2A}$ receptors has been undertaken to determine how important a role this plays.

N-methyl-D-aspartate (NMDA) depolarizes cortical neurons and this depolarization is enhanced by 5-HT acting at 5-HT$_{2A}$ receptors. Using this facilitation as a measure of 5-HT$_{2A}$ receptor activity the functionality of 5-HT$_{2A}$ receptors on cortical neurons was assessed following acute and chronic exposure to imipramine, fluoxetine or mianserin. These results were compared with those following a course of electroconvulsive treatment (ECT).

The results showed that following chronic (14 day), but not acute (2 day) exposure to imipramine and fluoxetine, the 5-HT concentration response relationship was shifted to the right and exhibited a lower maximum response compared to controls. In contrast, mianserin, a 5-HT$_{2A}$ receptor antagonist which also exhibits antidepressant efficacy, produced a similar shift and reduced maximum following acute exposure. ECT did not alter the 5-HT concentration-

response relationship. Neither drug exposure nor ECT significantly altered NMDA responses.

Based on these results it is concluded that cortical 5-HT_{2A} receptor down-regulation may be an important event in mediating the therapeutic response achieved following chronically administered antidepressants. 5-HT_{2A} receptor down-regulation may shift the balance from excitatory 5-HT_{2A} receptors in favor of inhibitory 5-HT_{1A} receptors, co-localized on postsynaptic cortical neurons. Thus, a rise in synaptic 5-HT levels following chronic antidepressant treatment increases the activity of postsynaptic 5-HT_{1A} receptors and results in an increase in inhibitory 5-HT neurotransmission. A rise in inhibitory 5-HT neurotransmission could counteract the increase in excitatory 5-HT activity thought to occur in depressed individuals. This represents a return in net 5-HT activity to a homeostatic level, alleviating symptoms associated with depression.

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Chapter 1

Introduction

1.1 Depression:

Depression can be divided into two types, exogenous and endognous (for review see Kandel et al., 1991). Exogenous depression is thought to result from a specific stress, such as the loss of a family member, loss of a job, or transient loss of health. It reflects an intensification of the normal response to a disturbing circumstance. Individuals who suffer from this type of depression appear to have an existing predisposition to depressive behavior. An important aspect of exogenous depression that differentiates it from endogenous depression is that the former condition does not respond to antidepressant treatment.

Endogenous depression is a debilitating disease which affects approximately 30% of the adult population during their lifetime (Klerman, 1987). Approximately 40 to 60% of individuals suffering from endogenous depression require hospitialization. Unlike exogenous depression, endogenous depression does not appear to result from an external event. There are five common features associated with endogenous depression: 1) diurnal variations in depression;

2) alteration of sleep patterns; 3) appetite changes and weight gain or loss; 4) psychomotor difficulties; 5) lack of interest.

Individuals who suffer from endogenous depression have symptoms which persist from 4 to 12 months and gradually abate if left untreated (Richelson, 1994). Endogenous depression appears to be cyclic, depressive episodes have a high probability of recurrence.. The severity of the condition is indicated by statistics showing that 30% of those suffering from endogenous depression commit suicide (Richelson, 1994). In addition to individual suffering, people who are afflicted with endogenous depression have a negative impact on families, friends and employment (Mendels, 1992).

The likelihood of an individual incurring a depressive episode is 2-3 times higher in women than in men suggesting an underlying biological component. (Mendels, 1992; Richelson, 1994). Additional evidence for a genetic component is that those who have first degree relatives that suffer from depression have a higher incidence of developing depression than individuals who do not (Richelson, 1994).

The annual cost of depression in the United States has been estimated to be 43.7 billion dollars, which includes direct treatment costs and indirect costs of suicide and loss in the workplace (Greenberg et al., 1993). Given the population

size of Canada, this would equate to an impact of approximately 4 billion dollars annually in Canada.

1.2 Treatment of Depression:

Although depression (melancholia) has been recognized for a long time, effective pharmacological treatment only began in the early 1960's (Pletscher, 1991). The first generation of antidepressant drugs were discovered serendipitously. Iproniazid, which was used in the treatment of tuberculosis (Fox et al, 1953), was observed to reverse the depression exhibited by patients treated with reserpine for hypertension (Brodie et al., 1966). Iproniazid inhibits monoamine oxidase (MAO) which metabolizes monoamines such as norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (Pletscher, 1991). Shortly thereafter another group of drugs, known as the tricyclics,were identified. These drugs undergoing evaluation for the treatment of schizophernia were observed to reverse the depressive symptoms induced by reserpine in the treatment of schizophernia (Meltzer, 1992). Research into both these agents led to the development of the first generation of antidepressant drugs.

Although both monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) are efficacious in the treatment of depression there are serious side effects which limit their use. Cardiotoxicity and overdose, leading to tissue damage and sometimes death, are associated with the use of TCAs. Hypertension and hepatic necrosis are associated with the use of MAOIs (Leonard, 1996). These side effects are due, in part, to the wide spectrum of action exhibited by these agents on multiple neurotransmitter systems including the cholinergic, histaminergic, serotonergic and adrenergic systems (Hollister and Claghorn, 1993). Side effects and the lack of therpeutic effectiveness in some patients prompted researchers to re-evaluate the use of MAOIs and TCAs and search for more efficacious treatment paradigms.

Selective serotonin re-uptake inhibitors (SSRIs) formed the second generation of antidepressants and became clinically important in the early 1980s (Hyttel, 1994). As the name implies these drugs were more selective for the serotonin transporter than either the norepinephrine or dopamine transporter. A major benefit of these drugs is their low affinity for muscarinic, adrenergic, histaminergic, GABAergic and serotonergic receptors (Hollister and Claghorn, 1993). Thus, many of the side effects associated with the use of MAOIs and TCAs are reduced or eliminated.

Continuing research on MAO revealed that MAO could be divided into two subtypes, MAO-A and MAO-B (Leonard, 1996). They are distinguished by their substrate preference and inhibitor specificity. Thus, NE and 5-HT are catabolized by MAO-A while dopamine is catabolized by both forms of MAO (Blier et al., 1990; Yang and Neff, 1974). With the advent of inhibitors selective for the MAO subtypes and, as a consequence, a reduction in side effects, there has been a resurgence of the use of MAOIs in the treatment of depression (Leonard, 1996).

Other treatment paradigms for depression include lithium and electroconvulsive shock therapy (ECT). Lithium has been used in the treatment of depression since the observation in the early 1970s that depressed patients show improvement in their mood when treated with lithium (Goodwin and Post, 1974). Lithium, however, is associated with serious side effects and high toxicity (Gelenberg, 1988).

Although ECT is used for the treatment of depression it is associated with side effects of which the most serious is memory impairments (Coleman et al., 1996). These side effects prevent its widespread use.Though ECT has been used for over 50 years, the basis of its therapeutic action remains unknown (Fink, 1990).

Despite the success of MAOIs, TCAs, SSRIs, lithium and ECT there remains a 14 to 21 day time-lag between the start of therapy and the onset of therapeutic effect despite an apparent immediate pharmacological effect of the drugs, e.g. blocking the 5-HT uptake or MAO, (Blackshear and Sanders-Bush,1982; Borsini, 1994; Oswald et al., 1972).

From this brief review it may be concluded that several neurotransmitter systems are potentially involved in the pharmacological treatment of depression. This offers the possibility that a combination of approaches may result in more efficacious treatment and, possibly, a reduction in the time lag between the start of therapy and the reduction in symptoms. The key to more efficacious treatment lies with a better understanding of the mechanisms underlying the present treatment paradigms.

1.3 Neurotransmitters and Depression:

Although depression is an age old medical condition the bridge between the onset of depression and the mechanisms that underlie depression only began to be understood in the early 1960's, when the benefits of using chemotherapy to treat depression were first recognized (Pletscher, 1991). These chemotherapeutic treatments affected a number of neurotransmitter systems which include NE (Pare and Sandler, 1959; Schildkraut, 1965); dopamine (Randrup et al., 1975) and 5-HT (Coppen, 1967). Although these were the first neurotransmitter systems investigated for their potential roles in depression, more recently a possible link has been shown with others, including GABAergic (Emrich et al., 1980), histaminergic (Green and Maayani, 1977), muscarinic (Sydner and Yamamura, 1977) and glutaminergic systems (Trullas and Skolnick, 1990).

1.4 Catecholamine Hypothesis of Depression:

A role for NE and dopamine, both catecholamines, in the etiology of mental illnesses has been postulated since the late 1950's. Based on observations with reserpine, MAOIs and imipramine-like drugs, Schildkraut (1965) advanced the catecholamine hypothesis. He proposed that some, if not all, forms of depression are associated with an absolute or relative deficiency of catecholamines, particularly NE, at functionally important adrenergic receptor sites in the brain.

Reserpine, which is isolated from the Indian plant *Rauwolfia serpentinia*, was formerly used clinically in the treatment of hypertension (Pletscher, 1991). Reserpine facilitates the release of NE, dopamine and 5-HT from storage vesicles (Sugrue, 1983). Thus, release of NE by reserpine and its subsequent enzymatic degradation by MAO ultimately results in a deficiency of endogenous NE (Kopin, 1964; Kopin, 1982; Von Euler et al., 1964). The association between reserpine and depression is supported by the observation that approximately 15% of all individuals treated for hypertension with reserpine exhibit depressive symptoms (Faucett et al., 1957; Jensen, 1959; Lemieux et al., 1956). Further evidence linking the effects of reserpine treatment to depression came from the

observations that: 1) a higher incidence of depression is seen in patients taking reserpine than is seen with other antihypertensives; 2) the larger the dose of reserpine, the higher the incidence of depressive reactions; 3) the depression usually clears when reserpine treatment is stopped, but returns if the drug is restarted (Bunney and Davis, 1965).

The focus of the catecholamine hypothesis on NE results, in part, from the effects of NE in the brain. The principal site of NE synthesis is the locus coeruleus which has a dense projection to many areas of the brain including the hypothalamus, hippocampus and cerebral cortex (Bremner et al., 1996), areas important for mood, arousal and psychomotor functions (Moore and Bloom, 1979). Given this, a potential involvement of NE in depression is not surprising.

Iproniazid was first synthesized in the early 1950s from isoniazid (Fox et al., 1953). It was developed to be a more effective treatment paradigm for tuberculosis but, as noted earlier, became very important in the treatment of depression (Pletscher, 1991). Use of iproniazid as an antidepressant began serendipitously when it was observed that iproniazid blocked the reserpine response (Pletscher, 1991). Thus, the sedation and lethargy produced with reserpine was replaced by excitation when iproniazid was administered (Brodie et al., 1966). Moreover, non-depressed patients receiving iproniazid for the

treatment of tuberculosis experienced euphoria, psychomotor stimulation and psychostimulation (Pletscher et al., 1960). Clinical studies using iproniazid as a treatment for depression were conducted on individuals diagnosed with stable depression for more than twenty years. Approximately 70% of the patients treated with iproniazid showed improvement. Thus, the first clinical drug for the treatment of depression was established (Pletscher, 1991).

Imipramine was discovered in the 1950s as a derivative of the tricyclic compound chlorpromazine, used in the treatment of schizophrenia (Pletscher, 1991). Though imipramine was ineffective in the treatment of schizophrenia it was found to be beneficial in treating depression (Kuhn, 1958). By blocking reuptake of NE, imipramine acts to increase the amount of NE in the synaptic cleft by inhibiting re-uptake into NE terminials (Sugrue, 1983). Moreover, like iproniazid, imipramine blocks reserpine-induced depression (Pletscher, 1991).

Although observations with reserpine, iproniazid and imipramine implicated the noradrenergic system with respect to the onset and treatment of depression, the notion of a simple deficiency of NE had to be abandoned. A major problem with the catecholamine hypothesis is its failure to explain the delayed therapeutic response (Borsini, 1994; Oswald et al., 1972). Fourteen to 21 days are required to observe the beneficial effects of drug therapy, whereas the blockade

of re-uptake of NE or the inhibition of MAO occurs almost immediately. This resulted in a shift away from the pre-synaptic focus of the catecholamine hypothesis to a focus on the importance of post-synaptic effects.

One of the most consistently observed responses to chronic antidepressant treatment has been the down-regulation of the β- adrenergic receptors (Sulser et al, 1983). Virtually every class of antidepressant treatment including ECT has this effect in the cortex (Caldecott-Hazard et al., 1991).

1.5 5-HT and Depression:

The idea that the onset of depression results from an irregularity of the 5-HT system was first examined in the late 1960s. Based on the disturbances in amine metabolism it was postulated that depression resulted from a deficiency in the 5-HT system (Coppen, 1967; Lapin and Oxenkrug, 1969). Coppen (1967) observed that tryptophan, a precursor of 5-HT, potentiates the antidepressant effect seen with MAOIs. Coppen (1967) also noted that in depression, tryptamine excretion decreases and lower levels of 5-hydroxyindole acetic acid (5-HIAA), a metabolite of 5-HT, are found in the cerebrospinal fluid (CSF). Post-mortem studies on depressed patients revealed decreased levels of both 5-HT and 5-HIAA when compared to individuals who were not diagnosed with depression when they died (Lloyd et al., 1974). Examination of CSF using baseline measures of 5-HIAA and postprobenecid 5-HIAA levels (probenecid inhibits efflux of 5-HIAA from CSF), revealed that the level of 5-HIAA was reduced in patients suffering from depression (Asberg et al., 1976; Goodwin and Post, 1977; Sjostrom, 1973). These results suggest that 5-HT turnover is reduced. More importantly, the decreased levels of 5-HIAA were only observed in individuals who were diagnosed with endogenous depression (Van Pragg et al., 1971).

Precursor depletion experiments also support the involvement of 5-HT in depression. Tryptophan, an essential amino acid, is the precursor for 5-HT synthesis (Gal and Dress, 1963; Tagliamonte et al., 1973). Dietary depletions of tryptophan reduce 5-HT activity (Delgado et al., 1990). Moreover, acute depletion of tryptophan reversed the therapeutic benefits of antidepressant treatment in depressed patients, but they returned to the remitted state on return to regular food 24 to 48 hours later (Delgado et al., 1990).

Studies examining the effects of TCAs and MAOIs show that they have effects just as profound on 5-HT transmission as they have on NE transmission (Caldecott-Hazard et al., 1991). MAOIs reduce 5-HT catabolism and hence increase 5-HT transmission, due to the increased 5-HT concentration in the synaptic cleft (Caldecott-Hazard, 1991). In the same vein, imipramine inhibits both NE and 5-HT re-uptake from the synaptic cleft (Byrant and Brown, 1986).

Two important experiments in the 1970s showed that parachlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, appears to reverse the antidepressant effectiveness of both imipramine (Shopsin et al., 1975) and tranylcypromine (Shopsin et al., 1976) within 24 hours of administration. This suggests that 5-HT must be available for antidepressants to exert their therapeutic effect.

The continued development of new antidepressants with increasingly greater selectivity for the 5-HT transporter over the NE transporter is consistent with a role for 5-HT in depression. Importantly, all serotonin selective re-uptake inhibitors (SSRIs) are efficacious in the treatment of depression and the only common feature among the SSRIs is their ability to block the uptake of 5-HT (Blier and De Montigny, 1994).

1.6 Role of 5-HT Receptors:

As with the catecholamine hypothesis, the failure of a simple monoamine deficiency theory to explain the delayed clinical response to antidepressants has shifted the focus of antidepressant research from presynaptic to postsynaptic events (Yates et al., 1990).

It has been proposed that 5-HT_{1A} receptors are involved in both the onset of depression and its treatment (Stahl, 1994). Physiologically, 5-HT_{1A} receptors mediate the 5-HT induced release of adrenocortotrophic hormone (ACTH) and cortisol along with a pronounced hypothermic response (Lesch, 1992; Lesch et al., 1990; Stahl, 1992). Individuals diagnosed with depression have a blunted

hypothermic response and decreased release of ACTH in response to the administration of 5-HT_{1A} partial agonists, such as buspirone or gepirone (Lesch, 1992; Stahl, 1992). This suggests that 5-HT_{1A} receptors may be down-regulated or desensitized in clinically depressed patients. There is a partial reversal of the neuroendocrine impairment following chronic antidepressant treatment (Newman et al., 1993; Stahl, 1994). Moreover, partial 5-HT_{1A} receptors agonists are effective antidepressants (Rausch et al., 1990). Finally, following chronic antidepressant treatment presynaptic somatodendritic 5-HT_{1A} receptors are down-regulated (Charney et al., 1981; Stahl, 1992). Somatodendritic 5-HT_{1A} receptors are inhibitory receptors which regulate the firing rate of the dorsal raphe neurons. These observations suggest a possible role for the 5-HT_{1A} receptor in the onset and treatment of endogenous depression but further research is needed to distinguish whether the receptor has a role in mediating the therapeutic response to antidepressants or whether the changes are merely a by-product of chronic antidepressant treatment.

Another receptor of interest with regard to depression is the 5-HT_{2A} receptor. In 1980, Peroutka and Snyder proposed that a common feature of chronic, but not acute, exposure to antidepressants was a down-regulation of 5-HT_2 binding sites in the brain. Post-mortem studies revealed a marked increase in

the density of frontal cortex 5-HT_2 receptors in patients who were diagnosed with major depression or had committed suicide (Arango et al., 1990; Arora and Meltzer, 1989; McKeith et al., 1987; Yates et al., 1990). Moreover, there are higher levels of 5-HT_{2A} receptors on platelets of suicidal depressed individuals, when compared to normal or non-suicidal subjects (Biegon et al., 1990; Pandey et al., 1990).

Following chronic treatment with antidepressants there is a marked decrease in 5-HT-stimulated inositol phosphate (IP) formation in rat cortex (Kendall and Nahorski, 1985). The 5-HT-stimulated IP formation is mediated via the 5-HT_{2A} receptor (Conn and Sanders-Bush, 1987). Finally, in rats, chronic antidepressant treatment reduces wet dog shakes, a behavior mediated by the activation of 5-HT_{2A} receptors (Eison et al., 1991).

Taken together these results suggest that chronic exposure to antidepressants modifies 5-HT responses. However there are several lines of evidence indicating that modification of the 5-HT receptor system may not be the only important event mediating depressive onset and, later, responses to therapy.

First, there appears to be a functional link between 5-HT, NE and dopamine that is evident following 5-HT depletion. Thus, cortical neurons exhibit decreased responsiveness to microiontophoretically applied NE and dopamine following

5-HT depletion (Ferron et al., 1982; Janowsky et al., 1982). Second, there appears to be a link between 5-HT and GABA which is also implicated in the therapeutic effectiveness of antidepressants (Blier et al., 1987). There is a 40% down-regulation of benzodiazepine binding in the brain following chronic treatment with buspirone (Gobbi et al., 1991). Third, ECT up-regulates 5-HT_{2A} binding which is in contrast to the usual observation with chronic exposure to antidepressants (Sanders-Bush et al., 1990; Sanders-Bush, 1990; Stockmeier and Kellar, 1986; Vetulani et al., 1981).

1.7 Rationale For Experiments:

Following this review on the involvement of 5-HT in depression, it is clear that there is an alteration of the 5-HT system that is manifested by an increase in 5-HT neurotransmission mediated by an increase in 5-HT_{2A} receptor activation. More specifically, there is an apparent up-regulation of the 5-HT_{2A} receptor in individuals who suffer from depression. Assuming that this up-regulation of the 5-HT_{2A} receptor is important, the treatment of patients with depression should involve a reduction of 5-HT_{2A} receptor function which would restore the brain to a homeostatic state. The hypothesis of this research is that following chronic, but

not acute, treatment with antidepressants or a course of ECT the 5-HT_{2A} receptor is functionally down-regulated. This would support the importance of down-regulating the 5-HT_{2A} receptor in reversing the effect of a depressive episode and in mediating the therapeutic response.

Previous studies examining the role of 5-HT_{2A} receptors in depression including biochemical and behavioral measurements or binding studies have not fully addressed the functional role of 5-HT_{2A} receptors in depression. Biochemical tests examining IP turnover measured IP accumulation 60 min after application of agonists (Kendall and Nahorski, 1985), whereas 5-HT_{2A} receptors are rapidly desensitized (Rahman and Neuman, 1993a). Therefore, measurement of IP accumulation at 60 min would not reflect activity of the undesensitized receptor. Wet dog shakes reflect 5-HT_{2A} receptor activity in the brainstem and not the frontal cortex (Bedard et al, 1977). The 5-HT_{2A} receptors located in the frontal cortex are the receptors up-regulated in depressed individuals. Thus, effects on behaviors mediated by 5-HT_{2A} receptors in the brainstem are not relevant. Finally, binding studies lack the ability to ascertain whether the 5-HT_{2A} receptor remains functional following chronic antidepressant treatment (Wamsley et al., 1987).

In the present study the functionality of 5-HT_{2A} receptors in the frontal cortex is directly assessed in brain slices using grease-gap methodology (Harrision

and Simmonds, 1985).This method has been employed to assess responses mediated by various G-protein coupled receptors including the 5-HT_{2A} receptor (Rahman and Neuman, 1993). Application of 5-HT alone does not provoke a response (Rahman and Neuman, 1993). However, N-methyl-D-aspartate induces a depolarization of cortical neurons and 5-HT enhances this depolarization via an action mediated through the 5-HT_{2A} receptor (Rahman and Neuman, 1993). Thus, changes in 5-HT_{2A} function can be readily determined following both acute and chronic administration of antidepressants or following the administration of ECT.

Chapter 2

Methods and Materials

2.1 Animals:

All procedures involving animals were in accordance with the guidelines of the Canadian Council of Animal Care and the Institutional Animal Care Committee of Memorial University.

Male Sprague Dawley rats, 150 to 350g, were purchased from the Animal Care Facility. The animals were housed at the animal care unit and kept on a 12 hour light cycle with controlled humidity and temperature. Food (Prolab Rat Chow) and water were provided *ad libitum*.

2.2 Wedge Preparation and Recording:

Wedges from sensorimotor cortex were prepared with minor modifications, as described by Harrison and Simmonds (1985). Rats were anaesthetized with 20% urethane (1.5mg kg^{-1} i.p.) and killed with a heavy blow to the base of the skull. The brain was rapidly removed and placed in ice cold (<5°C) modified artificial cerebrospinal fluid (M-ACSF). The brain was transferred to moist filter paper lying on a petri dish containing ice. The brain was blocked and fixed at its caudal end to a block using cyanoacrylate glue (Instant Crazy Glue). Coronal slices 500μm thick were cut at 0-4°C using a Viboslicer (Campden Instruments Ltd., U.K.). Slices were transferred to an incubation chamber containing cooled M-ACSF and bubbled with 95% O_2/ 5% CO_2. Five slices were retained starting slightly anterior to bregma (Paxinos and Watson, 1986) and proceeding caudally. Following 30 min of incubation, allowing the cooled M-ACSF to reach room temperature (20-24°C), the medium was replaced with ACSF.

After an additional 60 min incubation, a slice was transferred to a petri dish containing ACSF. One wedge of sensorimotor cortex (approximately 1.5 mm wide at pial surface and 1 mm wide at corpus callosum) was cut from a slice and

mounted in a two compartment recording bath (Harrison and Simmonds, 1985). Only one wedge of sensorimotor cortex from the same dorsal-lateral position was used from each hemisphere. The region of the wedge containing cell bodies was isolated electrically from corpus callosum and projecting axons by means of a grease seal (high vacuum silicone grease, BDH) (Fig 1). The two compartments were perfused separately at 2 ml/min via a peristaltic pump (Masterflex Pump, Cole Palmer, USA). Application of NMDA to the cell body/dendrite containing compartment resulted in a depolarization which was recorded with respect to the corpus callosum using Ag/AgCl electrodes embedded in 3% agar containing 1M NaCl. The electrodes were connected to a high impedance amplifier (3db down at 1 Hz) and the recorded potential displayed on a Kipp and Zonnen chart recorder.

2.3 Composition of ACSF

ACSF had the following composition (mM): NaCl 126, KCl 3.5, $CaCl_2$, 2, $MgCl_2$, 1.3, NaH_2PO_4, 1.2, $NaHCO_3$ 25, glucose 11, myo inositol 5. In M- ACSF,

Figure 1: Schematic diagram of cortical wedge postioned in two compartment recording chambers (Rahman, 1994). The stippled area on cortex is the grease seal separating the two compartments. Drugs were only applied to the side labled cortex. C.C. Corpus Callosum. See text for details.

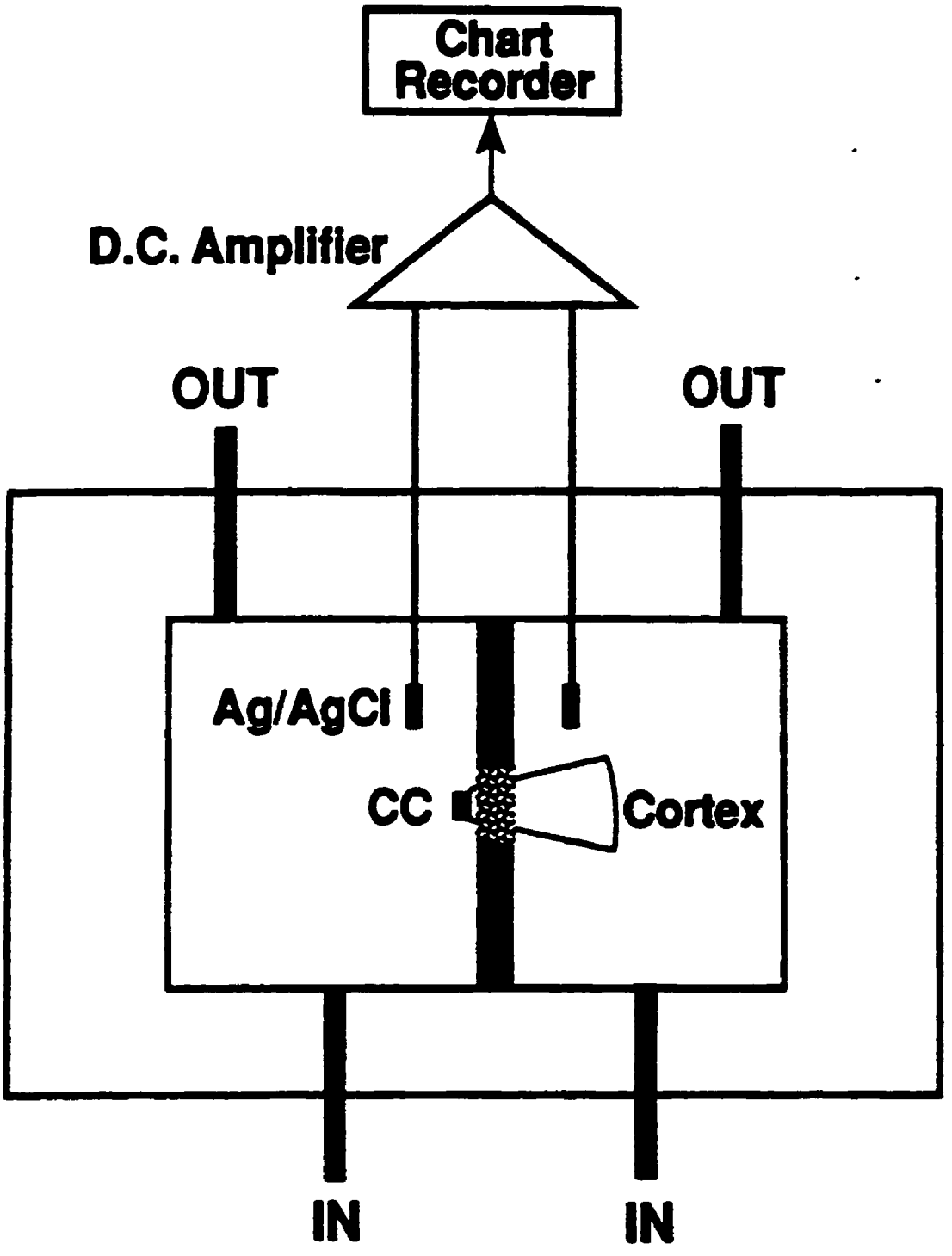


Chart Recorder
D.C. Amplifier
OUT
OUT
Ag/AgCl
CC
Cortex
IN
IN

NaCl was replaced by iso-osmotic sucrose and no myo inositol was present. (Aghajanian and Rasmussen, 1989). ACSF was aerated with 95% O_2/5% CO_2 and had a pH of 7.4.

2.4 Drug Application:

Both 5-HT and NMDA were dissolved in ACSF and applied via a 3 way valve system. NMDA applications were controlled by a computer program such that NMDA was applied for 2 min at 20 min intervals. Previous work has shown that 50 μM NMDA yielded the optimal facilitation when co-applied with 5-HT (Rahman and Neuman, 1993) and this concentration was used throughout the study. Co-application of 5-HT and NMDA was achieved by manually switching the valve for 2 min. 5-HT was diluted in ACSF just prior to application to reduce oxidation. The enhancement induced by 5-HT exhibits long term desensitization (Rahman and Neuman, 1993a). Therefore, 5-HT was administered once to each wedge. Drug concentrations were calculated as a salt. Stock solutions were kept frozen until use.

2.5 Antidepressant Administration:

Antidepressants were delivered by osmotic mini-pumps (Alzet, Palo Alto, CA) at doses comparable to those reported in the literature. Antidepressants examined were imipramine, 10 mg/kg, mianserin, 2 mg/kg and fluoxetine, 10 mg/kg. Imipramine and mianserin were dissolved in saline and fluoxetine was dissolved in dimethly sulfoxide (DMSO). Antidepressant treatment was acute (2 days) or chronic (14 days). Control groups consisted of 14 day treatment with vehicle (saline or DMSO) to insure that simply implanting the osmotic mini-pump did not alter the response to 5-HT. The dose delivered by the pump was based on a final weight of 300 grams for both acute and chronic treatment groups The osmotic pump was removed and one day was allowed for washout before the animal was sacrificed and cortical slices prepared.

2.6 Osmotic Mini-Pumps:

Using sterile procedures osmotic mini-pumps (Alzet, Palo Alto, CA) were filled with appropriate solutions and then re-weighed to insure that the pump was

properly filled. After filling the pump was warmed to 37 °C in a vial containing saline for four hours prior to implantation to allow the pump to start pumping before implantation.

Pump implantation was accomplished in a rat anaesthetized with sodium pentobarbital (60 mg/kg). An incision was made along the dorsal neckline and a subcutaneous pocket was made between the shoulders. The osmotic pump was placed in the pocket and the incision was sutured (4-0 Sofsilk Sutures). The animal was placed under a heat lamp and allowed to recover. Following recovery the rat was returned to the animal care facility.

Following either 2 or 14 days the animal was again anaesthetized and the osmotic pump removed. All treatment groups were re-weighed following the removal of the pump and exhibited normal weight gain over the time course of treatment with the antidepressant (see table 2 at end of results). The animal then was returned to the animal care facility for one more day prior to preparation of slices and recording.

2.7 Electroconvulsive Treatment (ECT):

Animals were lightly anaesthetized with sodium pentobarbital (60mg/kg) prior to ECT. After placing two clips on the animal's ears, a 100 volt A.C shock was administered for a duration of 1.5 sec. This was sufficient to induce tonic or tonic-clonic seizure activity. Treatments were administered every three days over 15 days. One day following the last treatment 5-HT_{2A} receptor functionality was examined. For the sham group the animals were anaesthetized and the clips were applied but no shock was administered.

2.8 Data Analysis :

Drug responses were quantified using the amplitude of the agonist-induced depolarization. Each response to the co-application of NMDA and 5-HT was normalized to percent of control, i.e. [(Treatment/Control) x 100]. The data were then log-transformed for analysis. This is necessary as the untransformed data are not normally distributed (Rahman and Neuman, 1993a). Multiple planned comparisons were analyzed by one way analysis of variance (Instat, Graph Pad

Software) followed by the Bonferroni test if the F value was significant. Data are presented as the antilog of the geometric mean. The standard error of the mean of the logarithmic data is not symmetrical when transformed so the larger value was used. Differences between means with "p" values less than 0.05 were considered significant.

2.9 Drugs:

The following drugs and chemicals were used: 5-hydroxytryptamine bimaleate (5-HT), N-methyl-D-aspartate (NMDA) and dimethyl sulfoxide (DMSO) (Sigma); fluoxetine hydrochloride (gift from Eli Lilly); imipramine hydrochloride and mianserin hydrochloride, (Research Biochemicals Inc.).

Chapter 3

Results

3.1 Effects of 5-HT on NMDA Depolarization:

Co-application of 5-HT with NMDA enhances the NMDA induced depolarization (Mally et al., 1991; Nedergaard et al., 1986; Rahman and Neuman, 1993; Reynolds et al., 1988). This enhancement is mediated via the 5-HT_{2A} receptor (Rahman and Neuman, 1993). Application of 5-HT alone does not result in a depolarization (Rahman and Neuman, 1993). In keeping with previous studies, co-application of 5-HT (30μM) and NMDA (50μM) for 2 minutes resulted in a depolarization significantly larger in amplitude than the NMDA control (Fig 2). The NMDA response returned to control level following a 20 min wash.

Construction of a concentration-response curve for 5-HT yielded a biphasic curve in which the facilitation increased from 10 to 30 μM 5-HT, but was reduced at 50 μM (Fig 3). The extent of the facilitation and the biphasic nature of the

Figure 2: 5-HT facilitates the NMDA induced depolarization of rat cortical neurons. Agonist application is for 2 minutes which is indicated by box under record. Note that NMDA depolarization returns to control magnitude following 20 minute washout. Note the delay between drug application and response results from the dead volume of the bath and associated tubing.

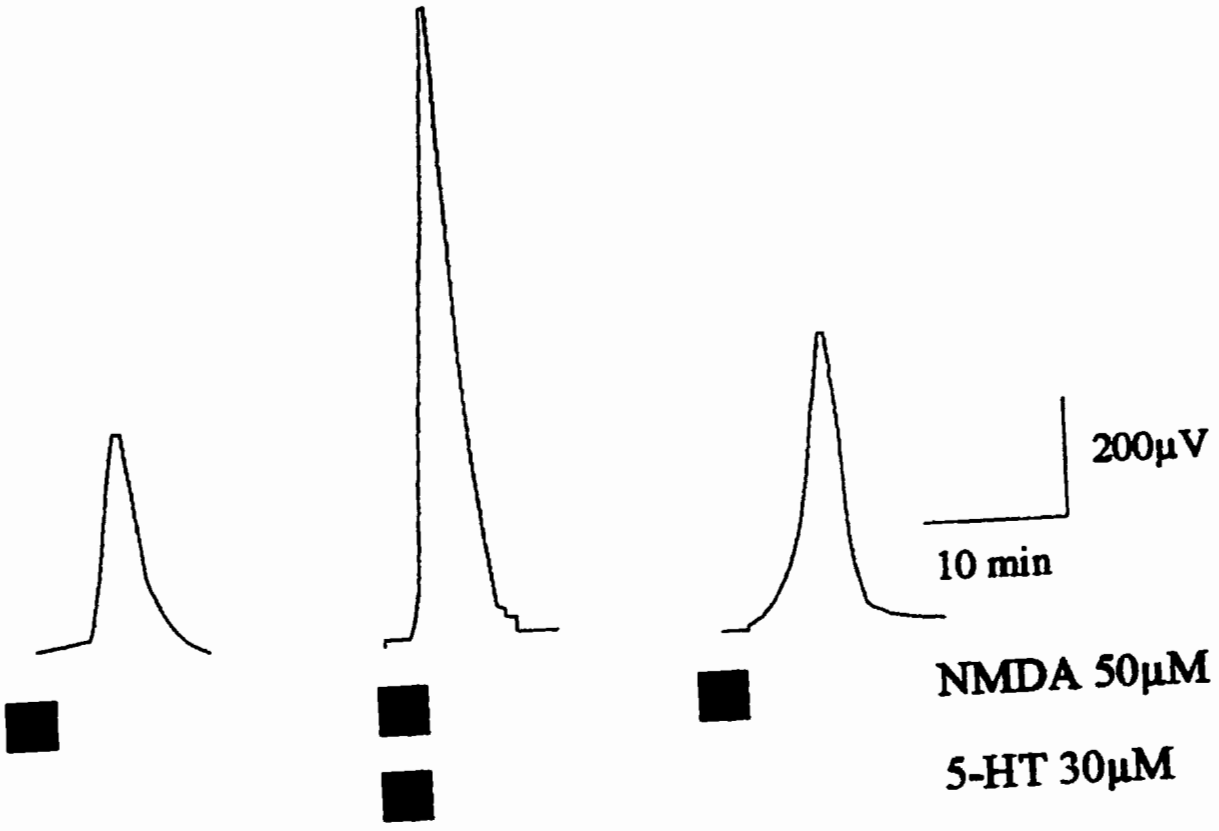
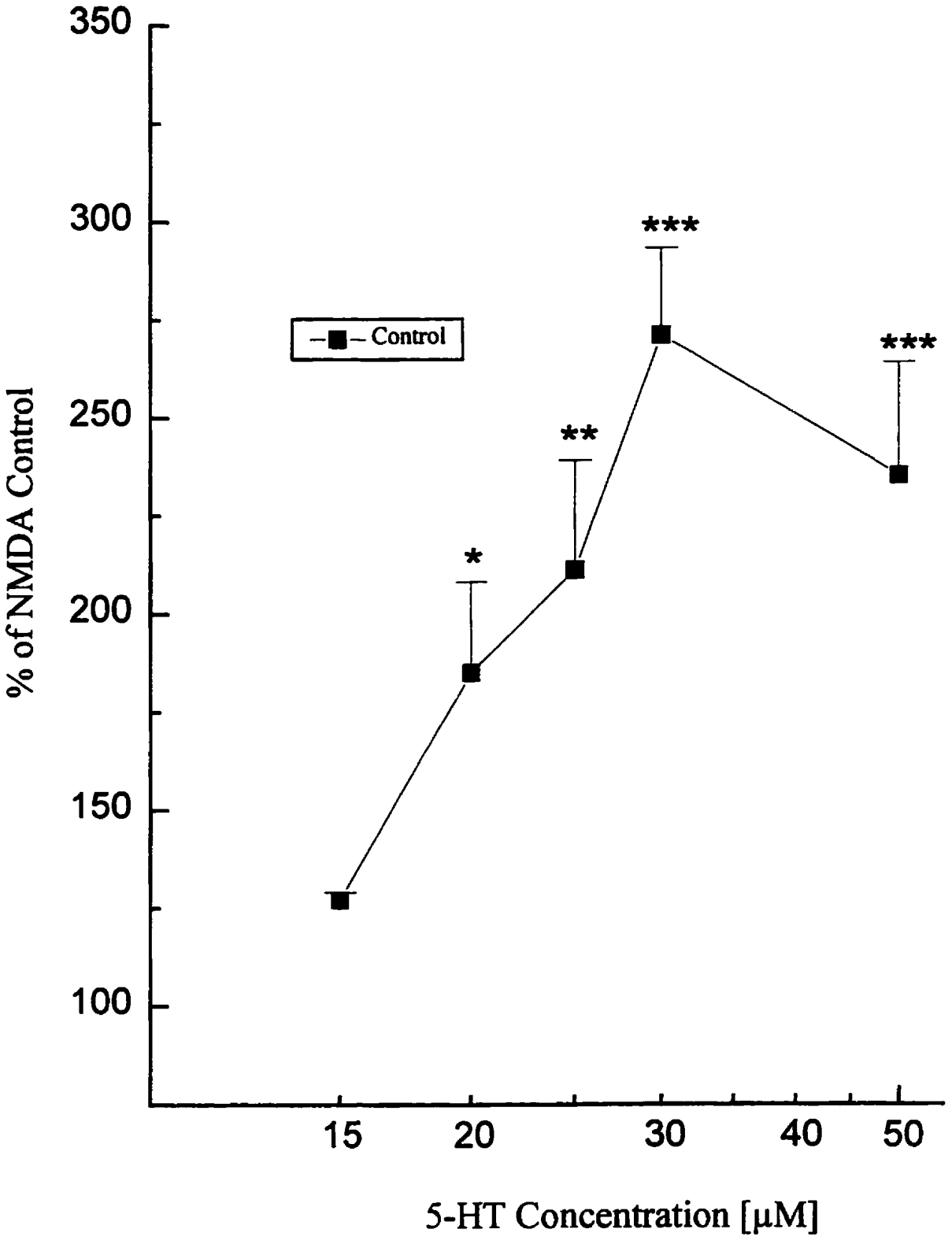

200µV
10 min
NMDA 50µM
5-HT 30µM

Figure 3: Magnitude of the 5-HT induced facilitation of NMDA depolarizing response is concentration dependent. Concentration-response relationship for 5-HT was determined at fixed test concentration of NMDA (50μM). 3 to 15 wedges (animals, n=20) were used for each point. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, treatment vs. control. The abscissa is logarithmic scale


350
300
250
200
150
100
% of NMDA Control
Control
*
**
***
***
15
20
30
40
50
5-HT Concentration [μM]

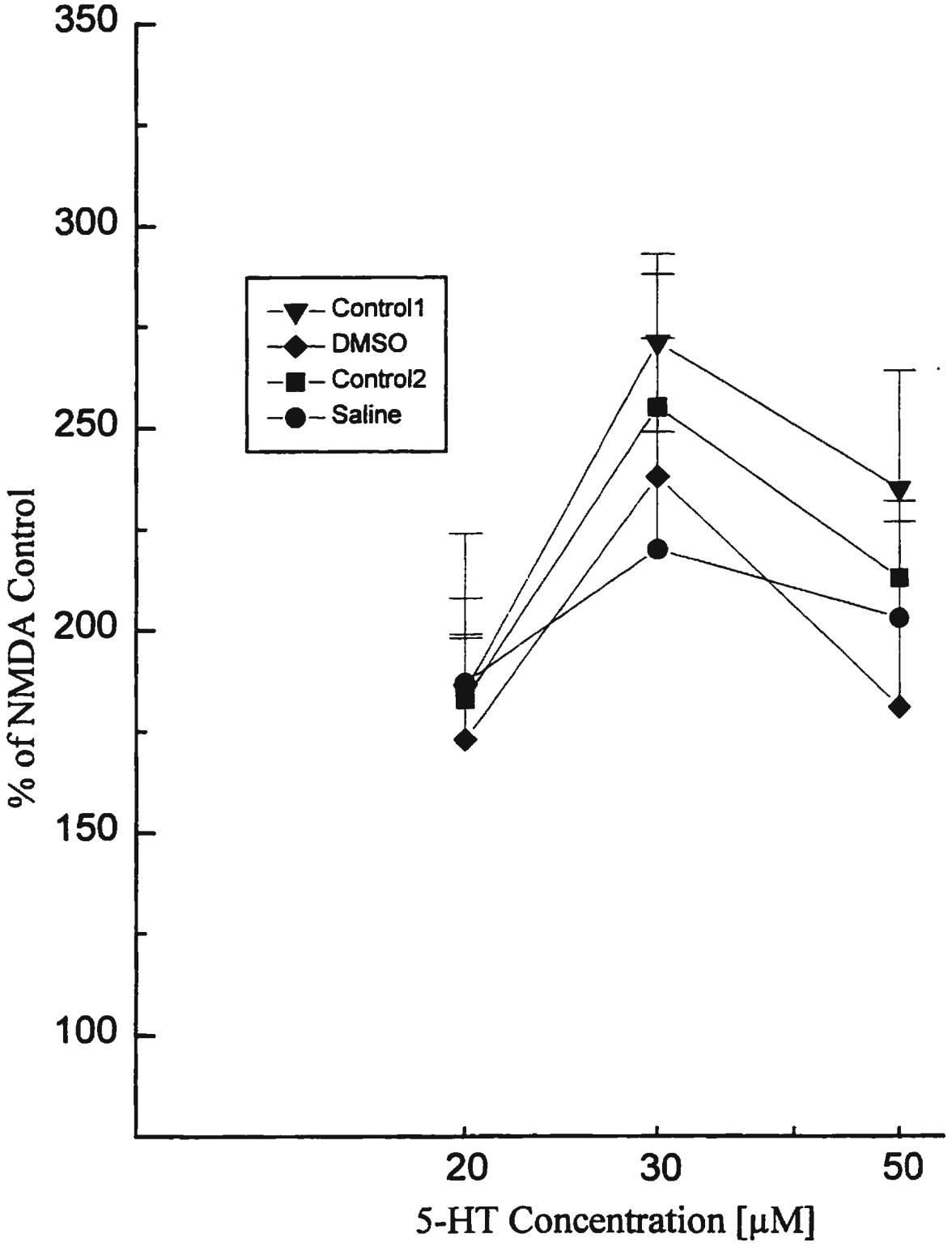
concentration-response relationship is consistent with previous observations (Rahman and Neuman, 1993). The downward shift in the curve at elevated concentrations of 5-HT reflects desensitization of the 5-HT_{2A} receptor (Rahman and Neuman, 1993a).

3.2 Imipramine:

Since imipramine was diluted in saline and administered by osmotic pump, experiments were conducted to examine the effect of the pump and vehicle alone over a 14 day administration. The results showed that over a selected 5-HT concentration range (20 μM to 50 μM) there was no significant difference between exposure to 14 days of saline or the matched controls (Fig 4). The other vehicle used in this study, DMSO, also had no significant effect following 14 day exposure (Fig 4). Since no significant difference was found between the two vehicle groups and the control group the data were combined to construct a new concentration-response control curve labeled control 2 (Fig 4). This control was used for comparison with imipramine and the other drugs used in this study.

In slices of cortex prepared from animals treated for 14 days with

Figure 4: 5-HT enhancement of the NMDA induced depolarizations is not signignificantly altered by 14 days exposure to osmotic mini-pumps filled with DMSO or Saline. Combination of untreated control (3 to 15 wedges) and vehicle groups (4 wedges for each vehicle) were used in the construction of a new concentration-response curve (Control 2). 3 to 23 wedges were used in new concentration-response curve titled Control 2 which is used as comparision to NMDA facilitation by 5-HT following antidepressant exposure. 5 to 7 animals were used in vehicle groups. Abscissa is logarothmic scale.

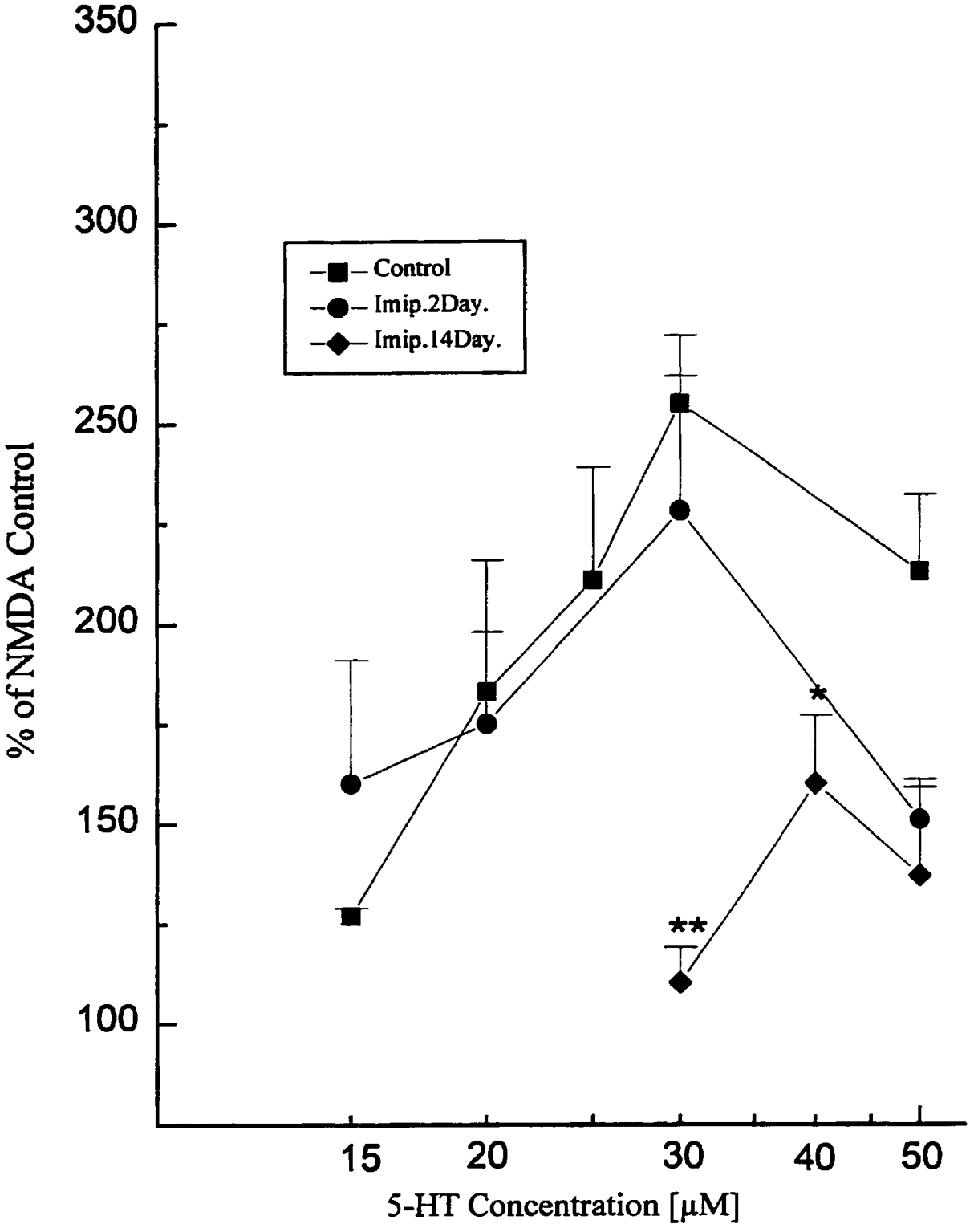

350
300
250
200
150
100
% of NMDA Control
Control1
DMSO
Control2
Saline
20
30
50
5-HT Concentration [μM]

imipramine there was a significant reduction observed in the 5-HT facilitation of NMDA responses from imipramine treated animals compared to that of controls. Exposure to imipramine for 14 days elicited a dramatic shift to the right and a lower maximum of the 5-HT concentration-response relationship (Fig 5).

Despite the lower maximum and shift to the right the biphasic nature of the concentration-response relationship was maintained. In contrast to chronic treatment, imipramine treatment for 2 days did not shift the 5-HT concentration -response relationship or significantly lower the maximum response (Fig 5).

Imipramine has been shown to reduce 5-HT_2 binding following chronic treatment, but not acute treatment (Peroutka and Synder, 1980). Thus, modification of the response to 5-HT might simply reflect a change in available 5-HT_{2A} receptors.

Figure 5: Fourteen, but not two day, treatment with imipramine shifts the concentration response relationship for the facilitation of NMDA responses induced by 5-HT. 3 to 5 wedges were used for each point. **, $p<0.01$.(significant from matched control) *, $p<0.05$. (significant from baseline). 4 to 6 animals used in imipramine group. Abscissa is logarithmic scale.

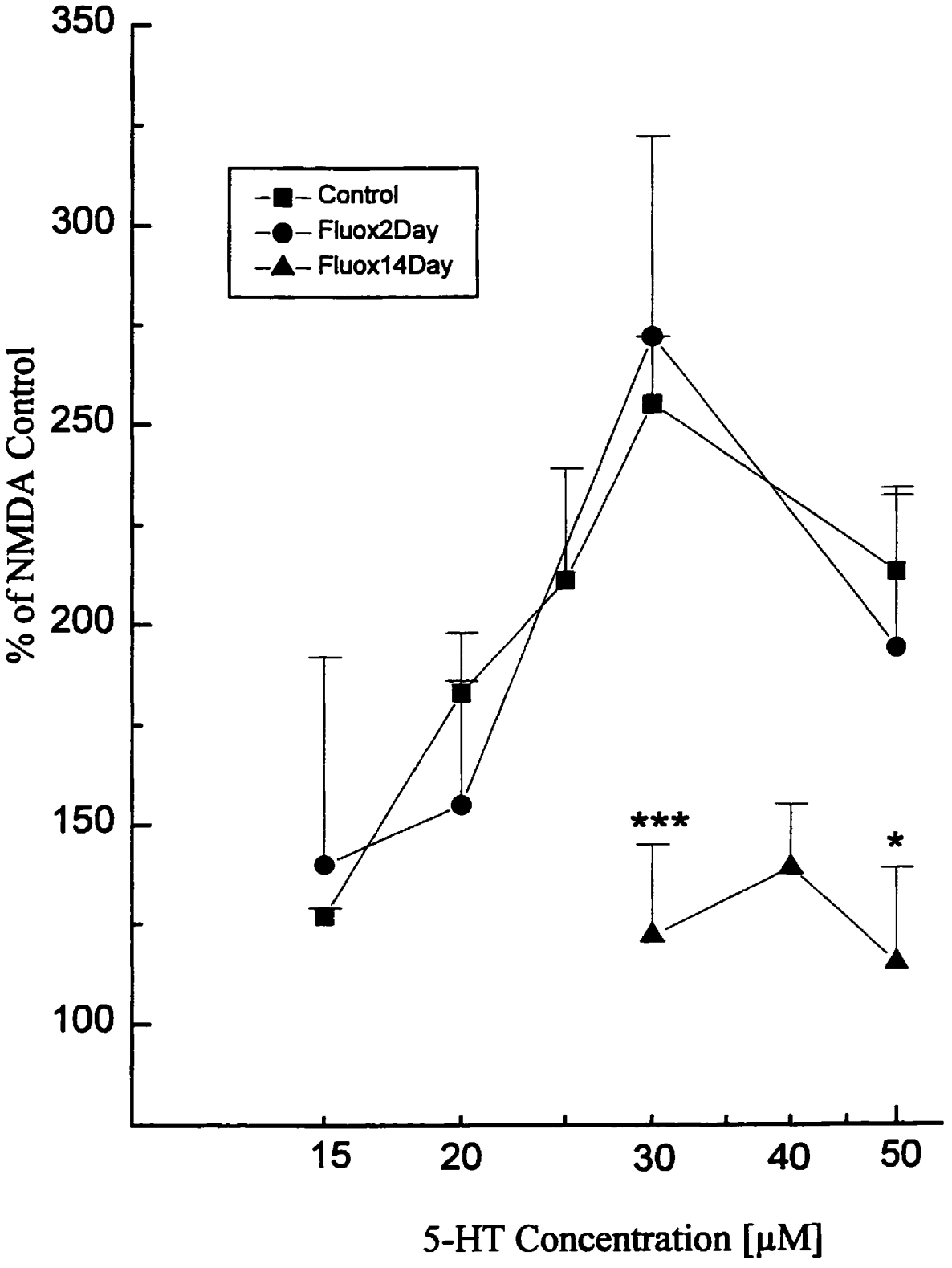

350
300
250
200
150
100
% of NMDA Control
Control
Imip.2Day.
Imip.14Day.
*
**
15
20
30
40
50
5-HT Concentration [μM]

3.3 Fluoxetine:

Not all antidepressants alter the binding to 5-HT_{2A} receptors as does imipramine. If down-regulation of functional 5-HT_{2A} receptor activity is important for mediating the therapeutic response of antidepressants then antidepressants which fail to significantly alter 5-HT_{2A} binding should still be effective in down-regulating functional activity at the 5-HT_{2A} receptor. Fluoxetine is one of the most commonly prescribed antidepressants and yet in binding studies it produces either small decreases or no effect on binding at 5-HT_{2A} receptors (Fuxe et al., 1983). Fluoxetine was examined using the same paradigm as used for imipramine.

Slices of cortex prepared from animals treated for 14 days with fluoxetine exhibited a profound shift to the right and a significant reduction in the maximum of the 5-HT concentration-response relationship (Fig 6). Thus, the response to fluoxetine resembled the response to imipramine despite the fact that fluoxetine has little effect on 5-HT_{2A} binding (Fuxe, 1983). The response to 30 μM and 50 μM 5-HT following chronic fluoxetine exposure was significantly different from that of their matched controls (Fig 6). As well, none of the 5-HT concentrations employed resulted in a significant enhancement of the NMDA response after fluoxetine exposure (Fig 6).

Figure 6: Fourteen day treatment with fluoxetine, but not two days shifted the concentration response relationship for the facilitation of NMDA responses induced by 5-HT. 5 to 8 wedges were used for each point. *, $p<0.05$, ***, $p<0.001$. Significant from matched controls (control curve 2). 5 to 6 animals were used in fluoxetine group. Abscissa is logarithmic scale.


350
300
250
200
150
100
% of NMDA Control
Control
Fluox2Day
Fluox14Day
***
*
15
20
30
40
50
5-HT Concentration [μM]

In contrast to chronic treatment, fluoxetine treatment for 2 days did not shift the 5-HT concentration-response relationship or result in the lowering of the maximum response (Fig 6). The washout time was 24 hours after the pump was removed in both the acute as well as the chronically treated rats. Thus, the shift in the concentration-response curve observed in chronically treated animals does not represent a residual action of fluoxetine on 5-HT uptake since the curve for acutely treated animals did not shift.

Since the functionality of the 5-HT_{2A} receptor was assessed by examining enhancement of the NMDA response it remains possible that the NMDA receptor complex was altered by chronic antidepressant treatment (Nowak et al., 1993) and not modification of signal transduction related to the 5-HT_{2A} receptor. However, neither fluoxetine nor imipramine exposure altered the amplitude of the NMDA response when compared to the NMDA response in untreated animals (Table 1). This suggested that the NMDA receptor was not altered by chronic exposure to the imipramine and fluoxetine.

Furthermore, in a few wedges, muscarinic facilitation of NMDA responses was examined following chronic fluoxetine treatment (Payne and McNeil, unpublished) and did not appear to differ from that reported previously (Rahman and Neuman, 1993). Therefore, effects observed following the exposure to

Table 1: NMDA (50 μM) Depolarization Following Treatment with Imipramine or Fluoxetine.

Treatment	Size (mV)±S.E.	N
Untreated Control	3.9±0.4	62
2 Day Imipramine	3.4±0.8	19
14 Day Imipramine	3.8±0.6	13
2 Day Fluoxetine	3.1±0.5	24
14 Day Fluoxetine	4.2±0.9	22

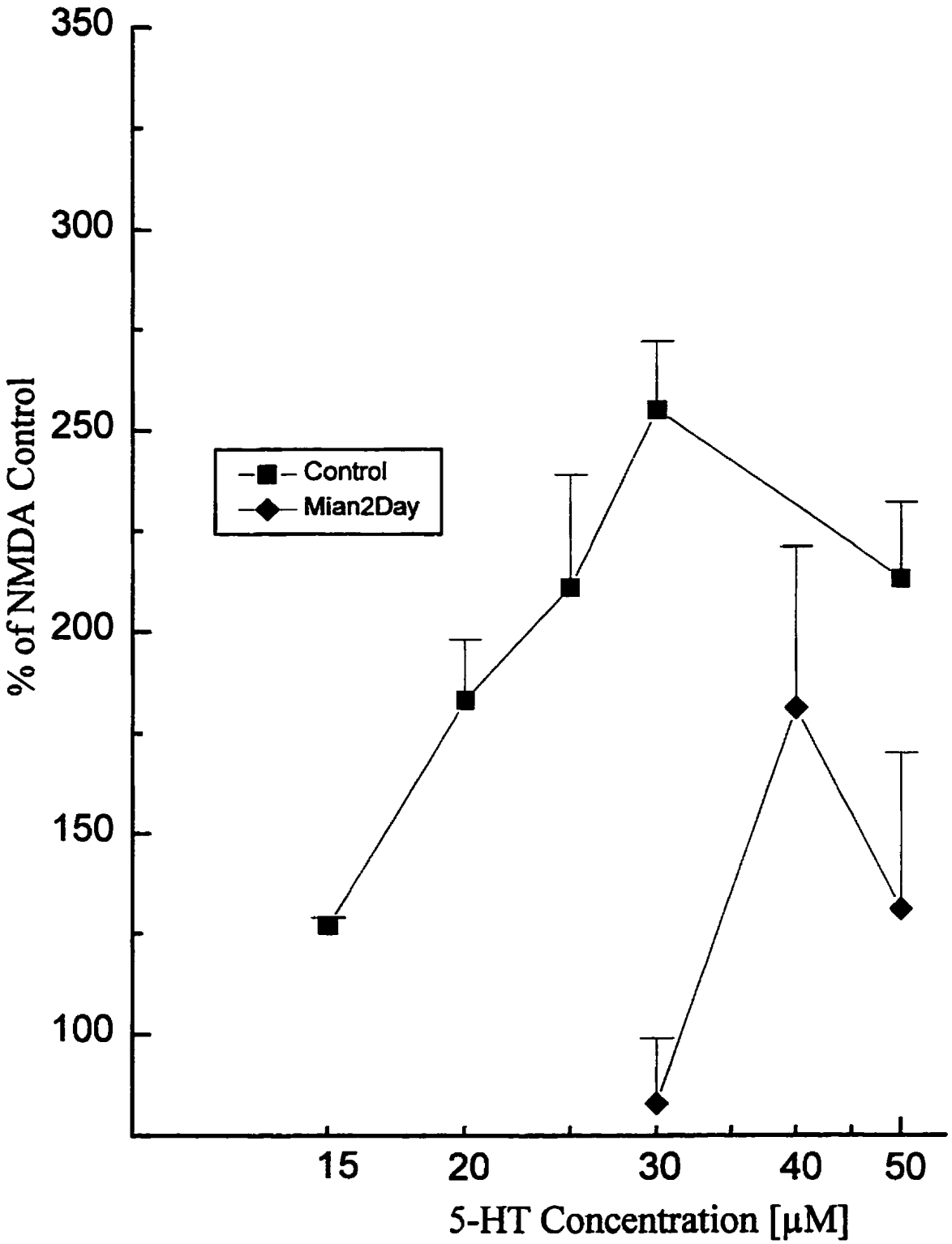
fluoxetine and imipramine are thought to result from alterations of 5-HT_{2A} receptor signal transduction and not from changes in the NMDA receptor complex.

3.5 Mianserin:

Mianserin is an antidepressant which antagonizes the action of 5-HT (Maj et al, 1978 ; Vargaftig et al., 1971) and has a high affinity for the 5-HT_2 binding site (Blackshear et al., 1981; Peroutka and Snyder, 1981). Previous studies with mianserin showed a rapid down-regulation of 5-HT_2 receptor function following exposure (Blackshear and Sanders-Bush, 1982). Mianserin, like other antidepressants, requires 14 to 21 days before effectively reducing the symptoms of depression (Blackshear and Sanders-Bush, 1982). Thus, it was important to assess whether mianserin rapidly down-regulated cortical 5-HT_{2A} function as measured in the present paradigm.

In slices of cortex exposed to mianserin for two days there was a dramatic shift to the right of the concentration-response relationship and a significant lowering of the maximum facilitation (Fig 7). Indeed, co-application of 5-HT, 30

Figure 7: Failure of 5-HT to significantly enhance the NMDA response following acute treatment with minaserin. Four wedges were used for each point (animals, n=5). None of the responses to 5-HT in tissue prepared from mianserin exposure were significantly different from control. Abscissa is logarithmic scale.


350
300
250
200
150
100
% of NMDA Control
Control
Mian2Day
15
20
30
40
50
5-HT Concentration [μM]

μM, to 50 μM with NMDA following 2 day exposure to mianserin was not significantly different from the NMDA control response (Fig 7). In fact the response to 30 μM 5-HT was lower than the control, although not significantly. This may reflect activation of another 5-HT receptor subtype and will be discussed later. As in the case of imipramine and fluoxetine, treatment with mianserin did not significantly alter the response to NMDA compared to the untreated control.

The results with mianserin are consistent with the observations of Blackshear and Sanders-Bush (1982) who observed a reduction in 5-HT_2 binding sites following acute exposure to mianserin. The results also suggest that there is a direct modification of 5-HT_{2A} receptor signal transduction rather than a change in NMDA receptor function.

3.6 Electroconvulsive Treatment:

ECT is effective in reducing the symptoms of depression (Fink, 1990) and is unique in that it reportedly increases 5-HT_2 binding sites following a course of shocks over 15 days (Sanders-Bush et al., 1990). Since an increase in 5-HT_{2A} activity does not fit with the down-regulation of 5-HT_{2A} receptor activity observed

with imipramine, fluoxetine or mianserin it was important to assess whether ECT had any effect, functionally, on the $5\text{-}HT_{2A}$ receptor following chronic administartion.

In cortical slices prepared from animals exposed to 5 episodes of ECT administration over 15 days, no shift was observed in the concentration-response relationship or in the maximum facilitation induced by 5-HT compared to that of the sham group (Fig 8). Comparison of the NMDA response in treated versus sham animals revealed no significant difference.

Figure 8: 5-HT facilitation following ECTor Sham treated animals for 15 days(5 treatments). 4 to 6 wedges were used for each point (animals n=4). Abscisssa is logarithmic scale.

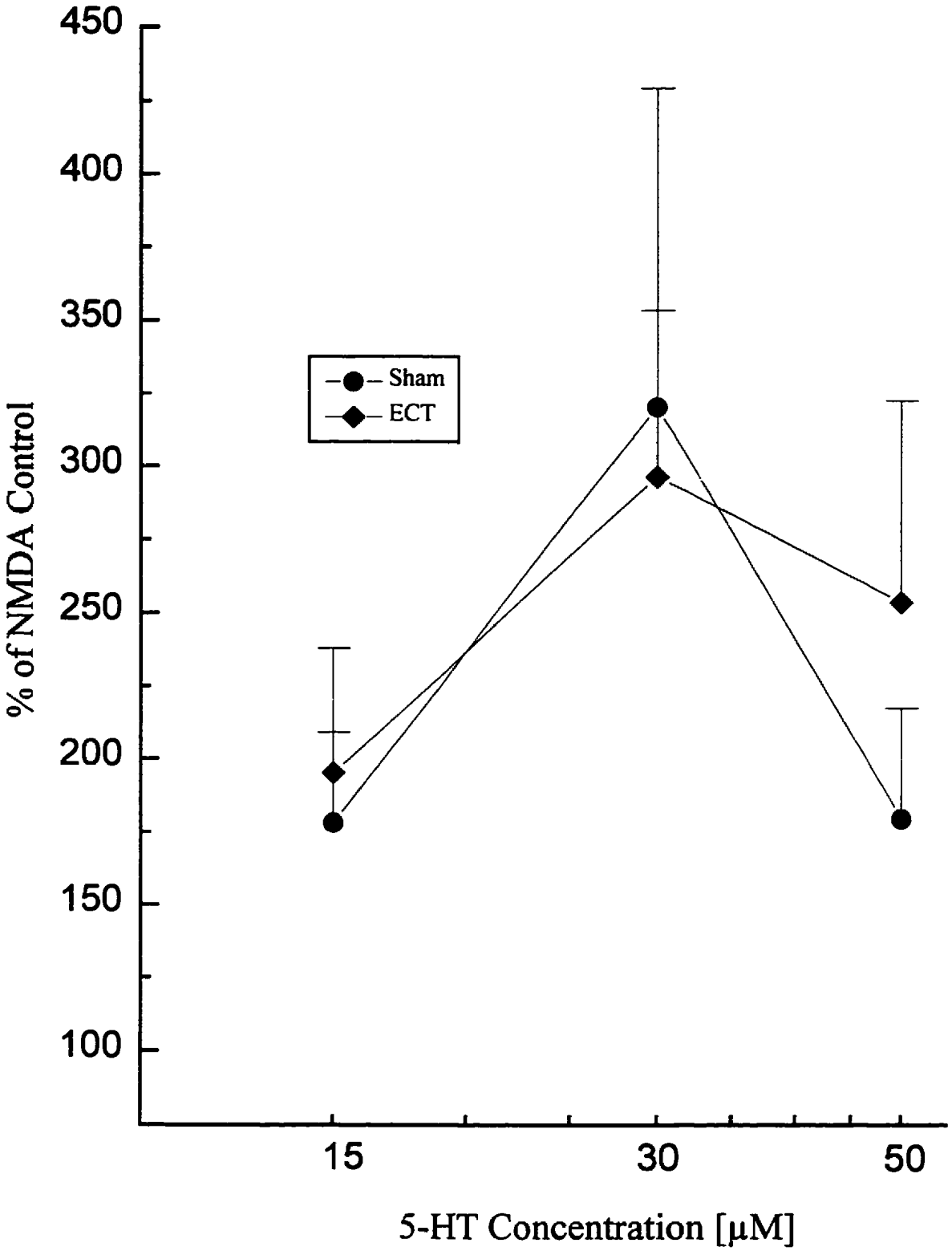

450
400
350
300
250
200
150
100
% of NMDA Control
Sham
ECT
15
30
50
5-HT Concentration [μM]

Table 2: Weight gain in animals following chronic exposure to imipramine, fluoxetine, saline or DMSO.

Treatment	Start(g)± SEM	Final (g)± SEM	Average Weight Gain(g)±SEM
Fluoxetine (10mg/kg)	172±18	272±21	101±5
Imipramine (10mg/kg)	114±4	227±7	112±8
Saline	190±21	294±19	104±4
DMSO (50%)	223±18	334±25	111±8

Chapter 4

Discussion

The aim of the present study was to assess whether chronic treatment with antidepressants and ECT functionally modulate 5-HT_{2A} receptor activity in the frontal cortex. The results show that antidepressants reduce the facilitation mediated by 5-HT_{2A} receptor activity, whereas ECT does not.

4.1 5-HT_{2A} Receptors and Antidepressants:

Peroutka and Snyder (1980) showed that chronic treatment with many antidepressants down-regulated 5-HT_2 binding. This down-regulation of 5-HT_2 binding sites following antidepressant treatment is consistent with observations that 5-HT_2 receptors are up-regulated in individuals diagnosed with depression. Therefore, antidepressants may work by counteracting the effects mediated via an up-regulation of the 5-HT_{2A} receptor that is found in individuals diagnosed with depression.

Results from the present study show that following chronic exposure to imipramine or fluoxetine, 5-HT induced facilitation of the NMDA depolarization is reduced. The apparent reduction in 5-HT_{2A} functional activity following chronic treatment with imipramine or fluoxetine cannot be attributed to residual drug action as the animals had a 24 hour washout period prior to sacrifice and the tissue was washed extensively before testing. Moreover, no loss of 5-HT activity was observed following acute exposure to either imipramine or fluoxetine. This suggests that down-regulation of the 5-HT_{2A} receptor following chronic antidepressant exposure occurs by 14 days and is consistent with the 14 day delayed onset of the therapeutic response exhibited by all antidepressants (Borsini, 1994; Oswald , 1972).

Although superficially similar, my results are not consistent with the observations of Peroutka and Snyder (1980) who found that chronic treatment with imipramine or fluoxetine reduced 5-HT_2 binding sites by approximately 40% and 13%, respectively. In contrast, loss of functionality of the 5-HT_{2A} receptor following chronic exposure to either imipramine or fluoxetine is substantially greater than the moderate reductions in 5-HT_2 binding sites reported by Peroutka and Synder (1980). Since a simple reduction in 5-HT_{2A} binding cannot account for my observations, a mechanism distal to the 5-HT_{2A} receptor must be involved.

Chronic antidepressant treatment alters the ligand binding properties of the glycine site located on the NMDA receptor complex (Nowak et al, 1993). Since assessment of 5-HT_{2A} functionality was obtained by measuring the 5-HT induced facilitation of the NMDA depolarization, a change in the NMDA receptor might account for the reduced facilitatory response observed with 5-HT. However, no significant difference was observed in the magnitude of the NMDA-induced depolarization between the control and the treatment groups. From this it is concluded that the decline in the 5-HT-induced facilitation of NMDA response observed following chronic imipramine or fluoxetine administration probably results in the decline in signal transduction associated with the 5-HT_{2A} receptor. Consistent with this interpretation, carbachol, acting at muscarinic receptors (Rahman and Neuman, 1993), continues to enhance NMDA responses in tissue from rats treated chronically with fluoxetine (Payne and McNeil, unpublished observations). Moreover, mianserin is known to rapidly reduce signal transduction at 5-HT_2 receptors (Sanders-Bush et al., 1987) and acute administration of mianserin significantly reduced the 5-HT induced facilitation.

The results with mianserin are quite different from those with imipramine and fluoxetine. Thus, significant down-regulation of the 5-HT_{2A} receptor was evident following acute exposure to mianserin. This acute response cannot be

attributed to a change in the NMDA response since there was no alteration in NMDA depolarization following acute exposure to mianserin. Mianserin is an antagonist at 5-HT_{2A} receptors. However, 24 hrs were allowed for drug washout after pump removal so that a persistent presence of drug is unlikely to account for loss of the response to 5-HT. The present findings are consistent with those of Blackshear and Sanders-Bush (1982) and Sanders-Bush's group (1987) who reported that mianserin rapidly decreased the number of 5-HT_2 binding sites and functionally uncoupled signal transduction at the 5-HT_{2A} receptor as measured by PI turnover. Mianserin requires 14 days before it reduces symptoms associated with depression (Borsini , 1994; Oswald et al., 1972) so that a rapid loss of 5-HT_2 activity alone is insufficient to account for its efficacy as an antidepressant.

One possible explanation for delayed onset in the therapeutic response of mianserin may be an indirect effect on 5-HT neurons in the dorsal raphe (DR). Pharmacological studies have suggested that the firing rate of 5-HT neurons located in the DR is dependent on tonic activation by noradrenergic input mediated via α_1-adrenoreceptors (Haddjeri et al., 1997; Svensson et al., 1975). Acute exposure to mianserin may inhibit excitatory 5-HT receptors located on locus coeruleus neurons or neurons excitatory to locus coeruleus neurons, which in turn would reduce the firing rate of the DR. If this proposal is correct, then only

after the DR adapts and returns to normal firing (Blier et al, 1990) would the antidepressant effect be achieved. A more detailed description of this mechanism is found in the section on 5-HT_{1A}-5-HT_{2A} receptor interaction.

4.2 5-HT_{2A} Receptors and ECT:

ECT increases the number of 5-HT_2 binding sites following chronic treatment (Fuxe et al., 1983). This is surprising since 5-HT_{2A} binding sites are reportedly increased in many, but not all, studies on depressed individuals (Arango et al., 1990; Arora and Meltzer, 1989; McKeith et al., 1987; Yates et al., 1990). In contrast to the binding studies, there was no significant change in the 5-HT enhancement following a chronic course of ECT over 15 days. Once again the present results show a dissociation between binding and functional activity (see also Wamsley). Moreover, these results suggest that functional down-regulation of the 5-HT_{2A} receptor is not necessary for antidepressant efficacy. However, the efficacy of ECT as an antidepressant treatment has been questioned. Based on meta analysis, Uebersax (1987) suggests that no clear empirical evidence exists to suggest that six weeks following treatment with ECT that there

is any difference between patients receiving ECT and those who did not. Therefore, based on my results with ECT, the role of 5-HT_{2A} receptors in the events mediating the therapeutic response can not be excluded since questions about its validity as an effective antidepressant exist.

4.3 Alterations in 5-HT_{2A} Receptor Signal Transduction:

It is well known that 5-HT_{2A} receptors are positively coupled to the phospholipase C (PLC)/ phosphoinositide (PI) hydrolysis second messenger system via a guanylate nucleotide binding protein (G-protein) (Chaung, 1989). Activation of 5-HT_{2A} receptors in turn activate PLC which metabolizes phosphoinositide 4,5- biphosphate (PIP_2) to produce two second messengers, inositol 1,4-5 triphosphate (IP_3) and diacylglycerol (DAG) (Chaung ,1989). IP_3 acting at IP_3 receptors located on smooth endoplasmic reticulum releases calcium (Taylor et al., 1991) and DAG which activates protein kinase C (PKC) (Nishizuka et al., 1991).

It has been postulated that the basis for the down-regulation of the 5-HT_{2A} receptor following chronic treatment with antidepressants occurs at target sites

distal to the 5-HT_{2A} receptor along its signal transduction pathway (Rasenick et al., 1996). Possible sites of alteration following chronic antidepressant treatment include: 1) receptor G-protein coupling; 2) G-protein expression; 3) G-protein effector coupling to PLC and; 4) effector expression (Rasenick et al., 1996). Facilitation of NMDA responses by 5-HT appears to depend on a rise in the intracellular Ca^{2+} concentration of cortical neurons (Rahman and Neuman, 1996). Thus, the loss of 5-HT_{2A} receptor activity following chronic treatment with imipramine, fluoxetine or acute mianserin exposure could result from the 5-HT_{2A} receptor becoming un-coupled from its G-protein system. This could account for the substantial reduction in functional 5-HT_{2A} receptor activity compared to moderate reductions of 5-HT_2 binding with imipramine and mianserin and little or no reduction in binding with fluoxetine. Preliminarily observations indicate that chronic fluoxetine does not alter the facilitation of NMDA responses induced by carbachol (Payne and McNeil, unpublished observations). This suggests that loss of signal transduction following antidepressant exposure is associated with the 5-HT_{2A} receptor and is not a generalized phenomenon. On the other hand I have observed a reduction in histamine H_1 receptor-mediated facilitation of NMDA responses following chronic, but not acute, exposure to fluoxetine (Payne, unpublished observation). This may again suggest an un-coupling of the

receptor from its associated G-protein.

Alterations in 5-HT_{2A} mediated responses associated with PKC, which could be important in the mechanism underlying the therapeutic response of antidepressants have been observed. PKC phosphorylates cellular proteins (Mann et al., 1995) and is associated with 5-HT_{2A} receptor-triggered signals (Nishizuka, 1988). PKC activity is significantly altered following chronic fluoxetine or desipramine exposure as well the PKC response to the 5-HT_{2A} agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Mann et al., 1995). In senescent animals, the 5-HT_{2A} receptor mediated facilitation is absent whereas the facilitation by α- adrenoceptors, muscarinic receptors and metabotrophic glutamate receptors remains intact (Rahman et al., 1995). The 5-HT_{2A} facilitation is restored following application of PKC inhibitors (Rahman et al., 1995). Following the chronic administration of antidepressants such as an SSRI, PKC activity may be reduced, thus, the inhibitory feedback of PKC on 5-HT_{2A} receptors (Roth et al., 1990) would be lost. Therefore, overstimulation of the 5-HT_{2A} receptor may result in receptor down-regulation.

4.4 5-HT_{1A}-5-HT_{2A} Receptor Interactions:

Based on results from the present study one possible mechanism mediating the therapeutic response of antidepressants is an interaction between cortical 5-HT_{1A} and 5-HT_{2A} receptors. It is well known that 5-HT_{1A} and 5-HT_{2A} receptors are co-localized on the same cortical neurons (Araneda and Andrade, 1991). Therefore, increases in 5-HT concentration would not exert any stable change in the firing rate of the postsynaptic neuron (Borsini, 1994) and a balance must be achieved between the effects mediated by each receptor (Araneda and Andrade, 1991). Stimulation of inhibitory neurotransmission in the cortex by activation of postsynaptic 5-HT_{1A} receptors may not be sufficient to reduce the enhanced 5-HT_{2A} activity found in depressed individuals.

Another aspect of chronic antidepressant treatment is the desensitization of somatodendritic 5-HT_{1A} autoreceptors 5-HT_{1A} terminal receptors which mediate the amount of 5-HT released (Blier and de Montigny, 1994). When imipramine or fluoxetine is first administered there is an increase of 5-HT in the synaptic cleft due to blockade of 5-HT uptake (Blier et al., 1994). This increased 5-HT activates somatodendritic 5-HT_{1A} autoreceptors that in part control the firing rate of the DR. DR firing is reduced and hence less 5-HT is released in the

forebrain. Over approximately 14 days the DR adapts and returns to its normal firing rate (Blier et al., 1994). Moreover, following administration of fluoxetine and imipramine, desensitization of both somatodendritic and terminal 5-HT_{1A} autoreceptors coupled with a normal firing DR results in greater 5-HT release per impulse and increased amounts of 5-HT therefore act at postsynaptic sites (Borsini, 1994). Increased synaptic 5-HT may result in chronic activation of cortical 5-HT_{2A} receptors and therefore down-regulation (Paul et al., 1988). In this state there would be a shift in the balance between the 5-HT_{1A} receptor mediated hyperpolarization and the 5-HT_{2A} receptor mediated depolarization favouring the 5-HT_{1A} hyperpolarization (Borsini, 1994).

It is proposed that the net result of all three antidepressants examined is an increase in inhibitory 5-HT neurotransmission. Some evidence for this suggestion is seen in the experiments with mianserin. Following acute administration of mianserin, facilitation of the NMDA response by 5-HT (30μM) is reduced to less than the NMDA control response. This is consistent with previous reports that (±)-8-hydroxy-dipropylaminotetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, reduces the NMDA induced depolarizations (Rahman and Neuman, 1993). Thus, the smaller facilitation following mianserin may reflect the activation of 5-HT_{1A} receptors , i.e., the balance shifts away from excitatory cortical 5-HT_{2A} receptors

towards inhibitory 5-HT_{1A} receptors. A possible explanation of why this is not observed with pretreatment with imipramine or fluoxetine is that mianserin acts directly on the 5-HT_{2A} receptor. In contrast, imipramine and fluoxetine down-regulate the receptor due to increased release of 5-HT. The later phenomenon may be insufficient to show the 5-HT_{1A} receptor effect in my paradigm.

Thus, with reduced functionality at postsynaptic 5-HT_{2A} receptors and increased 5-HT release due to desensitization of presynaptic autoreceptors or direct 5-HT_{2A} down-regulation would result in increased activity at postsynaptic 5-HT_{1A} receptors. The net result would be an increase in 5-HT inhibitory neurotransmission, reversing the excitatory deficiency mediated via an up-regulation of postsynaptic 5-HT_{2A} receptors and thereby returning 5-HT activity to the "normal" level.

Recently, a new antidepressant, BIMT 17, has begun clinical trials. BIMT 17 is novel in that it acts as both a 5-HT_{1A} agonist and 5-HT_{2A} antagonist (Borsini, 1994). It might be expected that since these actions are occurring simultaneously a faster onset of the therapeutic response would be achieved. Preliminary clinical trials with BIMT 17 demonstrate a faster onset in the therapeutic response (Peter Thompson, personal communication).

4.5 Conclusions:

Chronic antidepressant exposure to imipramine or fluoxetine or acute exposure to mianserin reduces cortical 5-HT_{2A} receptor activity as determined by a loss of 5-HT stimulated facilitation of the NMDA depolarization. This functional loss appears to reflect changes at the 5-HT_{2A} receptor and appears unrelated to a change in the NMDA receptor. Chronic ECT exposure appears to have no functional effect on cortical 5-HT_{2A} receptors. This suggests that down-regulation of 5-HT_{2A} receptors may be involved in mediating the therapeutic response and that the mechanism involved may be via enhancement of inhibitory 5-HT neurotransmission through cortical 5-HT_{1A} receptors.

4.6 Future Research:

Although this study did address the question of whether 5-HT_{2A} receptors were functionally altered following treatment with antidepressants, further experiments are needed to fully answer the question of what role 5-HT_{2A} receptors and other receptors play in depression.

1. Despite the use of antidepressants, these rats were not depressed and presumably had a normal 5-HT system prior to the administration of the antidepressant. In order to eliminate questions about the validity of the 5-HT_{2A} functional down-regulation following antidepressant exposure in normal subjects, a definitive model of depression is required for full assessment of the functional role of 5-HT_{2A} receptors.

2. There are a number of limitations associated with using cortical wedges in investigating the functionality of 5-HT_{2A} receptors. They include: 1) receptor desensitization which allows for only one agonist exposure per wedge; 2) indirect effects due to the grease-seal; 3) the number of NMDA receptors activated with each exposure of drug and; 4) the use of facilitation of NMDA responses as an

assessment of 5-HT_{2A} receptor activity. A more specific electrophysiological technique would reduce these problems.

3. It would be naive to think that only the 5-HT system is involved in the mediation of the therapeutic response to antidepressants. Thus, a careful functional assessment of the interaction of 5-HT_{2A} receptors with other receptor systems would further our understanding of the mechanisms underlying the therapeutic response of antidepressants.

4. A concentration-response curve addressing the time course of changes in 5-HT_{2A} functionality over a 14 day period would further the understanding of when 5-HT_{2A} receptor down-regulation occurs and how it relates to the onset of the therapeutic response.

5. Finally, the mechanism underlying the possible antidepressant effects of ECT need to be addressed to determine if indeed ECT is an valid antidepressant and if so what are the mechanisms that underly its action.

Chapter 5

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