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Short Communication

Elevated Serotonin and 5-HIAA in the Brainstem and Lower Serotonin Turnover in the Prefrontal Cortex of Suicides

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Low serotonin (5-hydroxytryptamine, 5-HT) neurotransmission is hypothesized in the pathophysiology of suicide (for review see Bach and Arango, 2012; Mann, 2003; Träskman et al., 1981). Cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) in depressed suicide attempters is lower compared to controls (Lidberg et al., 2000; Mann et al., 1996; Mann and Malone, 1997; Placidi et al., 2001; Träskman et al., 1981). In four out of five postmortem studies of brainstem (Beskow et al., 1976; Bourne et al., 1968; Lloyd et al., 1974; Pare et al., 1969; Shaw et al., 1967), suicides had less 5-HT or 5-HIAA compared to controls. In contrast, most postmortem studies report no differences in cortical 5-HT or 5-HIAA in suicides compared to controls (Arango and Mann, 1992; Arato et al., 1987; Arranz et al., 1997; Beskow et al., 1976; Cochran et al., 1976; Mahadik et al., 1988).

5-HT in the forebrain is synthesized by brainstem dorsal and median raphe nuclei (DRN and MRN) 5-HT neurons. Tryptophan hydroxylase 2 (TPH2) is the neuron-specific, rate-limiting enzyme for the synthesis of 5-HT (Alenina et al., 2009; Patel et al., 2004; Zhang et al., 2004). We found more 5-HT neurons (Underwood et al., 1999), more TPH2 protein (Boldrini et al., 2005; Underwood et al., 1999), and mRNA (Bach-Mizrachi et al., 2006, 2008) in DRN and MRN of suicides. We sought to reconcile these three sets of observations in suicides: low 5-HIAA or 5-HT in brainstem, unchanged levels in the cortex, and more 5-HT neurons containing more TPH2. We, therefore, performed a pilot study measuring 5-HT and 5-HIAA by high pressure liquid chromatography (HPLC), sampling the brainstem along the axis of DRN and MRN, and in dorsolateral prefrontal cortex (PFC), in unmedicated controls and depressed suicides, with postmortem intervals of less than 24 h.

All procedures for brain collection and psychological autopsy were approved by the applicable Institutional Review Boards. This study included eight non-psychiatric sudden death controls and six suicides (Table I). Psychiatric diagnosis in the suicides and absence of diagnoses in the controls was determined by the Structured Clinical Interview for DSM IV (SCID-I and II) as part of a psychological autopsy described elsewhere (Kelly and Mann, 1996). All brains were free of gross neuropathology and had negative brain toxicology for psychoactive and neurotoxic drugs. Postmortem interval (PMI) was not different between groups (C: 12.4 ± 3.7 h; S: 10.5 ± 6.1 h, $P = 0.5$).

The brainstem was dissected at autopsy, frozen immediately and stored at −80°C until sectioning in a cryostat. Transverse sections were cut at 60 μm thickness and stored in Eppendorf tubes at −80°C until assayed. pH was measured in cerebellar tissue (Harrison et al., 1995). Sections were collected every millimeter corresponding to 16–20 sections per case. Sets of adjacent sections, stained for Nissl substance, were used to help identify the neuroanatomical levels examined. The goal was to ensure comparable coverage of the raphe along the rostrocaudal axis to account for its anatomical variability. Brodmann Area 9 (BA9) was dissected frozen from coronal slabs of the hemisphere, meninges were removed and

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TABLE I. Subject demographics

Case	Subject	Age	Sex	PMI	Brain pH	Manner of death	Brain toxicology	Axis1
1	Control	18	Male	16	6.8	MVA-pedestrian	Clear	None
2	Control	18	Male	11	7.07	Accidental fall from height	Clear	None
3	Control	19	Male	13	6.84	CV disease	Clear	None
4	Control	32	Male	13	6.75	CV disease	Lidocaine	None
5	Control	51	Female	7	6.7	MVA-pedestrian	Clear	None
6	Control	54	Male	13	6.34	cv disease	Clear	None
7	Control	69	Male	8	5.8	Gun shot wound	Clear	None
8	Control	75	Female	18	6.2	MVA-pedestrian	Clear	None
9	Suicide	26	Male	22	6.8	Hanging-suicide	Clear	Pathological gambling
10	Suicide	33	Male	6	6.7	Hanging-suicide	Clear	Adjustment disorder
11	Suicide	42	Male	7	6.55	Hanging-suicide	Clear	MDD
12	Suicide	46	Male	6	6.6	Jump from height-suicide	Clear	Schizophrenia
13	Suicide	17	Male	12	6.4	Acid ingestion-suicide	Clear	MDD
14	Suicide	64	Male	10	6.8	Acid ingestion-suicide	Clear	MDD

CV: cardiovascular disease; MVA: motor vehicle accident; MDD: major depressive disorder.

samples were dissected as much as possible from gray matter.

5-HT and 5-HIAA were measured in brainstem sections and BA9 using reverse-phase HPLC with electrochemical detection. The samples were homogenized in 0.5 ml ice-cold 0.4M perchloric acid, centrifuged (5 min at 14,000*g*) and a 50 μl aliquot of the supernatant was injected over a Waters HPLC system. The mobile phase contained 0.75 mM sodium phosphate (pH 3.1), 1.4 mM 1-octanesulfonic acid, 10M ethylenediaminetetraacetic acid (EDTA), and 8% acetonitrile. The flow rate was 1.0 ml/min. A standard curve was generated with external standards. Values were calculated based on peak area and compared to the standard calibration. The inter- and intra-assay coefficients of variation of the assay were <5%. The sensitivity was < 0.5 pmol.

Analysis of Variance (ANOVA, SPSS Statistics Version 17.0 software) was used to determine the statistical significance of differences in total amount of 5-HT or 5-HIAA with fixed variables being group (control or suicide) and covariates being age, PMI, pH, or neuroanatomical level after matching based on adjacent Nissl stained sections. Effects of age, PMI, or pH were determined using regression analysis.

Higher 5-HT in rostral brainstem was consistent with the greater number of 5-HT synthesizing neurons in the midbrain. 5-HT correlated with 5-HIAA along the rostrocaudal axis ($r = 0.837$, $P = 0.001$) of the raphe nuclei measured. Compared with nonpsychiatric control cases, suicides had four times as much total 5-HT (nonpsychiatric controls: 271 ± 58 vs. suicides: 1091 ± 280 pmol/mg protein, $t = -2.87$, $P = 0.017$) and 1.5 times more 5-HIAA (nonpsychiatric controls: 4158 ± 534 vs. suicides: 6404 ± 898 pmol/mg protein $t = -2.15$, $P = 0.05$). The difference in 5-HT between controls and suicides was present throughout the rostrocaudal extent of the brainstem samples (Fig. 1; $F = 95.9$, $P < 0.0001$). The differences in 5-HT and 5-HIAA amounts in suicides and controls were independent of age and postmortem interval ($P > 0.05$). The sample size was too small to determine a relationship of MDD or other Axis 1 diagnoses to either 5-HT or 5-HIAA. To index 5-HT turnover, we calculated the ratio of 5-HIAA to 5-HT. The 5-HIAA:5-HT ratio in the brainstem was 15.3 in controls and 5.9 in suicides (co-varying for age and sex, $t = 2.76$, $P = 0.02$).

5-HT in prefrontal cortex (BA9) of suicides was comparable to nonpsychiatric controls (controls: 1.6 ± 0.5 vs. suicides: 2.1 ± 1.9 pmol/mg protein; $F = 0.8$, $P = 0.376$). Mean 5-HIAA in suicides was 42% of controls but was not statistically significant (controls: 11.6 ± 9.6 vs. suicides: 5.6 ± 4.4 pmol/mg protein, $F = 2.1$, $P = 0.174$), however, the mean 5-HIAA:5-HT ratio in BA9 was much lower in suicides (6.8 in controls vs. 2.8 in suicides, $t = 2.1$, $P = 0.05$). Cortical 5-HIAA levels are comparable with published values (Cheetham et al., 1989; Stanley et al., 1985) suggesting that assay sensitivity and tissue quality are comparable to other studies. Our observed difference in mean cortical 5-HIAA between suicides and controls, although not statistically significant, is comparable to differences observed by others (Beskow et al., 1976; Crow et al., 1984; Owen et al., 1983). In suicides, brainstem 5-HIAA and 5-HT were negatively correlated with cortical 5-HIAA and 5-HT (5-HT: $r = -0.79$, $P = 0.02$, 5-HIAA: $r = -0.85$, $P = 0.007$). The same was true for controls (5-HT: $r = -0.85$, $P = 0.007$; 5-HIAA: $r = -0.79$, $P = 0.02$).

We found more brainstem 5-HT and 5-HIAA in suicides compared with nonpsychiatric, sudden death controls. The difference was found throughout the rostrocaudal extent of the brainstem sampled, suggesting that 5-HT synthesis in suicides is greater within all DRN subnuclei and the MRN compared with controls. In both brainstem and prefrontal cortex (PFC), the ratio of 5-HIAA:5-HT was lower in suicides compared with controls suggesting a lower rate of turnover in suicides. However, since brainstem 5-HIAA is still 50% higher in suicides than controls, the results do not indicate lower 5-HT neurotransmission in the brainstem of suicides. In contrast to the brainstem, there was no difference in 5-HT in PFC between

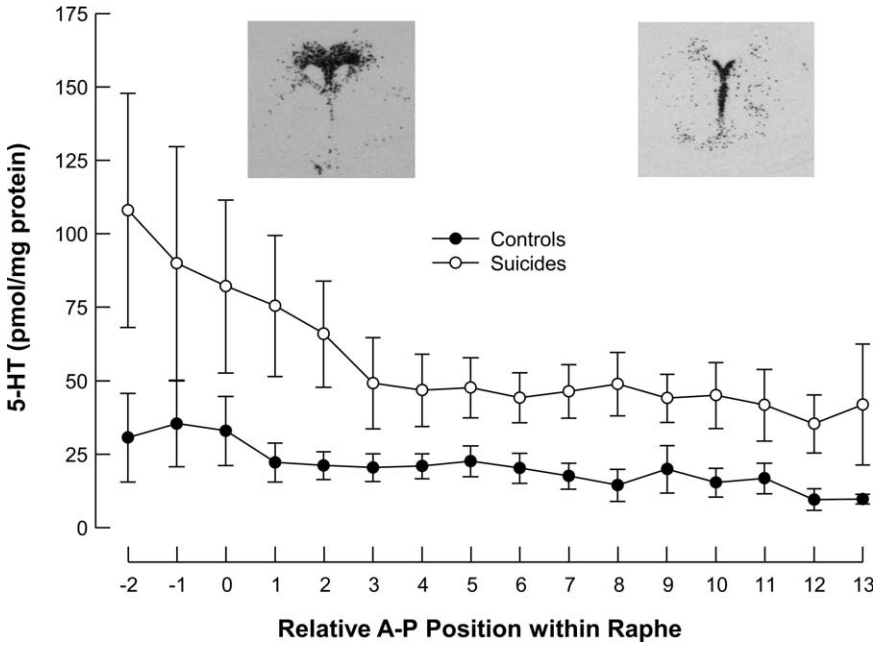

Fig. 1. Distribution of 5-HT in the brainstem. Note that suicides (empty circles) have more 5-HT throughout the rostrocaudal extent of the DRN than controls (filled circles). Rostral is to the left, 0 mm is the DRN peak area, and caudal is to the right; the inserts are corresponding autoradiograms of TPH2 mRNA. These plots include the male subjects only; the two control females were excluded from the plot but not the analyses because their values were markedly higher than the males.

suicides and controls suggesting that the extra brainstem 5-HT in suicides is not transported to or released from nerve terminals in the PFC.

Our finding of more brainstem 5-HT and 5-HIAA in suicides is not in agreement with most previous studies. Unlike those studies, we excluded subjects with long postmortem interval (>24 h) and either a positive history of psychotropic use or toxicology screen of brain tissue, factors that can lower 5-HT or 5-HIAA levels. We also assayed multiple anatomical levels in the brainstem to encompass the rostral-caudal extent of the DRN and MRN to avoid the risk of systematic, or even random, errors in tissue dissection. The suicide group in our study has a 1.9 h shorter mean PMI than controls, which was not statistically different and does not likely explain the differences between groups in 5-HT or 5-HIAA. Postmortem studies report that 5-HT decreases initially (Kontur et al., 1994; McIntyre and Stanley, 1984; Palmer et al., 1988) and is largely stable thereafter; 5-HIAA was reported to either increase or remain unchanged. Therefore, the 5-HT and 5-HIAA measures with the 6–22 h range of PMIs in our study are likely stable. Though postmortem degradation would presumably affect both groups equally, we used PMI as a statistical covariate.

Of note, one study found more CSF 5-HT and no difference in CSF 5-HIAA in MDD compared to nonpsychiatric controls (Gjerris et al., 1987) raising the possibility that the diagnosis of MDD is associated with elevated 5-HT levels. Our study included only three cases with MDD, and we saw comparable 5-HT and 5-HIAA levels in the non-MDD and MDD cases. Nevertheless, the comparable variability in the measures of 5-HT in the suicides to that of controls suggests that the levels of 5-HT and 5-HIAA in the suicide group are relatively homogeneous regardless of the different diagnoses. The higher 5-HT in the brainstem of suicides we find is consistent with our observations of more 5-HT neurons, and greater TPH2 mRNA and protein in the brainstem of suicides (Bach-Mizrachi et al., 2006, 2008; Boldrini et al., 2005; Underwood et al., 1999). There is other evidence of more 5-HIAA in MDD (reviewed in Andrews and Thomson, 2009). Patients with MDD have higher 5-HIAA in jugular venous blood, argued to reflect higher brain 5-HT neurotransmission and turnover (Barton et al., 2008). While findings of more 5-HT or 5-HIAA in blood and CSF of depressed subjects are not anatomically specific, and certainly not directly comparable to our measures in postmortem brain tissue, they lend support to a possible contribution of MDD to the elevated 5-HT that we detected. More cases are needed to distinguish effects of major depression from effects of suicide.

The main limitation of this pilot study is small sample size. Some of the previous studies in brainstem had comparable sample sizes: (Lloyd et al., 1974, five suicides, diagnosis not provided) and, (Cochran et al., 1976, 10 depressed suicides). Other studies (Beskow

et al., 1976; Bourne et al., 1968; Pare et al., 1969; Shaw et al., 1967) had more cases, but also had a mixture of diagnoses and were further confounded by either long PMIs or positive toxicology. None of these studies sought to distinguish potential effects of suicide from those of MDD or other diagnoses. In fact, many of the suicides in these studies had diagnoses other than MDD, suggesting that the reported reduction in 5-HT or 5-HIAA in the brainstem in these studies was associated with suicide rather than MDD (Mann et al., 1989). In PFC, we did not find significant differences in 5-HT or 5-HIAA in suicides; this was not likely explained by the small number of cases since a separate analysis of PFC in 35 controls and 37 suicides in our database also did not demonstrate a significant difference. More 5-HT in the brainstem and no change in the cortex of suicides, independent of diagnosis, are suggestive of deficits in serotonergic neurotransmission. A larger number of cases including suicides with MDD and MDD subjects that did not suicide would confirm that the upregulation of 5-HT demonstrated here is a biological correlate of suicide.

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