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1 **TITLE:**

2 Animal Models of Depression - Chronic Despair Model (CDM)

3

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24

25 **SUMMARY:**

26 The chronic despair mouse model (CDM) of depression consists of repetitive forced swim  
27 sessions and another delayed swim phase as a read-out. It represents a suitable model for  
28 induction of a chronic depressive-like state stable for at least 4 weeks, amendable to evaluate  
29 subchronic and acute treatment interventions.

30

31 **ABSTRACT:**

32 Major depressive disorder is one of the most prevalent forms of mental illness and causes  
33 tremendous individual suffering and socioeconomic burden. Despite its importance, current  
34 pharmacological treatment is limited, and novel treatment options are urgently needed. One  
35 key factor in the search for potential new drugs is evaluating their anti-depressive potency in  
36 appropriate animal models. The classical Porsolt forced swim test was used for this purpose for  
37 decades to induce and assess a depressive-like state. It consists of two short periods of forced  
38 swimming; the first to induce a “depressed state” and the second on the following day to  
39 evaluate the antidepressant effect of the agent given in between the two swim sessions. This  
40 model might be suitable as a screening tool for potential antidepressant agents but ignores the  
41 delayed onset of action of many antidepressants. The CDM was recently established and  
42 represented a modification of the classical test with notable differences. Mice are forced to  
43 swim for five consecutive days, following the idea that in humans, depression is induced by  
44 chronic rather than by acute stress. In a resting period of several days (1–3 weeks), animals

45 develop sustained behavioral despair. The standard read-out method is the measurement of  
46 immobility time in an additional delayed swim session, but several alternative methods are  
47 proposed to get a broader view of the mood status of the animal. Multiple analysis tools can be  
48 used targeting behavioral, molecular, and electrophysiological changes. The depressed  
49 phenotype is stable for at least 4 weeks, providing a time window for rapid but also subchronic  
50 antidepressant treatment strategies. Furthermore, alterations in the development of a  
51 depressive-like state can be addressed using this approach. CDM, therefore, represents a useful  
52 tool to better understand depression and to develop novel treatment interventions.

53

#### 54 **INTRODUCTION:**

55 Affective disorders, like major depressive disorder, are among the most frequent and  
56 challenging mental illnesses and are associated with high individual suffering<sup>1</sup>, an increase of  
57 suicide risk<sup>2</sup>, and cause a considerable socioeconomic burden<sup>3</sup> for society. Despite its impact,  
58 treatment options are limited, and there is an urgent need for the development of novel  
59 antidepressive interventions, especially due to the innovation crisis in psychopharmacology  
60 over the last decades. In order to understand the pathophysiology of depression and test  
61 potential new agents, rational and valid animal models are urgently needed<sup>4</sup>. For almost half a  
62 century, the classical forced swim test (FST), originally described by Porsolt<sup>5</sup>, was used as  
63 induction and read-out for screening of potential novel antidepressants. It consists of a forced  
64 swim period for 5–15 min on day one, subsequent one-time drug application, and evaluation of  
65 the portion mice spend immobile in water in another swim period on the following day. The  
66 immobility time was considered to represent a missing natural escape behavior and was  
67 thought to correlate with the degree of a depression-like state in the mice<sup>5</sup>.

68

69 The classical FST has been heavily criticized, not only in the scientific community<sup>6–8</sup> but also in  
70 public media<sup>8</sup>. Most controversies around the FST are due to the short induction and treatment  
71 periods of only one day in the classical paradigm. It was argued that FST represents rather an  
72 acute trauma model than a state comparable to human depression. Moreover, the Porsolt test  
73 might be suitable as a screening tool for potential antidepressive agents, but it ignores the  
74 delayed onset of action of many antidepressants.

75

76 The chronic despair model (CDM)<sup>9–15</sup>, which is derived from the original FST, represents a more  
77 appropriate animal model for depression. In CDM, repeated swim stress over five consecutive  
78 days avoids acute traumatic effects. By failing to escape from a repeated and ongoing stressful  
79 situation, mice are thought to develop a state of helplessness, surrender, and ultimately  
80 despair. This paradigm is more comparable to current psychological theories for the  
81 development of depression in humans than a single acute trauma, which is commonly  
82 experienced at the onset of a posttraumatic stress disorder. The resulting depression-like state  
83 in CDM is stable for up to 4 weeks<sup>9</sup> and therefore opens the possibility for longer treatment  
84 periods, which are better comparable to clinical conditions, where antidepressants usually need  
85 2–4 weeks to show a benefit<sup>16</sup>.

86

87 The evaluation of the depressive-like state should then be multidimensional. The measurement  
88 of immobility time, like in the classical FST, is useful, but should not be used as the only

89 outcome parameter. Various methods, which are described below, should be able to map  
90 different dimensions of a depressive state in line with symptoms usually found in depressed  
91 humans. Suitable read-out assessments could include escape behavior (immobility time<sup>9,10,17</sup>),  
92 tail suspension test (TST)<sup>9</sup>, anhedonia (classical sucrose preference test (SPT)<sup>18</sup>), motivation-  
93 oriented behavior (nose-poke sucrose preference test (NPSPT)<sup>10</sup>), expectation/exploration-  
94 behavior (response to ambiguous signal<sup>19</sup>; Y-maze test<sup>9</sup>), electrophysiology (measurements of  
95 long-term plasticity (long-term potentiation; LTP, long-term depression; LTD)<sup>20</sup>), molecular  
96 assessments (activation patterns of immediate early genes (IEGs); further stress patterns<sup>21</sup>).

97  
98 Theoretically, a repeated swim test can be used to induce a depressed state without any  
99 assessment of immobility time. However, it is strongly recommended to provide at least a  
100 proof-of-concept experimental series with immobility times. Additionally, CDM represents a  
101 suitable model to assess the development of a depressive-like state by measuring immobility  
102 time during the induction phase. Specific mouse strains or mice treated before swimming can  
103 be evaluated with respect to resilience or vulnerability to stress and the induction of behavioral  
104 despair.

105  
106 **PROTOCOL:**

107  
108 All experiments were performed in agreement with European guidelines (EU 2010/63) and in  
109 accordance with the German animal protection law (TierSchG), FELASA  
110 ([www.felasa.eu/guidelines.php](http://www.felasa.eu/guidelines.php)), the national animal welfare body GV-SOLAS ([www.gv-solas.de/index.html](http://www.gv-solas.de/index.html)) guide for the care and use of laboratory animals, and were approved by  
111 the animal welfare committee of the University of Freiburg and by the “Comite d’Ethique en  
112 Matiere d’Experimentation Animale de Strasbourg”(CREMEAS, CEEA35), as well as local  
113 authorities. Both sexes of C57Bl6N wild-type mice aged 10–14 weeks (70–98 post-natal days;  
114 PND) were used for wild-type (WT) indicated experiments. As a stress-resilient line, the  
115 transgenic mouse line with enhanced expression of adenosine A<sub>1</sub> receptors under the forebrain  
116 neuronal CaMKII promoter was used<sup>9,15</sup>. After the experiments, mice were sacrificed by cervical  
117 dislocation.  
118

119  
120 **1. Preparation**

121  
122 1.1. Obtain an animal research license, including thorough experimental planning.

123  
124 1.2. Arrival: Raise the animals in the animal facility to perform the CDM. In case the animals  
125 are bought from an external supplier, allow them to adapt for at least 2 weeks to the new  
126 environment.

127  
128 1.3. To house the animals, ensure that the cages are not occupied with the maximum  
129 number of animals to avoid additional stress. Guarantee that housing conditions are in line with  
130 international recommendations of mouse housing (for further information, see<sup>22</sup>) and  
131 constantly maintain them at all times.

132

133 NOTE: The most important standard housing conditions includes individually ventilated cages  
134 with 25–120 air changes per hour, 12-h light-dark cycle, temperature as stable as possible (at  
135 least constant between 20–24 °C), humidity as stable as possible (at least between 45%–65%),  
136 gnawing material and shelter present, no individual housing.

137

138 1.4. Perform all experiments at the same time of the day.

139

140 NOTE: No direct assessment has been made to verify the influence of the daytime on CDM, but  
141 most behavioral tests evaluating depressive-like states show variations depending on the time  
142 of the day<sup>23–25</sup>, and it is highly probable that daytime also influences CDM.

143

144 1.5. Reduce the nesting material to a minimum. Ensure there no running wheels etc.,  
145 present in the cage.

146

147 NOTE: Enriched environment prevents the induction of a depressed state.

148

149 1.6. Allow the animals to remain in the same group throughout the whole experiment.  
150 Group the female mice together even from different litters; group the male mice together with  
151 littermate male animals. Due to upcoming aggressiveness, especially of males, biting and  
152 barbering may become a problem, therefore give special emphasis to the group composition.  
153 Avoid single housing as deprivation is a major additional stressor.

154

155 1.7. Use different mouse strains, even though specific differences have been observed<sup>9,10</sup>. A  
156 frequently used mouse strain is C57Bl6N. Label mice in order to perform paired statistical  
157 analysis (see 3.2.4).

158

159 1.8. Equally use both male and female mice.

160

161 1.9. Ensure that the animals are at least 10 weeks (70 PND) old. Do not use younger animals  
162 due to the exhaustion caused by swimming.

163

164 1.10. Use a transparent glass cylinder/beaker with a capacity of at least 2 L, a diameter of 24–  
165 26 cm, and a minimum height of 30 cm. Further requirements include a thermometer to check  
166 the water temperature, paper towels, red light heating lamp/heating mat or comparable source  
167 of heating, timer, stopwatch, quiet surroundings. Videotape the swim sessions for offline  
168 analysis and documentation. Ensure that the date and the time are continuously visible on the  
169 tape/file, together with an identification code number for the individual animal. Store the files  
170 for later analysis and further reference. Film from the side of the glass cylinder, not from above,  
171 to facilitate analysis.

172

## 173 **2. Induction phase**

174

175 2.1. Before Starting

176

177 2.1.1. Visually observe the animals for abnormalities, including signs of biting or barbering.  
178 Exclude the whole cage from the experimental series if an animal shows any minimal injuries.  
179 Ensure that a veterinarian is available at any time as injuries will worsen during the experiment  
180 and will prevent continuation as mice become more aggressive under the influence of stress.

181

182 2.1.2. Obtain the body weight for each animal before starting the experiment. Ensure that the  
183 weight loss often observed does not exceed 20% of the initial body weight. Exclude animals  
184 with a weight loss of over 20% and immediately euthanize them, due to the assumed high  
185 suffering.

186

187 2.1.3. Fill up a beaker or cylinder with water at room temperature (22–23 °C) to a height of at  
188 least 20 cm from the bottom, leaving a minimum of 10 cm between the water surface and the  
189 upper border of the vessel.

190

191 2.2. Performance

192

193 2.2.1. Gently transfer the animals into the water. During the swim phase, observe the animal  
194 permanently to prevent drowning. Observe from a position where the animal cannot see the  
195 experimenter (for instance, video observation from a room next door).

196

197 2.2.2. Set a chronometer at the beginning of the experiment. Take out the animals of the  
198 water after 10 min by simply grabbing their tails. Gently dry them with a paper towel and put  
199 them either under a heating light or on a heating mat.

200

201 2.2.3. Evaluate only one animal at a time. Ensure that animals cannot see each other (for  
202 example, separate the housing cage from the experimental set-up by a room-divider).

203

204 2.2.4. Perform the forced swim session for a duration of 10 min each day for five consecutive  
205 days.

206

207 2.3. Finishing

208

209 2.3.1. Transfer the animals back to their home cages after five swim sessions and allow them  
210 to rest for at least 2 days. Start specific treatment interventions subsequently.

211

212 **3. Evaluation of an anti-depressive treatment**

213

214 3.1. Time course

215

216 3.1.1. Assess the acute and subchronic treatments with the CDM. Depending on the scientific  
217 question, adapt the resting period between the induction phase and the read-out.

218

219 3.1.2. To evaluate the acute and rapid-acting potency of ketamine, choose a short resting  
220 period (a few days) after the induction phase of CDM. Apply the treatment (i.e., intraperitoneal

221 injection), and then perform the evaluation (additional swim session or different evaluation  
222 method) shortly afterward.

223

224 3.1.3. To evaluate the effects of a subchronic treatment, increase the treatment period up to 4  
225 weeks (there is no data available for longer treatment periods). For example, give the oral  
226 treatment with imipramine to the animals during 4 weeks after the induction phase and  
227 evaluate thereafter.

228

229 3.1.4. Start to evaluate the depressed state right after the end of the treatment period, e.g.,  
230 the following day. Always choose an identical time period for control and experimental  
231 conditions.

232

233 3.2. Immobility time

234

235 3.2.1. Proof-of-concept

236

237 3.2.1.1. To use immobility time as a read-out method, evaluate each day of the induction  
238 phase and the test day to provide a proof-of-concept (see **Figure 1**). For further experimental  
239 series, reduce the assessments to day 1, day 5, and the test day (see **Figure 1C**).

240

241 3.2.1.2. Videotape each experiment. Allow two trained observers who are blinded to the  
242 experimental conditions to perform the analysis independently. Video analysis enables the  
243 experimenter to observe the behavior from a different room, therefore minimizing the  
244 interference with the test (for an example, see the video file in the **supplementary material**).

245

246 3.2.2. Conditions: Note that there are three different behavioral conditions during the swim  
247 test: struggling, swimming and immobility. Most researchers focus on immobility; a further  
248 differentiation between struggling and swimming is rarely useful and dramatically increases the  
249 complexity and duration of analysis.

250

251 3.2.2.1. Struggling: The animal actively tries to escape from the threatening situation.  
252 This involves pawing the side of the cylinder with the head oriented towards the wall and  
253 movements of all limbs. The water surface is typically slightly turbulent.

254

255 3.2.2.2. Swimming: The animal moves at least both hind paws and travels a distance  
256 throughout the water. It actively searches for a way out, but does not try to overcome the glass  
257 wall of the vessel. Swimming does not involve lifting the paws above the water surface, and the  
258 body is usually oriented parallel to the walls of the cylinder. In this condition, animals  
259 frequently turn around or move in circles.

260

261 3.2.2.3. Immobility: The animal keeps still, in a freezing-like position and does not move  
262 at all or only moves the tail or the forepaws to keep its head above the water surface. No  
263 distance is actively travelled except for passive floating, and no directed movement of the front  
264 paws observed.

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### 3.2.3. Tracking:

3.2.3.1. Perform the assessment using offline video recordings. Use blinded ratings by two independent and experienced examiners and calculate averages between the two ratings.

3.2.3.2. Repeat the ratings if the results of the two raters differ above a previously determined range. Continuously observe the mice as the different conditions change frequently between struggling, swimming, and immobility.

3.2.3.3. Use a stopwatch to measure the total time spent in a focused stage (usually immobility) over the 10 min the mouse stays in the water. Consider a short latency of about a second before changing the ongoing time measurement (e.g., if an animal remains for 20 s in immobility and only moves once for less than a second and returns to immobility for another 10 s, the total immobility time is 30 s).

3.2.4. Statistics: Due to the relatively high inter-individual standard deviation (probably caused by a transfer of hierarchy-depending behavior from the cage to the swim test), mark or label the animals to perform paired (instead of unpaired) parametric tests afterward. Evaluate the normality distribution and, depending on the specific question, perform analysis of variance (ANOVA) with post-hoc t-tests or paired t-tests to compare the different groups. Do the analysis in absolute values of immobility time (s) or as normalized values.

3.2.4.1. Absolute values: Give mean values of the immobility time from day 1 to day 5 and for the test day  $\pm$  SEM (see **Figure 1A**). Compare the averaged values for day 1 and day 5, preferably by a paired t-test to validate the induction of a depressed state. If there is a significant difference between day 1 and 5, compare the mean values of day 5 to the averaged results of the test day. Ensure that a typical group size in one experiment is between 6 and 10 animals and expect significant differences between baseline and post-induction immobility times in wild-type animals. Comparing different groups with an unpaired t-test is difficult if absolute values are used because of baseline differences; therefore, use normalized values.

3.2.4.2. Relative/Normalized values: Compare the different treatment effects by normalization to the individual result on day 5 and then express the values as a percentage of day 5 (see **Figure 1B**).

### 3.2.5. Control experiments

NOTE: The swimming performance might be correlated with locomotion. Substances that cause a hyper-locomotion could induce false-positive results (namely, a decrease of immobility time); as well as sedative agents could artificially increase immobility time.

3.2.5.1. Evaluate the changes in locomotion for unknown substances before performing



309 the swim analysis. Use Open Field Test (OFT) in a separate group of animals for at least 10 min.

310

311 3.2.5.2. Choose the same observation time (10 min) in the OFT as in the CDM to detect  
312 unspecific hyper-locomotive effects of the tested compound that might influence CDM read-  
313 out via measurement of immobility-time with high validity.

314

315 3.2.5.3. In case of significant hyper-locomotive effects, do not evaluate the swim session  
316 to assess the anti-depressive potency but use different read-out methods (for instance: sucrose  
317 preference, tail suspension test etc.).

318

#### 319 **4. Evaluation of the development of a depressive-like state**

320

321 4.1. To evaluate the development of a depressive disorder, assess each day of the induction  
322 phase to measure the immobility-time.

323

324 NOTE: In this case, a minor increase of immobility-time between each day describes resilience,  
325 whereas a stronger and earlier increase compared to untreated or wild-type animals represent  
326 an enhanced vulnerability to stress-induced despair. By treating mice before the swimming  
327 event, the preventive intervention or transgenic mouse lines could be assessed concerning the  
328 development of behavioral despair.

329

#### 330 **REPRESENTATIVE RESULTS:**

331 In the first swim session of the induction phase of CDM, mice usually show a mean immobility  
332 time between 190 s and 230 s, which constantly rises with every additional swim session  
333 (**Figure 1A**). This increase is more pronounced in the first three days and reaches a plateau-like  
334 phase during the last 2–3 days. The immobility-time measured on day 5 remains stable over up  
335 to 4 weeks, indicating a stable behavioral despair. The antidepressant potency of an  
336 intervention can be evaluated by treating the animal between the last day of the induction  
337 phase and the test day. Note that the absolute scoring time during the swim sessions is quite  
338 subjective and depends on the experimenter, age, sex and the mouse-line used. However, the  
339 relative difference between the sessions is fairly stable with only small interrater differences.

340 In **Figure 1**, several representative treatments are shown. Imipramine, sleep deprivation and  
341 ketamine significantly reduced the immobility-time, whilst sleep deprivation combined with a  
342 recovery sleep did not show a significant change of the depressive-like phenotype. These  
343 results are concordant with an anti-depressive potency of the applied treatments and similar to  
344 effects observed in human patients. The treatment involved ingestion of imipramine 20  
345 mg/kg/day for 3 weeks via drinking water, 3 mg/kg of ketamine by a single intraperitoneal  
346 injection 24 h before testing, and sleep deprivation for 6 h before testing.

347

348 Depending on the research question, various representations may be displayed. A  
349 representation of absolute values can give a real data overview and allows a good evaluation of  
350 the induction phase and of a single treatment (**Figure 1A,D**). However, the differences of  
351 various treatments cannot be directly compared; hence each treatment group has different  
352 mean values of immobility-time on day five. Therefore, it is recommended to use the

353 representation of normalized mean values in this case (**Figure 1B**). A reduced representation  
354 may be chosen due to space limitations (**Figure 1C**). Note that it is mandatory to show at least  
355 the results of day one, day five, and the test day.

356

357 [Place **Figure 1** here]

358

359 In case of an unchanged immobility time during all 5 days (**Figure 2**), the applied stress was not  
360 able to change the behavior relevantly, and no treatment effects can be evaluated; animals  
361 need to be sacrificed and must not be used further.

362

363 [Place **Figure 2** here]

364

365 Further read-out methods must be used to describe a broader view of the behavioral despair of  
366 the animals. A variety of behavioral tests, electrophysiological measurements, and molecular  
367 assessments of stress-induced changes are available. Exemplary results for Tail Suspension Test  
368 (TST), with CDM, imipramine and ketamine treatment, Nose-poke-Sucrose Preference Test  
369 (NPSPT) and assessment of long-term potentiation using the patch-clamp technique are given  
370 in **Figure 3**. These results encourage using CDM induction phase as a general tool for the  
371 induction of behavioral despair. For further detail of the used techniques (TST, NPSPT, LTP-  
372 assessment) see<sup>9,10,17,20</sup>.

373

374 [Place **Figure 3** here]

375

#### 376 **FIGURE AND TABLE LEGENDS:**

377

378 **Figure 1: Successful Results in absolute and normalized values.** (A) Successful induction of a  
379 depressed-like state in 30 mice can be observed. Each dot represents the immobility time of a  
380 single animal on a specific day and bars represent the mean values of the tested animals.  
381 Immobility time is represented for each day of the induction phase (day 1 to day 5) and for the  
382 test day (after the dotted line) with or without treatment. Note that in this sample, a significant  
383 increase can be observed between day 1 and day 2. In some cases, significance levels are first  
384 achieved between day 1 and day 3. For the continuation of the experiment, a statistically  
385 significant increase between day 1 and day 5 is mandatory. Note the typical ceiling effect  
386 (increase between day 1, 2, and 3, compared to the difference between day 4 and 5). Between  
387 day 5 and the test days, animals were housed for four weeks in their home cages, either  
388 without further treatment (CDM) or treated with imipramine (Imip.); sleep deprivation (SD);  
389 sleep deprivation and recovery sleep (RS), and ketamine (Ket). (B) Exemplary time course of  
390 the performance of individual animals are given for each day. (C) Normalized representation of  
391 the same results already shown in **Figure 1A**. The immobility time of each animal and day was  
392 normalized to its corresponding immobility time on day 5 and expressed in percentage. Post-  
393 treatment values of different groups can be better displayed and compared using this  
394 approach. (D) Representation of normalized values for day 1, day 5, and the test day. After a  
395 successful proof of concept, evaluation time points may be reduced to day (one,) five and the  
396 test day. These time points are needed because a significant increase between day 1 and day 5

397 is necessary to demonstrate a successful induction, and day 5 needs to be compared to the test  
398 day to give a statement on treatment efficacy. (E) Comparison of the immobility time of three  
399 different mouse lines: Wildtype (WT) shows a successful induction; an exemplary resilient-line  
400 (RL) shows a significantly decreased depression-like behavior on the first three days and on the  
401 test day. One-way ANOVA with Bonferroni *post hoc* test: \*/#p < 0.05, \*\*/##p < 0.01, \*\*\*/###p <  
402 0.001, \*\*\*\*/####p < 0.0001. (#indicate difference to mean values of day 1, \*indicate difference to  
403 mean values of day 5 in **Figure 1A,C** and to WT mouse line in **Figure 1E**). Data are expressed as  
404 the means  $\pm$  SEM.

405  
406 **Figure 2: Unsuccessful Results.** A representation of an ineffective induction is shown in the  
407 figure. Note that no significant increase in immobility time between day 1 and day 5 occurs.  
408 Therefore, criteria for continuation of the experiment were not achieved, and no further  
409 prolongation is rational (in this case, only male mice were tested, and after retrospective  
410 investigation, it was found that they were not littermates).

411  
412 **Figure 3: Additional results with CDM mice.** (A) An exemplary representation of the effects of  
413 CDM in the Tail Suspension Test. Mice were suspended by their tail, and the time spent  
414 immobile was recorded (for methodological details see<sup>9</sup>). Each dot represents the immobility  
415 time of a single animal, and bars represent the mean values of the tested animals. One-way  
416 ANOVA with Bonferroni *post hoc* test: \*\*\*p < 0.001. Data are expressed as the means  $\pm$  SEM. (B)  
417 Representative results of the recently established nose-pokes sucrose preference test in CDM  
418 mice. In this task, sucrose preference was measured with gradual increasing effort to reach the  
419 sucrose bottle (number of nosepokes) (for methodological details see<sup>10</sup>). Note that sucrose  
420 preference was decreased in CDM and that the difference between CDM and control mice  
421 gradually increases with the effort (mean values of nose pokes on each day indicated as Nspk1-  
422 7) mice had to apply to drink the sweet solution. Two-way ANOVA with Bonferroni *post hoc*  
423 test: \*\*p < 0.01, \*\*\*p < 0.001. Data are expressed as the means  $\pm$  SEM. (C) CDM-dependent  
424 changes in long-term synaptic plasticity are presented as changes of mean values of EPSPs after  
425 the application of an associative LTP induction protocol in hippocampal brain slices of WT mice.  
426 Data were obtained by stimulation of the CA3-CA1 synapse (for details see<sup>17,20</sup>). Unpaired *t*-test,  
427 \*\*p < 0.01, Data are expressed as the means  $\pm$  SEM.

## 428 429 **DISCUSSION:**

430 The CDM model represents a relevant and established model for testing the anti-depressive  
431 potency of new interventions and opens an extended time window for molecular or  
432 electrophysiological experiments to elucidate the pathophysiology of depression. Especially  
433 when combined with other tests to assess a depression-like state, CDM has a high face and  
434 concept validity. It combines subchronic stress and acquired helplessness for induction and  
435 produces a long-lasting depressive-like state. It is insensitive to the single application of classical  
436 antidepressants but responds to subchronic application and therefore mimics the situation in  
437 humans. In a time window of four weeks, many different antidepressive interventions show  
438 efficacy, ranging from different classes of antidepressants, non-invasive brain stimulation, sleep  
439 deprivation to rapid-acting antidepressants<sup>9-11</sup>. Furthermore, the measurement of immobility  
440 time during the induction phase could be used as a marker of stress resilience or vulnerability,

441 in case of testing transgenic animals or mice treated before the induction phase. All in all, the  
442 CDM is economical in terms of cost, duration, standardization, and reproducibility between  
443 labs. Even though the performance seems fairly simple -“you drop a mouse into a water vessel  
444 and take it out after 10 min”- there are several critical points that must be kept in mind in order  
445 to obtain reasonable and stable results. Most problems are due to insufficient accuracy during  
446 preparation or analysis.

447  
448 A commonly experienced problem is that mice, especially males, do not show a significant  
449 increase in immobility time in the induction phase. In these cases, mice might have been  
450 already stressed before the induction starts; therefore, additional stress during the swim  
451 protocol does not cause a relevant increase of despair. Note that immobility time seems to  
452 have a ceiling effect since the increase between day 1 and 2 is larger than between day 2 and 3,  
453 respectively. After day 3, usually, no further significant increase can be expected. Common  
454 reasons for excessive baseline stress might include recent transportation of the animals or  
455 cohabitation of adolescent/adult male animals, a condition that never occurs in nature.  
456 Therefore, the experimenter should be cautious and always assure that animals are littermates,  
457 that they had enough time to acclimatize to the new surroundings and that there are no signs  
458 of biting or barbering before the experiment starts. Furthermore, the animals must be weighed  
459 each day and weight loss must be controlled to not exceed 20% of the initial body weight. A  
460 greater weight loss is considered critical, due to the fact that repetitive swimming is exhausting  
461 and animals that are not capable of maintaining their body weight suffer too much from this  
462 exhaustion. A critical point here is that animals suffering too much from exhaustion are  
463 probably not able to swim or struggle for ten minutes during the test. When immobility times  
464 of those animals are analyzed, they tend to show a false negative outcome due to physical  
465 exhaustion.

466  
467 Another problematic circumstance that sometimes occurs, especially when longer treatment  
468 periods are required, is a spontaneous decrease of immobility time in the test evaluation. After  
469 four weeks, immobility time usually decreases compared to assessments performed two days  
470 after the end of the induction period (N.B. this corresponds, although with a different time  
471 scale, to the situation in humans where depressed episodes are usually self-limiting). To  
472 minimize this pitfall, it should be guaranteed that no unnecessary nesting material is applied to  
473 the animal’s home cage, which can be regarded as an effective antidepressive intervention  
474 (enriched environment). Furthermore, an increase in group size might help to decrease  
475 variance. If necessary, an additional swim session may be added as a modification of the  
476 standard protocol described above. For instance, an increase from five to seven swim sessions  
477 on seven consecutive days could be performed and should result in a more stable depressed  
478 state of the animals. It is not recommendable to further increase the duration of the individual  
479 swim session to avoid excessive exhaustion.

480 NOTE: There is no agreement within the scientific community about the most sensible time  
481 frame to be analyzed. Whilst some groups consider all 10 min important<sup>9,10</sup>, others argue that  
482 the behavior within the first minutes represent an “acute stress” situation and analyze only the  
483 last 4 min or 6 min<sup>18</sup>. The latter assumption is mainly derived from the common practice in the  
484 evaluation process of the classical FST. Experimental evidence addressing the question of the

485 most rational time frame to be analyzed in CDM is missing. Various high-ranked publications  
486 used the analysis of the whole 10 min in CDM<sup>9,10</sup>.

487  
488 Despite increasing numbers of commercially available software for automated video analysis,  
489 no set-up has demonstrated sufficient accuracy to replace a trained observer. Most software  
490 relies on tracking of locomotion of mice in the water and requires a camera position from  
491 above. Assessments by skilled humans have the advantage that not only locomotion but also  
492 assumed intention of more complex movements can be assessed, including the intensity of paw  
493 movements. For instance, mice frequently move by turning around their body or by subtle tail  
494 movements to keep their head above the water, which software usually tracks as swimming.  
495 Another example is the movement directed towards the glass wall of the vessel, including  
496 frequent nose poked from a short distance. Despite the clear intention to escape by vertical  
497 movements, the software frequently tracks immobility due to little locomotion. However,  
498 accurate and reliable assessments remain difficult and time-consuming. It is recommendable to  
499 train a rater by an experienced experimenter and prepare joint assessments of sample videos  
500 by the two independent raters to discuss common definitions and ambiguities. Moreover, the  
501 first results of a laboratory with the CDM should be compared to previously published results  
502 from other groups.

503  
504 Researchers using the CDM might frequently experience the notion that increased immobility is  
505 a rather “intelligent” end energy-saving learned reaction of mice to an inescapable but  
506 temporary stressful situation. In our opinion, this overrates the cognitive flexibility of mice;  
507 however, it emphasizes the necessity for further assessments of a depressed state independent  
508 of immobility time. It can further be argued that other well-established animal models of  
509 depression as the Chronic Mild Stress test, produce similar outcomes; and that a depressed  
510 state or strong stressors impede, not increase learning both in humans and in animals<sup>17,20,26–30</sup>.

511 The burden of the animals is usually rated as high to extreme in animal research applications.  
512 Experimental series should be thoroughly planned to minimize the number of animals, and  
513 animals should be treated with care and respect before and after the swim sessions. However,  
514 in some countries, it might not be possible to obtain an animal research license for the CDM.  
515 The CDM allows the assessment of anti-depressive efficacy of a wide range of interventions and  
516 the induction of a relatively stable depressed state. The heterogeneity and complexity of major  
517 depressive disorder in humans cannot be replicated in any animal model. Most animal models  
518 of depression are based on stress-induced/trauma-like experience in mice, which is not  
519 necessarily the case in humans, where childhood deprivation, complex learning history and/or  
520 sociocultural risk factors also seem to be important. Mouse models of depression should  
521 therefore be recognized as what they are: a simplified model for a highly complex disorder.  
522 However, if performed adequately and if multiple read-out methods are used, the CDM is a  
523 suitable tool in the search for novel Insights and targets in depression research.

524  
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531

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533 All authors declare no conflict of interests.

534

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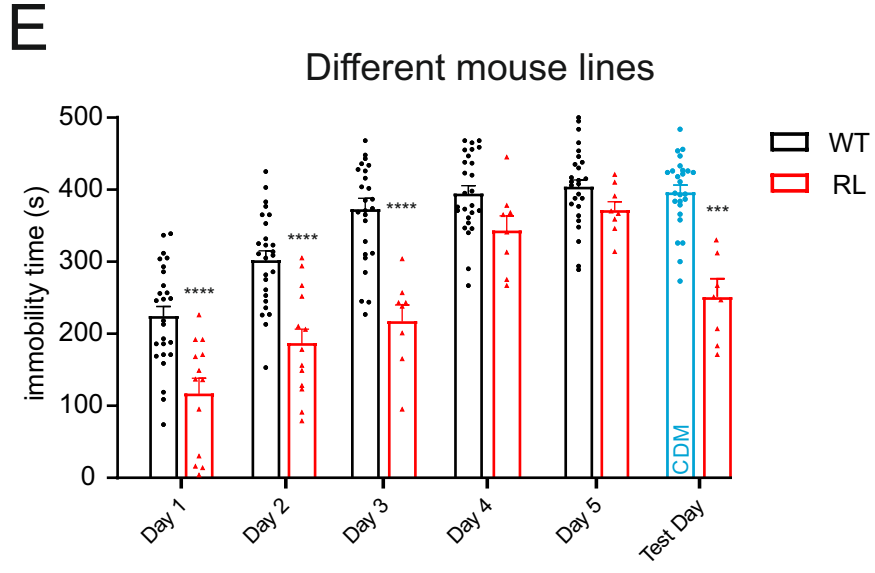
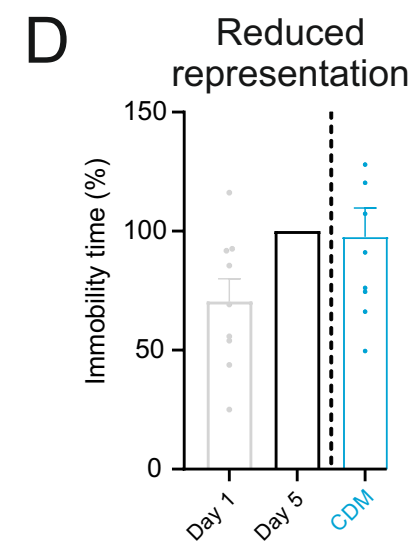
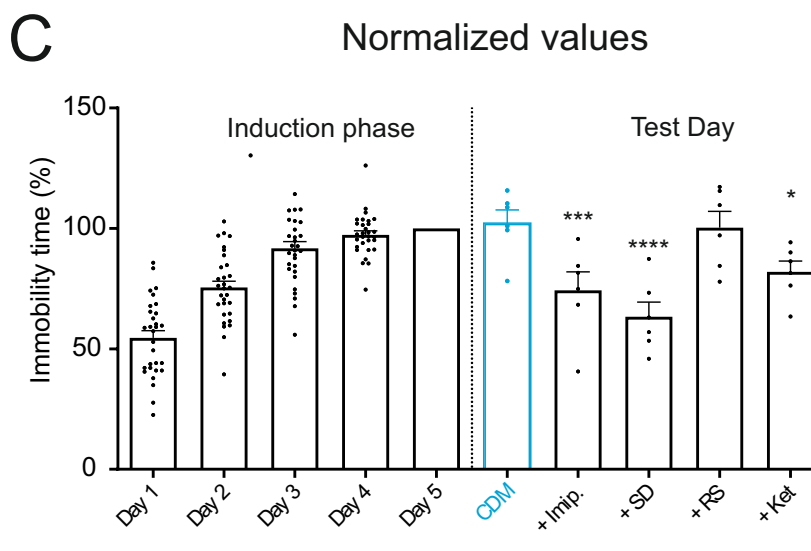
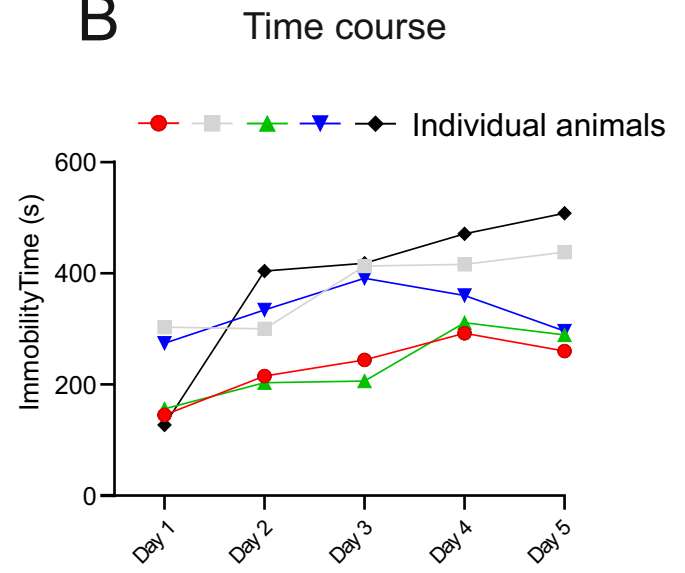
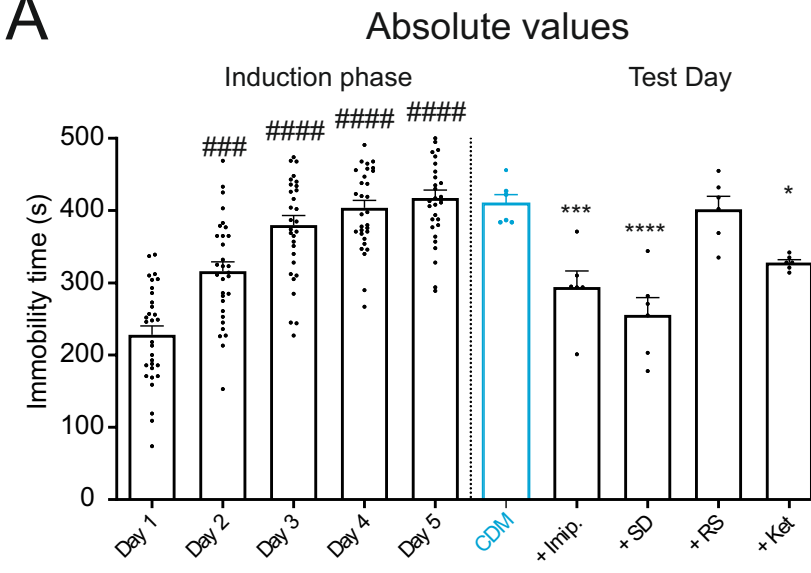
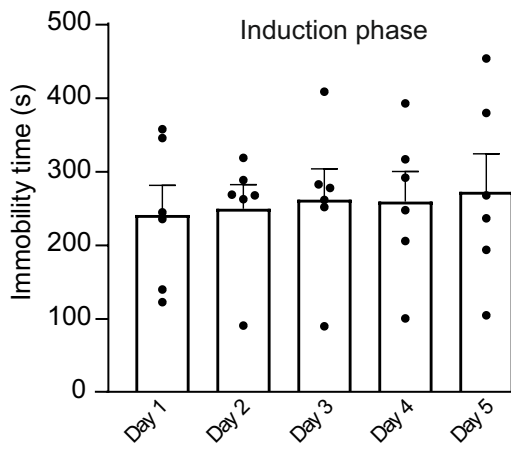
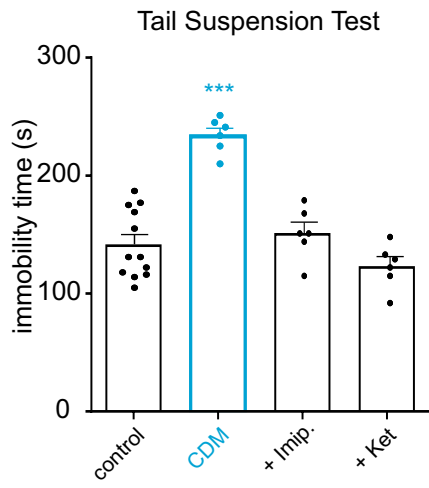
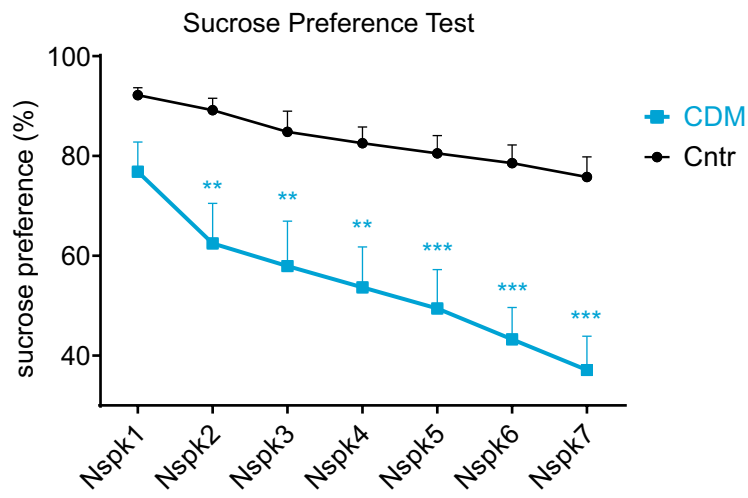
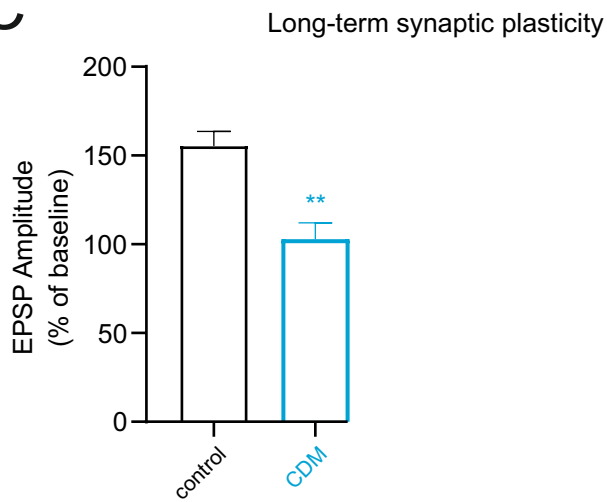


Figure 1



# Absolute values



**A****B****C****Figure 3**