Automatic analysis of treadmill running to estimate times to fatigue and exhaustion in rodents

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Introduction. The determination of fatigue and exhaustion in experimental animals is complicated by the subjective nature of the measurement. Typically, it requires an observer to watch exercising animals, e.g. rats running on the treadmill, and to identify the time of the event. In this study, we hypothesized that automatic analysis of the time-averaged position of a rat on a treadmill could be an objective way for estimating times to fatigue and exhaustion. To test this hypothesis, we compared these times measured by a human observer to the results of an automated video tracking system.

Methods. Rats, previously familiarized to running on the treadmill, ran at a fixed speed with zero incline, until exhaustion. The experiments were performed at either room temperature (24˚C) or in a hot environment (32˚C). Each experiment was video recorded. A trained observer estimated the times to fatigue and exhaustion. Then, video tracking software was used to determine the position of the animals on the treadmill belt. The times to fatigue and exhaustion were determined, based on the position on the treadmill using predefined criteria.

Results. Manual scores and the average position on the treadmill had significant correlation. Both the observer and the automated video tracking determined that exercise in a hot environment, compared with the exercise at room temperature, results in shorter times to exhaustion and fatigue. Also, estimates of times made by the observer and the automated video tracking were not statistically different from each other.

Discussion. A similarity between the estimates of times to fatigue and exhaustion made by the observer and the automated technique suggests that video tracking of rodents running on a treadmill can be used to determine both parameters in experimental studies. Video tracking technique allows for a more objective measure and would allow for an increased performance in experimentation. The Supplemental information to this manuscript contains an Excel file, which includes the code in Virtual Basic with freeware license, to process and visualize running data and automatically estimate the times to fatigue and exhaustion. Instructions for the software are also included.

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4 **rodents**

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16 **Running head:** Video tracking of running in rodents

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24 **ABSTRACT**

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27 watch exercising animals, e.g. rats running on the treadmill, and to identify the time of the event.

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30 hypothesis, we compared these times measured by a human observer to the results of an

31 automated video tracking system.

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34 (24˚C) or in a hot environment (32˚C). Each experiment was video recorded. A trained observer

35 estimated the times to fatigue and exhaustion. Then, video tracking software was used to

36 determine the position of the animals on the treadmill belt. The times to fatigue and exhaustion

37 were determined, based on the position on the treadmill using predefined criteria.

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40 environment, compared with the exercise at room temperature, results in shorter times to

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51

52   **INTRODUCTION**

53

54   Fatigue and exhaustion are a part of everyday life. For athletes, the ability to overcome them

55   can be the difference between winning and losing an endurance event. A similar need for

56   thwarting fatigue and exhaustion extends to military personnel, who must remain alert to avoid

57   jeopardizing their missions (Hursh et al. 2004; Shattuck & Matsangas 2015). Problems with

58   fatigue and exhaustion, however, extend beyond the healthy individual. Cancer patients and

59   persons with a variety of chronic illnesses, e.g., arthritis, multiple sclerosis, consistently state that

60   fatigue dramatically decreases their quality of life (Bang et al. 2015; Gossec et al. 2014; Patejdl

61   et al. 2016; Winger et al. 2015). However, the lack of understanding of the central and peripheral

62   mechanisms underlying fatigue and exhaustion hampers the development of new treatments.

63   This growing issue in both healthy and chronically ill individuals creates a clear need for

64   research into its mechanisms. While fatigue and exhaustion are recognized as a significant health

65   issues, they are difficult to diagnose and treat, in part due to their subjectivity (Heckman et al.

66   2015; Jason et al. 2015). Both terms, "fatigue" and "exhaustion", often are used interchangeably

67   (Hasegawa et al. 2008; Walters et al. 2000). In fact, the Merriam-Webster dictionary defines

68   fatigue as "weariness or exhaustion from labor, exertion, or stress" (2003). However, many

69   researchers separate them by stating that exhaustion is synonymous with fatigue but is more

70   intense. Cancer patients who are fatigued experience difficulty concentrating, anxiety, and a

71   gradual decrease in stamina. People who suffer from exhaustion report frank confusion that

72   resembles delirium, emotional numbness, sudden loss of energy, and difficulty in staying awake

73   (Saligan et al. 2015). Fatigue indicates declining ability to respond to stressors, while exhaustion

74   indicates almost complete inability to respond to stressors (Olson et al. 2008).

75   Based on the previous definitions of fatigue and exhaustion, exercise (e.g., treadmill,

76   swimming) is a common model used to study it. In the animal model of exertional fatigue, the

77   repeated slipping from front running position to the middle is a reflection of the inability to

78   maintain optimal physical performance, and will be consistent with fatigue. The rat always

79   restores front running position after receiving electric shocks, which in turn, corresponds to the

80   ability to respond to stressors. However, with time, the ability of stress to restore running

81   performance declines, and repeated shocks do not help rat to keep pace with the moving belt.

82   This stage phenomenologically corresponds to an inability to respond to stressors. The status, in

83   which the animal no longer responds to prodding to keep with the moving belt, is translationally

84   similar to the exhaustion. Here, the major feature of exhaustion is an inability of animal to

85   continue to exercise despite prodding. In treadmill experiments, when an animal is nearing

86   exhaustion, it has a sustained inability to keep the pace with the belt. This means that the animal

87   is permanently located near the back of the treadmill, and unable to make it back to the front

88   running position despite any unpleasant or painful stimuli such as electric shocks. The definition

89   of exhaustion, therefore, could be defined exclusively by the position on the treadmill. In

90   contrast, the definition of fatigue is usually more complex than just the position of the animal on

91   the treadmill. To make it translationally valid, the researchers usually include clearly

92   recognizable and characteristic behavioral pattern that reflects the loss of the optimal physical

93   performance. If this pattern is observed for more than some predefined period of time, it is

94   considered as an objective sign of fatigue (Fuller et al. 1998). This behavior is also accompanied

95   by the animal having transient periods when it cannot keep pace with the treadmill so that the

96   animal moves toward the back of the treadmill, may receive a prodding stimulus, and then

97   returns to the front of the treadmill. This transient inability to keep pace with the treadmill is

98   used by some authors as the definition of fatigue (Rodrigues et al. 2009; Soares et al. 2004). The

99   recurrent pattern of an animal sliding to the back of the treadmill, followed by a return to the

100   front of the treadmill would result in the average position of the rat on the belt of the treadmill

101   shifting from the front to the middle.

102   Based on the above, we proposed that in animal experiments the average position of the

103   animal on the treadmill can be measured and used to define fatigue and exhaustion. Fatigue

104   would correlate with the shift of average position at the treadmill from the front to the middle of

105   the lane and exhaustion to the back of the lane. To test this approach, we recorded the average

106   position of rats running on a treadmill at room temperature and in hot environment. We

107   compared results of obtained by a human observer with that obtained using video tracking

108   software.

METHODS

Animals

Male adult Sprague-Dawley rats (weight 300 ± 20 g; Harlan, Indianapolis, IN) were used in this study. All procedures were approved by the Indiana University Animal Care and Use Committee (#10093). Experiments were performed using single-housed rats that were maintained in a 12 h light/dark cycle and fed ad libitum. We conducted experiments on fully conscious rats between the hours of 10:00 a.m. and 4:00 p.m. The experiments were performed in a custom-made environmental chamber, which housed a treadmill.

Experimental protocol

To validate the use of automatic video tracking software for estimation of the times to fatigue and exhaustion, we had two groups of rats (N=6 each) run until exhaustion at either room temperature (ambient temperature 24 ± 1^oC, humidity 30-70%) or in a hot environment (ambient temperature 32 ± 1^oC, humidity below 20%). Initially, the home cage with the rat was placed outside the environmental chamber at room temperature. The rat was allowed to adapt in the home cage before being transferred to the treadmill lane.

The rats were subjected to constant-speed exercise, and ran until exhaustion at a fixed treadmill speed of 18 m/min with zero incline. The runs were video recorded and simultaneously manually scored by one of the authors (HK). Video files were analyzed using video tracking software Anymaze (Stoelting Co., Wood Dale, IL) by a researcher blinded to the results of the manual scoring. Manual and automated scores were compared. Automated scores were used to determine times to fatigue and exhaustion in further experiments.

Treadmill running

Familiarization to treadmill running

 Prior to all treadmill experiments, the rats were familiarized to running on a motorized rodent treadmill (Columbus Instruments, Columbus, OH) set with zero incline. On the first day

140   of familiarization, the rats were placed on the treadmill with the belt speed on standby. They

141   were given 10 min to explore their surroundings. After this, the treadmill speed was set to 6

142   m/min for 5 min. For next four days, the rats were subjected daily to five-minute sessions that

143   included running at progressively increased speeds up to a maximum of 18 m/min by day 5.

144   Previous studies have shown that the workloads of familiarization protocols accustom rats to

145   treadmill running but do not induce training adaptations (Lambert & Noakes 1989). Mild electric

146   stimuli (1 mA, 3 Hz) at the back of the treadmill chamber promoted the learning of running

147   behavior.

148

149   *Treadmill running*

150   On the day of the experiment, the cage with the animal was brought to the research lab. The

151   rats were allowed to acclimate to the new environment for at least 60 min. The temperature of

152   the custom-made environmental chamber, which housed the treadmill, was set to either room

153   temperature or a high temperature.

154   After the period of acclimation, the rat was placed on the treadmill lane. The treadmill

155   opening was closed with a terminal plate that contained a fan providing air circulation from the

156   chamber, and the belt was turned on at 18 m/min with zero incline. When a rat was consistently

157   unable to maintain the pace of the treadmill and received three continuous electric shocks (1 mA,

158   3 Hz) on the grid at the back of the treadmill, the belt was stopped. The rat was taken

159   immediately from the lane and was transferred back to the home cage at room temperature.

160

161   *Video recording of treadmill experiments*

162   Video recordings were obtained using a USB-camera connected to a laptop running

163   Xtrasense software (surveillance computer-based system, XmediaStudio, Dawang group) using a

164   proprietary codec. The timer of the computer was embedded into the video stream, which was set

165   at the resolution 640x480. The analysis can be performed as described using recordings with

166   lower resolutions of 352x288 or 320x240. Files were stored until analysis could be done.

167   The video camera was positioned to capture the entire treadmill lane (Columbus Instruments

168   Inc., Columbus, OH) from the side; the camera could also be positioned looking down from the

169   top of the treadmill. To increase contrast between the animal and the background, the opposite

170 face of the treadmill lane was covered from the outside with dark paper. The same effect could

171 be achieved by recording a transparent treadmill lane positioned in front of a black wall.

172

173 **Data analysis**

174

175 *Manual scoring of the quality of treadmill running*

176 An observer scored the quality of the animal's running every minute using a previously

177 described technique (Guasch et al. 2013) with minor modifications. The animal was scored a 5 if

178 it ran the majority of a 1-min epoch in the front of the treadmill (proximal third). The rat with

179 this score had a normal running gait with the tail raised. As animals became fatigued they would

180 lay down flat on the belt, and ride to the back of the treadmill. As they approached the back of

181 the treadmill and before they touched the shock grid, they would right themselves and run to the

182 front of the treadmill. This cycle would then repeat. When the animal spent the majority of the

183 time in a 1-min epoch in this fashion, they were scored a 3. This behavior that we call "belt-

184 riding" resulted in an average position in the middle third of the treadmill. Finally, the animals

185 lost the ability to return to the front of the treadmill after riding to the back. If this was consistent

186 during the time epoch, the average position would be in the low third of the treadmill length, and

187 the animal was scored 1. When the animal refused to run, or was not able to avoid repeated

188 shocks, the score was 0.

189 Similar to other researchers, we defined the time to fatigue as the time until an animal

190 became unable to continuously keep pace with the treadmill and stay in the front (Rodrigues et

191 al. 2009; Soares et al. 2004). In our scoring system, this corresponds to the time the animal

192 transitioned from a running score of 5 to 3. To assure that this was not a transient phenomenon,

193 but rather represented true fatigue, a single score of 3 immediately followed by a recovery to

194 score 5 did not qualify as fatigue. Only three consecutive scores of 3 or below counted as fatigue.

195 Exhaustion in our scoring system was defined by a score of 0.

196

197 *Analysis with Anymaze*

198 To quantitate the running quality and to automate the analysis, we used video tracking

199 system software (Anymaze, Stoelting Co., Wood Dale, IL). We created an experimental protocol

200 based on the built-in function to measure the distance of the animal's center from a predefined

201 reference point, which for our experiments was defined as the back of the treadmill. The video

202 file was selected as the source of the video stream. The observation area ("apparatus" in

203 Anymaze) was defined in the configuration as required, and the length of the treadmill was

204 calibrated where appropriate. The analysis of the video file was performed, and the data were

205 exported as a file in comma separated values format. The resulting file was imported into

206 Microsoft Excel.

207

208 *Data processing using Excel template with the code in virtual basic*

209 Video tracking generates a variable number of data points per unit of time. On a typical

210 laptop, Anymaze is able to estimate 3-8 positions per second, which is equivalent to 10000-

211 30000 pairs of (time; position) per hour. At room temperature, a non-trained rat can run up to 90

212 minutes at the speed and incline used in our experiments. To process such large datasets, and to

213 obtain averages over selected time intervals (e.g., 1 min), we programmed and used a template in

214 Excel, which is included as supplemental information to this manuscript and can be used as

215 freeware.

216 The difference between actual recording time and time during video play was corrected by a

217 coefficient which was calculated by comparing the elapsed time on video player timer during

218 playback and the timer embedded into the video stream. This coefficient was stable within each

219 recording and ranged from 0.9 to 1.33. More details and the method to calculate the coefficient is

220 provided in the manual for the template.

221 Initially, the template calculated only averages. The user could select the time interval for the

222 averaging. We then added graphic representations. Finally, we implemented the algorithm to

223 estimate the time to fatigue and the time to exhaustion using the rules similar to ones used by the

224 observer. Time to fatigue was defined as the earliest time when the average animal position over

225 temporal step fell below upper threshold and did not recover for predefined number of steps.

226 Unless noted, temporal step was 1 min, and predefined number of steps was 3. Similarly, the

227 time to exhaustion was defined as the earliest time when the average animal position over

228 temporal step fell below lower threshold and did not recover for at least a predefined number of

229 steps. Unless noted, the upper threshold was 0.7 and the lower threshold was 0.35.

230   A detailed user manual for the template is included into the Supplemental materials.

231   Instructions on how to use the template are in the sheet of the Excel file, and are included as a

232   separate file in the Supplemental materials.

233

234   *Correlation of scoring by human observer and video tracking software*

235   The one-minute averages of the normalized position on the treadmill calculated by video

236   tracking software (scores ranged from 0 to 1) and manual scoring (scores ranged from 0 to 5)

237   were aligned in columns in an Excel spreadsheet. For each rat we calculated a coefficient of

238   correlation between the manual score and the normalized position obtained by video tracking

239   software. To determine the applicability of this method in different environments, we compared

240   the video tracking software data with manual scoring for rats running at both room temperature

241   and in a hot environment.

242   To fully automate the quantification of fatigue and exhaustion, we used a least-square test to

243   determine the boundaries between zones which will best correspond to scores 5, 3, and 1 in the

244   manual scoring for each rat. To do that, we set two adjacent cells in MS Excel (D2 and D3 in the

245   Fig.1) filled with our initial estimates of what the boundaries would be: the upper boundary

246   between running zones 3 and 5 was set at 0.7, while the lower boundary between running zones

247   1 and 3 was set at 0.35. Then, two extra columns were added for the data for each rat. In the first

248   extra column (E in the Fig.1) the automated running score was calculated using the estimated

249   boundaries between running zones. The second column (column F, Fig.1) contained squared

250   difference between the manual and the automated score. The target cell (F2) contained a sum of

251   differences for all running times. By changing the cells containing upper and lower boundaries

252   (D2 and D3) we minimized the value in the target cell (F2). Unfortunately, due to the discrete

253   nature of the target function, algorithms from the Solver tool-kit were ineffective. To our

254   advantage, the function allows for minimizing the target cell by sequentially finding the best

255   estimate for the first variable, followed by determining another variable. We restricted the

256   precision of finding the estimates to two digits after the comma. The estimates of the boundaries

257   between the zones for each rat were averaged.

258   For processing experimental data, we selected 0.7 and 0.35 for the upper and lower

259   boundaries, accordingly. Using these preset values, we calculated the correlation coefficients for

260   each rat between manual score values and automatic score values. Data was averaged for rats

261   running at both room temperature and in a hot environment.

262

263   *Statistical procedures and graphing*

264   Statistical analysis and graphing were performed using Microsoft Excel 2010 (Microsoft,

265   Redmont, WA) and Prism 4.0 (GraphPad Software, San Diego, CA). The comparisons between

266   groups were performed using Student's t-test or the analysis of variance (ANOVA), followed by

267   Fisher's least significant difference (LSD) post hoc test, where appropriate. Significance was

268   defined as a *p*-value <0.05. Values are presented as means ±SEM.

269

270

271   **RESULTS**

272

273   *Video tracking software provides an objective measure of running quality*

274   The automatic tracking analysis of the video recording by Anymaze creates a continuous

275   measure of a rat's position on the treadmill relative to the back of the treadmill (Fig.2A). The

276   length of the treadmill belt is 0.4 m, however due to the length of rat itself, the range of possible

277   positions was between 0.1 m and 0.33 m. These minimal and maximal values are estimated from

278   the completed recording and were used to calculate the normalized position on the treadmill

279   (with values between 0 and 1).

280   At the start of exercise, rats run at the front of the treadmill. The automated scoring of

281   individual runs captures more temporal details of the changes in the rat's movement compared to

282   manual scoring. For instance, recordings often show that before fatigue or exhaustion occurs,

283   some rats transiently appear at the back of the treadmill; in most of these cases they appear to be

284   exploring. In the first 15 min of recording, the lowest points on the curve do not fall below 0.15

285   m. This means that early on during exercise, when an animal slides back, it does not reach the

286   electric grid and is readily able to recover its position at the front of the treadmill (Fig.2A, Zone

287   5). Animals running in zone 5 have a low variability of position (Fig.2A, SD graph). Over time,

288   as fatigue develops, there is a change in running quality (that we referred as "belt riding"). Due

289   to more time spent closer to the back, the average position slips to the middle of treadmill or 0.5

290   in relative value (Zone 3). Zone 3 running is characterized by the highest variability in the

291   animal's position (Fig.2C). Finally, when the rat approaches exhaustion, it is incapable of

292   reaching the front of the treadmill; it often fails to keep up with the treadmill while positioned

293   near the back of the belt. This corresponds to an automated average position that falls below 0.5.

294   This zone 1 running also has decreased variability (Fig.2D).

295   Reaching the averaged position around zero or below zero corresponds to the exhaustion

296   determined by the observer.

297

298   *Manual and automated running scores correlate*

299   Fig.3 compares the results of manual scoring for animals to the results of videotracking.

300   Manual scoring has obvious visual similarity with the results of automatic analysis for

301   experiments performed at room temperature (Fig.3A) and in hot conditions (Fig.3B). Manual

302   scores and average positions correlated significantly with an average coefficient of 0.75±0.03.

303   The time to reach averaged position close to zero was exactly the same as the time of exhaustion

304   determined by the observer in the case of each rat.

305   Based on these results, we used the position on the treadmill to calculate the time to fatigue

306   and exhaustion. Using the method of least squares (see Methods) we defined the boundaries that

307   correspond to subjective scores of 1, 3 and 5 for each rat (named zones 1, 3, and 5,

308   correspondingly). Using such boundaries would result in an automatic score closest to the

309   manual score for this rat. On average, the boundary between zones 5 and 3 appeared at

310   0.73±0.02, and between zones 3 and 1 at 0.35±0.04. This is not surprising as the direct

311   application of instructions to the observer implies the upper boundary at 0.67, and the lower

312   boundary at 0.33.

313   For further calculations, we selected 0.7 and 0.35 for the boundaries between zones 3 and 5,

314   and 1 and 3, accordingly. In some individual rats the average values fell below 0.7 for a short

315   period of time, and then scores quickly returned to above 0.7 for prolonged time. In these

316   situations, the posture of the rat was typical for pre-fatigue status. To avoid underestimation of

317   time to fatigue, we introduced an additional rule similar to that used in manual scoring (Guasch

318   et al. 2013): time to fatigue was determined when the average position for one minute fell below

319   0.7 and did not recover for at least three minutes.

320

321

322   *Exercise in a hot environment results in faster fatigue and exhaustion*

323   In rats familiarized to treadmill running, but otherwise untreated, the estimates of time to

324   fatigue made by a human observer and video tracking were not statistically different. At room

325   temperature, fatigue developed after 35 ± 4 min according to the human observer or after 30 ± 3

326   min according to the video tracking ($p>0.05$ when two approaches were compared). In a hot

327   environment, fatigue occurred statistically significantly earlier according to both the human

328   observer and the video tracking (22 ± 2 min for both, $p<0.05$ when compared to time to fatigue at

329   room temperature). The coefficient of correlation between the times to fatigue determined by the

330   observer and the ones determined by video tracking was 0.79.

331   Further dynamics differed dramatically between the two environments. At room temperature,

332   exhaustion developed at 88 ± 5 min, which was 58 ± 5 min after fatigue if determined by the

333   observer or 52 ± 2 min if determined by the video tracking ($p>0.05$). In contrast, in the hot

334   environment, exhaustion occurred at 42 ± 2 min (only 21 ± 2 min after the development of the

335   fatigue according to both the observer and the video tracking, both values are statistically

336   different from those at room temperature, $p<0.001$).

337   The difference between the time to exhaustion and the time when the average position

338   dropped below 0.35 was 4 ± 1 min or 7 ± 1 % of the time to exhaustion. The percentage was not

339   different between two environments. The coefficient of correlation between the time to transition

340   from score 3 to score 1 determined by the video tracking and the time to exhaustion was 0.99.

341

342 **DISCUSSION**

343

344   This study outlines a system to objectively measure fatigue and exhaustion in animals

345   running on a treadmill. In animal studies, the major feature of exhaustion is an inability of an

346   animal to keep pace with the treadmill belt despite prodding, by either physical means or an

347   electric shock. There is, however, variability in how researchers define this. For example, some

348   researchers add the requirement that animals must be unable to move for period of time after

349   exhaustion occurs (Hasegawa et al. 2008). Likewise, fatigue has been defined as the inability of

350   an animal to keep pace with the treadmill (Rodrigues et al. 2009; Soares et al. 2004). But how

351   does one objectively measure this?

352   Our study showed that the subjective grading of running by an observer correlates with the

353   position of an animal on a treadmill as measured by the video tracking software. Therefore, the

354   averaged position of the animal on the treadmill, measured by video tracking software, can

355   provide an objective estimate of the quality of running. Using the same measure, the time at

356   which fatigue and the exhaustion occurs can be estimated.

357   We tested video analysis technique using rats running on the treadmill in two environmental

358   conditions: at room temperature and in hot environment. Significant differences in times between

359   two environments, the phenomenon which is well-known from studies (Drummond et al. 2016)

360   and from personal experience of athletes (Molkov & Zaretsky 2016), allowed for the

361   visualization of descriptive power of automatic analysis.

362   The data recorded by the human observer clearly correlated with the results of automatic

363   processing, as shown in Fig. 3, in both of these environments. Considering that the parameters

364   of analysis were tuned so that results of the video tracking resemble the estimations of the

365   observer, it is not surprising that the time to fatigue and the time to exhaustion determined by

366   both the observer and automatic analysis were not different.

367   Both approaches revealed that the hot environment statistically significantly decreases the

368   times to both fatigue and exhaustion when compared with the same times at room temperature.

369   The difference between two environments can be interpreted using known core body temperature

370   dynamics. Hyperthermia suppresses the ability to perform physical work. Exercise-related heat

371   production, which drives an increase of body temperature, is not dependent on ambient

372   temperature (Yoo et al. 2015). However, in the hot environment the dissipation of heat is less

373   effective, and thermoregulatory responses to exercise are not effective (Yoo et al. 2015).

374   Therefore, it takes less time for body temperature to reach thresholds affecting the ability to run.

375   Both the human observer and the video tracking system identified the occurrence of fatigue at

376   earlier times in the hot environment. Importantly, the automatic measurements of the time to

377   fatigue were not statistically different from human estimates in both environments.

378   In the hot environment, rats became unable to run shortly after showing that they are

379   fatigued. At fatigue, the body temperature of rats is in the range of 39-40°C. It leaves very little

380   reserve for further increase, while body temperature is raising at 0.1-0.2°C/min (Zaretsky et al.

381   2015). In the absence of effective heat dissipation, animals quickly reach the thermal threshold

382   for the exhaustion, which was estimated at 42.2-42.5 °C (Walters et al. 2000). A fast rise of body

383   temperature is reflected in a progressive loss of performance with the corresponding average

384   sliding to the back of the treadmill.

385   In contrast, at room temperature, after rats became fatigued, they still were able to run for a

386   long time. At room temperature, heat loss mechanisms have sufficient power to dissipate the heat

387   generated by exercise at relatively low power which was used in this study (Tanaka et al. 1988).

388   After hyperthermia induces cutaneous vasodilation, body temperature stabilizes at elevated

389   levels. As a result, the average position on the treadmill drifts to the end of the treadmill slowly.

390   As one of the potential advantages of having the record of running quality over time, we

391   found that the time to exhaustion can be predicted before the exhaustion itself occurs. Most

392   researchers consider exhaustion when the animal is not able to move; a subjective score of 0 in

393   our manual grading system. Unfortunately, reaching such a score results in the animal receiving

394   multiple shocks.

395   We found that the time of transition from running with score 3 to running with score 1

396   correlates with the time to exhaustion with a correlation coefficient of 0.99. However, the

397   transition occurs a few minutes before actual exhaustion, which allows to reliably and

398   objectively estimate the time to exhaustion, but to spare the animal of the most stressful

399   experience during the test. Importantly, the difference corresponds to the similar percentage of

400   the total time in both environments, which explains such a high correlation.

401

402

403

404 **CONCLUSION**

405

406   An objective nature of estimation of times to fatigue and exhaustion has the advantage of

407 eliminating potential observer bias. Importantly, this technique can also dramatically increase the

408 throughput of experiments as multiple animals can be run and recorded at the same time. In

409 addition, universal scoring, performed via automatic means, can standardize the definitions of

410 fatigue and make the studies done by independent groups more comparable. Lastly, this

411 technique may open a way for a more humane way of studying exhaustion. The time to the

412 decrease in the average running position below 0.35 provides a reliable estimate of time to

413 exhaustion, while eliminating the stressful component of repeated shocks. We consider that the

414 use of objective measures of running quality may help to improve animal care in experiments

415 focusing on studying exercise, endurance and exhaustion.

416

417

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419

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421 Anymaze software which was used for video tracking in this study.

422

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486   hypothalamus. *Brain Research* 1608:75-81.
487

488   **FIGURE LEGENDS**

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491

492   **Figure 1.** The screenshot of an example calculating optimal borders between zones for one

493   rat.

494   Portions of a spreadsheet corresponding to the beginning (rows 8-16), the middle (rows 35-

495   40) and the end of run (rows 85-90) are shown. Rectangles contain the formulas to calculate

496   corresponding cells.

497

498

499   **Figure 2.** Typical result of automatic tracking of a rat running on the treadmill.

500   **A:** The record of complete run. Top row – original data; middle row – data averaged every

501   minute; bottom row – standard deviation for the position for each minute. **B, C, D:** Scaled

502   portions of the recording representing rats running with scores 5 (B), 3 (C), and 1 (D) with

503   corresponding average distance for shown time interval.

504

505

506   **Figure 3.** The comparison of the results of the manual scoring and automatic analysis of the

507   position on the treadmill.

508   Rats ran at room temperature (**A**, 24°C, N=6) or in a hot environment (**B**, 32°C, N=6).

509   Averages were calculated as long as at least three rats were still running. Open circles – the

510   results of the observer (manual); closed circles – the results of the video tracking (video).

511

512

513

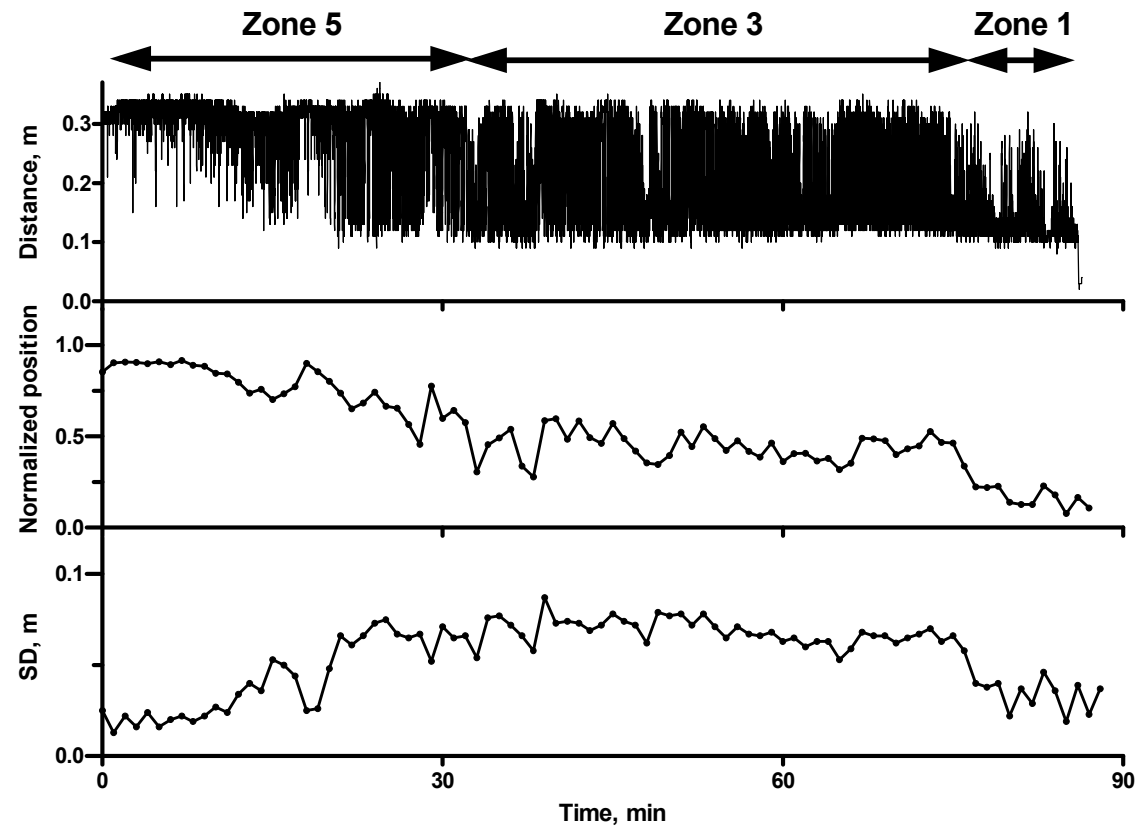
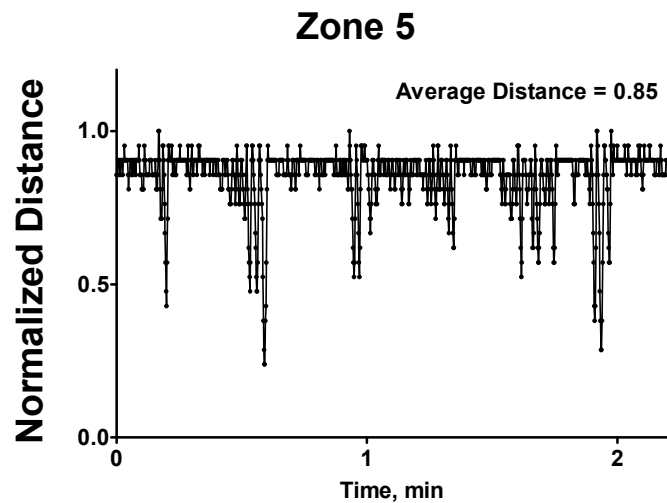
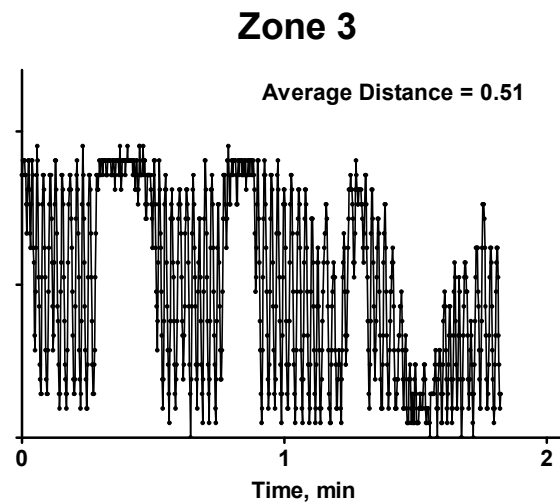
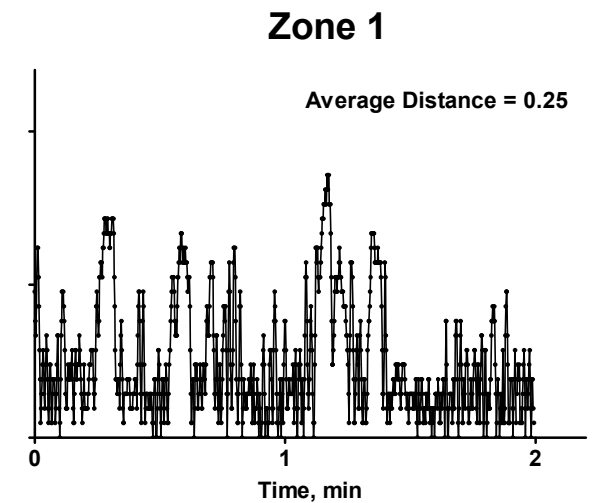
Figure 1

The screenshot of an example calculating optimal borders between zones for one rat.

Portions of a spreadsheet corresponding to the beginning (rows 8-16), the middle (rows 35-40) and the end of run (rows 85-90) are shown. Rectangles contain the formulas to calculate corresponding cells.

	A	B	C	D	E	F	G	H	I
1									
2			Upper -->	0.76		85		= SUM(F8:F100)	
3			Lower -->	0.37					
4									
5									
6	Rat # 123A								
7			Automatic score	Manual score	Calculated score	Difference squared			
8		0	0.9175	5	5	0		=(D8-E8)^2	
9		1	0.9031	5	5	0			
10		2	0.8105	5	5	0		=(IF(C8>D$2, 5, IF(C8<D$3, 1, 3)))	
11		3	0.7909	5	5	0			
12		4	0.4804	3	3	0			
13		5	0.8559	5	5	0			
14		6	0.4544	3	3	0			
15		7	0.4010	5	3	4			
16		8	0.8446	5	5	0			
35		27	0.8880	5	5	0			
36		28	0.6905	5	3	4			
37		29	0.6240	3	3	0			
38		30	0.5882	3	3	0			
39		31	0.6273	3	3	0			
40		32	0.6910	3	3	0			
85		77	0.4081	1	3	4			
86		78	0.5200	1	3	4			
87		79	0.3690	1	1	0			
88		80	0.3308	1	1	0			
89		81	0.2243	1	1	0			
90		82	0.0165	0	1	1			
91									

Typical result of automatic tracking of a rat running on the treadmill.

A: The record of complete run. Top row – original data; middle row – data averaged every minute; bottom row – standard deviation for the position for each minute. B, C, D: Scaled portions of the recording representing rats running with scores 5 (B), 3 (C), and 1 (D) with corresponding average distance for shown time interval.

The comparison of the results of the manual scoring and automatic analysis of the position on the treadmill.

Rats ran at room temperature (A, 24°C, N=6) or in a hot environment (B, 32°C, N=6). Averages were calculated as long as at least three rats were still running. Open circles – the results of the observer (manual); closed circles – the results of the video tracking (video).