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Genes Involved in Long-Term Memory Are Expressed in Testis of Cryptorchid Boys and Respond to GnRHa Treatment

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Abstract
It has been known for many years that boys with unilateral or bilateral undescended testis (cryptorchidism) tend to have a low IQ, and those who belong to the high infertility risk (HIR) group perform less well at school than low infertility risk (LIR) patients. However, the molecular biological processes underlying this phenomenon are not understood. In this study, we report the outcome of testicular RNA profiling for genes involved in long-term memory formation. We analyzed the histology and the transcriptome of testicular biopsies from bilateral HIR cryptorchid boys, comparing those who received GnRHa treatment for 6 months after the first surgery with those who did not receive GnRHa before the second surgery. We found that GnRHa treatment alters the testicular mRNA levels of neuronal genes that are involved in long-term memory and testosterone synthesis. These data highlight a possible molecular link between cryptorchidism, impaired mini-puberty, and diminished cognitive functions. Our results are consistent with the hypothesis that hypogonadotropic hypogonadism in cryptorchid boys with altered mini-puberty may affect neuronal genes important for memory and learning, which could help explaining the negative correlation between cryptorchidism and intellectual abilities.

During mini-puberty, which occurs at 30 to 90 days after birth, increased levels of luteinizing hormone (LH) and testosterone induce the transition of gonocytes into Ad (dark) spermatogonia that are stem cells for sperm development [Hadziselimovic et al., 2005, 2016]. Ad spermatogonia are a key marker of potential fertility, since in the testis of cryptorchid boys who belong to the high infertility risk (HIR) group, the transition of gonocytes into Ad spermatogonia is lacking due to impaired LH and testosterone secretion [Hadziselimovic and Hoecht, 2008]. Recently, strong evidence was postulated that LH deficiency originates from impaired *PROK2* gene

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expression controlled through the master regulatory genes *EGR4* and *PITIX1* [Hadziselimovic et al., 2016]. Among patients who had orchidopexy during childhood, sperm counts were normal in 94% of the cases whose prepubertal testis contained Ad spermatogonia (low infertility risk, LIR), while an abnormally low sperm count was found in 92% of men who lacked Ad spermatogonia at the time of orchidopexy [Hadziselimovic and Hoecht, 2008]. Noticeably, treatment during childhood of HIR patients with the GnRH antagonist Buserelin substantially increased their fertility during adult life [Hadziselimovic, 2008].

It was reported that cryptorchid boys tend to have a low IQ as determined by the Stanford-Binet intelligence test [Depue, 1988]. The caveat of this study is that developmental syndromes were not excluded, and the limitations of the IQ test concerning psychomotoric skills were not appropriately taken into account. However, in a prospective study of 65 cryptorchid men using 3-dimensional analysis of variance (ALLOC), a significantly poorer school performance and lower education levels were found among infertile cryptorchid men as compared to fertile cryptorchid patients [Hadziselimovic and Herzog, 1990]. Furthermore, cryptorchid boys show lower testicular mRNA levels for *EGR4, FMR2,* and *VCX3A*, which are involved in signaling pathways that regulate cytoskeletal organization, synaptic vesicle transport, and the establishment of connections between neuronal cells. These reduced gene expression patterns may reflect an altered expression in brain cells that could contribute to the impaired intellectual and cognitive functioning in infertile cryptorchid males [Hadziselimovic et al., 2014]. It is not understood, however, to what extent GnRHa treatment influences the expression of genes involved in memory and learning.

RASGRF1 is a guanine nucleotide exchange factor that activates Ras or Rho GTPases and is highly expressed in the brain and strongly in the testis. *RASGRF1* is paternally expressed and imprinted and shows a more limited pattern of expression across tissues than *RASGRF2*. *RasGrf1* mutant mice have a normal short-term memory but display a deficient long-term memory [Giese et al., 2001; Silingardi et al., 2011].

We carried out RNA sequencing analyses of testicular biopsies from HIR patients who received a 6-month GnRHa treatment and compared them to those who were untreated before the second orchidopexy. We found that GnRHa alters testicular mRNA concentrations for several genes that are involved in long-term memory formation including *RASGRF1*, which is expressed at a low level in testis from HIR patients and upregulated by GnRHa treatment. This is the first indication that GnRHa, which was initially used to prevent infertility in HIR patients, also influences neuronal genes. This may have implications about altered neuronal gene expression in treated HIR patients due to possible positive effects of GnRHa on cognitive functions.

Materials and Methods

Patient Cohort and Biopsy Sample Collection

We selected 15 patients with isolated cryptorchidism based on histological results and divided them into 2 groups. Seven belonged to the HIR and 8 to the LIR group. Data from HIR bilateral cryptorchid boys treated either with GnRHa following the first orchidopexy (4 patients) or with surgery alone (3 patients) were retrieved from an ongoing randomized study. Initial biopsies revealed no Ad spermatogonia, indicating a defective mini-puberty (HIR group). Patients were age and ethnicity matched. The second testis was managed by orchidopexy and biopsied 6 months after the initial surgery. Thus, results from 22 biopsies were compared. Over a period of 6 months, 10 μg GnRHa (Buserelin) was administered as a nasal spray in the evening of every second day. The patients had a median age of 18.5 months (range 8–59 months).

A cryptorchid testis is defined as a testis localized outside of the scrotum and incapable of being brought into a stable scrotal position. All undescended testes in this study were located in the inguinal region. The cryptorchid boys entering the study underwent an extensive examination with no clinical signs of developmental malformations or syndromes.

Performing clinical examination in accordance with STROBE criteria for case-controlled studies, we excluded patients with small testes, a small penis, or a lack of normal scrotal rugae, pigmentation and gynecomastia [von Elm et al., 2014]. The testicular biopsy samples were split with 1 fragment fixed in glutaraldehyde for histological processing and the other immediately immersed in RNAlater and stored at –25°C until further processing.

RNA-profiling analyses of human male gonads are challenging, because they contain many somatic- and germ cell types. It is, however, possible to compare normal and pathological testis to extract meaningful information about mRNA levels associated with specific cell populations [Hadziselimovic et al., 2009, 2016; Chalmel et al., 2012]. In this study, samples from HIR patients before and after GnRHa treatment were analyzed and compared to the samples from untreated LIR patients (controls). All samples from the HIR patients belong to a randomized study, which means that their distribution into groups of treated and untreated patients was not influenced by any parameter other than undescended testes that were operated. The sample size is small but sufficient for our pilot study that is meant to pave the way for further work with larger cohorts.

Histological Analyses

Biopsies were fixed in 3% glutaraldehyde in PBS and then embedded in Epon resin. Semi-thin sections (1 μm) were cut using a Reichert Om-U3 ultramicrotome. Sections were mounted on glass slides, stained with 1% toluidine blue, and examined under a light microscope at a total magnification of ×600. Biopsies were histo-

Table 1. The strongest signals of the antibody-based profiling of the tissue-specific long-term memory proteins and mRNAs from normal tissue

Gene ID	Name	Highest RNA expression[a]	Protein highly enriched[b]	Reference
RASGRF1	Ras-protein specific nucleotide releasing factor 1	brain/testis	brain/testis	Giese et al., [2001]; Faisal et al. [2014]
EGR2	early growth response 2	endocrine tissue/testis	–	DeSteno and Schmauss [2008]
BDNF	brain derived neutrotrophic factor	brain/testis	brain/testis	Roberts et al. [2006]
GABRA4	gamma-amino butyric acid A receptor alpha4 subunit	ubiquitary/brain/testis	brain	Smith [2013]
GHSR	growth hormone secretagogue receptor	brain	brain	Davis et al. [2011]
PDYN	prodynorphin	brain/testis	brain/testis	Bilkei-Gorzo et al. [2012]
GRIN2A	glutamate receptor inotropic N-methyl D-aspartate 2A	ubiquitary/brain/testis	ubiquitary	Bharadwaj et al. [2014]
GRIN2B	glutamate receptor inotropic N-methyl D-aspartate 2B	brain/testis	brain/testis	Bharadwaj et al. [2014]
EGR3	early growth response 3	ubiquitary/brain/testis	ubiquitary/GI	Li et al. [2007]
ACTL6B	actin like 6B	brain/testis	brain	Vogel-Ciernia and Wood [2014]
HDAC1	histone deacetylase 1	ubiquitary	ubiquitary	Song et al. [2016]
HDAC2	histone deacetylase 2	ubiquitary	ubiquitary	Guan et al. [2009]
HDAC3	histone deacetylase 3	ubiquitary	brain/testis	McQuown et al. [2011]
HAT1	histone acetyltransferase	ubiquitary	ubiquitary	Peleg et al. [2010]
BAIAP2	Bai1-associated protein 2	ubiquitary/brain/testis	ubiquitary	Luksys et al. [2014]
EGR4	early growth response 4	brain/testis	brain/testis	Li et al. [2005]
SATB2	special AT-rich sequence binding protein homeobox 2	brain/GI/testis	brain/GI	Jaitner et al. [2016]
MSI1	Musashi RNA binding protein 1	brain/testis	brain	Hadziselimovic et al. [2014]

Expression data were extrapolated from the Human Gene Database (GeneCards)[Stelzer et al., 2016], the Human Protein Atlas and for each gene analyzed [Uhlén et al., 2015], and Genevestigator [Hruz et al., 2008].

Noticeably, in human and mice, the highest similarity in gene expression profiles is between brain and testes [Guo et al., 2003]. GI, gastrointestinal. [a] (100xFPKM)1/2; [b] log10(ppm).

logically examined by the same pathologists with expertise in the interpretation of semi-thin sections of prepubertal testes. During histological analyses, at least 100 tubular cross sections per biopsy were evaluated with regard to their number and absence of dark-type (Ad) spermatogonia.

RNA Preparation, Sequencing, Data Analyses, and RNA Expression Levels

Library preparation was performed from 300 ng total RNA using the TruSeq Stranded Total RNA Kit with Ribo-Zero Gold (Cat# RS-122–2301, Illumina, San Diego, CA, USA). The workflow from RNA isolation, purification, library preparation, sequencing, and data analysis was described previously in a HIR patient RNA profiling study [Hadziselimovic et al., 2017].

Expression Data Analysis

To determine genes differentially expressed before and after GnRHa treatment, the counts per gene samples from patients treated with GnRHa were statistically analyzed in R using the edgeR package [Robinson et al., 2010] by fitting a quasi-likelihood negative binomial generalized log-linear model for each gene [Lund et al., 2012]. The model was designed with 2 additive categorical factors: (1) patient, which identifies measurements from the same patient, before and after GnRHa treatment, and controls for gene expression differences between individuals, and (2) treatment, which models gene expression changes induced by GnRHa. Only genes with at least 1 read per million in at least 2 samples were included. *p* values and fold-changes were calculated for the treatment factor, and differentially expressed genes were defined as those displaying a false discovery rate (FDR) of <0.05 and an absolute change in expression of at least 2-fold. FDR-controlling procedures are designed to estimate the expected proportion of "discoveries" (rejected null hypotheses) that are false (incorrect rejections). This is one way of conceptualizing the rate of type I errors in null hypothesis testing when conducting multiple comparisons. FDR-controlling procedures provide a less stringent control of Type I errors compared to familywise error rate (FWER)-controlling procedures (such as the Bonferroni correction), which control

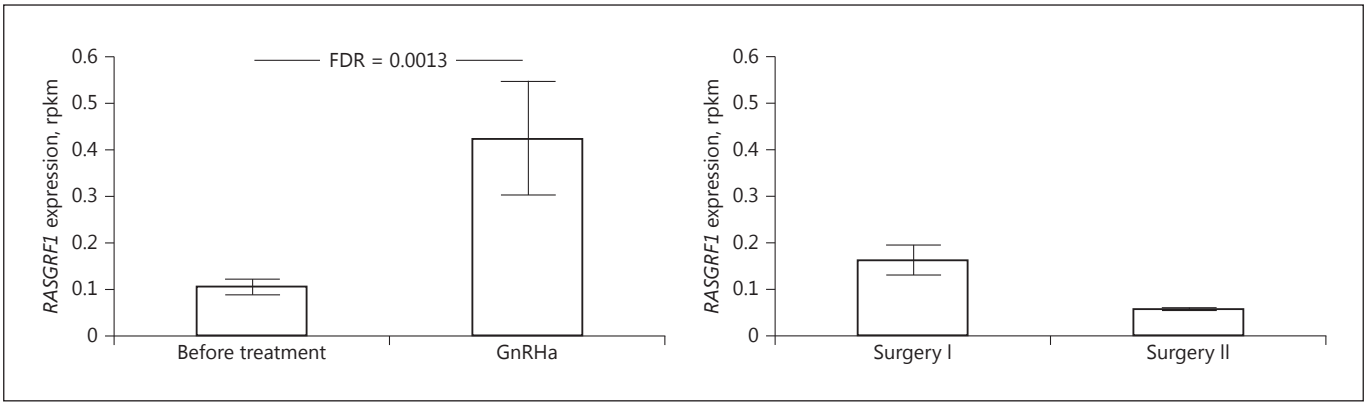

Fig. 1. RNA sequencing data for *RASGRF1* expression signals for samples before and after GnRHa treatment and for samples taken from untreated patients at time points corresponding to the treated samples (surgery I = before, surgery II = after). The standard deviation is given, and the FDR is shown at the top.

the probability of at least 1 Type I error. Thus, FDR-controlling procedures have greater power at the cost of increased rates of Type I errors. Cut-off was not applied to raw *p* values, but significant values of <0.05 were corrected for multiple testing, namely FDR was calculated after the Benjamini and Hochberg [1995] approach.

Results

Among 28,645 genes for which RNA was detected, 6,469 significantly differed in pre- and post-treatment concentrations (FDR <0.05; absolute signal change ≥2-fold). Approximately 90% (5,823/6,469) showed increased expression following GnRHa treatment [Hadziselimovic et al., 2017]. In the present analysis, we re-interpreted the data with emphasis on selected genes known to be relevant for learning and memory or directly implicated in memory formation (Table 1). We first verified that the target genes are expressed both in testis and brain using information provided by the Human Protein Atlas [Uhlen et al., 2015], Genevestigator [Hruz et al., 2008], and GeneCards [Stelzer et al., 2016] (Table 1). Next, we grouped the genes into 4 different response patterns.

Lower Expression in HIR before Treatment and Positive Response to GnRHa

RASGRF1 showed a 2-fold lower expression in the HIR group prior to treatment and a 1.5-fold induction in response to GnRHa (log transformed data). The gene is expressed in testis and shows significant induction upon GnRHa treatment, while no positive effect is detectable in "surgery only" patients (Fig. 1). *RASGRF1* plays a key role in regulating the RAS signaling pathway and is important for long-term memory formation (Tables 1, 2) [Giese et al., 2001; Faisal et al., 2014].

We also found that *EGR2* is 2-fold downregulated in HIR patients and 1.23-fold upregulated after GnRHa treatment. The expression of individual members of the EGR family is differentially induced during different cognitive processes [DeSteno and Schmauss, 2008]. *EGR2* recognizes EGR response elements in promotor regions of a number of neuronal genes, which are likely to play a role in mediating plastic changes that occur in response to cognitive demand [DeSteno and Schmauss et al., 2008].

Equal Expression between HIR and LIR and Positive Response to GnRHa

The following genes were equally expressed in the HIR and LIR groups before treatment and showed a positive response to GnRHa treatment: *ACTL6B*, *BDNF*, *GABRA4*, *GHSR* [Davis et al., 2011], *GRIN2A*, *GRIN2B*, *PDYN* [Bilkei-Gorzo et al., 2012], and *EGR3* (Tables 1, 2). The brain-derived neurotrophic factor *(BDNF)* gene has a central regulating role in modulating learning and memory function and is also responsible for *RASGRF1* regulation [Roberts et al., 2006]. Human studies support the concept that polymorphisms and mutations in loop-bound DNA can influence working memory and *GRIN2B* expression [Bharadwaj et al., 2014]. The *ACTL6B* gene encodes a subunit of the BRG1/brm-associated factor (BAF) complex in mammals. Mice harboring certain genetic manipulations of *BAF53b* exhibit severe long-term memory defects and long-lasting forms

Table 2. GnRHa treatment affects genes important for long-term memory formation

Gene ID	HIR median (MAD)	LIR median (MAD)	$logFC^{HIR/LIR}$	$FDR^{HIR/LIR}$	Before treatment median (MAD)	GnRHa-treated median (MAD)	$logFC^{GnRHa}$	FDR^{GnRHa}
RASGRF1	0.06 (0.01)	0.14 (0.05)	–1.09	0.01	0.10 (0.03)	0.42 (0.24)	**+1.55**	1.3E-03
EGR2	0.34 (0.07)	0.84 (0.17)	–1.18	1.3E-03	0.38 (0.15)	1.61 (0.56)	**+1.23**	2.2E-03
BDNF	0.18 (0.03)	0.22 (0.05)	ns	ns	0.22 (0.02)	0.98 (0.56)	**+1.71**	1.7E-05
GABRA4	0.19 (0.08)	0.19 (0.08)	ns	ns	0.20 (0.1)	1.25 (0.65)	**+2.34**	1.4E-08
GHSR	0.08 (0.01)	0.09 (0.06)	nd	nd	0.07 (0.02)	0.47 (0.36)	**+2.56**	4.7E-03
PDYN	0.02 (0.03)	0.08 (0.03)	ns	ns	0.08 (0.02)	0.53 (0.24)	**+2.39**	2.6E-03
GRIN2A	0.18 (0.06)	0.28 (0.09)	ns	ns	0.22 (0.03)	0.88 (0.4)	**+1.45**	5.0E-06
GRIN2B	0.16 (0.05)	0.17 (0.07)	ns	ns	0.15 (0.03)	1.00 (0.37)	**+1.88**	7.7E-05
EGR3	0.21 (0.11)	0.35 (0.08)	ns	ns	0.23 (0.04)	0.95 (0.22)	**+1.60**	3.5E-04
ACTL6B	0.05 (0.04)	0.22 (0.06)	nd	nd	0.18 (0.06)	0.97 (0.39)	**+1.50**	0.04
HDAC1	18.47 (1.03)	17.51 (0.73)	ns	ns	18.59 (1.28)	14.91 (1.22)	–0.81	8.6E-04
HDAC2	10.77 (0.98)	13.58 (2.05)	ns	ns	12.27 (1.18)	11.83 (1.11)	–0.67	4.4E-03
HDAC3	17.42 (1.02)	17.87 (0.62)	ns	ns	18.41 (0.74)	18.71 (0.79)	–0.45	0.05
HAT1	10.66 (0.97)	13.91 (1.81)	ns	ns	12.62 (1.88)	8.44 (1.10)	–1.05	6.2E-05
BAIAP2	1.39 (0.11)	2.69 (0.65)	–0.99	4.7E-04	1.52 (0.78)	2.37 (0.88)	nd	nd
EGR4	0.07 (0.05)	0.84 (0.50)	–3.35	3.2E-04	0.18 (0.61)	0.31 (0.50)	nd	nd
SATB2	0.84 (0.17)	1.91 (0.76)	–1.37	1.7E-03	1.00 (0.56)	2.00 (0.67)	nd	nd
MSI1	0.12 (0.08)	0.43 (0.14)	–1.54	9.9E-03	0.27 (0.23)	0.54 (0.16)	nd	nd

Gene expression differences between the high (HIR) and low infertility risk (LIR) groups are presented as logFC and false discovery rate (FDR), and the median values are given. Values in bold are genes that positively responded to GnRHa. MAD, median absolute deviation; ns, not significant; nd, not determined.

of hippocampal synaptic plasticity. Among *BAF53b* mutant mice, memory impairments can be rescued by reintroducing BAF53b in the adult hippocampus, suggesting that BAF53b plays roles beyond neuronal development [Vogel-Ciernia and Wood, 2014]. The *EGR3* gene plays essential roles in learning and memory processing, which appear to be partly distinct from the actions of *EGR1* [Li et al., 2007]. Both *EGR3* and *GABRA4* genes are involved in memory formation [Roberts et al., 2006; Smith, 2013].

Lower Expression in HIR and No Response to GnRHa

Four genes that are important for long-term memory formation – *BAIAP2*, *EGR4*, *MSI1*, and *SATB2* – showed lower pre-treatment expression in the HIR group compared to the LIR group, which is why we included them in our analysis. However, no significant response to GnRHa treatment was detected.

Signaling by *SATB2* and *EGR4* is involved in long-term memory formation. Based on the highly specific expression pattern of *SATB2* in the adult brain and the severe learning disabilities and mental retardation observed in patients diagnosed with SAS (SATB2-associated syndrome), it was hypothesized that *SATB2* is critical for human learning and memory [Jaitner et al., 2016]. Interestingly, while *SATB2* showed no post-treatment response, its lncRNA, *SATB2* antisense, did respond to GnRHa treatment (+1.78 logFC, FDR 0.019). At the molecular level, *BDNF* upregulates *SATB2*, which itself binds to promoters of coding and noncoding genes [Jaitner et al., 2016]. It was recently shown that the *MSI* (Musashi) gene induces forgetting and represents a novel mechanism of memory decay by linking translational control to the structure of the neuronal actin cytoskeleton [Hadziselimovic et al., 2014].

Equal Expression in HIR and LIR and Negative Response to GnRHa

The expression of *HAT1*, *HDAC1*, *HDAC2*, and *HDAC3* did not differ between the HIR and LIR groups, but all genes were downregulated following GnRHa treatment (Tables 1, 2). Altered histone acetylation is associated with age-dependent memory impairment in mice [Peleg et al., 2010]. Moreover, *HDAC2* and *HDAC3* are reportedly critical negative regulators of learning memory formation [Guan et al., 2009; McQuown et al., 2011]. Interestingly, *HDAC1* inhibition induces increased *RASGRF1* gene expression [Song et al., 2016].

Discussion

In this study, we interpreted RNA-profiling data from testis of operated cryptorchid patients that were treated with GnRHa as compared to cryptorchid boys that did not receive a treatment. We manually inspected a list of genes that are differentially expressed in testis between HIR and LIR patients and/or differentially expressed after GnRHa treatment with emphasis on selected genes known to be involved in cognitive functions.

The regulation of chromatin structure through post-translational modification of histone proteins – primarily histone H3 phosphorylation and acetylation, as well as methylation – is a basic early step in the induction of synaptic plasticity and the formation of long-term memory [Gupta et al., 2010]. Thus, the observed negative post-treatment responses of *HDAC2* and *HDAC3* may imply an additional mechanism towards stabilization of long-term memory formation.

GnRHa treatment induced a positive response in brain-derived neurotrophic factor (*BDNF*), which plays an important role in the function and maintenance of several neuronal systems and stimulates *RASGRF1* gene expression. *BDNF* mediates *EGR3*-induced *GABRA4* regulation in developing neurons [Roberts et al., 2006; Smith, 2013]. Both *EGR3* and *GABRA4* are involved in memory formation [Roberts et al., 2006; Smith, 2013]. Interestingly, both glutamate receptors *GRIN2A* and *GRIN2B* also positively responded to GnRHa treatment. Changes in *GRIN2B* expression profoundly affect memory and cognition [Bharadwaj et al., 2014]. It is puzzling why we detected no GnRH-dependent stimulation of *EGR1* and *EGR4* expression. It is possible that these genes, which can potentially be epigenetically downregulated, were not responsive to the administered GnRHa treatment regimen.

In healthy men, testosterone enhances memory by increasing the biological salience of incoming information [Ackermann et al., 2012]. GnRHa induces positive gene responses among several genes that regulate testosterone synthesis, including protein-coding genes that are involved in pituitary and hypothalamic-pituitary-gonadal axis development. Treatment with GnRHa stimulates LH secretion and the development of juvenile Leydig cells [Hadziselimovic et al., 1984]. This therapy increased, as expected, the abundance of several genes that regulate testosterone synthesis (*HSD3B1*, *HSD17B2*, *CYP19A1*, *CYP11B2*, and *CYP11B1*) [Hadziselimovic et al., 2017]. The LH increase observed after 6 months of therapy suggests that LH secretion results from an alternate pathway stimulation via *EGR2* and *EGR3*, indicating that both genes compensate for *EGR1* and *EGR4*.

In conclusion, GnRHa treatment augments LH and testosterone secretion and induces testicular expression of various genes involved in long-term memory formation, in particular *RASGRF1* and *EGR2*. Our present RNA sequencing analysis results may help to explain, at the molecular level, the decreased IQ and poor school performance observed in cryptorchid boys at a high risk of infertility, since *RASGRF1* and *EGR2* have been identified as important genes for long-term memory and cognition formation. In future studies, it will be exciting to determine if and how GnRHa treatment permanently rescues long-term memory performance by influencing gene expression in the brain.

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Statement of Ethics

Investigations were carried out following the rules of the Declaration of Helsinki of 1975, revised in 2008. The Institutional Review Board and the Independent Ethics Committee of Vilnius University approved all aspects of this study. Approval was also provided for research involving the use of material (data records or biopsy specimens) that had been collected for non-research purposes (Vilnius Regional Biomedical Research Ethics Committee, No. 158200-580-PPI-17).

Disclosure Statement

The authors have no conflicts of interest to disclose. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

References

Ackermann S, Spalek K, Rasch B, Gschwind L, Coynel D, et al: Testosterone levels in healthy men are related to amygdala reactivity and memory performance. Psychoneuroendocrinology 37:1417–1424 (2012).

Benjamini Y, Hochberg Y: Controlling for false discovery rate: a practical and powerful approachto multiple testing. J R Stat Soc B 57: 289–300 (1995).

Bharadwaj R, Peter CJ, Jiang Y, Roussos P, Vogel-Ciernia A, et al: Conserved higher-order chromatin regulates NMDA receptor gene expression and cognition. Neuron 84:997–1008 (2014).

Bilkei-Gorzo A, Erk S, Schürmann B, Mauer D, Michel K, et al: Dynorphins regulate fear memory: from mice to men. J Neurosci 32: 9335–9343 (2012).

Chalmel F, Lardenois A, Evrard B, Mathieu R, Feig C, et al: Global human tissue profiling and protein network analysis reveals distinct levels of transcriptional germline-specificity and identifies target genes for male infertility. Hum Reprod 27:3233–3248 (2012).

Davis JF, Choi DL, Clegg DJ, Benoit SC: Signaling through the ghrelin receptor modulates hippocampal function and meal anticipation in mice. Physiol Behav 103:39–43 (2011).

Depue RH: Cryptorchidism, an epidemiologic study with emphasis on the relationship to central nervous system dysfunction. Teratology 37:301–305 (1988).

DeSteno DA, Schmauss C: Induction of early growth response gene 2 expression in the forebrain of mice performing an attention-set-shifting task. Neuroscience 152:417–428 (2008).

Faisal M, Kim H, Kim J: Sexual differences of imprinted genes' expression levels. Gene 533: 434–438 (2014).

Giese KP, Friedman E, Telliez JB, Fedorov NB, Wines M, et al: Hippocampus-dependent learning and memory is impaired in mice lacking the Ras-guanine-nucleotide releasing factor 1 (Ras-GRF1). Neuropharmacology 41: 791–800 (2001).

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, et al: HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60 (2009).

Guo J, Zhu P, Wu C, Yu L, Zhao S, Gu X: In silico analysis indicates a similar gene expression pattern between human brain and testis. Cytogenet Genome Res 103:58–62 (2003).

Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, et al: Histone methylation regulates memory formation. J Neurosci 30:3589–3599 (2010).

Hadziselimovic F: Successful treatment of unilateral cryptorchid boys risking infertility with LH-RH analogue. Int Braz J Urol 34:319–326 (2008).

Hadziselimovic F, Herzog B: Hodenerkrankungen im Kindesalter (Hippokrates Verlag, Stuttgart 1990).

Hadziselimovic F, Hoecht B: Testicular histology related to fertility outcome and postpubertal hormone status in cryptorchidism. Klin Padiatr 220:302–307 (2008).

Hadziselimovic F, Hoecht B, Herzog B, Girard J: Does long term treatment with buserelin improve the fertility chances of cryptorchid testes? in Labrie F, Belanger A, Dupont A (eds): LH-RH and Its Analogues, p 457 (Elsevier, Amsterdam 1984).

Hadziselimovic F, Zivkovic D, Bica DTG, Emmons LR: The importance of mini-puberty for fertility in cryptorchidism. J Urol 174: 1536–1539 (2005).

Hadziselimovic F, Hadziselimovic NO, Demougin P, Krey G, Hoecht B, Oakeley EJ: EGR4 is a master gene responsible for fertility in cryptorchidism. Sex Dev 3: 253 –263 (2009).

Hadziselimovic F, Hadziselimovic NO, Demougin P, Oakeley EJ: Decreased expression of genes associated with memory and X-linked mental retardation in boys with non-syndromic cryptorchidism and high infertility risk. Mol Syndromol 5:76–80 (2014).

Hadziselimovic F, Gegenschatz-Schmid K, Verkauskas G, Docampo-Garcia MJ, Demougin P, et al: Gene expression changes underlying idiopathic central hypogonadism in cryptorchidism with defective mini-puberty. Sex Dev 10:136–146 (2016).

Hadziselimovic F, Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, et al: GnRHa treatment of cryptorchid boys affects genes involved in hormonal control of the HPG axis and fertility. Sex Dev, DOI: 10.1159/000471937 (2017).

Hadziselimovic N, Vukojevic V, Peter F, Milnik A, Fastenrath MS, et al: Forgetting is regulated via musashi-mediated translational control of the Arp2/3 complex. Cell 156:1153–1166 (2014).

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, et al: Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics 2008:420747 (2008).

Jaitner C, Reddy C, Abentung A, Whittle N, Rieder D, et al: Satb2 determines miRNA expression and long-term memory in the adult central nervous system. Elife pii:e17361 (2016).

Li L, Carter J, Gao X, Whitehead J, Tourtellotte WG: The neuroplasticity-associated *arc* gene is a direct transcriptional target of early growth response (Egr) transcription factors. Mol Cell Biol 25:10286–10300 (2005).

Li L, Yun SH, Keblesh J, Trommer BL, Xiong H, et al: Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. Mol Cell Neurosci 35:76–88 (2007).

Lund SP, Nettleton D, McCarthy DJ, Smyth GK: Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. Stat Appl Genet Mol Biol doi: 10.1515/1544-6115.1826 (2012).

Luksys G, Ackermann S, Coynel D, Fastenrath M, Gschwind L, et al: BAIAP2 is related to emotional modulation of human memory strength. PLoS One 9:e83707 (2014).

McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, et al: HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31:764–774 (2011).

Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, et al: Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 328:753–756 (2010).

Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ: Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor α4 subunits in hippocampal neurons. J Biol Chem 281:29431–29435 (2006).

Robinson MD, McCarthy DJ, Smyth GK: edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140 (2010).

Silingardi D, Angelucci A, De Pasquale R, Borsotti M, Squitieri G, et al: ERK pathway activation bidirectionally affects visual recognition memory and synaptic plasticity in the perirhinal cortex. Front Behav Neurosci 5:84 (2011).

Smith SS: The influence of stress at puberty on mood and learning: role of the $\alpha_4\beta\delta$ GABAA receptor. Neuroscience 249:192–213 (2013).

Song JM, Sung YM, Nam JH, Yoon H, Chung A, et al: A mercaptoacetamide-based class II histone deacetylase inhibitor increases dendritic spine density via RasGRF1/ERK pathway. J Alzheimers Dis 51:591–604 (2016).

Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, et al: The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics 54: 1.30.1–1.30.33 (2016).

Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, et al: Proteomics. Tissue-based map of the human proteome. Science 23:347 (2015).

Vogel-Ciernia A, Wood MA: Neuron-specific chromatin remodeling: a missing link in epigenetic mechanisms underlying synaptic plasticity, memory, and intellectual disability disorders. Neuropharmacology 80:18–27 (2014).

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC: The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg 12:1495–1499 (2014).