Effect of anti-depressants on neuro-behavioural consequences following impact accelerated traumatic brain injury in rats

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Disruption of normal neuronal networks and neurotransmitters like serotonin and norepinephrine levels in post traumatic brain injury (TBI) are observed to be the primary causative agent for depression/anxiety. This communication reports the efficacy of various classes' anti-depressants in the treatment of depression/anxiety following TBI in rats. Chronic treatment with anti-depressants (escitalopram and venlafaxine) leads to improvement in the depressive/anxiogenic-like behaviour in the TBI rat and corroborates the notion of the involvement of serotonin and norepinephrine in the behavioural consequences of post-TBI. Chronic treatments with escitalopram and venlafaxine significantly reversed the effect of TBI as compared to vehicle-treated TBI group. The results showed a quantitative battery of neuro-behavioural functional assessments that correlates with neuronal damage following traumatic brain injury.

Keywords: Anti-depressants, Anxiety, Depression, Traumatic brain injury

Traumatic brain injury (TBI) is a sort of neurological impairment which originates from any mechanical or infectious injury to the brain and manifests as a major handicap to the affected individual. Among the human survivors of TBI, changes in the emotional and social behaviour are considered to be the most common and debilitating consequences of TBI including cognitive or memory impairment, apathy, aggressiveness, and mood disorders1. Depression and other neurological diseases that occur post TBI are now thought to be associated with bilateral dysfunction of the frontal and temporal lobes and disruption of deep diencephalic structures and networks. It was postulated that premorbid psychiatric disorder and social impairments may contribute to major depression following TBI2. Disruption of normal neuronal networks and neurotransmitter levels in these anatomical areas could be associated with depression3. In addition to disrupting neuronal circuits directly, TBI results in the disruption of neurotransmitter systems such as nor-adrenaline, serotonin (5-HT), dopamine and acetylcholine4. Such disruption is part of the secondary injury cascade following TBI5 and is thought to account for much of the mortality and morbidity after the traumatic insult.

Synaptic plasticity in brain regions such as hippocampus, amygdala and pre-frontal cortex are associated with mood disorders and anxiety5-8. Anxiety symptoms may be attributed to trauma of the amygdala, which, like the hippocampus, is prone to injury because of its medial temporal location9,10. In the present study, impact acceleration models used for mimicking a typical injury includes direct head impaction using a piston, human stunner or captive bolt pistol, calibrated pendulum, or weight drop onto the skull11,12. These models simulate head injury in motor vehicle accidents or falls where there is rapid acceleration/deceleration of the head after impact to an intact skull, but sometimes fail to produce a highly repeatable injury13-15. A critical link in successful translational research in the development of animal models of traumatic brain injury is required that can provide a platform to correlate meaningful functional outcome measures with well-characterized behaviour viz depression and anxiety is envisaged. In the light of preceding information, the study was devised to describe more completely, the nature of the relationship between particular neuro-behavioural symptoms in post-TBI. Another goal of this work is to provide additional information regarding the neuro-psychological and functional out come of anti-depressants in a rodent test battery involving exposure to novel stimuli following TBI. The...
development of animal models to estimate parameters from behavioural data simulating to human behaviour post traumatic brain injury is the focus of the current study. Activity and parameters in TBI and treated rats were compared. Using parameter differences the effect of four anti-depressants drugs-escitalopram, venlafaxine, bupropion and amitriptyline was studied. Escitalopram (ESC) is an anti-depressant of the selective serotonin re-uptake inhibitor (SSRI) class, prescribed for the treatment of major depressive disorder and generalized anxiety disorder\textsuperscript{16}. Venlafaxine (VLF) is an anti-depressant of the serotonin-norepinephrine re-uptake inhibitor (SNRI), prescribed for the treatment of clinical depression and anxiety disorders\textsuperscript{17}. Bupropion (BUP) is an atypical anti-depressant that acts as a norepinephrine and dopamine re-uptake inhibitor\textsuperscript{18}. Amitriptyline (AMI) a tricyclic anti-depressant is well known to block the re-uptake of nor-adrenaline and 5-HT\textsuperscript{19}. The tested dose was selected from the pilot study i.e acute and chronic antidepressant study in laboratory and assessed for anti-depressants/anxiolytic profiles in TBI induced depression/anxiety. The aim of the study is to develop these animal models simulating neuro-behavioural changes post-TBI, including the assessment of standard anti-depressants in rodents behavioural test battery of depression and anxiety.

**Materials and Methods**

**Animals**—For optimal behavioural responsiveness, male Wistar rats (250-300 g, 12-14 weeks old) were obtained from Hisar Agricultural University, Hisar, Haryana, India and maintained in standard laboratory conditions with food (standard pellet chow feed) and filtered water \textit{ad libitum}. Experiments on animals were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani, India (Protocol No. IAEC/RES/4/1, IAEC/RES/7/1). The animals were housed in colony cages under standard lighting condition (lights on from 0700 to 1900 hrs), 22\textdegree \pm 2\textdegree C, and 60 \pm 10\% RH for at least one week before the experimental sessions. **Drugs**—Escitalopram, bupropion, amitriptyline and venlafaxine, were obtained from Glenmark Pharmaceutical Ltd, IPCA Laboratories, Mumbai and Ranbaxy Research Lab, Delhi India respectively. Ketamine and xylazine were purchased from Reidel (Neon Lab, Indian Immunological) Mumbai, India. All the drugs were dissolved in distilled water and prepared immediately before administration. All the drugs were administered orally once a day. The schedule of drug administration and behavioural test is shown in Table 1. The administration of anti-depressants was started 10 days after the injury and continued for 14 days as TBI-induced depression presents a problem requiring long-term treatment. After the rats were treated with anti-depressants or vehicle for 14 days, the first behavioural test was performed.

**Induction of injury**

\textit{Impact accelerated model of traumatic brain injury}—Rats were anesthetized using a mixture of Ketamine and xylazine (75 and 5 mg/kg, ip). Once adequate anesthesia was achieved, a 1 cm midline scalp incision was made and tissues were retracted to expose the skull. A stainless steel disc (10 mm in diam. and 3 mm in depth) was firmly positioned over rat’s skull, centrally between the lambda and bregma (Fig. 1A). Injury was then induced using the modified impact-acceleration model of TBI as described elsewhere\textsuperscript{20,21}. A 400 g weight was dropped from a height of 1 m onto the metal disc fixed over the rat’s skull guided through a straight pipe (length 1 meter diameter 35 mm) without wobbling. A 10 cm foam bed was placed underneath the animal which helped to absorb the impact. Before dropping the weight the animal was positioned in the centre of pipe so that the
weight falls exactly over the metal disc placed over the rats head (Fig. 1B). After impact, the metal disc was removed and skin was sutured with absorbable surgical catgut. Povidone-iodine (10% w/v, Betadine) was applied undiluted following wound closure for post-operative surgical wound disinfection. During 10 days post-injury, the wounds were daily inspected to ensure complete healing. Sham operated rats were treated in the same way, including mid-line incision, except brain injury.

**Behavioural assays**

Behavioural models, incorporating repeated exposure to stress have been widely used as experimental models for depression because stress is thought to play an important role in the etiology of depression and anxiety\(^{22}\). Dosing and behavioural tests were performed with substantial modification as described earlier\(^ {23}\). All the anti-depressants were administered per-oral once a day for 14 days. Alternate test of depression and anxiety was performed during two days (26\(^{th}\)-27\(^{th}\)) behavioural test first and second half of the day respectively (Table 1).

**Depression assessment**

*Open field test*—The open field exploration was conducted as described\(^ {24,25}\) with slight modifications. The apparatus consisted of a circular (90 cm diam.) arena with 75cm high aluminum walls and floor equally divided into 10 cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. During the test each animal was individually placed in the center of the open field apparatus and the following parameters were noted for 5 min by a trained observer unaware of the specific treatments. Ambulation scores (number of outer squares crossed), number of rearing episodes and number of pellet were recorded. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly to eliminate the residual odor.

*Hyper-emotionality*: Hyper-emotionality was measured using modifications of the procedure described\(^ {26,27}\), measurement included the scoring of responses to the following stimuli: (i) Startle response: Startle response to a stream of air directed at the dorsum was scored. The air was delivered using 10-ml syringe, (ii) Struggle response: Struggle response was scored by handling with a gloved hand and (iii) Fight response: Fight response was scored by pinching the tail with a forceps (i.e the rat tail is gently held from the back of a rat using forceps). A trained researcher performed these operations. These responses were graded as follows: 0, no reaction; 1, slight; 2, moderate; 3, marked; or 4, extreme response. For each emotional response, audible vocalization were also scored and graded as follows, 0, no vocalization; 1, occasional vocalization; or 2, marked vocalization. The vocal score were added to each emotional response score. The score for each animal in emotional response was given within 5 min.

**Anxiety assessment**

*Social interaction test*—The protocol was adopted (with slight modifications as mentioned below) from File and Hyde\(^ {28}\). The apparatus consisted of a circular (90 cm diam.) arena with 75cm-high aluminium wall and a floor equally divided into 10cm squares. A 15 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. On the day of test, pairs of rats, of the same treatment/surgery group housed in different cages, were put into two different corners of the open field arena. The social interaction behaviour, including the forward running, probing, grooming, mounting and crawling under the other rat, was recorded for 5 min.

*Marble burying behaviour*—The method of Broekkamp \textit{et al.}\(^ {29}\), was used with substantial modification. The test apparatus was a \(40 \times 25 \times 12\) cm plastic rat cage similar to the animals home cage. The floor of the cage was evenly covered with 5 cm of bedding material. Each rat was assigned to a particular test cage for the duration of the experiment. The animal was placed singly in the test cage for 40 min with glass marbles placed in a triangular formation at one end of the cage. The number of marbles covered in a 40 min

<table>
<thead>
<tr>
<th>Table 1—Schedule of treatments and behavioural assessments of TBI/sham rats in chronic study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>Surgery</td>
</tr>
<tr>
<td>26(^{th}) day</td>
</tr>
<tr>
<td>27(^{th}) day</td>
</tr>
</tbody>
</table>
Statistical analysis—All analysis was performed using graph pad prism for Windows. All the results of experiments are expressed as mean ± SE. Data were analyzed by one way ANOVA. Where ever ANOVA was significant, further comparisons between vehicle and drug treated TBI groups were done using post hoc bonferroni test. The level of statistical significance was fixed at $P < 0.05$.

Results

Open field behaviour—TBI resulted in significant increase in the ambulation (crossing), number of fecal pellets and rearing behaviour in an open field compared to sham operated rats (Table 2). Chronic (14 days) ESC and VLF (10 mg/kg) showed significant ($P < 0.05$) reduction in number of ambulation, fecal pellets and rearing in TBI rats compared to vehicle treated group. ESC and VLF showed antidepressant like effects in TBI but BUP and AMI failed to reach the level of significance.

Hyper-emotionality—TBI rats showed significantly higher hyper-emotionality scores, compared with sham group (Fig. 2.) Treatment with ESC and VLF (10 mg/kg) significantly ($P <0.05$) attenuated the hyper-emotionality as compared to the vehicle treated TBI group.

Social interaction test—The results of social interaction tests are summarized in Fig. 3. The social interaction studies were done in pairs of rats from the same experimental treatment and time spent in active social interaction (sniffing, following, grooming, kicking, jumping or crawling under or over the partner), time spent in passive social interaction (the rats sitting or lying with their bodies in contact, but without interacting with each other), were analysed. TBI rats exhibited a significant decrease in total interaction time and an increases in the number of passive interactions (crossing each other in open field), as compared to sham rats (Fig. 3a and b). ESC and VLF (10 mg/kg) significantly ($P <0.05$) increased the total interaction time and reduced the number of passive interactions, compared with vehicle treated TBI group. BUP (20) and AMI (10 mg/kg) failed to show significant changes (Fig. 3a and b).

Discussion

The use of animal model of human mental disorder, despite of their oblivious limitation have

Table 2—Effects of anti-depressants (ESC, VLF, BUP and AMI) on open field behaviour. Number of square crossed, fecal pellets and rearing in open field compared to vehicle treated TBI rats. All drugs / vehicle were administered once a day, per-oral. Results are expressed as mean ± SE from 6 rats in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, po)</th>
<th>Crossing</th>
<th>Pellet</th>
<th>Rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0</td>
<td>90.16±8.06</td>
<td>2±0.66</td>
<td>17±1.56</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>84.66±4.92</td>
<td>1.33±0.45</td>
<td>15.33±1.32</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>90.83±6.29</td>
<td>1.5±0.39</td>
<td>13.55±1.44</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>153.21±12.23</td>
<td>3.5±0.77</td>
<td>19.45±2.30</td>
</tr>
<tr>
<td>TBI</td>
<td>0</td>
<td>172.66±22.03</td>
<td>4.66±0.93</td>
<td>24.13±2.36</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>136.23±8.13</td>
<td>2.33±0.50</td>
<td>15.23±1.48</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>168.35±15.15</td>
<td>3.83±0.79</td>
<td>22.33±2.23</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>153.21±12.23</td>
<td>3.5±0.77</td>
<td>19.45±2.30</td>
</tr>
</tbody>
</table>

$P$ values: $<0.05$; a vs sham operated, b vs vehicle treated TBI rats
ESC= Escitalopram; VLF= Venlafaxine; BUP= Bupropion; AMI= Amitriptyline
proven to be of value in the pre-clinical behavioral analysis for experimental validation of psycho-pharmacological assessment. In the present study, it has been demonstrated that long-term increase in depressive disorder in rats which occurs following traumatic brain injury is consistent with the development of anxiety. The present results suggest that the novel impact accelerated TBI is the best, most sensitive and reproducible test instrument with sensitivity to detect alterations in neuro-behavioural function post traumatic brain injury. The use of anti-depressants in the treatment of depression is an accepted protocol in the clinic, however the effects of anti-depressants on behavioural changes post accelerated impact TBI were investigated in the present study using rodent’s test battery that was constructed for the purpose. Chronic drugs administration is necessary in order to completely reverse the effect of TBI.

Depression assessment in TBI rats—Depression, post TBI is most likely caused by disruption of multiple neuro-transmitter homeostasis. However, it is not clear which brain regions are involved in the behavioural effects. Depressive symptoms in TBI rats were assessed using the open field test and hyperemotionality which is a well characterized rodent model of depression. TBI rats exhibited a specific abnormal behaviour pattern in the brightly lit, circular, open field arena. The results demonstrated a significant hyperactivity in open field in TBI viz. increased ambulation, defecation and rearing compared to sham control. This hyperactivity reflects the psychomotor agitation that occurs post TBI. Such exploratory hyperactivity is reversed by chronic treatment with anti-depressants of pharmacological classes. ESC (10mg/kg) and VLF (10mg/kg) showed a similar significant decrease in each hyper-emotional response (struggle, fight, and startle) of TBI rats. Hyper-emotionality manifested by violent flight reactions in response to previous neutral or innocuous stimuli, have been observed in rats with lesions. Thus, it is possible that TBI induced hyper-emotional behaviour may resemble psychomotor symptoms, a diagnostic criterion for depression. Furthermore, as reflected by the total score of hyperemotional responses, it was demonstrated from the present study that the inhibitory effects of ESC and VLF were significantly greater than those of BUP and AMI. On the basis of these findings, it is assumed that changes in emotional reactivity following lesions of the brain regions could likely be manifested because of emotional imbalance. The mechanisms by which anti-depressants produces these beneficial effects in impact accelerated TBI are unknown, although they are likely to involve actions on neurotransmitter systems, particularly serotonin.

Behaviour of TBI rats in anxiety—Anxiety disorders occur in a significant proportion of patients...
with TBI and frequently coexist with depressive disorders. There is a significant degree of comorbidity between mood and anxiety disorders among patients with TBI. The purpose of the social interaction test was to investigate whether social behaviour was affected by TBI. Vouimba et al. observed that, serotonergic system was disrupted by TBI which is consistently implicated in animal and human studies on social behaviour. The isolated neurobehavioural symptoms (e.g., social withdrawal) may be strongly related to severity of injury. In the social interaction test, it has been found that the treated TBI pair of rats spends more active social interaction. Further, it was demonstrated that obsessive-compulsive disorder in TBI rats, as one of many anxiety-related sequelae of brain injury assessed using marble burying behaviour, which reflects both compulsiveness and fear of novelty. TBI rats display a neophobic response by burying objects associated with aversive stimulation. In the present study, sparkling glass marbles provided the novel aversive stimulus in a familiar environment. It is clear from the results that marked differences between sham operated and TBI rats appear under such conditions. Increased marble burying behaviour by TBI rat reflects fear of novelty and compulsiveness which indicates indices of anxiety. Chronic ESC and VLF treatment showed positive response by inhibiting marble burying behaviour in TBI rats. A number of medications that were originally approved for treatment of depression have been found to be effective for anxiety disorders. In line with this observation, in the present experiments, chronic administration of escitalopram and venlafaxine significantly reversed the marble-burying behavior in TBI rats. The present study showed that ESC and VLF significantly reversed the anxiogenic behaviour, post TBI in all the aversive condition in anxiety, consistent with the previous reporting that chronic treatment with SSRI and SNRI significantly decreased the frequency of or extinguishes panic attacks, lessens anticipatory anxiety.

Depressive illness often represents a disorder that occurs after a traumatic event, but is seen by some as requiring a differential diagnosis to exclude or confirm comorbidity with depression and anxiety correlated with monoamines such as serotonin and nor-adrenaline. The classes of anti-depressants used in the present, appeared to exert their effect principally by increasing serotonin and norepinephrine level in the brain. All though bupropion and amitriptyline were ineffective, escitalopram and venlafaxine were found to significantly attenuate the behavioural changes associated with TBI. Since only single dose of each anti-depressant was assessed in behavioural test battery, it cannot be discerned whether variation in the ability of the different anti-depressants to modify habituation rates resulted from differences in potency or reflected actual drug efficacy. Studies using different dose levels may come up with some justification for the ineffectiveness of amitriptyline and bupropion. The repeated testing of the TBI rats in the successive tasks may affect the performance from one test to the other. However, both the sham and TBI group were subjected to the same series of tests, assuming that the effects of repeated testing were controlled. On the other hand, it cannot be excluded that this successive testing could have interfered with treatment effects.

The present study contains an extensive behavioural data characterizing the effect of TBI and action of several prototypical anti-depressants: escitalopram, venlafaxine, amitriptyline and bupropion on behavioural consequences post TBI. The tests were developed specifically with the goal of redressing the inattention paid to neuro-behavioural symptoms in the assessment of patients with TBI. The study showed that depression and anxiety are frequent complications of impact accelerated TBI that exerts a deleterious effect on the recovery process and psychological outcome in brain injury, and that chronic administration of anti-depressants attenuates depression and anxiety following severe TBI. Finally, it is proposed that the reproducible test instruments used and standardized in the present study for neurobehavioural outcomes have wide applications in studies of traumatic brain injury where rat models are widely utilized. Future research will proceed with multiple doses, larger sample sizes and longer duration of time to determine the efficacy and effectiveness of different anti-depressants in treating depressive symptoms following TBI.

Acknowledgement
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