Alkali metals levels in the human brain tissue: Anatomical region differences and age-related changes

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A B S T R A C T

The link between trace elements imbalances (both "toxic" and "essential") in the human brain and neurodegenerative disease has been subject of extensive research. More recently, some studies have highlighted the potential role of the homeostasis deregulation of alkali metals in specific brain regions as key factor in the pathogenesis of neurodegenerative diseases such as multiple sclerosis and Alzheimer's disease.

Using flame atomic emission spectrometry and inductively coupled plasma-mass spectrometry after microwave-assisted acid digestion of the samples, alkali metals (Na, K, Li, Rb and Cs) were determined in 14 different areas of the human brain (frontal cortex, superior and middle temporal gyri, caudate nucleus, putamen, globus pallidus, cingulated gyrus, hippocampus, inferior parietal lobule, visual cortex of the occipital lobe, midbrain, pons, medulla and cerebellum) of adult individuals (n = 42; 71 ± 12, range: 50–101 years old) with no known history and evidence of neurodegenerative, neurological or psychiatric disorder.

Potassium was found as the most abundant alkali metal, followed by Na, Rb, Cs and Li. Lithium, K and Cs distribution showed to be quite heterogeneous. On the contrary, Rb and Na appeared quite homoge-neously distributed within the human brain tissue. The lowest levels of Na, K, Rb and Li were found in the brainstem (midbrain, medulla and pons) and cerebellum, while the lowest levels of Cs were found in the frontal cortex. The highest levels of K (mean ± sd; range 15.5 ± 2.5; 8.9–21.8 mg/g) Rb (17.2 ± 6.1; 3.9–32.4 μg/g and Cs (83.4 ± 48.6; 17.3–220.5 ng/g) were found in putamen. The highest levels of Na and Li were found in the frontal cortex (11.6 ± 2.4; 6.6–17.1 mg/g) and caudate nucleus (7.6 ± 4.6 2.2–21.3 ng/g), respectively.

Although K, Cs and Li levels appear to remain largely unchanged with age, some age-related changes were observed for Na and Rb levels in particular brain regions (namely in the hippocampus).

Keywords: Human brain;Alkalimetals; Postmortem analysis; Ageing

Introduction

Neurodegenerative diseases (ND), such as Alzheimer's and Parkinson's diseases, are chronic multifactorial diseases and it is assumed that they involve a complex interaction between (natu-ral) ageing, and genetic and environmental factors [1–3]. Growing evidence indicates a role for oxidative stress [4–6] and some dis-turbances in transition metals levels (e.g., Fe, Cu, Zn) [7,8]. More recently, imbalances in alkali metals levels (Na and K) have also been associated with some ND [9,10].

Potassium and Na are the most important cations of intra and extracellular fluids, respectively, playing a major role in many phys-

iological processes, such as electric impulse conduction in excitable cells (*e.g.*, neurons, cardiac cells) [11,12]. The concentration of these electrolytes is maintained by the Na^+/K^+-ATPase pump and imbalances of Na and K concentration in the extracellular fluid cause the movement of water into or out of the cells, altering the intracellular fluid osmotic pressure and causing cells swelling or shrinkage [13]. Osmotic shifts affect all cells and tissues but the brain is particularly susceptible to damage from changes in intracranial pressure [11].

Although Li does not have a known biological role and does not appear to be an essential element for humans, lithium salts (*e.g.*, carbonate, acetate) have been extensively used in the treatment of manic-depressive disorders for more than 50 years [14]. In the last two decades, several studies have further suggested that Li may also have neuroprotective effects against amyotrophic lateral sclerosis, and Alzheimer's and Parkinson's diseases [15].

Rubidium and Cs are also regarded as non-essential elements for humans and have no known biological role. In many animals, including humans, the distribution of both Rb and Cs resembles that of K and it has been shown that Rb and, to a lesser extent, Cs can replace K as an essential nutrient for the growth of bacteria, yeast and rats [16]. Although generally less readily transported, Cs has been shown to compete with K and Rb for both active and passive membrane transport due to their similar physicochemical properties [17].

It must be highlighted that the brain is a highly heterogeneous organ, with anatomically and physiologically very different areas, which may be affected in different manners by the ageing and neurodegenerative processes [18]. So, as a first step, a detailed study of the anatomical distribution of alkali metals in the "normal" brain is indispensable to clarify their role both in the normal human brain physiology and neurodegenerative diseases. To date, alkali metals (K, Na, Rb, Li and Cs) have been a poorly studied and data on their topographical distribution in the human brain are scarce, mostly limited to a few brain regions, to large or not specified brain regions, and/or involving a reduced number of subjects [19–22].

Based on this background, the main goal of the present study was to contribute to the establishment of reference levels for alkali metals in the different anatomical and functional regions of "normal" human brain. We directly determined Na, K, Rb, Cs and Li levels in postmortem human brain tissue in order to evaluate: (a) the regional anatomic differences across the brain and (b) their change in relation to age.

Materials and methods

Subjects

Brain samples were obtained from men (n = 27; 67 ± 11 years old) and women (n = 15; 77 ± 12 years old) not registered in the Portuguese *National Registry of Refusal to Organ Donation database* and complying with all the current regulations regarding human tissue collection for scientific research purposes.

Samples were collected from individuals submitted to forensic autopsy during the first semester of 2012 at the North Branch (Porto) of the Portuguese National Institute of Legal Medicine and Forensic Science (INMLCF). Individuals from each of the following age groups were studied: 50–59 ($n = 10$), 60–69 ($n = 10$), 70–79 ($n = 10$), 80–89 ($n = 9$) and ≥90 ($n = 3$) years old. Inclusion criteria were: (a) absence of known neurodegenerative, neurological or psychiatric disorder history; (b) absence of injuries involving CNS; (c) macroscopically normal brain tissue.

Samples from two individuals with documented Alzheimer's disease (women, 73 and 85 years old) and one individual with documented Parkinson's disease (woman, 91 years old) were also collected and results are also presented.

Sample collection

Samples were collected by INMLCF pathologists following a standard protocol. In order to prevent sample contamination, all materials contacting with the samples, including the stainless steel dissection tools, were previously decontaminated with 5% (v/v) nitric acid solution (prepared from concentrated HNO_3 ≥69%; Sigma-Aldrich, Germany) and thoroughly rinsed with ultrapure water (resistivity >18.2 MΩ cm at 25 °C), produced by an arium® pro (Sartorius, USA) water purification system.

After removing the brain from the cranium, the excess of blood was thoroughly washed with ultrapure water. Meninges were removed with plastic tweezers and the brain tissue was washed again with ultrapure water to minimize samples contamination with blood or cerebrospinal fluid.

In order to establish an accurate diagnosis and study the relationship between the disease process and either the clinical features seen in life or the cause of death, Paine and Lowe [18] have proposed a post-mortem approach where 14 key brain areas should be studied individually (Fig. 1). Using decontaminated plastic knives, tissue fragments (approximately 1 cm^3) were collected from the following brain areas: frontal cortex (1), superior (2A) and middle (2B) temporal gyri, caudate nucleus (3A), putamen (3B), globus pallidus (3C), cingulated gyrus (4), hippocampus (5), inferior parietal lobule (6), visual cortex of the occipital lobe (7), midbrain (including the *substantia nigra* at the level of the third nerve) (8), pons-locus coeruleus (9), medulla (10) and cerebellum-dentate nucleus (11). Samples were stored in decontaminated polypropylene tubes (Sarstedt, Germany) at −4 °C until analysis.

Sample pre-treatment

After thawing, brain samples were thoroughly washed with ultrapure water and placed in a dry oven (Raypa, Spain) at 110 °C until constant weight (ca. 24 h). Dried samples (ca. 100–500 mg) were weighed directly in the microwave digestion vessels previously washed with 10% (v/v) HNO_3 and rinsed with ultrapure water. Samples were digested with 2.5 mL of HNO_3 ≥65% and 1.0 mL of H_2O_2 ≥30% (both *Trace*SELECT® Ultra, Sigma-Aldrich, France). The digestion was performed in a MLS-1200 mega microwave oven (Milestone, Italy), equipped with a HPR 1000/10 rotor, using the following power (W)/time (min) program: 250/1, 0/2, 250/5, 400/5 and 600/5. After cooling, samples digests were made up to 50 mL with ultrapure water and stored in closed propylene tubes at 4 °C until analysis.

Potassium and sodium determination

Determination of K and Na was performed by flame atomic emission spectrometry (FAES), using a PerkinElmer (Germany) Model 3100 instrument according to the standard conditions recommended by the manufacturer (air/acetylene flame; K: 766.5 nm, slit 0.7 nm; Na: 589.0 nm, slit 0.2 nm).

Calibration curves were obtained with five standard solutions with concentrations ranging from 0.2 to 1.0 mg/L for Na and 0.4–2.0 mg/L for K, prepared by adequate dilution of 1000 mg/L single element commercial standard solutions (Sigma-Aldrich, Switzerland) with 0.2% v/v HNO_3. Sample solutions were also diluted (100-fold) with 0.2% v/v HNO_3. A 1% (w/v) Cs solution, prepared by dissolution of Cs_2CO_3 (Sigma-Aldrich, France) in ultrapure water, was added to all samples and calibration standards (final concentration: 0.1% (w/v)) in order to suppress analytes ionization.

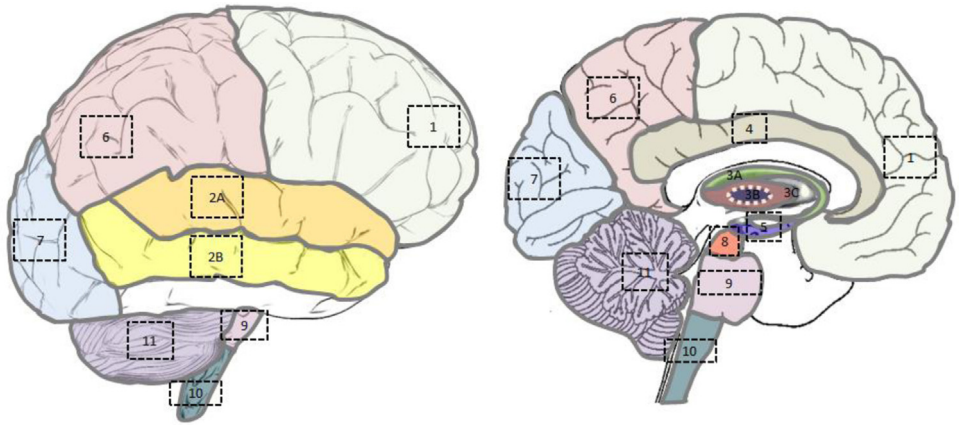

Fig. 1. Sampled brain regions, as suggested by Paine and Lowe [18]: frontal cortex (1), superior (2A) and middle (2B) temporal gyri, basal ganglia including the caudate nucleus (3A), putamen (3B), globus pallidus (3C), cingulate gyrus (4), hippocampus (5), inferior parietal lobule (6), visual cortex of the occipital lobe (7), midbrain (including the substantia nigra at the level of the third nerve) (8), pons-locus coeruleus (9), medulla (10), and cerebellum-dentate nucleus (11).

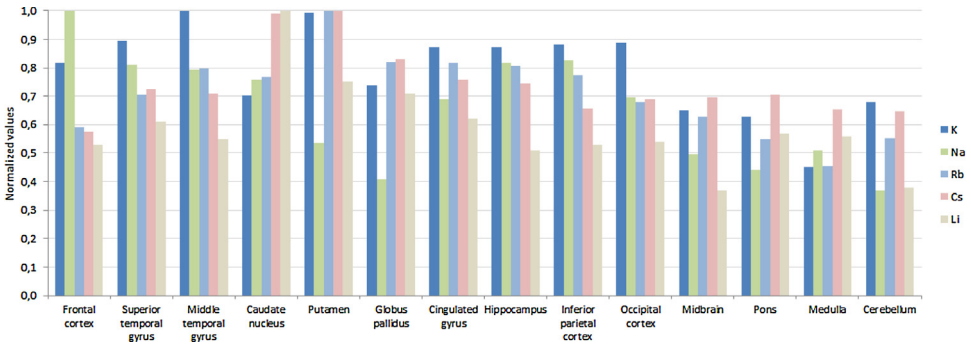

Fig. 2. Distribution of alkali metals according to the brain region. The metal content (mass of metal/g of dry weight tissue) were "normalized" using the specific region value to maximum value ratio.

Lithium, cesium and rubidium determination

Lithium, Cs and Rb quantification was performed by ICP-MS using an iCAP-Q instrument (Thermo Scientific, UK), equipped with MicroMist™ (Glass Expansion, Australia) type A concentric nebulizer, a Peltier-cooled impact-bead spray chamber, a standard quartz tube torch, and nickel sampling and skimmer cones. Sampling interface was cooled to 20 °C by circulating water. For sample introduction, an ASX-520 autosampler (Cetac Technologies, USA) was used. High-purity argon (BIP, 99.99997%; supplied by Gasin, Portugal) was used as the nebulizer and plasma gas. The equipment control and data acquisition were made through the Qtegra software (Thermo Scientific). Elemental isotopes (*m*/*z* ratios) ^{9}Li, ^{85}Rb and ^{133}Cs were monitored for analytical determinations, and ^{45}Sc, ^{89}Y, ^{115}In and ^{159}Tb were monitored as internal standards. The instrument was daily checked for maximum signal sensitivity and stability with Tune B iCAP solution (1.0 μg/L Ba, Bi, Ce, Co, In, Li and U) from Thermo Scientific.

All the solutions were prepared with 2% (v/v) HNO_3. Calibration curves were obtained with seven solutions of element concentrations within the 1–100 μg/L range (Cs range: 0.1–10 μg/L). Calibration solutions were prepared by adequate dilution of SCP-33-MS multi-element standard solution (100 mg/L, SCP Science, Canada) and a 1000 mg/L Cs standard solution (Sigma-Aldrich, Switerzland). An internal standards (Sc, Y, In, Tb, and Bi) solution was prepared by dilution of a commercial solution (AccuTrace™ Reference Standard, ICP-MS 200.8-IS-1; AccuStandards, USA). It was added to all samples and standard solutions in order to obtain a 10 μg/L final concentration. Sample solutions were diluted (5-fold) before analysis.

Analytical quality control

Because human brain tissue is not available as certified reference material (CRM) for alkali metals determination, DOLT-4 Dogfish Liver CRM for Trace Metals (National Research Council, Canada), Standard Reference Material 1640 Trace Elements in Natural Water (National Institute of Standards and Technology, USA) and ERM-CA022 Soft Drinking Water UK—Metals (European Reference Materials, UK) were used for analytical quality control purposes. DOLT-4 was subjected to the same pre-treatment as brain samples.

For contamination control during the microwave-assisted acid digestion procedure, a sample blank was performed in each digestion cycle (10 samples). In total, 77 sample blanks were run, and the mean value was subtracted from the sample values. All the samples solutions, after the adequate dilution, were analyzed in triplicate.

Statistical evaluation

Descriptive statistical parameters (mean and standard deviation) were calculated using Microsoft Office Excel 2013 (Microsoft Co., USA). Pearson correlation calculation and mean comparisons ($\alpha = 5\%$) were performed using StatPlus:mac version v5 (AnalystSoft Inc., USA).

For statistical calculations, sample values below the detection limit (DL) were replaced by DL divdided by the square root of 2.

Results and discussion

Potassium [mean ± sd (range): 12.3 ± 1.2 (8.9–15.4) mg/g dry weight] was shown to be the most abundant alkali metal in the human brain, followed by Na [7.6 ± 1.1 (5.7–10.5) mg/g], Rb [12.5 ± 4.1 (2.9–22.5) μg/g], Cs [69.2 ± 27.9 (36.0–168.5) ng/g] and Li [4.5 ± 1.6 (2.1–9.0) ng/g].

Inter-individual variability, characterized as the mean of the percent relative standard deviation (RSD%) representative of each region [22], is higher for non-essential elements Li (60%), Cs (51%) and Rb (39%) than for essential elements Na (20%) and K (17%). Saiki et al. [23] reported higher inter-individual variability for Rb (20%) than for Na (18%) and K (11%), and Bélavári et al. [21] found greater inter-individual variability for Rb (44%) and Cs (30%) than for K (12%) and Na (10%). This high variability in brain tissue levels between individuals for non-essential elements such as Cs and Rb is probably the result of the different levels of exposure, particularly through the diet, which strongly depends on the geological characteristics of the area where the individual lives [21]. Moreover, homeostatic mechanisms that ensure the maintenance of brain levels within narrow limits are not expected to exist for these non-essential elements. Increased measurement errors might also have contributed to the higher inter-individual variability of less abundant metals (Cs and Li).

Anatomical region differences on alkali metals levels

Results (mean ± sd; mass of element per mass of dry tissue) of alkali metals levels obtained for each of the 14 different brain regions studied are summarized in Table 1.

Regarding the inter-regional variability (characterized as the RSD% of the mean values for each brain region) [22], results showed that Li, K and Cs distribution is quite heterogeneous (inter-regional variability: 27%, 20%, 17% respectively). As previously referred, increased inaccuracy of Cs and Li measurements might have contributed to the higher inter-regional variability, since these metals levels were at six order of magnitude lower than Na and K.

For clarity of presentation, data (mass of metal/g of dry weight tissue) were "normalized" using the specific region value to maximum value ratio (Fig. 2).

Regardless of age group, the highest levels of K were found in the middle temporal gyrus [15.6 ± 1.8 (11.5–19.1) mg/g] and putamen [15.5 ± 2.5 (8.9–21.8) mg/g]. The highest levels of Cs were found in the putamen [93.0 ± 49.8 (32.6–250.3) ng/g], a brain structure mainly involved in motor control [24].

The lowest levels of K and Cs were found in the medulla [7.0 ± 1.8 (2.8–10.6) mg/g] and frontal cortex [53.4 ± 21.1 (20.1–117.3) ng/g], respectively. The pons [9.8 ± 1.8 (4.4–13.8) ng/g], midbrain [10.2 ± 1.5 (6.7–13.9) ng/g] and cerebellum [10.7 ± 1.4 (8.3–14.3) ng/g] were also regions with low K content. Low levels of Cs were also found in the cerebellum [60.2 ± 34.3 (18.2–180.6) ng/g] and medulla [60.7 ± 25.4 (15.0–155.5) ng/g].

The highest levels of Li were found in the caudate nucleus [7.6 ± 4.6 ng/g (2.2–21.3)] and the lowest in the cerebellum [2.9 ± 1.3 ng/g (1.1–6.6)].

Rubidium (inter-regional variability: 8%) and Na (15%) seems to be more homogeneously distributed in the human brain than the other alkali metals. The highest levels of Rb were found in the putamen [17.4 ± 6.1 (3.9–32.4) μg/g] and the lowest in the medulla [7.9 ± 4.3 (1.5–20.6) μg/g]. The highest levels of Na were found in the frontal cortex [11.6 ± 2.4 (6.6–17.1) mg/g] and the lowest in the cerebellum [4.3 ± 0.8 (2.4–7.9) mg/g].

The heterogeneous regional distribution of several major and trace elements in the brain is well known [25] and it has been assumed that the distribution pattern is probably related to the specific physiological environment and functions of the different

Table 1
Alkali metals levels (mean ± SD) in the 14 different regions of the human brain (see footnote) of non-diseased individuals (n = 42; 71 ± 12 years old).

Element	Brain regions[a]															
	1	2A	2B	3A	3B	3C	4	5	6	7	8	9	10	11	mean ± sd	IRV (%)	IIV (%)
K (mg/g)	12.6 ± 2.5	14.0 ± 2.4	15.6 ± 1.8	10.9 ± 3.3	15.5 ± 2.5	11.5 ± 2.4	13.7 ± 1.6	13.6 ± 2.5	13.8 ± 2.1	13.9 ± 1.7	10.1 ± 1.5	9.7 ± 1.8	7.1 ± 1.8	10.7 ± 1.4	12.3 ± 1.2	20	17
Na (mg/g)	11.6 ± 2.4	9.4 ± 1.9	9.2 ± 2.0	8.8 ± 2.6	6.2 ± 1.7	4.7 ± 1.1	8.0 ± 1.9	9.5 ± 2.2	9.6 ± 12.0	8.1 ± 1.6	5.8 ± 1.1	5.1 ± 1.0	5.9 ± 1.4	4.3 ± 0.8	7.6 ± 1.1	15	20
Rb (μg/g)	10.3 ± 4.1	12.3 ± 4.1	13.9 ± 5.0	13.4 ± 5.4	17.4 ± 6.1	14.3 ± 5.1	14.2 ± 5.2	14.0 ± 5.7	13.5 ± 6.1	11.8 ± 4.8	10.9 ± 3.4	9.6 ± 3.1	7.9 ± 4.3	9.6 ± 3.8	12.5 ± 4.1	8	39
Cs (ng/g)	53.2 ± 21.4	67.6 ± 30.8	65.9 ± 31.0	92.0 ± 44.9	93.0 ± 49.8	77.3 ± 34.8	70.5 ± 40.1	69.4 ± 36.0	61.2 ± 29.4	64.0 ± 45.9	64.7 ± 35.2	65.8 ± 33.2	60.7 ± 25.4	60.2 ± 34.3	61.5 ± 28.6	17	51
Li (ng/g)	4.1 ± 1.7	4.7 ± 2.8	4.2 ± 1.8	7.6 ± 4.6	5.7 ± 3.5	5.4 ± 2.9	4.7 ± 3.6	3.9 ± 1.8	4.0 ± 2.1	4.7 ± 3.9	2.8 ± 1.7	4.3 ± 3.4	4.3 ± 1.8	2.9 ± 1.3	4.5 ± 1.6	27	60

IRV: inter-regional variability; IIV: inter-individual variability.
[a] 1-Frontal cortex; 2A-superior temporal gyrus; 2B-middle temporal gyrus; 3A-caudate nucleus, 3B-putamen; 3C-globus pallidus; 4-cingulated gyrus; 5-hippocampus; 6-inferior parietal lobule; 7-visual cortex of the occipital lobe; 8-midbrain, 9-pons 10-medulla; 11-cerebellum.

brain areas. The heterogeneous distribution of elements across the human brain may be partly due to differences in cellular density, cell volumes and cell types between the different brain regions [21]. While the white matter is mainly composed of glial cells and myelinated axons, the grey matter contains the cell bodies, dendrites and axon terminals of neurons, so it is where all synapses are [26]. Since Na^+/K^+-ATPase is present in the synaptic membranes [13], higher levels of alkali metal in regions with higher content of grey matter can be expected. Except for Na, the highest levels of alkali metals were found in the deep grey matter nuclei, which include the basal ganglia, a structure comprising caudate nucleus, putamen and globus pallidus. The lowest alkali metals levels were found mainly in the brainstem (pons, medulla, midbrain), regions predominantly composed of white matter.

In general, our results regarding alkali metals distribution across the brain are in good agreement with previously published data (Table 2). Saiki et al. [23] also reported that Rb (5.4%) is more homogeneously distributed than K (12.3%) and Na (13.3%). Bélavári et al. [21] also found a heterogeneous distribution of alkali metals, although they reported that K (9%) is more homogeneously distributed than Na (22%), Cs (21%) and Rb (18%).

As a result of their chemical similarity and metabolic interchangeability, a correlation would be expected between K and Rb and K and Cs. Our results showed a positive correlation (p-value < 0.001) between K and Rb ($r = 0.597$), Cs and Li ($r = 0.452$), Rb and Cs ($r = 0.437$) and K and Na ($r = 0.415$). Several authors found similar results. Panayi et al. [29] also found a positive correlation for Rb-Cs ($p < 0.001$), K-Rb and K-Cs ($p < 0.005$). Stedman and Spyrou [28] found a positive correlation for Na-K ($r = 0.5448$; $p < 0.01$). Bélavári et al. [21] found a positive correlation only for Rb-Cs ($r = 0.864$; $p < 0.001$) and Duflou et al. [25] only for K-Rb ($r = 0.88$; $p < 0.05$). On the other hand, Rajan et al. [19] found a negative correlation between mole% of Na and mole% of K ($r = -0.82$).

Age-related changes on alkali metals levels

Considering the mean value of the results obtained for the 14 brain regions, a tendency for an age-related increase of Na levels was found ($r = 0.37$; $p = 0.02$) (Fig. 3a). This positive correlation was particularly significant in some specific regions, namely in the hippocampus ($r = 0.53$; $p < 0.001$) and, although not reaching statistical significance, in the middle temporal gyrus ($r = 0.28$; $p = 0.08$) (Fig. 3b), two brain regions involved in memory functions [41].

On the other hand, a tendency for an age-related decrease of Rb levels was found ($r = -0.36$; $p = 0.02$) (Fig. 4a). Excluding the pons, where Rb levels remained quite unchanged with age, this negative correlation was observed in all regions studied, the most significant being the frontal cortex ($r = -0.46$; $p < 0.01$) and hippocampus ($r = -0.41$; $p = 0.01$) (Fig. 4b and c).

No age-related changes were found for K, Cs and Li levels in the 14 studied brain regions (Fig. 5).

A limited number of studies evaluating the age-related changes of alkali metals in human brain are available. Tohno et al. [35] evaluated the relationship between age and Na levels in the caudate nucleus, putamen and globus pallidus but no correlation was found. Duflou et al. [25] also did not find any clear correlation between Rb levels and age. These authors reported an age-related decline in K levels but this conclusion was supported on extreme values obtained in two (out of 12) subjects. Hebbrecht et al. [42] studied five regions (basal ganglia, cerebral cortex, cerebral white matter, brainstem and cerebellar cortex) of human brain ($n = 18$ individuals, 7–79 years old) and found an age-related decrease in Rb and K levels in the basal ganglia and cerebral white matter. An age-related decrease was also found for Rb in the other three areas studied.

Gender-related differences on alkali metals levels and the effect of smoking habits

Considering the mean metal levels (*i.e.*, the mean value for the 14 regions of each subject), no gender-related differences were found for any of the alkali metals. Also, no gender-related differences were found in the alkali metals levels at any individual brain region. Although scarce, previous studies also showed no gender-related differences on alkali metals levels in the human brain [28,35].

In a separate analysis by gender, the tendency for an age-related increase of Na levels was only observed for females ($r = 0.56$; $p = 0.03$), and was particularly significant in the hippocampus ($r = 0.61$; $p = 0.01$) and middle temporal gyrus ($r = 0.66$; $p < 0.001$). The tendency for an age-related decrease of Rb levels was still present although not reaching statistical significance in both male ($r = -0.27$; $p = 0.17$) and female ($r = -0.34$; $p = 0.20$). No age-related changes were observed in either gender for the other alkali metals.

Also considering the mean metal levels, significantly higher levels of K (15.4 ± 5.1 mg/g *vs.* 11.1 ± 3.8 mg/g; $p = 0.006$), Rb (15.2 ± 5.0 μg/g *vs.* 11.1 ± 3.8 μg/g; $p = 0.007$) and Cs (82.3 ± 34.9 ng/g *vs.* 61.9 ± 21.3 ng/g; $p = 0.034$) were found in smokers. The presence of alkali metals in tobacco has been reported [43]. Higher levels of alkali metals in smokers could be explained by the transference of these elements into tobacco smoke and consequent absorption after inhalation. The transport by the olfactory nerve directly to the brain is another potential route of exposure [44]. Inhaled in cigarette smoke, nicotine quickly reaches the brain. Chronic cigarette smoking has shown neuronal membrane damage arising from decreased activity of membrane-bound enzymes (*e.g.*, Na^+/K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase) in rat brain [45] and reduced expression of functional Na^+/K^+-ATPase at the blood-brain barrier [46]. Increased levels of K, Rb and Cs found in brain tissue of smokers may be a result of a decreased activity and/or expression of Na^+/K^+-ATPase, leading to imbalances of electrolytes.

To our knowledge, no studies regarding the effect of smoking habits in alkali metals levels in the human brain are available.

Disease-related changes on alkali metals levels

Besides elevated levels of reactive oxygen species and protein aggregation, depressed Na^+/K^+-ATPase levels have been reported in AD brain [47]. A decrease in Na^+/K^+-ATPase activity and protein levels may lead to disturbances in the ionic equilibrium of neuronal cells and play a role in the development of several disorders, including ND such Alzheimer's and Parkinson's diseases [13].

Brain samples from two AD patients (73 and 85 years old) and one PD patient (91 years old) were also obtained during the sample collection period (first semester of 2012). Although no definite conclusions can be drawn with such a small number of cases, the ratio between alkali metals levels in those patients and age-matched control subjects are summarized in Table 3.

Increased Na levels were found in the globus pallidus of both AD patients, by a factor of 2.1 and 2.3, respectively. In AD#1 patient, decreased K levels were found in 7 of the 14 brain regions, including the medulla (4.9 *vs.* 9.2 mg/g) and caudate nucleus (6.9 *vs.* 12.1 mg/g). Although normal Rb and Cs levels were found in all of the brain regions of AD#2, decreased levels of these elements were found in most of the studied regions of the AD#1.

Regarding PD patient, increased Cs levels were found in 9 of the 14 brain regions by a factor ranging from 1.4-fold (in the superior globus pallidus) to 16.7-fold (in the frontal cortex). Increased Rb levels were also found in all the brain regions of this patient, ranging from 1.4-fold (in the cerebellum) to 2.4-fold (in the inferior parietal lobe).

Table 2
Levels (mass of element per mass of dry tissue weight) of alkali metals in the different human brain regions obtained in previous studies.

	Ref	IT	n	Age (mean ± sd; range)	Brain regions												
					1	2A	2B	3A	3B	3C	4	5	6	7	8	9	10	11
Cs	[27][a]	NAA	4	(30–73)					149 ± 31									
(ng/g)	[28][a]	NAA	15	72 ± 13	38 ± 13													
	[29][a]	NAA	8	64 ± 19(38–87)			53.8 ± 21.5											
	[30][a]	NAA	1					5.13		4.61		5.68						4.56
	[21]	NAA	5	75				60 ± 20	70 ± 20	60 ± 20				40 ± 10				
	[22]	NAA	5					57 ± 22	65 ± 23	59 ± 23			45 ± 20	44 ± 20				
	[31]	NAA	30	61(52–69)								73.9						
	[31]	NAA	30	63(55–72)								65.8						
K	[27][a]	NAA	4	(30–73)					7.69 ± 2.70									
(mg/g)	[28][a]	NAA	15	72 ± 13	12.77 ± 2.72													
	[29][a]	NAA	8	64 ± 19(38–87)			15.78 ± 3.93											
	[21]	NAA	5	75				11.8 ± 2.1	14.0 ± 1.3	11.8 ± 1.0		14.8 ± 1.6						
	[22]	NAA	5					10.73 ± 2.31	14.0 ± 1.53	11.82 ± 1.16			11.38 ± 3.86	13.99 ± 1.53				
	[31]	NAA	30	61(52–69)								17.29						
	[31]	NAA	30	63(55–72)								18.24						
	[20]	ICP-AES	3					14.3 ± 1.0		12.6 ± 1.0								
	[19][a]	ICP-AES	8	(50–60)	12.8 ± 1.7	14.9 ± 6.4					10.4 ± 4.3	19.3 ± 2.2	15.3 ± 4.3	11.2 ± 3.6	9.1 ± 4.0	10.9 ± 4.3	12.1 ± 2.8	
	[34]	NAA	21	79(51–94)	11.59 ± 1.36							12.01 ± 1.39						
	[23]	NAA	31	78 ± 9(51–95)	12.23 ± 1.30	13.34 ± 1.60					11.38 ± 1.64	12.87 ± 1.42	12.37 ± 1.24				15.87 ± 1.59	
	[25]	PIXE	12	36 ± 21(7–69)				16.8 ± 1.0	18.1 ± 1.5	15.3 ± 15.3	17.9 ± 1.9	17.0 ± 2.2						11.2 ± 2.0
Li(ng/g)	[22]	ICP-AES	1							9.08 ± 0.51								7.80 ± 0.38
Na	[27][a]	NAA	4	(30–73)					11.48 ± 4.84									
(mg/g)	[28][a]	NAA	15	72 ± 13	6.30 ± 1.19													
	[29][a]	NAA	8	64 ± 19(38–87)			8.94 ± 3.02											
	[21]	NAA	5	75				6.40 ± 0.50	5.00 ± 0.40	4.30 ± 0.30		10.2 ± 1.0						
	[22]	NAA	5					6.40 ± 0.57	4.98 ± 0.43	4.62 ± 0.30			8.62 ± 1.32	6.82 ± 0.39				
	[20]	ICP-AES	3					7.3 ± 0.8		12.2 ± 1.0								
	[19][a]	ICP-AES	8	(50–60)	8.5 ± 1.7	9.4 ± 3.0					11.2 ± 2.5	10.5 ± 1.7	10.5 ± 2.9	12.7 ± 6.9	7.6 ± 1.1	14.1 ± 5.1	7.7 ± 0.6	
	[34]	NAA	21	79(51–94)	8.67 ± 1.86							7.99 ± 1.43						
	[23]	NAA	31	78 ± 9(51–95)	7.96 ± 1.69	8.20 ± 1.35					7.51 ± 1.32	6.30 ± 1.09	6.02 ± 1.01				7.07 ± 0.94	
	[35]	ICP-AES	45	83 ± 8(70–101)				0.056 ± 0.043	0.052 ± 0.089	0.037 ± 0.026								
	[36]	ICP-AES	45	83 ± 8(70–101)								0.028 ± 0.05						
Rb	[25]	PIXE	12	36 ± 21(7–69)				18.4 ± 2.9	18.7 ± 2.9	15.7 ± 3.2	17.0 ± 2.6	15.1 ± 2.2						8.9 ± 1.7
(μg/g)	[37][a]	NAA	7	50 ± 19(23–60)				22.7 ± 1.3	26.3 ± 2.8	19.8 ± 1.2		21.1 ± 3.3						18.2 ± 4.5
	[34]	NAA	21	79(51–94)	19.4 ± 5.1							21.6 ± 5.5						
	[38]	NAA	1					1.5	1.0	1.2		1.8		1.4				1.6
	[27][a]	NAA	4	(30–73)					42.7 ± 11.3									
	[28][a]	NAA	15	72 ± 13	10.47 ± 2.77													
	[29][a]	NAA	8	64 ± 19(38–87)			11.5 ± 4.35											
	[30][a]	NAA	1					12.3		8.5		8.1						6.4
	[39][a]	ICP-MS	12	79 ± 9(63–89)								2.86 ± 0.87						
	[21]	NAA	5	75				14 ± 5	16 ± 6	14 ± 6				10 ± 4				
	[22]	NAA	5					15 ± 4	16 ± 6	13 ± 6			13 ± 6	12 ± 6				
	[31]	NAA	30	61(52–69)								7.73						
	[31]	NAA	30	63(55–72)								8.57						
	[23]	NAA	31	78 ± 9(51–95)	22.2 ± 5.2	21.6 ± 4.1					22.2 ± 5.2	23.2 ± 5.0	21.1 ± 3.7				24.5 ± 5.0	

Brain regions: 1: frontal cortex; 2A: superior temporal gyrus; 2B: middle temporal gyrus; 3A: caudate nucleus; 3B: putamen; 3C: globus pallidus; 4: cingulated gyrus; 5: hippocampus; 6: inferior parietal lobule; 7: visual cortex of the occipital lobe; 8: midbrain; 9: pons; 10: medulla; 11: cerebellum.

[a] Results originally reported as per wet weight basis. Converted into a dry weight basis by using dry to wet weight conversion ratios published for the specific brain regions [29,21,23,40].

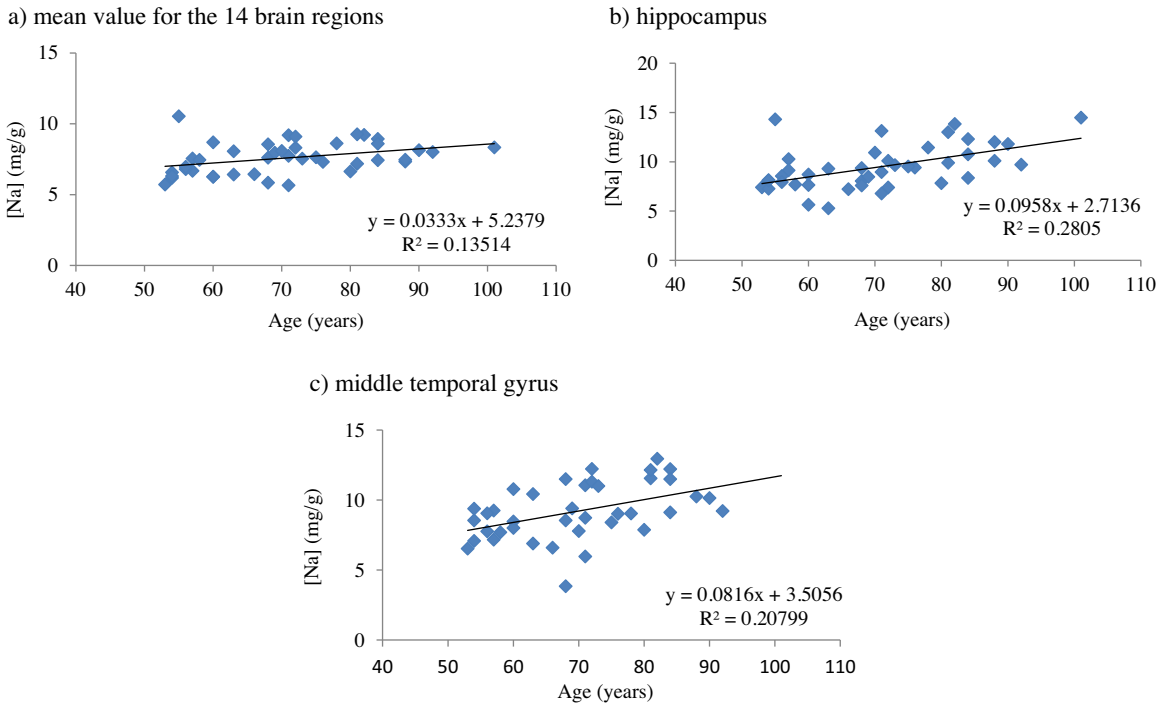

Fig. 3. Relationship between Na levels (mg/g) and age (years). Mean Na level (a) for each subject (mean value for the 14 brain regions) and Na levels in the hippocampus (b) and middle temporal gyrus (c).

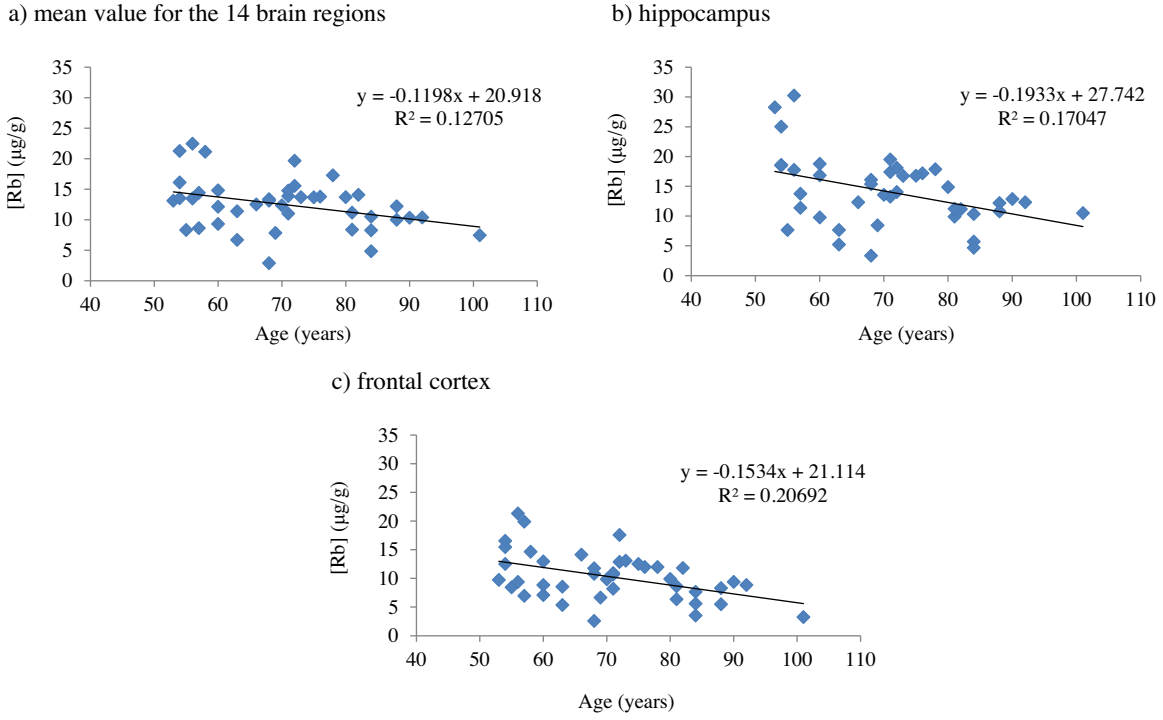

Fig. 4. Relationship between Rb levels (µg/g) and age (years). Mean Rb level (a) for each subject (mean value for the 14 brain regions) and Rb levels in the hippocampus (b) and frontal cortex (c).

Conclusion

This study, involving the direct determination of alkali metals in 14 different brain regions of 42 non-diseased individuals, provides evidence on the heterogeneous distribution of alkali metals across the human brain: the lowest levels of K and Li were found in the brainstem (midbrain, medulla and pons) and cerebellum, while the lowest levels of Cs were found in the frontal cortex. The highest levels of K and Cs were found in putamen. Rubidium and Na seem to be more homogeneously distributed than the other alkali metals.

Considering the whole brain mean level, K, Cs and Li seem to remain quite unchanged with age, but some age-related changes were observed in Na and Rb levels in particular regions (namely in the hippocampus).

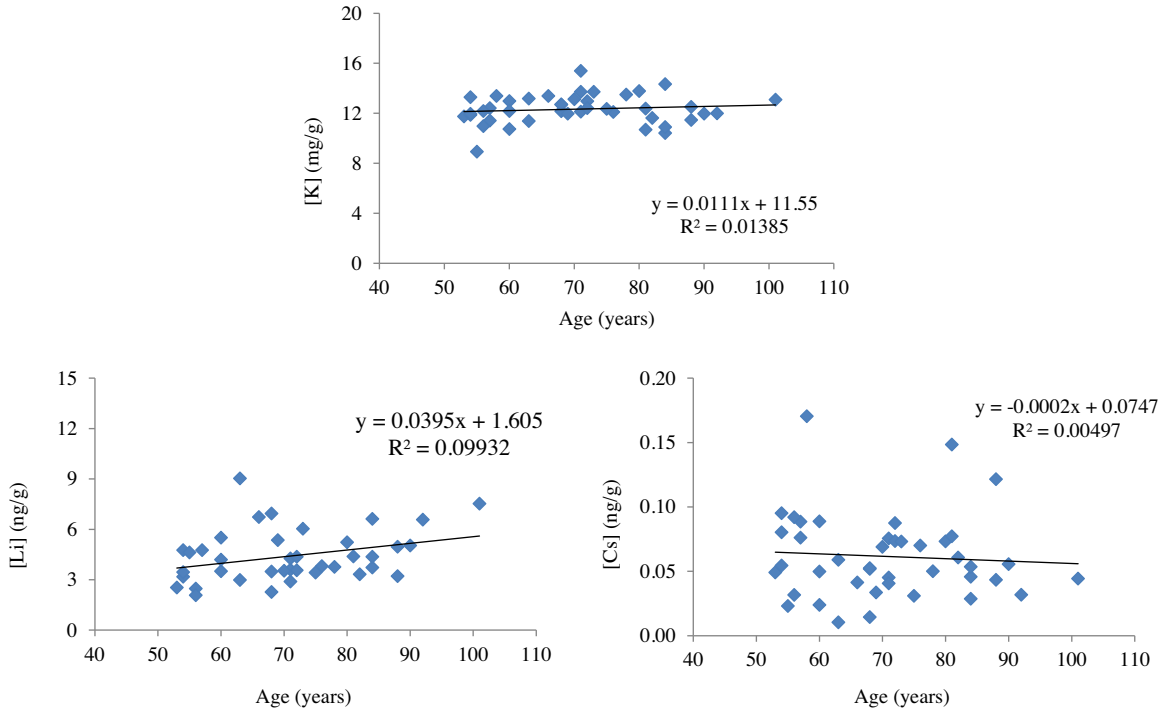

Fig. 5. Relationship between the mean value of K (mg/g) (a), Li (ng/g) (b) and Cs (ng/g) levels (c) in the 14 brain regions studied and age (years).

Table 3
Ratio between alkali metals levels in ND patients and age-matched control subjects.

Patient	Element	Brain regions													
		1	2A	2B	3A	3B	3C	4	5	6	7	8	9	10	11
AD#1	K	0.9	0.9	0.9	0.6	0.9	0.9	0.7	0.6	0.9	0.8	0.8	0.6	0.5	0.9
	Na	1.0	1.3	1.2	1.2	1.0	2.1	1.3	1.0	1.3	0.9	1.0	0.9	0.7	0.8
	Rb	0.7	0.7	0.8	0.6	0.8	0.7	0.5	0.6	0.7	0.7	0.7	0.5	0.7	0.8
	Cs	0.3	0.3	0.3	1.5	0.4	1.4	0.6	0.9	0.3	0.4	0.4	0.5	1.0	0.1
	Li	0.3	0.3	0.4	1.6	0.5	1.7	1.0	1.5	0.5	0.3	0.5	1.0	1.2	1.2
AD#2	K	1.2	0.9	1.0	1.1	1.1	1.0	1.2	1.1	1.1	1.1	1.1	1.2	1.6	1.0
	Na	1.2	1.2	1.0	1.1	1.0	2.3	1.1	1.0	1.0	1.0	1.3	1.2	1.0	0.9
	Rb	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.1	1.2	1.4	1.3	1.3	1.5	1.3
	Cs	0.8	0.7	0.8	0.8	0.8	1.0	0.7	0.8	1.0	1.2	0.9	0.9	0.9	1.0
	Li	1.1	1.0	1.4	1.1	0.8	1.1	1.5	1.1	1.5	1.1	1.8	1.3	1.4	0.7
PD	K	1.1	1.3	1.0	0.6	1.0	1.1	1.2	1.1	1.3	0.9	1.0	1.0	1.0	0.9
	Na	1.1	0.8	0.7	1.1	0.9	0.7	1.1	0.9	0.9	0.9	0.7	0.9	0.8	1.1
	Rb	2.0	2.0	1.4	1.8	1.7	1.7	1.9	1.7	2.4	1.5	1.6	1.5	1.8	1.4
	Cs	16.7	3.9	1.3	3.6	1.1	1.4	2.0	1.7	4.3	1.7	1.9	0.9	1.2	1.3
	Li	14.2	2.4	1.4	5.3	0.9	1.1	0.9	1.9	2.9	1.7	2.7	0.3	0.9	1.6

No differences in alkali metals levels were found between women and men. Higher levels of K, Rb and Cs were found in the brain of smokers compared to non-smokers.

Although differences in alkali metals levels in several brain regions of ND patients were found, no conclusions can be drawn due to reduced number of diseased subjects.

This work provides comprehensive and updated data about alkali metals levels in non-diseased human brain, thus contributing for defining the “normal” levels, in order to allow future comparisons with data obtained from patients affected by ND. Furthermore, this work showed that the distribution of alkali metals in the normal human brain is heterogeneous, namely for Li, Cs and K, highlighting the importance of defining the specific brain regions when studying the elemental composition on ND and other brain diseases.

References

[1] J. Campdelacreu, Parkinson disease and Alzheimer disease: environmental risk factors, Neurologia 29 (9) (2014) 541–549 (Barcelona, Spain).
[2] C. Reitz, R. Mayeux, Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers, Biochem. Pharmacol. 88 (4) (2014) 640–651.
[3] Schapira A.H.V. Etiology, Pathogenesis of parkinson disease, Neurol. Clin. 27 (3) (2009) 583–603.
[4] E. Radi, P. Formichi, C. Battisti, A. Federico, Apoptosis and oxidative stress in neurodegenerative diseases, J. Alzheimers Dis. 42 (Suppl. 3) (2014) S125–52.
[5] A.H. Bhat, K.B. Dar, S. Anees, M.A. Zargar, A. Masood, M.A. Sofi, et al., Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight, Biomed. Pharmacother. 74 (2015) 101–110.
[6] E. Niedzielska, I. Smaga, M. Gawlik, A. Moniczewski, P. Stankowicz, J. Pera, et al., Oxidative stress in neurodegenerative diseases, Mol. Neurobiol. (2015).
[7] K.A. Jellinger, The relevance of metals in the pathophysiology of neurodegeneration, pathological considerations, Int. Rev. Neurobiol. 110 (2013) 1–47.
[8] S. Rivera-Mancia, I. Perez-Neri, C. Rios, L. Tristan-Lopez, L. Rivera-Espinosa, S. Montes, The transition metals copper and iron in neurodegenerative diseases, Chem. Biol. Interact. 186 (2) (2010) 184–199.
[9] V.M. Vitvitsky, S.K. Garg, R.F. Keep, R.L. Albin, R. Banerjee, Na+ and K+ ion imbalances in Alzheimer's disease, Biochim. Biophys. Acta 1822 (11) (2012) 1671–1681.
[10] D. Paling, B.S. Solanky, F. Riemer, D.J. Tozer, C.A. Wheeler-Kingshott, R. Kapoor, et al., Sodium accumulation is associated with disability and a progressive course in multiple sclerosis, Brain 136 (Pt 7) (2013) 2305–2317.
[11] A.R. Michell, Sodium: physiology, in: B. Caballero (Ed.), Encyclopedia of Human Nutrition, third edition, Waltham: Academic Press, 2013, pp. 200–203.
[12] L.J. Appel, Potassium, in: B. Caballero (Ed.), Encyclopedia of Human Nutrition, third edition, Waltham: Academic Press, 2013, pp. 52–55.
[13] G.R. de Lores Arnaiz, M.G.L. Ordieres, Brain Na(+), K(+)-ATPase activity in aging and disease, Int. J. Biomed. Sci. 10 (2) (2014) 85–102.
[14] H. Aral, A. Vecchio-Sadus, Lithium: environmental pollution and health effects, in: J.O. Nriagu (Ed.), Encyclopedia of Environmental Health, Elsevier, Burlington, 2011, pp. 499–508.
[15] O.V. Forlenza, V.J. De-Paula, B.S. Diniz, Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders, ACS Chem. Neurosci. 5 (6) (2014) 443–450.
[16] A.S. Relman, The physiological behavior of rubidium and cesium in relation to that of potassium, Yale J. Biol. Med. 29 (3) (1956) 248–262.
[17] R.W. Leggett, L.R. Williams, D.R. Melo, J.L. Lipsztein, A physiologically based biokinetic model for cesium in the human body, Sci. Total Environ. 317 (1-3) (2003) 235–255.
[18] S.M.L. Paine, J.S. Lowe, Approach to the post-mortem investigation of neurodegenerative diseases: from diagnosis to research, Diagn. Histopathol. 17 (5) (2011) 211–216.
[19] M.T. Rajan, K.S. Jagannatha Rao, B.M. Mamatha, R.V. Rao, P. Shanmugavelu, R.B. Menon, et al., Quantification of trace elements in normal human brain by inductively coupled plasma atomic emission spectrometry, J. Neurol. Sci. 146 (2) (1997) 153–166.
[20] E. Andrasi, L. Orosz, L. Bezur, L. Ernyei, Z. Molnar, Normal human brain analysis, Microchem. J. 51 (1–2) (1995) 99–105.
[21] C. Bélavári, E. Andrási, Z. Molnár, D. Gawlik, Determination of Na, K, Rb and Cs distribution in human brain using neutron activation analysis, Microchim. Acta. 146 (3-4) (2004) 187–191.
[22] C. Bélavári, E. Andrási, Z. Molnár, É. Bertalan, Determination of alkali metals in control and AD brain samples by different techniques, Microchem. J. 79 (1–2) (2005) 367–373.
[23] M. Saiki, R.E.P. Leite, F.A. Genezini, L.T. Grinberg, R.E.L. Ferretti, J.M. Farfel, et al., Trace element concentration differences in regions of human brain by INAA, J. Radioanal. Nucl. Ch. 296 (1) (2013) 267–272.
[24] A.K. Afifi, The basal ganglia: a neural network with more than motor function, Semin. Pediatr. Neurol. 10 (1) (2003) 3–10.
[25] H. Duflou, W. Maenhaut, J. De Reuck, Regional distribution of potassium, calcium, and six trace elements in normal human brain, Neurochem. Res. 14 (11) (1989) 1099–1112.
[26] P. Brodal, The Central Nervous System, 4th ed., University Press, New York: Oxford, 2010.
[27] L. Zecca, R. Pietra, C. Goj, C. Mecacci, D. Radice, E. Sabbioni, Iron and other metals in neuromelanin, substantia nigra, and putamen of human brain, J. Neurochem. 62 (3) (1994) 1097–1101.
[28] J.D. Stedman, N.M. Spyrou, Elemental analysis of the frontal lobe of normal brain tissue and that affected by Alzheimer's disease, J. Radioanal. Nucl. Ch. 217 (2) (1997) 163–166.
[29] A.E. Panayi, N.M. Spyrou, P. Part, Differences in trace element concentrations between Alzheimer and normal human brain tissue using instrumental neutron activation analysis (INAA), J. Radioanal. Nucl. Ch. 249 (2) (2001) 437–441.
[30] G. Henke, H. Möllmann, H. Alfes, Vergleichende untersuchungen über die konzentration einiger spurenelemente in menschlichen hirnarealen durch neutronenaktivierungsanalyse, Z. Neurol. 199 (4) (1971) 283–294.
[31] N.I. Ward, J.A. Mason, Neutron activation analysis techniques for identifying elemental status in Alzheimer's disease, J. Radioanal. Nucl. Ch. 113 (2) (1987) 515–526.
[34] R.E.P. Leite, W. Jacob-Filho, M. Saiki, L.T. Grinberg, R.E.L. Ferretti, Determination of trace elements in human brain tissues using neutron activation analysis, J. Radioanal. Nucl. Ch. 278 (3) (2008) 581–584.
[35] Y. Tohno, S. Tohno, C. Azuma, T. Minami, L. Ke, N. Ongkana, et al., Mineral composition of and the relationships between them of human basal ganglia in very old age, Biol. Trace Elem. Res. 151 (1) (2013) 18–29.
[36] Y. Tohno, S. Tohno, N. Ongkana, P. Suwannahoy, C. Azuma, T. Minami, et al., Age-related changes of elements and relationships among elements in human hippocampus, dentate gyrus, and fornix, Biol. Trace Elem. Res. 138 (1-3) (2010) 42–52.
[37] A. Höck, U. Demmel, H. Schicha, K. Kasperek, L.E. Feinendegen, Trace element concentration in human brain. Activation analysis of cobalt, iron, rubidium, selenium, zinc, chromium, silver, cesium, antimony and scandium, Brain 98 (1) (1975) 49–64.
[38] M. Ördögh, S. Fazekas, E. Horváth, I. Óváry, L. Pogány, I. Sziklai, et al., The regional distribution of copper and other trace elements in the human brain with special reference to Wilson's disease, J. Radioanal. Nucl. Ch. 79 (1) (1983) 15–21.
[39] F. Corrigan, G. Reynolds, N. Ward, Hippocampal tin, aluminum and zinc in Alzheimer's disease, Biometals 6 (3) (1993) 149–154.
[40] L. Wuyts, J. Smeyers-Verbeke, D.L. Massart, Atomic absorption spectrophotometry of copper and zinc in human brain tissue: a critical investigation of two digestion techniques, Clin. Chim. Acta 72 (3) (1976) 405–407.
[41] B. Opitz, Memory function and the hippocampus, Front Neurol. Neurosci. 34 (2014) 51–59.
[42] G. Hebbrecht, W. Maenhaut, J.D. Reuck, Brain trace elements and aging, Nucl. Instrum. Methods Phys. Res. Sect. B 150 (1–4) (1999) 208–213.
[43] A. Rodgman, T.A. Perfetti, The Chemical Ocmponents of Tobacco and Tobacco Smoke, 2nd ed., CRC Press, Boca Raton, 2013.
[44] P. Zatta, Metal Ions and Neurodegenerative Diseases, World Scientific, Singapore, 2003.
[45] K. Anbarasi, G. Vani, K. Balakrishna, C.S. Devi, Effect of bacoside A on membrane-bound ATPases in the brain of rats exposed to cigarette smoke, J. Biochem. Mol. Toxicol. 19 (1) (2005) 59–65.
[46] L. Wang, J.G. McComb, M.H. Weiss, A.A. McDonough, B.V. Zlokovic, Nicotine downregulates alpha 2 isoform of Na, K-ATPase at the blood-brain barrier and brain in rats, Biochem. Biophys. Res. Commun. 199 (3) (1994) 1422–1427.
[47] N. Hattori, K. Kitagawa, T. Higashida, K. Yagyu, S. Shimohama, T. Wataya, et al., CI-ATPase and Na+/K(+)-ATPase activities in Alzheimer's disease brains, Neurosci. Lett. 254 (3) (1998) 141–144.