RESEARCH ARTICLE

Right cerebral hemispheric sensitivity to pH and physiological ions in fixed post-mortem Wistar rat brains

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Abstract Post-mortem human neural tissues fixed in ethanol and aldehyde-based solutions express modulated frequency-dependent microvolt potentials when probed by chemical and electrical stimuli. These observations run contrary to the assumption that basic tissue functions are irreversibly impaired upon fixation, in the absence of nutrients and sufficient concentrations of physiological ions. The aim of the current study was to investigate the relative effects of pH and specific charged particles relevant to normal cell physiology upon electric potentials associated with fixed post-mortem rat brain tissue. We identified a positive relationship between the total time the brains had been immersed in ethanol–formalin–acetic acid and high-frequency microvolt potentials within the dorsal right hemisphere of the rat cerebrum. Measuring the pH of the fixative solution surrounding the brains indicated that as time increased, a logarithmic trend toward alkalinity could be observed. Further experiments revealed that high-frequency microvolt potentials were related to pH changes within the right hemisphere only. The right ventral cerebrum displayed a unique response to potassium chloride in ways uncounted for by pH alone. The results suggest that the fixed post-mortem right cerebrum of the rat is particularly sensitive to pH and physiological ions which explains a subset of previous findings with respect to stimulus–response patterns in human coronal brain sections. A concluding hypothesis is presented which suggests

that brain tissue expresses material properties independent of metabolic activity though perhaps relevant to living brain function.

Keywords Post-mortem brain · Microvolt potential · pH · Ethanol–formalin–acetic acid · Cerebrospinal fluid

Introduction

Various methods have been developed which allow researchers to extract live tissues from organisms so as to observe their functions within controlled settings. Organotypic slice cultures represent one such in vitro method by which cellular and molecular investigations can be undertaken to further understand the mechanisms underlying the function of neural tissue (Bui and Glavinović 2013, 2014; Kamijo et al. 2014; Noraberg et al. 2005; Yoneyama et al. 2011). As experiments are typically performed shortly after the animal is sacrificed (Opitz-Araya and Barria 2011), the method can be considered near in vivo, where proportions of cells continue to live by means of nutrient supplementation (Humpel 2015). Without sufficient nutrients or the presence of physiological ions which are integral to the maintenance of membrane potential differences, the explant withers until normal function is lost entirely upon death. Assuming optimal conditions, cell survival in slice preparations is temporary where the typical culture period can last from hours to a few months (Egert et al. 1998; Humpel 2015; Østergaard et al. 1990).

If the specimens are treated with ethanol or aldehyde-based fixative solutions, the structure of the tissue explant can remain intact for years—presumably at the cost of function. It is assumed that cell and tissue functions are irreversibly impaired upon fixation—stripped of their

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capacity to process electrochemical signals. Therefore applied stimuli would not be expected to elicit physiologically relevant responses post-fixation. If, however, some residual response capacity could be detected within fixed neural tissue by means of chemical and electric probes, the assumption of lost function would be subject to re-examination. Such observations would, in all likelihood, violate the basic principles governing normal cell physiology. Alternatively, the detection of stimulus–response patterns in post-mortem tissue would represent a novel observation, separate and distinct from those observed in living organisms and their tissues. Rather than a violation of established principles, the phenomenon could represent a general property of brain tissue in the absence of metabolic function.

Several experiments have recently demonstrated that chemically fixed post-mortem human and rat brains exhibit regional, frequency-dependent electric potential differences which vary systematically in response to applied stimuli. Coronal sections of human brain tissue were selectively responsive to electrical (Rouleau and Persinger 2016) and chemical (Rouleau et al. 2016a, b) stimuli with evidence of dose-dependence and habituation. Most notably, opposing deviations of high-frequency spectral power were noted for applications of glutamate and ketamine—ligands which act upon the NMDA receptor as an agonist and antagonist respectively (Rouleau et al. 2016c). Stimulus–response patterns were observed in addition to baseline low-frequency spectral power cerebral hemispheric asymmetries (Rouleau et al. 2016a). In combination with the observation that proportions of theta spectral power (4–7.5 Hz) differentiated rat brains which were, several years earlier, experimentally induced to seize by a single system injection of lithium–pilocarpine and controls (Rouleau et al. 2016b), it was clear that a mechanism underlying post-mortem brain tissue responses should be pursued. Whereas the physical–chemical environment imposed upon the tissue by the fixative solution should have, in principle, inhibited any capacity for the tissue to respond to applied stimuli, the evidence ran contrary to this prediction. Rather, brains fixed in ethanol–formalin–acetic acid reliably demonstrated non-random, living-like signal processing capacities.

Our current approach was to investigate the fixation process with respect to changes of electric potential differences within the neural tissues in addition to the relative contribution of pH and physiological ions to regional spectral power differences across the neural axis. In search of a mechanism, we systematically investigated how pH changes over time were related to spectral power derived from microvolt fluctuations within the tissues. Further, we re-hydrated fixed tissues to determine whether or not morphologically preserved brains could be induced to

express changes in electric potential differences within select regions when exposed to physiological ions typical of the neural environment.

Methods

Brain specimens

Forty-eight ($n = 48$) brains were extracted from Wistar (albino) rats and placed within solutions of ethanol–formalin–acetic acid (72% ethanol, C_2H_6O ; 18% distilled water, dH_2O ; 5% acetic acid, $C_2H_4O_2$; 5% formaldehyde, CH_2O , $pH = 2.9$) post-excision. Of the forty-eight, thirty-six ($n = 36$) were examined previously by Rouleau et al. (2016b), where it was determined that lithium–pilocarpine seized rats ($n = 13$) expressed reduced brain mass and proportional decreases of 4–7.5 Hz spectral power as inferred by microvolt fluctuations obtained within the tissue relative to non-seized rats ($n = 23$). Though sexually dimorphic brain masses were noted, male–female differences could not be identified when examining microvolt fluctuations within the tissue, indicating that simple differences of mass or diffuse, sexually dimorphic nuclei were not driving the observed effects. Rather, regional pathologies were identified as the proximal cause of theta differences (Rouleau et al. 2016b). Eleven ($n = 11$) additional brains were utilized which were originally collected during neurobiology laboratory demonstrations associated with the Behavioural Neuroscience Program at Laurentian University between 1996 and 2008. A final brain ($n = 1$) was harvested as a longitudinal case in order to experimentally validate observations which indicated temporal effects associated with the fixation process. Brains were stored in a darkened environment at room temperature ($23\text{ }^{\circ}C$) and remained undisturbed, some for over 20 years in solution.

Artificial cerebrospinal fluid

Tissues were subjected to a re-hydration protocol with supplemented physiological ions. The brains were immersed within different formulations of artificial cerebrospinal fluid (CSF) which are outlined in Table 1. The standard formula (134 mM NaCl, 26 mM $NaHCO_3$, 10 mM D-Glucose, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.25 mM K_2HPO_4), which will be referred to as CSF 1, was experimentally manipulated by excluding compounds from the mixture in order to investigate the relative contribution of each component to any effects observed during tissue re-hydration. Alternative formulae which deviated from the standard CSF 1 solution were labelled CSF 2–CSF 6 for brevity (Table 1). All solutions

Table 1 Artificial cerebrospinal fluid (CSF) condition formulae

Cerebrospinal fluid condition (osmolarity)	Formula	
	Included	Excluded
CSF 1 (349 mOsm/L)	134 mM NaCl, 26 mM NaHCO ₃ , 10 mM D-Glucose, 2.5 mM KCl, 2 mM CaCl ₂ , 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	
CSF 2 (81 mOsm/L)	26 mM NaHCO ₃ , 10 mM D-Glucose, 2.5 mM KCl, 2 mM CaCl ₂ , 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	134 mM NaCl
CSF 3 (343 mOsm/L)	134 mM NaCl, 26 mM NaHCO ₃ , 10 mM D-Glucose, 2.5 mM KCl, 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	2 mM CaCl ₂
CSF 4 (344 mOsm/L)	134 mM NaCl, 26 mM NaHCO ₃ , 10 mM D-Glucose, 2.5, 2 mM CaCl ₂ , 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	2.5 mM KCl
CSF 5 (297 mOsm/L)	134 mM NaCl, 10 mM D-Glucose, 2.5 mM KCl, 2 mM CaCl ₂ , 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	26 mM NaHCO ₃
CSF 6 (18 mOsm/L)	10 mM D-Glucose, 1.3 mM MgCl ₂ , 1.25 mM K ₂ HPO ₄	134 mM NaCl, 26 mM NaHCO ₃ , 2.5 mM KCl, 2 mM CaCl ₂

were gassed with 5% CO₂, 95% O₂ for 20 min before they were stored at 4 °C. Solutions were not bubbled continuously throughout recordings. Before immersion, CSF solutions were brought to room temperature or 23 °C.

Electric potential measurements

The brains were probed by needle electrodes inserted strategically into regions along the neural axis as was first attempted by Rouleau et al. (2016b). Recently, similar methods were applied to human brain sections (Rouleau and Persinger 2016; Rouleau et al. 2016c). Four needle electrodes were inserted into the spinal cord and caudal medulla, spatially in parallel (Fig. 1b, c). These sensors constituted the electrical reference point for electric potential (μ V) measurements. A fifth electrode, which served as the primary probe from which electric potentials were obtained, was inserted 6.6 mm into the dorsal or ventral components of the left and right cerebral hemispheres. When inserted dorsally, the probe was positioned at equidistance between the rostral and caudal poles of the cerebrum as well as between the interhemispheric fissure and the lateral convexity of the hemisphere. When inserted ventrally, the probe was positioned at the caudal pole, inferior to the rhinal fissure, and just anterior to the cerebellum. The four resulting quadrants of measurement (i.e. left dorsal, left ventral, right dorsal, and right ventral) were observed for all brains over the course of the study.

Data (microvolt potentials) were collected using a Mit-sar 201 quantitative electroencephalography (QEEG) amplifier which streamed live data to a laptop computer (HP Envy) running WinEEG version 2.93.59 (07.2013) software with a Windows 8 operating system. The sampling rate of the device was 250 Hz. A low-cut filter of 1.6 Hz and a high-cut filter of 50 Hz were applied while

recording. An additional notch filter was toggled to eliminate frequency contributions between 50 and 70 Hz as well as between 110 and 130 Hz. The gain of the device was set to 150 μ V. Raw microvolt potentials were converted to spectral power densities (μ V² Hz⁻¹) within classical EEG bandwidths: delta (1.5–4 Hz), theta (4–7.5 Hz), alpha (7.5–14 Hz), beta1 (14–20 Hz), beta2 (20–30 Hz), and gamma (30–40 Hz).

pH measurement

It was necessary to measure the pH of the fixative solution as well as other solutions which served as experimental manipulations of the tissues. A glass pH probe was inserted into volumes of solution at room temperature while coupled to a DrDAQ board streaming live data to a ThinkPad laptop computer running Pico Technology (UK) software. The recording rate of the device was set to 1 Hz with an intrinsic refresh rate of 50 ms. The device was calibrated using standard buffered solutions between 4.0 and 10.0 pH before use.

Data analysis

Electrodynamic data were extracted within the WinEEG interface as spectral power densities within the aforementioned frequency bands. Averaged spectral power measures (e.g., alpha to gamma, beta 1 to gamma) were computed in order to observe generalized high-frequency modulations which were readily manipulated over the course of the experiments. Once extracted, data were imported to SPSS v20 and analyzed. Analyses are specified where results are presented though generally consisted of simple measures of differences in addition to both parametric and non-parametric correlational analyses.

Fig. 1 Rat brains (a) were probed (b, c) by needle electrodes inserted into the left and right dorsal and ventral cerebrum referenced to the caudal medulla and spinal cord

Results

Brains fixed in EFA

Examining thirty-six ($n = 36$) brains which could be identified as seized or non-seized animals based upon microvolt potentials and brain mass (Rouleau et al. 2016b), a weak positive relationship was identified between an average of high-frequency (14–40 Hz) spectral power densities and the number of days that the tissue had been immersed in EFA or, implicitly, time post-mortem, $r = .23$, $p < .005$, $\rho = .20$, $p < .05$ (Fig. 2). Isolating spectral power within the right dorsal quadrant from other probed regions increased the strength of the relationship, $r = .365$, $p < .05$, $\rho = .40$, $p < .05$. This result suggested that cerebral quadrants were independently sensitive to unique factors. When comparing cerebral dorsal measurements for non-seized ($n = 23$) and seized rats ($n = 13$), no significant difference could be discerned ($z < 1.96$, $p < .05$), though in both cases the strength of the association further increased ($r = .50 - .70$).

We then measured the pH of the fixative solution (EFA) within randomly selected containers holding rat brains from our collection extracted between 1996 and 2016. Brains were always immersed within EFA after the animal was sacrificed. Therefore all brains received the same post-mortem “treatment” of fixation with EFA. The data, presented in Fig. 3, suggest that there was a logarithmic relationship between the pH of the fixative solution surrounding the rat brains and time. Over a relatively short period of time (12 days), the EFA (pH 2.9) became slightly more alkaline (pH 3.2). An

Fig. 2 Averaged high-frequency (14–40 Hz) spectral power densities for all quadrants (top) and for right dorsal quadrant (bottom)

inflection in the curve was noted around 3 years, which was followed by a weak linear relationship. At 7467 days post-mortem (20.5 years), the solution surrounding the tissue was associated with a pH of ~ 4.3 . In other words, EFA surrounding brain tissue was associated with a relatively sharp trend toward neutral pH over the course of the 2 first months post-fixation followed by a relatively gradual trend subsequently which persisted over several years.

Fig. 3 pH of EFA within containers surrounding rat brains as a function of time (days) that the brains were immersed in fixative solution and stored

Our observations that pH and microvolt potentials were both related to time—though likely physical–chemical processes operating over time—suggested that longitudinal observations of the same brain would reveal that, in fact, these factors could be observed to systematically change in concert with each other. To this end, we extracted the brain of an aged (1315 days old) male Wistar rat ($n = 1$) on October 6th, 2016. The brain weighed (wet) 2.58 g and was anatomically unremarkable at a gross structural level. It was subjected to immediate measurement (Fresh) of electric potential differences between cerebral hemispheric loci and the spinal cord, after which it was immersed within a 100 mL solution of EFA and stored at room temperature. Subsequent measurements were obtained at 30 min, 24, 48, 72 h, and several other times 1–9 weeks post-fixation (Fig. 4). The net result of the serial measurement protocol was a longitudinal case study of electric potential differences over the fixation period of the rat brain which could be compared to the greater ($n = 47$) sample of electric potential measurements (Fig. 2) and the pH phenomenon observed in Fig. 3. Figure 4 demonstrates that high-frequency (14–40 Hz) spectral power increased over the course of the 9 weeks period with a cumulative positive shift of .6 pH units. The average (smoothed, dashed line) trend over time expressed an amplitude peak between 3 and 6 weeks post-fixation.

Re-hydrating fixed brains

Fixation is generally treated as a unidirectional phenomenon. That is, once the tissue is perfused by the fixative, the physiological mechanisms which were once present within the biological substrata are assumed to be

irreversibly impaired. To evaluate this assumption, we re-hydrated rat brains ($n = 8$) using different formulations of artificial CSF (Table 1): CSF 1 ($n = 3$), CSF 2 ($n = 3$), CSF 3 ($n = 3$), CSF 4 ($n = 3$), CSF 5 ($n = 3$), and CSF 6 ($n = 3$). Each rat brain was subjected to three of the possible 7 conditions (CSF conditions plus EFA condition). Our original measurements indicated that global (1.5–40 Hz) right hemispheric spectral power could be experimentally altered by consecutively (24 h cycles) immersing the brains in EFA and CSF over a 5 days period (Fig. 5a). Combined trials indicated that immersion within CSF 1 produced significantly different high-frequency (7.5–30 Hz) spectral power excluding gamma frequencies (30–40 Hz) over time, $F(4, 14) = 4.56$, $p < .05$, $\eta^2 = .65$ (Fig. 5b). The major source of variance or most notable effect was a significant increase of 7.5–30 Hz power within the right dorsal quadrant when the brains were re-introduced to the relatively acidic fixative solution (EFA) after 24 h of CSF 1 immersion, $t(4) = 4.07$, $p < .05$, $r^2 = .81$. The difference between power during the 24 h CSF 1 immersion and baseline measures involving fixative solution (pre-EFA) was equivalent, $r^2 = .80$. The most notable observation was that high-frequency spectral power returned to baseline levels as is observed when comparing the means of pre-EFA and post-EFA ($p > .05$). Based upon these observations, we conducted a series of counterbalanced between-subject experiments in order to investigate the potential relative contribution of components of CSF 1 to the re-hydration effect.

An analysis of variance (ANOVA) identified high-frequency spectral power differences between CSF formulations and control conditions (EFA, dH₂O) after 1 h immersions within the right hemisphere only, $F(8, 71) = 2.10$, $p < .05$, $\eta^2 = .21$ (Fig. 6). Low-frequency power was unaffected ($p > .05$). The left hemisphere did not respond differentially to alternative formulations of CSF and control conditions ($p > .05$) as seen in Fig. 7. It was subsequently identified that dH₂O was driving the right hemispheric effect, however, further investigation revealed that there was a significant positive relationship (Fig. 6) between pH and beta 2 (20–30 Hz) spectral power within the ventral ($r = .36$, $p < .005$, $\rho = .39$, $p = .001$) right hemisphere which was not observed within the dorsal component of the same hemisphere ($r = .10$, $p > .05$, $\rho = .12$, $p > .05$). Examining differences between expressed 7.5–40 Hz power within the ventral and dorsal components of the right hemisphere, only CSF 1 (standard CSF, $r^2 = .53$) and CSF 3 (CaCl₂ is removed, $r^2 = .61$) produced reliable disparities ($p < .05$). The effects associated with CSF 2 (NaCl is removed) were ambiguous, $t(6) = 2.31$, $p = .06$, demanding further investigation. These results suggested that CaCl₂ was not driving the dorsal–ventral differences produced by CSF 1 which were

Fig. 4 High-frequency (14–40 Hz) spectral power densities within each cerebral quadrant for serial measurements of a rat brain undergoing fixation in EFA solution. A smoothed average is represented by the *dashed line*

Fig. 5 Global right hemispheric power of a single rat brain varied systematically when immersed for 24 h within alternating EFA and CSF solutions over five consecutive days (a). Averaged high-frequency (7.5–30 Hz) spectral power excluding gamma (30–40 Hz) within the right dorsal quadrant of rat brains ($n = 3$) measured serially over time indicates that power returns to baseline conditions once re-immersed in the fixative solution (b)

unobserved in the absence of physiological ions (EFA and dH_2O). However, we conducted further tests to experimentally demonstrate the contribution of the physiological ions. Rather than removing components from the CSF 1 standard formula, we simply added CaCl_2 , KCl , or NaCl (or a combination of these) to dH_2O and immersed the tissue for 1 h before assessing microvolt potentials.

An ANOVA revealed that significant differences of beta 2 (20–30 Hz) spectral power densities within the right hemisphere differed as a function of the solution within which the brains were immersed, $F(5, 75) = 4.86$, $p < .001$, $\eta^2 = .26$ (Fig. 8). Homogeneous subsets identified three groups which differed significantly: EFA, dH_2O , and all other conditions. However, when examining

Fig. 6 The relationship between right hemispheric ventral and dorsal beta 2 (20–30 Hz) spectral power and pH of the solution within which the tissue was immersed. Averaged wide-band high-frequency (7–40 Hz) spectral power densities for *dorsal* and *ventral*

measurements within the right hemisphere demonstrated significant differences within the CSF 1 (no compound excluded) and CSF 3 (CaCl₂ excluded) conditions. *Significant differences* are indicated ($p < .05$)

Fig. 7 Averaged wide-band high-frequency (7.5–40 Hz) spectral power densities for *dorsal* and *ventral* measurements within the left hemisphere demonstrating no *significant differences* between any of the conditions ($p > .05$)

7.5–40 Hz spectral power differences between ventral and dorsal components of the left and right hemispheres, only right hemispheric effects could be discerned, $t(4) = 3.10$,

$p < .05$, $r^2 = .71$ (Fig. 8a). The major source of variance, which explained the results presented in Figs. 6 and 8a, was a significant difference between dorsal and ventral components of the right hemisphere in brains exposed to KCl, $t(4) = 3.62$, $p < .05$, $r^2 = .77$. Whereas the removal of KCl (CSF 4) from the standard cerebrospinal fluid formula (CSF 1) abolished the effects in Fig. 6, the addition of KCl to water produced dorso-ventral disparities within the right hemisphere. This confirmed our original suspicion that KCl was driving the CSF 1 effect.

Discussion

The data indicate that the right cerebral hemispheres of Wistar rats are sensitive to pH changes within the fixative solution (the medium) as well as the presence of KCl in ways unaccounted for by pH. The findings reported in Fig. 1 demonstrate the relationship between the time that

Fig. 8 Averaged wide-band high-frequency (7.5–40 Hz) spectral power densities within the dorsal and ventral components of the left and right cerebral hemispheres when exposed to a combination of NaCl, CaCl₂, and KCl (a). Beta 2 (20–30 Hz) spectral power

increases within the dorsal component of the right hemisphere relative to the ventral component when exposed to KCl. Significant differences are indicated ($p < .05$)

the brains had been immersed in ethanol–formalin–acetic acid (EFA) and the high-frequency (14–40 Hz) spectral power derived from microvolt potentials measured within the four quadrants of the cerebrum. The data suggested that this relationship was accentuated within the right dorsal cerebrum. Suspecting the medium was perhaps changing over time and influencing microvolt potentials within the tissue, we measured the pH of the fixative material surrounding the brains while noting the total time that the vat containing both the tissue and fixative solution had remained in storage. A logarithmic relationship was apparent, where trends toward neutral pH were sharp within the first ~60 days, followed by a relatively gradual increase over time (Fig. 3). A total pH shift of ~1.5 units over 20.5 years was noted.

Having identified a process involving pH trends toward alkalinity with proportional increases of high-frequency microvolt potentials within the right cerebral hemisphere, we endeavored to observe the early phases of full brain fixation by serial measurement of a longitudinal case ($n = 1$). Figure 4, which summarizes the 9 weeks data collection period, demonstrates that high-frequency (14–40 Hz) spectral power derived from individual quadrants become increasingly disparate over time—suggesting non-isotropic distribution of the fixative material, or stated otherwise, unequal perfusion (or the direct molecular consequences of perfusion). The right ventral cerebral hemisphere displayed consistently elevated high-frequency spectral power relative to other quadrants over the fixation period. Also of note was the averaged (dashed lines) trend which suggested an amplitude peak between 3 and 6 weeks post-fixation. This temporal period is significant as it represents the approximate time required for the solidification

of methylene cross-bridges in aldehyde-based fixation (Kiernan 2000; Bjorksten 1951). We would therefore expect to see a relative reduction in the rate of change of spectral power after the first month of fixation. If methylene bridges ($-CH_2-$) continued to form at a reduced rate during the month following initial fixation, one might expect to observe the gradual liberation of hydroxyl ions into the medium which would manifest as increased alkalinity of the solution surrounding the tissue over long periods of time.

The process of hydroxyl liberation begins with formaldehyde dissolved in water which produces methylene hydrate:

Within an environment rich in amino acids, such as a brain, methylene cross-links ($-CH_2-$) would form between reactive atoms in proteins that are very close together, liberating free hydroxyl ions (OH^-) into the medium surrounding the tissue proper. The net result would be increased fixation of brain matter paired with an increase of pH within the medium due to the liberation of OH^- . If free protons were also sequestered by the brain or masked by protein cross-linking, pH increases would be amplified.

The next logical step was to experimentally manipulate the medium within which the tissues were immersed by varying pH, and the presence or absence of physiological ions. Re-hydrating the tissue in artificial CSF produced changes within 1 h of immersion with the most pronounced effects emerging after 24 h. If the brains were re-immersed in EFA after re-hydration, microvolt potentials returned to baseline conditions (Fig. 5b). It was apparent that re-hydration effects could only be observed for high-frequency

spectral power within the right cerebral hemisphere. We observed that the right ventral quadrant was particularly sensitive to our experimental manipulations of pH (Fig. 6) in ways unobserved within the dorsal component of the same hemisphere. The spectral power relationship centered upon the narrow 20–30 Hz band. Brain immersion within CSF 1, the standard formula containing all essential components, as well as CSF 3, which involved the removal of CaCl_2 , was associated with high-frequency dorso-ventral disparity of spectral power within the right hemisphere only. The likely sources of variance were NaCl and KCl as their removal from solution abolished or partially abolished dorso-ventral power disparities. In order to experimentally demonstrate the relative effects of CaCl_2 , KCl, and NaCl, each compound was added to water and served as solutions within which rat brains were placed for 1 h before testing. This approach represented the inverse of what was presented in Fig. 6 wherein compounds were systematically removed from solution. The results indicated that only solutions containing KCl reliably reproduced the high-frequency dorso-ventral spectral power disparities observed initially with CSF 1.

Together, our findings indicate that post-mortem rat brain measurements of electric potential differences are influenced by pH of the medium as well as by the presence of certain ion-producing compounds. In combination with results reported by Rouleau et al. (2016c), these experiments further approximate mechanisms underlying post-mortem tissue responsiveness. Though receptor-mediated membrane events paired to ionic flow remain unlikely, further work should aim to investigate residual molecular capacities at the level of the cellular membrane in order to establish a mechanism which explains post-mortem brain responses as have been reported here and elsewhere. However, some explanatory heavy lifting is currently required of molecular mechanisms to address why the right cerebral hemisphere as a gross structural aggregate is particularly sensitive to charged molecules, neurotransmitters (Rouleau et al. 2016c), and applications of direct current (Rouleau and Persinger 2016). Likewise, it is still unclear how particular frequencies emerge as reliable responses to particular stimuli. Future studies should aim to shift frequency responses contingent upon controlled applications of molecular agents.

Asymmetric hemispheric distributions of pH- or charge-sensitive molecules embedded within the tissues could explain why different neuroanatomical loci express degrees of microvolt fluctuation independence as well as the capacity to display dose-dependent “responses” in the presence of neurotransmitters. Known cortical and dimensional asymmetries between the left and right cerebral hemispheres in humans (Galaburda et al. 1978) and rats (Kolb et al. 1982) could account for some proportion of

the variance reported here. Indeed, heterogeneous perfusion of fixative material across post-mortem brain space could, in principle, produce voltage gradients across the tissues by altering regional conductivity. In all likelihood, the source of the phenomenon is likely to involve some interaction between charged particles associated with the fixative solution and structural features of the cerebrum—particularly those in proximity to the hippocampi as reported in several of the aforementioned studies. Some ambiguity of effects associated with CSF 2 and CSF 6 should warrant further investigation into potential contributions of osmotic pressure as these conditions were significant outliers in this regard. However, our experiments examining the relative contribution of physiological ions to the CSF effects involved several conditions associated with low osmolarity (<10 mOsm/L) and high osmolarity (>100 mOsm/L) which were not paired to obvious differences as a function of this parameter alone when isolating individual components.

Further studies should target likely protein candidates which might be contributing to the observed effects using immunohistological and other methods. Alternatively, fixed post-mortem tissue can be thought of as an intricate scaffold of microstructures with intrinsic resonant capacities. Perhaps the presence of charged particles within or across residual circuits of preserved brain material structures otherwise noisy electrical signals within the tissues into more coherent oscillations which are measured as reliable spectral power modulations. The former hypothesis assumes conventional mechanisms while the latter considers the possibility that subsections of preserved, post-mortem brain tissue can be observed and treated as a material with physical properties independent of biology though intimately related to physics and chemistry. If the electrical properties of brain material independent of life (e.g., metabolism, cell function, etc.) do impinge upon normal neurophysiological functions, a thorough study of post-mortem brain tissue and its material properties is now warranted.

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