

Producing a database of protein expression changes in human Alzheimer's Disease

Dr Richard Unwin

Senior Lecturer

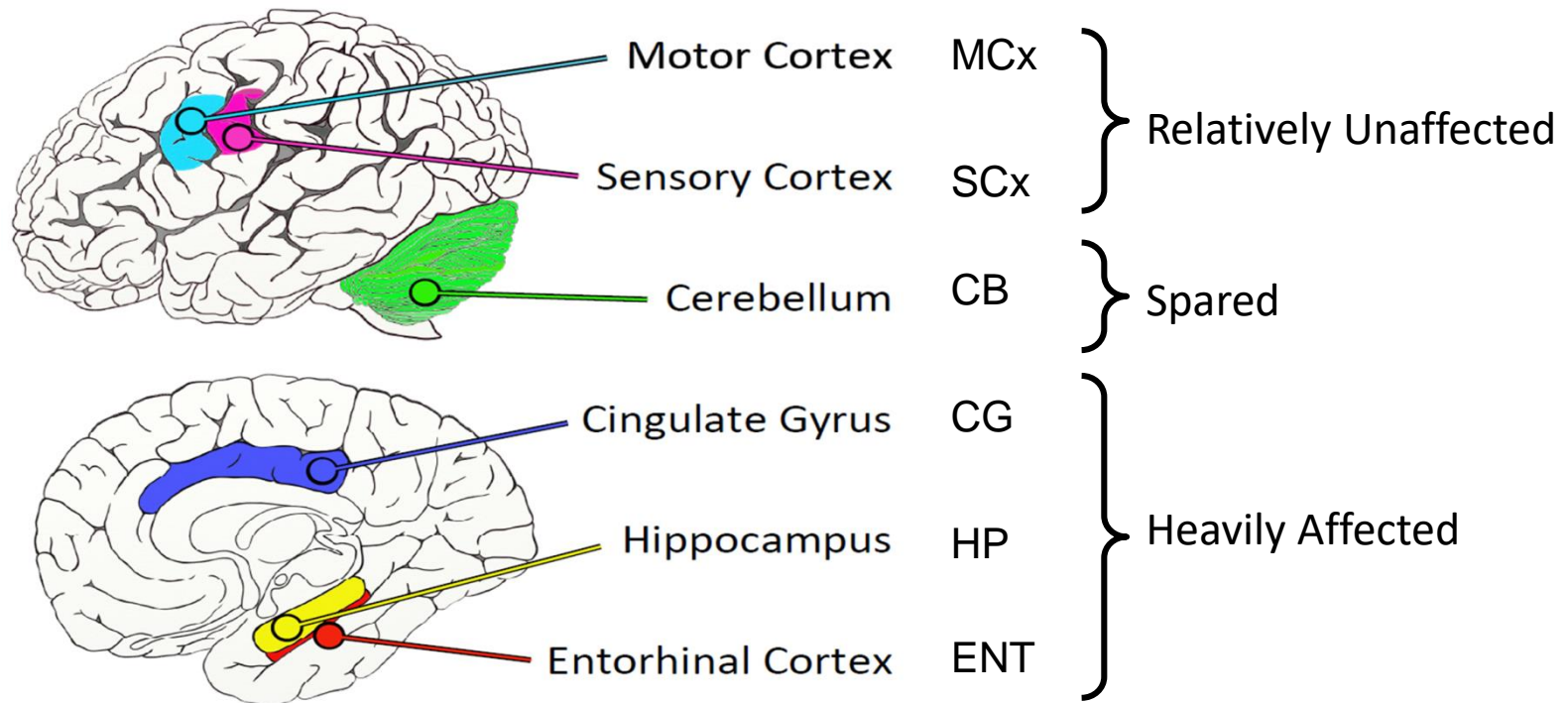
Division of Cancer Sciences



The University of Manchester

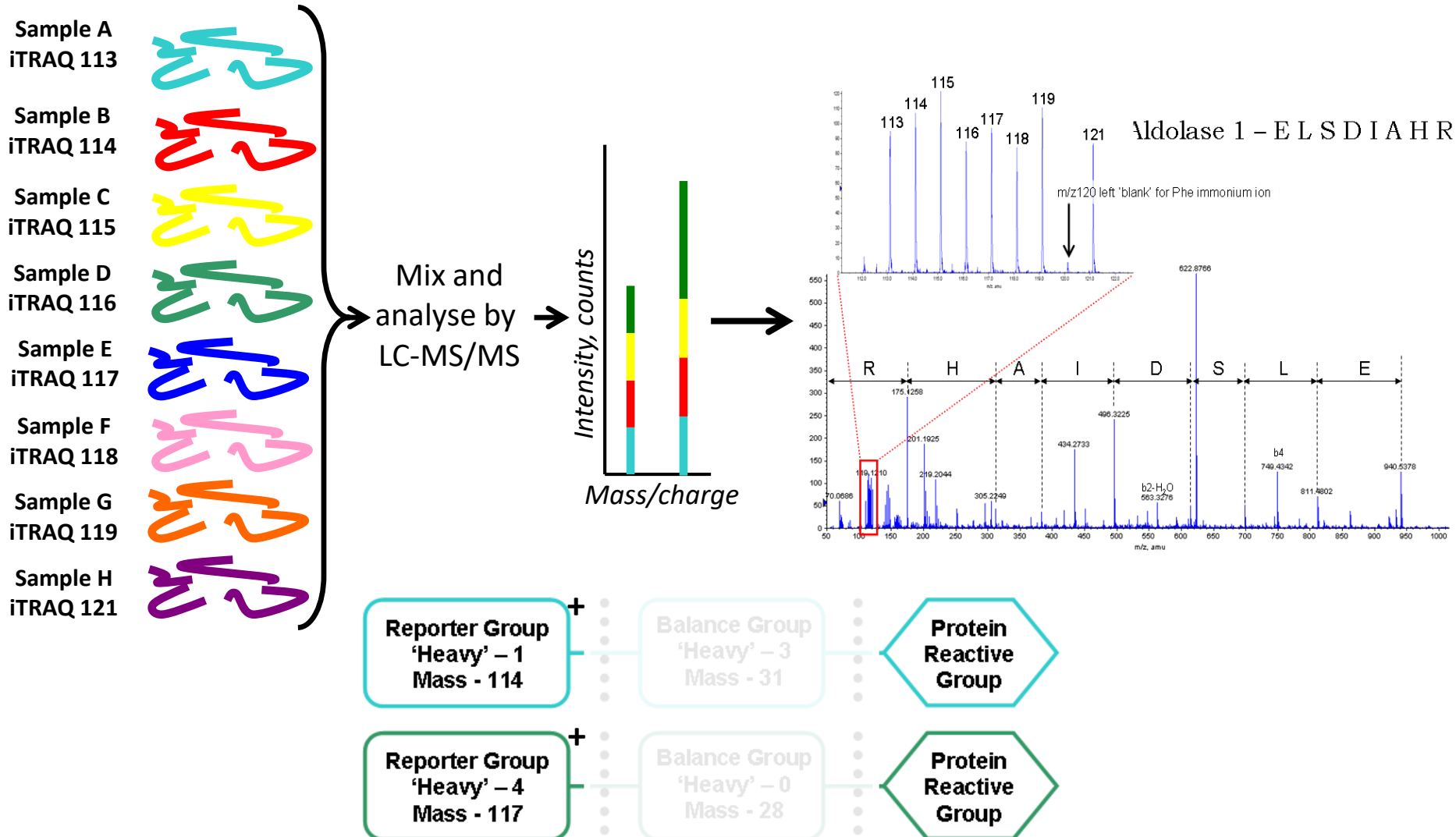
Alzheimer's Disease

- Aim: To perform a proteomic, metabolomic and metallomic analysis of human brain tissue from patient with AD and age-matched healthy controls.
- Collaboration with Jingshu Xu and Richard Faull (Auckland).
- Analyse SIX distinct tissue regions in Alzheimer's:



iTRAQ for relative quantification

- isobaric Tags for Relative and Absolute Quantification (c.f. TMT 10-plex)
- Used for comparing 8 samples simultaneously

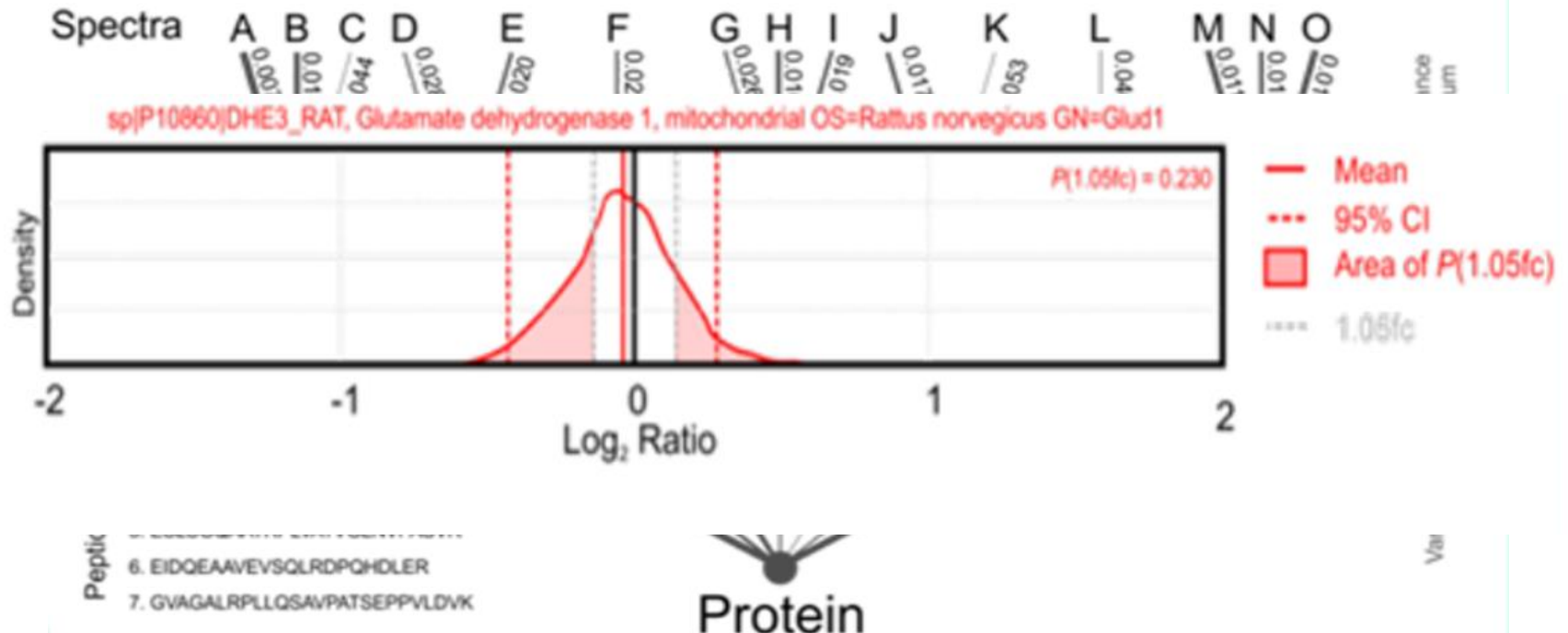


BayesProt

- To overcome difficulties merging ratiometric data from individual 8-plexes, we have developed a Bayesian framework for combining data from multiple 8plexes and samples into a single statistical model:

BayesProt

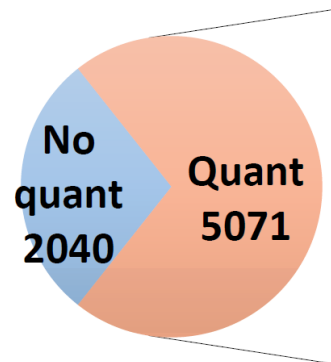
- To overcome difficulties merging ratioetric data from individual 8-plexes, we have developed a Bayesian framework for combining data from multiple 8plexes and samples into a single statistical model:
- Collaboration with Prof Andrew Dowsey, University of Bristol.



Data summary

Region	#Proteins quantified	#Proteins quantified spectra>3
Hippocampus	4444	3548
Motor Cortex	2992	2188
Sensory Cortex	4362	3412
Cerebellum	5279	4198
Entorhinal Cortex	4641	3241
Cingulate Gyrus	3900	2722

>25,000 datapoints



Data sharing

- This study provides a large amount of data that is useful to many groups. It needs to be freely available, not least because:
 - There's no way we can follow up all of the leads
 - It provides extra information for researchers studying specific disease aspects.
 - Might help garner extra citations.
 - The funders would like to see it used.
 - Journal will expect all data to be shared.
 - We spend 3+ years gathering it – WE'd like to see it used.

The 'usual' way

File

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Formulas

Data

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View

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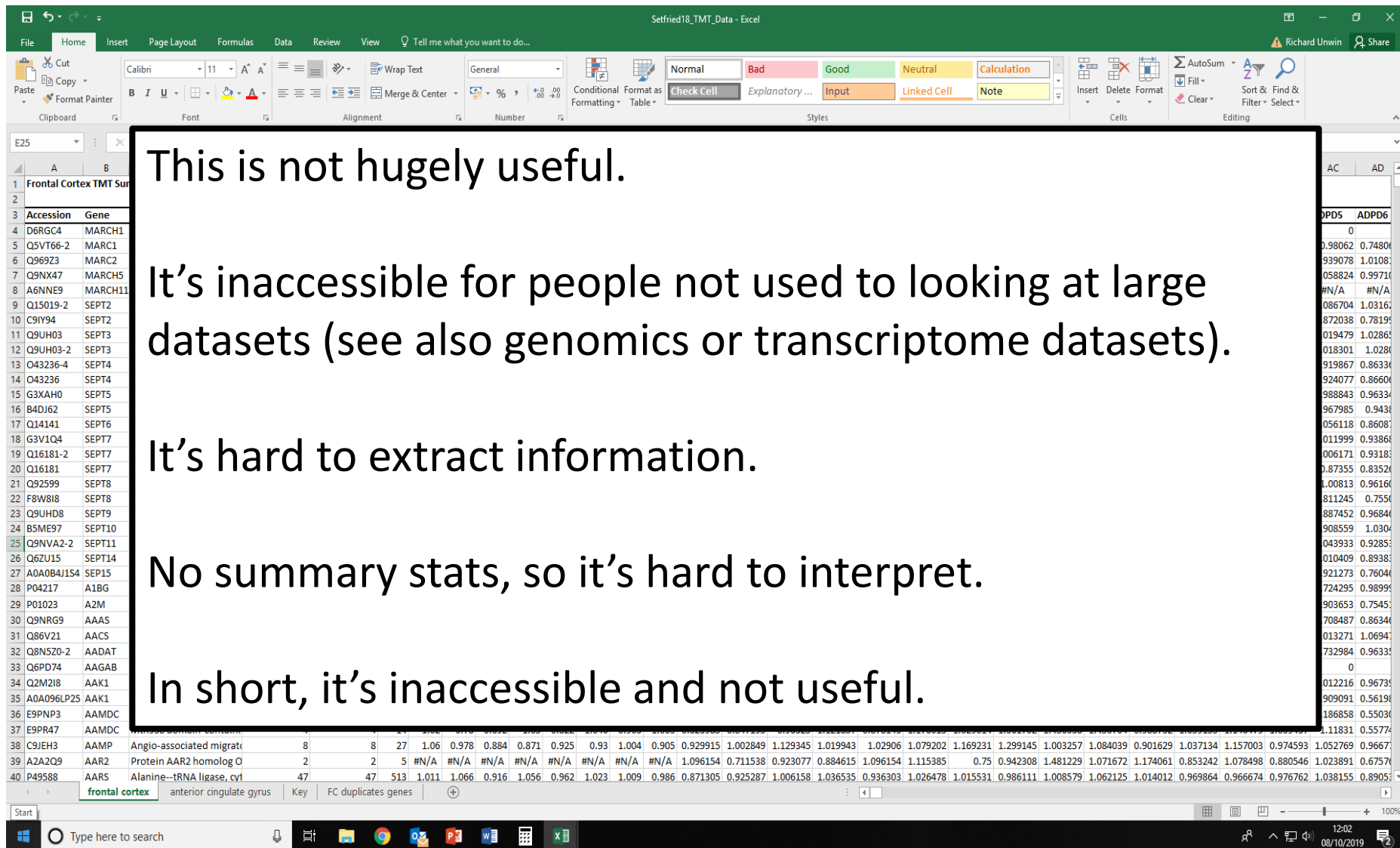
Format Painter

Calibri

11

A

The 'usual' way



This is not hugely useful.

It's inaccessible for people not used to looking at large datasets (see also genomics or transcriptome datasets).

It's hard to extract information.

No summary stats, so it's hard to interpret.

In short, it's inaccessible and not useful.

Research IT brief

Build a way to make this more accessible.

- Web accessible via a unique UoM URL.
- Searchable, so that researchers can easily find proteins of interest.
- Provide an immediate and easily interpretable result.
- Provide the ability to 'delve deeper' into a result to see the underlying data.

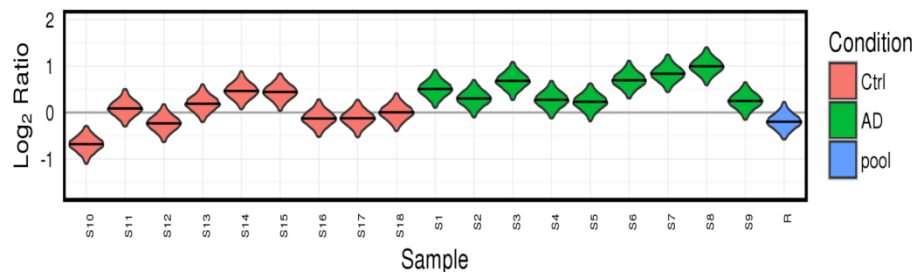
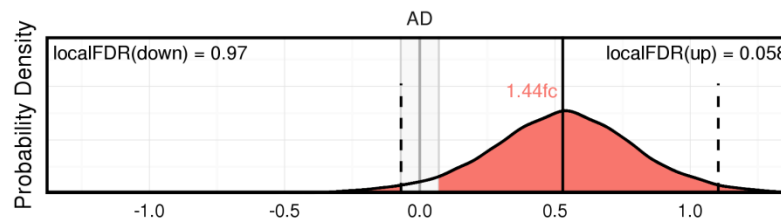
Research IT – Our data

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	
1	ProteinID	N	Protein				Peptides	Spectra	MinConf	MinPrecu	Condition	Test	itt_pred	itt_ok	burnin_pr	burnin_ok	dic	me	
2	SPTAN1	1	1 sp [Q13813] SPTN1_HUMAN: Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTN1 PE=1 SV=3	Q13813	SPTN1_HUMAN	Spectrin alpha chain, non-er	215	1740	15.16	21.5486	AD	Down	51292	No	600	Yes	116991.3	-	
3	PLEC	4	2 sp [Q15149] PLEC_HUMAN: Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3	Q15149	PLEC_HUMAN	Plectin	225	1118	17.38	17.0688	AD	Down	5354	Yes	100	Yes	74364.61	-	
4	DYNC1H1	7	3 sp [Q14204] DYHC1_HUMAN: Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYHC1 PE=1 SV=3	Q14204	DYHC1_HUMAN	Cytoplasmic dynein 1 heavy	198	911	15.89	12.9352	AD	Down	6532	Yes	200	Yes	57226.93	-	
5	SPTBN1	3	4 sp [Q10182] SPTB2_HUMAN: Spectrin beta chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTB2 PE=1 SV=3	Q10182	SPTB2_HUMAN	Spectrin beta chain, non-ery	165	1127	16.82	26.8085	AD	Down	10427	Yes	200	Yes	76872.26	-	
6	GFAP	0	5 sp [P14136] GFAP_HUMAN: Glial fibrillary acidic protein OS=Homo sapiens GN=P14136	P14136	GFAP_HUMAN	Glial fibrillary acidic protein	95	1829	15.09	8.9846	AD	Up	2283	Yes	200	Yes	125305	0	
7	MAP1B	13	6 sp [P46821] MAP1B_HUMAN: Microtubule-associated protein 1B OS=Homo sapiens GN=MAP1B PE=1 SV=3	P46821	MAP1B_HUMAN	Microtubule-associated pro	109	607	16.29	25.7969	AD	Down	5513	Yes	200	Yes	41940.9	-	
8	CLTC	6	7 sp [Q00610] CLH1_HUMAN: Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC	Q00610	CLH1_HUMAN	Clathrin heavy chain 1	95	918	17.28	23.9908	AD	Down	11988	Yes	600	Yes	59618.93	-	
9	MAP2	15	8 sp [P11137] MTAP2_HUMAN: Microtubule-associated protein 2 OS=Homo sapiens GN=MTAP2 PE=1 SV=3	P11137	MTAP2_HUMAN	Microtubule-associated pro	101	580	20.13	15.2525	AD	Down	7085	Yes	200	Yes	39757.51	-	
10	DPYL2	5	9 sp [Q16555] DPYL2_HUMAN: Dihydropyrimidinase-related protein 2 OS=Homo sapiens GN=DPYL2 PE=1 SV=3	Q16555	DPYL2_HUMAN	Dihydropyrimidinase-relate	48	963	16.18	32.7329	AD	Up	7092	Yes	200	Yes	59351.29	0	
11	AHNAK	23	11 sp [Q09666] AHNK_HUMAN: Neuroblast differentiation-associated protein AH OS=Homo sapiens GN=AHNK PE=1 SV=3	Q09666	AHNK_HUMAN	Neuroblast differentiation-i	140	470	15.48	56.0356	AD	Up	286	Yes	200	Yes	32300.82	-	
12	TUBB4A	260	13 sp [P04350] TBB4A_HUMAN: Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=3	P04350	TBB4A_HUMAN	Tubulin beta-4A chain	7	103	58.97	89.7103	AD	Down	6004	Yes	100	Yes	6947.057	-	
13	PKM	8	14 sp [P14618] KPYM_HUMAN: Pyruvate kinase PKM OS=Homo sapiens GN=PKM F14618	P14618	KPYM_HUMAN	Pyruvate kinase PKM	62	860	15.88	10.7199	AD	Up	47142	No	900	Yes	52799.17	0	
14	GAPDH	2	15 sp [P04406] G3P_HUMAN: Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=G3P PE=1 SV=3	P04406	G3P_HUMAN	Glyceraldehyde-3-phosphat	49	1169	15.36	23.6527	AD	Up	24282	No	600	Yes	75260.96	0	
15	ANK2	31	16 sp [Q01484] ANK2_HUMAN: Ankyrin-2 OS=Homo sapiens GN=ANK2 PE=1 SV=4	Q01484	ANK2_HUMAN	Ankyrin-2	103	382	15.04	24.6719	AD	Down	7981	Yes	200	Yes	25048.84	-	
16	MAP1A	26	17 sp [P78559] MAP1A_HUMAN: Microtubule-associated protein 1A OS=Homo sapiens GN=MAP1A PE=1 SV=3	P78559	MAP1A_HUMAN	Microtubule-associated pro	90	434	15.3	30.5056	AD	Down	7483	Yes	200	Yes	30044.37	-	
17	ENO1	16	18 sp [P06733] ENOA_HUMAN: Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=3	P06733	ENOA_HUMAN	Alpha-enolase	38	572	15.89	19.0355	AD	Up	19276	No	1200	Yes	36633.82	0	
18	SYN1	22	19 sp [P17600] SYN1_HUMAN: Synapsin-1 OS=Homo sapiens GN=SYN1 PE=1 SV=3	P17600	SYN1_HUMAN	Synapsin-1	36	493	22.21	29.3997	AD	Down	7014	Yes	200	Yes	31135.84	-	
19	CNP	24	20 sp [P09543] CN37_HUMAN: 2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Homo sapiens GN=CN37 PE=1 SV=3	P09543	CN37_HUMAN	2',3'-cyclic-nucleotide 3'-ph	52	461	15.09	22.0104	AD	Down	8346	Yes	200	Yes	29979.34	-	
20	CKB	19	21 sp [P12277] KCRB_HUMAN: Creatine kinase B-type OS=Homo sapiens GN=CKB	P12277	KCRB_HUMAN	Creatine kinase B-type	43	543	15.22	11.439	AD	Down	6693	Yes	200	Yes	34050.91	-	
21	ATP1A1	70	22 sp [P05023] AT1A1_HUMAN: Sodium/potassium-transporting ATPase subunit alpha OS=Homo sapiens GN=ATP1A1 PE=1 SV=3	P05023	AT1A1_HUMAN	Sodium/potassium-transport	41	237	16.86	36.2169	AD	Down	6429	Yes	200	Yes	16769.63	-	
22	ATP5B	10	23 sp [P06576] ATPB_HUMAN: ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3	P06576	ATPB_HUMAN	ATP synthase subunit beta, i	43	685	20.72	13.9268	AD	Up	8360	Yes	200	Yes	39895.41	0	
23	MBP	12	24 sp [P02686] MBP_HUMAN: Myelin basic protein OS=Homo sapiens GN=MBP PE=1 SV=3	P02686	MBP_HUMAN	Myelin basic protein	27	642	15.18	12.8273	AD	Up	5114	Yes	100	Yes	42993.06	-	
24	HSPD1	9	25 sp [P10809] CH60_HUMAN: 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=3	P10809	CH60_HUMAN	60 kDa heat shock protein, n	52	760	17.53	24.4854	AD	Down	10048	Yes	200	Yes	43334.31	-	
25	HSPA8	46	26 sp [P11142] HSP7C_HUMAN: Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=3	P11142	HSP7C_HUMAN	Heat shock cognate 71 kDa p	38	318	16.42	46.8514	AD	Down	6088	Yes	200	Yes	21804.43	-	
26	ATP5A1	18	27 sp [P25705] ATPA_HUMAN: ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=3	P25705	ATPA_HUMAN	ATP synthase subunit alpha,	54	556	15.2	24.6985	AD	Down	6700	Yes	200	Yes	34498.72	-	
27	GD1I	82	28 sp [P31150] GDIA_HUMAN: Rab GDP dissociation inhibitor alpha OS=Homo sapiens GN=GD1I PE=1 SV=3	P31150	GDIA_HUMAN	Rab GDP dissociation inhibi	21	214	16.46	89.9831	AD	Up	6743	Yes	200	Yes	14169.46	0	
28	ALDOA	25	29 sp [P04075] ALDOA_HUMAN: Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=3	P04075	ALDOA_HUMAN	Fructose-bisphosphate aldo	25	438	18.06	45.575	AD	Up	9531	Yes	300	Yes	26309.98	0	
29	HK1	29	30 sp [P19367] HXK1_HUMAN: Hexokinase-1 OS=Homo sapiens GN=HK1 PE=1 SV=3	P19367	HXK1_HUMAN	Hexokinase-1	53	398	17.13	31.3413	AD	Down	6047	Yes	200	Yes	28288.26	-	
30	MYH10	58	31 sp [P35580] MYH10_HUMAN: Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3	P35580	MYH10_HUMAN	Myosin-10	61	261	16.13	23.5077	AD	Down	7749	Yes	200	Yes	15671.21	-	
31	NEFM	35	32 sp [P07197] NFM_HUMAN: Neurofilament medium polypeptide OS=Homo sapiens GN=NEFM PE=1 SV=3	P07197	NFM_HUMAN	Neurofilament medium pol	52	359	15.39	21.0107	AD	Down	7457	Yes	300	Yes	24771.17	-	
32	SPTBN2	61	33 sp [Q15020] SPTN2_HUMAN: Spectrin beta chain, non-erythrocytic 2 OS=Homo sapiens GN=SPTBN2 PE=1 SV=3	Q15020	SPTN2_HUMAN	Spectrin beta chain, non-ery	70	253	18.41	50.757	AD	Down	8306	Yes	200	Yes	16250.47	-	
33	HBB	34	34 sp [P68871] HBB_HUMAN: Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=3	P68871	HBB_HUMAN	Hemoglobin subunit beta	15	373	15.35	14.3396	AD	Up	7036	Yes	200	Yes	24849.27	-	
34	CNTN1	32	35 sp [Q12860] CNTN1_HUMAN: Contactin-1 OS=Homo sapiens GN=CNTN1 PE=1 SV=3	Q12860	CNTN1_HUMAN	Contactin-1	54	382	16.01	11.903	AD	Down	9290	Yes	200	Yes	25026.85	-	
35	ATP6V1A	14	36 sp [P38606] VATA_HUMAN: V-type proton ATPase catalytic subunit A OS=Homo sapiens GN=ATP6V1A PE=1 SV=3	P38606	VATA_HUMAN	V-type proton ATPase cataly	47	583	17.03	7.9533	AD	Down	6431	Yes	200	Yes	33354.21	-	
36	ALB	21	37 sp [P02768] ALBU_HUMAN: Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=3	P02768	ALBU_HUMAN	Serum albumin									200	Yes	32854.74	-	
37	FASN	64	38 sp [P49327] FAS_HUMAN: Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3	P49327	FAS_HUMAN	Fatty acid synthase									600	Yes	14911.37	-	
38	ANXA6	30	39 sp [P08133] ANXA6_HUMAN: Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	P08133	ANXA6_HUMAN	Annexin A6									100	Yes	24962.89	-	
39	STXB1	20	40 sp [P61764] STXB1_HUMAN: Syntrophin-binding protein 1 OS=Homo sapiens GN=STXB1 PE=1 SV=3	P61764	STXB1_HUMAN	Syntrophin-binding protein 1									300	Yes	34180.13	-	
40	ACO2	42	41 sp [Q99798] ACON_HUMAN: Aconitate hydratase, mitochondrial OS=Homo sapiens GN=ACO2 PE=1 SV=3	Q99798	ACON_HUMAN	Aconitate hydratase, mitochond									100	Yes	23588.54	-	
41	DNM1	81	42 sp [Q05193] DYN1_HUMAN: Dynamin-1 OS=Homo sapiens GN=DNM1 PE=1 SV=3	Q05193	DYN1_HUMAN	Dynamin-1									200	Yes	14091.54	-	

x6

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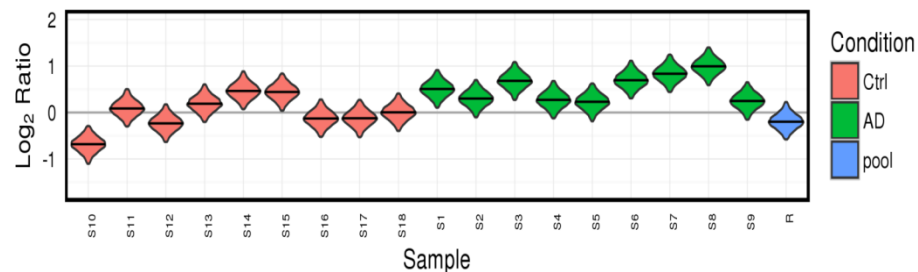
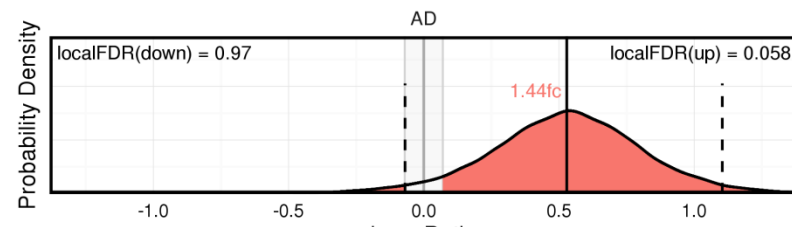


Research IT – Our data Take 2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1							HIPPOCAMPUS		MOTOR CORTEX		SENSORY CORTEX		CEREBELLUM		ENTORHINAL CORTEX		DOLLORE TEMPORAL GYR		CINGULATE GYRUS			HIP
2	Gene Name	SwissProt	SwissProt	Name			ProteinID	N	ProteinID	N	ProteinID	N	ProteinID	N	ProteinID	N	ProteinID	N	ProteinID	N		Peptides
3	GFAP	P14136	GFAP_HU	Glial fibr			0	5	2	15	5	10	4	7	1	5	0	11	1	8		95
4	SPTAN1	Q13813	SPTN1_HU	Spectrin a			1	1	0	1	0	1	0	2	0	1	1	1	0	1		215
5	GAPDH	P04406	G3P_HUM	Glyceral			2	15	5	41	3	14	2	17	7	16	3	13	5	14		49
6	SPTBN1	Q01082	SPTB2_HU	Spectrin b			3	4	1	2	1	4	3	4	2	4	5	2	4	3		165
7	PLEC	Q15149	PLEC_HUN	Plectin			4	2	6	5	9	3	7	3	6	2	27	5	17	5		225
8	DPYSL2	Q16555	DPYL2_HU	Dihydrop			5	9	10	11	7	13	9	9	8	9	6	9	6	12		48
9	CLTC	Q00610	CLH1_HUN	Clathrin h			6	7	3	3	2	5	5	5	3	6	4	3	3	4		95
10	DYNC1H1	Q14204	DYHC1_HU	Cytoplasm			7	3	7	4	4	2	1	1	5	3	26	4	9	2		198
11	PKM	P14618	KPYM_HU	Pyruvate			8	14			12	15	12	12								62
12	HSPD1	P10809	CH60_HUM	60 kDa he			9	25	36	40	11	30	6	41	21	50	45	39	24	37		52
13	ATP5B	P06576	ATPB_HUM	ATP synth			10	23	24	28	8	25	13	29	32	33	30	24	30	43		43
14	HBA1	P69905	HBA_HUM	Hemoglob			11	48	15	66	15	53	8	57	11	58	8	152	11	64		16
15	MBP	P02686	MBP_HUM	Myelin ba			12	24	4	71	6	34	23	49	4	30	2	28	2	26		27
16	MAP1B	P46821	MAP1B_H	Microtubu			13	6	18	9	20	11	15	10	10	11	21	12	22	13		109
17	ATP6V1A	P38606	VATA_HU	V-type pr			14	36	27	30	22	35	30	36	28	37	25	23	23	36		47
18	MAP2	P11137	MTAP2_HU	Microtubu			15	8	21	7	18	7	26	13	16	14	19	8	19	9		101
19	ENO1	P06733	ENO1_HU	Alpha-en			16	18	13	70	17	63	16	22	22	18	11	27	14	22		38
20	ENO2	P09104	ENO2_HU	Gamma-en			17	67	26	43	14	26	17	50	38	62	20	57	21	66		25
21	ATP5A1	P25705	ATPA_HU	ATP synth			18	27	20	33	16	21	14	23	35	32	23	25	27	28		54
22	CKB	P12277	KCRB_HU	Creatine k			19	21	16	35	10	16	11	15	15	22	13	30	12	21		43
23	STXBP1	P61764	STXB1_HU	Syntaxin-			20	40	14	29	13	29	10	32	17	31	12	18	18	24		56

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x1

Online database

Developed in collaboration with Research IT

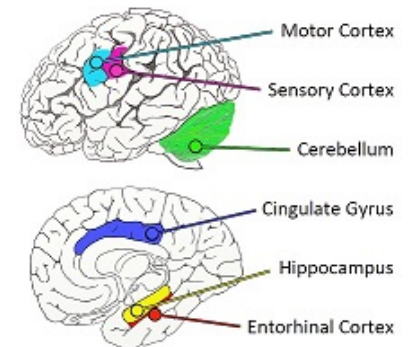
www.manchester.ac.uk/dementia-proteomes-project

Explore the Alzheimer's Disease Proteome

The database contains the results of a series of proteomics experiments performed using Liquid-chromatography-Mass spectrometry (LC-MS) on human brain samples to compare protein expression levels between individuals with Alzheimer's Disease and age-matched controls.

Data have been analysed using a validated Bayesian statistical pipeline which produces a probability distribution for the relative expression of each protein between classes. These distributions, along with the data for each individual sample in the experiment, are also provided from the search results.

Full details of this study, including patient metadata, methodology and key findings, as well a description of this resource, can be found in [the associated paper](#) (Xu et al, "Localised protein expression changes in human Alzheimer's brain correlate with disease progression" Submitted.).



GFAP



Enter your protein here – will search by gene name or free text in protein name

Online database

Developed in collaboration with Research IT

www.manchester.ac.uk/dementia-proteomes-project

1 Proteins returned

Clicking on the column headers will reorder the table according to the data in that column (toggling between asc/desc order). Selecting a Proteome by clicking its name will take you to the data collected for that specific item.

Name	Description	SwissProt_AC	SwissProt_ID
GFAP	Glial fibrillary acidic protein	P14136	GFAP_HUMAN

Previous 1 Next

Select protein of interest from list

Online database

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www.manchester.ac.uk/dementia-proteomes-project

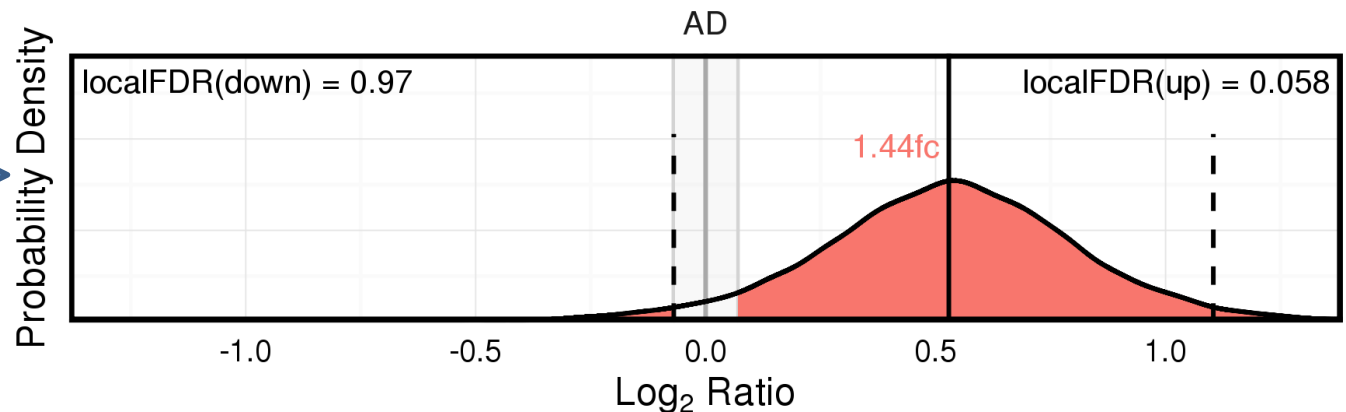
GFAP

AC : **P14136**

ID : **GFAP_HUMAN**

Description : **Glial fibrillary acidic protein**

Brain Area	Peptides	Spectra	Log ₂ (fc)	Lower	Upper	LocalFDR	GlobalFDR
Hippocampus (HP)			0.529149485	-0.066167874	1.103791236	0.0573	0.021314341
Motor Cortex (MCx)	44	590	0.198755814	-0.247789602	0.661911914	0.2655	0.140215328
Sensory Cortex (SCx)							
Cerebellum (CB)							
Entorhinal Cortex (ENT)							
Cingulate Gyrus (CG)							



[Back to Results](#)

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GFAP

AC : **P14136**

ID : **GFAP_HUMAN**

Description : **Glial fibrillary acidic protein**

Brain Area	Peptides	Spectra	Log ₂ (fc)	Lower	Upper	LocalFDR	GlobalFDR
Hippocampus (HP)	95	1829	0.529149485	-0.066167874	1.103791236	0.0573	0.021314341
Motor Cortex (MCx)	44	590	0.198755814	-0.247789602	0.661911914	0.2655	0.140215328

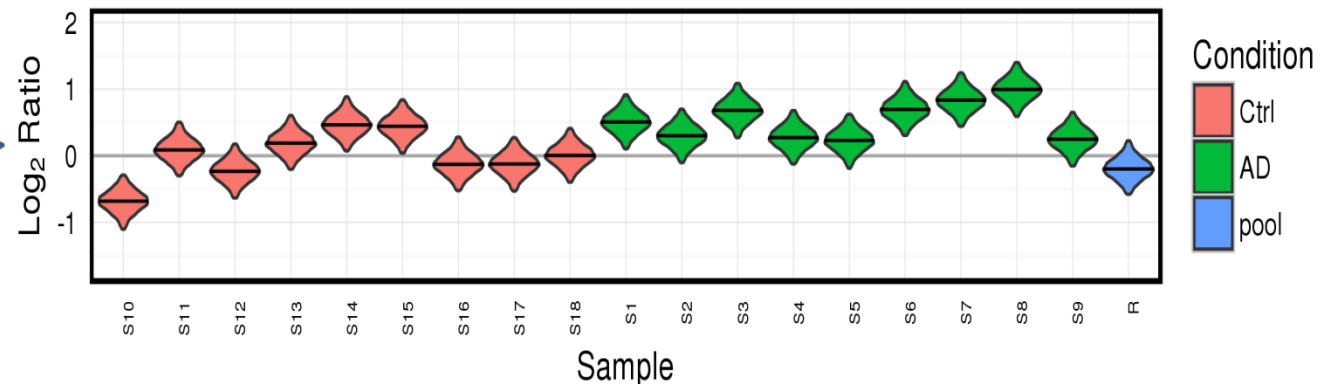
Sensory Cortex (SCx)

Cerebellum (CB)

Entorhinal Cortex (ENT)

Cingulate Gyrus (CG)

Back to Results



Outcomes

- Fantastic tool for me to be able to get easy access to our data – web page works on my phone!
- Great feedback from reviewers of our paper. (Reviewer 2: “I was impressed with ... the authors’ commitment to publicly sharing this data both in this paper and on the online resource they developed.”)
- Great example for inclusion into future grant applications, and good feedback.
- Easy access to the data by others several other groups at UoM and round the UK I know are looking through this DB to get data to support their own work.

Lessons learned

- Approach Research IT early!
- We redesigned bits of our data output sheets to fit with their needs, including reformatting to only include key data columns, all on one sheet etc.
- Also reformatted data processing to output images as .png, not .pdf as originally written.
- Think about impact – we should have included a ‘counter’ for visits, or searches?
- Road test it. Made a few minor changes after feedback from lab members – image appearing in same rather than a new tab or as a file download, for example....

Acknowledgements



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Stephanie Church

Adam Stevens

Rob Haines (Research IT)
Phil Bradbury (Research IT)



Alex Phillips



Andrew Dowsey



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