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SUMMARY

The purpose of the report this year is to evaluate stereological data of the postmortem human brain within the framework of complexity theory. Last year, we applied a similar analysis to living brains (Bolender, 2012) using MRI data from the Internet Brain Volume Database (Kennedy et al., 2012). Specifically, we would like to know the extent to which stereological data can contribute to creating a parallel complexity - one that faithfully mirrors the complexity of living brains. Such a question represents a fundamental first step toward the larger goal of making the transition from reductionism to an evidenced-based medicine based on complexity theory. Stereology, which includes a collection of elegant mathematical methods, enjoys the remarkable distinction of being able to quantify and connect biological parts of all sizes. As such, it creates mathematical pathways that can take us into the realm of biological complexity. The results of the report begin to explain why the remarkable potential of this method has failed to reach its full potential. It turns out that the problem lies not with stereology, but rather with the source of stereological data. When we apply stereological methods to biology postmortem, the resulting data typically carry a wide range of distortions (Rule Book: Bolender, 2007-2013). Of these, volume appears to be the most important. In contrast to the MRI database, which operated successfully at both local and global levels (Bolender, 2012), the postmortem data of the stereology database worked reasonably well locally, but not globally. Evidence of mathematical patterns – detected as identical mathematical markers across many MRI studies of the living brain - failed to appear across similar stereological studies of postmortem brains. This tells us that the parts and connections of postmortem brains differ importantly from those of living brains. After running a battery of tests with roughly a million mathematical markers, it appears that postmortem brains retain only about five percent of the patterns found in living brains. Apparently, when biological parts make the transition from living to nonliving, their volumes distort unequally. In effect, the parts and connections of postmortem brains become largely chaotic when interpreted within the framework of reductionism. The report considers the implications of these finding and offers new solutions to this problem based on the application of complexity theory.

INTRODUCTION

Understanding complexity and using it to solve a wide array of challenging problems will ultimately define the future of the biology enterprise. Since we now know that it takes a complexity to study a complexity, the Enterprise Biology Software Project actively supports this new direction by introducing and testing a new theory structure for a data-driven biology. The strategy is simple and straightforward. We use the biology literature to construct complexi-

ties that capture the biological patterns needed to play the complexity game. As is the case for all games, winning and losing represent the expected outcomes. However, a well-defined strategy at the outset markedly improves our chances of winning.

When dealing with biology as a complexity, finding patterns is tantamount to finding solutions. A mathematical marker, for example, represents a biological pattern consisting of parts (names) and connections (ratios). These markers conveniently scale from data pairs (2 parts; 2 connections - AX:BY) to triplets (3 parts; 3 connections – AX:BY:C:Z) to the entire organism (n parts; n connections). Increasing the size of a marker increases the sensitivity of its patterns.

Armed with a new theory structure (Bolender, 2011-2013), we can begin to pursue general solutions to problems currently resistant to such outcomes under reductionism. Consider clinical diagnosis. When we calculate mathematical markers from patient data across a wide range of brain disorders, the resulting patterns combine to produce a powerful diagnostic tool (Bolender, 2012). Moreover, these patterns show us how the brain creates a disease by altering the relationship of its parts to connections. Although each disease displays a unique pattern, the modular components of that pattern often appear in other diseases. Such observations become helpful because they allow us to generalize disease within the framework of complexity theory. In short, the study of complex biological phenomena seems to require access to a data set having a complexity parallel to that of biology.

The current report will apply complexity theory to an unsolved problem that has plagued biological stereology since its inception, namely the affect of specimen preparation on experimental results. We will ask, "Can we use the postmortem data of stereology to diagnose disorders of the brain?" To answer this question, we will assemble an appropriate complexity consisting of about a million mathematical markers derived from published data. This new complexity, which exists as database tables in the software package, allows us to compare the patterns of living and postmortem brains quantitatively.

METHODS AND RESULTS

The software package for 2013/2014 includes new data and software tools for assembling, analyzing, and interpreting mathematical markers derived from published data. The report will show that problems widely assumed unsolvable yield by simply switching to a more powerful theory structure.

Enterprise Biology Software Package

The software includes eight screens offering ready access to programs, databases, and documents (Figure 1).

Figure 1. Enterprise Biology Software Package – 2013/14.

Vetting Stereological Data

The task before us is to test the effectiveness of stereological data in detecting the complex patterns of biology. We already know that MRI volume data collected from the living human brain can detect the same mathematical markers in many different studies (Bolender, 2012). This demonstrates that patient data generalize across individuals according to a well-defined stoichiometry. In effect, biology plays by a set of rules based on ratios.

If we now apply a similar analysis to the data of postmortem brains using data from the stereology literature database, will we get the same result? The answer is yes and no. The same markers tend to appear in different papers coming from the same lab, but not in papers coming from different labs. This tells us that preparing specimens for a stereological analysis disrupts the mathematical markers, by altering the volumes of the brain parts differently. Differently because uniform increases or decreases in the volumes of all the parts would not affect the ratios of the mathematical markers.

Next, we compare mathematical markers for brain volumes identified by source – stereology (postmortem) and MRI (living). Once again, we discover that only a few matches occur, offering further evidence that specimen preparation leads to distortions in the parts, connections, markers, and patterns. Indeed, the tests show repeatedly that specimen preparation obscures the underlying biological rules by physically changing the stoichiometry of the parts. These unnatural changes call into question our ability to play the complexity game on a playing field defined by biology. Fortunately, by offering a helping hand, complexity theory will keep us in the game.

The vetting process consists of submitting stereological data from postmortem brains to a battery of tests, using gold standards based on the MRI data of living brains and on volume independent cell counts. The results of each test leads to the next until we eventually arrive at our goal of extracting a parallel complexity from postmortem data.

Test 1 (triplets): Demonstrate that postmortem volume data coming from the stereology literature database can diagnose a disorder of the brain (schizophrenia) correctly. Results: Unsatisfactory – diagnosis incorrect.

Method: This test consisted of running mathematical markers (AX:BY:CZ) derived from postmortem brains with schizophrenia (stereology) against all the markers of the MRI diseases database. The diagnostic procedure consisted of mixing the markers of 24 known disorders (MRI) with markers for schizophrenia coming from postmortem stereology and counting the duplicates that occurred between the two data sets. The diagnosis went to the disorder with the largest number of duplicate markers (MRI = Stereology), as described earlier (Bolender, 2012).

Results: The stereological data failed the test. Figure 2 indicates that the postmortem data clearly missed the correct diagnosis of schizophrenia, giving it instead to bipolar disorder.

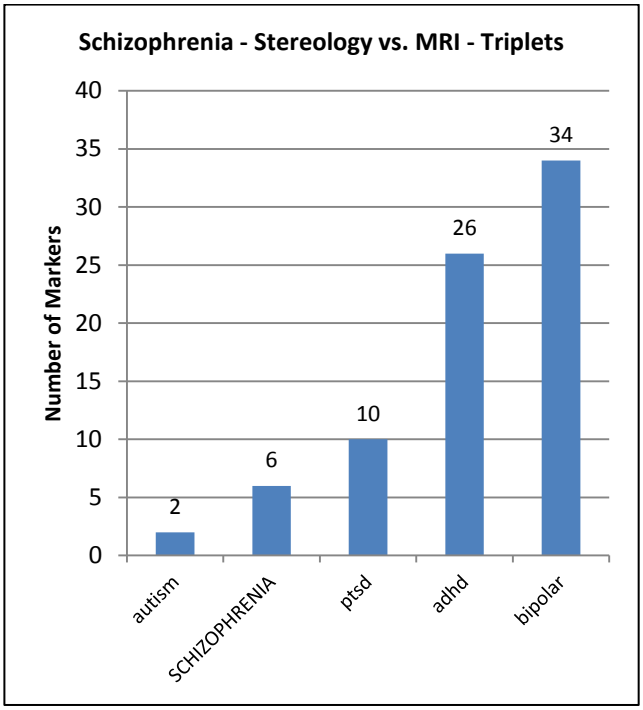

Figure 2. When mathematical markers for schizophrenia coming from postmortem brains (stereology) were run against those of living brains (MRI), the resulting diagnosis (bipolar) was incorrect. Notice that the correct diagnosis - schizophrenia – was not even close. Moreover, relatively few markers were in play.

Retest 1 (data pairs): Make the test easier. Decrease its sensitivity by reducing the number of variables in the mathematical markers from six to four. Results: better but still inconclusive.

Method: Calculate a new set of markers for the stereology and MRI data sets based on data pairs (AX:BY).

Results: Unfortunately, the stereological data still failed the test. Figure 3 shows that the results are too close to call. The diagnosis went to both schizophrenia and Alzheimer disease with bipolar a close second. These results suggest – once again - that the postmortem brains have lost many of the quantitative patterns found in living brains (Bolender, 2012).

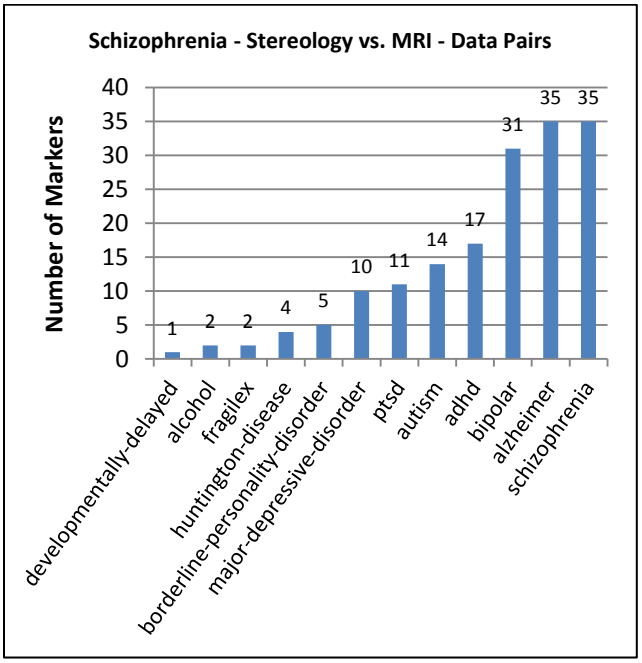

Figure 3. Using mathematical markers based on data pairs, stereological data still cannot diagnose schizophrenia unambiguously as the disorder.

Figure 4 includes the expected pattern of markers, as seen for schizophrenia in living brains. Notice that it does not resemble the histogram in Figure 3. For the convenience of the reader, Appendix II includes reference data for a wide range of disorders – based on data pairs.

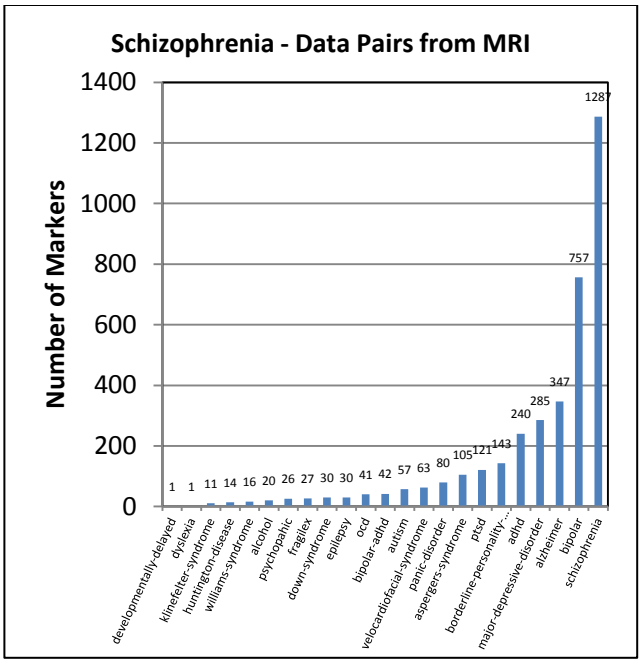

Figure 4. The MRI data of living brains provided 1287 mathematical markers for schizophrenia – based on data pairs (AX:BY). Notice, for example, that of these 1287 markers, 757 were shared with bipolar, 347 with Alzheimer, and 285 with major depressive disorder.

Test 2 (triplets): How much information – in the form of global patterns - has been lost by the postmortem brains? Results: Roughly, 80%.

Method: This test relied on counting duplicate mathematical markers (triplets) for control volumes found in the stereology and MRI databases. First, markers were identified as a duplicate if they appeared in at least two publications, which meet the minimum requirement of a global marker. For each data set (stereology or MRI), a given duplicate marker was counted only once. The amount of global information lost in postmortem brains was assessed by comparing the counts of duplicates found in the MRI and stereology data sets.

Results: Using the approach described previously for the MRI data (Bolender, 2012), mathematical markers (AX:BY:CZ) for the controls were generated for each paper in the stereology database containing volume data for the human brain. This produced 53,298 markers, of which 1,060 (2%) were duplicated in more than one paper (Table 1). In contrast, the MRI data had 160,736 markers with 17,048 (10.6%)

duplicates. Table 2 and Figure 5 summarize the results.

Table 1. Mathematical markers (triplets) – based on stereological data - characterize control volumes of postmortem brains.

Control-Volume-Stereology			
Stereology	**No. Volume Markers**	**No. Duplicate Markers**	**No. Unique Parts**
Triplets	53,298	1060 (2%; 1060/53,298)	179

Table 2. Mathematical markers (triplets) – based on stereological and MRI data - characterize control volumes of postmortem and living brains.

Control-Volume-Stereology vs. Control-Volume-MRI			
	No. Volume Markers	**No. Duplicate Markers**	**No. Unique Parts**
Stereology	53,298	1,060 (2%; 1060/53,298)	179
MRI	160,736	17,048 (10.6%; 17048/160,736)	126
Stereology vs. MRI	196,410	64 (0.033%; 64/196,410)	42 parts in common

Next, the duplicate markers for control volumes (stereology) were run against those of MRI (Table 2, Figure 5). Exact matches between the two data sets identified experimental settings where the ratios (X:Y:Z) of three parts (A, B, C) remained unaltered by the stereological methods of specimen preparation and data collection. Notice that this measure of compatibility between the two data sets was found to be less than 1% ((64/196,410)x100%=0.03%).

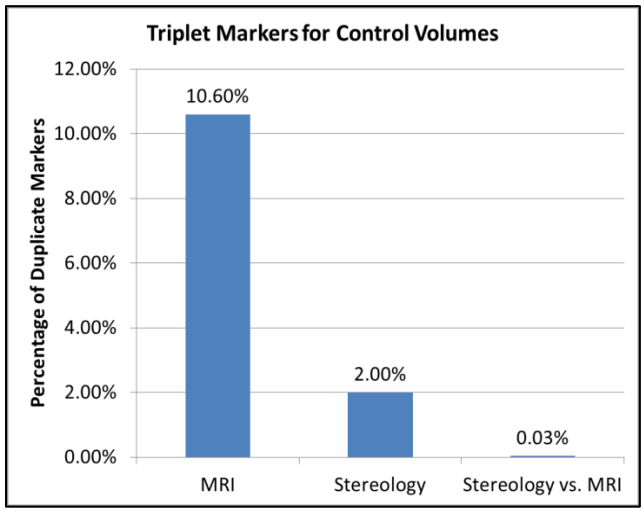

Figure 5. In the MRI database, brains of patients – across publications - display a common order in their parts and connections as suggested by the almost 11% incidence of duplicate mathematical markers. In contrast, this measure of order in postmortem brains of the stereology database was only 2%. This difference amounts to an 81% loss of information - ((1-(2%/10.6%))x100%=81%).

The effectiveness of the MRI volume database in forming duplicate mathematical markers was 10.6%, whereas the stereology database was considerably less capable at 2% (Figure 5). This gives the MRI data more than a five-fold advantage over the stereological data as a source of global biological patterns. Moreover, it tells us that the expected consequence of collecting data from postmortem brains is an 81% loss of the information needed to study of biology as a complexity. Note that a three-fold difference in the number of markers (MRI (160,736) vs. stereology (53,298)) produced a sixteen-fold difference (17,048/1060) in the number of duplicate markers that these two data sources produced.

The results shown in Figure 5 more or less tell the story. If living brains represent reality, then lifeless ones seem to forfeit a considerable slice of reality – at least for stereological volumes. This becomes an important finding because without true-to-life data we cannot deliver a parallel complexity with data coming from postmortem brains.

Test 3 (triplets): In schizophrenia, to what extent do the distortions in postmortem brains diminish the biological patterns seen in living brains? Results: By roughly 32%.

Method: This test consisted of combining control data with those of schizophrenic patients into a single table and then counting the number of duplicate and nonduplicate markers – paper by paper. Duplicate markers occur when both control and experimental markers are the same; they signal no change (Co=Ex). Nonduplicate markers exist when a control marker does not have an experimental counterpart; they signal a change (Co≠Ex). If schizophrenia had no effect on the brain, then 100% of the markers would be duplicates. The test was applied separately to markers coming from living (MRI) and postmortem (stereology) brains.

Results: Table 3 and Figure 6 illustrate the results. The number of duplicate markers went from 44.8% in living brains to 30.6% in postmortem brains. This represented a 32% loss of information postmortem ((1-(30.6/44.82))x100%=32%).

Table 3. Mathematical markers (triplets) – based on stereological and MRI data – compare normal and schizophrenic brains – living and postmortem.

Normal + Schizophrenia – MRI (living) and Stereology (postmortem)		
Triplets	**No. Duplicate Markers**	**No. Nonduplicate Markers**
MRI	159,814	196,730
Stereology	26,808	60,810

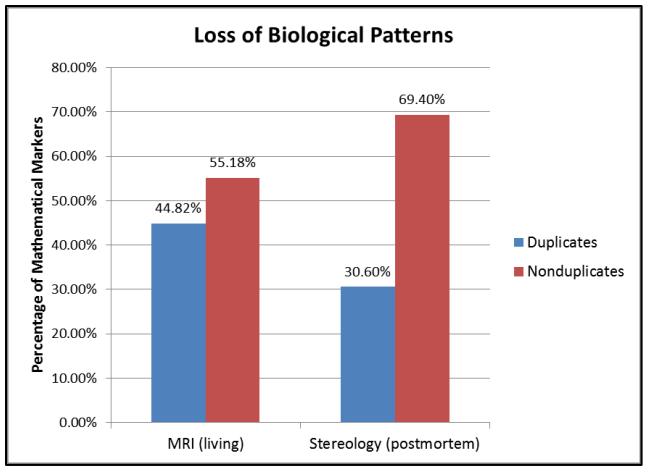

Figure 6. Schizophrenia changes quantitative patterns in the human brain by transforming duplicate markers into nonduplicates. Duplicates occur when the control and experimental markers are the same, indicating that a change has not occurred. In the living brain, schizophrenia decreased the percentage of duplicates to 44.82%, whereas in postmortem brains the value fell to 30.6%. Postmortem brains contained 32% less information. This finding triggers the key question. Why does the same experiment produce different results?

Figure 6 illustrates the profound effect of schizophrenia on the human brain. It changed 55.18% of the markers of living brains to nonduplicates, a value that soars to 69.4% in postmortem brains. Clearly, this disorder involves a major remodeling in the parts and connections of the brain.

The test showed that the complexity of living and postmortem brains differs. Although both share the complexity of the disease, postmortem brains carry a second complexity associated with death and specimen preparation. The next test considers the source and magnitude of this second complexity.

Test 4 (data pairs): Identify similar mathematical markers that occur in both living and postmortem brains and determine their compatibility. Results: Duplicate markers shared by the two data sources fall within the range of .03% to 5.6%.

Method: The test, which used data pair markers coming from living (MRI) and postmortem (stereology) brains, looked for specific patterns shared by these two data sources; control and experimental markers were treated separately. It determined the compatibility of living and postmortem data.

Results: By calculating data pairs for the data in the stereology and MRI databases and then combining them, we get 46,246 mathematical markers - including control and experimental volumes and numbers (Table 4). This combined stereology-MRI data set included 33,130 markers with duplicates - 5,814 came from stereology and 27,316 from MRI. From this group, 2,763 duplicates were shared by the two data sources (stereology=MRI) with 709 coming from stereology and 2,054 from MRI. For Volumes, there were 156 duplicates (stereology=MRI) for controls and 107 for experimentals.

Table 4. Mathematical markers (data pairs) – based on stereological and MRI data – compared control (c = green) to experimental (e = red) duplicates for numbers (n) and volumes (v). *Matched duplicates occurred when identical mathematical markers existed for both living (MRI) and postmortem (stereology) brains. See text for details.

Mathematical Markers	Count	Stereology	MRI	c	e	n	v
Total DP Markers	46,246	11,195	35,051	X	X	X	X
Markers with duplicates	33,130	5,814	27,316	X	X	X	X
MRI + Stereology duplicates	2,763	709	2,054	X	X	X	X
Stereology + MRI*	524	X	X	X		X	X
MRI markers*	320		X	X			X
Stereology=MRI*	204	X		X		X	X
Stereology=MRI*	48	X		X		X	
Stereology=MRI*	156	X		X			X
Stereology + MRI*	649	X	X		X	X	X
MRI markers*	492		X		X		X
Stereology=MRI*	157	X			X	X	X
Stereology=MRI*	50	X			X	X	
Stereology=MRI*	107	X			X		X

Figure 7 summarizes the results. It shows that the compatibility between the stereology and MRI markers was only 5.6% for controls and 3.9% for experimentals. If, as an alternative, we divide by 33,130 instead of 2,763, we get 0.5% for controls and 0.3% for experimentals. Once again, the test showed that the postmortem data of stereology resemble - only very slightly – those of the living brain.

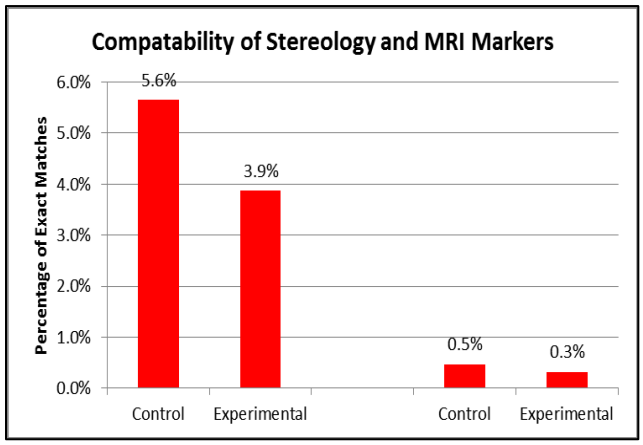

Figure 7. Only a small percentage of the postmortem markers of stereology duplicate those in the living brain. In effect, these two different versions of the brain are largely incompatible.

Using the data set of Table 4, connectivity plots show the extent of the volume disruptions (Figures 8, and 9). Notice how the connectivity of the controls largely disappears in brains with schizophrenia.

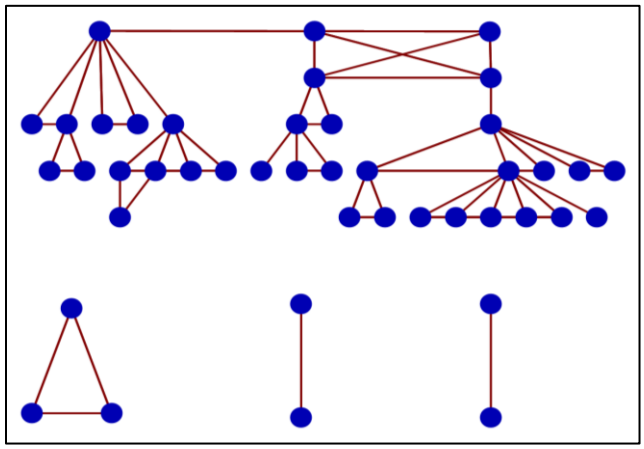

Figure 8. In the controls, these parts (blue dots) form duplicate (identical) markers in both living and postmortem brains. Notice the partial loss of connectivity, evidenced by the three isolated groups in the lower portion of the figure. Appendix III identifies the parts (blue dots).

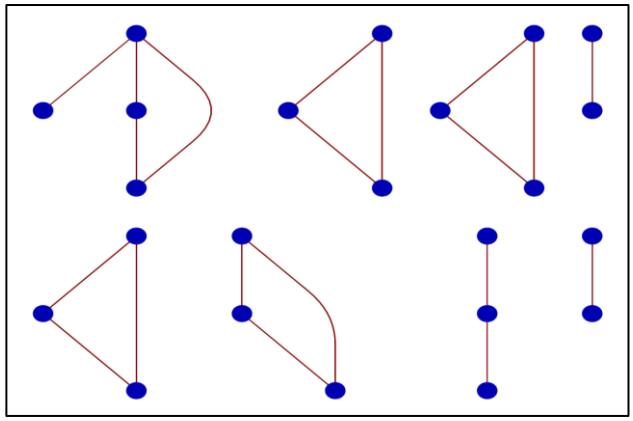

Figure 9. In brains diagnosed for schizophrenia, these parts (blue dots) form duplicate markers in both living and postmortem brains. Notice that the complex pattern of connectivity seen in the control (Figure 8) diminished to eight disconnected groups - most of which are local (See Appendix III). This figure helps to explain why the postmortem data of stereology lacks the critical information needed to diagnose disorders of the brain (Figures 2 and 3). The distortions in volume have largely destroyed the original biological complexity.

Test 5: Since the mathematical markers of postmortem brains carry volume distortions, can we identify them and apply corrections? Results: Yes.

Problem: Consider the worst-case scenario. In post mortem brains, the volume of each part may have increased (swollen), decreased (shrunken), or remained the same. Moreover, the same part may have a different volume distortion in normal and abnormal brains. Given such a scenario, the standard method of calculating absolute values with hierarchy equations - which ignores such distortions - becomes questionable. Furthermore, the distortions associated with each volume term in the hierarchy equation become multiplicative. Consider a simple example. If each of three volumes carries a similar distortion of 10%, distortion alone can generate enough change to suggest a significant difference (1.1 x 1.1 x 1.1 = 1.33). This puts us in a difficult position. Whenever stereological data collected from postmortem brains carry two distinct complexities, one attributed to biology and the other to the methods of data collection, our data and interpretations become ambiguous and thus unreliable.

Since the results shown in Figures 10 and 11 indicate that the worst-case scenario actually exists, we now

have the incentive to fix this long-standing problem. Fortunately, complexity theory offers several workable solutions.

Solution 1: The ideal solution to the problem would be to make the before (living) and after (postmortem) volumes the same, or nearly so. One way of doing this is to determine the volumes of brain parts in a living patient and then determine the volumes of the same parts in the same patient postmortem. This gives – for a given individual – a correction factor – part by part - for the volume distortions:

$$\text{Correction Factor} = V_{part,before}/V_{part,after} \quad (1)$$
$$= 80\ mm^3 / 70\ mm^3 = 1.143.$$

To fix the volume distortion in the postmortem brain, simply multiply the postmortem volume (after) by the correction factor.

$$V_{part,before} = V_{part,after} \times \text{Correction Factor} \quad (2)$$
$$= 70\ mm^3 \times 1.143 = 80\ mm^3 .$$

Solution 2: Since before and after estimates for the volumes of Solution 1 were unavailable, we can make rough estimates for these correction factors using our current data set. After identifying the parts that existed in both the stereology and MRI data tables, average values were calculated and used to evaluate equation (1). Figure 10 includes correction factors for 37 disrupted parts found in the control brains and Figure 11 does the same for brains with schizophrenia (See Appendix IV). These figures begin to explain why the mathematical markers of postmortem and living brains shared so few duplicates (Figures 2, 3, 5 and 6). Moreover, they provide new information about the source and magnitude of the second complexity.

The histograms of Figures 10 and 11 indicate that the correction factors are both variable and subject to change. This unexpected array of distorted volumes bears the responsibility for superimposing a second complexity on the intrinsic complexity of biology. In the human brain, it now appears that the volume of each part typically responds uniquely to its postmortem environment.

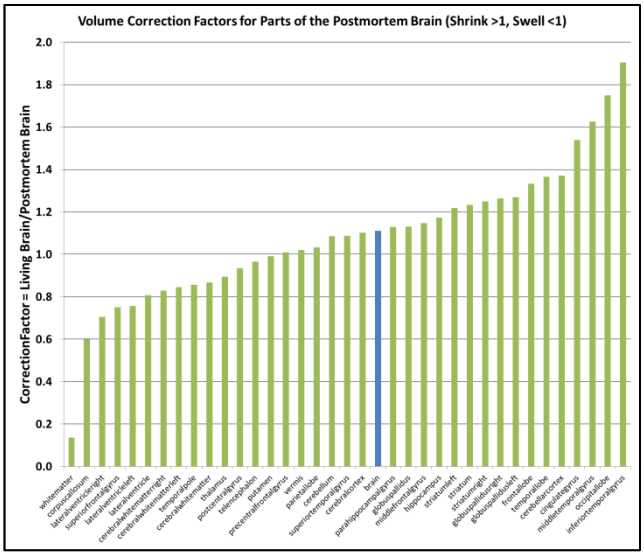

Figure 10. Volume correction factors for specific parts of the postmortem human brain display a wide range of values. A value of 1 indicates no change, >1 shrinkage, and <1 swelling. The blue column identifies the brain, which requires a correction factor of 1.11 to account for shrinkage of about 11%. See Appendix IV for further details.

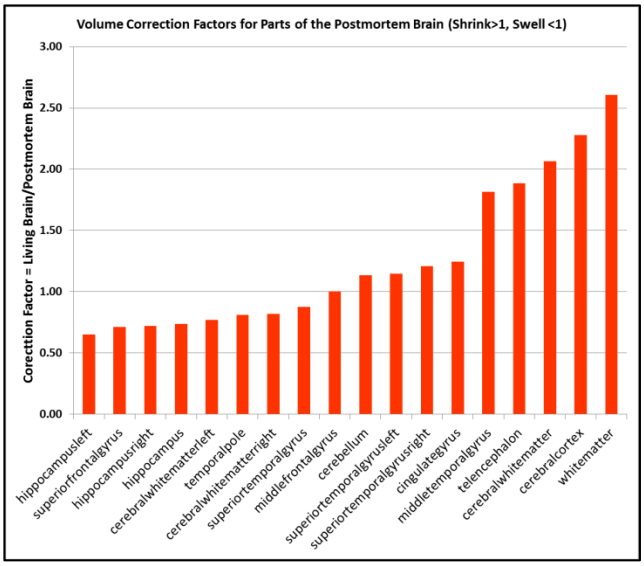

Figure 11. Volume corrections for parts of the postmortem brain with schizophrenia display a wide range of values. For details, see Figure 10 and Appendix IV.

This observation has consequence. In the real world, biology comes with the expected complexity, but in our postmortem world, we – as investigators - have to deal simultaneously with biological complexity and with the complexity produced by distorted volumes. Consider the enormous advantage of our competition. MRI data, which are not compelled to support the postmortem burden, already interpret

biological complexity as mathematical patterns – almost effortlessly - and at the same time offer an inviting array of new and potentially disruptive technologies (Bolender, 2012). Since MRI can classify brain disorders objectively, it already represents a formidable diagnostic tool. Has stereology with all its postmortem baggage lost its competitive edge?

Still unconvinced? Run the numbers. Look, for example, at the correction factors for the hippocampus (Appendix IV). The control hippocampus (0.74) showed a swelling of 26%, whereas the one in the brain with schizophrenia (1.17) had a shrinkage of 17%. If, for the purpose of illustration, we assume a constant volume of 100 mm^3 for the living hippocampus, this gives us a postmortem volume of 126 mm^3 in the normal brain and 83 mm^3 in the brain with schizophrenia. Divide 83 by 126 to get 0.658 – a decrease of 34.2%. Although such a difference may be statistically significant, to infer that the hippocampus is 34% smaller in schizophrenic brains ignores the underlying fact that volume disruptions alone produced 100% of the change – not the brain. Moreover, it seems likely that this problem of interpretation extends to all volume-dependent estimates coming from all postmortem material.

Indeed, the data of Figures 10 and 11 strike at the very heart of reductionism – the principal theory structure in experimental biology. Our practice of collecting control and experimental data from postmortem tissue and demonstrating a significance difference is difficult to defend when change is a function of both the biology and the experimental methods applied thereto. By demonstrating the existence of two complexities postmortem, complexity theory begins to explain why different people doing the same experiment often get different results. With two complexities in play, reductionism as a theory structure for biology becomes largely ineffective.

Solution 3: The stereological data failed Test 1 repeatedly because the MRI data standards were trying to diagnose schizophrenia in postmortem brains that carried one complexity related to biology and a second complexity related to specimen preparation. If, however, we repeat Test 1 (Figure 3) after applying the correction factors for schizophrenia to remove the offending second complexity (Figure 11), we arrive at the correct diagnosis (Figure 12). Now our stereological data from postmortem brains can work hand-in-hand with MRI data when playing the complexity game. In effect, both stereological data of postmortem brains and MRI data of living brains can contribute to building a complexity parallel to the one of biology.

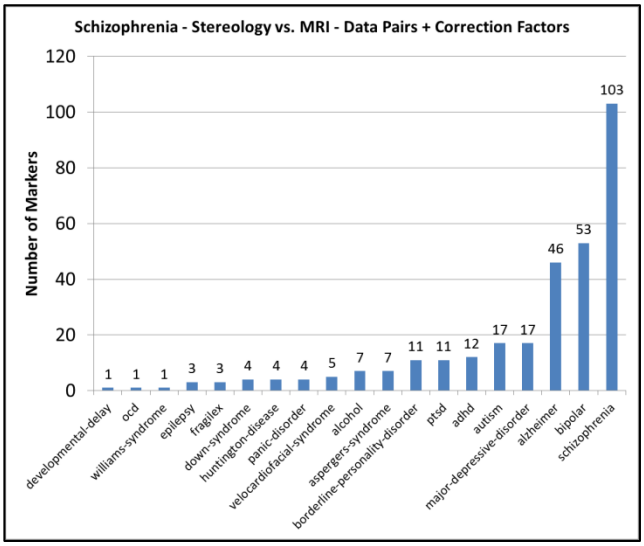

Figure 12. After applying the correction factors to the distorted volumes, we get the correct diagnosis - schizophrenia. This postmortem distribution compared to that of Figure 4 (living brains) reveals striking similarities. Indeed, this distribution shows that we can remove the second complexity from postmortem data and thereby gain access to biology with the remaining parallel complexity. In effect, stereology is back in the game.

Mathematical Markers

If complexity keeps a lock on biology's secrets, then mathematical markers represent a key that can open that lock. The secrets, which often take the form of patterns, exist in both postmortem (Bolender, 2011) and living brains (Bolender, 2012). Although these mathematical patterns appear wherever we look, they tell very different stories for the two different types of brains. Until now, we have tacitly assumed an equivalency between the data of postmortem and living brains. As soon as we treat biology as a complexity, however, we quickly discover that this is not the case (Figures 10 and 11).

Mathematical markers allow us to phenotype an organism in remarkable detail using published data from the basic and clinical sciences. As such, they offer a gentle transition from a research model based on reductionism to one based on complexity. This new resource – included in the software package - offers the reader hands-on experience with a digital representation of the biomedical literature expressed as a complexity.

Consider the immediate payback of this arrangement. Each paper actively contributes to and benefits from all the other papers in the database tables. In this way, we optimize the effectiveness of our research by allowing our data to contribute to the success of everyone – now and for many years to come. At the same time, we benefit from the data of everyone else. In effect, patterns in our data – and in the literature - begin to appear everywhere both locally and globally. Complexity theory – with its remarkable ability to tap into biology – repeatedly produces such win-win outcomes.

The software package includes tables of mathematical markers for data pairs X:Y (Figure 13) and data triplets X:Y:Z (Figure 14). The All In table contains all the markers in one place: data pair (X:Y), data triplet (X:Y:Z), MRI, stereology, control (c), experimental (e), volume (v), and number (n).

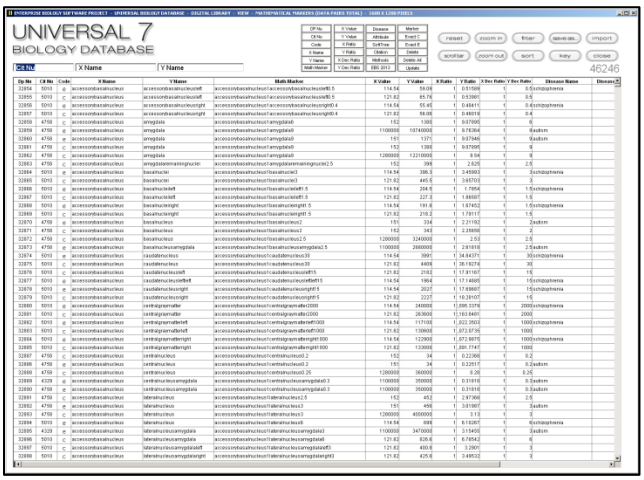

Figure 13. Mathematical markers based on data pairs. Notice that this single table includes data from control and experimental data, volume, number, stereology, and MRI. Command buttons simplify the task of analyzing data collected from postmortem brains

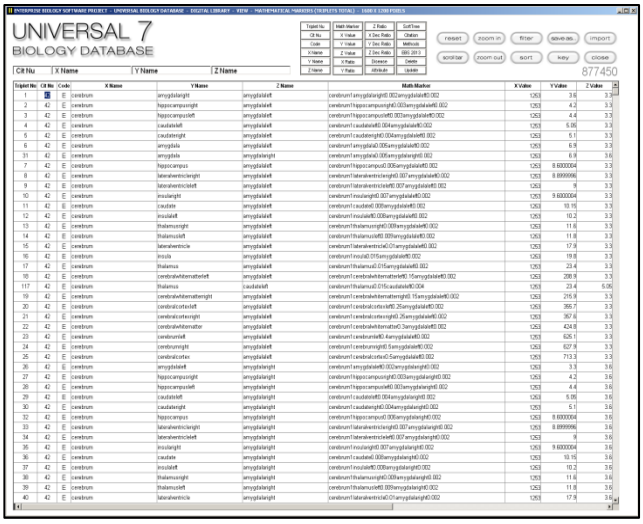

Figure 14. Mathematical markers for triplets include data from both living (MRI) and postmortem (stereology) brains.

The familiar QBE interface simplifies the task of finding patterns with mathematical markers in the data pair (Figure 15), data triplet, and all in tables.

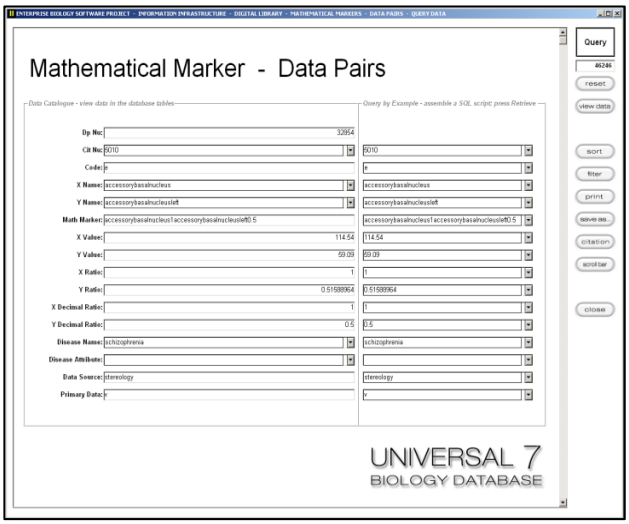

Figure 15. The QBE (query by example) interface speeds the task of finding complex patterns with mathematical markers.

DISCUSSION

The complexity of biology derives from it parts and connections arranged into well-defined patterns. When quantified, these patterns reveal the presence of stoichiometries defining the relationship of parts to connections. We can capture these patterns from

living brains with mathematical markers, which, in turn, offer a host of new insights and opportunities (Bolender, 2012). In postmortem brains, however, these orderly patterns become disrupted or lost altogether because the volumes of many parts no longer resemble those of the living brain. This troubling picture emerged after analyzing the data of living and postmortem brains with mathematical markers.

These finding are important because they alter – in a fundamental way - our perception of stereology as a primary gateway method for studying biology as a complexity. While stereological data from postmortem brains can detect patterns locally, most of the biological patterns have been lost and the ones remaining typically carry distortions. In short, MRIs of living brains can deliver data consistent with complexity theory, whereas most stereological data from postmortem brains cannot. After coming to this unhappy conclusion, the report – with the help of complexity theory – looked for and found ways to repair this serious shortcoming of biological stereology.

The Tests

The tests described in this report relied solely on data contained in two databases: MRI and stereology. Although we know that complexity theory requires large numbers of patterns, we do not know whether the numbers of patterns used for the purposes described herein represented a fair test. This applies especially to the stereological data set, which included data fewer in number. Nonetheless, the tests revealed – consistently - patterns of loss and a widespread disruption of information in postmortem brains. When tested with MRI data from living brains, stereological data repeatedly failed to supply the correct clinical diagnoses, even after switching from data triplets to data pairs (Figure 3). The explanation for such an outcome appears directly related to an important loss in quantitative patterns that occurs in postmortem brains (Figure 6). Figure 16 summarizes the problem.

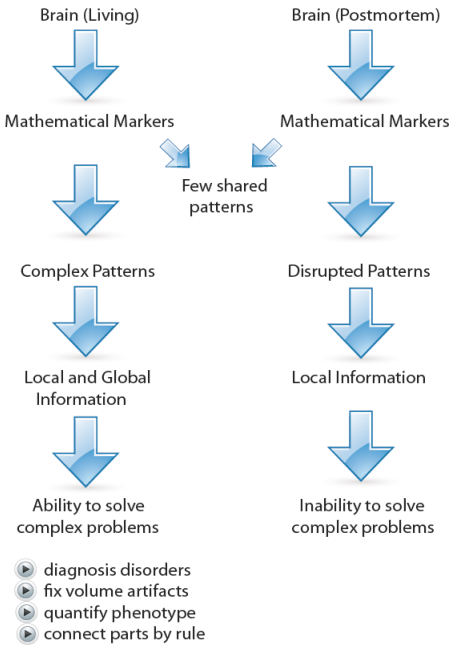

Figure 16. A quantitative rift exists between living and postmortem brains. Data collected from postmortem brains differ importantly from those of living brains.

Old Assumptions Challenged

Stereological methods estimate the volumes, surfaces, lengths, and numbers of parts as densities (i.e., concentrations): volume density (V/V), surface density (S/V), length density (L/V), and numerical density (N/V). In turn, these concentrations become absolute values (V, S, L, and N) by multiplying a concentration by the total volume of the containing part. Herein lies a problem. Since most stereological data come from postmortem material, volume distortions corrupt our data and introduce uncertainty.

Consider, if you will, the limitation imposed by these volume distortion on our use of hierarchy equations. In their simplest form, they convert concentrations into absolute values:

$$V(part) = Volume(reference) \times V(part)/V(reference), \quad (3)$$

where, for example,

$$V(part) = 7\ \frac{cm^3}{} \times 4\ cm^3/\frac{cm^3}{} = 28\ cm^3. \quad (4)$$

Notice that equation (4) works mathematically because the two cubic centimeters highlighted in blue

(cm^3) cancel. If, however, one or more of the volume compartments of equation 4 carry a distortion, then the cubic centimeters will not cancel biologically and we cannot evaluate the equation (Rule Book, 2007-2013).

If we expand equation (4) by adding another hierarchical level, we exacerbate the problem:

$$V(part) = 7\ cm^3 \times 4\ cm^3/cm^3 \times 0.5\ cm^3/cm^3 = 14\ cm^3. \quad (5)$$

Now we have multiple volume compartments, all of which may display unique distortions – such as those seen in Figures 10 and 11. Unfortunately, the problem gets worse. Since the histograms in these two figures show that the volume of a given part can distort differently in controls and experimentals, absolute values and significant differences based on hierarchy equations ultimately carry a tangle of distortions. Consequently, results loose credibility.

In short, postmortem estimates of absolute values based on hierarchy equations become unstable because distortions probably exist in all or most of the variables. In fact, hierarchy equations can work correctly only when the data are entirely free of volume distortions. This creates a dilemma. To use hierarchy equations, we must assume that our postmortem data are free of volume distortions, which everyone already knows is not the case. This means that to get absolute data with hierarchy equations we have to break the rules and suffer the obvious consequences. In short, the hierarchy equation trap forces us to accept a lose-lose situation. Our absolute values and significant differences cannot hold up because they refer to a proxy that does not correctly represent biology.

Where do we stand? If we compare concentrations (i.e., densities), we fall into the concentrations trap. If we compare absolute values, we fall into the hierarchy equation trap. What's left? In fact, we may have access to only two largely unimpeachable sources of data – MRI volumes from living brains (Kennedy et al., 2012) and postmortem cell counts from the fractionator (Gundersen et al., 1988). Fortunately, these data can become our lifelines.

Fixing Postmortem Stereology

There is a basic truth. If we apply unbiased sampling methods to a biased specimen (e.g., postmortem biology), we get a biased result (Figures 2, 3, 5-11). Thus far, the report explains how we can avoid this unfortunate outcome by enlisting the help of MRI data coming from living brains (Figures 10-12).

Solution 4: To be more inclusive, however, we would prefer a solution to this problem of distorted volumes that requires only postmortem data and generalizes across all types of parts, settings, and species. Consider this. If we estimate – using the unbiased sampling methods of stereology – the same parameter with and without a volume distortion, then the ratio of the two should give us a correction factor for the distortion.

Figure 17 plots cell counts – estimated with the disector (Sterio, 1984) - against the volumes of the parts containing the cells. R^2s close to one can occur because both estimates share the same reference compartments and consequently the same volume distortions. Recall that the disector method estimates a numerical density (N/V), which when multiplied by a volume gives an absolute value (Rule Book, 2007-2013; Rule 6). Such an estimate is volume dependent (vd). It carries a volume distortion.

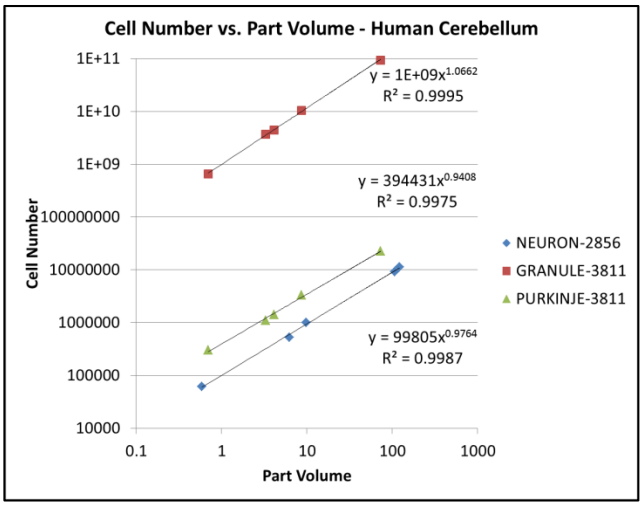

Figure 17. Cell numbers plotted against part volumes in the human cerebellum display R^2s close to 1.0. Citation numbers identify the original papers. These data come from postmortem material and hierarchy equations.

Next, consider the fractionator (Gundersen et al., 1988). Recall that this method gives a volume independent (vi) estimate for the total number of cells in a biological part (N(cell,vi)). It qualifies as a lifeline because we can be reasonably confident that estimates for the number of cells pre and postmortem remain the same. It avoids a volume distortion.

The correction method works as follows. Using the same set of sections collected for fractionator-counting, estimate the numerical density of the cells with the disector (Sterio, 1984) and the volume of the biological part containing the cells with the optical volume fractionator (Bolender and Charleston, 1993; Bolender et al., 1993). (N.B., the OVF estimates all of the variables used to make the correction.) Since these estimates for cell number and part volume both carry the same volume distortion, they give a volume dependent (vd) estimate for cell number:

$$N(cell,vd) = V(part,vd) \times ((Ncell,vd)/V(part,vd)). \quad (6)$$

Finally, calculate a correction factor (CF) for a postmortem part by dividing the volume independent cell count by the volume dependent one:

$$CF = N(cell,vi)/N(cell,vd). \quad (7)$$

To correct the volume of a postmortem part, simply multiply the volume dependent part by the correction factor:

$$V(part,vi) = V(part,vd) \times CF. \quad (8)$$

The correction factor (CF) applies to all volume dependent estimates, including volume, surface, length, and number. Note that this method generalizes across the hierarchy up to and including the nucleus of the cell currently counted. Remember that each part requires a separate correction factor, coming from the fractionator and equations 6 and 7. This means that we can get absolute data free from the volume distortions associated with hierarchy equations (equations 3, 4, and 5) - without breaking the rules.

Notice how complexity theory continually rewards us with surprisingly simple solutions to problems previously unsolvable. In return, it merely asks us to play by the rules. Although reductionist theory allows us to access postmortem data with stereological methods, it – in contrast - extracts a hefty price. Indeed, the closer one looks, the more unfriendly this theory appears. By distorting the volumes of parts, for example, reductionist methods effectively prevent us from detecting biological changes – the most important thing it promises to do. Moreover, when estimating absolute values with hierarchy equations and Cavalieri estimators (Gundersen and Jensen, 1987), we are often compelled – albeit unwittingly - to break the rules and end up in a trap.

Complexity Games

Many consider complexity games to be among the most difficult because to play them successfully, one must first figure out the rules and then make the right moves. We find ourselves today in the process of learning how to play an entry-level game, namely one that consists of a complexity wherein the rules and procedures have already been well defined and thoroughly tested. Biology, for example, qualifies as such a game. Our challenge merely consists of finding biology's rules and figuring out what they do. The rewards for playing such a game can be – more or less - whatever we want them to be because we choose the moves … the questions.

Before making the first move, however, we have to get the right pieces in the right places on the right playing field. In our case, the right pieces include credible data, the right places data tables, equations, patterns, and the right playing field complexity. Recall that an empirical equation with an R^2 close to one identifies a rule and a repeated set of parts and connections a pattern. This initializing process generates a theory structure for the game, which in turn guides our moves. Finally, by transforming the game into a software package, everyone interested in taking biology on - as a partner - can become a player. The field of play, which now consists of a single da-

tabase table, comes with more than 18,000,000 data fields (see the "all in" table).

One of the moves in the report last year included the unraveling of a complexity, which was the result of simply asking: "How does the brain create disorders?" We now know that it rearranges its parts and connections into patterns specific to a given disorder (Bolender, 2012). In making this move, however, it became apparent that the brain employs a modular strategy wherein it assembles different diseases by using different combinations of similar patterns. Our move, which consisted of creating a complexity parallel to that of biology, included the application of mathematical markers, a software tool that allows us to detect quantitative patterns in complex data sets.

Another move from last year addressed a long-standing problem in clinical medicine – diagnosing disorders of the brain using a data driven approach (Bolender, 2012). This move consisted of rearranging the data of an MRI database into mathematical markers, assembling diagnostic patterns therefrom, and then simply running unknowns against standards. The question asked, "Can we use MRI data to diagnose disorders of the brain?" The answer came back yes.

This year, we combined the data of living and postmortem brains and asked, "Can we use the postmortem data of stereology to diagnose disorders of the brain?" Unfortunately, the answer came back as a no. It was the wrong move. This unexpected result told us that important differences existed between living and postmortem brains. In fact, we soon discovered that most of the diagnostic information found in the living brain had been lost. The right move was to recover the lost information first and then ask the question. This move proved to be more successful. In addition to answering the question with a provisional yes, complexity theory delivered four practical solutions to our postmortem data problem. Winning, it would appear, seems to be a behavior fundamental to complexity theory.

Concluding Comments

Notice what happened. Using stereological data of postmortem brains, we set out to demonstrate a complexity parallel to that of biology. Instead of discovering this parallel complexity, we found an altered reality shaped by the mischief of distorted volumes. Such a finding limits the ability of stereological data to play the complexity game. If stereology is not a major player, then reaching our long-term goal of assembling phenotypes - extending from organisms to genes – quickly becomes implausible. The only option was to fix the problem.

What – exactly - is the problem? Stereological data collapse into a chaotic state when they attempt to carry two complexities; one from biology and the other from the methods we use to collect data from postmortem material (see Rule Book, Enterprise Biology Software, 2007-2013). Fortunately, complexity theory can separate these two complexities and remove the offending one by generating correction factors. If this approach continues to be successful, then biological stereology – in all likelihood – will continue to be a major player going forward.

By compelling us to look very carefully at biology, as it actually exists, complexity theory becomes a new and disruptive technology. The application of the theory to basic research and patient care uses a parallel complexity to work its magic. Local patterns provide for the needs of individual patients, while global patterns supply the generalizations basic to diagnosis and prediction. By establishing a parallel complexity, we will eventually gain access to the relationship of phenotype to genotype and promote the emergence of a single, unified set of connected patterns. Such patterns will help to accelerate progress in biology to unprecedented speeds.

Observe that this parallel complexity does for us what the original does for biology – it supplies emergent properties. Diagnosing disorders of the brain, assembling a new theory structure for health and disease, and repairing the postmortem data of stereology (Bolender, 2012-2013) are all emergent properties of a parallel complexity. Since we now

understand – even only a little - the intricate relationship of data to complexity to emergent properties, we can use our new knowledge and software technology to rethink the way we do business. The stereology community – perhaps more than any other – has the wherewithal to invent an exciting future for the biology enterprise because it enjoys a robust mathematical foundation that can deliver some of the best data and the most credible results. With stereology, we can ask and answer many of the most challenging questions facing biology now and for many years to come.

A key point to emerge from the report and software package is that our understanding of the human brain comes from two remarkably different worlds, one alive the other dead. Each world tells a different story, one is real and the other what we imagine real to be. This creates a curious dilemma for the reader. Will it be the red pill or the blue?

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APPENDIX I

Theory of Biological Complexity

The overarching principle of the new theory is that it takes a complexity to solve a complexity. This means that to test the theory empirically we need to construct a parallel complexity as close to the original as possible, relying exclusively on the rules that exist first in biology and then in our data. Stereology with its unbiased sampling methods plays an essential role in this building process by providing the most reliable access to these data and by supplying the equations that estimate and connect the data of this parallel complexity. This holds true even when the data sources carry biases. The parallel complexity captures the phenotype, which the genome continually updates.

Complexity is an unfamiliar place. New rules apply, our perceptions change, and we get to ask and answer questions differently. The first order of business is to learn the rules of the game, which in science consists of developing a new theory structure. This represents an ongoing process wherein the theory evolves in step with the discovery process.

Recall that the fundamental building blocks of a biological complexity include parts and connections. Volumes, surfaces, lengths, or numbers define the parts quantitatively and ratios derived therefrom the connections. From this simple beginning, the complexity of an organism grows as the parts and connections cascade throughout the hierarchical levels of an organism. Since everything consists of the same basic building blocks and all the blocks are connected, our parallel complexity begins to resemble the original biology – at least on a limited scale. Testing the theory consists of looking for persistent patterns - locally and globally – and then using these patterns to define the rules of the game.

A collection of working lists, including Goals, Requirements, Basic Principles and Definitions, Derivatives, and Rationale summarize recent progress in constructing this new theory structure.

Theory of Biological Complexity: In its simplest form, the theory states that it takes a complexity to solve a complexity. We can define a biological complexity mathematically as a distinct set of elements (parts and connections) that combine to form patterns (e.g., mathematical markers) capable of scaling at both local and global levels. Typically, biology displays its complexity as a stoichiometry based on the ratios of it parts. Biology uses this simple rule to create both order and disorder.

Theory Structure: The accompanying theory structure includes a current set of guidelines for exploring biology as a complexity. **Items highlighted in red identify recent additions.**

Goals

- Generalize the data of the biology literature.
- Define and assemble a data-driven approach to the basic and clinical sciences.
- Identify mathematical patterns in biology.
- Explore biology as a rule-based system.
- Use published data to create a parallel complexity using rules intrinsic to biology.
- **Remove postmortem distortions by harmonizing pre and postmortem data.**
- Demonstrate the effectiveness of a new approach to problem solving based on empirical data and guided by the rules of biology.
- Develop software that can accelerate productivity by transforming biological data into problem-solving tools.
- Capture biological phenotypes mathematically and use them to diagnose and predict outcomes.
- Connect phenotypes to genotypes.
- Evaluate current methods.
- Optimize outcomes.

Requirements

- Collect data with unbiased sampling methods.
- Express data as volumes, surfaces, length, or numbers.
- Assemble data as connected sets.
- Integrate data within and across hierarchical levels.
- Use a common format to organize and generalize data.
- Configure data to detect local and global patterns.
- **Operate within the bounds of a complexity parallel to the one of biology.**
- **Correct the volume distortions of postmortem data.**
- Configure data to display diagnostic and predictive properties.
- Store and distribute data in digital form.
- Encourage open access to data.

Basic Principles and Definitions

- A biological complexity consists of parts and connections distributed hierarchically.
- Complexities can be both local and global.
- A biological complexity can unfold into smaller patterns or fold into larger ones.
- Parts and connections define the organizational framework of biology as distinct patterns. As such, they represent a rule-based management system.
- A parallel complexity represents a data-driven construct designed specifically to capture biological complexity.
- Ratios and derivatives thereof (i.e., mathematical markers) serve as the basic units of information in a parallel complexity.
- Mathematical markers include parts (names) and connections (ratios).
- **A second complexity exists in the postmortem data of stereology, produced by the methods of data collection.**
- Parts display quantitative (volume, surface, length, number) and qualitative properties (names, locations).
- All parts are connected or connectable by forming ratios.
- A ratio defines the relationship of one part to another. Moreover, ratios define nested and modular sets of connections within and across hierarchical levels.
- Parts and connections form patterns that scale in size, beginning with a ratio of two parts and ending with a ratio of n parts - where n represents the entire organism.
- Patterns captured as mathematical markers increase their specificity as the number of parts in the marker increases.
- In living subjects, mathematical markers detect both local and global patterns.
- **In postmortem subjects, mathematical markers can detect both local and global patterns when correction factors for volume distortions are applied.**
- Valances describe the ability of the same set of parts to display different numerical ratios (connections).

Derivatives

A derivative includes - as a minimum - the names of two parts and their corresponding values formed into a ratio. In forming a ratio, the original published values may be used directly (repertoire value) or converted to a decimal step (decimal repertoire value). Data pair ratios take the form X:Y and data triplets X:Y:Z. Mathematical markers add the names of the parts: AX:BY and AX:BY:CZ. The theory structure currently considers only data pairs and triplets.

Data Pairs

- A data pair consist of two parts (names) and two connections (ratios) expressed as repertoire and a decimal repertoire values. Data pairs can be formed by inspection or by taking all possible permutations of the names of the two parts – to which numerical values are assigned.
  - Data pair values – expressed as a decimal step (decimal repertoire value) – combine with names to form mathematical markers.
  - **A data pair can use data with or without corrections for the volume distortions of postmortem material.**

- Data pairs display valences in that the same two parts can occur in different proportions.

Data Triplets

- A data triplet consists of three parts and three connections with the ratios expressed as repertoire and decimal repertoire values. Triplets are formed by inspection or by taking all possible permutations of the three names of the parts – to which numerical values are assigned. Mathematical markers use decimal repertoire values.
  - **A data triplet can use data with or without corrections for the volume distortions of postmortem material.**
  - Triplets display valences in that the same three parts can occur in different proportions.

Properties of Data Pairs and Triplets

- Data pairs and triplets form general, unique, and diagnostic patterns.
- Conservation of patterns occurs within and across animal species.
- Globally, disorders of the brain display a distinct collection of markers. Locally, however, different disorders share many of the same markers.
- All patterns and their antecedents can be stored in a single database table.
- Mathematical markers offer a general solution to the problem of biology as a complexity.
- **Mathematical markers can detect the distorted volumes of postmortem brains.**

Rationale

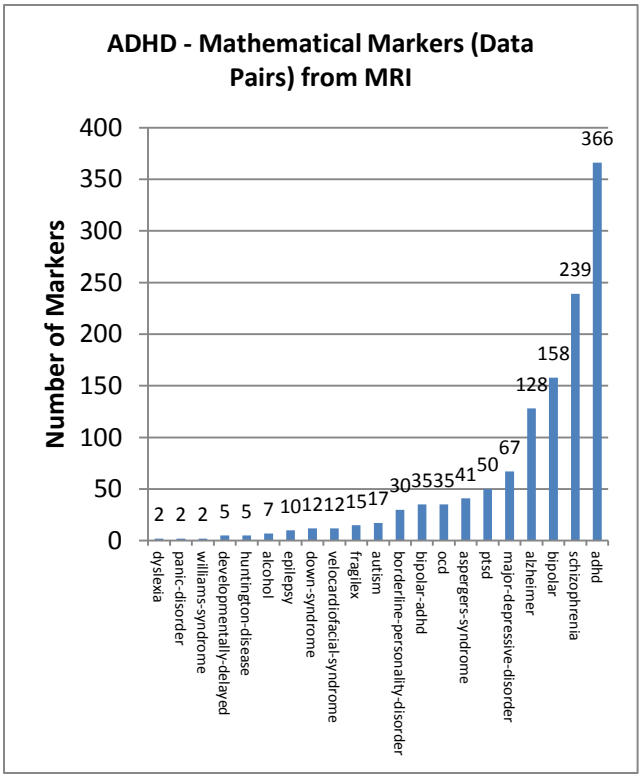
- Complexity theory represents a long overdue response to the limitations of our current theory structure based on reductionism.
- Reductionist theory takes biology apart, studies parts in isolation, and applies statistical tests to detect changes. It purports to simplify biology, but instead adds a second complexity, often making reliable interpretations difficult to impossible. This second complexity includes a wide range of distortions caused by death and by the methods of specimen preparation and data collection. Concentrations, which are the most common form of biological data, fail to detect biological changes because they ignore complexity. Hierarchy equations, which are used to convert concentrations into absolute values, fail when the variables used to evaluate the equations carry volume distortions.
- The methods of reductionist theory minimize the effectiveness of published data, obscure biological patterns, and substitute reproducibility and significant differences for accuracy. By corrupting biological data, such methods actively inhibits learning, discovery, and innovation.
- Complexity theory addresses most of the limitations imposed by reductionism and adds a host of new capabilities. A principal argument for studying biology within the framework of complexity theory is that it simplifies everything and provides a tent large enough to accommodate all parts of the biology enterprise.
  - **Absolute values can be estimated independent of hierarchy equations.**
  - Mathematical markers transform old forms of biological data into new patterns consistent with complexity.
  - All mathematical markers can be stored in a single database table, searched for patterns, and used directly for problem solving.
  - By defining phenotypes robustly, mathematical markers support diagnosis and prediction.
  - Quantitative phenotypes can provide mathematical pathways to and from the genome.
  - Biological patterns exist both locally and globally.
  - Global patterns lead to generalizations.
  - Patterns provide access to biological rules.
  - Large, integrated data sets – fundamental to complexity theory – can be extracted from the biology literature.
  - Forming data ratios (data pairs, triplets) minimizes bias.
  - Outcomes can be tested rigorously.
  - Patterns frequently provide multiple solutions to the same problem.
  - New data formats capture the complexity of biology as patterns.
  - **Data distortions can be identified and removed.**

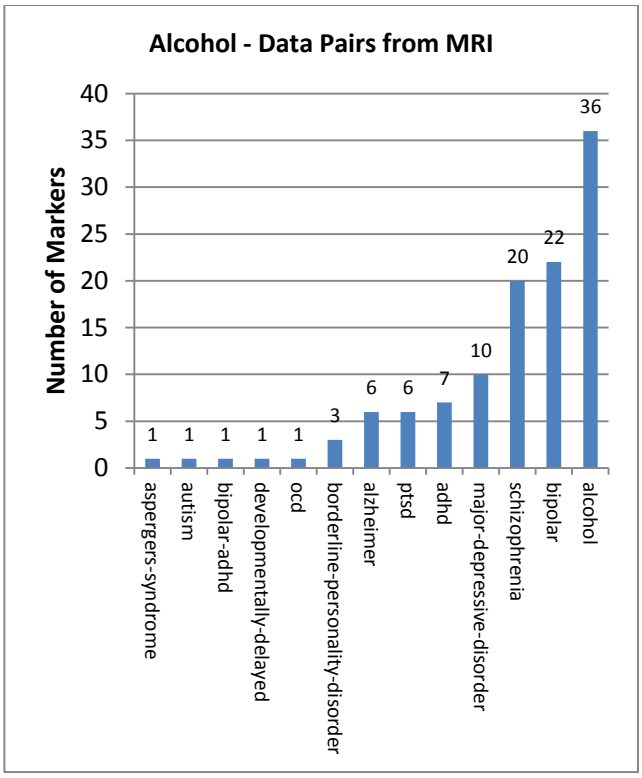
APPENDIX II

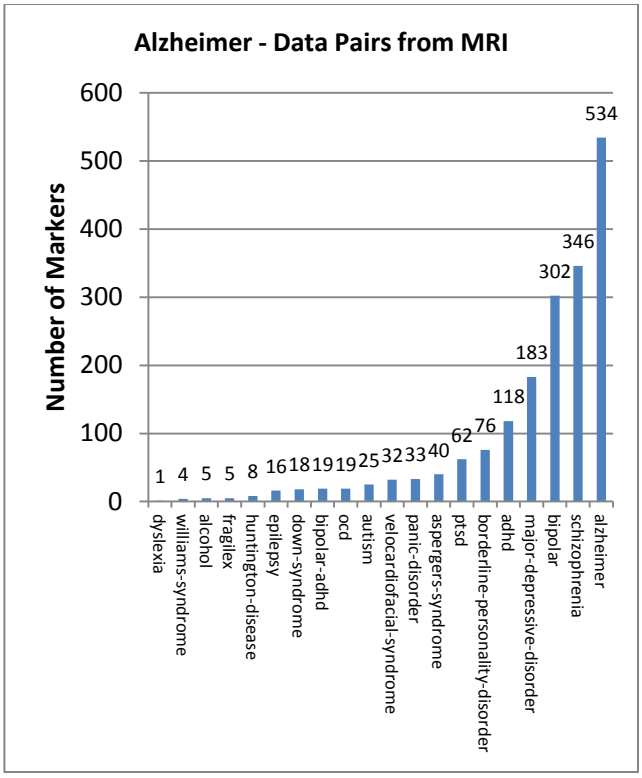
Mathematical Markers: MRI Data Pairs

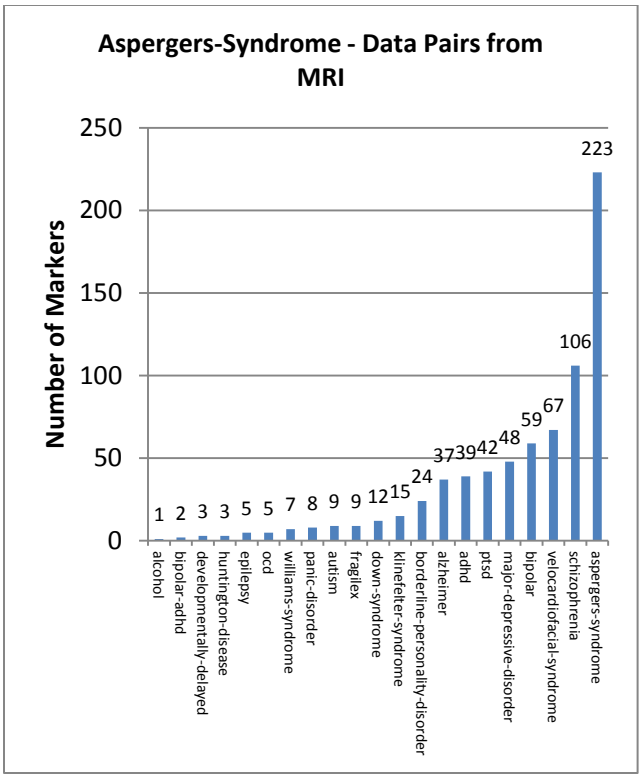
MRI data from living brains, expressed as data pairs, and translated into mathematical markers show extensive overlap of in their quantitative patterns. ADHD, for example, has 366 mathematical markers - 239 of which it shares with bipolar, 158 with major-depressive-disorder, etc.

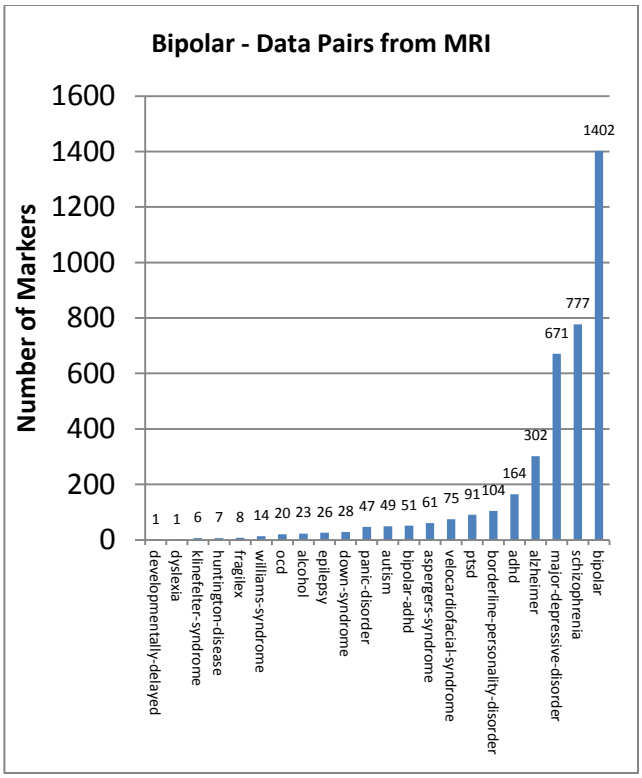
These plots reveal an underlying complexity in disorders of the brain - based on distinct and quantifiable patterns.

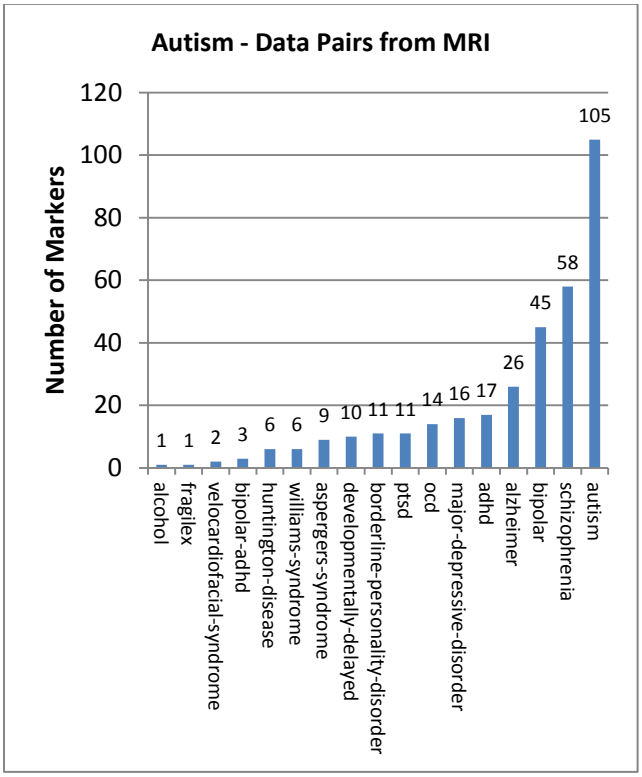


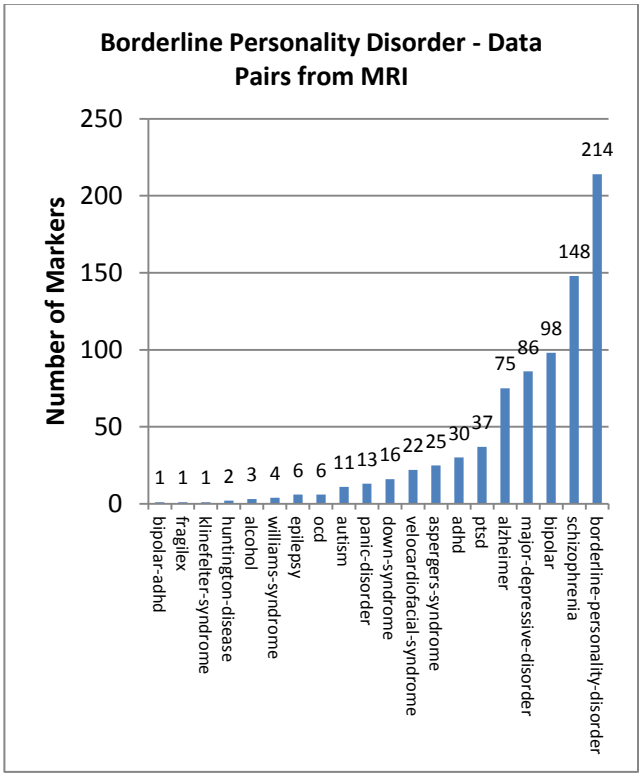


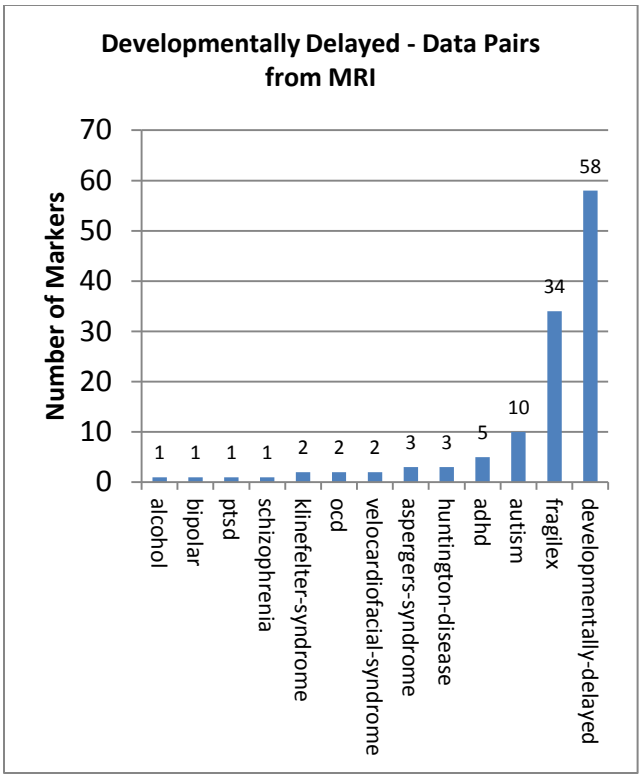


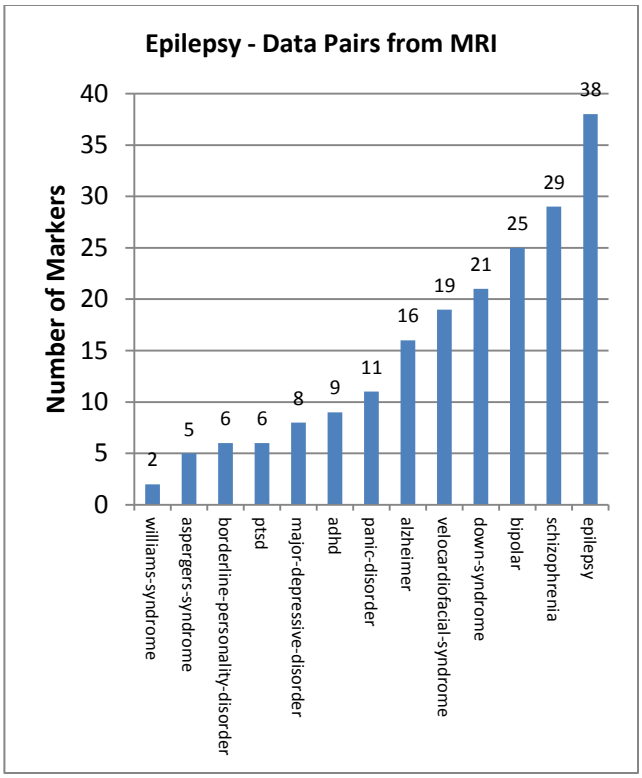

Aspergers-Syndrome - Data Pairs from MRI
Number of Markers
0
50
100
150
200
250
1 2 3 3 5 5 7 8 9 9 12 15 24 37 39 42 48 59 67 106 223
alcohol
bipolar-adhd
developmentally-delayed
huntingtton-disease
epilepsy
ocd
williams-syndrome
panic-disorder
autism
fragilex
down-syndrome
klinefelter-syndrome
borderline-personality-disorder
alzheimer
adhd
ptsd
major-depressive-disorder
bipolar
velocardiofacial-syndrome
schizophrenia
aspergers-syndrome

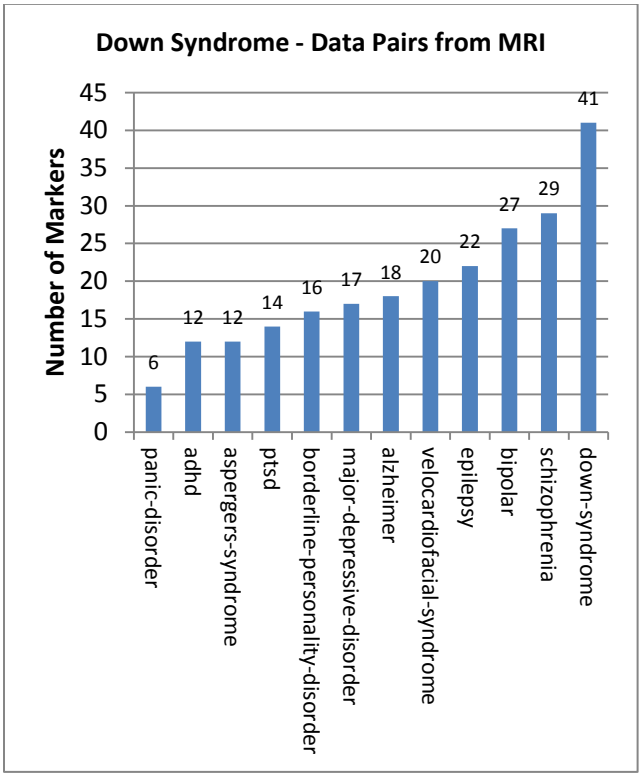

Bipolar - Data Pairs from MRI
Number of Markers
0
200
400
600
800
1000
1200
1400
1600
1 1 6 7 8 14 20 23 26 28 47 49 51 61 75 91 104 164 302 671 777 1402
developmentally-delayed
dyslexia
klinefelter-syndrome
huntingtton-disease
fragilex
williams-syndrome
ocd
alcohol
epilepsy
down-syndrome
panic-disorder
autism
bipolar-adhd
aspergers-syndrome
velocardiofacial-syndrome
ptsd
borderline-personality-disorder
adhd
alzheimer
major-depressive-disorder
schizophrenia
bipolar

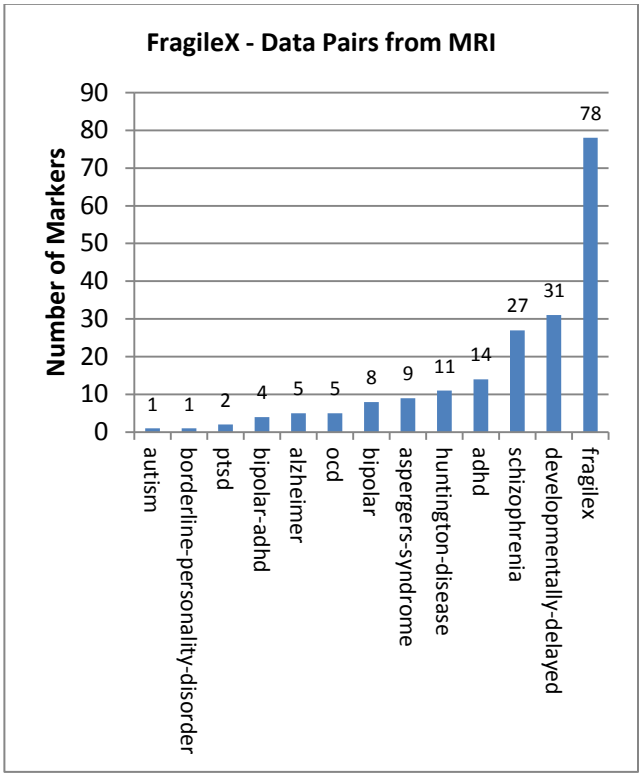

Autism - Data Pairs from MRI
Number of Markers
0
20
40
60
80
100
120
1 1 2 3 6 6 9 10 11 11 14 16 17 26 45 58 105
alcohol
fragilex
velocardiofacial-syndrome
bipolar-adhd
huntington-disease
williams-syndrome
aspergers-syndrome
developmentally-delayed
borderline-personality-disorder
ptsd
ocd
major-depressive-disorder
adhd
alzheimer
bipolar
schizophrenia
autism

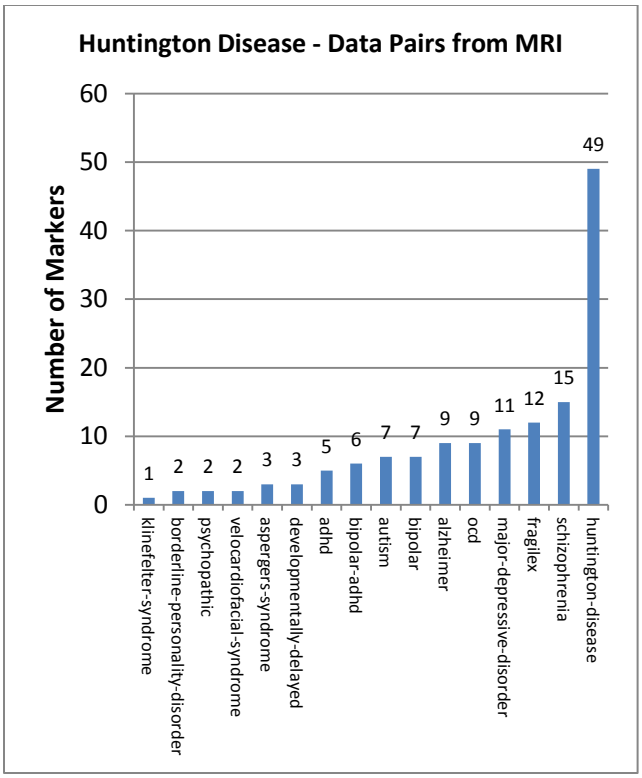

Borderline Personality Disorder - Data Pairs from MRI
Number of Markers
0
50
100
150
200
250
1 1 1 2 3 4 6 6 11 13 16 22 25 30 37 75 86 98 148 214
bipolar-adhd
fragilex
klinefelter-syndrome
huntington-disease
alcohol
williams-syndrome
epilepsy
ocd
autism
panic-disorder
down-syndrome
velocardiofacial-syndrome
aspergers-syndrome
adhd
ptsd
alzheimer
major-depressive-disorder
bipolar
schizophrenia
borderline-personality-disorder

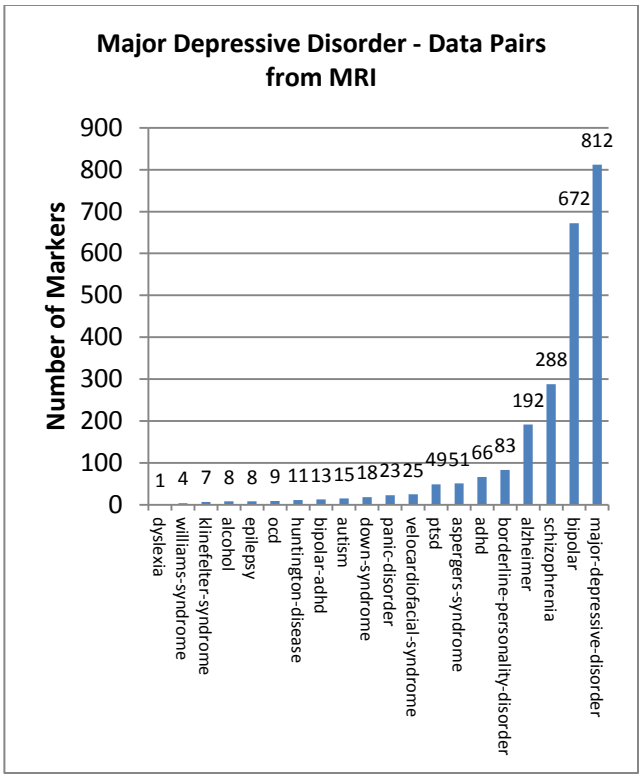

Developmentally Delayed - Data Pairs from MRI
Number of Markers
70
60
50
40
30
20
10
0
1 1 1 1 2 2 2 3 3 5 10 34 58
alcohol
bipolar
ptsd
schizophrenia
klinefelter-syndrome
ocd
velocardiofacial-syndrome
aspergers-syndrome
huntington-disease
adhd
autism
fragilex
developmentally-delayed

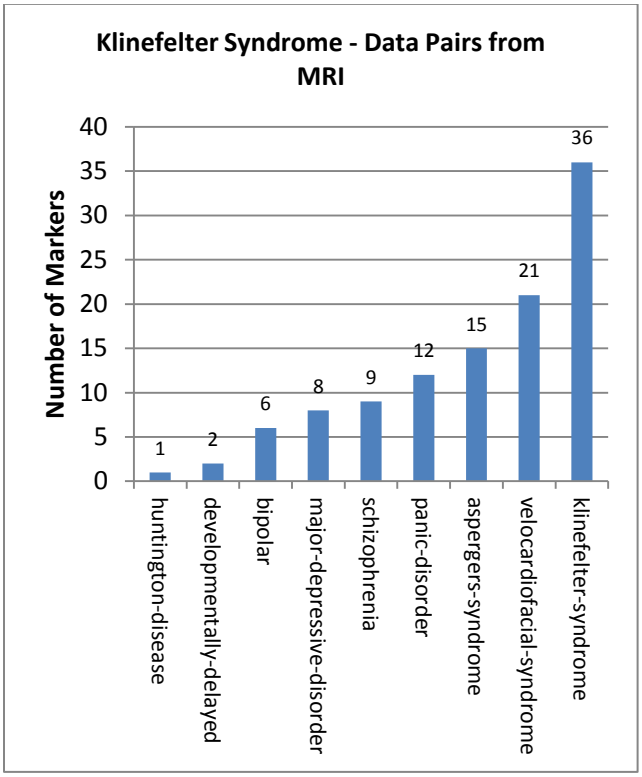

Epilepsy - Data Pairs from MRI
Number of Markers
40
35
30
25
20
15
10
5
0
2 5 6 6 8 9 11 16 19 21 25 29 38
williams-syndrome
aspergers-syndrome
borderline-personality-disorder
ptsd
major-depressive-disorder
adhd
panic-disorder
alzheimer
velocardiofacial-syndrome
down-syndrome
bipolar
schizophrenia
epilepsy

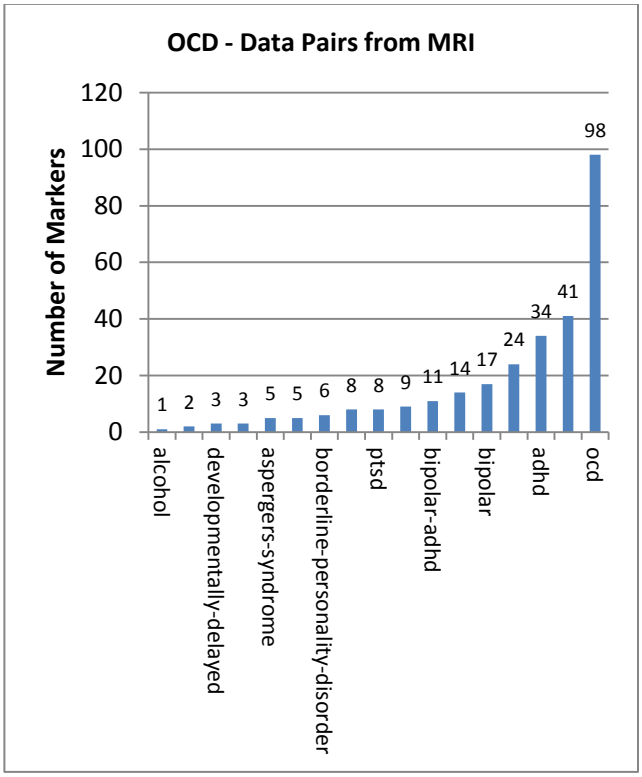

Down Syndrome - Data Pairs from MRI
Number of Markers
45
40
35
30
25
20
15
10
5
0
6 12 12 14 16 17 18 20 22 27 29 41
panic-disorder
adhd
aspergers-syndrome
ptsd
borderline-personality-disorder
major-depressive-disorder
alzheimer
velocardiofacial-syndrome
epilepsy
bipolar
schizophrenia
down-syndrome

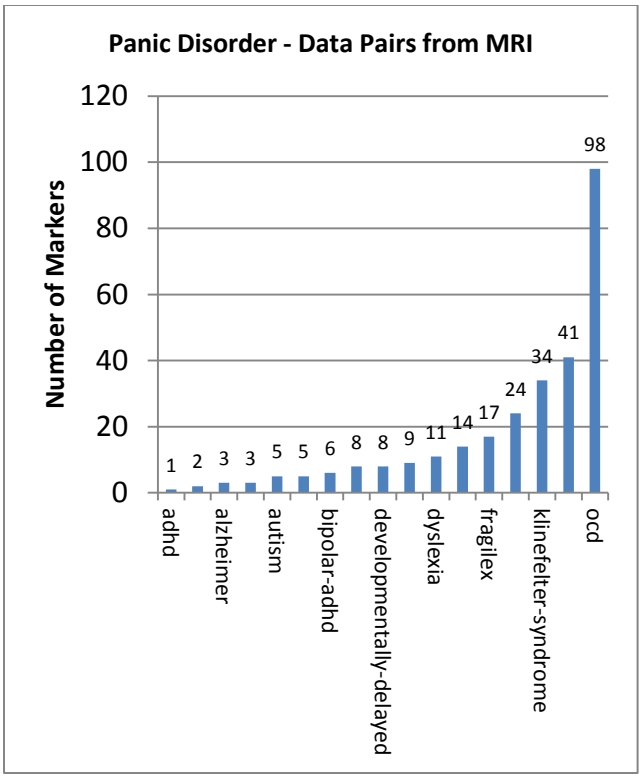

FragileX - Data Pairs from MRI
Number of Markers
90
80
70
60
50
40
30
20
10
0
1 1 2 4 5 5 8 9 11 14 27 31 78
autism
borderline-personality-disorder
ptsd
bipolar-adhd
alzheimer
ocd
bipolar
aspergers-syndrome
huntington-disease
adhd
schizophrenia
developmentally-delayed
fragilex

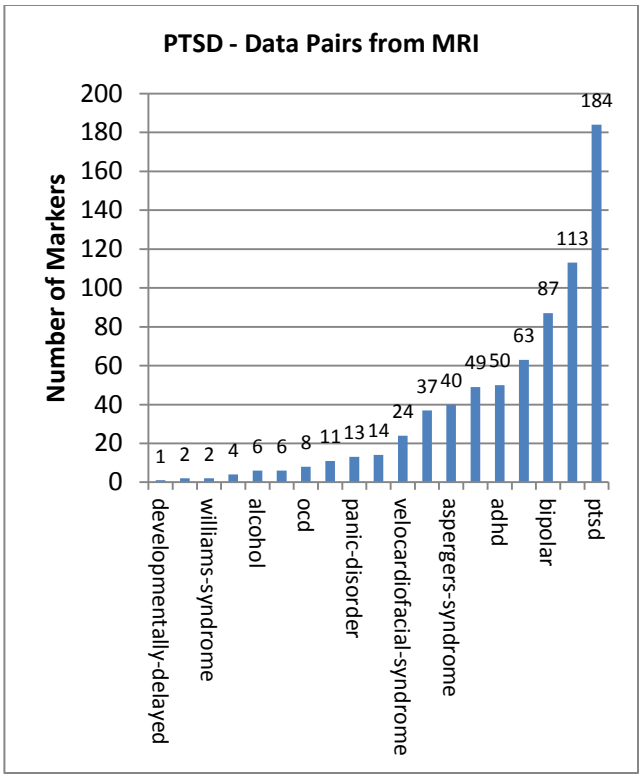

Huntington Disease - Data Pairs from MRI
Number of Markers
0
10
20
30
40
50
60
1 2 2 2 3 3 5 6 7 7 9 9 11 12 15 49
klinefelter-syndrome
borderline-personality-disorder
psychopathic
velocardiofacial-syndrome
aspergers-syndrome
developmentally-delayed
adhd
bipolar-adhd
autism
bipolar
alzheimer
ocd
major-depressive-disorder
fragilex
schizophrenia
huntington-disease

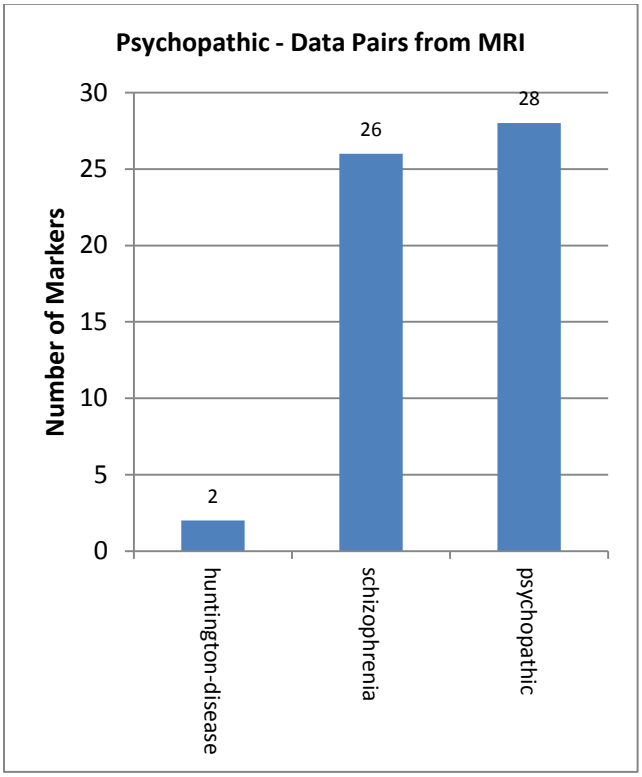

Major Depressive Disorder - Data Pairs from MRI
Number of Markers
0
100
200
300
400
500
600
700
800
900
1 4 7 8 8 9 11 13 15 18 23 25 49 51 66 83 192 288 672 812
dyslexia
williams-syndrome
klinefelter-syndrome
alcohol
epilepsy
ocd
huntington-disease
bipolar-adhd
autism
down-syndrome
panic-disorder
velocardiofacial-syndrome
ptsd
aspergers-syndrome
adhd
borderline-personality-disorder
alzheimer
schizophrenia
bipolar
major-depressive-disorder

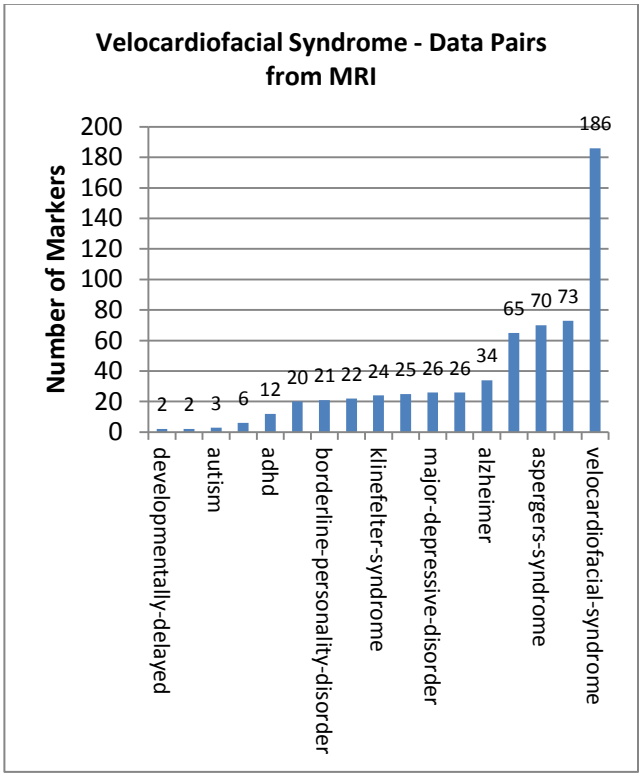

Klinefelter Syndrome - Data Pairs from MRI
Number of Markers
0
5
10
15
20
25
30
35
40
1 2 6 8 9 12 15 21 36
huntington-disease
developmentally-delayed
bipolar
major-depressive-disorder
schizophrenia
panic-disorder
aspergers-syndrome
velocardiofacial-syndrome
klinefelter-syndrome

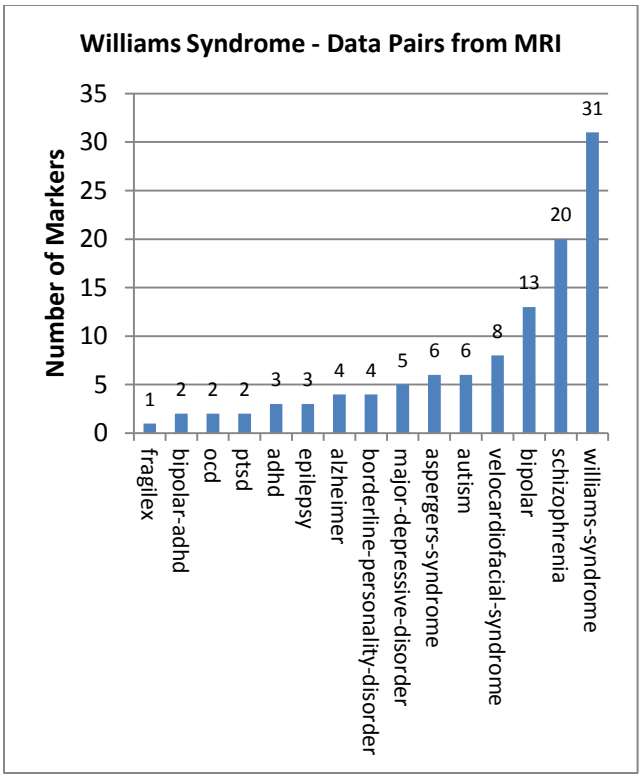

OCD - Data Pairs from MRI
Number of Markers
0
20
40
60
80
100
120
1 2 3 3 5 5 6 8 8 9 11 14 17 24 34 41 98
alcohol
developmentally-delayed
aspergers-syndrome
borderline-personality-disorder
ptsd
bipolar-adhd
bipolar
adhd
ocd


Panic Disorder - Data Pairs from MRI
Number of Markers
120
100
80
60
40
20
0
1 2 3 3 5 5 6 8 8 9 11 14 17 24 34 41 98
adhd
alzheimer
autism
bipolar-adhd
developmentally-delayed
dyslexia
fragilex
klinefelter-syndrome
ocd


PTSD - Data Pairs from MRI
Number of Markers
200
180
160
140
120
100
80
60
40
20
0
1 2 2 4 6 6 8 11 13 14 24 37 40 49 50 63 87 113 184
developmentally-delayed
williams-syndrome
alcohol
ocd
panic-disorder
velocardiofacial-syndrome
aspergers-syndrome
adhd
bipolar
ptsd


Psychopathic - Data Pairs from MRI
Number of Markers
30
25
20
15
10
5
0
2 26 28
huntington-disease
schizophrenia
psychopathic


Velocardiofacial Syndrome - Data Pairs from MRI
Number of Markers
200
180
160
140
120
100
80
60
40
20
0
2 2 3 6 12 20 21 22 24 25 26 26 34 65 70 73 186
developmentally-delayed
autism
adhd
borderline-personality-disorder
klinefelter-syndrome
major-depressive-disorder
alzheimer
aspergers-syndrome
velocardiofacial-syndrome


Williams Syndrome - Data Pairs from MRI
Number of Markers
35
30
25
20
15
10
5
0
1
2
2
2
3
3
4
4
5
6
6
8
13
20
31
fragilex
bipolar-adhd
ocd
ptsd
adhd
epilepsy
alzheimer
borderline-personality-disorder
major-depressive-disorder
aspergers-syndrome
autism
velocardiofacial-syndrome
bipolar
schizophrenia
williams-syndrome

APPENDIX III

Duplicate Mathematical Markers: Parts and connections shared by living and postmortem brains

Figures 1 and 2 replace the blue dots of Figure 8 and 9 with the names of the parts.

In the controls (Figure 1), some of the parts form smaller groups disconnected from the main group. Compared to the living brain, however, far fewer parts and connections are in play (Bolender, 2012).

Figure 2 shows that most of the connectivity of the control disappears postmortem in schizophrenic brains, with fewer duplicate parts in play. This helps to explain the inability of stereological data to diagnose this disorder (Report - Figures 2 and 3). In effect, the parallel complexity needed to make the diagnosis was obscured in the postmortem brain.

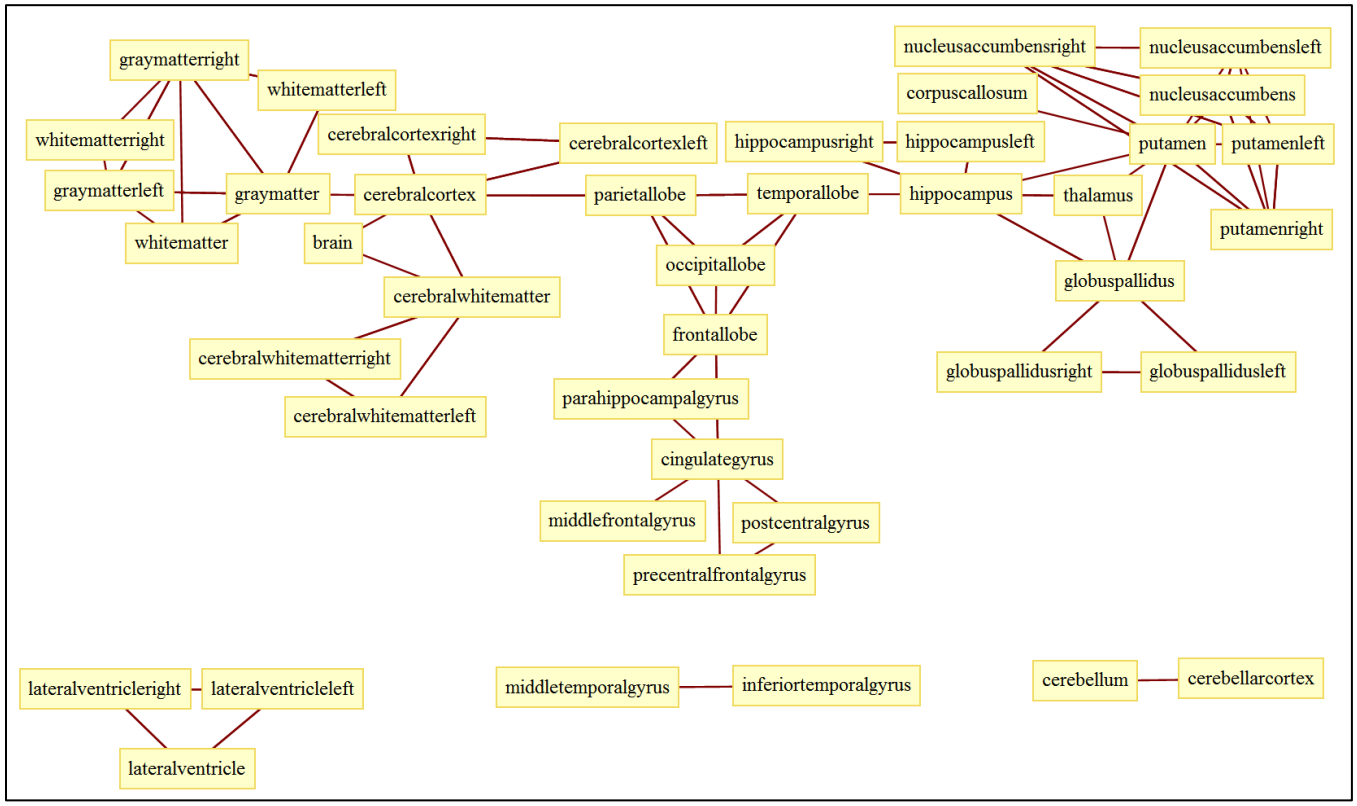


Figure 1. Control data for the human brain wherein living and postmortem brains share duplicate mathematical markers.

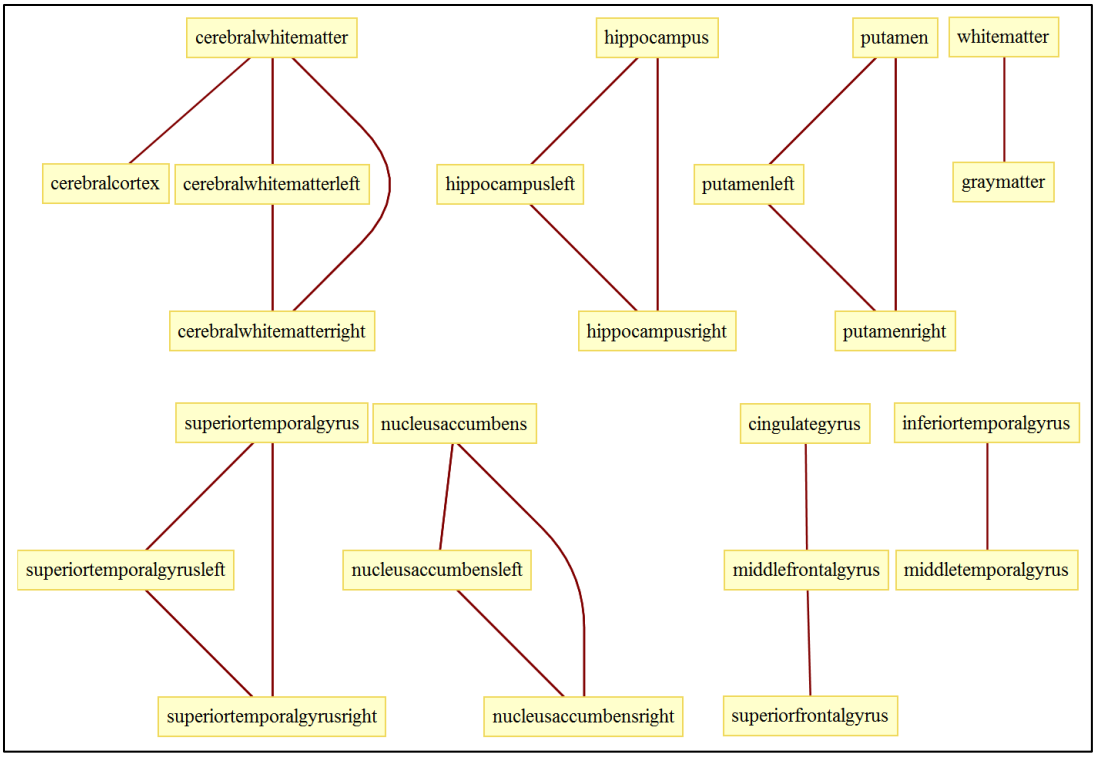


Figure 2. Experimental data (schizophrenia) for the human brain wherein living and postmortem brains share duplicate mathematical markers. Notice that the connectivity seen in Figure 1 no longer exists. Instead, we see an extensive disruption, displayed as eight isolated groups – most of which involve subsets of the same part.

APPENDIX IV

Volume Correction Factors for Distorted Parts in Postmortem Brains

Figures 1 and 2 show that each part of the postmortem brain carries a unique distortion in its volume. These distortions, which insert a second complexity into stereological data, limit the effectiveness of stereological data – both locally and globally. By applying these correction factors to the parts of postmortem brains, the second complexity retreats and the resulting data begin to resemble those of living brains (Report - Figure 12). The correction factors for white matter seem puzzling.

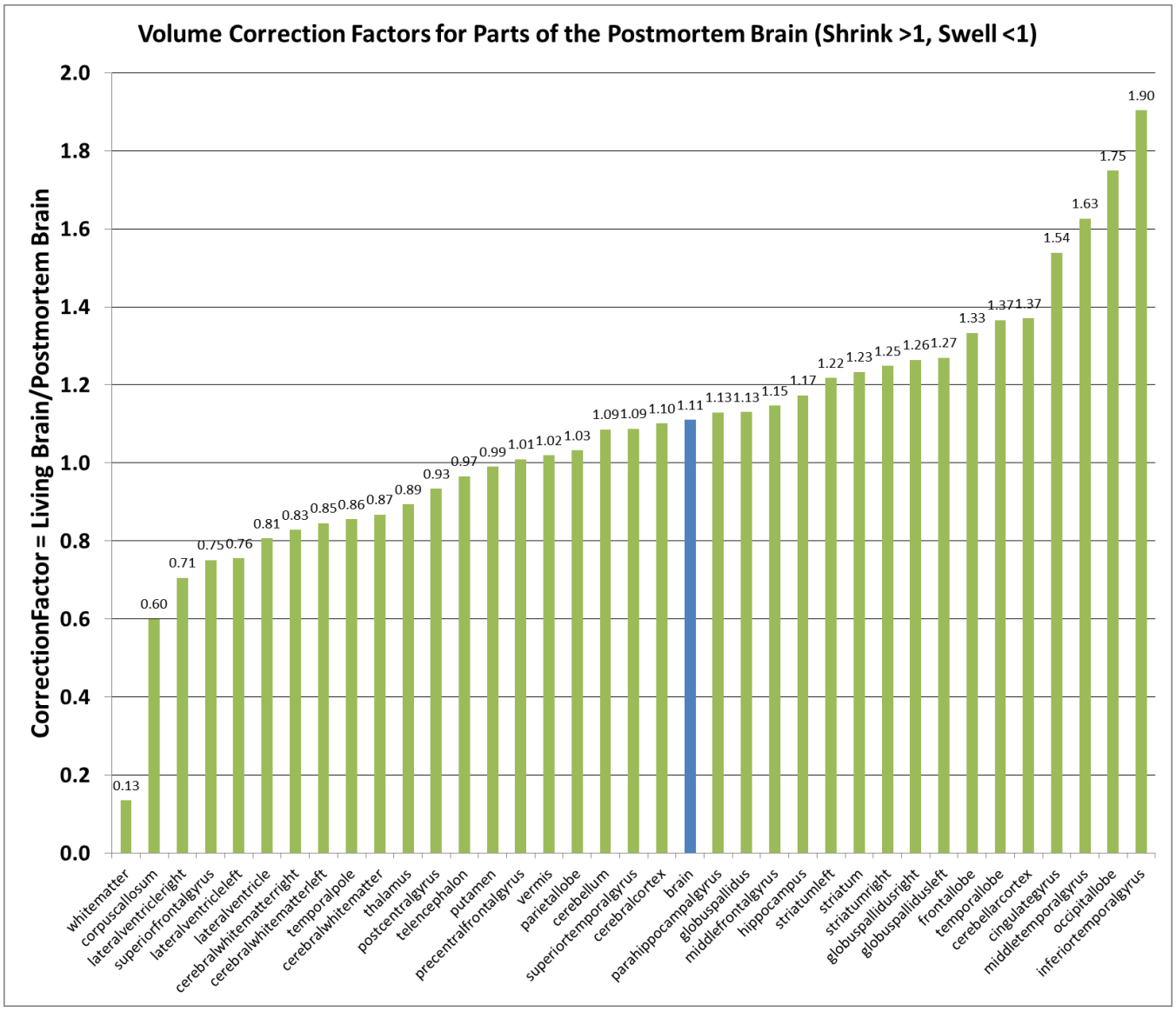


Figure 1. Correction factors for the postmortem brain (control) display a broad range of values, one for each part. The blue column, which represents the human brain, has a correction factor of 1.11. It corrects for a shrinkage of about 11%. See the text for a worked example.

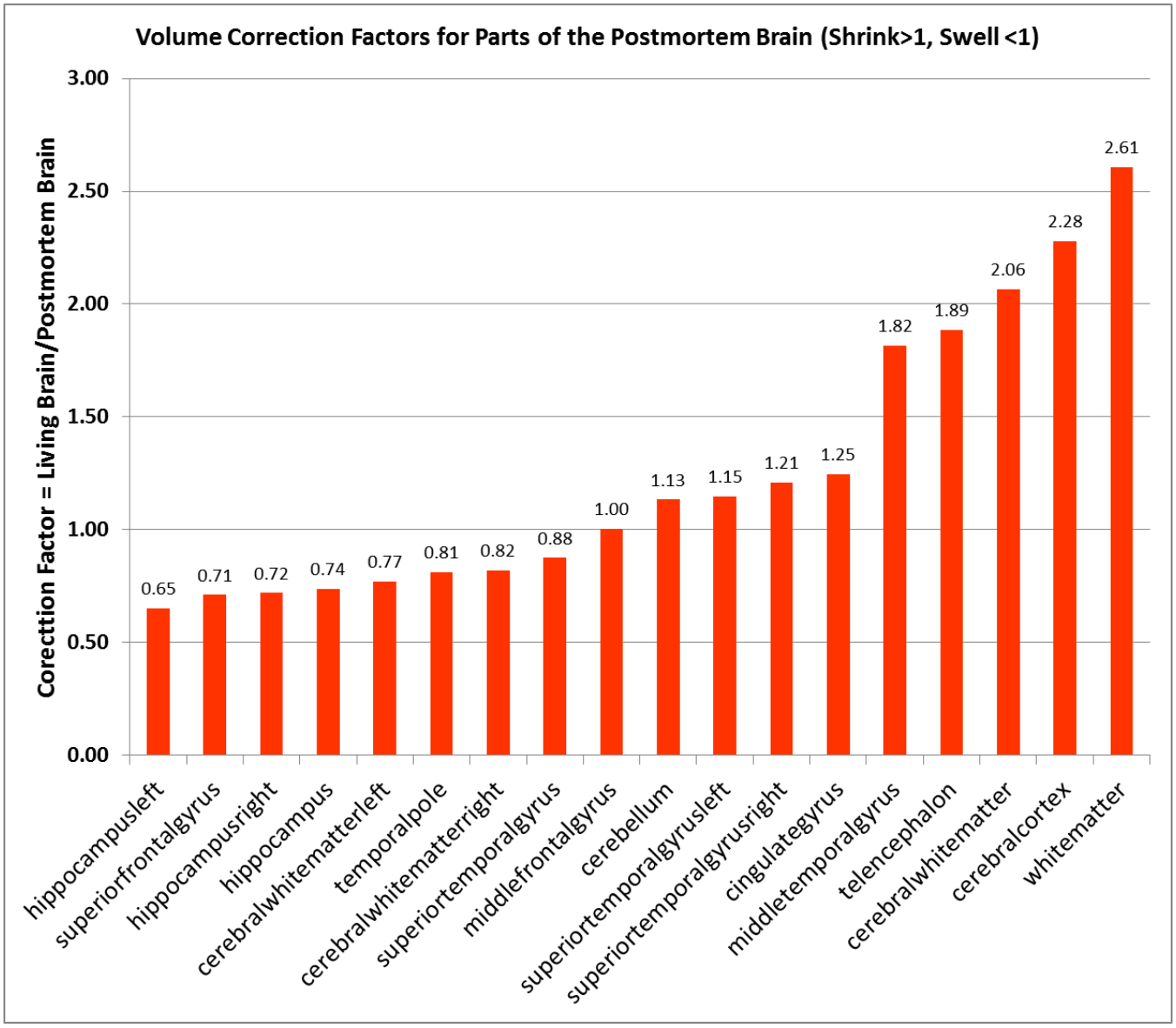


Figure 2. Correction factors for parts of the human brain with schizophrenia can differ markedly from those of the controls (Figure 1).