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Anterior cingulate pyramidal neurons display altered dendritic branching in depressed suicides

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ABSTRACT

Background: It is hypothesized that mood disorders are accompanied by altered wiring and plasticity in key limbic brain regions such as the anterior cingulate cortex (ACC). To test this hypothesis at the cellular level, we analyzed basilar dendritic arborizations extended by layer VI pyramidal neurons in silver-impregnated postmortem ACC samples from well-characterized depressed suicide subjects ($n = 12$) and matched sudden-death controls ($n = 7$).

Methods: One cm^3 tissue blocks were stained using a Golgi preparation, cut on a microtome, and mounted on slides. Basilar dendritic arbors from 195 neurons were reconstructed, and the number, length, and diameter of branches were determined at each branch order. The size and number of spines borne by these branches were also assessed.

Results: Third-order branches were significantly reduced in number (24% fewer; $p = 0.00262$) in depressed suicides compared to controls. The size and average length of these branches, as well as their number of spines/length were unaltered. On average, for each pyramidal neuron analyzed in depressed subjects, the fewer third-order branches resulted in a significant reduction in branch length (28% shorter; $p = 0.00976$) at this branch order.

Conclusions: These results provide the first evidence of altered cortical dendritic branching in mood disorders. Given that proximal dendritic branches grow during perinatal development, and that they are generally less plastic at maturity than distal segments, we speculate that these differences in dendritic branching may reflect a biological predisposition to depression and suicide.

1. Introduction

In recent years, there has been growing support to the view that neuronal connectivity and plasticity are altered in the brain of individuals with mood disorders. In particular, postmortem evidence of perturbed neurotrophin signaling has been provided by several research groups. For instance, Evans and collaborators (2004) have described major depressive disorder (MDD)-related dysregulations in the cortical fibroblast growth factor (FGF) system that were attenuated by antidepressant treatment. Other neurotrophin pathways also appear to be at play, since region-specific alterations in the expression of brain-derived neurotrophic factor (BDNF) (Dwivedi et al., 2003), nerve growth factor (NGF), neurotrophin (NT)-3, and NT-4/5 (Dwivedi et al., 2005) have been reported in the brains of suicide subjects. More recently, decreases in the expression and activity of tropomyosin receptor kinases (Trks) in suicide brains have also been documented (Ernst et al., 2009; Dwivedi et al., 2009). These findings are in accordance with the neurotrophic hypothesis of depression (Nestler et al., 2002), according to which severe stressors can lead to deficiencies in neurotrophic support and affect neuronal function and survival.

Despite inconsistencies, neuroanatomical studies have gathered morphological evidence suggesting that the fine structural organization of cortico-limbic circuitries is affected in mood disorders (reviewed in Hercher et al. (2009a) and Rajkowska and Miguel-Hidalgo (2007)). Interestingly, the atrophy of dendrites and of other components of the neuropil have been suggested to occur in these psychiatric conditions (Chana et al., 2003; Stockmeier et al., 2004). Dendrites are dynamic neuronal extensions that are often highly branched, giving rise to a tree-like network of processes acting as the main sites of input from other cells. The geometry of the dendritic arbor as well as the number and shape of its spines (Fiala et al., 2002) have been proposed to determine many functional neuronal properties (Koch and Segev, 2000). It is thus likely that significant dendritic alterations could result in behavioral disturbances (Selemon and Goldman-Rakic, 1999; Stockmeier et al.,

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2004). This hypothesis is supported by a series of elegant postmortem studies having demonstrated important changes in cortical dendritic arborizations and spine densities in schizophrenic brains (Garey et al., 1998; Glantz and Lewis, 2000; Broadbelt et al., 2002; Sweet et al., 2009). Unfortunately, fine neuroanatomical studies of this kind have been limited for mood disorders. To our knowledge, a single study has been published, showing decreases in dendritic arbors and spines in the subiculum of individuals with mood disorder (2 MDD and 4 bipolar disorder [BPD] subjects) having at least two first-degree relatives with definite or probable mood disorders (Rosoklija et al., 2000). Whether dendritic arborizations in cortical regions thought to be involved in mood disorders display similar structural alterations remains to be investigated.

The anterior cingulate cortex (ACC), a region involved in the stress response (Herman et al., 2005; Pruessner et al., 2008), has been repeatedly implicated in mood disorders (Ebert and Ebmeier, 1996; Nitschke and Mackiewicz, 2005; Drevets et al., 2008). Being "the seat of dynamic vigilance by which environmental experiences are endowed with an emotional consciousness" (Papez, 1937), the ACC is a major target for glucocorticoids and acts as a bridge between limbic structures and the frontal lobe, integrating cognitive activity with affective experience. Dysfunctions within this network have been described in depressed individuals showing impaired problem solving, maladaptive behavioral regulation and abnormal error monitoring (Davidson et al., 2002). Macroscopic alterations occurring in the ACC of subjects with mood disorders have also been described *in vivo* in spectroscopic (Auer et al., 2000; Mirza et al., 2004; Frye et al., 2007), structural (Ballmaier et al., 2004; Konarski et al., 2008; Wang et al., 2008), and functional studies (Bremner et al., 2004; George et al., 1997; Holmes and Pizzagalli, 2008; Mannie et al., 2008). Microscopic changes related to cell densities and somal sizes have also been reported in this region by some groups (Ongur et al., 1998; Cotter et al., 2001; Chana et al., 2003) but not by others (Bouras et al., 2001; Hercher et al., 2009b).

In the present study, postmortem ACC samples (Brodmann area 24; BA24) from depressed suicides and matched sudden-death controls were silver impregnated, and dendritic arborizations extended by layer VI pyramidal neurons were reconstructed and measured using the centrifugal method. This approach allows for detailed analyses of dendritic branching. Our working hypothesis was that in depressed suicides, these principal ACC neurons would display structural differences in the form of reduced dendritic branching.

2. Methods

2.1. Subjects

This study was approved by the Douglas Hospital Research Ethics Board, and written informed consent from next-of-kin was obtained for each subject. Postmortem brain tissues were obtained from the Quebec Suicide Brain Bank, which is part of the McGill Group for Suicide Studies (www.douglasrecherche.qc.ca/suicide). Depressed suicides ($n = 12$) and sudden-death controls without mental illness ($n = 7$) were matched for age, pH, postmortem interval (PMI) and storage time (see Section 3). The depressed suicide group was comprised of 7 subjects with MDD and 5 with BPD (see Table 1 for demographic and clinical informations). All had committed suicide while depressed, 11/12 subjects were prescribed antidepressants in the last three months of life, and 4/12 were alcohol-dependent. In each case, the cause of death was ascertained by the Quebec Coroner office. For both case and control subjects, psychological autopsies were performed as described previously (Dumais et al., 2005), thus allowing access to detailed information on psychiatric and medical history, as well as other relevant clinical and socio-demographic data. In brief, a trained interviewer conducted the *Structured Clinical Interview for DSM-IV* Psychiatric Disorders (SCID-I) with one or more informants of the deceased. A panel of clinicians reviewed SCID-I assessments, case reports, coroner's notes and medical records to obtain consensus psychiatric diagnoses.

Table 1
Summary of individual demographic and clinical informations.

Group	Gender	Age	Cause of death	Diagnosis
C	M	72	Cardiac arrest	–
C	M	63	Accidental fall	–
C	M	42	Car accident	–
C	M	18	Cardiac arrest	–
C	F	66	Car accident	–
C	F	49	Accidental fall	–
C	M	52	Cardiac arrest	–
DS	M	48	Fall	MDD
DS	M	28	Hanging	BPD-I
DS	M	22	Hanging	MDD
DS	F	46	Intoxication	MDD
DS	F	55	Hanging	MDD
DS	M	51	Hanging	BPD-I
DS	M	72	Hanging	BPD-I
DS	F	61	Intoxication	BPD-II
DS	M	47	Intoxication	MDD
DS	F	49	Intoxication	MDD
DS	F	55	Hanging	BPD-I
DS	M	39	Hanging	MDD

C: control; DS: depressed suicide; M: male; F: female; MDD: major depressive disorder; BPD: bipolar disorder (type I or II).

2.2. Golgi impregnations

Previously defined macroscopic criteria (Gittins and Harrison, 2004; Vogt et al., 1995) were used to dissect 1 cm^3 formalin-fixed tissue samples from the right hemisphere of an anterior region within the ACC immediately dorsal to the genu of the corpus callosum (BA24). A Golgi impregnation protocol was used to visualize BA24 pyramidal neurons. This "black reaction" stain involves two basic steps: a chromation stage using potassium dichromate ($K_2Cr_2O_7$), and an impregnation stage using silver nitrate ($AgNO_3$). Tissue samples were contained in separate opaque glass jars wrapped in aluminum foil and placed on a shaker at room temperature. On the first day, samples were immersed in a solution of 3% $K_2Cr_2O_7$ and 10% formalin for 24 h, followed by 24 h in a fresh solution of 3% $K_2Cr_2O_7$. Samples were then washed in distilled water and then in 2% $AgNO_3$ until the solution ran transparent. Tissue blocks were next placed in clean opaque glass jars with 2% $AgNO_3$ for 48 h before being dehydrated through a graded series of ethanol solutions, cleared in xylene, and embedded in paraffin. Stained tissues were cut on a microtome in serial sections ranging in thickness between 40 and 50 μm, with one sample being cut at 35 μm. No significant post-processing thickness differences were observed between groups [$t(17) = 0.650$, $p = 0.524$]. All samples were coded and reconstructed in random order by a single researcher (C.H.) who remained blind to diagnosis and subject number throughout the study.

2.3. Dendritic analyses

Silver-impregnated pyramidal neurons were identified by their characteristic triangular somal shape, and apical dendrite extending toward the pial surface (Fig. 1). A preliminary survey of the tissues revealed that for all subjects, the silver impregnation in