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Investigating the role of Histone Deacetylase HDAC4 in long-term memory formation

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ABSTRACT

Epigenetic mechanisms are emerging as master regulators of cognitive abilities such as learning and memory. It has been previously shown that the histone deacetylase HDAC4 plays a critical role in memory formation in both mammals and insects although the specific mechanisms through which it acts have not yet been elucidated. HDAC4 undergoes nucleocytoplasmic shuttling and, in neurons, it is largely cytoplasmic implying it may play both nuclear and non-nuclear functions. To identify upstream regulators and downstream targets of *HDAC4*, a genetic interaction screen was performed in the fruit fly *Drosophila melanogaster*, a powerful model system to study the genetic mechanisms of neurological disease. Twenty-nine genes were found to interact with *HDAC4* suggesting they are part of the same molecular pathway. Functional network analysis revealed that many of the genes could be grouped into three biological categories comprising transcriptional factors, SUMOylation machinery enzymes and cytoskeletal regulators/interactors. Within the latter, *Ankyrin2* was selected for further analysis as it is implicated in synaptic stability and in human intellectual disability. In addition HDAC4 harbours a conserved ankyrin binding domain. Immunohistochemical analyses showed widespread distribution of *Ankyrin2* throughout the adult brain and coincident distribution with HDAC4 was observed in the axons of the mushroom body, a key structure for memory formation in flies. Both *HDAC4* and *Ankyrin2* were also found to regulate mushroom body development. RNAi-mediated depletion of *Ankyrin2* in the adult brain impaired long-term memory in the courtship suppression assay, a model of associative memory and preliminary evidence of a physical association between HDAC4 and *Ankyrin2* was also demonstrated. The genes identified in the screen provide new avenues for investigation of the mechanisms through which *HDAC4* regulates memory formation and preliminary analyses suggest that interaction with the cytoskeletal adaptor *Ankyrin2* may involve remodelling of the actin/spectrin cytoskeleton, phenomenon that underlies memory related processes like synaptic plasticity and neuronal excitability.

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TABLE OF CONTENTS

Abstract	i
Acknowledgements	ii
Table of contents	iv
List of Figures	ix
List of Tables	xiii
Abbreviations	xiv
1 Introduction	1
1.1 Neurological disorders: a burden of our times	2
1.2 Learning and Memory: a historical journey	3
1.3 The use of animal models to study neurodegenerative processes: <i>Drosophila melanogaster</i> the model system	9
1.3.1. The mushroom body of <i>Drosophila melanogaster</i> : a brain structure involved in memory	10
1.3.2 <i>Drosophila</i> and its genetic tractability	14
1.3.3 The use of behavioural paradigms to test learning and memory	18
1.4 The role of epigenetics in learning and memory formation	23
1.4.1 Histone Acetyltransferases (HATs)	23
1.4.2 Histone Deacetylases (HDACs)	25
1.4.2.1 HDACs in <i>Drosophila melanogaster</i>	27
1.4.2.2 Inhibition of HDAC activity improves memory	28
1.5 Histone Deacetylase HDAC4 and its role in memory formation	29
1.5.1 <i>Drosophila</i> HDAC4.	31
1.5.2 HDAC4: a master regulator of memory	32
1.6 Ankyrin proteins	34
1.7 Aims and objectives	42
2 Materials and methods	44
2.1 <i>Drosophila melanogaster</i> strains	45
2.2 Maintenance of fly strains	45
2.2.1 Genetic crosses	45
2.3 Rough eye phenotype screen.....	46
2.3.1 Scanning electron microscopy.....	46

2.4 Isolation of <i>Drosophila</i> heads	47
2.5 Transcriptome analysis.....	47
2.6 Immunohistochemistry on whole mount <i>Drosophila</i> brains.....	48
2.7 Polymerase Chain Reaction (PCR)	50
2.7.1 Standard PCR amplification	50
2.7.2 High Fidelity PCR	51
2.8 Sequencing	52
2.9 DNA purification.....	53
2.9.1 PCR purification	53
2.9.2 Agarose gel purification	53
2.10 DNA manipulation	53
2.10.1 Restriction digest	53
2.10.2 Ligation.....	54
2.10.3 Plasmid transformation.....	54
2.10.4 Plasmid DNA purification	54
2.10.4.1 Mini preparation of plasmid DNA.....	54
2.10.4.2 Midi-scale preparation of plasmid DNA	55
2.10.5 Addition of MYC epitope tag to the C-terminus of Ankyrin1	55
2.10.6 Cloning of HDAC4 ankyrin binding region into pGEX-2TK.....	57
2.11 Generation of transgenic flies	58
2.11.1 Genetic crosses to establish lines.....	60
2.12 Preparation of cell lysates	61
2.13 SDS-PAGE and western blotting	62
2.14 Coomassie Brilliant Blue staining.....	64
2.15 GST pull-down assay	64
2.15.1 HDAC4-GST Fusion construct expression and induction	64
2.15.2 GST pull-down	66
2.16 RNA manipulation	67
2.16.1 RNA extraction.....	67
2.16.2 cDNA synthesis	68
2.16.3 Quantitative PCR.....	68
2.17 Courtship suppression assay.....	69
2.18 Statistical analysis	71
3 Results	72

3.1 Identification of genes transcriptionally regulated by <i>HDAC4</i>	73
3.2 A genetic screen for modifiers of the <i>HDAC4</i>-induced rough eye phenotype ...	81
3.2.1 Development and validation of the method	84
3.2.2 A genetic screen for modifiers of the <i>HDAC4</i> -rough eye phenotype detected genes involved in transcriptional regulation, cytoskeleton regulation and SUMOylation pathway	88
3.3 Characterisation of <i>Ankyrin1</i> and <i>Ankyrin2</i> expression in the adult brain and their roles in brain development and memory formation	95
3.3.1 <i>Ankyrin2</i>	96
3.3.1.1 <i>Ankyrin2</i> is highly expressed throughout the fly brain and it is an axonal protein.....	96
3.3.1.2 <i>Ankyrin2</i> is required for normal development of the mushroom body	103
3.3.1.3 <i>Ankyrin2</i> is required for long-term memory formation both during development and in adulthood.....	110
3.3.1.3.1 Depletion of <i>Ankyrin2</i> during development impairs long-term memory formation but does not affect learning and immediate recall of memory	111
3.3.1.3.2 Knockdown of <i>Ankyrin2</i> in the adult brain impairs long-term memory	114
3.3.1.3.2.1 Decreased expression of <i>Ankyrin2</i> in all neurons of the brain impairs long-term memory formation	114
3.3.1.3.2.2 Knockdown of <i>Ankyrin2</i> in the mushroom body impairs long-term memory formation.....	116
3.3.1.3.2.3 <i>Ankyrin2</i> is required in the γ lobes for long-term memory formation	120
3.3.2 <i>Ankyrin1</i>	126
3.3.2.1 <i>Ankyrin1</i> is localised in the mushroom body lobes and calyces	126
3.3.2.2 Expression of <i>Ankyrin1</i> is dispensible for development of the mushroom body lobes.....	128
3.3.2.3 Depletion of <i>Ankyrin1</i> during development is not required for long-term memory formation	131
3.4 Investigation of the relationship between <i>HDAC4</i> and <i>Ankyrin2</i>.....	132
3.4.1 <i>HDAC4</i> co-localises with <i>Ankyrin2</i> in mushroom body lobes	133
3.4.2 <i>HDAC4</i> is required for normal development of the mushroom body lobes.....	136
3.4.3 Investigation of a physical interaction between <i>HDAC4</i> and <i>Ankyrin2</i>	142
3.4.4 Examining an interaction between <i>HDAC4</i> and <i>Ankyrin2</i> in long-term memory formation in <i>Drosophila</i> : preliminary data	147

4 Discussion.....	150
4.1 Identification of genes that interact with <i>HDAC4</i>.....	151
4.1.1 Transcriptome analysis in the head of <i>Drosophila</i> reveals that <i>HDAC4</i> does not have a global effect on gene expression.....	151
4.1.2 Transcription factors, SUMOylation machinery enzymes and cytoskeletal regulators interact with <i>HDAC4</i>	153
4.1.2.1 The rough eye phenotype screen detected conserved interactions in the <i>HDAC4</i> genetic pathway	154
4.1.2.2 Novel interactions were detected by the <i>HDAC4</i> -induced rough eye phenotype screen	157
4.1.2.3 <i>HDAC4</i> interacts with the SUMOylation machinery	158
4.1.2.4 <i>HDAC4</i> interacts with regulators of the cytoskeleton.....	159
4.1.3 Limitations of the analysis	163
4.1.4 Future directions.....	165
4.2 Analysis of the roles of <i>Ankyrin2</i> and <i>Ankyrin1</i> in mushroom body development and long-term memory formation	166
4.2.1 <i>Ankyrin2</i> is broadly distributed within the adult brain	166
4.2.2 <i>Ankyrin2</i> is required for maturation of the mushroom body lobes and long-term memory formation.....	166
4.2.2.1 Future directions	168
4.2.3 <i>Ankyrin1</i> is distributed in the mushroom body and it is not dispensable for brain development and long-term memory formation.....	170
4.3 Investigating the interaction between <i>HDAC4</i> and <i>Ankyrin2</i>.....	171
4.3.1 <i>HDAC4</i> and <i>Ankyrin2</i> co-localise in the axons of the mushroom bodies as well as with Neuroglian suggesting a possible interaction among these factors	171
4.3.2 A pull-down assay suggests a physical interaction between <i>Ankyrin2</i> and <i>HDAC4</i> ankyrin repeat-binding domain	173
4.3.3 <i>HDAC4</i> and <i>Ankyrin2</i> may interact during long-term memory formation.	175
5 Summary and future perspectives.....	177
5.1 Overexpression of <i>HDAC4</i> in the whole fly head has minimal effect on global changes in gene expression.	178
5.2 A genetic screen for modifiers of the <i>HDAC4</i>-induced rough eye phenotype detected genes involved in transcription, SUMOylation and cytoskeletal organisation	179

5.3 Ankyrin2 is a cytoplasmic protein required for <i>Drosophila</i> mushroom body development and long-term memory formation in both developing and post-mitotic phases	180
5.4 A preliminary study on a putative interaction between Ankyrin2 and HDAC4	180
6 References	182
7 Appendices	218
7.1 Supplemental tables	219
7.2 Supplemental figures	229
7.2.1 Subcloning of DNA (Ankyrin1-MYC)	229

LIST OF FIGURES

Figure 1.1 Synaptic connectivity.....	4
Figure 1.2 Conserved molecular mechanisms of memory storage in <i>Aplysia</i> sensory neuron (A) and in mouse CA1 hippocampal neuron (B)	8
Figure 1.3 The mushroom body of <i>Drosophila melanogaster</i>	13
Figure 1.4 The GAL4/UAS binary system in <i>Drosophila</i>	16
Figure 1.5 TARGET system.	17
Figure 1.6 Aversive odour conditioning assay.....	19
Figure 1.7 Sequence of courtship behaviour steps undertaken by male fruit flies.	20
Figure 1.8 Conditioned courtship suppression assay.	22
Figure 1.9 Acetylation-deacetylation mechanism.....	24
Figure 1.10 Human HDAC family members.	27
Figure 1.11 HDAC4 translocation regulatory domains.	31
Figure 1.12 Domain organisation and alignment of <i>Drosophila</i> and human HDAC4 proteins.....	31
Figure 1.13 Domain structure of HDAC4 highlighting the ankyrin repeats binding domain	35
Figure 1.14 Schematic representation of the domains organisation of canonical ankyrins.	36
Figure 1.15 The axon initial segment.....	37
Figure 1.16 Schematic representation of ANK-G variants within the vertebrate nervous system.....	39
Figure 1.17 Ankyrin repeat region alignment between human ANK-G and <i>Drosophila</i> Ank2.....	41
Figure 2.1 Transgenic insertion mechanisms	59
Figure 2.2 Injection procedure to generate transgenic flies	61
Figure 3.1 The <i>elav-GAL4; tub-GAL80^{ts}</i> construct drives transgene expression in all neurons during adulthood.....	73
Figure 3.2 Representation of the genetic scheme to generate <i>HDAC4OE</i> and control flies for transcriptome analysis	74
Figure 3.3 Boxplots showing the FPKM distribution of the sample replicates	75
Figure 3.4 Wild-type eye of <i>Drosophila</i>	82

Figure 3.5 The GMR-GAL4/UAS system.	82
Figure 3.6 Cartoon showing the premise of the genetic screen for modifiers of the <i>HDAC4</i> -induced rough eye phenotype	84
Figure 3.7 Eye images showing the impact of different dose of <i>HDAC4</i> on the eye surface phenotype and the validation of the screen	86
Figure 3.8 Enhancers of the <i>HDAC4</i> -induced rough eye phenotype.	89
Figure 3.9 STRING analysis of the enhancers of the <i>HDAC4</i> -induced rough eye phenotype	93
Figure 3.10 <i>Ank2</i> is broadly expressed in the adult brain of <i>Drosophila</i>	97
Figure 3.11 <i>Ank2</i> co-localises with <i>Nrg</i> in the axons of the adult brain.	98
Figure 3.12 Colour-blind friendly version of Figure 3.11.	99
Figure 3.13 <i>Ank2</i> localises to axons in the brain.	100
Figure 3.14 <i>Ank2</i> does not localise in the dendritic regions of the <i>Drosophila</i> brain. .	101
Figure 3.15 <i>Ank2</i> localises to distinct nuclear compartment in the nuclei of the Kenyon cells.	102
Figure 3.16 <i>Ank2</i> does not localise in glial cells	103
Figure 3.17 <i>Ank2</i> knockdown phenotypes at 22°C.	105
Figure 3.18 <i>Ank2</i> knockdown phenotypes at 25°C.	107
Figure 3.19 <i>Ank2</i> knockdown phenotypes at 27°C.	109
Figure 3.20 <i>Elav-GAL4</i> pan neuronal knockdown of <i>Ank2</i> in the brain during development abolishes LTM formation.	112
Figure 3.21 <i>Elav-GAL4</i> pan-neuronal knockdown of <i>Ank2</i> throughout development has no impact on learning and immediate memory	113
Figure 3.22 Pan-neuronal knockdown of <i>Ank2</i> induced during adulthood impairs LTM formation.	115
Figure 3.23 Pan-neuronal knockdown of <i>Ank2</i> is not induced by the TARGET system during development.....	116
Figure 3.24 <i>OK107-GAL4</i> driver labelling profile.....	117
Figure 3.25 Decreased expression of <i>Ank2</i> in the mushroom body severely compromises LTM formation in adult flies.	118
Figure 3.26 <i>MB247-GAL4</i> driver expression profile.	119
Figure 3.27 RNAi-mediated decreased expression of <i>Ank2</i> in α/β and γ lobes negatively affects LTM formation in adult flies.....	119
Figure 3.28 <i>1471-GAL4</i> driver expression profile.	120

Figure 3.29 <i>Ank2</i> knockdown driven by <i>1471-GAL4</i> in the γ neurons does not affect LTM significantly.....	121
Figure 3.30 <i>NP1131-GAL4</i> driver expression profile.....	122
Figure 3.31 <i>NP1131-GAL4</i> ; <i>tub-GAL80ts</i> driven expression of <i>UAS-Ank2RNAi</i> affects LTM formation.....	122
Figure 3.32 <i>c739-GAL4</i> driver expression profile.	123
Figure 3.33 Knockdown of <i>Ank2</i> in α/β neurons does not affect LTM formation.	124
Figure 3.34 <i>c305a-GAL4</i> driver expression profile.	125
Figure 3.35 Knockdown of <i>Ank2</i> does not affect LTM formation in α'/β' neurons.....	125
Figure 3.36 <i>Ank1</i> is distributed in the mushroom body lobes and in the calyces.	127
Figure 3.37 <i>Ank1</i> knockdown phenotypes at 25°C.....	129
Figure 3.38 <i>Ank1</i> knockdown phenotypes at 27°C.....	130
Figure 3.39 Decreased expression of <i>Ank1</i> during developmental phases does not impair LTM formation.....	131
Figure 3.40 HDAC4 harbours an ankyrin-repeat-binding domain.	132
Figure 3.41 <i>Ank2</i> and HDAC4 are distributed in the same brain regions.	134
Figure 3.42 Schematic representation of Nrg structural domains.....	135
Figure 3.43 HDAC4 strongly co-localises with Nrg in the lobes of the mushroom body.	136
Figure 3.44 <i>HDAC4</i> overexpression phenotypes.	137
Figure 3.45 Knockdown of <i>Ank2</i> and overexpression of <i>HDAC4</i> have similar detrimental effects on mushroom body lobes development.	138
Figure 3.46 Illustration of the genetic mating scheme employed to generate the <i>UAS-Ank2RNAi</i> ; <i>UAS-HDAC4OE</i> fly line for epistasis studies	139
Figure 3.47 Combination of <i>Ank2</i> knockdown and <i>HDAC4</i> overexpression in the developing brain causes additive effects to the mushroom body lobe phenotype	141
Figure 3.48 PCR Confirmation of EGFP insert into the <i>Ank2-EGFP</i> line of <i>Drosophila</i>	143
Figure 3.49 Western blotting showing <i>Ank2-EGFP</i> band.	144
Figure 3.50 GST pull-down assay to investigate potential physical interaction between HDAC4 and <i>Ank2</i>	146
Figure 3.51 Putative role of <i>HDAC4</i> and <i>Ank2</i> in the regulation of 24 hours courtship memory.	149
Figure 4.1 Dendritic spines location and morphology	160

Figure 7.1 Physical map of the pUASTattB plasmid.....	229
Figure 7.2 Ank1-MYC DNA gels.....	230
Figure 7.3 Physical map of pUASTattB-Ank1-MYC vector.....	231
Figure 7.4 pGEX-2TK-HDAC4-GST DNA gels.....	231
Figure 7.5 Physical map of pGEX-2TK-HDAC4-GST vector	232
Figure 7.6 Protein gel showing IPTG induction of pGEX-2TK-HDAC4-GST	232
Figure 7.7 Standard curves from qPCR experiments.....	233
Figure 7.8 Assesment of RNAi knockdown via qPCR.....	234

LIST OF TABLES

Table 2.1 List of primary antibodies and respective dilutions used for immunohistochemistry	49
Table 2.2 List of secondary antibodies and dilutions used for immunohistochemistry ..	49
Table 2.3 Primers used for PCR and quantitative Real Time PCR experimental procedures	50
Table 2.4 Primers used to confirm the identity of the Ank2-EGFP line.....	52
Table 2.5 Primers used for sequencing of the Ankyrin1-MYC construct.....	52
Table 2.6 Primers used for sequencing of pGEX-2TK-HDAC4-GST	52
Table 2.7 Restriction endonucleases used for Ankyrin1-MYC subcloning.....	55
Table 2.8 Primers used for Ankyrin1-MYC subcloning.....	55
Table 2.9 Restriction endonucleases used for pGEX-2TK-HDAC4-GST subcloning...	57
Table 2.10 Primers used for amplification of HDAC4-GST.	57
Table 2.11 Primary antibodies and corresponding dilutions used for western blot analyses	62
Table 2.12 Secondary antibodies and corresponding dilutions used for western blot analyses.	63
Table 3.1 Reads alignment percentages.	76
Table 3.2 Genes whose transcripts are significantly altered in abundance by overexpression of <i>HDAC4</i>	79
Table 3.3 RNA expression levels in <i>Drosophila</i> head, eyes and brain of the genes transcriptionally regulated by <i>HDAC4</i>	80
Table 3.4 Genes excluded from further analysis after the rough eye phenotype screen.	91
Table 3.5 Conserved interactions detected by the <i>HDAC4</i> -induced rough eye phenotype	91
Table 3.6 Novel interactions detected via the <i>HDAC4</i> -induced rough eye phenotype screen.....	92
Table 7.1 <i>Drosophila melanogaster</i> GAL4-driver lines and control strains used in this study	219
Table 7.2 <i>Drosophila melanogaster</i> RNAi strains used in this study.....	224
Table 7.3 Genes that resulted in additive effects in the <i>HDAC4</i> -induced rough eye phenotype screen.	228

ABBREVIATIONS

°C	Degree Celsius
AIS	Axon initial segment
Ank1	Ankyrin1
Ank2	Ankyrin2
Ank3	Ankyrin3
ANK-B	Ankyrin B
ANK-G	Ankyrin G
ANK-R	Ankyrin R
Arc1	Activity-regulated cytoskeleton associated protein 1
Att	Arginine tolerance test
A β	Amyloid-beta
BDSC	Bloomington Drosophila Stock Centre
bp	Base pair
Ca ⁺⁺	Calcium
CaMK	Calcium/calmodulin-dependent kinase
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA
CI	Courtship index
CIP	Calf intestinal alkaline phosphatase
Cm	Centimeters
CRE	cAMP response element
CrebB	cAMP response element binding protein B
CS	Canton special
Cy	Curly
DNA	Deoxyribonucleic acid
DroID	Drosophila interactions database
dsRNA	Double stranded RNA
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
EGTA	Ethylene glycol tetraacetic acid
Elav	Embryonic lethal abnormal visual system

FasII	Fasciclin II
FLIM	Fluorescence lifetime imaging microscope
FPKM	Fragments per kilobase of transcript per million mapped
FRET	Fluorescence resonance energy transfer
GFP	Green fluorescent protein
GMR	Glass multimer reporter
GST	Glutathione S-transferase
H ⁺	Hydrogen
HAT	Histone acetyltransferase
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HDAC4	Histone deacetylase 4
HEK293	Human embryonic kidney 293 cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
INTACT	Isolation of nuclei tagged in specific cell types
IPTG	Isopropyl- β -D-thiogalactoside
K ⁺	Potassium
KCl	Potassium chloride
kDa	Kilodalton
L	Litre
LI	Learning index
LoxP	Locus of X-over P1
LTM	Long-term memory
M	Molar
mA	Milliampere
MAPK	Mitogen-activated protein kinase
Mef2	Myocyte enhancer factor 2
mg	Milligram
MgCl ₂	Magnesium chloride
MI	Memory index
ml	Millilitre
mm	Millimeters
mM	Millimolar
mRNA	Messenger RNA

Na ⁺	Sodium
NES	Nuclear export signal
ng	Nanogram
NLS	Nuclear localisation signal
nm	Nanometers
NMDARs	N-Methyl-D-Aspartic acid receptors
Nrg	Neuroglial
OE	Overexpression
PCR	Polymerase chain reaction
PKA	Protein kinase A
qPCR	Quantitative Real Time PCR
Repo	Reversed polarity
RFP	Red fluorescent protein
RNA	Ribonucleic acid
RNAi	RNA interference
RNAseq	RNA sequencing
Rpm	Revolution per minute
Sb	Stubble
SDS-PAGE	Sodium dodecyl sulphate – polyacrylamide gel electrophoresis
STM	Short-term memory
STRING	Search tool for the retrieval of interacting genes/proteins
SUMO	Small ubiquitin-like modifier
SV40	Simian virus 40
TARGET	Temporal and regional gene expression targeting
Ts	Temperature sensitive
UAS	Upstream activating sequence
Ubc9	Ubiquitin Carrier Protein 9
V	Volt
VDRC	Vienna Drosophila Resource Centre
Wt	Wild-type
µg	Microgram
µl	Microlitre
µm	Micrometer