CHAPTER 2
ANTIDEPRESSANT-LIKE EFFECT OF BUPROPION IN BEHAVIORAL PARADIGMS OF DESPAIR: POSSIBLE MECHANISM OF ACTION

Part-1: Involvement of nitric oxide (NO) signaling pathway in the antidepressant action of bupropion, a dopamine reuptake inhibitor

2.1.1. INTRODUCTION
The catecholamine and indoleamine hypothesis of depression (Schildkraut 1965; Berton and Nestler, 2006) spurred research into abnormalities of noradrenergic and serotonergic transmission as causes of depression. Subsequently, a dopamine hypothesis of depression was put forward (Randrup et al., 1975). Since then, multiple lines of investigations have explored the role of dopaminergic systems in depression (Kapur and Mann, 1992; Ebert and Lammers, 1997). Bupropion [(±)-α-t-butylamino-3-chloropropiophenone] also known as amfebutamone is reported to have antidepressant activity in man (Wilkes, 2006). Bupropion, an atypical antidepressant, is a potent inhibitor of dopamine reuptake with subtle activity on noradrenergic reuptake also (Cooper et al., 1980). Bupropion being a centrally acting catecholamine (dopamine and norepinephrine)-transporter blocker has shown to improve chronic fatigue symptoms (Schonfeldt-Lecuona et al., 2006). The drug is also under clinical examination for the treatment of obesity (Palamara et al., 2006) and drug addiction (Mooney and Sofuoglu, 2006). One of our earlier studies has shown that bupropion could reverse the withdrawal effects from benzodiazepine tolerance (Joshi et al., 2004).

The forced swimming and tail suspension-induced state of immobility in animals claimed to represent a condition similar to human depression (Renard et al., 2003) and amenable to reversal by
antidepressant drugs (Porsolt et al., 1977a; Kulkarni and Mehta, 1985; Steru et al., 1985; Parale and Kulkarni, 1986). These test models are based on the despair or helplessness behavior to some inescapable and confined space (Porsolt et al., 1977a; 1977b; 1978a; 1978b) in animals and are sensitive to various antidepressant drugs viz. tricyclics, selective serotonin reuptake inhibitors, dual reuptake inhibitor of serotonin and norepinephrine, monoamine oxidase inhibitors and atypicals. However, the effect of dopamine reuptake inhibitors (e.g. bupropion) is not properly standardized in these animal models. In vivo brain microdialysis studies have demonstrated that, after chronic administration, there was an enhancement of bupropion-induced increases in extracellular dopamine in the nucleus accumbens and hippocampus region (Nomikos et al., 1992; Piacentini et al., 2003). However, a recent study has cast partial doubt upon the therapeutic effect of bupropion with its dopaminergic modulation (Argyelan et al., 2005). This raises the question about the exact mechanism of action of bupropion in relieving the symptoms of depression.

L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Nitric oxide, a messenger molecule in the brain, synthesized from L-arginine by nitric oxide synthase (NOS), and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidences have shown that the reduction of nitric oxide levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal nitric oxide in the neurobiology of stress and depression (Joca and Guimaraes, 2006). Nitric oxide is also known to modulate the levels of cyclic guanosine monophosphate (cGMP) which in turn known to produce depression like state in animals (Kaster et al., 2005b). Studies have shown the possibility that the inhibition of nitric oxide synthase enzyme could be used as a strategy to enhance the clinical
efficacy of serotonergic antidepressants (Harkin et al., 2004). In one of the studies, NOS inhibitors and methylene blue increased extracellular levels of serotonin and dopamine in the rat ventral hippocampus and L-arginine has opposite effect thus showing that endogenous nitric oxide may exert a negative control over the levels of serotonin and dopamine in the hippocampus (Wegener et al., 2000). As bupropion is known to increase the levels of dopamine in hippocampus (Piacentini et al., 2003), it could be expected that nitric oxide system may play an important role in mediating its antidepressant action and further NOS inhibitors may have potentiating effect on its activity. However, the involvement of L-arginine-NO-cGMP pathway in the antidepressant activity of bupropion has not been explored. With this background, the present study attempts to explore the various behavioral and neurochemical aspects of bupropion with respect to its antidepressant activity and the participation of L-arginine-NO-cGMP pathway in the antidepressant activity of bupropion in the forced swim test in mice.

2.1.2. MATERIALS AND METHODS

2.1.2.1 Animals

Male albino mice (Laca strain) weighing between 22-30 g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh were used. For measurement of rectal temperature, male albino rats (wistar strain) weighing between 150-200 g were used. For other details, refer to Chapter 1 (1.2.1).

2.1.2.2. Forced Swim Test: Refer to Chapter 1 (1.2.2.1.)

2.1.2.3. Tail Suspension Test: Refer to Chapter 1 (1.2.2.2.)

2.1.2.4. Reserpine-induced behavioral despair in mice: The procedure was conducted according to a previously validated method in our laboratory (Kulkarni and Mehta, 1985; Shaji and Kulkarni, 1988). In
brief, reserpine (2 mg/kg., i.p.) was administered 4 h before challenging the animals to forced swim. Bupropion was administered in different doses in reserpinised mice 30 min before recording the immobility period. The immobility period was calculated for a total of six min duration.

2.1.2.5. **Body temperature in rats:** Variation in rectal temperature was recorded using a telethermometer (Yellow Springs Instrument Co., Inc. USA) by inserting the thermister probe to a depth of 2.5 cm into the rectum of rat. The rectal temperature of each animal was recorded just before drug administration and at 0, 30, 60, 90 and 120 min after administration, respectively. The temperature of each animal was recorded for 1 min. Rectal temperature recorded at 0 time served as control for each animal in addition to a separate control group being included in the study. Bupropion was administered at different doses and rectal temperature was noted at different time intervals.

2.1.2.6. **Activity monitoring in animals:** Locomotor activity (ambulations) was measured by using computerized actophotometer (IMCORP). An array of 16 infrared emitter/detector pairs (spaced at 6.5 cm intervals; beam wave length= 875 nm; distance between emitter and detector = 45 cm) measured the animal activity along single axis of motion, the digital data being displayed on the front panel meters as ambulatory movements. Briefly, after drug treatment mice were individually placed in a transparent plastic cage (30x 23x 22 cm$^3$) and mice were allowed to acclimatize to the observation chamber for a period of 2 minutes. The activity was continuously monitored for a period of 5 min. The locomotion was expressed in terms of total photobeams counts per 5 min per animal (Dhir et al., 2005).

2.1.2.7. **Measurement of biogenic amines:** Biogenic amines (dopamine, norepinephrine, and dopamine metabolite homovanillic acid) were estimated by High Performance Liquid Chromatography (HPLC) with
Chapter 2

Electrochemical Detector (ECD) by the method of Beyer et al., 2002. Waters® standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column and electrochemical detector were used. Data were recorded and analyzed with the help of Empower® software. Mobile phase consisting of 0.15 M sodium dihydrogen phosphate, 0.25mM ethylenediaminetetraacetic acid, 1.75mM 1-octane sulfonic acid, 2 % v/v isopropanol and 4 % v/v methanol (pH 4.8). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 1-100 nA. Separation was carried out at a flow rate of 1 ml/min. Samples (20 µl) were injected manually. Brain samples were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that samples were centrifuged at 24,000 g for 15 min. The supernatant was further filtered through 0.25 micron nylon filters before injecting in the High Performance Liquid Chromatography injection pump. Data were recorded and analyzed with the help of Empower® software provided by Waters®.

2.1.2.8. Drugs and treatment schedule

The following drugs were used: bupropion hydrochloride (GlaxoSmithKline Beecham, Pennsylvania, USA), reserpine hydrochloride (Sigma Aldrich, St. Louis, MO, USA), L-arginine (Loba-Chemie, Mumbai, India), methylene blue (S.D.-Fine Chem Ltd., Gujarat, India.), 7-nitroindazole (Tocris Bioscience, Missouri, USA), sildenafil (Panacea Biotec, New Delhi, India). All the drugs were dissolved in distilled water except 7-nitroindazole which was dissolved in few drops of Tween 80 and volume was made with distilled water. The doses of the drugs used were selected according to the previous studies conducted in our laboratory (Patil et al., 2005) and as reported in the literature (Harkin et al., 2004; Almeida et al., 2006). Different doses were administered intraperitoneally in a fixed volume of 1 ml/100g body weight 30 min before the animals were subjected to test. Mice were pretreated with L-arginine, a precursor of nitric oxide (750 mg/kg, i.p., a dose that produces no effect in the forced swim test), or
vehicle. Thirty min after L-arginine, bupropion (20 mg/kg, i.p., a dose active in the forced swim test) or vehicle was injected and 30 min later animals were subjected to forced swim test. In another set of experiments, we investigated the synergistic effect of bupropion (10 mg/kg, i.p., a subeffective dose) with a subeffective dose of 7-nitroindazole (25 mg/kg, i.p., a specific neuronal nitric oxide synthase inhibitor) or methylene blue (10 mg/kg, i.p., an inhibitor of nitric oxide synthase and an inhibitor of soluble guanylate cyclase). These modulators were administered 30 min before bupropion or vehicle and 30 min later challenged with forced swim test. To observe the role of cGMP in the antidepressant action of bupropion, mice received an injection of sildenafil (5 mg/kg, i.p., phosphodiesterase 5 inhibitor) or vehicle 30 min before bupropion (20 mg/kg, i.p.). Thirty min post bupropion administration the animals were subjected to forced swim test.

2.1.2.9. Statistical analysis
Results expressed as mean (s) ± S.E.M and the significance of the difference in the responses of treatment groups in comparison to the control was determined by either One Way Analysis of Variance or Two Way Analysis of Variance followed by post-hoc test wherever appropriate. The rectal temperature data were analyzed by One way ANOVA for repeated measurements. P<0.05 was considered statistically significant. 

ED50 value was calculated by using the Litchfield and Wilcoxon method (Litchfield and Wilcoxon, 1949). (Chi)² test was applied for checking the homogenous distribution of the data.

2.1.3. RESULTS
2.1. Effect of bupropion on behavioral despair in mice
Bupropion (20 mg/kg, i.p.) time dependently decreased the immobility period in forced swim test in mice with maximum effect observed at 30 min of its treatment and the effect was reduced at 60 and 120 min (Fig.2.1.1). Therefore, bupropion at different doses were administered 30 min before
recording any behavioral or neurochemical parameters. Bupropion dose-
dependently (10, 15, 20 and 40 mg/kg., i.p.) decreased immobility period
(in seconds) with respect to vehicle control group (Control: 222.60 ±
19.65, 10 mg/kg: 198.0 ± 33.47, 15 mg/kg: 97.80 ± 5.64: 20 mg/kg: 70.33
± 12.23, 40 mg/kg: 7.0 ± 0.89 ) [F=17.044, p<0.001] in forced swim test
(Table 2.1.). The ED50 value of bupropion in forced swim test was
calculated to be 18.5 mg/kg., i.p. (7.34-46.6). Similarly, bupropion
treatment (10, 15, 20 and 40 mg/kg., i.p.) resulted in the decrease of
immobility period (in seconds) with respect to vehicle control group
(Control: 194.7 ± 12.85, 10 mg/kg: 155.0 ± 14.63, 15 mg/kg: 81 ± 27.24:
20 mg/kg: 68.0 ± 10.23, 40 mg/kg: 0 ± 0 ) [F=17.044, p<0.001] in the tail-
suspension test (Table 2.1.1, Fig. 2.1.2.). Bupropion at 40 mg/kg., i.p. fully
abolished the immobility phase in animals in tail-suspension test (Table
2.1.1). The ED50 value of bupropion in tail-suspension test was found to be
18 mg/kg., i.p. (7.23-44.90).

Reserpine at 2 mg/kg produced severe depression in mice as
shown by an increase in immobility period in forced swim test as
compared to vehicle control group (Fig. 2.1.3.). Bupropion (10 mg/kg., i.p)
reversed the reserpine-induced immobility in forced swim test. A two way
ANOVA showed no effect of pretreatment [F (1,20)=1.39, p=0.256] but
significant effect of treatment [F (1, 20)=12.37, p<0.05] and pretreatment ×
treatment [F (1, 20)=23.39, p<0.001] (Fig. 2.1.3.).

Bupropion when administered at higher dose of 20 mg/kg., i.p.
reversed the reserpine-induced immobility period. A two way ANOVA
showed significant effect of pretreatment [F (1,20)=306.41, p<0.001]
treatment [F (1, 20)=2606.25, p<0.001] and pretreatment × treatment [F (1,
20)=35.24, p<0.001] (Fig. 2.1.3.).

Similarly, bupropion at a highest dose of 40 mg/kg., i.p. reversed
the effect of reserpine in forced swim. A two way ANOVA showed
significant effect of pretreatment [F (1,20)=402.51, p<0.001] treatment [F
Chapter 2

(1, 20)= 3506, p<0.001] and pretreatment × treatment [F (1, 20)=35.24, p<0.001] (Fig. 2.1.3.).

2.1.3.2. Effect of bupropion on locomotor activity in mice: Bupropion (15, 20 and 40 mg/kg., i.p.) treatment increased locomotor activity. Both ambulations [F=56.50, p<0.05] and rearing movements [F=12.69, p<0.05] were increased. The lower dose of bupropion (10 mg/kg., i.p.) did not affect the locomotor activity in mice (Fig 2.1.4.).

2.1.3.3. Effect of bupropion on rectal temperature in rats: Bupropion (20 and 40 mg/kg., i.p.) produced hypothermia at 60 and 90 min of the treatment [F=12.619, p<0.05]. The body temperature returned normal after 120 min (Fig.2.1.5.).

Fig. 2.1.1. Time-dependent reduction in immobility period by bupropion (20 mg/kg., i.p.) in forced-swim test in mice. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analysed by One Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.001 compared with 0 min time period, #p < 0.001 compared with 20 min time period.
Table 2.1.1. Comparison of ED50 values in two models of depression mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test</th>
<th>Treatment</th>
<th>Dose</th>
<th>% Reduction in immobility period</th>
<th>ED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Forced swim test</td>
<td>Bupropion</td>
<td>10</td>
<td>11.05 ± 2.35</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>50.61±3.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>68.40 ±5.56&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>96.86 ±3.44&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tail-suspension test</td>
<td>Bupropion</td>
<td>10</td>
<td>20.38 ± 9.70</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>47.61 ± 4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>64.66 ± 8.65&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>100.0 ± 0&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.05 as compared to bupropion (10 mg/kg., i.p.), <sup>b</sup>p<0.05 as compared to bupropion (15 mg/kg., i.p.), <sup>c</sup>p<0.05 as compared to bupropion mg/kg., i.p.).

![Recordings of immobility period in TS](image)

Fig. 2.1.2. Recording of immobility period and its modification in tail suspension test (TST) in mice
2.1.3. Effect of bupropion on immobility period in reserpinised mice as compared to vehicle treated group. Vehicle or bupropion were administered 30 min before the test. Data were analyzed by Two way Analysis of Variance (ANOVA) \((n = 6–8)\) followed by Dunnett’s test. *\(p<0.05\) as compared to vehicle treated group, #\(p<0.05\) as compared to reserpine treated group; ♦\(p<0.05\) as compared to bupropion (10 mg/kg., i.p.), §\(p<0.05\) as compared to bupropion (20 mg/kg., i.p.) (for treatment schedule, see text). Res: Reserpine

2.1.3.4. Effect of bupropion on brain neurotransmitter levels in mice:
When different standards of biogenic amines were run through HPLC column, the retention times for norepinephrine (NE), dopamine (DA), homovanillic acid (HVA) were found to be 1.9, 2.286, 7.932, respectively. Bupropion dose dependently (10, 20 and 40 mg/kg., i.p.) increased the concentration of free dopamine [F = 43.69, \(p<0.001\)] and its metabolite homovanillic acid [F = 17.95, \(p<0.05\)] (Fig. 2.1.6.) in the brain homogenate. Bupropion at 10 mg/kg resulted in slight increase in the dopamine levels but there was marked increase in its levels at 40 mg/kg., i.p. Further, bupropion treatment (20 mg/kg., i.p.) increased the levels of norepinephrine in the brain homogenate [F = 23.69, \(p<0.001\)] (Fig 2.1.6.).
2.1.3.5. Involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway in the antidepressant activity of bupropion

Pretreatment with a subeffective dose of L-arginine (750 mg/kg., i.p., nitric oxide precursor) reversed the antidepressant action of bupropion (20 mg/kg., i.p.) as shown by an increase in immobility period compared to bupropion (20 mg/kg., i.p.) per se group \( [F(3,23)=21.254, p<0.001] \) (Fig. 2.1.7.). A two-way ANOVA revealed significant differences of pretreatment \( [F (1, 20) = 18.13, P < 0.001] \), treatment \( [F (1, 20) = 35.54, p < 0.001] \) and of pretreatment × treatment interaction \( [F (1, 20) = 89.59, p < 0.001] \) on immobility period in the forced swim test. Post hoc analyses indicated that the antidepressant-like effect of bupropion (20 mg/kg., i.p.) was prevented by pretreatment of animals with L-arginine.

Similarly, 7-nitroindazole (25 mg/kg., i.p., a specific neuronal nitric oxide synthase inhibitor) enhanced the antidepressant effect of lower dose of bupropion (10 mg/kg., i.p.) (Fig. 2.1.8.). A two-way ANOVA revealed significant differences of pretreatment \( [F (1, 20) = 49.34, p < 0.001] \), treatment \( [F (1, 20) = 57.19, p < 0.001] \) and of pretreatment × treatment interaction \( [F (1, 20) = 47.71, p < 0.001] \) on immobility period in the forced swim test. Post hoc analyses indicated that the antidepressant-like effect of bupropion (10 mg/kg., i.p.) was enhanced by pretreatment of animals with 7-nitroindazole.

Methylene blue (10 mg/kg., i.p.) did not affect the immobility period per se. However, methylene blue significantly enhanced the antidepressant effect of lower dose of bupropion (10 mg/kg., i.p.) (Fig.2.1.9.). A two-way ANOVA revealed significant differences of pretreatment \( [F (1, 20) = 59.07, p < 0.001] \), treatment \( [F (1, 20) = 49.14, p < 0.001] \) and of pretreatment × treatment interaction \( [F (1, 20) = 38.58, p < 0.001] \) on immobility period in the forced swim test. Post hoc analyses indicated that the antidepressant-like effect of bupropion (10 mg/kg., i.p.) was enhanced by pretreatment of animals with methylene blue.
Sildenafil (5 mg/kg., i.p., a phosphodiesterase 5 inhibitor) did not affect immobility per se, but pretreatment with sildenafil reversed the antidepressant effect of bupropion (20 mg/kg., i.p.) (Fig. 2.1.10.). A two-way ANOVA revealed significant differences of pretreatment \[ F (1, 20) = 23.01, p < 0.001 \], treatment \[ F (1, 20) = 14.25, p < 0.05 \] and of pretreatment × treatment interaction \[ F (1, 20) = 41.19, p < 0.001 \] on immobility period in the forced swim test. Post hoc analyses indicated that the antidepressant-like effect of bupropion (20 mg/kg., i.p.) was prevented by pretreatment of animals with sildenafil.

Combination studies with all the nitric oxide modulators with bupropion did not affect the locomotor activity of mice as compared to effect per se (Data not shown).

![Fig. 2.1.4. Effect of bupropion on the locomotor activity (ambulation and rearing) in mice. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test \( n = 6-8 \). \( p<0.05 \) as compared to vehicle treated group.](image-url)
Fig. 2.1.5. Effect of bupropion on the rectal temperature of rats (n = 6-8). Data were analyzed by One Way Analysis of Variance (ANOVA) repeated measurements. *p<0.05 as compared to vehicle treated group, #p<0.05 as compared to bupropion (10 mg/kg treated group, $p<0.05 as compared to bupropion (20 mg/kg treated group).

Fig. 2.1.6. Effect of bupropion (10, 20 and 40 mg/kg i.p.) on the biogenic levels in the mice brain homogenates. Data were analyzed by One Way of Variance (ANOVA) followed by Dunnett's test (n = 6–8). *p<0.05 as compared to vehicle treated group, #p<0.05 as compared to bupropion (10 mg/kg treated group, $p<0.05 as compared to bupropion (20 mg/kg, i.p.) treated group.
2.1.4. DISCUSSION

In our experiments, bupropion dose-dependently reversed the immobility period in both forced swim test and the tail suspension test in mice. The ED$_{50}$ values in both the tests were found to be comparable. Bupropion also dose dependently increased the locomotor activity in mice. Further, bupropion increased concentration of free dopamine content and its metabolite homovanillic acid with subtle increases in noradrenaline levels. The antidepressant action of bupropion was attenuated by pretreatment with L-arginine and the action of lower dose of bupropion was potentiated by subeffective doses of 7-nitroindazole (neuronal nitric oxide synthase inhibitor) or methylene blue (nitric oxide synthase inhibitor and soluble guanylate cyclase inhibitor). Another important observation of the study was the reversal of antidepressant action of bupropion by sildenafil (phosphodiesterase 5 inhibitor). The present study for the first time demonstrated the antidepressant-like effect of bupropion in the forced swim test is linked to modulating the L-arginine-NO-cGMP pathway.

The forced swim-induced state of immobility in animals claimed to represent a condition similar to human depression (Renard et al., 2003) and amenable to reversal by antidepressant drugs (Kulkarni and Mehta, 1985). This model is widely accepted to screen antidepressant drugs belonging to all major classes of antidepressants including tricyclics, selective serotonin-reuptake inhibitors, monoamine oxidase inhibitors, and atypicals (Porsolt et al., 1977b; 1978a). The monoamine hypothesis of depression is based on the deficiency of one or other monoamine is commonly evoked to explain the pathophysiology of mental depression (Maj and Rogoz, 1999; Joca et al., 2000).

This hypothesis, initially based on noradrenaline and serotonin deficiency, is recently extended to dopamine (Kapur and Mann, 1992; Ebert and Lammers, 1997).
Fig. 2.1.7. Effect of bupropion (20 mg/kg., i.p.) and its modification by L-arginine (750 mg/kg., i.p.) on the mean immobility period in mouse forced swim test. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analysed by Two Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.001 compared with the vehicle-treated control; #p < 0.001 compared with the same group pretreated with vehicle (For treatment schedule, see text).

Immobility in the swim test may be reversed not only by antidepressants, but also by dopamine D2/D3 receptor agonists (Siuciak and Fujiwara, 2004; Basso et al., 2005) applied systemically or to the nucleus accumbens. Conversely, a number of studies have reported that antidepressant effects in the swim test were reversed by dopamine antagonists, these include studies in which antidepressants were administered chronically (Yamada et al., 2004). The effects of chronically administered tricyclic antidepressants were reversed by the administration of sulpiride in the nucleus accumbens, but not in the dorsal striatum (Yamada et al., 2004).
Fig. 2.1.8. Effect of subeffective dose of bupropion (10 mg/kg., i.p.) and its modification by 7-nitroindazole (25 mg/kg., i.p.) on the mean immobility period in mouse forced swim test. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analysed by Two Way Analysis of Variance (ANOVA) followed by Dunnett's test. *p < 0.05 compared to 7-nitroindazole per se group (For treatment schedule, see text).

Bupropion and nomifensine are selective dopamine reuptake inhibitors (Yamada et al., 2004). Bupropion [(±)-α-t-butylamino-3-chloropropiophenone] is clinically used in humans as an antidepressant or in the withdrawal therapy of nicotine (Wilkes et al., 2006). Animal studies suggested that antidepressants enhance dopamine neurotransmission in mesolimbic system (D’Aquila et al., 2003). Fluoxetine (a selective serotonin re-uptake inhibitor) or desipramine (a potent inhibitor of the noradrenaline re-uptake carrier) are also reported to increase the extracellular dopamine concentration in the prefrontal cortex (Ainsworth et al., 1998). In the present study, bupropion dose dependently inhibited the immobility period in both the behavioral paradigms of despair i.e. forced swim test and tail-suspension test, and also enhanced brain dopamine contents thereby showing that the drug which enhances the dopamine levels are also active in these models.
Reserpine produced depression in animals by depleting monoamines in the brain. Various antidepressant treatments reversed reserpine-induced immobility period (Sharma and Kulkarni, 1994). Bupropion administration reversed reserpine-induced immobility in a dose-dependent manner.

As bupropion is known to enhance the dopamine levels in the brain, it is expected to increase the locomotor activity in mice. Bupropion at 15, 20 and 40 mg/kg when injected intraperitoneally increased the locomotor activity in mice. In one of the studies, rats with damaged dopamine neural systems failed to display significant locomotor stimulation after bupropion treatment, while rats with large depletions of primarily norepinephrine responded like control animals to various doses of bupropion by producing locomotor activity (Cooper et al., 1980).

![Fig. 2.1.9. Effect of subeffective dose of bupropion (10 mg/kg., i.p.) and its modification by methylene blue (10 mg/kg., i.p.) on the mean immobility period in mouse forced swim test. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analysed by Two Way Analysis of Variance (ANOVA) followed by Dunnett's test. *p < 0.001 compared to methylene blue group.](image-url)
Our studies are consistent with the findings of Redolat et al., who has demonstrated that bupropion (at doses 10, 15 and 20 mg/kg) induced a significant increase in locomotor activity in mice (Redolat et al., 2005). Similarly, pramipexole, a dopamine D2/D3 receptor agonist showed enhanced locomotor activity when administered at a dose of 0.3 mg/kg which is considered to be its antidepressant dose (Maj et al., 1997) in mice. On the contrary, the drugs which increases the norepinephrine or serotonin levels in the brain either by inhibiting its reuptake or by acting as agonists of receptors respectively, should not affect the locomotor activity in animals. However, bupropion is devoid of any other autonomic actions which differentiated this compound from amphetamine like compounds and dopamine uptake blockade seems to be the sole mechanism of its antidepressant action (Cooper et al., 1980).
The effect of bupropion on the body temperature is not clear. There are various controversial reports available in the literature. Some studies have indicated that bupropion increased the body temperature in rats (Liu et al., 2004; Hasegawa et al., 2005), while others indicated a hypothermic effect (Zarrindast et al., 1992). In the present study, bupropion (20 and 40 mg/kg., i.p.) produced hypothermia in rats only after 60 min of its treatment and abates at 120 min. The hypothermic effect of bupropion may be mediated through dopamine D2 receptor activation. Inhibition of dopamine reuptake may result in an increase in free availability of dopamine and may act on central dopamine D2 receptors producing hypothermia (Verma and Kulkarni., 1991). Nonselective dopamine receptor agonists R (-) apomorphine and R (-)-N-n-propylnorapomorphine are reported to elicit dose- and time-dependent hypothermia in mice (Meller et al., 1989).

Attention has been focused on cerebral monoaminergic systems, the dysfunction of which is thought to underlie the depressive symptomatology. Biochemical evidences in patients with depression is derived from the study of homovanillic acid, a dopamine metabolite. Reduced venoarterial plasma concentration gradients of homovanillic acid and increased plasma levels of a serotonin metabolite, hydroxyindoleacetic acid, were found to be increased in the depressed patients (Mitani et al., 2006). Anhedonia is a frequent symptom of depression and it is commonly associated with a dysfunction of the dopaminergic reward system (Nestler and Carlezon, 2006). In the present study, neurochemical parameters have shown the increased levels of dopamine and its metabolite homovanillic acid by bupropion, thus further confirming the role of dopamine in depression. These neurochemical changes were well correlated with the behavioral paradigms. Bupropion has been shown to alleviate both the negative affective and somatic effects of nicotine withdrawal. Nicotine is known to stimulate dopamine release in the brain and there is clear evidence of dopamine and noradrenaline reuptake inhibition, and nicotinic receptor antagonism with bupropion (Warner and Shoaib, 2005). Bupropion, through it mimicking effect of nicotine, exerted its main effect in nicotine withdrawal and relapse (Wilkes, 2006).
Similarly, the involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) system in mediating the antidepressant effect of venlafaxine, a dual reuptake inhibitor of serotonin and norepinephrine has also been elucidated (reported in Chapter 3) in mouse forced swim test was elucidated. Nitric oxide plays a significant neuromodulatory role in the CNS. Any pharmacological manipulation of nitric oxide pathway may be considered as a novel therapeutic approach for the management of CNS disorders, more so for mental depression (Heiberg et al., 2002). Several in vivo studies have shown a modulatory role of nitric oxide in the extracellular levels of serotonin re-uptake mechanism in the central nervous system (Harkin et al., 2004). Various NOS inhibitors have been reported to possess antidepressant-like behavioral properties at doses that are without any effect on locomotor activity (Wegener et al., 2003). These effects are dose-dependent and stereoselective and can be reversed by co-treatment with the nitric oxide precursor, L-arginine. L-arginine is reported to exert a U-shape effect in the forced swim test, doses ranging from 30, 100 to 1000 mg/kg with lower dose causing no alteration, middle dose causing statistically significant reduction, and higher doses causing no alteration in the immobility period, respectively (Ergun and Ergun, 2007). We have chosen the dose of L-arginine (750 mg/kg., i.p.) that did not affect the immobility period and the locomotor activity per se. In our study, pretreatment of subeffective dose of L-arginine (750 mg/kg., i.p., NO precursor) resulted in the reversal of the antidepressant action of bupropion (20 mg/kg., i.p.) as shown by increased in immobility period as compared to bupropion (20 mg/kg., i.p.) per se group. Similarly, the antidepressant effects of imipramine were also blocked by pre-treatment with L-arginine and contrary to this, NOS inhibitor, NO-nitro-L-arginine augmented the behavioral effect of imipramine or fluoxetine in the forced swim test (Harkin et al., 2004). The studies carried out in our laboratory have demonstrated that fluoxetine suppressed the dependence and development of tolerance to the antinociceptive effect of morphine (Singh et al., 2003). Fluoxetine-induced
suppression was potentiated by \( \text{N}^\text{G}\)-nitro-L arginine methyl ester (L-NAME) and accentuated by L-arginine, thus demonstrating NO modulation of drug effects (Singh et al., 2003). These studies argue for the possibility of inhibition of nitric oxide synthase could be a strategy to enhance the clinical efficacy of various antidepressants. In one of the study carried out in our laboratory, venlafaxine is demonstrated to have antidepressant activity by acting through L-arginine-NO-cGMP system (reported in Chapter 3).

As expected, when the lowest subeffective dose of bupropion (10 mg/kg) was combined with 7-nitroindazole or methylene blue it exhibited a synergistic effect. On the contrary, an inhibitory effect was observed when it (20 mg/kg) was combined with L-arginine or sildenafil.

Previous reports have suggested that 7-nitroindazole (50 mg/kg), a specific neuronal nitric oxide synthase inhibitor and methylene blue (15 and 30 mg/kg), a direct inhibitor of both NOS and soluble guanylate cyclase (sGC) (Patil et al., 2005), inhibit the immobility period in forced swim test. The effect is comparable to standard antidepressants like imipramine (Eroglu and Caglayan, 1997; Volke et al., 2003). Similar studies have shown the anti-immobility effect of a specific inhibitor of sGC i.e. ODQ [1H-[1, 2, 4] Oxadiazole [4, 3-a] quinoxalin-1-one] (Heiberg et al., 2002; Ergun and Ergun, 2007) in forced swim test and its immobility period can be reversed by pretreatment with L-arginine, suggesting that NO-sGC pathway may play an important role in the mediation of its behavioral effect in the forced swim test (Heiberg et al., 2002).

Our results are in accordance with the findings of Wegener et al (2000) that NOS inhibitors increased extracellular levels of serotonin and dopamine in the rat ventral hippocampus after local or systemic administration, whereas the nitric oxide precursor L-arginine had the opposite effect. They concluded that the endogenous nitric oxide may exert a negative control over the levels of serotonin and dopamine in the hippocampus (Wegner et al., 2000). Further, retrodialysis of the ventral hippocampus with 7-nitroindazole induced a very large magnitude of
increase in extracellular dopamine compared with an increase in serotonin (Wegner et al., 2000). In our study, 7-nitroindazole (a neuronal nitric oxide synthase inhibitor) potentiated the effect of bupropion which is a dopamine reuptake inhibitor and the effect was antagonized by L-arginine, a precursor of nitric oxide. Thus, these results indicate that the inhibition of nitric oxide synthesis may underlie the reduction in the immobility period in the forced swim test elicited by bupropion. However, contradictory evidence also exists concerning the role of nitric oxide on the levels of the monoamines. L-Arginine has been shown to induce dopamine release from the striatum and increase the extracellular levels of serotonin and dopamine in the medial preoptic area in vivo (Lorrain and Hull, 1993; Strasser et al., 1994). We believe that nitric oxide serves different roles in regulating transmitter levels in distinct brain areas and, at least in the ventral hippocampus, increased nitric oxide synthesis may result in suppression of serotonin and dopamine overflow. Furthermore, hippocampus region is majorly involved in the depression and bupropion is known to increase the levels of various neurotransmitters such as norepinephrine, dopamine and serotonin in hippocampus area of the brain (Piacentini et al., 2003).

Another interesting observation of the present study was the reversal of the antidepressant-like effect of bupropion by sildenafil, a phosphodiesterase (PDE) 5 inhibitor (Kulkarni and Patil, 2004). This indicates that bupropion exerts its effect in the forced swim test probably by decreasing cGMP levels. The intracellular cGMP concentrations are regulated not only by sGC, but also by PDE5, which catalyses the hydrolysis of the second messengers cAMP and cGMP to yield AMP and GMP, respectively (Denninger and Marletta, 1999). The duration and magnitude of an NO-induced cGMP signal is determined by the activity of phosphodiesterase 5. Phosphodiesterase 5 is expressed in several brain areas, particularly in the neurons of the Purkinje cell layer in the cerebellum and in the pyramidal neurons of the hippocampus (Bender and Beavo, 2004).
Chapter 2

It is concluded that bupropion showed antidepressant activity in different animal models of depression possibly acting through dopaminergic and/or L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway.

Part-2: Possible involvement of sigma-1 (σ₁) receptors in the anti-immobility action of bupropion, a dopamine reuptake inhibitor

2.2.1. INTRODUCTION

Sigma receptors have recently been the target of drug development in psychiatric disorders, including schizophrenia, depression and Alzheimer's (Ishihara and Sasa, 2002). Of the established two subtypes of sigma receptors (sigma-1 and sigma-2), sigma-1 receptors are known to be involved in learning and memory, response to stress and depression, psychostimulant-induced sensitization, vulnerability to addiction and pain perception (Hashimoto and Ishiwata, 2006). Sigma-1 receptor agonists modulated the release of serotonin and dopamine in the brain (Kobayashi et al., 1997; Bermack and Debonnel, 2005). Further, sigma-1 receptors alleviated behavioral despair in both forced swim test (FST) (Skuza and Rogoz, 2006) and tail suspension tests (TST) (Ukai et al., 1998). Sigma receptors may play, in some way, a role in the antidepressant actions of selective serotonin reuptake inhibitors such as fluoxetine, fluvoxamine or escitalopram (Narita et al., 1996). The earlier study from our laboratory has indicated the involvement of sigma-1 receptors in modulating the antidepressant-like effect of neurosteroids in mouse FST (Reddy et al., 1998). One of the studies has also demonstrated the sigma modulatory activity of ropinirole, a dopamine D2/D3 receptor agonist in mice (See Chapter 6).
It has been therefore, hypothetized that sigma receptor modulation may be involved in the antidepressant action of drugs like bupropion. The present study explores the possible involvement of central sigma-1 receptors in the anti-immobility effect of bupropion in mouse FST. (+)-Pentazocine (a sigma-1 receptor agonist), progesterone (a sigma-1 receptor antagonist neurosteroid), rimcazole (another sigma-1 receptor antagonist) or BD 1047 (a novel sigma-1 receptor antagonist) were used in the study to examine the involvement of these receptors.

2.2.2. MATERIALS AND METHODS

2.2.2.1. Animals: Refer to Chapter 1 (1.2.1)

2.2.2.2. Forced Swim Test (FST): Refer to Chapter 1 (1.2.2.1)

2.2.2.3. Measurement of locomotor activity: Refer to Chapter 2 (2.1.2.6)

2.2.2.4. Drugs and treatment

The following drugs were used: (+)-pentazocine (Ranbaxy Co., Gurgaon, India), progesterone (Sigma Aldrich Co., MO, USA), BD 1047 (Tocris Co., Missouri, USA), rimcazole (Sigma Aldrich Co., MO, USA). All the drugs except progesterone were dissolved in distilled water and different doses were administered intraperitoneally in a fixed volume of 1 ml per 100g of body weight. Progesterone was dissolved in vegetable oil and administered subcutaneously. Bupropion was administered 30 minutes before challenging the animals to FST. In case of interaction studies, various agonists or antagonists were given 15 minutes before administering bupropion. The experimental protocol comprised of various groups, each consisting of six to eight animals. Different set of animals were used for measuring the locomotor activity. All the doses were chosen according to the literature available and the previous studies done in our laboratory (Reddy et al., 1998; Skuza and Rogoz, 2003).

2.2.2.5. Statistical analysis: Refer to Chapter 2 (2.1.2.9)
2.2.3. RESULTS
Effect of various sigma receptor modulators on the action of bupropion in FST
The subeffective dose of (+)-pentazocine (2.5 mg/kg., i.p.) enhanced the anti-immobility effect of subeffective dose of bupropion (10 mg/kg., i.p.) [F=5.260, p<0.05] (Fig.2.2.1.A.).

Pretreatment with a subeffective dose of progesterone (10 mg/kg., s.c.) [F=30.352, p<0.001] (Fig. 2.2.1B.), rimcazole (5 mg/kg., i.p.) [F=17.375, p<0.001] (Fig. 2.2.1C.) or BD 1047 (1 mg/kg., i.p.) [F=13.049, p<0.001] (Fig. 2.2.1D.), reversed the anti-immobility action of bupropion (20 mg/kg., i.p.) as shown by an increase in immobility period compared to bupropion (20 mg/kg., i.p.) per se group.

Combination of bupropion with various agonists or antagonists of sigma-1 receptors did not affect the locomotor activity as compared to the effect per se.

2.2.4. DISCUSSION
Bupropion exhibited anti-immobility activity in the forced swim test. Co-administration of sigma-1 receptor agonist potentiated the effects of subeffective dose of bupropion, while the sigma receptor antagonists particularly the sigma-1 subtype reversed the anti-immobility action of bupropion thereby demonstrating the involvement of sigma-1 receptor modulation in mediating the anti-immobility (antidepressant-like) effect of bupropion for the first time.

The forced swim test (FST) is considered as major and recognized paradigm in animals for screening of antidepressant drugs (Wang et al., 2007; Kulkarni and Mehta, 1985; Porsolt et al., 1977a). The paradigm is based on the observation that rats or mice when forced to swim in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves (despair or helplessness) to the experimental condition (Porsolt et al., 1977a). Both first and second generation of antidepressants drugs are known to reverse this state of despair in animals.
Immobility in the swim test is reversed not only by antidepressants, but also by dopamine D2/D3 receptor agonists (Siuciak and Fujiwara, 2004; Basso et al., 2005) administered systemically or directly applied in the nucleus accumbens. Conversely, a number of studies have reported that antidepressant effects in the swim test were reversed by dopamine antagonist sulpiride when applied directly in the nucleus accumbens, but not in the dorsal striatum (Yamada et al., 2004).

Bupropion is known to increase the contents of dopamine in the brain of mice (For details, see Chapter 2, Part-1). Bupropion showed antidepressant-like effects in all the age groups of mice (Bourin et al., 1998). It also reduced the firing rates of noradrenergic and dopaminergic neurons not that of serotonergic neurons in the dorsal raphe nucleus (Ascher et al., 1995). However, bupropion is reported to potentiate the release of dopamine and norepinephrine in hypothalamus and nucleus accumbens regions of the brain when co-administered with fluoxetine and may show better and rapid onset of antidepressant actions (Li et al., 2002). Recently, the involvement of 5HT2A and 5HT6 serotonin receptors in the antidepressant-like effect of bupropion in mouse forced swim test has also been demonstrated (Kitamura et al., 2008; Wesolowska and Nikiforuk, 2008). The fact that bupropion is effective in reducing symptoms of nicotine withdrawal (Fossati et al., 2007), it may block nicotinic acetylcholine receptors in vitro, which might contribute to its efficacy in smoking cessation (Portugal et al., 2007), besides other reported mechanisms.

Animal studies suggested that some of the other antidepressants not belonging to the category of dopamine reuptake inhibitors also enhance dopamine neurotransmission in mesolimbic system (D’Aquila et al., 2003). Fluoxetine (a selective serotonin reuptake inhibitor) or desipramine (a potent inhibitor of the noradrenaline reuptake carrier) is also reported to increase the extracellular dopamine concentration in the prefrontal cortex (Ainsworth et al., 1998). This strongly calls for the involvement of dopamine to a significant extent in mediating the actions of standard antidepressant agents.
Fig. 2.2.1. Effect of bupropion and its modulation by (A) (+)-pentazocine (2.5 mg/kg, i.p.) (B) progesterone (10 mg/kg, s.c.) (C) rimcazole (5 mg/kg, i.p.) or (D) BD 1047 (1 mg/kg, i.p.) on the immobility period (seconds) induced by FST. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05 as compared to vehicle treated group, **p<0.05 as compared to respective bupropion treated group (For treatment schedule, see text).
Chapter 2

In the present study, bupropion increased the locomotor activity in mice. Our results are similar to the findings by Redolat et al., who have also demonstrated an increase in locomotor activity in mice following acute administration of bupropion (Redolat et al., 2005). However, bupropion is devoid of any other autonomic actions which differentiated this compound from amphetamine like compounds and dopamine uptake blockade seems to be one of the major mechanisms of its antidepressant action (Cooper et al., 1994).

Sigma receptors, non-opioid, non-phencyclidine sensitive are of two subtypes: sigma-1 and sigma-2 receptors. Sigma-1 receptors are expressed in specific regions of the brain such as layers of the cortex, hippocampus, hypothalamic nuclei, substantia nigra and purkinje cells in the cerebellum (Heroux et al., 1992; Ainsworth et al., 1998) and have recently been the target of drug development of anti-schizophrenic and antidepressant drugs (Ishihara and Sasa, 2002). Many pharmacological and physiological actions have been attributed to sigma-1 receptors. Sigma-1 receptors modulate the release of various neurotransmitters (Su and Hayashi, 2003) like serotonin (Bermack and Debonnel, 2005), dopamine (Kobayashi et al., 1997) or glutamate (Yagasaki et al., 2006) and known to alleviate the symptoms of depression. Sigma-1 agonists have been then tested in various behavioral tests classically used to predict an antidepressant activity. SA 4503 [1-(4-fluoromethoxy-3-methoxyphenethyl)-4-(3-phenylpropyl)piperazine], (+) - pentazocine, DTG (1,3-di-o-tolylguanidine), JO-1784 (igmesine), and SKF-10047 (N-allyl normetazocine) (all sigma-1 receptor agonists) dose dependently decrease immobility in the FST (Matsuno et al., 1996; Kinsora et al., 1998; Skuza and Rogoz, 2003). These effects were blocked by the sigma-1 receptor antagonist NE-100 or BD1047. Further, SA 4503 and (+)-pentazocine (both sigma-1 receptor agonists) also decreased immobility period in the tail-suspension test, an effect also antagonized by NE-100 (Ukai et al., 1998).
Although, various studies have reported the involvement of sigma receptors in the action of antidepressants, none of them have explored the involvement of this receptor system in the antidepressant outcome of bupropion. Many antidepressant drugs interact with sigma receptors and accumulating evidence suggests that these proteins mediate antidepressant-like effects in animals and humans (Wang et al., 2007). Antidepressants like fluvoxamine, fluoxetine, citalopram, sertraline, clorgyline, and imipramine all possessed moderate to high affinity for sigma-1 sites (Bermack and Debonnel, 2005) and it has been proposed that certain differences in the clinical effects of the antidepressants may, in part, be explained on their distinct influence on cerebral sigma receptors. For example fluvoxamine is known to have high affinities for sigma-1 receptors, while tricyclic antidepressants have less (Narita et al., 1996; Takebayashi et al., 2004; Stahl, 2005); the effect can be antagonized by the administration of progesterone or BD 1047 (a sigma-1 receptor antagonist) (Rogoz and Skuza, 2006). The sigma ligands are hypothesized to increase the firing activity of dorsal raphe nucleus. Treatment with sigma ligands may rapidly modulate N-methyl-D-aspartate (NMDA) receptor-mediated transmission in the hippocampus, and potentially other forebrain regions, which in turn would lead to a modulation of serotonin neurotransmission in the dorsal raphe nucleus via feedback loops (Bermack and Debonnel, 2005). Combined treatment with sigma ligands and amantadine or memantine (NMDA receptor antagonist) may be an alternative in the treatment of antidepressant-resistant depressive patients in the future (Skuza and Rogoz, 2006).

To elucidate the interaction of bupropion with sigma receptors, we have used (+)-pentazocine, a selective sigma-1 receptor agonist and found that pretreatment with subeffective dose (the dose which did not affect the immobility period in FST) potentiated the antidepressant activity of subeffective dose of bupropion. Further, the antidepressant effect of effective dose of bupropion was antagonized by progesterone, a sigma-1 receptor antagonist neurosteroid, rimcazole, a sigma-1 receptor antagonist
antipsychotic agent, or BD 1047, a novel selective sigma-1 receptor antagonist, respectively. Further, there were no alterations in the locomotor activity when bupropion was combined with various agonists or antagonists. This implies that the antidepressant-like effect in forced swim test is independent of locomotor activity of bupropion.

Many brain regions have been implicated in regulating emotions and there is an increasing recognition of the role played by particular subcortical structures in depression such as nucleus accumbens (Malkesman et al., 2007; Nestler et al., 2002). These areas have been found to play a critical role in domains which are prominently affected in most depressed patients such as regulation of motivation, sleep and response to pleasurable and aversive stimuli (Malkesman et al., 2007). Bupropion is known to increase extracellular dopamine and norepinephrine concentrations in several mesocorticolimbic areas including nucleus accumbens which may have an impact on the antidepressant actions of bupropion (Li et al., 2002). In vivo brain microdialysis studies demonstrate that administration of bupropion enhances the increases in extracellular dopamine in the nucleus accumbens (Ascher et al., 1995). Sigma -1 receptors are known to modulate the dopamine release in the nucleus accumbens region of the brain as systemic administration of JO-1784, (+)-pentazocine, both selective sigma-1 receptor ligand, and di-tolyl-guanidine (20 microg/i.v.), a non selective sigma receptor ligand is known to produce a significant increase of N-methyl-D-aspartate (NMDA)-induced dopaminergic neuronal activation (Gronier et al., 1999). In the present study, it is hypothesized that sigma receptors may modulate the bupropion-induced dopaminergic release in the nucleus accumbens region; thus displaying anti-immobility action in forced swim test.

In conclusion, these findings suggest that sigma receptors (sigma-1 site) may play a role in the anti-immobility activity of bupropion in despair paradigm of mice.