Please cite this article in press as: Rajkowska G et al. Length of axons expressing the serotonin transporter in orbitofrontal cortex is lower with age in depression. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.07.006

Neuroscience xxx (2017) xxx–xxx

LENGTH OF AXONS EXPRESSING THE SEROTONIN TRANSPORTER IN ORBITOFRONTAL CORTEX IS LOWER WITH AGE IN DEPRESSION

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Abstract—Studies of major depressive disorder (MDD) in postmortem brain tissue report enhanced binding to inhibitory serotonin-1A autoreceptors in midbrain dorsal raphe and reductions in length of axons expressing the serotonin transporter (SERT) in dorsolateral prefrontal cortex. The length density of axons expressing SERT in the orbitofrontal cortex (OFC) was determined in 18 subjects with MDD and 17 age-matched control subjects. A monoclonal antibody was used to immunohistochemically label the SERT in fixed sections of OFC. The 3-dimensional length density of SERT-immunoreactive (ir) axons in layer VI of OFC was estimated. The age of subjects with MDD was negatively correlated with SERT axon length ($r = -0.77$, $p < 0.0005$). The significant effect of age persisted when removing four depressed subjects with an antidepressant medication present at the time of death, or when removing nine depressed subjects that had a recent prescription for an antidepressant medication. Neither gender, tissue pH, postmortem interval, 5-HTTLPR genotype, time in fixative, nor death by suicide had a significant effect on axon length. The age-related decrease in SERT-ir axon length in MDD may reflect pathology of ascending axons passing through deep white matter hyperintensities. Greater length of axons expressing SERT in younger subjects with MDD may result in a significant deficit in serotonin availability in OFC.

Key words: serotonin transporter, major depressive disorder, orbitofrontal cortex, age, postmortem, morphometry.

INTRODUCTION

Clinical and basic research implicates the serotonin neurotransmitter system in the pathophysiology and treatment of major depressive disorder (MDD). Extracellular serotonin levels are regulated by the serotonin transporter (SERT), which is located either on the cell membranes presynaptically or along axons (Zhou et al., 1998). Although the most widely used treatments for MDD inhibit the SERT, there is no consistent evidence for altered radioligand binding to the SERT in cerebral cortex in MDD, as determined in postmortem brain tissue or with neuroimaging (see review by Stockmeier, 2003; and meta-analyses by Gryglewski et al., 2014; Kambeitz and Howes, 2015).

The recent meta-analyses of neuroimaging studies in MDD by Gryglewski et al. (2014) and Kambeitz and Howes (2015) establish consistent decreases in SERT binding in subcortical regions but not in the frontal cortex. However, other studies note greater SERT binding in prefrontal cortical and subcortical regions in a subgroup of depressed patients with highly negativistic dysfunctional attitudes (Meyer et al., 2004) or a greater seasonal increase in SERT binding in patients with seasonal affective disorder (Tyrer et al., 2016a). In a comprehensive review of studies of neuroimaging in humans, rodent models, and neuroimmunology, Savitz and Drevets (2013) found support for the hypothesis that function at the SERT is increased in depression. Further, in neuroimaging studies, it appears that alterations in SERT binding are found only in subgroups of depressed patients and in discrete brain regions.

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Abbreviations: DSM, Diagnostic and Statistical Manual of Mental Disorders; ir, immunoreactive; MDD, major depressive disorder; OFC, orbitofrontal cortex; PMI, postmortem interval; SERT, serotonin transporter; TF, storage time in formalin.

http://dx.doi.org/10.1016/j.neuroscience.2017.07.006
0306-4522/

In contrast to studies examining living subjects with depression, research in postmortem tissues from younger suicide victims with MDD that were antidepressant drug-free shows a significant decrease in radioligand binding to SERT across all cortical layers in the orbitofrontal cortex (OFC) vs. normal control subjects (Underwood et al., 2012) or OFC and dorsolateral prefrontal cortex in subjects with MDD where no monoamine-related antidepressant drug was present at death (Mann et al., 2000). In an immunohistochemical assessment of regional expression of SERT, there was a reduction in the overall length of axons immunoreactive for SERT in one layer only (VI) of the dorsolateral prefrontal cortex of suicide victims with MDD (Austin et al., 2002).

The present study was undertaken to test the hypothesis that the length of SERT immunoreactive (-ir) axons is lower in OFC in subjects with MDD, some of whom died by suicide. Orbitofrontal cortex was also selected as a region of interest in this study because of previous observations in OFC of reduced neuronal density and sizes in subjects with MDD (Rajkowska et al., 1999, 2005; Underwood et al., 2012). In addition, there was a significant age-related decrease in neuronal density in OFC in subjects with MDD but not in control subjects (Rajkowska et al., 2005). Most subjects examined in the present study were the same as those studied in Rajkowska et al. (1999, 2005). The 5HTTLPR genotype was also assessed as there is evidence that the 5HTTLPR polymorphism affects SERT expression (Lesch et al., 1996). Older subjects with MDD have more frontal deep white matter hyperintensities than age-matched controls (Krishnan et al., 1988; Rabins et al., 1991; O'Brien et al., 1996; Thomas et al., 2002; Tupler et al., 2002; van Agtmaal et al., 2017). Increases with age in deep white matter hyperintensities in depression may induce pathology in ascending serotonergic axons from the midbrain raphe system projecting to the OFC.

EXPERIMENTAL PROCEDURES

Human subjects

The Declaration of Helsinki was adhered to for all experiments involving human subjects. The Institutional Review Boards of University Hospitals Case Medical Center, Cleveland, OH, and the University of Mississippi Medical Center approved the research protocol for recruitment of next-of-kin, collection of brain tissue, and informant-based interviews. The left orbitofrontal cortex was sampled from 17 psychiatrically normal control subjects and 18 age-matched subjects that met clinical criteria for MDD at autopsy at the Cuyahoga County Medical Examiner's Office (Cleveland, OH). Informed consent was acquired from all legally defined next-of-kin to permit tissue collection and informant-based retrospective diagnostic interviews. The Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV; APA, 1994) was administered regarding all subjects by a trained interviewer using the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1995), as described (Cobb et al., 2013). Consensus diagnosis with the aid of medical records was used to determine lifetime and recent Axis I psychopathology. Information on psychoactive substance use and history of medications was collected from informants and medical records. Head trauma, neurologic or neuropathological disease were exclusion criteria. Eighteen subjects met criteria for a lifetime diagnosis of MDD and 16 met criteria for a major depressive episode in the last month of life. Psychotic features were also present in two subjects with MDD. Four subjects with depression also experienced another Axis I disorder (Table 1). None of the control subjects ever

Table 1. Demographic and disease characteristics of control and MDD subjects

Parameter	Controls (n = 17)	MDD (n = 18)
Age (years) (range)	55 ± 5 (27–86)	57 ± 4 (30–87)
Gender (F:M)	7:10	9:9
PMI (hrs) (range)	20 ± 1 (11–27)	20 ± 1 (10–26)
Tissue pH (range)	6.74 ± 0.05 (6.32–7.01)	6.57 ± 0.05 (6.24–6.97)
TF (months) (range)	36 ± 7 (7–103)	19 ± 2 (7–43)
Time in Ethanol (years) (range)	11 ± 0.6 (7–14)	13 ± 0.4 (10–16)
Cause of death	Cardiovascular disease n = 13; asthma n = 1; accidental electrocution n = 1; homicide by firearm n = 1; pulmonary thromboembolism n = 1	Suicide n = 8 (firearm n = 2; CO poisoning n = 2; hanging n = 2; drowning n = 1; drug overdose n = 1) Other causes n = 10 (cardiovascular disease n = 7; pulmonary thromboembolism n = 1; homicide by firearm n = 1; undetermined n = 1)
Psychiatric Diagnosis	None (n = 15) Remote history of alcohol dependence (n = 1) Remote history of alcohol abuse (n = 1)	MDD (n = 18) MDD plus alcohol dependence (n = 2) MDD plus polysubstance dependence (n = 2)
Duration of MDD (years) (range)	Not applicable	16.8 ± 3.5 (0.17–50)
Antidepressant drug history	None	n = 9
Postmortem toxicology	None	n = 4 (amitriptyline n = 1; nortriptyline n = 1; sertraline n = 2; chlorpromazine n = 1)

Data represent the mean ± S.E.M. Abbreviations: CO – carbon monoxide; F – female; M – male; MDD – major depressive disorder; PMI – Postmortem interval; TF – Time in fixative.

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