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1 # *Morc1* knockout evokes a depression-like phenotype in mice
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Abstract

Morc1 gene has recently been identified by a DNA methylation and genome-wide association study as a candidate gene for major depressive disorder related to early life stress in rodents, primates and humans. So far, no transgenic animal model has been established to validate these findings on a behavioral level. In the present study, we examined the effects of a *Morc1* loss of function mutation in female C57BL/6N mice on behavioral correlates of mood disorders like the Forced Swim Test, the Learned Helplessness Paradigm, O-Maze and Dark-Light-Box. We could show that *Morc1*$^{-/-}$ mice display increased depressive-like behavior whereas no behavioral abnormalities regarding locomotor activity or anxiety-like behavior were detectable. The baseline CORT plasma levels did not differ significantly between *Morc1*$^{-/-}$ mice and their wildtype littermates, yet – surprisingly - total BDNF mRNA-levels in the hippocampus were up-regulated in *Morc1*$^{-/-}$ animals. Although further work would be clarifying, *Morc1*$^{-/-}$ mice seem to be a promising epigenetically validated mouse model for depression associated with early life stress.

Keywords: depression; *Morc1*; transgenic mice; early life stress; epigenetics; BDNF.

1 Introduction

Clinical studies show that early life stress has profound and persistent effects on brain functions and is one of the major risk factors for developing a depressive disorder later in life [1], [2], [3]. The fundamental role of epigenetics mediating this process became clear in the groundbreaking animal study of Weaver et al. [4]: life-long epigenetic modifications of the glucocorticoid receptor gene in the rat hippocampus induced by early life stress were followed by a stable enhanced HPA response to stress. In the meantime, several studies identified effects of early life stress on DNA methylation for further genes in rodents [5], [6], [7], [8], [9]. For some of these genes, like BDNF, the serotonin transporter and the glucocorticoid receptor, altered DNA methylation was also revealed in humans after experiencing early life stress [10], [11], [12]. All of the above-cited studies focused on a candidate gene approach. Transgenic mice models with altered expression of the examined genes only partially show a consistent depressive-like phenotype [13], [14], [15], [16], [17]. Two more recent studies used a genome-wide methylation analysis and reported a wide range of epigenetic alterations as a result of early life stress [18], [19], but none of them led to a behaviorally validated mouse model for depression.

In a novel systematic translational genome-wide epigenetic approach, we recently succeeded in detecting an epigenetic marker of early life stress that is present in blood cell progenitors at birth in humans and monkeys, and also detectable in the prefrontal cortex of adult rats: Microrchidia (MORC) 1 – alias MORC family CW-type zinc finger 1 [20]. Moreover, we were able to verify an association between *Morc1* and major depressive disorder in a gene-set based analysis of an already available genome-wide association study [21].

MORC was first described in mammals by Watson et al. [22], who found that it is mainly expressed in male germ cells where it regulates mammalian germ cell development and meiosis. Male *Morc1*$^{-/-}$ mice are infertile due to a disrupted spermatogenesis, whereas male

110 *Morc1*$^{+/–}$ and female *Morc1*$^{–/–}$ mice do not show deficits in their reproductive system, *Morc1*
111 knockout mice were seen exclusively as a model for male fertility defects. The human MORC
112 protein consists of 984 amino acids and is 66% identical to the mouse MORC. Mutations in
113 humans might also cause male infertility and be involved in testicular germ cell tumors [23].
114 More recently Pastor et al. [24] reported that the male infertility in *Morc1* mutant mice is
115 caused by defects of DNA methylation of specific classes of transposons, resulting in failed
116 transposon silencing at these sites. Further analysis revealed that the MORC family is not only
117 decisive for male reproduction, but is also involved in the pathophysiology of numerous
118 forms of cancer: *Morc1* e.g. is frequently expressed in multiple myeloma [25] and mutated in
119 estrogen receptor-positive lobular breast cancer [26]. Additionally, *Morc1* has been related to
120 diabetes traits in a genome-wide complex trait analysis [27], which could play a role in the
121 association of major depressive disorder with type 2 diabetes mellitus. In plants, *Morc1*
122 influences immunological processes by different gene silencing mechanisms and
123 heterochromatin condensation [28], [29], [30]. Recent evidence suggests that *Morc1* plays a
124 more general biological role as part of a highly conserved nuclear protein superfamily that
125 serves as epigenetic regulators in diverse nuclear processes that are not yet fully understood
126 [31], [32], [33]. Hence *Morc1* appears to be primarily involved in gene silencing and changes
127 of the chromatin structure [34], [35]. We were the first to report a different methylation of
128 *Morc1* in brain tissue – the prefrontal cortex of adult rats – and thus strongly support the
129 hypothesis of an epigenetic influence of this gene in the brain [20].
130
131 Animal models are still valuable tools in preclinical research, yet no rodent model of *Morc1*
132 regarding depressive-like behavior has been established. Thus, in the present study we aimed
133 to implement a transgenic mouse model to validate the role of *Morc1* expression in affective
134 disorders. For this purpose we used *Morc*$^{Tg(Tyr)1Az/J}$ mice, in which exons 2-4 of *Morc1* gene
135 on chromosome 16 had been deleted with the help of a transgenic insert [36]. We

136 characterized MORC$^{Tg(Tyr)1Az/J}$ mice behaviorally in a test battery for locomotion and
137 exploratory, anxiety-like and despair behavior. Plasma corticosterone levels were analyzed as
138 a possible indicator of a depression-like HPA-system dysfunction. Furthermore, we
139 determined total BDNF mRNA-levels known to be decreased in hippocampus in depressive
140 patients and animal models of depression [37], [38], [39] as well as in closely connected and
141 presumably affected structures, such as prefrontal cortex and amygdala [40], [41]. Due to
142 difficult breeding and an increased mortality of male *Morc1*$^{-/-}$ mice, we only used female
143 animals for our experiments, which is also the sex more predisposed to develop depression in
144 humans.
145

146 # 2 Materials and Methods
147
148 ## 2.1 Animals
149
150 *Morc*$^{Tg(Tyr)1Az/J}$ mice had been generated by introducing a transgenic construct containing a
151 tyrosinase gene under the control of an RNA polymerase II 1 promoter into FVB/N fertilized
152 mouse eggs. Sequences adjacent to the transgenic insert are disrupted resulting in the deletion
153 of *Morc1* exons 2-4 [36]. Heterozygous *Morc1*$^{+/-}$ on the original FVB/N background were
154 purchased from the Jackson Labs (Bar Harbour, Maine, USA) and crossed with C57BL/6N
155 (Charles River, Sulzfeld, Germany) mice to obtain an F1 generation. Heterozygous F1
156 offspring were then inter-crossed to generate an F2 generation, which was used for behavioral
157 and molecular analyses. Male *Morc1*$^{-/-}$ mice were infertile and had smaller testes than
158 wildtypes, whereas male *Morc1*$^{+/-}$ mice and female *Morc1*$^{-/-}$ mice showed no reproductive
159 deficits. Animals were genotyped by PCR as recommended by the Jackson Labs (Bar Harbour,
160 Maine, USA). Female *Morc1*$^{-/-}$ mice and their wild-type littermates were housed individually
161 2 weeks before the first behavioural test started in macrolon type II cages with nesting
162 material under a reversed day-night cycle (lights on from 19.00-07:00 hrs with 12h dark and
163 12-h light phase) and supplied with food and water *ad libitum*. All procedures complied with
164 the regulations covering animal experimentation within the EU (European Communities
165 Council Directive 2010/63/EU). They were conducted in accordance with the institutions'
166 animal care and use guidelines and approved by the national and local authorities
167 (Regierungspräsidium Karlsruhe).

168
169
170 ## 2.2 Behavioral tests
171
172 At the age of 3, 5 and 6 months, 11 female *Morc1*$^{-/-}$ mice and 12 of their wild-type littermates
173 were tested behaviorally in the dark phase, i.e., in the animals` active phase. Mice were
174 acclimatized to the experimental room for at least 30 minutes prior to each test and tested by
175 an investigator, who was blinded regarding their genotype. The order of the tests followed

176 earlier recommendations ranking the tests from least stressful to more stressful. Mice were
177 sacrificed 2-3 weeks after the last experiment at an age of 21 - 25 weeks.
178
179 *2.2.1 Novel Cage Test*
180
181 Explorative behaviour was measured by counting the number of rearings within the first 5
182 minutes after placing the animal into a fresh standard type II macrolon cage as described
183 earlier [42].
184
185 *2.2.2 Open Field Test*
186
187 To evaluate locomotor and exploratory behavior, mice were individually placed into an open
188 arena measuring 50x50 cm^2 under dimmed light conditions (25 Lux). Activity monitoring was
189 conducted 15 min via a Video camera (Sony CCD IRIS). The resulting data were analyzed
190 using the image processing system EthoVison 1.96 (Noldus Information Technology,
191 Wageningen, the Netherlands) as described earlier [43].
192
193 *2.2.3 Dark-Light-Box*
194
195 Anxiety-related behavior was tested in the Dark-Light Box consisting of two plastic chambers
196 connected by a small tunnel. Mice were placed into the dark chamber, which was covered by
197 a lid and measured 20x15 cm^2. Latency to first exit, number of exits and total time in the
198 aversive light compartment (30x15 cm^2, illuminated with 600 Lux) was recorded for 5 min as
199 described earlier [44].
200
201 *2.2.4 O-Maze*
202
203 The Elevated Zero-Maze analyzes anxiety-related behavior by assessing avoidance of the
204 aversive unsheltered compartment of the arena. A grey plastic annular runway (width 6 cm,
205 outer diameter 46 cm, 50 cm above ground level, illuminated with 25 Lux) was covered with
206 black cardboard paper to prevent animals slipping off the maze. Two opposing sectors were
207 protected by inner and outer walls with a height of 10 cm. Animals were placed in one of the

protected sectors and latency to first exit, number of exits and total time spent in the open compartments was measured for 5 minutes as described earlier [45].

2.2.5 Forced Swim Test

The Forced Swim Test was applied to measure depressive-like behavior. For this purpose mice were placed into a glass cylinder (23 cm height, 13 cm diameter) filled with water (22°C) up to a height of 8 cm. Within a period of 6 min the onset and the percentage of floating was determined as described earlier [46]. 24 hours later the animals were tested again under the same conditions as before.

2.2.6 Hot Plate Test

To exclude altered pain sensitivity as a confounding factor for Learned Helplessness, the Hotplate Test (ATLab, Vendargues, France) was applied. Temperature was set at 53 °C (±0.3 °C) and a 45s cut-off was determined to prevent injury. Latency to first reaction, i.e. licking hind paws or jumping, was assessed as described earlier [47].

2.2.7 Learned Helplessness

In the Learned Helplessness Paradigm mice were placed in a transparent plexiglas shock chamber (18×18×30cm³) with a stainless steel grid floor (Coulborn Instruments, Düsseldorf, Germany) through which they received 360 unpredictable and unavoidable footshocks (0.150mA) on 2 consecutive days. The footshocks applied varied regarding shock-duration (1–3s) and interval-episodes (1–15s) and lasted approximately 52 min in total. 24 hours after the second shock procedure, learned helplessness was assessed by testing shuttle box performance (Graphic State Notation, Coulborn Instruments, Düsseldorf, Germany) as described earlier [48]. Spontaneous initial shuttles from one compartment to the other were counted during the first 2min. Performance during 30 shuttle escape trials each starting with a

236 light stimulus of 5s, announcing a subsequent footshock (intensity 0.15mA) of maximum 10s
237 duration was analyzed. Inter-trial interval was 30s and total testing time about 20min.
238
239 **2.3 CORT levels**
240 All animals were sacrificed at the age of 21-25 weeks by decapitation between 8.00 and 11.00
241 hrs in the morning, and trunk blood was collected within 30s after the animal's removal from
242 the cage. Baseline plasma corticosterone levels – without applying any acute stress or
243 intervention before taking the samples – were determined using commercially available
244 radioimmunoassay kits (ICN Biomedicals, Eschwege, Germany) as described earlier [49].
245
246 **2.4 RNA Preparation and Gene Expression Analysis by Quantitative Real-Time PCR**
247 After decapitation of the animals, the brains were immediately extracted from the skull. The
248 hippocampus, prefrontal cortex and amygdala were rapidly dissected from the whole brain,
249 frozen on dry ice and stored for later analyses.
250 Total RNA was isolated by single step of guanidinium isothiocyanate/phenol extraction using
251 PureZol RNA isolation reagent (Bio-Rad Laboratories) according to manufacturer's
252 instructions and quantified by spectrophotometric analysis. Following total RNA extraction,
253 the samples were processed for real-time PCR (RT-PCR) to assess total BDNF mRNA levels.
254 An aliquot of each sample was treated with DNase to avoid DNA contamination. RNA was
255 analyzed by TaqMan qRTPCR instrument (CFX384 real time system; Bio-Rad Laboratories)
256 using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories). Samples were
257 run in 384 well formats in triplicate as multiplexed reactions with a normalizing internal
258 control (36B4). Primers and probes sequences of total BDNF (forward primer:
259 AAGTCTGCATTACATTCCTCGA, reverse primer:
260 GTTTTCTGAAAGAGGGACAGTTTAT and probe: TGTGGTTTGTTGCCGTTGCCAAG)
261 and 36B4 (forward primer: AGATGCAGCAGATCCGCAT, reverse primer:

262 GTTCTTGCCCATCAGCACC and probe: CGCTCCGAGGGAAGGCCG) were purchased
263 from Eurofins Genomics (Vimodrone, Italy), while probe and primer sequences for *Morc1*
264 (Assay ID: Mm00501711_m1) were purchased from Life Technologies (Monza, Italy) and
265 are available on request.
266
267 **2.5 Statistical analyses**
268 Intergroup comparisons were calculated by one-sided t-tests assuming that knockout-mice
269 display higher levels of depressive and anxiety-like behavior as well as higher CORT-levels,
270 less body weight, less locomotor and exploratory behavior and decreased total BDNF mRNA-
271 levels in hippocampus, prefrontal cortex and amygdala than wild-types. A one-way repeated
272 measurements ANOVA was used to analyze the open field test. Calculations regarding
273 mRNA levels were run with fold change values. The correlation between Learned
274 Helplessness Escape Latency and Failures was analyzed applying spearman rho correlation. A
275 p-value ≤ 0.05 was seen as the level of statistical significance in all tests. The statistical
276 analyses were performed using the SPSS 21.0 software package for Windows.
277

278 ## 3 Results

279 *3.1 Morc1 knockout mice display regular locomotor and exploratory behavior*

280 *Morc1*$^{-/-}$ mice showed normal vertical locomotor activity (rearing) in the Novel Cage Test
281 (T(21)=0.874; p=0.196; data not shown). The horizontal locomotor activity and exploratory
282 behavior as measured by the Open Field Test also did not differ significantly from control
283 animals: e.g. the total distance moved by *Morc1*$^{-/-}$ mice was similar to those of the control
284 group (time*genotype: $F_{1,21}$=1.125, p=0.301; between subject-factor genotype $F_{1,21}$=0.032;
285 p=0.859). Both genotypes moved significantly less in the second half of the test, showing that
286 habituation had taken place as expected (Time: $F_{1,21}$=8.792; p=0.007; data not shown).

287

288 *3.2 Morc1 knockout mice show unaltered anxiety-related behavior*

289 *Morc1*$^{-/-}$ mice did not display increased anxiety-like behavior as monitored in the Dark-Light
290 Box (latency: T(21)=1.023; p=0.159; end exploration: T(14.011)=0.914; p=0.188; exits:
291 T(21)=-1.289; p=0.106; light time: T(21)=-0.074; p=0.471; data not shown). In the Elevated
292 O-Maze, again *Morc1*$^{-/-}$ mice exhibited similar latencies to enter the aversive compartment as
293 their littermate controls (T(21)=0.314; p=0.3785; data not shown). They also did not exit
294 (T(21)=-0.128; p=0.4495; data not shown) or fully cross (T(21)=0.095; p=0.4625; data not
295 shown) the maze less than controls and spent about the same amount of time in the open arms
296 of the maze (T(21)=-0.862; p=0.199; data not shown).

297

298 *3.3 Morc1 knockout mice demonstrate increased depressive-like behavior*

299 Depressive-like behavior as measured by the Forced Swim Test showed that *Morc1*$^{-/-}$ mice
300 display a significant lower latency to float (T(21) =2.346; P=0.015) on day 1 than wild-types
301 (s. Fig 1A). Additionally, on day 1 immobility times were increased in *Morc1*$^{-/-}$ mice, which
302 resulted in a statistical tendency from minute 4 to 6 of the test (minute 0 to 2: T18.051=-0.819;

303 p=0.216; minute 2 to 4: T21=-0,567; p=0.289; minute 4 to 6: T21=-1.343; p=0.097; s. figure
304 1B).
305 24 hours later (on day 2), the difference in the latency to start floating was not detectable any
306 more (T21=0.113; p=0.456; s. fig. 1C.) Furthermore, *Morc1*$^{-/-}$ mice showed a significant
307 increase in immobility on day 2 from minute 2-4 compared to wild-types (T(21)=-2.009;
308 p=0.029) and a statistical tendency also from minute 4-6 (T21=-1.431; p=0.084) as depicted
309 in fig. 1D. From minute 0 to 2 there was no significant difference in immobility time between
310 the two groups (T21=-0.508; p=0.308).
311
312 In the Learned Helplessness Paradigm of depression, *Morc1*$^{-/-}$ mice displayed significantly
313 more failures to escape in comparison to littermate controls (T(12.573)=-1.844; p=0.045) (s.
314 Fig 2A). As a tendency, *Morc1*$^{-/-}$ mice had a higher latency to escape than controls (T
315 (16.093)=-1.539; p=0.072) as shown in fig. 2B. A clear correlation between escape latency
316 and number of failures with the *Morc1*$^{-/-}$ mice showing the highest values (spearman
317 rho=0.961, p<0.000) is depicted in fig. 2 C.
318
319 *3.4 Morc1 knockout mice reveal regular CORT levels, but increased total BDNF in*
320 *hippocampus*
321 *Morc1*$^{-/-}$ mice showed similar plasma corticosterone levels as their control littermates
322 (T(21)=-0.941; p=0.1785; s. fig. 3A). In the hippocampus however, total BDNF mRNA-levels
323 of *Morc1*$^{-/-}$ mice were significantly increased compared to those of wild-types (T(21)= -3.538;
324 p=0.001; s. Fig. 3B). As a tendency, the total BDNF mRNA-levels in the prefrontal cortex
325 (T(14.968)=1.428; p=0.087; data not shown) and in the amygdala (T(18)=1,337; p=0.099;
326 data not shown) were down-regulated.

327 As expected, *Morc1* mRNA was not expressed in *Morc1*$^{-/-}$ mice, whereas we found its full
328 mRNA expression in the wildtype animals in hippocampus, prefrontal cortex and amygdala
329 (data not shown).
330

331 ## 4 Discussion

332 In the present study, we characterized for the first time *Morc1*$^{-/-}$ mice in a test battery for
333 emotional behavior. *Morc1*$^{-/-}$ mice floated earlier in the Forced Swim Test and showed more
334 immobility in general. In the Learned Helplessness Paradigm, they exhibited a higher latency
335 to escape and more escape failures, two parameters that are seen as classical indicators for
336 increased learned helplessness in rodents [50]. *Morc1*$^{-/-}$ mice showed a significant increase of
337 immobility in the Forced Swim Test only on day 2 and just as a statistical tendency on day 1.
338 Although day 1 is regarded as more relevant for mice than day 2, the consistently increased
339 means of immobility on both days taken together with the results of the Learned Helplessness
340 Test, allow to postulate a clear increase in depressive-like behavior of *Morc1*$^{-/-}$ mice.
341 Anxiety is often concomitant to depression in psychiatric patients. Up to now, the question if
342 *Morc1* is also involved in anxiety disorders has not been examined in clinical trials. In our
343 animal model we did not find any evidence of increased anxiety in *Morc1*$^{-/-}$ mice in the Dark-
344 Light Box Test and Elevated O-Maze, indicating that *Morc1* may specifically be involved in
345 depression but not anxiety. We were able to exclude reduced locomotion and exploratory
346 behavior as possible confounding factors in our study, as *Morc1*$^{-/-}$ mice did not show any
347 difference to their wild-type littermates in the Novel Cage and Open Field Test.
348 All in all, this new transgenic mutation seems to represent a promising model to further
349 investigate the depressive phenotype and its underlying neurochemical, genetic and epigenetic
350 pathophysiology.
351

352 Besides a deficient spermatogenesis, small testical size and aberrant eye pigmentation,
353 Watson et al. [22] described no phenotypic abnormalities in male *Morc1*-mutant mice. In our
354 breeding, we found increased mortality in male *Morc1*$^{-/-}$ mice, and the small sample size of
355 males in our cohort constrained us to focus only on females for our behavioral testing.
356 Although we bred only for two generations, a random effect cannot be definitely dismissed,

357 yet this increased mortality might be another confirmation of *Morc1* – as already stated above
358 – not only being involved in spermatogenesis but serving a more general biological function
359 [31], [32], [33].
360 This leads to the more general question why *Morc1* might be involved in spermatogenesis as
361 well as in mood disorders – two biological processes seemingly independent at first glance.
362 Soumillon et al. [51] come to the conclusion that a considerable part of genes expressed in
363 testes do not have testes-specific functions. Shen et al. [52] could demonstrate that testes-
364 specific genes usually have a fast evolutionary rate and therefore are more likely to gain new
365 functions. In their phylogenetic analysis they illustrate that some open reading frames were
366 first expressed in testis and later in evolution got expressed in other tissues. The authors
367 assume that testis may play a role in producing new genes and even in supporting testes-
368 specific genes in gaining new functions for other organs/tissues. Blendy et al. [53] for
369 example demonstrated that the *Crem* gene is involved in spermatogenesis. Aguado et al. [54]
370 found the same gene being involved in hippocampal synaptogenesis. Wang et al. [55] proved
371 another gene - *hsf-2* – to be involved in sperm production as well as central nervous
372 development.
373
374 As women are more often affected by major depressive disorder than men [56], [57], we saw
375 the restriction to female $^{-/-}$ mice in the present study as a possibility for validly modeling
376 depression. Nevertheless, sex differences with respect to the development of depressive-like
377 behaviors in mice with targeted mutagenesis have also been reported for other genes such as
378 BDNF [58], [59], [60]. Therefore another study on male *Morc1*$^{-/-}$ mice and their conceivable
379 depressive-like behavior seems appealing. A possible sex effect in our animal model could
380 also shed more light on the differing pathophysiology of depression in women and men.
381 Moreover, additional behavioral tests concerning e.g. social behavior and cognition that are

382 often correlated to depressive-like behavior, are warranted to further characterize the novel
383 *Morc1* mouse model of depression.
384
385 We did not detect any difference regarding baseline corticosterone levels in *Morc1*$^{-/-}$ mice
386 suggesting that the HPA-axis in these animals is not disrupted under resting conditions. This
387 does not exclude the possibility that the hormonal responsiveness is affected under
388 challenging conditions.
389
390 We also investigated BDNF expression that represents a prototype marker of neuronal
391 plasticity, which has often been associated with a depressive phenotype, its expression being
392 reduced in the brain of depressed subjects as well as in different animal models of depression
393 [61], [62] – summarized in the so-called neurotrophin hypothesis of depression. Contrary to
394 these previously found changes, the hippocampal expression of BDNF was upregulated in
395 *Morc1*$^{-/-}$ mice, whereas a trend toward a reduction was found in the prefrontal cortex. Despite
396 the complex interactions between glucocorticoids and BDNF, this finding might be narrowed
397 down to the undisturbed HPA-Axis in our animals, as BDNF is mostly suppressed by
398 glucocorticoids [63], [64].
399 An alternative explanation for this unexpected result may be sex differences. Interestingly, the
400 decrease of BDNF in depressed patients was found to be more pronounced in men than in
401 women [65]. Hayley et al. [66] demonstrated that in female depressed patients who committed
402 suicide BDNF protein levels were reduced in the frontopolar prefrontal cortex, but not in the
403 hippocampus. Conversely, males displayed significantly decreased BDNF protein levels only
404 in the hippocampus yet not in the prefrontal cortex. Jaworska et al. [67] showed a significant
405 decrease of BDNF in the hippocampus of male, but not of female gerbils after early life stress.
406 As we used only female mice for our experiment, this sex-specific regulation could be the
407 reason for the lack of a BDNF down-regulation in our study. Furthermore, there is growing

408 evidence that estradiol can induce BDNF expression and vice versa that estradiol effects in
409 the hippocampus are mediated by BDNF [68], [69], [70], [71].
410
411 In a recent study, Calabrese et al. [72] demonstrated that BDNF in serotonin transporter
412 knock-out rats, which had been exposed to early life stress was down-regulated in the ventral
413 hippocampus and the ventromedial prefrontal cortex, but was significantly increased in the
414 dorsal hippocampus and the dorsomedial prefrontal cortex. A similar region-specific process
415 might underlie the detected increase of BDNF in hippocampus in the present study with the
416 amount of BDNF in the dorsal parts outweighing the downregulation in the ventral parts. We
417 did not differentiate between the dorsal and ventral regions of hippocampus and prefrontal
418 cortex, but this issue would be very interesting to address in future experiments.
419
420 However, all these aspects fail to entirely explain the highly significant increase of BDNF we
421 found in hippocampus of *Morc1*$^{-/-}$ mice. In contrast, the trend of reduced BDNF in prefrontal
422 cortex and amygdala of *Morc1*$^{-/-}$ mice is more in line with previous findings showing either
423 no change of BNDF in these two regions [73] or a decrease in depressive-like animals [74]
424 and depressive humans [75].
425
426 Last, increased BDNF levels in the hippocampus might also be the consequence of
427 compensatory mechanisms set in motion in *Morc1* mutants. Thus, Faure et al. [76], Marais et
428 al. [77] and Daniels et al. [78] found a significant increase of BDNF in (dorsal) rat
429 hippocampus after maternal separation during early life. Hellweg et al. [79] also found a
430 significant increase of BDNF in bulbectomized depressive-like mice. According to the
431 authors this finding in addition to the fact that bulbectomized animals display different
432 behavioral abnormalities than glucocorticoid receptor compromised mice as well as a
433 serotonergic dysfunction possibly defines a new endophenotype of depression. Although

434 *Morc1*$^{-/-}$ mice show the same depressive-like phenotype with deficiencies in the FST and LH
435 paradigm as described by the animals used for the neurotrophin hypothesis of depression,
436 their serotonergic function has not been evaluated yet. Maybe *Morc1*$^{-/-}$ mice constitute an
437 intermediate endophenotype situated between the two previously described animal models.
438 Nonetheless, as BDNF-levels in the brain have never been analyzed before in *Morc1*$^{-/-}$ mice,
439 the regulation of BDNF in regard to *Morc1* and depression remains a matter of pure
440 speculation. Systematic investigation of this question is required.
441
442 One of the most intriguing issues to address in the future will be the role of epigenetic
443 modulation by *Morc1* in the brain. We were the first to detect a different methylation of
444 *Morc1* in the prefrontal cortex of the rat brain [20]. In our cross-species and cross-tissues
445 approach, we could prove the particular relevance of *Morc1* methylation after early life stress.
446 However, to date the specific cerebral subtypes that are affected by *Morc1* and the concrete
447 epigenetic function of *Morc1* in the brain is still completely unknown.
448 A further implication of this finding is the question if the methylation pattern will be
449 replicable in the mouse brain or in other regions of the brain like e.g. the hippocampus. As
450 epigenetic processes are always highly sex-specific, it would also be most interesting to
451 examine in which way the epigenetic mark will be expressed differently in male individuals.
452
453 One limitation of the presented study is certainly that only females have been used so that the
454 model might be a sex-specific. Additionally, as we analyzed BDNF and CORT levels after
455 applying stressful behavioral tests to the animals, we cannot exclude that e.g. the FST or the
456 LH paradigm has had some influence on these parameters. It is thinkable that the increase of
457 BDNF in the hippocampus of *Morc1*$^{-/-}$ mice is due to some compensatory mechanism
458 induced by the higher amount of electric shocks these animals received in the LH paradigm
459 due to their bad performance in this behavioral test.

460 Our study results are further limited by the fact that we only used *Morc1* homozygous
461 knockout mice as heterozygous knockouts are usually regarded as a better model for the
462 human condition. We are actually planning to also behaviorally analyze *Morc1* heterozygous
463 - and male - mice after backcrossing our animals for a few more generations into the c57/bl6
464 background.
465
466 In conclusion, although further work still has to be accomplished, the animal model of *Morc1*$^{-/-}$
467 mice will be useful in future studies on the role of *Morc1* in the pathophysiology and
468 therapy of depression.
469

470 5 Conflict of interest

471 The authors declare no conflict of interest.
472

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481 # 7 References

482 [1] Agid O, Shapira B, Zislin J, Ritsner M, Hanin B, Murad H, et al. Environment and
483 vulnerability to major psychiatric illness: a case control study of early parental loss in major
484 depression, bipolar disorder and schizophrenia. Molecular psychiatry. 1999;4:163-72.
485 [2] Wiersma JE, Hovens JG, van Oppen P, Giltay EJ, van Schaik DJ, Beekman AT, et al. The
486 importance of childhood trauma and childhood life events for chronicity of depression in
487 adults. The Journal of clinical psychiatry. 2009;70:983-9.
488 [3] Shamseddeen W, Asarnow JR, Clarke G, Vitiello B, Wagner KD, Birmaher B, et al.
489 Impact of physical and sexual abuse on treatment response in the Treatment of Resistant
490 Depression in Adolescent Study (TORDIA). Journal of the American Academy of Child and
491 Adolescent Psychiatry. 2011;50:293-301.
492 [4] Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al.
493 Epigenetic programming by maternal behavior. Nature neuroscience. 2004;7:847-54.
494 [5] Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, Meaney MJ. Maternal care
495 associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-
496 alpha expression in the medial preoptic area of female offspring. Endocrinology.
497 2006;147:2909-15.
498 [6] Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmuhl Y, Fischer D, et al. Dynamic
499 DNA methylation programs persistent adverse effects of early-life stress. Nature neuroscience.
500 2009;12:1559-66.
501 [7] Roth TL, Lubin FD, Funk AJ, Sweatt JD. Lasting epigenetic influence of early-life
502 adversity on the BDNF gene. Biological psychiatry. 2009;65:760-9.
503 [8] Chen J, Evans AN, Liu Y, Honda M, Saavedra JM, Aguilera G. Maternal deprivation in
504 rats is associated with corticotrophin-releasing hormone (CRH) promoter hypomethylation
505 and enhances CRH transcriptional responses to stress in adulthood. Journal of
506 neuroendocrinology. 2012;24:1055-64.
507 [9] Jensen Pena C, Monk C, Champagne FA. Epigenetic effects of prenatal stress on 11beta-
508 hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. PloS one. 2012;7:e39791.
509 [10] Toledo-Rodriguez M, Lotfipour S, Leonard G, Perron M, Richer L, Veillette S, et al.
510 Maternal smoking during pregnancy is associated with epigenetic modifications of the brain-
511 derived neurotrophic factor-6 exon in adolescent offspring. American journal of medical
512 genetics Part B, Neuropsychiatric genetics : the official publication of the International
513 Society of Psychiatric Genetics. 2010;153b:1350-4.
514 [11] Vijayendran M, Beach SR, Plume JM, Brody GH, Philibert RA. Effects of genotype and
515 child abuse on DNA methylation and gene expression at the serotonin transporter. Frontiers in
516 psychiatry. 2012;3:55.
517 [12] Labonte B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, et al. Differential
518 glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide
519 completers with a history of childhood abuse. Biological psychiatry. 2012;72:41-8.
520 [13] Zorner B, Wolfer DP, Brandis D, Kretz O, Zacher C, Madani R, et al. Forebrain-specific
521 trkB-receptor knockout mice: behaviorally more hyperactive than "depressive". Biological
522 psychiatry. 2003;54:972-82.
523 [14] Chourbaji S, Hellweg R, Brandis D, Zorner B, Zacher C, Lang UE, et al. Mice with
524 reduced brain-derived neurotrophic factor expression show decreased choline
525 acetyltransferase activity, but regular brain monoamine levels and unaltered emotional
526 behavior. Brain research Molecular brain research. 2004;121:28-36.
527 [15] Holmes A, Murphy DL, Crawley JN. Abnormal behavioral phenotypes of serotonin
528 transporter knockout mice: parallels with human anxiety and depression. Biological
529 psychiatry. 2003;54:953-9.

530 [16] Lira A, Zhou M, Castanon N, Ansorge MS, Gordon JA, Francis JH, et al. Altered
531 depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin
532 transporter-deficient mice. Biological psychiatry. 2003;54:960-71.
533 [17] Boyle MP, Kolber BJ, Vogt SK, Wozniak DF, Muglia LJ. Forebrain glucocorticoid
534 receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. The
535 Journal of neuroscience : the official journal of the Society for Neuroscience. 2006;26:1971-8.
536 [18] Labonte B, Suderman M, Maussion G, Navaro L, Yerko V, Mahar I, et al. Genome-wide
537 epigenetic regulation by early-life trauma. Archives of general psychiatry. 2012;69:722-31.
538 [19] Yang BZ, Zhang H, Ge W, Weder N, Douglas-Palumberi H, Perepletchikova F, et al.
539 Child abuse and epigenetic mechanisms of disease risk. American journal of preventive
540 medicine. 2013;44:101-7.
541 [20] Nieratschker V, Massart R, Gilles M, Luoni A, Suderman MJ, Krumm B, et al. MORC1
542 exhibits cross-species differential methylation in association with early life stress as well as
543 genome-wide association with MDD. Translational psychiatry. 2014;4:e429.
544 [21] Rietschel M, Mattheisen M, Frank J, Treutlein J, Degenhardt F, Breuer R, et al. Genome-
545 wide association-, replication-, and neuroimaging study implicates HOMER1 in the etiology
546 of major depression. Biological psychiatry. 2010;68:578-85.
547 [22] Watson ML, Zinn AR, Inoue N, Hess KD, Cobb J, Handel MA, et al. Identification of
548 morc (microrchidia), a mutation that results in arrest of spermatogenesis at an early meiotic
549 stage in the mouse. Proceedings of the National Academy of Sciences of the United States of
550 America. 1998;95:14361-6.
551 [23] Inoue N, Wei F, Seldin MF, Zinn AR, Watson ML. Assignment of microrchidia (Morc)
552 to mouse chromosome 16 by interspecific backcross linkage analysis and human chromosome
553 3q13 using somatic cell hybrids and in situ hybridization. Cytogenetics and cell genetics.
554 2000;90:123-5.
555 [24] Pastor WA, Stroud H, Nee K, Liu W, Pezic D, Manakov S, et al. MORC1 represses
556 transposable elements in the mouse male germline. Nature communications. 2014;5:5795.
557 [25] Condomines M, Hose D, Raynaud P, Hundemer M, De Vos J, Baudard M, et al.
558 Cancer/testis genes in multiple myeloma: expression patterns and prognosis value determined
559 by microarray analysis. Journal of immunology (Baltimore, Md : 1950). 2007;178:3307-15.
560 [26] Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, et al. Mutational
561 evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature.
562 2009;461:809-13.
563 [27] Zheng JS, Arnett DK, Lee YC, Shen J, Parnell LD, Smith CE, et al. Genome-wide
564 contribution of genotype by environment interaction to variation of diabetes-related traits.
565 PloS one. 2013;8:e77442.
566 [28] Moissiard G, Bischof S, Husmann D, Pastor WA, Hale CJ, Yen L, et al. Transcriptional
567 gene silencing by Arabidopsis microrchidia homologues involves the formation of heteromers.
568 Proceedings of the National Academy of Sciences of the United States of America.
569 2014;111:7474-9.
570 [29] Liu ZW, Shao CR, Zhang CJ, Zhou JX, Zhang SW, Li L, et al. The SET domain proteins
571 SUVH2 and SUVH9 are required for Pol V occupancy at RNA-directed DNA methylation
572 loci. PLoS genetics. 2014;10:e1003948.
573 [30] Langen G, von Einem S, Koch A, Imani J, Pai SB, Manohar M, et al. The compromised
574 recognition of turnip crinkle virus1 subfamily of microrchidia ATPases regulates disease
575 resistance in barley to biotrophic and necrotrophic pathogens. Plant physiology.
576 2014;164:866-78.
577 [31] Li DQ, Nair SS, Kumar R. The MORC family: new epigenetic regulators of transcription
578 and DNA damage response. Epigenetics : official journal of the DNA Methylation Society.
579 2013;8:685-93.

580 [32] Iyer LM, Abhiman S, Aravind L. MutL homologs in restriction-modification systems and
581 the origin of eukaryotic MORC ATPases. Biology direct. 2008;3:8.
582 [33] Moissiard G, Cokus SJ, Cary J, Feng S, Billi AC, Stroud H, et al. MORC family
583 ATPases required for heterochromatin condensation and gene silencing. Science (New York,
584 NY). 2012;336:1448-51.
585 [34] Perry J, Zhao Y. The CW domain, a structural module shared amongst vertebrates,
586 vertebrate-infecting parasites and higher plants. Trends in biochemical sciences. 2003;28:576-
587 80.
588 [35] Iyer LM, Anantharaman V, Wolf MY, Aravind L. Comparative genomics of
589 transcription factors and chromatin proteins in parasitic protists and other eukaryotes.
590 International journal for parasitology. 2008;38:1-31.
591 [36] Inoue N, Hess KD, Moreadith RW, Richardson LL, Handel MA, Watson ML, et al. New
592 gene family defined by MORC, a nuclear protein required for mouse spermatogenesis.
593 Human molecular genetics. 1999;8:1201-7.
594 [37] Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders.
595 Biological psychiatry. 2006;59:1116-27.
596 [38] Sirianni RW, Olausson P, Chiu AS, Taylor JR, Saltzman WM. The behavioral and
597 biochemical effects of BDNF containing polymers implanted in the hippocampus of rats.
598 Brain research. 2010;1321:40-50.
599 [39] Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A. Resilience to chronic
600 stress is mediated by hippocampal brain-derived neurotrophic factor. The Journal of
601 neuroscience : the official journal of the Society for Neuroscience. 2011;31:4475-83.
602 [40] Luoni A, Berry A, Calabrese F, Capoccia S, Bellisario V, Gass P, et al. Delayed BDNF
603 alterations in the prefrontal cortex of rats exposed to prenatal stress: preventive effect of
604 lurasidone treatment during adolescence. European neuropsychopharmacology : the journal of
605 the European College of Neuropsychopharmacology. 2014;24:986-95.
606 [41] Legge RM, Sendi S, Cole JH, Cohen-Woods S, Costafreda SG, Simmons A, et al.
607 Modulatory effects of brain-derived neurotrophic factor Val66Met polymorphism on
608 prefrontal regions in major depressive disorder. The British journal of psychiatry : the journal
609 of mental science. 2015;206:379-84.
610 [42] von Bohlen und Halbach O, Zacher C, Gass P, Unsicker K. Age-related alterations in
611 hippocampal spines and deficiencies in spatial memory in mice. Journal of neuroscience
612 research. 2006;83:525-31.
613 [43] Domanskyi A, Geissler C, Vinnikov IA, Alter H, Schober A, Vogt MA, et al. Pten
614 ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models.
615 FASEB journal : official publication of the Federation of American Societies for
616 Experimental Biology. 2011;25:2898-910.
617 [44] Berkel S, Tang W, Trevino M, Vogt M, Obenhaus HA, Gass P, et al. Inherited and de
618 novo SHANK2 variants associated with autism spectrum disorder impair neuronal
619 morphogenesis and physiology. Human molecular genetics. 2012;21:344-57.
620 [45] Fuss J, Ben Abdallah NM, Hensley FW, Weber KJ, Hellweg R, Gass P. Deletion of
621 running-induced hippocampal neurogenesis by irradiation prevents development of an
622 anxious phenotype in mice. PloS one. 2010;5.
623 [46] Weber T, Vogt MA, Gartside SE, Berger SM, Lujan R, Lau T, et al. Adult AMPA
624 GLUA1 receptor subunit loss in 5-HT neurons results in a specific anxiety-phenotype with
625 evidence for dysregulation of 5-HT neuronal activity. Neuropsychopharmacology : official
626 publication of the American College of Neuropsychopharmacology. 2015;40:1471-84.
627 [47] Chourbaji S, Brandwein C, Vogt MA, Dormann C, Hellweg R, Gass P. Nature vs.
628 nurture: can enrichment rescue the behavioural phenotype of BDNF heterozygous mice?
629 Behavioural brain research. 2008;192:254-8.

630 [48] Vogt MA, Inta D, Luoni A, Elkin H, Pfeiffer N, Riva MA, et al. Inducible forebrain-
631 specific ablation of the transcription factor Creb during adulthood induces anxiety but no
632 spatial/contextual learning deficits. Frontiers in behavioral neuroscience. 2014;8:407.
633 [49] Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, et al. Mice with
634 genetically altered glucocorticoid receptor expression show altered sensitivity for stress-
635 induced depressive reactions. The Journal of neuroscience : the official journal of the Society
636 for Neuroscience. 2005;25:6243-50.
637 [50] Chourbaji S, Zacher C, Sanchis-Segura C, Dormann C, Vollmayr B, Gass P. Learned
638 helplessness: validity and reliability of depressive-like states in mice. Brain research Brain
639 research protocols. 2005;16:70-8.
640 [51] Soumillon M, Necsulea A, Weier M, Brawand D, Zhang X, Gu H, et al. Cellular source
641 and mechanisms of high transcriptome complexity in the mammalian testis. Cell reports.
642 2013;3:2179-90.
643 [52] Shen L, Liu G, Zou Y, Zhou Z, Su Z, Gu X. The evolutionary panorama of organ-
644 specifically expressed or repressed orthologous genes in nine vertebrate species. PloS one.
645 2015;10:e0116872.
646 [53] Blendy JA, Kaestner KH, Weinbauer GF, Nieschlag E, Schutz G. Severe impairment of
647 spermatogenesis in mice lacking the CREM gene. Nature. 1996;380:162-5.
648 [54] Aguado F, Diaz-Ruiz C, Parlato R, Martinez A, Carmona MA, Bleckmann S, et al. The
649 CREB/CREM transcription factors negatively regulate early synaptogenesis and spontaneous
650 network activity. The Journal of neuroscience : the official journal of the Society for
651 Neuroscience. 2009;29:328-33.
652 [55] Wang G, Zhang J, Moskophidis D, Mivechi NF. Targeted disruption of the heat shock
653 transcription factor (hsf)-2 gene results in increased embryonic lethality, neuronal defects, and
654 reduced spermatogenesis. Genesis. 2003;36:48-61.
655 [56] Kornstein SG. Gender differences in depression: implications for treatment. The Journal
656 of clinical psychiatry. 1997;58 Suppl 15:12-8.
657 [57] Maciejewski PK, Prigerson HG, Mazure CM. Sex differences in event-related risk for
658 major depression. Psychological medicine. 2001;31:593-604.
659 [58] Urani A, Chourbaji S, Gass P. Mutant mouse models of depression: candidate genes and
660 current mouse lines. Neuroscience and biobehavioral reviews. 2005;29:805-28.
661 [59] Autry AE, Adachi M, Cheng P, Monteggia LM. Gender-specific impact of brain-derived
662 neurotrophic factor signaling on stress-induced depression-like behavior. Biological
663 psychiatry. 2009;66:84-90.
664 [60] Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, et al. Brain-
665 derived neurotrophic factor conditional knockouts show gender differences in depression-
666 related behaviors. Biological psychiatry. 2007;61:187-97.
667 [61] Lindholm JS, Castren E. Mice with altered BDNF signaling as models for mood
668 disorders and antidepressant effects. Frontiers in behavioral neuroscience. 2014;8:143.
669 [62] Stepanichev M, Dygalo NN, Grigoryan G, Shishkina GT, Gulyaeva N. Rodent models of
670 depression: neurotrophic and neuroinflammatory biomarkers. BioMed research international.
671 2014;2014:932757.
672 [63] Vaidya VA, Duman RS. Depresssion--emerging insights from neurobiology. British
673 medical bulletin. 2001;57:61-79.
674 [64] Shi CG, Wang LM, Wu Y, Wang P, Gan ZJ, Lin K, et al. Intranasal administration of
675 nerve growth factor produces antidepressant-like effects in animals. Neurochemical research.
676 2010;35:1302-14.
677 [65] Jiang C, Salton SR. The Role of Neurotrophins in Major Depressive Disorder.
678 Translational neuroscience. 2013;4:46-58.

679 [66] Hayley S, Du L, Litteljohn D, Palkovits M, Faludi G, Merali Z, et al. Gender and brain
680 regions specific differences in brain derived neurotrophic factor protein levels of depressed
681 individuals who died through suicide. Neuroscience letters. 2015;600:12-6.
682 [67] Jaworska N, Dwyer SM, Rusak B. Repeated neonatal separation results in different
683 neurochemical and behavioral changes in adult male and female Mongolian gerbils.
684 Pharmacology, biochemistry, and behavior. 2008;88:533-41.
685 [68] Scharfman HE, Maclusky NJ. Similarities between actions of estrogen and BDNF in the
686 hippocampus: coincidence or clue? Trends in neurosciences. 2005;28:79-85.
687 [69] Allen AL, McCarson KE. Estrogen increases nociception-evoked brain-derived
688 neurotrophic factor gene expression in the female rat. Neuroendocrinology. 2005;81:193-9.
689 [70] Franklin TB, Perrot-Sinal TS. Sex and ovarian steroids modulate brain-derived
690 neurotrophic factor (BDNF) protein levels in rat hippocampus under stressful and non-
691 stressful conditions. Psychoneuroendocrinology. 2006;31:38-48.
692 [71] Sun MK, Alkon DL. Differential gender-related vulnerability to depression induction and
693 converging antidepressant responses in rats. The Journal of pharmacology and experimental
694 therapeutics. 2006;316:926-32.
695 [72] Calabrese F, van der Doelen RH, Guidotti G, Racagni G, Kozicz T, Homberg JR, et al.
696 Exposure to early life stress regulates Bdnf expression in SERT mutant rats in an
697 anatomically selective fashion. Journal of neurochemistry. 2015;132:146-54.
698 [73] Patki G, Solanki N, Atrooz F, Allam F, Salim S. Depression, anxiety-like behavior and
699 memory impairment are associated with increased oxidative stress and inflammation in a rat
700 model of social stress. Brain research. 2013;1539:73-86.
701 [74] Takeda H, Tsuji M, Yamada T, Masuya J, Matsushita K, Tahara M, et al. Caffeic acid
702 attenuates the decrease in cortical BDNF mRNA expression induced by exposure to forced
703 swimming stress in mice. European journal of pharmacology. 2006;534:115-21.
704 [75] Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB
705 and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with
706 schizophrenia, bipolar disorder, and major depressive disorder. Neurobiology of disease.
707 2015;77:220-7.
708 [76] Faure J, Uys JD, Marais L, Stein DJ, Daniels WM. Early maternal separation alters the
709 response to traumatization: resulting in increased levels of hippocampal neurotrophic factors.
710 Metabolic brain disease. 2007;22:183-95.
711 [77] Marais L, van Rensburg SJ, van Zyl JM, Stein DJ, Daniels WM. Maternal separation of
712 rat pups increases the risk of developing depressive-like behavior after subsequent chronic
713 stress by altering corticosterone and neurotrophin levels in the hippocampus. Neuroscience
714 research. 2008;61:106-12.
715 [78] Daniels WM, Fairbairn LR, van Tilburg G, McEvoy CR, Zigmond MJ, Russell VA, et al.
716 Maternal separation alters nerve growth factor and corticosterone levels but not the DNA
717 methylation status of the exon 1(7) glucocorticoid receptor promoter region. Metabolic brain
718 disease. 2009;24:615-27.
719 [79] Hellweg R, Zueger M, Fink K, Hortnagl H, Gass P. Olfactory bulbectomy in mice leads
720 to increased BDNF levels and decreased serotonin turnover in depression-related brain areas.
721 Neurobiology of disease. 2007;25:1-7.
722
723
724
725
726
727