

Behavioural responses of anxiety in aversive and non-aversive conditions between young and aged Sprague-Dawley rats

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Highlights

1. Aged and young animals display different severity of anxiety when tested in behavioural assays modified with aversive and non-aversive conditions.
2. Aged animals demonstrate greater anxiety than young animals.
3. Different behavioural assays measure different aspects of anxiety.
4. Future studies should employ various behavioural assays to better characterise the degree of anxiety-like responses.

51 **ABSTRACT**

52 Measures of anxiety in behavioural tests remain largely unclear even decades after
53 their establishment. Differences in the severity of anxiety measured by anxiety tests is
54 an important issue that must be addressed. To test the hypothesis that the addition of
55 light as an aversive stimulus will elicit a difference in behaviour between aged and
56 young animals, we compared the responses of aged and young animals in the home
57 cage emergence test (HCET) and elevated plus maze (EPM), in high aversive bright
58 light and low aversive dim light conditions. In the HCET, our results demonstrated that
59 young animals escaped with shorter latency and greater frequency than aged animals
60 in both bright and dim light conditions, indicating that young animals display greater
61 exploratory tendencies than aged animals. In the EPM, bright light conditions induced
62 anxiogenic effects in both age groups. Interestingly, two-way ANOVA showed a
63 significant interaction effect of age and light on the number of entries into the open
64 arms of the EPM as well as frequency of escape in the HCET. These results show that
65 the addition of light as an aversive stimulus in the EPM and HCET produced different
66 responses in aged versus young animals in each test. In conclusion, significant
67 interactions between age and light affected aged and young animals differently in the
68 HCET and EPM, indicating that the two tests measure different aspects of anxiety.

69
70 **Keywords:** Anxiety, Home Cage Emergence Test, Elevated Plus Maze, Aged, Young
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75 INTRODUCTION

76 Anxiety-related disorders affect 40 million adults in the United States every year [1].
77 Preclinical studies examining anxiety-like behaviours assess behavioural and
78 physiological responses to simulated naturalistic environments containing anxiogenic
79 stimuli. These tests vary in **procedure** and methodology and are largely classified into
80 two **categorizations**, unconditioned response tests and conditioned response tests [2].
81 Various behavioural testing paradigms have been developed to measure anxiety-like
82 responses in rodents. These tests depend on established preferences and
83 behavioural responses that correlate with anxiety and psychological stress indicators.
84 The approach-avoidance paradigms make use of natural behavioural phenotypes of
85 rodents, such as affinity to dark environments, aversion to height and avoidance to
86 novel objects to determine approach-avoidance behavioural responses, **which are**
87 **examples of unconditioned responses** of anxiety-like behaviour [3, 4]. **Conditioned**
88 **response tests commonly use stimuli associated with a predator or fear conditioning**
89 **to respond to a light or sound cue, with long delays between cue introduction and**
90 **shock administration thought to reflect anxiety-like response [3, 5]. Defensive**
91 **behaviours in rodents, such as freezing, have also been used to assess anxiety-like**
92 **behaviour.** The validity of these tests have been verified by consistent behavioural
93 changes observed in response to antidepressant and pharmacological compound
94 treatments, using behavioural assays such as elevated plus maze (EPM) [6, 7], social
95 interaction test [8] and shock probe burying test [5, 9].

96

97 The EPM and home cage emergence test (HCET) are categorised as approach-
98 avoidance test paradigms that examine the exploratory behaviours of animals as a
99 measure of anxiety-like behaviour. The EPM consists of closed arms, which provides

100 dark enclosures that rodents prefer, and open arms, which elicits an unconditioned
101 fear of heights and open spaces [10]. The EPM is used to assess anxiety by comparing
102 the amount of time spent by the animal in the open arms and closed arms. The HCET
103 is a measure of neophobia, which is the tendency to avoid novel objects and places
104 [11]. The latency of animals to exit their home cage is an indicator of anxiety-like
105 behaviour. Ramos has previously acknowledged that each test does not necessarily
106 capture the full scope of anxiety, and that a battery of tests might distort the accuracy
107 of results in each test. Ramos proposes a 'triple test' wherein several tests of anxiety
108 were combined into a single setup [12]. However, it remains to be seen whether the
109 nature of such a protocol would not affect animals in the same way as a battery of
110 tests.

111

112 Compelling evidence in human studies have suggested that the prevalence of anxiety
113 disorders increases throughout the adult lifespan until the age of 70 [13-15]. Preclinical
114 studies examining aging and anxiety have analysed animal responses to novel
115 environments by placing them in an open field, with some findings indicate that aged
116 animals exhibit less exploratory behaviour than young animals but no reduction in
117 locomotion [16, 17] while others show the opposite [18, 19]. However, it is noted that
118 the open field does not exclusively test for anxiety. Thus, it is suggested that the EPM
119 may be a more reliable test of anxiety-like behaviour for rodents [6]. Some studies
120 reported that aged animals spent a great amount of time in the open arms of EPM
121 compared to that of young animals [20, 21] whereas others reported the opposite [22-
122 24]. Previous studies have also attempted to modify the protocol of testing to achieve
123 a more accurate and consistent representation of anxiety-like behaviours throughout

124 age groups, taking such measures as reducing pre-test handling, and imposing mild
125 feeding restrictions [18, 25].

126

127 Aversive stimuli have the capacity to alter behaviour in animals and humans by
128 suppressing or encouraging coping behaviours [26]. Indeed, certain behavioural tests
129 utilize aversive stimuli to measure approach-avoidance behaviours of animals.
130 Exploratory behaviour of animals is attenuated in novel environments and can be
131 reversed by administration of anxiolytic drugs [27], which indicates the anxiogenic
132 nature of adverse environments and their effects on emotional behaviour of animals.
133 Light has been established as an aversive stimulus in rodents in anxiety-related
134 behavioural paradigms [28-31]. Behavioural alterations in the presence of light such
135 as aversion to light when feeding, escape to burrows in the presence of light but not
136 in darkness, and corresponding hormonal activity such as increased noradrenergic
137 response indicate the anxiogenic properties of light [32]. Previous studies have also
138 used aversive stimuli such as odour and foot shock to shed light on learning processes
139 [33, 34] and biological processes [35-37].

140

141 **The present study** seeks to discern the differences between anxiety responses in
142 young and aged animals by including an additional aversive stimulus of lighting levels
143 in established paradigms. We hypothesize that the bright light condition will reveal
144 differences in behavioural responses between young and aged animals, which might
145 differentiate aspects of anxiety measured by HCET and EPM. The present study also
146 seeks to reduce the likelihood of generating a false positive result due to inflated effect
147 size by utilizing a large sample size [38]. Since each animal cannot be tested multiple

148 times in the same apparatus, we have taken the approach of increasing the sample
149 size for the test paradigms.

150

151 **MATERIALS AND METHODS**

152 *Subjects*

153 Young (n=58; 3 months old) and aged (n=56; 22 months old) male Sprague-Dawley
154 rats were housed in pairs under controlled temperature ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$), humidity (60% -
155 65%), and 12-hour dark/light cycles (light from 21:00 to 09:00). Food and water were
156 available ad libitum. Animals were randomly assigned to the following experimental
157 groups: 1) Young – Dim Light (n=29); 2) Young – Bright Light (n=29); 3) Aged – Dim
158 Light (n=28); and 4) Aged – Bright Light (n=28). All animal procedures were approved
159 by the Committee on the Use of Animals for Teaching and Learning, The University of
160 Hong Kong.

161

162 *Behavioural Testing*

163 This present study investigated the effects of light as an aversive stimulus on anxiety-
164 like behaviours of young and aged male animals using the EPM and HCET paradigms.
165 Animals were tested in the HCET, followed by EPM, with one day of rest in between.
166 This order of tests allows is supported by previous studies showing EPM results is not
167 affected by pre-exposure to other testing environments [10]. Furthermore, the EPM
168 has been noted to be more stressful than the home cage-based tests [39]; and thus,
169 in our study the EPM was conducted after the HCET. Previous studies on rats
170 assessing brightness as aversive stimuli in different behavioural paradigms have
171 utilised a range of illumination for the bright light/dim light conditions [29-31]. In our

172 study, we have used 5 lux and 450 lux for dim light and bright light conditions,
173 respectively in both the HCET and EPM test paradigms.

174

175 *Home Cage Emergence Test (HCET)*: The HCET was performed as previously
176 described with minor modifications [40]. In brief, a wire grid ramp was placed in the
177 animal's home cage leading to a new cage, each measuring 42.5 x 26.6 x 18.5 cm.
178 Behaviour was recorded over 5 mins and the escape latency from the home cage, as
179 well as the frequency of entry into the new cage were analysed. The animals were
180 tested in either a dim light (5 lux) condition or a bright light (450 lux) aversive condition
181 by shining a lamp on the new cage arena (Fig. 1).

182

183 *Elevated plus maze (EPM)*: The EPM test was conducted as previously described with
184 minor modifications [41]. In brief, the elevated plus maze contained two open arms
185 intersecting two closed arms made of black Plexiglas and elevated by 40 cm. Each
186 open and closed arm measured 50 x 15 cm, with the closed arms surrounded by 15-
187 cm high walls. Animals were placed in the centre of the EPM and recorded for 5 mins,
188 in either a dim light (5 lux) condition or a bright light (450 lux) aversive condition by
189 shining a lamp on the open arms (Fig. 2). The duration and frequency of entry into
190 both open and closed arms were video recorded and analysed as above.

191

192 *Statistical Analysis*

193 The behavioural data were presented as individual values for each animal and as the
194 mean \pm S.E.M. The normality of behavioural data was tested by the Shapiro-Wilk test.

195 All data were analysed using two-way ANOVA with simple effects analysis and

196 **Bonferroni corrections for pairwise comparisons.** All p-values ≤ 0.05 were considered
197 significant.

198

199

200 **RESULTS**

201 *Anxiety-like behaviour of aged animals in the HCET*

202 In the HCET, two-way ANOVA showed a significant age effect in the escape frequency
203 ($F_{(1,110)}=378.35$, $p<0.001$) (Fig. 1a) and escape latency ($F_{(1,110)}=360.56$, $p<0.001$) (Fig.
204 1b), with aged animals exhibiting longer escape latency and escape the home cage
205 **less frequently** compared to young animals. No significant difference was observed
206 for the light condition ($F_{(1,110)}=0.523$, $p=n.s.$) or interaction effects of age x light
207 ($F_{(1,110)}=0.393$, $p=n.s.$) in the escape latency (Fig, 1b). **HCET escape was** significantly
208 affected by age ($F_{(1,110)}=378.350$, $p<0.001$) and interaction of age x light
209 ($F_{(1,110)}=3.945$, $p=0.05$) with no significant effect of light conditions ($F_{(1,110)}=0.233$,
210 $p=n.s.$) (Fig, 1a). In the HCET, the interaction of age and light was found to be
211 significant; aged animals show greater escape frequency in bright light compared to
212 dim light ($F_{(1,110)}=152.515$, $p<0.001$) (Fig. 3a), whereas young animals escaped more
213 frequently in dim light compared to bright light ($F_{(1,110)}=229.779$, $p<0.001$) (Fig. 3a).

214

215 *Anxiety-like behaviour in the EPM induced by bright light conditions*

216 Aged animals spent more **time than the young animals** in the closed arms and less
217 time in the centre zone and open arms of the EPM. In the EPM, there was a significant
218 main effect of age found in the time spent in the closed arms ($F_{(1,110)}=11.754$, $p=0.001$)
219 (Fig, 2c) and centre zone ($F_{(1,110)}=10.733$, $p=0.001$) (Fig. 2e). A significant effect of
220 age was also observed for the **frequency of closed arm entries** ($F_{(1,110)}=71.463$,

221 $p < 0.001$) (Fig 2d), **open arm entries** ($F_{(1,110)} = 22.782$, $p < 0.001$) (Fig, 2b) **and centre**
222 **zone entries** ($F_{(1,110)} = 50.046$, $p < 0.001$) (Fig. 2f), with aged animals entering all zones
223 with decreased frequency. No significant effect of age was found for the time spent in
224 the open arms ($F_{(1,110)} = 2.81$, $p = n.s.$) (Fig 2a). Interestingly, in bright light, both groups
225 spent more time in the closed arms ($F_{(1,110)} = 87.962$, $p < 0.001$) (Fig. 2c), and less time
226 in the anxiogenic regions of open arms ($F_{(1,110)} = 60.718$, $p = 0.001$) (Fig, 2a) and centre
227 zone ($F_{(1,110)} = 25.801$, $p < 0.001$) (Fig. 2e). The significant effect of light condition was
228 also observed for the frequency of animals entering the open ($F_{(1,110)} = 44.551$,
229 $p < 0.001$) (Fig. 2b), closed ($F_{(1,110)} = 6.967$, $p < 0.05$) (Fig. 2d), and centre zones
230 ($F_{(1,110)} = 25.380$, $p < 0.001$) (Fig. 2f) of the EPM. Although significant effects of age and
231 light interactions were found for the frequency of animals entering the open arms
232 ($F_{(1,110)} = 5.579$, $p < 0.05$) and centre zone ($F_{(1,110)} = 4.147$, $p < 0.05$), no statistical
233 significance of age x light interaction was detected for frequency of animals entering
234 the closed arms ($F_{(1,110)} = 0.466$, $p = n.s.$) or the time spent in the open arms
235 ($F_{(1,110)} = 0.726$, $p = n.s.$), closed arms ($F_{(1,110)} = 0.124$, $p = n.s.$) and centre zone
236 ($F_{(1,110)} = 0.237$, $p = n.s.$) of the EPM. In the EPM, simple effects analysis showed that
237 there was no difference between young and aged animals in frequency of open arm
238 entries in the bright light condition ($F_{(1,110)} = 2.907$, $p = n.s.$) (Fig. 3b). However, under
239 dim light conditions, young animals entered the open arm of the EPM ($F_{(1,110)} = 25.454$,
240 $p < 0.001$) (Fig. 3a) with greater frequency compared to aged animals, which indicates
241 that young animals exhibit less anxiety compared with their aged counterparts in dim
242 light.

243

244 These analyses suggest the severity of anxiogenic stimuli in the EPM in dim light
245 condition is greatly reduced in young animals compared to aged animals. While in the

246 HCET, the behavioural differences observed in aged and young animals is less
247 affected by either dim or bright lighting condition. This indicates that light exerts a
248 greater aversive effect in the EPM test paradigm compared to the HCET paradigm.

249

250 **DISCUSSION**

251 The results show that aged animals exhibited significantly longer escape latency and
252 lower escape frequency in the HCET compared to young animals in both dim and
253 bright light conditions, indicating the aged animals exhibited greater anxiety in both
254 conditions. However, there was no significant difference in the time spent in the open
255 arms of EPM between aged and young animals in bright light conditions, with both
256 groups spending significantly more time in the open arms in the dim light condition
257 compared to the bright light condition. Young animals showed lower avoidance
258 response to light in the HCET compared to the EPM, indicating the light had more
259 aversive effects in the EPM than in the HCET test paradigms. Light aversion in rodents
260 has been previously reported [42], but this raises the question as to why the animal
261 behavioural responses to light varies in these two different behavioural paradigms.
262 Response to light is an unconditioned behaviour in rodents and is a measure of state
263 anxiety, which refers to anxiety that is transiently induced in an animal by an
264 anxiogenic stimulus. This unconditioned behaviour combined with the well-established
265 anxiogenic effects of the open arms in the EPM [10] is sufficient to reduce entry
266 frequency and time spent in the open arms in both young and aged rats in aversive
267 light conditions.

268

269 Different behavioural tests for anxiety-like behaviours examine different aspects of
270 anxiety. Certain tests introduce an aversive stimulus to induce an anxiety-like

271 response, whereas others introduce an approach-avoidance conflict to interfere with
272 instinctive animal behaviours [4]. The EPM is a classic example of the latter, in which
273 our data obtained on the frequency of entry to the open arms suggests that age and
274 light as well as the interaction between them are major factors in determining how the
275 animal responds in the EPM test paradigm. However, the anxiety-like behaviours of
276 young and aged animals in our study did not follow this pattern in the HCET test
277 paradigm, despite the HCET being a test that measures neophobia, which is an
278 unconditioned approach-avoidance response. The effect of light was not significant in
279 the HCET but was significant in the EPM, indicating that aversive stimuli affects
280 behaviour in the two tests differently.

281

282 In the HCET, when compared to the aged animals, simple effects analysis showed
283 that young animals escaped more frequently in the dim light condition as opposed to
284 the bright light condition. In the EPM, it was found that young animals entered the
285 open arm with higher frequency compared to aged animals in the dim light condition.
286 This indicates that the aspects of anxiety measured in the HCET may not be the same
287 as measured in the EPM. The EPM has long been used as a test for anxiety in rodents,
288 as the open arms act as a fear inducing stimulus [6] and are avoided by rodents in
289 most cases. Research on the EPM has shown that thigmotactic stimuli such as the
290 height of the walls of the open and closed arms greatly influence the time spent in
291 those zones [43], and that rodents avoid the open arms as a fear response [44]. On
292 the other hand, the HCET paradigm is described as a test for neophobia, which tests
293 animals' tendency to remain in a familiar environment or explore novel areas [11].
294 Exploratory behaviour is influenced by motivation to gather information about novel
295 environments and neophobia which encourages remaining in a familiar and secure

296 environment, thus exploratory behaviour has been studied as an indicator of anxiety
297 [45-48].

298

299 In the present study, aged and young animals display differences in behaviour when
300 tested in the HCET and EPM test paradigms. Interestingly, these findings are contrary
301 to previous studies using both EPM and HCET to measure anxiety-like behaviours in
302 rats found that results of both tests were largely analogous [49, 50]. One possible
303 explanation might be the variable expression of anxiety-like behaviours between rat
304 strains, as both these studies used Wistar rats compared to Sprague-Dawley rats used
305 in the present study. Different expressions of anxiety-like behaviours between these
306 two strains have been documented in a previous study, whereby Wistar rats exhibited
307 greater anxiety than Sprague-Dawley rats in the social interaction test and EPM,
308 respectively [51]. The shorter escape latency exhibited by the young animals in the
309 HCET in this study may be due to their high exploratory drive, as reported in a previous
310 study that showed young mature rats (90 days old) had higher exploratory drive than
311 their aged counterparts (12 and 24 months old) [52]. As in the EPM, exploratory drive
312 may also explain differences in the behaviour between young and aged rats in the
313 HCET, suggesting exploratory behaviour plays a greater role than the anxiogenic
314 response.

315

316 Aging has been associated with decline in learning and cognition, including the
317 learning of cues related to fear [53]. It has been shown that aged animals **that have**
318 **been exposed to the EPM twice would spend more time** in the open arms compared
319 to younger animals, despite spending less time than young animals in the first test.
320 Furthermore, repeated measures analysis of data showed that risk assessment

321 behaviour decreases with age in the initial trial in the EPM as well as the retest [54]. It
322 is for this reason that EPM was not repeated on an animal, rather, a larger sample
323 size was used. Aged animals also show a decrease in locomotion and exploratory
324 behaviour and are unable to form or recall memories about spatial environments [19,
325 55]. Aging has also been shown to be correlated with decreased locomotion with less
326 activity in the open field as well as reduced response to changes in the environment
327 and decreased exploratory behaviour relative to young animals [56-58]. The minutiae
328 observed in the behaviour of aged animals such as reduced fine movements and
329 ambulation during the dark cycle compared to young animals as well as immediately
330 following the transition between light and dark cycle [59] reinforces the notion that
331 aged animals exhibit attenuated exploratory tendency and response to novelty.
332 Indeed, reduction of locomotion is to be expected in aged animals, however this has
333 been shown to be independent of age dependent increase in anxiety [60, 61]. We
334 found that aged animals escape less frequently than young animals in both dim and
335 bright light conditions in the HCET, and in the EPM aged animals also spent less time
336 and entered the open arms less frequently than young animals. These results are in
337 line with previous work that described increase of anxiety-like behaviours in aging.

338

339 There has been relatively little research investigating the differences in multiple
340 anxiety assays in aged animals. Existing data suggests that the performance of
341 anxiety assays is relatively consistent regardless of age and strain [62, 63]. It has been
342 demonstrated that control animals tested in the EPM and open field show that aged
343 animals as well as animals of the same strain perform similarly in both tests. Another
344 study on anxiety in different rat strains found that behavioural trends in anxiety were
345 consistent between tests [64]. However, the same study noted that risk assessment

346 behaviours were not associated with measures of anxiety, but rather locomotion and
347 novel environments, and posited that differences in behaviour might have be caused
348 by increased aversiveness introduced in the different tests [64]. The aged animals
349 may have had decreased exploratory drive, as aged rats were observed to prefer
350 familiar environments over novel environments [17], but this needs further
351 investigation. We have found that young animals displayed greater anxiety in dim light
352 conditions in the EPM than in the HCET, whereas aged animals exhibited similar
353 degree of anxiety to young animals tested in high aversive conditions in bright and dim
354 light conditions in both HCET and EPM. This indicates that the HCET appears to
355 measure exploratory tendencies more than anxiety. Anxiety is a complex construct
356 that can be separated into subsets, such as fear and apprehension. The EPM
357 assesses fear-based anxiety using innate fear of elevation while the HCET poses a
358 choice between a familiar environment and a novel environment, thus it should be
359 expected that while the constructs measured in both tests fall broadly into anxiety,
360 there are differences that may be pertinent to behavioural testing. As novelty-seeking
361 and avoidance behaviours have been established to be distinct and independent of
362 one another [65], further research on the differences in the degree of anxiety
363 measured in the various anxiety assays might conclusively identify specific types of
364 anxiety that each test measures.

365

366 A limitation of our present study includes the limited scope of test paradigms employed
367 (i.e. only two behavioural tests were investigated) to study the anxiety-like behavioural
368 differences between young and aged animals across the various tasks. Other
369 measures of anxiety-like behaviour test paradigms could have included the light-dark
370 box test, social interaction test and novelty-suppressed feeding test [66-68]. Analyses

371 of a greater number of tests would have been more informative in terms of identifying
372 the differences that may exist between the range of aversive stimuli used in each
373 behavioural test. Another limitation is the use of only male rodents in our study. Further
374 studies could be performed on females, as it has been previously reported that
375 females have different behavioural responses to male test subjects [69-72].
376 Nonetheless, the data presented here represents the first step in clarifying the varying
377 degrees of anxiety-related behavioural responses in different test paradigms.

378

379 In conclusion, the present study revealed a significant interaction **between the** effect
380 of age and light in animal behaviour in escape frequency **in** the HCET and frequency
381 of entry to open arms in the EPM. This suggests these two behavioural test paradigms
382 measure different aspects of anxiety. This study found that testing animals of different
383 age groups in established behavioural tests with additional aversive stimuli may be
384 instrumental in accurately identifying specific aspects of anxiety measured by each
385 test, thus allowing researchers to achieve greater specificity in behavioural studies,
386 being able to better characterise the anxiety-like responses exhibited by animals.

387

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392

393 **CONFLICTS OF INTEREST**

394 All authors declare no conflicts of interest.

395

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573

574

575 LEGENDS

576 **Figure 1. Anxiety-like behaviour in aged animals.** Illustration of the HCET in dim
577 and bright light conditions, respectively. The scatter plots and bar graphs show escape

578 frequency (a) and escape latency (b) in both young and aged animals with and without
579 external bright light aversive stimuli. Note, there was a significant decrease in escape
580 frequency ($p < 0.001$) and increase in escape latency ($p < 0.001$) in aged animals in both
581 dim and bright light conditions compared to young animals, indicating an anxiogenic-
582 like response in aged animals.

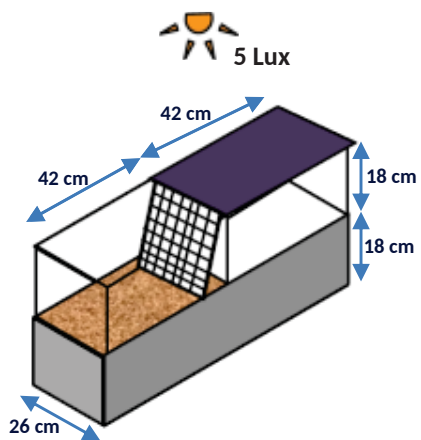
583

584 **Figure 2. Bright light conditions induced anxiety-like behaviour in both aged and**
585 **young animals.** Illustration of the EPM in dim and bright light conditions. A set of
586 scatter plots and bar graphs of the behavioural measures from EPM in young and
587 aged animals in dim and bright light conditions. Note that both aged and young animals
588 spent significantly less time in the open arms ($p < 0.001$) in the bright light condition
589 than in the dim light condition, indicating the anxiogenic effects of bright light.

590

591 **Figure 3.** Two-way analysis of variance was used to test the difference between
592 means for significance. (a) Mean escape frequency of aged and young animals from
593 the HCET in bright and dim light. Interestingly, simple effects analysis showed that
594 within group, young animals escape more frequently in the dim light condition
595 ($F_{(1,110)} = 229.779$, $p < 0.001$) while aged animals escaped more frequently in bright light
596 condition ($F_{(1,110)} = 152.515$, $p < 0.001$). This difference is reflected in significant age x
597 light interaction ($F_{(1,110)} = 3.945$, $p = 0.05$). (b) Mean open arm entry frequency in bright
598 and dim light conditions between aged and young animals. Interaction of age x light
599 was significant ($F_{(1,110)} = 5.579$, $p < 0.05$) and simple effects analysis indicates difference
600 between young and aged animals is notable in the dim light condition as young
601 animals entered with significantly greater frequency than aged animals
602 ($F_{(1,110)} = 25.454$, $p < 0.001$).

Figure 1



Home Cage Emergence Test

Familiar home cage environment with and without external bright light aversive stimulus.

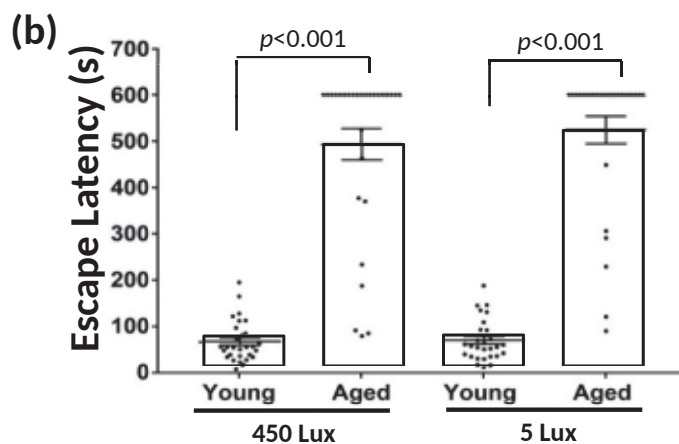
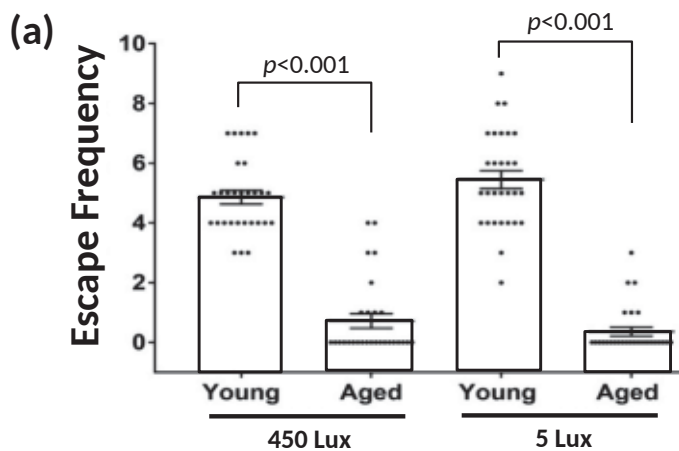
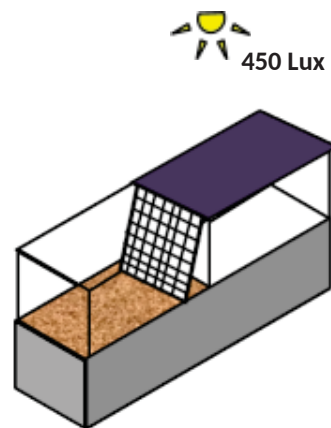
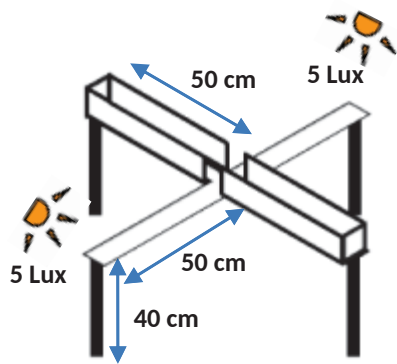


Figure 2



Elevated Plus Maze Test

Novel anxiogenic environment with and without external bright light aversive stimulus.

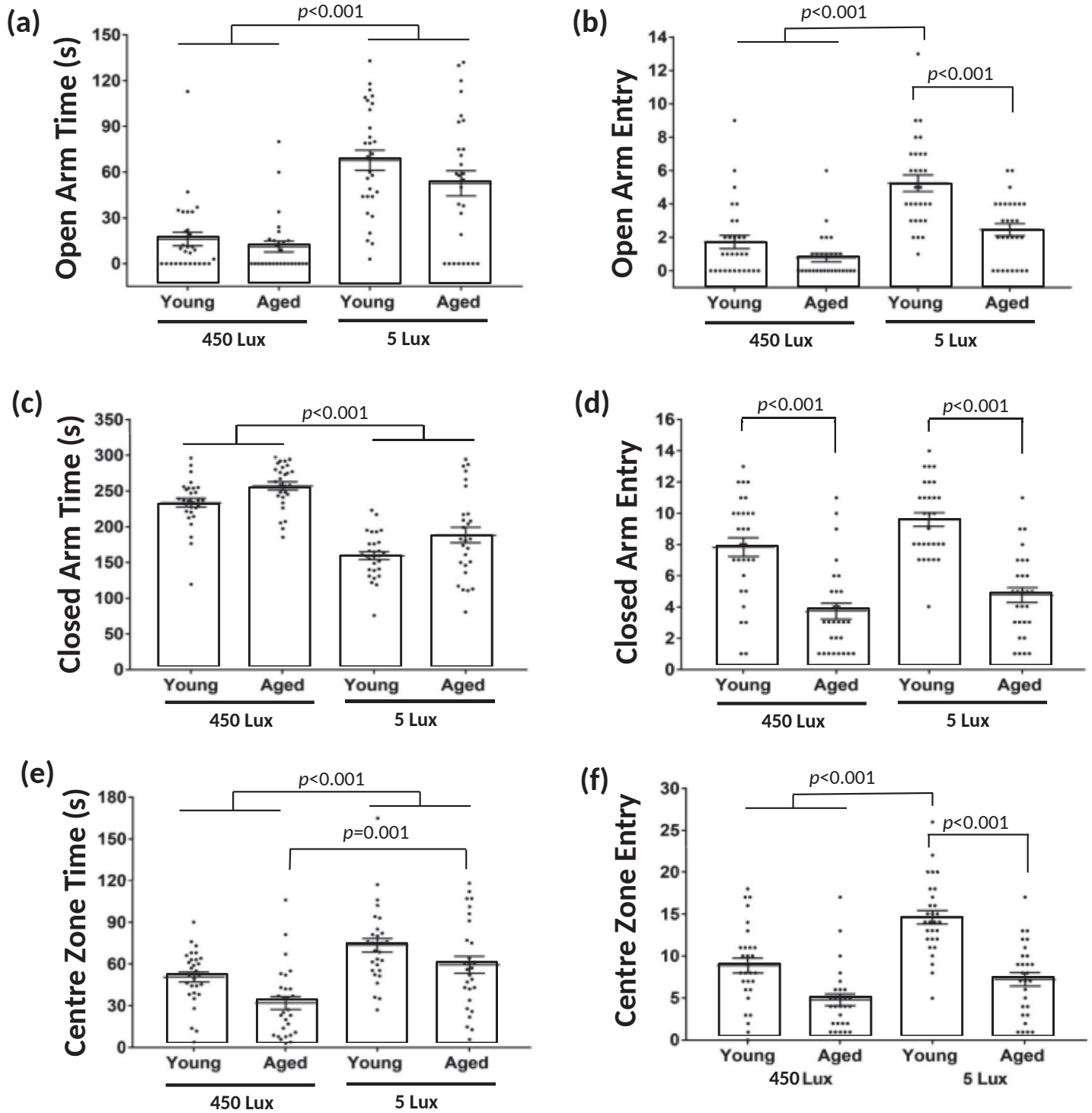
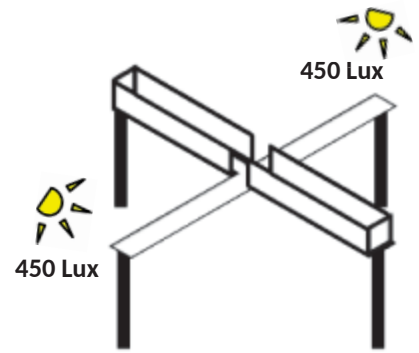
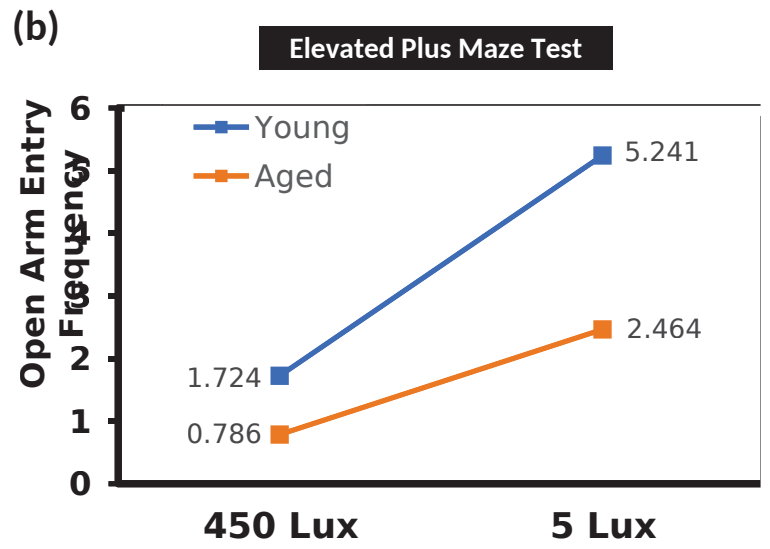
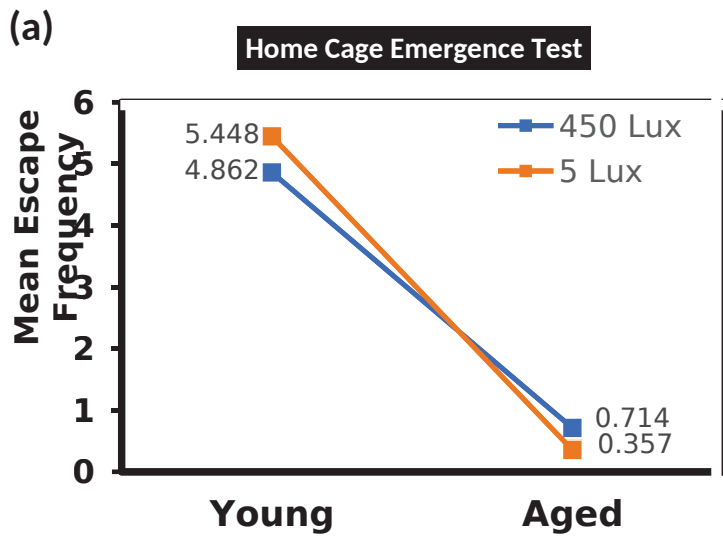


Figure 3



Author Statement

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Investigation, Resources, Visualization **Sharafuddin KHAIRUDDIN:** Writing-
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