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Research report

A morphometric, immunohistochemical, and in situ hybridization study of the dorsal raphe nucleus in major depression, bipolar disorder, schizophrenia, and suicide

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ABSTRACT

Background: Several lines of evidence implicate 5-hydroxytryptamine (5-HT, serotonin) in the pathophysiology of mood disorders and suicide. However, it is unclear whether these conditions include morphological involvement of the dorsal raphe nucleus (DRN), the origin of most forebrain 5-HT innervation.

Method: We used morphometric, immunohistochemical, and molecular methods to compare the DRN in post-mortem tissue of 50 subjects (13 controls, 14 major depressive disorder [MDD], 13 bipolar disorder, 10 schizophrenia; 17 of the cases died by suicide). NeuN and PH8 antibodies were used to assess all neurons and serotonergic neurons respectively; 5-HT_{1A} autoreceptor expression was investigated by regional and cellular in situ hybridization. Measurements were made at three rostrocaudal levels of the DRN.

Results: In MDD, the area of the DRN was decreased. In bipolar disorder, serotonergic neuronal size was decreased. Suicide was associated with an increased DRN area, and with a higher density but decreased size of serotonergic neurons. Total neuronal density and 5-HT_{1A} receptor mRNA abundance were unaffected by diagnosis or suicide. No changes were seen in schizophrenia.

Conclusion: The results show that mood disorders and suicide are associated with differential, limited morphological alterations of the DRN. The contrasting influences of MDD and suicide may explain some of the discrepancies between previous studies, since their design precluded detection of the effect.

1. Introduction

The 5-hydroxytryptamine (5-HT; serotonin) neurons that innervate the forebrain lie in the rostral raphe nuclei of the brainstem (Hornung, 2003; Molliver, 1987), with the dorsal raphe (DRN) being the largest nucleus (Baker et al., 1991). The DRN provides the main input to the frontal cortex (McQuade and Sharp, 1997) and an enlarged lateral subdivision characterises primates, including man (Hornung, 2003). In the DRN cells projecting to prefrontal cortex are preferentially found in more rostral, medial, and ventral subdivisions, and around half are non-serotonergic (Del Cid-Pellitero and Garzón, 2011; Wilson and Molliver, 1991). Afferent projections to the DRN are primarily from the limbic system (Hornung, 2003) but there is also a reciprocal innervation of the DRN from prefrontal cortex which modulates neuronal activity (Celada et al., 2001).

The 5-HT system has been implicated in many psychiatric disorders, including mood disorders (Barton et al., 2008; Deakin, 1998; Jans et al., 2007; Mahmood and Silverstone, 2001), and in suicide (Ernst et al., 2009; Mann et al., 1989; Placidi et al., 2001). The evidence is diverse, and includes alterations in 5-HT metabolism, 5-HT receptors and transporters, and associations with serotonergic gene polymorphisms. There is also evidence of decreased neuronal density and

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Table 1
Demographics of subjects studied.

	Control	Schizophrenia[a]	Bipolar disorder[b]	Major depression
Subjects	13	10	13	14
Age (years)	48.7 (10.8)	43.2 (13.0)	42.4 (12.2)	46.6 (9.7)
Sex (M, F)	8, 5	8, 2	8, 5	8, 6
Brain pH	6.25 (0.24)	6.12 (0.29)	6.17 (0.25)	6.19 (0.22)
Fresh brain weight (g)	1521 (167)	1516 (98)	1444 (180)	1439 (133)
Freezer storage (months)[c]	92.7 (8.1)	102.1 (5.6)	102.8 (4.7)	95.6 (10.0)
Suicides	0	3	8	6
Onset of illness (years)	–	22.9 (8.0)	21.4 (8.9)	34.4 (13.7)
Duration of illness (years)	–	21.0 (10.7)	21.6 (9.7)	12.3 (11.4)
Lifetime antipsychotic dose[d]	0	47,800 (54,788)	23,492 (6868)	0
Antipsychotics ever	0	10	11	0
Antidepressants ever	0	3	6	9
Mood stabilisers ever	0	0	8	2
Current substance abuse or dependence	0	2	4	3

Values are mean (S.D.) where appropriate.
[a] Subtypes: paranoid (n = 2), undifferentiated (n = 8).
[b] 10 subjects had psychotic features.
[c] Differs between groups (ANOVA $p = 0.003$; controls < schizophrenia ($p = 0.005$) and bipolar disorder ($p = 0.001$).
[d] Fluphenazine equivalents (g).

serotonergic abnormalities in prefrontal cortex of depressed suicides (Underwood et al., 2011).

In contrast, the morphology and cytoarchitecture of the DRN in these disorders have received limited attention. The existing studies have utilised conventional stains (Baumann et al., 2002; Underwood et al., 1999), or antibodies detecting tryptophan hydroxylase (TPH) to identify 5-HT neurons (Hendricksen et al., 2004; Syed et al., 2005; Underwood et al., 1999); there are also studies which have used other 5-HT markers (e.g. 5-HT_{1A} autoreceptors; Arango et al., 2001; Boldrini et al., 2008; Stockmeier et al., 1998), or a marker of raphe neuron 'activation' (Bielau et al., 2005). The studies have produced variable results, likely reflecting both methodological and clinical factors. For example, as well as measuring different parameters, and using various DRN sampling strategies, the studies are small, and differ in the subjects' age, polarity of mood disorder, and presence of comorbid conditions.

This investigation was performed to help shed some further light on the involvement of the DRN in the neuropathology of mood disorder and suicide. It has a larger sample size than existing studies; it includes patients from three diagnostic groups (major depression [MDD], bipolar disorder, and schizophrenia, as well as suicides and non-suicides within each group), and uses three complementary techniques: NeuN to assess all neurons, and TPH immunohistochemistry and 5-HT_{1A}R mRNA in situ hybridization (ISH) as markers of serotonergic neurons.

2. Materials and methods

2.1. Subject and tissue characteristics

Unfixed frozen 14 μm sections of brainstem were provided by the Stanley Neuropathology Consortium from their series of 60 subjects diagnosed (by DSM-IV criteria) with schizophrenia, bipolar disorder or MDD, and controls (Torrey et al., 2000). In each diagnostic group some subjects died by suicide. The sections provided were quite rostral, and tissue from ten subjects did not contain sufficient clearly discernible DRN to be included. Table 1 summarises the details of the resulting 50 subjects. Adjacent sections were taken for NeuN and PH8 immunostaining every 1 mm, and 1 section every 500 μm for 5-HT_{1A}R ISH. The experiments described here, were carried out with ethical approval from Oxfordshire Research Ethics Committee B (#O02.040). All material was coded by the Stanley Medical Research Institute, and experiments and analyses conducted blind to diagnostic and other information.

2.2. Immunohistochemistry for NeuN and PH8

The NeuN antibody stains virtually all neuron populations and is widely used for morphometry (Mullen et al., 1992). It has the advantage over Nissl stains that glia are not labelled (Gittins and Harrison, 2004). Incubations were carried out at a concentration of 1:100 overnight at 4 °C. The PH8

Table 2
Density of NeuN-labelled neurons in the DRN.

	Controls		Schizophrenia		Bipolar disorder		Major depression		Suicides		Non-suicides[a]	
Level	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	N
1	26.1 (1.8)	8	20.2 (3.8)	8	22.0 (2.5)	11	30.5 (4.2)	7	27.3 (3.1)	12	20.7 (2.5)	14
2	28.2 (1.8)	10	21.2 (3.6)	5	26.4 (3.6)	6	30.1 (3.4)	7	26.7 (2.5)	8	26.1 (3.3)	10
3	31.7 (3.1)	7	29.5 (3.1)	6	27.8 (4.5)	5	35.7 (4.0)	5	32.1 (2.8)	7	30.0 (3.4)	9

Values are cells per mm^2. There are no significant differences between groups.
[a] Excludes normal controls.