



**ROLE OF ECLOSION HORMONE DURING ECDYSIS BEHAVIOR
IN *DROSOPHILA MELANOGASTER***

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TABLE OF CONTENTS

CHAPTER I	1
RESUMEN	2
ABSTRACT	4
INTRODUCTION	6
Figure 1: Activity pattern of ETH target neurons during <i>ex vivo</i> stimulation.....	10
REFERENCES	14
CHAPTER II	17
Role of the neuropeptide, Eclosion Hormone, during ecdysis behavior in <i>Drosophila melanogaster</i>	18
ABSTRACT	19
INTRODUCTION	20
RESULTS	23
CG10738 gene function is required for EH-induced ETH release from Inka cells.	23
Figure 1: Genetic lesions in CG10738 abolish EH-induced ETH release.....	24
CG10738-expressing cells are necessary for larval, pupal, and adult, ecdysis.	25
Figure 2: Ablating or inactivating EHR-expressing cells causes failures at larval, pupal, and adult ecdysis.	26
Mutations in EHR cause lethal phenotypes during ecdysis behavior.....	27
Figure 3: EHR mutant animals fail at the first larval ecdysis.....	28
EHR is expressed in Inka cells and in the CNS, as well as in other tissues during all developmental stages.	29
Figure 4: EHR is expressed in the Inka cells and the epithelial cells of the trachea as well in the leg imaginal disc of the third instar larva.....	30

Figure 5: EHR expression in the larval CNS.....	31
Figure 6: EHR expression in the pharate adult <i>Drosophila</i> CNS.	32
Downregulating EHR in specific subsets of EHR-expressing cells causes lethal phenotypes.....	33
Table 1: Percentage of ecdysial failure caused by downregulation of EHR in specific subsets of cells.	35
Table 2: Percentage of ecdysial failure caused by downregulation of ETHR in specific subsets of cells.	36
Table 3: Percentage of ecdysial failure caused by downregulation of both EHR and ETHR in specific subsets of cells.	36
EHR-expressing neurons show a complex pattern of activity during fictive pupal ecdysis.	37
Figure 7: Time course of activation of EHR neurons in response to ETH, stimulation <i>ex vivo</i>	38
Search for subsets of EHR-expressing cells that are sufficient to rescue the ecdysis behavior in EHR-mutants animals.....	39
Table 4: Success of genetic rescue of EHR lethal phenotype by expressing EHR in specific subsets of.	39
DISCUSSION	40
MATERIALS AND METHODS	43
Fly strains.....	43
UAS-EHR generation	43
EH synthesis	43
Stimulation with synthetic EH.....	44
Immunohistochemistry	44
Quantification of larval ecdysis behavior.....	45
Quantification of pupal ecdysis and adult eclosion	46
RNAi screening	46
Real-time GCaMP Imaging	46
Video analysis	47

Statistics.....	47
SUPPLEMENTARY FIGURES	48
Supplementary Figure 1: Location of the insertions within the HER gene used here.	48
Supplementary Figure 2: Activation of all EHR-expressing cells using <i>TrpA1</i> caused failures at pupal ecdysis and at adult eclosion.....	49
Supplementary figure 3: EHR is broadly expressed across developmental stages...50	
SUPPLEMENTARY TABLES	51
Table S1: List of all the genotypes and their source.....	51
Table S2: EHR RNAi screening	53
List of RNAi lines against EHR screened using tubulin-GAL4.	53
Table S3: Statistic analysis.....	54
ACKNOWLEDGMENTS	57
REFERENCES	57
APPENDIX.....	61
The gut microbiome influences memory and sleep in <i>Drosophila</i>.....	62
SUMMARY STATEMENT	63
ABSTRACT.....	63
INTRODUCTION	64
MATERIALS AND METHODS	66
Fly rearing and stocks	66
Generation of axenic flies.....	66
Olfactory learning and memory experiments	67
Anxiety-like behavior	69
Locomotor activity levels	70
Sleep, sleep deprivation and recovery	70
Circadian rhythmicity of locomotor activity	70
Statistical analyses.....	71

RESULTS.....	72
Learning and memory	72
Figure 1. Effect of the microbiome on learning and memory	73
Anxiety-like behavior	74
Figure 2. Effect of the microbiome on anxiety-like behavior	74
Circadian rhythmicity of locomotor activity	75
Figure 4. Effect of the microbiome on the circadian rhythm of adult locomotor activity	77
Sleep behavior	78
Figure 5. Effect of the microbiome on sleep and sleep consolidation	80
Figure 6. Effect of the microbiome on sleep recovery.....	81
DISCUSSION	82
ACKNOWLEDGMENTS	85
COMPETING INTERESTS.....	85
AUTHOR CONTRIBUTIONS.....	86
FUNDING	86
DATA AVAILABILITY	86
SUPPLEMENTARY FIGURES AND TABLES.....	87
Figure S1. Comparison of locomotor activity in isogenic sub-lines A and B.	87
Figure S2. Comparison of sleep behavior in isogenic sub-lines A and B.....	88
Figure S3. Profiles of locomotor activity of axenic and conventional flies.....	89
Figure S4. Effect of the microbiome on the daily distribution of locomotor activity ..	90
Figure S5. Sleep amount during sleep deprivation night, baseline and the recovery day	92
Table S1. Results of all statistical analyses performed	93
REFERENCES	94
Orcokinin neuropeptides regulate reproduction in the fruit fly, <i>Drosophila melanogaster</i>	98
RUNNING TITLE:	98

ABSTRACT.....	99
KEY WORDS	99
INTRODUCTION	100
MATERIALS AND METHODS	101
Fly strains and genetics	101
qPCR.....	102
Pupal ecdysis	102
Courtship behavior	103
Locomotor activity rhythms and levels	103
Sleep	104
Oviposition	104
Histochemistry.....	105
Statistics.....	105
RESULTS.....	106
Validation of GAL4 drivers and UAS-RNAi lines.	106
Figure 1. Knockdown efficiency of R RNAi transgenes	107
Pupal ecdysis.....	108
Figure 2. Effect of ok knockdown on the duration of ecdysis behaviors	108
Locomotor activity and sleep.....	109
Figure 3. Effect of OK knockdown on the daily pattern of locomotor activity	110
Figure 4. Circadian rhythmicity of locomotor activity was not affected by ok knockdown	111
Figure 5: Sleep behavior after ok knockdown.....	113
Figure 6: Courtship behavior of males towards females after ok knockdown.....	115
Figure 7: Courtship behavior of a male towards another male was disinhibited by ok knockdown without affecting sexual preference	116
Oviposition.....	117
Figure 8: Effect of ok knockdown on the number of eggs laid by mated females....	118

DISCUSSION	119
DECLARATION OF INTEREST	122
AUTHOR CONTRIBUTIONS	122
ACKNOWLEDGEMENTS	122
SUPPLEMENTARY FIGURES	123
Supplementary Figure 1: Orcokinin pattern of expression.....	123
Supplementary Figure 2: Effect of ok knockdown on locomotor activity speed.	124
Supplementary Figure 3: Effect of ok knockdown on sleep	126
Supplementary Figure 4: Courtship behavior of male towards female after ok knockdown using ok2-GAL4 driver	127
REFERENCES	128

LIST OF TABLES

CHAPTER I

Table 1: Percentage of ecdysial failure caused by downregulation of EHR in specific subsets of cells.....	35
Table 2: Percentage of ecdysial failure caused by downregulation of ETHR in specific subsets of cells.....	36
Table 3: Percentage of ecdysial failure caused by downregulation of both EHR and ETHR in specific subsets of cells.	36
Table 4: Success of genetic rescue of EHR lethal phenotype by expressing EHR in specific subsets of.....	39
Table S1: List of all the genotypes and their source.	51
Table S2: EHR RNAi screening.	53
List of RNAi lines against EHR screened using tubulin-GAL4.	53
Table S3: Statistic analysis	54

APPENDIX

Scientific paper 1

Table S1. Results of all statistical analyses performed	93
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LIST OF FIGURES

CHAPTER I

Figure 1: Activity pattern of ETH target neurons during <i>ex vivo</i> stimulation.....	10
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CHAPTER II

Figure 1: Genetic lesions in CG10738 abolish EH-induced ETH release.....	24
Figure 2: Ablating or inactivating EHR-expressing cells causes failures at larval, pupal, and adult ecdysis.....	26
Figure 3: EHR mutant animals fail at the first larval ecdysis.....	28
Figure 4: EHR is expressed in the Inka cells and the epithelial cells of the trachea as well in the leg imaginal disc of the third instar larva.....	30
Figure 5: EHR expression in the larval CNS.....	31
Figure 6: EHR expression in the pharate adult <i>Drosophila</i> CNS.....	32
Figure 7: Time course of activation of EHR neurons in response to ETH, stimulation <i>ex vivo</i>	38
Supplementary Figure 1: Location of the insertions within the HER gene used here.....	48
Supplementary Figure 2: Activation of all EHR-expressing cells using <i>TrpA1</i> caused failures at pupal ecdysis and at adult eclosion.....	49
Supplementary figure 3: EHR is broadly expressed across developmental stages.....	50

APPENDIX

Scientific paper 1

Figure 1. Effect of the microbiome on learning and memory	73
Figure 2. Effect of the microbiome on anxiety-like behavior	74
Figure 4. Effect of the microbiome on the circadian rhythm of adult locomotor activity	77
Figure 5. Effect of the microbiome on sleep and sleep consolidation	80
Figure 6. Effect of the microbiome on sleep recovery.....	81
Figure S1. Comparison of locomotor activity in isogenic sub-lines A and B.	87
Figure S2. Comparison of sleep behavior in isogenic sub-lines A and B.....	88
Figure S3. Profiles of locomotor activity of axenic and conventional flies.....	89
Figure S4. Effect of the microbiome on the daily distribution of locomotor activity	90
Figure S5. Sleep amount during sleep deprivation night, baseline and the recovery day	92

Scientific paper 2

Figure 1. Knockdown efficiency of R RNAi transgenes	107
Figure 2. Effect of ok knockdown on the duration of ecdysis behaviors	108
Figure 3. Effect of OK knockdown on the daily pattern of locomotor activity	110
Figure 4. Circadian rhythmicity of locomotor activity was not affected by ok knockdown.....	111
Figure 5: Sleep behavior after ok knockdown.....	113
Figure 6: Courtship behavior of males towards females after ok knockdown.....	115
Figure 7: Courtship behavior of a male towards another male was disinhibited by ok knockdown without affecting sexual preference	116
Figure 8: Effect of ok knockdown on the number of eggs laid by mated females.....	118
Supplementary Figure 1: Orcokinin pattern of expression.....	123
Supplementary Figure 2: Effect of ok knockdown on locomotor activity speed.	124
Supplementary Figure 3: Effect of ok knockdown on sleep	126
Supplementary Figure 4: Courtship behavior of male towards female after ok knockdown using ok2-GAL4 driver.....	127

Los insectos son el grupo más exitoso y diverso del reino animal. Una característica que podría contribuir a su éxito es su exoesqueleto (también conocido como cutícula), que los protege y evita su desecación. Sin embargo, su estructura rígida limita su crecimiento y, para crecer, el insecto debe mudar periódicamente. Durante este proceso, el insecto secreta una nueva cutícula y desecha la del estadio anterior mediante el comportamiento innato y vital llamado **ecdisis**. Este comportamiento está controlado a través de la acción secuencial y compleja de varias hormonas y neuropéptidos, incluyendo la *Ecdysis Triggering Hormone* (ETH) y la *Eclosion Hormone* (EH), que actúan sobre sistema nervioso central (CNS).

En este estudio, nos centramos en el papel de EH en la ecdisis, identificando los blancos de EH y determinando su función en el control de la ecdisis. Se cree que el receptor de EH (EHR) está codificado por el gen CG10738 de la mosca del vinagre, *Drosophila*. Aquí mostramos que las células epitraqueales (que expresan ETH y son blancos directos de EH) también expresan CG10738. Además, los mutantes de CG10738 no liberan ETH en respuesta a estímulo *in vitro* con EH sintético, lo que demuestra que este gen codifica para el receptor de EH.

Utilizando inmunocitoquímica en combinación con EHR-GAL4 y los *hemidriviers* "split"-GAL4, encontramos que EHR y ETHR (receptor de ETH) se co-expresan en neuronas que también expresan los neuropéptidos EH, CCAP (péptido cardíaco crustáceo), kinina, FMRFamidas, MIPs (péptidos mioinhibidores) y bursicon, que se sabe que participan en el control de la ecdisis. Además, EHR también se expresa en motoneuronas y en muchas otras neuronas cuya identidad y función en la ecdisis son desconocidas.

Para elucidar el papel de las células que expresan EHR, analizamos las consecuencias de su ablación o silenciamiento en diferentes etapas de desarrollo utilizando UAS-*reaper* y UAS-*2xKir2.1*, respectivamente. Encontramos que ambas manipulaciones provocaron fallos en la ecdisis larval, similares al fenotipo de los mutantes de *Eh* y *ehr*. También observamos fallos en la ecdisis en la etapa de pupa y en la eclosión. Curiosamente, la activación de todas las células EHR en la eclosión utilizando UAS-*TrpA1* también provocó fallos en la ecdisis, quedando las moscas atrapadas dentro del pupario. Luego, interrumpimos la expresión del gen CG10738 en subconjuntos de células que expresan EHR utilizando un RNAi específico y encontramos que la expresión de EHR en células que también expresan la proteína *dimmed*, *sinaptobrevina* neuronal, *breathless*, *eth* o *amontillado* es necesaria para el éxito del comportamiento de la ecdisis las diferentes etapas. Además observamos que algunas neuronas sensoriales requerían la expresión de EHR exclusivamente para la ecdisis pupal pero no así para otras ecdisis.

Finalmente, evaluamos el patrón de actividad neuronal de los blancos de EH utilizando el sensor de calcio GCaMP. Al estimular exógenamente la ecdisis en preparaciones *ex vivo* del sistema nervioso central utilizando ETH sintético, pudimos observar que, aunque todas las neuronas fueron expuestas a este neuropéptido simultáneamente, diferentes subgrupos de células que expresan EHR respondieron en momentos diferentes con patrones de actividad específicos, lo que demuestra la complejidad de este comportamiento.

Estos resultados sugieren en conjunto que las células blanco de EH son necesarias y desempeñan un rol crítico durante este complejo comportamiento de la ecdisis en *Drosophila*. Además, esta investigación contribuye a la comprensión de cómo los neuropéptidos actúan y controlan los comportamientos animales.