Biological Forum – An International Journal **15(1): 601-609(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Gender-related Alterations in Neurochemical Milieu of Suicide: An Analysis in Human Postmortem Brain

Ritabrata Banerjee[1]*, Madhusudan Das[2] and Anup Kumar Ghosh[3]
[1]Assistant Professor, Department of Zoology,
Parimal Mitra Smriti Mahavidyalaya (Affiliated to University of North Bengal),
Jalpaiguri (West Bengal), India.
[2]Professor, Department of Zoology, University of Calcutta,
Kolkata (West Bengal), India
[3]Professor, Department of Instrumentation Science, Jadavpur University,
Kolkata (West Bengal), India.

(Corresponding author: Ritabrata Banerjee)*
(Received: 09 December 2022; Revised: 15 January 2023; Accepted: 19 January 2023; Published: 23 January 2023)
(Published by Research Trend)

ABSTRACT: Depression is a wide spread, incapacitating psychiatric disorder, with 10-30% of women and 7-15% of men in a population being tremendously affected with this disease at any given time. Depression can lead to suicide, a tragic fatality at its worst. The neurobiology of suicide has been studied by numerous researchers, although the specific molecular and pathophysiologic pathways are still unclear. The goal of the current study was to determine whether there were any changes in the expressions of BDNF, TrkB, NGF, and/or TrkA in the postmortem brains of suicide participants' hippocampus and amygdala, and whether these changes were connected to gender-specific psychopathologic conditions of suicide. Expression profiles of neurotrophins and their cognate receptors were assayed by Western Blotting. mRNA levels were also measured by RT-PCR. In this study it was found that the protein and mRNA levels of neurotrophins and their receptors were much lower in the hippocampus and amygdala of male suicide subjects, whereas female suicide victims showed decreased levels of same factors only in the hippocampal area. It indicates a possible sex-specific effect in the regulation of BDNF and NGF expressions and important insights into the altered neurochemical milieu of suicide.

Keywords: Gender, Hippocampus, Amygdala, Suicide, BDNF, NGF, TrkB, TrkA.

INTRODUCTION

Every suicide is a tragedy. The term "suicide" refers to a purposeful attempt to self-harm that results in death. Somewhere in the World in every 40 seconds a person dies by suicide and many more attempt suicide (WHO, 2014). One of the most pressing challenges in mental and public health around the world is suicidal thoughts and attempts. According to the most recent data from the National Institute for Health Statistics (NCHS) and Center for Disease Control (CDC), suicide is the second greatest cause of death for people aged 15–19 and 20–24 in 2019 (Vargas-Medrano *et al.*, 2020). The prevalence of suicide rises with age in both men and women, and it exhibits a consistent trend across all age groups. In most nations, men die from suicide more frequently than women do (Brent and Moritz 1996; Chehil and Kutcher 2012). As the men are less likely to seek help for emotional problems, and express depression differently, used to choose more lethal methods of suicide (Rich *et al.*, 1988; Addis and Mahalik 2003). Particularly in high-income countries, where the average male-to-female ratio is 3.5:1, suicide rates for men are much greater than those for women (WHO, 2014). The disparity in suicide rates between men and women has been linked to psychosocial risk factors such unemployment, retirement, and being single, although no other major risk factors were identified for women except their mental illness (Qin *et al.*, 2000; Tóth *et al.*, 2014).

Previous research claimed that the different suicide methods used by men and women are what causes the variation in suicide mortality between the sexes. Drug overdose, carbon monoxide poisoning, self-cutting and drowning etc methods are often chosen by women while men tend to use firearms, jumping from height, railway runover and hanging etc. Guns, height jumps, and hanging offer relatively little chance of surviving compared to drug overdose, carbon monoxide poisoning, or self-mutilation, which may help to explain variances in suicide death rates (Denning *et al.*, 2000; Shenassa *et al.*, 2003). However, gender-related neurobiology of suicide is still in dearth. These studies have provided significant new information regarding the altered neurochemical milieu of suicide that differs according to gender.

In line with other impairments of higher mental functions, depression appears to be in the hippocampus and amygdala, according to several neuroimaging, neuropsychiatric, and brain stimulation therapy investigations. The hypothalamus, hippocampus, amygdale, cingulate cortex, and other medial brain

regions that create a ring around the inner border of the cortical mantle make up the limbic system of the brain, which is located on both sides of the thalamus. When a person experiences high amounts of stress, their hippocampus becomes more activated, which impairs their ability to encode declarative and spatial memories. In contrast, the amygdale, a different region of the limbic system, becomes more active during stress, improving recall of emotionally significant events (Maftoon *et al*., 2014). Depression is majorly indicated by both structural and functional anomalies in these areas (Pandya *et al.*, 2012; Schweitzer *et al.*, 2001). More and more researchers are beginning to understand how neurotrophins control brain development and neuronal activity. In addition to these functions, neurotrophins are crucial for cellular growth, migration, phenotypic differentiation, and the upkeep of central nervous system development. Structural integrity of neurons, neurogenesis and maintenance of neuronal functions in the adult central nervous system also required adequate presence of neurotrophins (Banerjee *et al.*, 2012a; McAllister, 2001), indicating that neurotrophins have physiologic importance throughout life.

Neurotrophin, BDNF (Brain-derived neurotrophic factor) binds to the tyrosine kinase receptor B (TrkB) and involves in neuronal development through maintaining neurite outgrowth, synthesis of differentiating factors and morphological plasticity. In adulthood, BDNF also regulates neural homeostasis, connectivity, including learning and memory, drug addiction, aggression, and anxiety-related behaviours (Banerjee *et al.*, 2012b). A precursor peptide to mature BDNF called pro-BDNF is encoded by the BDNF gene, which is located on chromosome 11p13 (Dwivedi, 2010). Another member of neurotrophin family, Nerve growth factor (NGF) specifically binds to tyrosine kinase receptor A (TrkA)to regulate the viability, growth, and proliferation of sympathetic and sensory neurons (Banerjee *et al.*, 2013a). The gene for nerve growth factor (NGF) is found at 1p13.1.NGF appears to play a role in the stress response and the hypothalamic-pituitary-adrenocortical axis in addition to its trophic effect (HPAA) (Richthofen *et al.*, 2003). The intracellular MAPK/ERK signalling pathway has been observed to be activated by mood stabilisers. Neurotrophins like BDNF and NGF utilise this route (Hunsberger *et al.*, 2009).

Numerous fundamental and clinical investigations showed that stress-induced regulation of BDNF and NGF in the brain may significantly contribute to the onset of depression and that these proteins are crucial components of the antidepressant mechanism of action (Dwivedi *et al.*, 2003; Banerjee *et al.*, 2014). The current study was designed to determine gender-specific expressional alterations of BDNF and/or TrkB along with NGF and/or TrkA in the hippocampus and amygdala of all suicide victims, irrespective of psychiatric diagnosis, compared to healthy control individuals. The significance of BDNF and NGF in preserving the structural integrity and synaptic plasticity of the brain, as well as their considerable contribution to stress and emotional disorders, led to this action.

MATERIALS AND METHODS

Subjects. Postmortem brain samples from 40 suicide victims and 40 healthy, non-psychiatric control participants of both sexes were taken from the hippocampus and amygdala regions and kept at -80 °C. Table 1 (for the male group) and Table 2 (for the female group) respectively provide detailed demographic information for participants in the control and suicide groups. The Institutional Review Board (Ref. No. 06/B/IEC/MCH) of the Calcutta Medical College Hospitalunder West Bengal University of Health Sciences, gave its approval to the entire study.

Diagnostic methodology. The psychological autopsy technique was used to make psychiatric diagnoses for the subjects. This method has been shown effective for Axis I and II diagnoses (Banerjee *et al.*, 2013b; Conner *et al.*, 2001). Information about the suicide victims were obtained from their family members who were most familiar with the deceased. The interview was carried out by using the Diagnostic Evaluation After Death (DEAD) (Salzman *et al.*, 1983), and the Schedule for Clinical Interviews for the DSM-IV (SCID) (First *et al.*, 2002) by a psychiatrist. Interviewers compiled a case history for each individual using data from SCID I and II interviews, the coroner's notes, and medical records. Similar consensus diagnostic techniques were used to confirm that the control samples were free of mental disease. Male controls ranged in age from 18 to 75 whereas male suicide victims were in the 19 to 72 age range. The age range for female control participants was 14–71 years, while for female suicide victims it was 17–69 years. Brain samples were taken during autopsy at the Calcutta Medical College Hospital Morgue within 13 to 28 hours of the subjects' passing (postmortem interval data is presented in Tables 1 and 2). Medical records of suicide victims were used to gather information on the age range, drug history, and cause of death of the subjects. Tables 1 and 2 provide a thorough description of the participants examined in this investigation.

Collection of hippocampus and amygdala from postmortem brains. The amygdaloid brain regions, which encompass all major nuclei (central, medial, and basolateral amygdala), as well as the dentate gyrus and areas CA1-4, were extracted from the middle part of the hippocampus. RIPA buffer was used for tissue extraction, which has the following ingradients like 20 mM Tris-HCL (pH 8), 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM Na_2MoO_4, 0.5 mM Na_3VO_4, 5 mM $Na_2P_2O_7$, 1% Triton X-100, 0.5% Na deoxycholate, 0.1% SDS, 10% glycerol, 10 µg/mL leupeptin, 10 µg/mL aprotinin, 0.01 mM phenylmethylsulfonyl fluoride, 1 mg/mL pepstatin A, and 10 mM benzamidine. Centrifugation (REMI, Mumbai, India) at 23792 × *g* for 10 min at 4 °C was used to prepare the supernatant. Protein samples were estimated using the Bradford technique (Bio-Rad, Hercules, CA, USA).

Table 1: Demographic characteristics of Male Suicide and Male Control Subjects.

Suicide Group						
Subject No.	**Sex (M/F)**	**Age (Yr.)**	**PMI (h)**	**Brain pH**	**Cause of death**	**Psychiatric Diagnosis**
1	M	66	28	6.91	Acid Poisoning	Drug and alcohol abuse
2	M	45	24	6.23	Hanging	Marital disharmony
3	M	34	18	6.72	Jumped from height	Marital disharmony
4	M	23	21	6.92	Run over in railway track	No Psychiatric illness
5	M	59	28	6.95	Hanging	Major depression, agoraphobia
6	M	55	23	6.1	Jumped from height	Major depression
7	M	27	26	6.4	Run over in Metro rail	Drug and alcohol abuse
8	M	47	18	5.69	Hanging	Schizoaffective disorder
9	M	19	24	6.44	Drug overdose	Major depression, adjustment disorder
10	M	38	20	6.3	Hanging	Major depression, alcohol abuse
11	M	27	19	6.65	Run over in railway track	Familial disharmony
12	M	19	26	6.77	$CuSO_4$ Poisoning	Drug and alcohol abuse
13	M	72	23	6.55	Hanging	Familialdisharmony
14	M	52	20	6.32	Jumped from height	Post-traumatic stress disorder
15	M	47	22	6.2	Wrist cutting	No Psychiatric illness
16	M	46	20	6.52	Acid Poisoning	Major depression, agoraphobia
17	M	35	21	7	Run over in Metro rail	Familial disharmony
18	M	21	18	6.66	Hanging	Bipolar disorder
19	M	44	22	7.06	Run over in Metro rail	Post-traumatic stress disorder
20	M	23	16	6.71	Drug overdose	Major depression, adjustment disorder
Control Group						
Subject No.	**Sex (M/F)**	**Age (Yr.)**	**PMI (h)**	**Brain pH**	**Cause of death**	**Psychiatric Diagnosis**
21	M	75	25	6.25	Anaphylaxis	Normal
22	M	35	22	5.8	Lung cancer	Normal
23	M	18	14	7.02	Hypertensive heart	Normal
24	M	32	13	6.23	Hypoplastic coronary artery	Normal
25	M	45	26	6.2	Motor vehicle accident	Normal
26	M	67	21	6.52	Hypertrophic cardiomyopathy	Normal
27	M	34	15	6.35	Motor vehicle accident	Normal
28	M	29	15	6.64	Ovarian cancer	Normal
29	M	27	26	6.22	Atherosclerotic cardiovascular disease	Normal
30	M	34	18	6.1	Cardiac arrhythmia	Normal
31	M	54	24	6.43	Pneumonia	Normal
32	M	32	23	6.75	Subarachnoid hemorrhage	Normal
33	M	29	23	6.92	Atherosclerotic cardiovascular disease	Normal
34	M	32	25	6.95	Accidental trauma	Normal
35	M	25	17	6.1	Motor vehicle accident	Normal
36	M	65	16	6.4	Hypertensive heart	Normal
37	M	43	20	6.23	Liver cirrhosis	Normal
38	M	50	17	6.11	Struck by lightning	Normal
39	M	48	28	5.7	Cardiac arrhythmia	Normal
40	M	40	26	6.47	Struck by lightning	Normal

Immunoprecipitation of BDNF and NGF. Antibodies against BDNF and NGF (100:1 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were incubated with supernatant containing 100 µg of protein for 2 h at 4 °C.

Protein-A sepharose beads (Amersham, NJ, USA)were suspended in Tris-buffered saline (TBS) and then incubated at 4 °C for 1h. The pellet was obtained by centrifuging it for 30 s at 2,500 rpm and 4°C, and then washing it four times in TBS containing 0.5 mM Na_3VO_4 and 0.01 mM phenylmethylsulfonyl fluoride. Before Western blot analysisthe pellet was resuspended in 2× sample buffer and boiled for 5 min.

Western blotting of BDNF, TrkB, NGF, and TrkA proteins. All proteins were transferred onto nitrocellulose membranes after being electrophoresed on a 7.5% polyacrylamide gel (Mini-PROTEAN® Tetra Cell with Mini-Trans Blot®, Bio-Rad). The membranes were blocked using 5% non-fat dried milk. Then the membranes were incubated overnight at 4 °C with primary anti-BDNF polyclonal antibodies (1:1000 dilution in 3% BSA, Chemicon), anti-TrkB polyclonal antibodies(1:500 dilution in 3% BSA, Chemicon), NGF polyclonal antibodies (1:1000 dilution in 3% BSA, Chemicon) and anti-TrkA polyclonal antibodies (1:400 dilution in 3% BSA, Santa Cruz Biotechnology). 0.1 % Tween-20 was used to wash the nitrocellulose membranes. Next those membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-sheep IgG (1:1000) for 2 h at room temperature. Enhanced chemiluminescence (ECL) (Santa Cruz Biotechnology) was used to visualize immuno-reactive bands. Membranes were cleaned with stripping solution before being probed with anti-β-actin monoclonal antibody (1:10,000 dilution in 3% BSA, Sigma, St. Louis, MO, USA).

Using the OD of the associated β-actin band, the electrophoresis image analysis system (Smart View Pro Imager System, USA) calculated the OD of each protein. As a percentage of the control, the values are displayed.

Table 2: Demographic characteristics of Female Suicide and Female Control Subjects.

Suicide Group						
Subject No.	**Sex (M/F)**	**Age (Yr.)**	**PMI (h)**	**Brain pH**	**Cause of death**	**Psychiatric Diagnosis**
1	F	48	26	6.54	Wrist cutting	Drug and alcohol abuse
2	F	57	21	6.98	Acid Poisoning	Alcohol abuse
3	F	28	24	6.72	Runover in railway track	Major depression, adjustment disorder
4	F	21	26	6.87	Hanging	No Psychiatric illness
5	F	17	18	6.95	Acid Poisoning	Familial disharmony
6	F	36	19	6.54	$CuSO_4$ Poisoning	Major depression
7	F	19	26	6.49	Hanging	Alcohol abuse
8	F	63	18	5.94	Jumped from height	Schizoaffective disorder
9	F	54	23	5.67	Wrist cutting	Major depression, adjustment disorder
10	F	69	27	6.3	Jumped from height	Major depression, alcohol abuse
11	F	29	24	6.73	Run over in Metro rail	Familial disharmony
12	F	38	26	6.77	Hanging	Drug and alcohol abuse
13	F	61	29	6.65	Jumped from height	Schizoaffective disorder
14	F	47	22	6.32	Drug overdose	Familial disharmony
15	F	39	22	6.21	Hanging	No Psychiatric illness
16	F	28	20	6.52	Jumped from height	Major depression, agoraphobia
17	F	36	26	7.03	Wrist cutting	Familial disharmony
18	F	58	28	6.66	Hanging	Post-traumatic stress disorder
19	F	28	19	7.06	Run over in railway track	Schizoaffective disorder
20	F	32	22	5.42	Wrist cutting	Major depression, adjustment disorder
Control Group						
Subject No.	**Sex (M/F)**	**Age (Yr.)**	**PMI (h)**	**Brain pH**	**Cause of death**	**Psychiatric Diagnosis**
21	F	64	24	6.21	Motor vehicle accident	Normal
22	F	53	21	5.97	Drowning	Normal
23	F	36	17	7.33	Congenital heart disease	Normal
24	F	25	25	6.23	Cardiac arrhythmia	Normal
25	F	51	27	7.04	Accidental trauma	Normal
26	F	38	28	6.52	Motor vehicle accident	Normal
27	F	59	21	6.35	Accidental trauma	Normal
28	F	38	26	7.19	Blood cancer	Normal
29	F	27	24	6.22	Motor vehicle accident	Normal
30	F	26	17	5.98	Congenital heart disease	Normal
31	F	34	19	6.77	Pneumonia	Normal
32	F	28	29	5.33	Drowning	Normal
33	F	56	27	6.92	Congenital heart disease	Normal
34	F	48	17	6.95	Accidental trauma	Normal
35	F	66	18	7.11	Cardiac arrhythmia	Normal
36	F	54	26	7.4	Struck by lightning	Normal
37	F	26	10	6.43	Motor vehicle accident	Normal
38	F	71	15	6.11	Struck by lightning	Normal
39	F	41	25	7.34	Cardiac arrhythmia	Normal
40	F	14	24	6.47	Motor vehicle accident	Normal

Total mRNA isolation and RT-PCR of BDNF, NGF, TrkB, and TrkA. TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract total mRNA from 50–100 mg tissue of hippocampus and amygdala. BDNF, NGF, TrkB, and TrkA mRNA in each extraction were determined by real-time RT-PCR (Applied Biosystems, Foster City, CA, USA). Primers used for BDNF:5′-ATTAGGTGGCTTCATAGGAGAC-3′ (sense) with 5′-GAACAGAACAGAACAGAACAGG-3′ (antisense) and TrkB: 5′-TCTCTCGG TCTATGCCGTGGTGG-3′ (sense) with 5′-TCCAGGCACTTCCTCGTTCAGT-3′ (antisense). Similarly, the primers required for NGF: 5′-AGCGTAAT GTCCATGTTGTTCTAC-3′ (sense) with 5′-TGCTATCTGTGTACGGTTCTGC-3′ (antisense) and TrkA: 5′-CTTGCGCCGCATCCTGTCGT-3′(sense) with 5′-GCAGGCCGCGGAGGGTATTC-3′ (antisense). As an internal control GAPDH was also co-amplified with BDNF, NGF, TrkB, and TrkA mRNAs. GAPDH was amplified using the primers 5′-TTGCCATCAATGACCCCTTCA-3′ (sense) with 5′-CGCCCCACTTGATTTTGGA-3′ (antisense). According to the serial number from Genebank the primers were designed by AuGCT-Technology Company (Beijing, China). Each primer contained comparable G/C content to minimize variability in hybridization efficiency at the annealing temperature. The PCR mixture was amplified using 32 cycles of denaturation (94 °C, 15 s), annealing (60 °C, 30 s), and elongation (72 °C, 30 s). The reaction was stopped after a last elongation phase that lasted 5 minutes at 72 °C. The PCR results were seen using 1.5% agarose gel electrophoresis, and the density of each band was examined using a gel image analysis system (Smartview 2001, S/N: SV-0002202). By measuring the density ratio of each band of BDNF, TrkB, NGF, and TrkA mRNA to GAPDH mRNA, the level of the mRNA was determined.

Statistical evaluation. The Statistical Package for the Social Sciences (SPSS) 15.0 was used for statistical analysis. Every piece of information has been statistically examined using the student's *t*-test, correlation-coefficient (r). Statistical significance was defined as *P* values below 0.001 and 0.05. The demographics of healthy controls of both sexes and suicide victims were also evaluated statistically using an analysis of variance.

RESULTS

Demographic characteristic of postmortem brain of suicide victims and non-psychiatric healthy controls

of both sexes. Table 1 provides a summary of the demographic data for male suicide victims (n = 20) and male non-psychiatric healthy controls (n = 20). Male control individuals and suicide victims had corresponding mean postmortem intervals of 20.70 ± 1.06 h and 21.85 ± 0.76 h. For male control participants and suicide victims, the mean brain pH levels were6.36 ± 0.08 and 6.55 ± 0.07 respectively. Male participants were not significantly different from each other in terms of age ($F_{1,38}$ = 0.20; t = 0.59; df = 19, *P*< 0.05), postmortem interval ($F_{1,38}$ = 0.78; t = 1.05; df = 19, *P*< 0.05), or pH of the brain ($F_{1,38}$ = 2.75; t = 1.52; df = 19, *P*< 0.05).

Table 2 lists the demographic information for female suicide victims (n = 20) and female non-psychiatric healthy controls (n = 20). For female control subjects and suicide victims, the mean postmortem intervals were 22.00 ± 1.14 h and 23.30 ± 0.76 h respectively. Female control participants had a brain pH of 6.60 ± 0.13, while female suicide sufferers had a pH of 6.52 ± 0.10. Additionally, there were no significant differences between the groups of female participants in terms of age ($F_{1,38}$ = 0.22; t = 0.50; df = 19, *P*< 0.05), postmortem interval ($F_{1,38}$ = 0.90; t = 0.82; df = 19, *P*< 0.05), or brain pH ($F_{1,38}$ = 0.22; t = 0.47; df = 19, *P*< 0.05).

Gender-specific expressional alterations of BDNF, NGF, TrkB, and TrkA proteins in the hippocampus and amygdala of suicide victims and non-psychiatric healthy controls. Western blotting was used to examine sex-specific variations in BDNF and NGF expression in the hippocampus and amygdala regions. Male suicide victims showed significantly lower levels of BDNF and NGF in the hippocampus (t_{BDNF}= 12.82; df = 19; *P*<0.001 and t_{NGF}= 12.05; df = 19; *P*<0.001 respectively; Fig. 1A) and amygdala (t_{BDNF}= 9.43; df = 19; *P*<0.001 and t_{NGF}= 13.05; df = 19; *P*<0.001 respectively; Fig. 1B). When compared to healthy controls, female suicide victims exclusively showed these changes in the hippocampus(t_{BDNF}= 14.74; df = 19; *P*<0.001 and t_{NGF}= 10.40; df = 19; *P*<0.001 respectively; Fig. 1A). During the study of their receptors, lower expressions were also seen in the hippocampus and amygdala of male suicide individuals, with t_{TrkB} = 14.31; df = 19; *P*<0.001 and t_{TrkA}= 18.24; df = 19; *P*<0.001 in hippocampus and t_{TrkB}= 12.73; df = 19; *P*<0.001 and t_{TrkA}= 13.59; df = 19; *P*<0.001 in amygdala (Fig. 1A and 1B). However, among female suicide subjects, there was only a substantial drop in neurotrophins' receptors in the hippocampus(t_{TrkB}= 9.84; df = 19; *P*<0.001 and t_{TrkA}= 20.00; df = 19; *P*<0.001, Fig. 1A). Expression of BDNF, NGF, TrkB, and TrkA proteins were normalized against the internal control β-actin to determine their expression.

Gender-related mRNA expressional alteration of BDNF, NGF, TrkB, and TrkA in the hippocampus and amygdala of suicide victims and non-psychiatric healthy controls. It was also measured gender-related mRNA expressions in both hippocampal and amygdaloid parts of brain. Among the male suicide victims mRNA expressions of BDNF, NGF, TrkB, and TrkA were reduced in hippocampus (t_{BDNF}= 16.17; df = 19; *P*<0.001; t_{TrkB}= 12.39; df = 19; *P*<0.001; t_{NGF}= 15.81; df = 19; *P*<0.001 and t_{TrkA} = 13.89; df = 19; *P*<0.001; Fig. 2A) as well as in amygdala (t_{BDNF}= 14.19; df = 19; *P*<0.001; t_{TrkB}= 12.68; df = 19; *P*<0.001; t_{NGF}= 16.20; df = 19; *P*<0.001 and t_{TrkA} = 11.53; df = 19; *P*<0.001; Fig. 2B) and the differences were statistically significant.

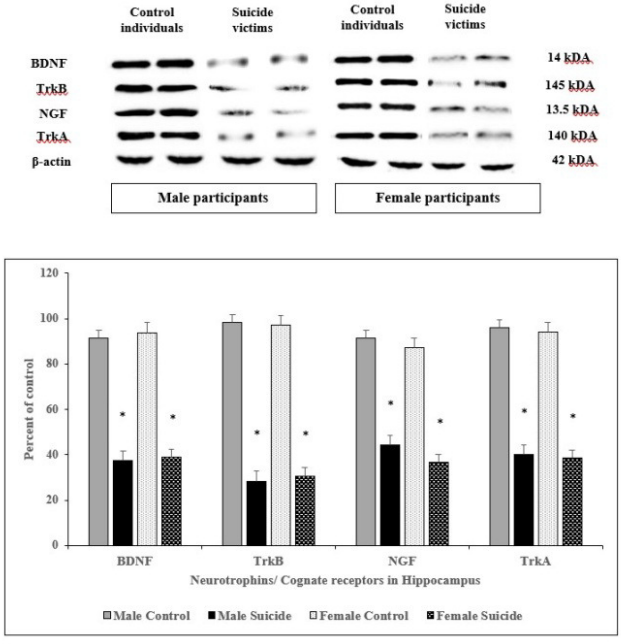

Fig. 1A. Representative bands of Western blot showing the protein levels of BDNF, TrkB, NGF, and TrkA in the hippocampus of suicide subjects of both sexes and non-psychiatric healthy controls. Data are the mean ± SD and **P*<0.001. Hippocampus samples were from 40 non-psychiatric healthy control individuals (20 males + 20 females) and 40 suicide victims (20 males + 20 females).

Among the female suicide victims altered mRNA expressions were restricted in hippocampus (t_{BDNF}= 15.17; df = 19; P<0.001; t_{TrkB}= 9.02; df = 19; P<0.001; t_{NGF}= 12.68; df = 19; P<0.001 and t_{TrkA} = 11.27; df = 19; P<0.001; Fig. 2A) only. The lengths of the amplified fragments for BDNF, NGF, TrkB, TrkA, and GAPDH were 178, 199, 79, 96, and 173 base pairs, respectively. As an internal control, the level of GAPDH mRNA was employed. Participants in the control and suicide groups did not exhibit any differences in GAPDH mRNA expression (t_{GAPDH} = 0.100917; df = 19; P = 0.46; Fig. 2A and 2B).

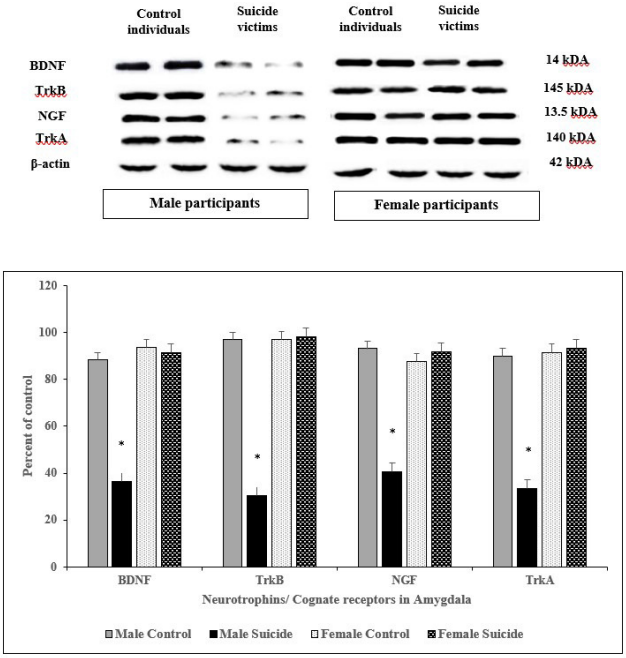

Fig. 1B. Representative bands of Western blot showing the protein levels of BDNF, TrkB, NGF, and TrkA in the amygdala of suicide subjects of both sexes and non-psychiatric healthy controls. Data are the mean ± SD and *P<0.001. Amygdala samples were from 40 non-psychiatric healthy control individuals (20 males + 20 females) and 40 suicide victims (20 males + 20 females).

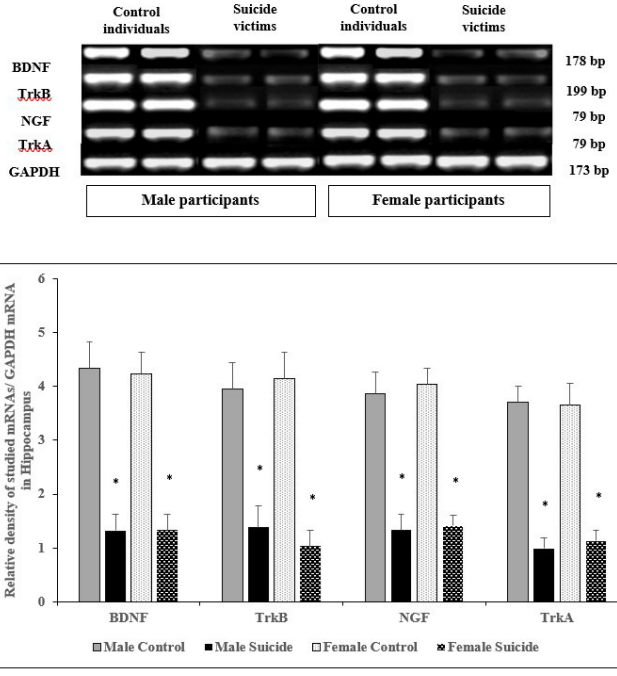

Fig. 2A. Representative mRNA levels of BDNF, TrkB, NGF, and TrkA in the hippocampus of male and female suicide subjects and normal controls. Data are the mean ± SD and *P<0.001. Hippocampus samples were from 40 non-psychiatric healthy control individuals (20 males + 20 females) and 40 suicide victims (20 males + 20 females).

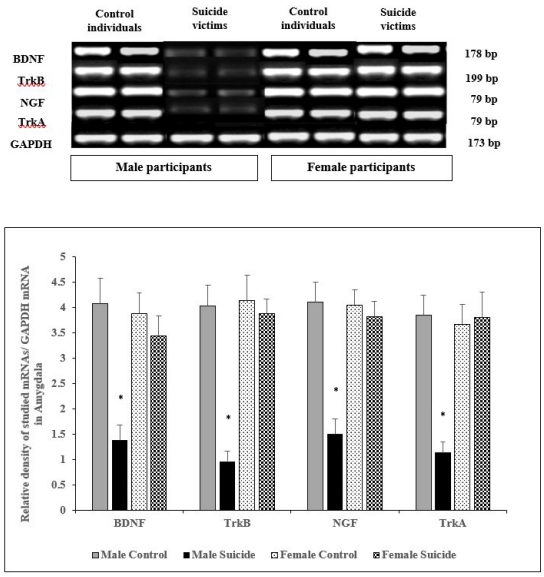

Fig. 2B. Representative mRNA levels of BDNF, TrkB, NGF, and TrkA in the amygdala of male and female suicide subjects and normal controls. Data are the mean ± SD and **P*<0.001. Amygdala samples were from 40 non-psychiatric healthy control individuals (20 males + 20 females) and 40 suicide victims (20 males + 20 females).

Correlations between BDNF, TrkB, NGF, and TrkA protein and mRNA levels in the hippocampus and amygdala of postmortem brains from suicide victims of both sexes. Positive correlations were observed between neurotrophins with their respective mRNA levels and the targeted receptors of neurotrophins with their respective transcript levels in both hippocampus and amygdala. In both male and female suicide victims, the BDNF and NGF protein levels in the hippocampus were positively linked with the corresponding mRNA levels(r_{BDNF} = 0.91; df = 19; *P*<0.001and r_{NGF} = 0.94; df = 19; *P*<0.001 and r_{BDNF} = 0.94; df = 19; *P*<0.001and r_{NGF} = 0.94; df = 19; *P*<0.001 respectively). Similarly, the positive correlations were also observed in the hippocampus among the neurotrophin receptors of both sexes(r_{TrkB}= 0.93, df = 19; *P*<0.001 and r_{TrkA} = 0.85; df = 19; *P*< 0.001 in male suicide subjects and r_{TrkB}= 0.94, df = 19; *P*<0.001 and r_{TrkA} = 0.96; df = 19; *P*< 0.001 in female suicide subjects). Interestingly we observed a different result in amygdala. Instead of female suicide victims only males were showing positive correlations between neurotrophins and their mRNA (r_{BDNF} = 0.92; df = 19; *P*<0.001 and r_{NGF} = 0.96; df = 19; *P*<0.001) as well as neurotrophin receptors and their respective mRNA(r_{TrkB}= 0.95, df = 19; *P*<0.001 and r_{TrkA} = 0.93; df = 19; *P*< 0.001).

DISCUSSION

Recent advances in the study of mood disorders have focused a lot of attention on the hippocampus region of the brain. Although it almost certainly isn't the only source of the wide range of symptoms associated with depression, the stress-sensitive hippocampal region may be crucial to the development of depressive illness (Banerjee *et al.*, 2012a; Banerjee *et al.*, 2014; Dwivedi *et al.*, 2009). Additionally known as the centres for emotion and anxiety, the amygdaloid structures are also thought to contribute to the motivating elements of alcohol consumption (Koob, 2003; Pandey, 2004).

In order to regulate structural, synaptic, and morphological plasticity as well as to regulate the strength and quantity of synaptic connections and neurotransmission, growth factors called neurotrophins are essential (Thoenen, 2000). Neurotrophins play a biological role throughout a person's whole lifespan by contributing to the preservation of neuronal functions, the structural integrity of neurons, and neurogenesis in the adult central nervous system (Cooper *et al.*, 1996). Due to the following factors, there is growing interest in researching how BDNF and NGF affect suicide in recent years:1) suicide has a significant component of depression, 2) stress has a substantial correlation with suicide, and 3) sadness and stress both affect the production of BDNF and NGF (Dwivedi, 2010).

In the current investigation, Western blot and real-time PCR were used to quantitatively assess the levels of BDNF, NGF, and their cognate receptors TrkB and TrkA in the hippocampus and amygdala of 20 male and 20 female participants who died by suicide as well as 20 male and 20 female non-psychiatric healthy controls. According to Western blotting analysis in the hippocampus region, both male and female suicide subjects in this study demonstrated a statistically significant decline in the protein levels of BDNF, NGF, and their corresponding receptors. This conclusion is consistent with our earlier findings (Banerjee *et al.*, 2012a; Banerjee *et al.*, 2013b). Though the protein levels of neurotrophins and their respective receptors reduced in male amygdaloid region but those remain unchanged in the amygdala of female suicide subjects. Such findings of present study interestingly corroborate with the results of Kozicz *et al.* (2008) who have observed the gender-specific expressional alteration of BDNF in suicide subjects and found that the BDNF level reduced markedly in the midbrain of male suicide

subjects than females, showing a potential sex effect in the control of neurotrophin expression in suicide subjects.

The mRNA transcription of BDNF and its receptor TrkB, as well as NGF and its receptor TrkA, were shown to be statistically significantly lower in the hippocampus and amygdala regions of solely male suicide participants than in female suicide subjects, with the changes being confined to the hippocampus. Hippocampal BDNF and NGF play a critical role in stress and depressive disorders, according to numerous studies. A reduction in hippocampus BDNF and NGF mRNA is linked to acute and chronic stress in rats. On the other hand, taking an antidepressant enhances the expression of BDNF and NGF as well as their corresponding receptors (Banerjee *et al.*, 2013b; Nibuya *et al.*, 1995). Hippocampal neurotrophins may also play a functional role in depression, according to human studies. BDNF, NGF, TrkB, and TrkA levels in the hippocampus region of postmortem suicide victims' brains were shown to be reduced (Banerjee *et al.*, 2012a; Banerjee *et al.*, 2013b).

The current work demonstrates male biasness in amygdala-specific expressional changes of neurotrophins and their corresponding receptors. Female suicide victims' amygdaloid regions continue to express BDNF, TrkB, NGF, and/or TrkA at both the protein and mRNA levels. Since the amygdala has allegedly been linked to stress-related illnesses like depression in humans, this is very intriguing (Drevets, 2000).

Since the amygdala has been demonstrated to play a key role in fear-dependent learning (Rattiner *et al.*, 2004) and it contains significant levels of BDNF mRNA and protein (Conner *et al.*, 1997), BDNF modulation has been discussed as a potential correlate of stress-dependent learning and the ensuing behavioural changes (Rattiner *et al.*, 2005; Williams *et al.*, 2005).

From the clinical correlations of the current study, it can be assumed that the suicidal tendency among both sexes results in (i) inadequate availability of BDNF and NGF, (ii) deficiency of neurotrophin receptors like TrkB and TrkA in the hippocampus of brain, and/or (iii) stress-induced dysregulation in neurotrophin mediated signalling milieuin hippocampus. Though among the males the depression induction phenomenon via amygdaloid BDNF-TrkB and NGF-TrkA signalling dysregulation possibly enhanced suicidal tendency but unchanged amygdaloid neurotrohins and their cognate receptors in female brain may ameliorate the vulnerability to suicidal behaviour in female population.

Though the present findings are of particular relevance for understanding the gender-related alterations in neurochemical milieu of suicide, it was beyond the scope of the present work to conduct an in-depth investigation of the molecular mechanisms of these neurochemical alterations associated with gender-specific suicidal vulnerability. Future research is required to not only comprehend the neurochemical factors that contribute to suicide in the hippocampus and amygdala, but also to develop therapeutic approaches that target those molecular pathways to stop suicidal consequences.

FUTURE SCOPE

We need to evaluate other critical factors of brain related to gender-specific depression induction towards suicide and sex-specific antidepressant response to ameliorate suicidal tendency among individuals.

Acknowledgement. We would especially like to thank the administrators of Parimal Mitra Smriti Mahavidyalaya, Malbazar, Jalpaiguri (WB), Calcutta Medical College Hospital under West Bengal University of Health Sciences, Kolkata (WB), Department of Zoology, University of Calcutta (WB) and Department of Instrumentation Science, Jadavpur University (WB).

Conflicts of Interest. None.

REFERENCES

Addis, M. E. and Mahalik, J. R. (2003). Men, masculinity, and the contexts of help-seeking. *American Psychologist*, *58*(1), 5-14.

Banerjee, R., Ghosh, A. K., Ghosh, B., Bhattacharya, S. and Mondal, A. C. (2012a). Reduced expression profile of neurotrophins and their cognitive receptors in the hip-pocampal region of postmortem suicidal brain. *Nerve*, *1*(1), 13–17.

Banerjee, R., Ghosh, A. K. and Mondal, A. C. (2012b). Effects of chronic stress and antidepressant treatment on behavioral, physiological and neurochemical aspects in male and female rats. *Al Ameen J Med Sci., 5*(2), 165-176.

Banerjee, R., Ghosh, A. K., Ghosh, B. and Mondal, A. C. (2013a). Role of NGF/Trk A signaling milieu in depressogenic induction of rat brain. *Al Ameen J of Med Sci., 6*(4), 355-363.

Banerjee, R., Ghosh, A. K., Ghosh, B., Bhattacharya, S. and Mondal, A. C. (2013b). Decreased mRNA and Protein Expression of BDNF, NGF, and their Receptors in the Hippocampus from Suicide: An Analysis in Human Postmortem Brain. *Clin Med Ins Path., 6*, 1-11.

Banerjee, R., Hazra, S., Ghosh, A. K. and Mondal, A. C. (2014). Chronic administration of BM increases BDNF protein and mRNA expressions: A study in CUS induced animal model of depression. *Psychiatry Investig.*, *11*(3), 297-306.

Brent, D. A. and Moritz, G. (1996). Developmental pathways to adolescent suicide In Cicchetti, D., & Toth, L. (Eds) *Adolescence: Opportunities and challenges. Rochester symposium on developmental psychopathology.* Rochester, NY: University of Rochester Press, *7,* 233-258.

Chehil, S. and Kutcher, S. (2012). *Suicide risk management: A manual for health professionals* Sussex: John Wiley & Sons, Ltd., *2,* 1-176.

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M. and Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J. Neurosci., 17,* 2295–2313.

Conner, K. R., Conwell, Y. and Duberstein, P. R. (2001). The validity of proxy-based data in suicide research: a study of patients 50 years of age and older who attempted suicide. II. Life events, social support and suicidal behavior. *Acta Psychiatr Scand.*, *104*(6), 452–457.

Cooper, J. D., Skepper, J. N., Berzaghi, M. D., Lindholm, D. and Sofroniew, M. V. (1996). Delayed death of septal

cholinergic neurons after excitotoxic ablation of hippocampal neurons during early postnatal development in the rat. *Exp Neurol., 139,* 143–155.

Denning, D. G., Conwell, Y., King, D. and Cox, C. (2000). Method choice, intent, and gender in completed suicide. *Suicide and Life-Threatening Behavior*, *30*(3), 282-288.

Drevets, W. C. (2000). Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Prog. Brain Res., 126*, 413–431.

Dwivedi, Y., Rizavi, H. S., Conley, R. R., Roberts, R. C., Tamminga, C. A. and Pandey, G. N. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry*, *60*(8), 804–815.

Dwivedi, Y., Rizavi, H. S., Zhang, H., Mondal, A. C., Roberts, R. C., Conley, R. R. and Pandey, G. N. (2009). Neurotrophin Receptor Activation and Expression in Human Postmortem Brain: Effect of Suicide. *Biol Psychiatry, 65*(4), 319–328.

Dwivedi, Y. (2010). Brain-derived neurotrophic factor and suicide pathogenesis. *Ann Med., 42*, 87–96.

First, M. B., Spitzer, R. L., Miriam, G. and Williams, J. B. W. (2002). Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P) New York: Biometrics Research, New York State Psychiatric Institute.

Hunsberger, J., Austin, D. R., Henter, I. D. and Chen, G. (2009). The neurotrophic and neuroprotective effects of psychotropic agents. *Dialogues Clin Neurosci., 11*(3), 333-348.

Koob, G. F. (2003) Alcoholism: allostasis and beyond. *Alcohol Clin Exp Res*., *27,* 232–243.

Kozicz, T., Tilburg-Ouwens, D., Faludi, G., Palkovits, M. and Roubos, E. (2008). Gender-related urocortin 1 and brain-derived neurotrophic factor expression in the adult human midbrain of suicide victims with depression. *Neurosci.,152,* 1015–1023.

Maftoon, P., Shakouri, N. and Nazari, O. (2014). Limbic System and Second Language Acquisition: Reconsidering the Role of Emotion. *Biological Forum – An International Journal, 6*(2), 398-403.

McAllister, A. K. (2001). Neurotrophins and neuronal differentiation in the central nervous system. *Cell Mol Life Sci*., *58*(8), 1054–1060.

Nibuya, M., Morinobu, S. and Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci., 15*, 7539–7547.

Pandya, M., Altinay, M., Malone Jr., D. A. and Anand, A. (2012). Where in the Brain Is Depression? *Curr Psychiatry Rep., 14*(6), 634–642.

Pandey, S. C. (2004). The gene transcription factor cyclic AMP responsive element binding protein: role in positive and negative affective states of alcohol addiction. *Pharmacol Ther., 104*, 47–58.

Qin, P., Agerbo, E., Westergard-Nielsen, N. and Eriksson, T. (2000). Gender differences in risk factors for suicide in Denmark. *Br. J. Psychiatry, 177*, 546–550.

Rattiner, L. M., Davis, M. and Ressler, K. J. (2004). Differential regulation of brain-derived neurotrophic factor transcripts during the consolidation of fear learning. *Learn. Mem., 11,* 727–731.

Rattiner, L. M., Davis, M. and Ressler, K. J. (2005). Brain-derived neurotrophic factor in amygdala-dependent learning. *Neuroscientist*., *11*, 323–333.

Rich, C. L., Ricketts, J. E., Fowler, R. C. and Young, D. (1988). Some differences between men and women who commit suicide. *Am J Psychiatry, 145*(6), 718-722.

Richthofen, S., Lang, U. E. and Hellweg, R. (2003). Effects of different kinds of acute stress on nerve growth factorcontent in rat brain. *Brain Res., 987*, 207–213.

Salzman, S., Endicott, J., Clayton, P. and Winokur, G. (1983). Diagnostic Evaluation After Death (DEAD). Rockville, MD, National Institute of Mental Health, Neuroscience Research Branch.

Schweitzer, I., Tuckwell, V., Ames, D. and O'Brien, J. (2001). Structural neuroimaging studies in late-life depression: a review. *World J Biol Psychiatry, 2*(2), 83–88.

Shenassa, E. D., Catlin, S. N. and Buka, S. L. (2003). Lethality of firearms relative to other suicide methods: a population-based study. *J Epidemiol Community Health*, *57*(2), 120-124.

Thoenen, H. (2000). Neurotrophins and activity-dependent plasticity. *Prog Brain Res., 128*, 183–191.

Tóth, M. D., Ádám, S., Birkás, E., Székely, A., Stauder, A. and Purebl, G. (2014). Gender differences in deliberate self-poisoning in Hungary analyzing the effect of precipitating factors and their relation to depression. *Crisis, 5*(3), 145–153.

Vargas-Medrano, J., Diaz-Pacheco, V., Castaneda, C., Miranda-Arango, M., Longhurst, M. O., Martin, S. L., Ghumman, U., Mangadu, T., Chheda, S., Thompson, P. M. and Gadad, B. S. (2020). Psychological and neurobiological aspects of suicide in adolescents: Current outlooks. *Brain, Behav., & Immun. – Health, 7*, 1-11.

WHO (2014). *Preventing Suicide: A Global Imperative.* WHO, Ed.; World Health Organization: Geneva, Switzerland, 1-89.

Williams, L. M., Kemp, A. H., Felmingham, K., Barton, M., Olivieri, G., Peduto, A., Gordon, E. and Bryant, R. A. (2005). Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. *Neuroimage, 29*(2), 347–357.

How to cite this article: Ritabrata Banerjee, Madhusudan Das and Anup Kumar Ghosh (2023). Gender-related Alterations in Neurochemical Milieu of Suicide: An Analysis in Human Postmortem Brain. *Biological Forum – An International Journal, 15*(1): 601-609.