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Data Article

# Measured data of *Drosophila melanogaster* (Diptera Drosophilidae) development and learning and memory behaviour after copper exposition

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A B S T R A C T

This article presents the statistical analysis data from *Drosophila melanogaster* development (from larvae to adult) and learning and memory retention behavior of a Pavlovian conditioning in male and female flies exposed to copper. While the full data sets are available In the article: Copper decrease associative learning and memory in *D. melanogaster*, this data-in-brief article includes the detailed statistical analysis performed. Data demonstrates Statistica Software analysis between the subject part of the analysis: 2 treatments x 2 sexes x 2 ages and within subject part of the analysis: 2 treatments x 2 sex x 2 ages x 4 times, repeated measures.



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Specifications table

| | |
|---|---|
| Subject | Toxicology |
| Specific subject area | Toxicology of metals in an *in vivo* model |
| Type of data | Table, Graphs and Images |
| How data were acquired. | Data were acquired manually/visually<br>Model: *Drosophila melanogaster*<br>Instruments: Microscope; Apparatus for conditioning and electric shock<br>Programs: GraphicPad Prism 6 Software and Statistica Software |
| Data format | Raw, Analyzed and Filtered |
| Parameters for data collection | Data were collected at constant temperature (22-24 °C) and relative humidity (60–70%).<br>For memory retention test, homogeneous light at all environments and absence of other significant odors that could unfeasible the behavior. |
| Description of data collection | Survival and Retention memory data were collected visually.<br>Graphs and statistical analysis were constructed using GraphPad Prism 6 Software and Statistica Software. |
| Data source location | Universidade Federal de Santa Maria<br>Santa Maria/Rio Grande do Sul<br>Brazil |
| Data accessibility | With the article |
| Related research article | Daniele C. Zamberlan, Paula T. Halmenschelager, Luis F. O. Silva, João B. T. da Rocha. Copper decrease associative learning and memory in *Drosophila melanogaster*. Science of the total environment. 2019. |

**Value of the Data**

- These data testify and reinforce with the insights of its related research paper: Copper decrease associative learning and memory in *Drosophila melanogaster*
- These data serve as a starting point for the scientific community that investigates the potential risks of metals contamination by different sources, like the use of metal-enriched chemicals, fertilizers, organic amendments, and others
- The detailed statically analysis described in this article can be used to compare different data, and more surely detect significant results.

## 1. Data description

In this article, we present the detailed statistical analysis performed data from *Drosophila melanogaster* development (from larvae to adult - Fig. 1) and learning and memory retention behavior (Table 1 and Figs. 2–4) after copper exposition. The full data sets are available on the research article: Copper decrease associative learning and memory in *D. melanogaster*.

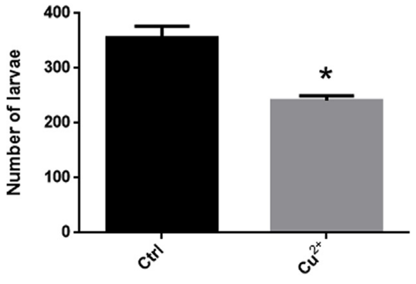
Fig. 1 demonstrates the detailed statistical analysis of data evidencing the effect of copper ($Cu^{2+}$) on *D. melanogaster* survival in larvae (A – unpaired T test), pupae (B – unpaired T test), total adults and adults divided in male and female (C and D – Two-way ANOVA followed by Newman-Keuls multiple comparisons test). Media ±SEM, R square and F test to compare variances are also demonstrated. Seven independent flasks were analyzed for control and copper-treated groups, the dependent variables were the number of larvae, pupae or adult male and female flies per each flask. Flies were exposed to control or $Cu^{2+}$-containing media from egg to adult life. GraphPad Prism 6 was used to construct the figures.

This article also demonstrates Statistica data of the effect of $Cu^{2+}$ on *D. melanogaster* memory retention test. Table 1 presents the Data within subject part of the analysis: 2 treatments (control/$Cu^{2+}$) x 2 sex (male/female) x 2 ages (4-days-old/11 days-old) x 4 times (0/15/30/60 min after shock) included in Statistica Software. Repeated Measures Analysis of Variance with Effect Size and Powers was performed using Statistica Software and is illustrated in Fig. 2. Data demonstrates a significant effect of treatment [$F = 54, 258$; $p < 0.001$], age [$F = 101, 621$; $p < 0.001$], time [$F = 817, 798$; $p < 0.001$], time x treatment [$F = 4, 094$; $p < 0.01$] and time x age [$F = 6313$; $p < 0.001$].

The current effect between subject part of the analysis - 2 treatments (control/$Cu^{2+}$) x 2 sexes (male/female) x 2 ages (4-days-old/11 days-old) are demonstrated in Fig. 3. It can be observed in Fig. 3 (A) a significant difference between treatments ($p < 0.05$), (B) significant difference between age

A

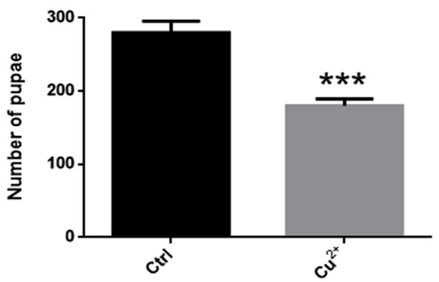
| | A | B |
|---|---|---|
| 1 | Ctrl | $Cu^{2+}$ |
| 2 | 370 | 247 |
| 3 | 407 | 250 |
| 4 | 350 | 262 |
| 5 | 413 | 237 |
| 6 | 327 | 194 |
| 7 | 372 | 243 |
| 8 | 250 | 254 |




| | t test | |
|---|---|---|
| 1 | Table Analyzed | larvae N |
| 2 | | |
| 3 | Column B | $Cu^{2+}$ |
| 4 | vs. | vs. |
| 5 | Column A | Ctrl |
| 6 | | |
| 7 | Unpaired t test | |
| 8 | P value | 0.0003 |
| 9 | P value summary | *** |
| 10 | Significantly different? (P < 0.05) | Yes |
| 11 | One- or two-tailed P value? | Two-tailed |
| 12 | t, df | t=5.079 df=12 |
| 13 | | |
| 14 | How big is the difference? | |
| 15 | Mean ± SEM of column A | 355.6 ± 20.94 N=7 |
| 16 | Mean ± SEM of column B | 241.0 ± 8.389 N=7 |
| 17 | Difference between means | -114.6 ± 22.56 |
| 18 | 95% confidence interval | -163.7 to -65.42 |
| 19 | R square | 0.6825 |
| 20 | | |
| 21 | F test to compare variances | |
| 22 | F,DFn, Dfd | 6.229, 6, 6 |
| 23 | P value | 0.0426 |
| 24 | P value summary | * |
| 25 | Significantly different? (P < 0.05) | Yes |

B

| | A | B |
|---|---|---|
| 1 | Ctrl | $Cu^{2+}$ |
| 2 | 270 | 147 |
| 3 | 280 | 180 |
| 4 | 230 | 164 |
| 5 | 262 | 178 |
| 6 | 310 | 170 |
| 7 | 356 | 193 |
| 8 | 251 | 228 |



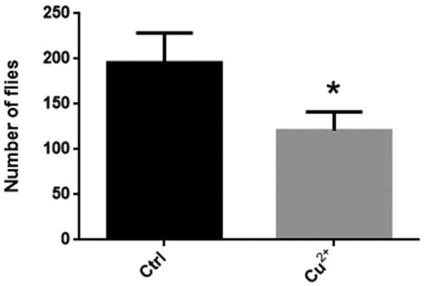

| | t test | |
|---|---|---|
| 1 | Table Analyzed | Pupae |
| 2 | | |
| 3 | Column B | $Cu^{2+}$ |
| 4 | vs. | vs. |
| 5 | Column A | Ctrl |
| 6 | | |
| 7 | Unpaired t test | |
| 8 | P value | 0.0002 |
| 9 | P value summary | *** |
| 10 | Significantly different? (P < 0.05) | Yes |
| 11 | One- or two-tailed P value? | Two-tailed |
| 12 | t, df | t=5.400 df=12 |
| 13 | | |
| 14 | How big is the difference? | |
| 15 | Mean ± SEM of column A | 279.9 ± 15.77 N=7 |
| 16 | Mean ± SEM of column B | 180.0 ± 9.663 N=7 |
| 17 | Difference between means | -99.86 ± 18.49 |
| 18 | 95% confidence interval | -140.1 to -59.57 |
| 19 | R square | 0.7084 |
| 20 | | |
| 21 | F test to compare variances | |
| 22 | F,DFn, Dfd | 2.662, 6, 6 |
| 23 | P value | 0.2586 |
| 24 | P value summary | ns |
| 25 | Significantly different? (P < 0.05) | No |

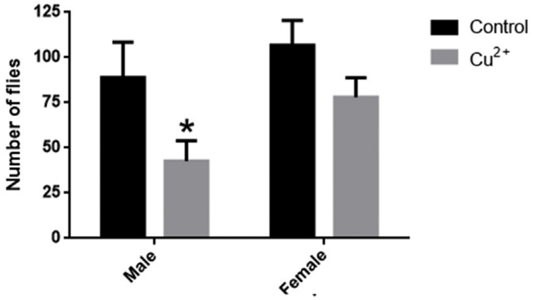
**Fig. 1.** Statistical analysis of *Drosophila melanogaster* survival in (A) larvae, (B) pupae, and (C–D) adults after 0.75 mM copper exposition. Data are expressed as mean + −SEM for N = 7 per group (the dependent variables were the number of larvae, pupae or adult male and female flies per each flask; seven independent flasks were analyzed for control and copper-treated groups, flies were exposed to control or $Cu^{2+}$-containing media from egg to adult life). *p < 0.05; ***p < 0.001 Student T test (compared to control).

C

| | A | B |
|---|---|---|
| 1 | Ctrl | $Cu^{2+}$ |
| 2 | 168 | 125 |
| 3 | 121 | 100 |
| 4 | 107 | 31 |
| 5 | 139 | 85 |
| 6 | 293 | 138 |
| 7 | 332 | 176 |
| 8 | 210 | 190 |

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Ctrl | | Cu2+ | |
| 2 | Male | Female | Male | Female |
| 3 | 78 | 90 | 26 | 99 |
| 4 | 38 | 83 | 24 | 76 |
| 5 | 39 | 68 | 7 | 24 |
| 6 | 58 | 81 | 27 | 58 |
| 7 | 155 | 138 | 51 | 87 |
| 8 | 162 | 170 | 79 | 97 |
| 9 | 93 | 117 | 85 | 105 |




| | 2way ANOVA | A | B | C | D | E |
|---|---|---|---|---|---|---|
| | | Data Set-A | Data Set-B | Data Set-C | Data Set-D | Data Set-E |
| | | Y | Y | Y | Y | Y |
| 1 | Table Analyzed | Male/Female | | | | |
| 2 | | | | | | |
| 3 | Two-way ANOVA | Ordinary | | | | |
| 4 | Alpha | 0.05 | | | | |
| 5 | | | | | | |
| 6 | Source of Variation | % of total variation | P value | P value summary | Significant? | |
| 7 | Interaction | 1.091 | 0.5440 | ns | No | |
| 8 | Sex | 9.924 | 0.0757 | ns | No | |
| 9 | Treatment | 19.87 | 0.0148 | * | Yes | |
| 10 | | | | | | |
| 11 | ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| 12 | Interaction | 540.3 | 1 | 540.3 | F (1, 24) = 0.3788 | P = 0.5440 |
| 13 | Sex | 4916 | 1 | 4916 | F (1, 24) = 3.446 | P = 0.0757 |
| 14 | Treatment | 9844 | 1 | 9844 | F (1, 24) = 6.901 | P = 0.0148 |
| 15 | Residual | 34233 | 24 | 1426 | | |

**Fig. 1.** (*continued*).

($p < 0.05$), (C) no significant difference between sex, (D) no significant difference comparing sex *versus* treatment, (E) no significant difference comparing sex *versus* age and (F) no significant difference comparing treatment *versus* age.

The Statistica data within subject part of the analysis: 2 treatments (control/$Cu^{2+}$) x 2 sex (male/female) x 2 ages (4-days-old/11 days-old) x 4 times (0/15/30/60 min after shock – repeated measure), are demonstrated in Fig. 4. Fig. 4A demonstrates a significant main effect of time [$F(3,72) = 817.8$;

**Table 1**
Raw data within subject part of analysis involving: 2 sex (1 = female and 2 = male), 2 treatments (1 = control and 2 = $Cu^{2+}$), 2 ages (1 = young and 2 = old) and 4 times (0, 15, 30 and 60 min after shock − dependent variables).

| Sex<br>1 = female<br>2 = male | Treatment<br>1 = control<br>2 = $Cu^{2+}$ | Age<br>1 = Young<br>2 = old | Time 0 | Time 15 | Time 30 | Time 60 |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 100 | 100 | 60 | 14,2 |
| 1 | 1 | 2 | 83 | 74 | 55 | 17,6 |
| 1 | 2 | 1 | 90 | 84 | 60 | 10 |
| 1 | 2 | 2 | 80 | 60 | 28 | 11 |
| 2 | 1 | 1 | 95 | 86 | 66 | 17,6 |
| 2 | 1 | 2 | 80 | 77 | 58 | 10 |
| 2 | 2 | 1 | 78 | 77 | 55 | 5,2 |
| 2 | 2 | 2 | 72 | 63 | 33 | 5,8 |
| 1 | 1 | 1 | 100 | 100 | 77 | 12,5 |
| 1 | 1 | 2 | 82 | 76 | 52 | 11 |
| 1 | 2 | 1 | 89 | 82 | 71 | 9 |
| 1 | 2 | 2 | 82 | 63 | 26 | 5,26 |
| 2 | 1 | 1 | 91 | 83 | 71 | 11 |
| 2 | 1 | 2 | 78,9 | 72 | 53 | 11 |
| 2 | 2 | 1 | 84 | 74 | 63 | 9 |
| 2 | 2 | 2 | 80 | 50 | 41 | 6,6 |
| 1 | 1 | 1 | 100 | 97 | 80 | 35 |
| 1 | 1 | 2 | 75 | 67 | 42 | 0 |
| 1 | 2 | 1 | 85 | 75 | 50 | 15 |
| 1 | 2 | 2 | 53 | 43 | 30 | 0 |
| 2 | 1 | 1 | 90 | 80 | 60 | 20 |
| 2 | 1 | 2 | 70 | 60 | 45 | 7 |
| 2 | 2 | 1 | 73 | 67 | 52 | 5 |
| 2 | 2 | 2 | 60 | 55 | 30 | 5 |
| 1 | 1 | 1 | 95 | 100 | 70 | 46 |
| 1 | 1 | 2 | 71 | 64 | 53 | 7 |
| 1 | 2 | 1 | 77 | 70 | 43 | 12 |
| 1 | 2 | 2 | 62 | 40 | 32 | 5 |
| 2 | 1 | 1 | 91 | 87 | 74 | 27 |
| 2 | 1 | 2 | 71 | 60 | 50 | 0 |
| 2 | 2 | 1 | 85 | 60 | 57 | 15 |
| 2 | 2 | 2 | 66 | 50 | 43 | 0 |

| Effect | Repeated Measures Analysis of Variance with Effect Sizes and Powers<br>Sigma-restricted parameterization<br>Effective hypothesis decomposition<br>SS | Degr. of Freedom | MS | F | p | Partial eta-squared | Non-centrality | Observed power (alpha=0.05) |
|---|---|---|---|---|---|---|---|---|
| Intercept | 375266.0 | 1 | 375266.0 | 4002.470 | 0.000000 | 0.994039 | 4002.470 | 1.000000 |
| Sex | 271.6 | 1 | 271.6 | 2.897 | 0.101664 | 0.107708 | 2.897 | 0.372403 |
| Treatment | 5087.1 | 1 | 5087.1 | 54.258 | 0.000000 | 0.693321 | 54.258 | 1.000000 |
| Age | 9527.9 | 1 | 9527.9 | 101.621 | 0.000000 | 0.808950 | 101.621 | 1.000000 |
| Sex*Treatment | 155.6 | 1 | 155.6 | 1.660 | 0.209901 | 0.064688 | 1.660 | 0.235590 |
| Sex*Age | 355.6 | 1 | 355.6 | 3.792 | 0.063275 | 0.136457 | 3.792 | 0.463965 |
| Treatment*Age | 83.3 | 1 | 83.3 | 0.888 | 0.355284 | 0.035698 | 0.888 | 0.147865 |
| Sex*Treatment*Age | 0.4 | 1 | 0.4 | 0.005 | 0.946273 | 0.000193 | 0.005 | 0.050490 |
| Error | 2250.2 | 24 | 93.8 | | | | | |
| TIME | 91302.7 | 3 | 30434.2 | 817.798 | 0.000000 | 0.971490 | 2453.395 | 1.000000 |
| TIME*Sex | 224.6 | 3 | 74.9 | 2.011 | 0.119963 | 0.077325 | 6.034 | 0.496632 |
| TIME*Treatment | 457.1 | 3 | 152.4 | 4.094 | 0.009665 | 0.145738 | 12.283 | 0.827584 |
| TIME*Age | 704.8 | 3 | 234.9 | 6.313 | 0.000731 | 0.208268 | 18.940 | 0.958387 |
| TIME*Sex*Treatment | 24.5 | 3 | 8.2 | 0.219 | 0.882872 | 0.009045 | 0.657 | 0.089322 |
| TIME*Sex*Age | 72.7 | 3 | 24.2 | 0.651 | 0.584674 | 0.026423 | 1.954 | 0.180409 |
| TIME*Treatment*Age | 226.4 | 3 | 75.5 | 2.028 | 0.117563 | 0.077917 | 6.084 | 0.500249 |
| TIME*Sex*Treatment*Age | 11.4 | 3 | 3.8 | 0.103 | 0.958312 | 0.004254 | 0.308 | 0.067696 |
| Error | 2679.5 | 72 | 37.2 | | | | | |

**Fig. 2.** Statistica Software global data of *D. melanogaster* memory retention test after 0.75 mM copper exposition. Repeated Measures Analysis of Variance with Effects size and Powers demonstrates a significant effect of treatment [$F = 54, 258$; $p < 0.001$], age [$F = 101, 621$; $p < 0.001$], time [$F = 817, 798$; $p < 0.001$], time x treatment [$F = 4, 094$; $p < 0.01$] and time x age [$F = 6313$; $p < 0.001$].

$p < 0,0001$]. Within subject analysis Fig. 4B indicated a significant difference in time *versus* treatment [$F (3,72) = 4.09$; $p < 0,01$] and Fig. 4. C in time *versus* age [$F (3,72) = 6,31$; $p < 0,001$] interaction. No significant effect comparing time *versus* sex (Fig. 4D), time *versus* sex *versus* treatment (Fig. 4E), time *versus* sex *versus* age (Fig. 4F), time *versus* treatment *versus* age (Fig. 4G) and time *versus* sex *versus* treatment *versus* age at all time points (Fig. 4 H−K).

## 2. Experimental design, materials, and methods

*D. melanogaster* (Harwich strain) was obtained from the National Species Stock Center (Bowling Green, OH, USA) and maintained following previously described [1] at constant temperature (22-24 °C) and relative humidity (60−70%) under 12-h dark/light cycle conditions. The offspring of approximately 70 flies (5 couples per each flask, in a total of 14 flasks divided into 2 groups) mated during 24 h were used in the present investigation. The flies were exposed to 0 or 0.75 mM $Cu^{2+}$ during the entire life cycle (from egg to adult age).

Data from official sources were manually acquired. The survival rates of larvae (5 days of exposure), pupae (9 days of exposure) and male and female adult (13 days of exposure) to $Cu^{2+}$ exposure were visually evaluated. Memory retention test was performed with protocols previously described [2,3].

Graphs were plotted using GraphPad Prism 6 Software and statistical analysis was performed using Statistica Software. Survival significance was assessed by Unpaired T test of student and two-way analysis of variance (ANOVA), followed by Newman-Keuls's post hoc test. Memory retention significance was assessed by a 2 (control/$Cu^{2+}$) x 2 (male/female) x 2 (4- or 11-days-old) x 4 (trials, immediately, 15, 30 or 60 min after the shock) ANOVA (with trials treated as repeated measure factor). For clarity, results from the between- and within-subject effects (repeated measures) were discussed separately. Differences were considered as statistically significant among groups when $p < 0.05$.

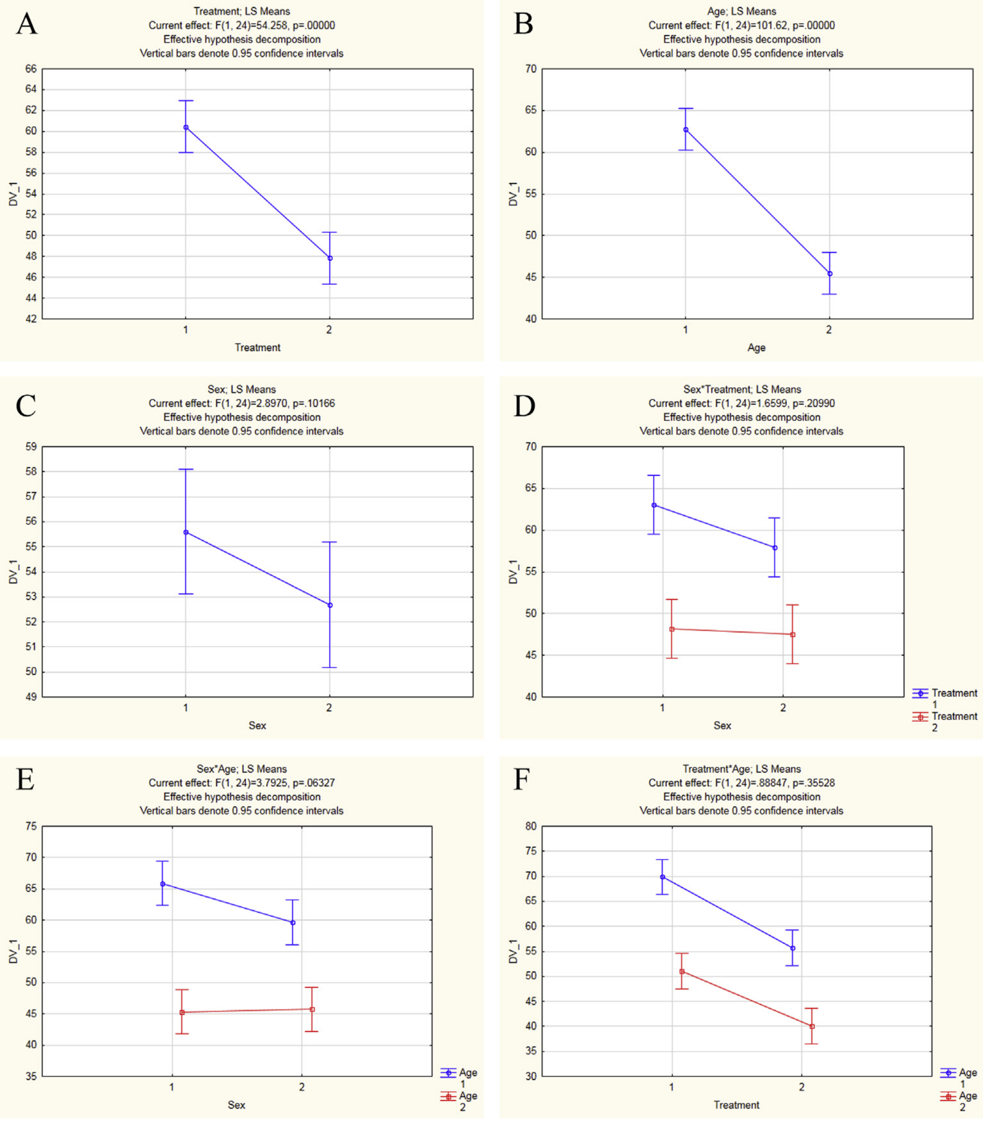



**Fig. 3.** Statistica data between subject part of the analysis - 2 treatments (control/$Cu^{2+}$) x 2 sexes (male/female) x 2 ages (4-days-old/11 days-old). Data demonstrates significant difference between (A) treatments [F (1,24) = 54,258; p < 0.05] and (B) age [F (1,24) = 101,62; p < 0.05], and no significant difference between (C) sex, (D) comparing sex *versus* treatment, (E) comparing sex *versus* age and (F) comparing treatment *versus* age.

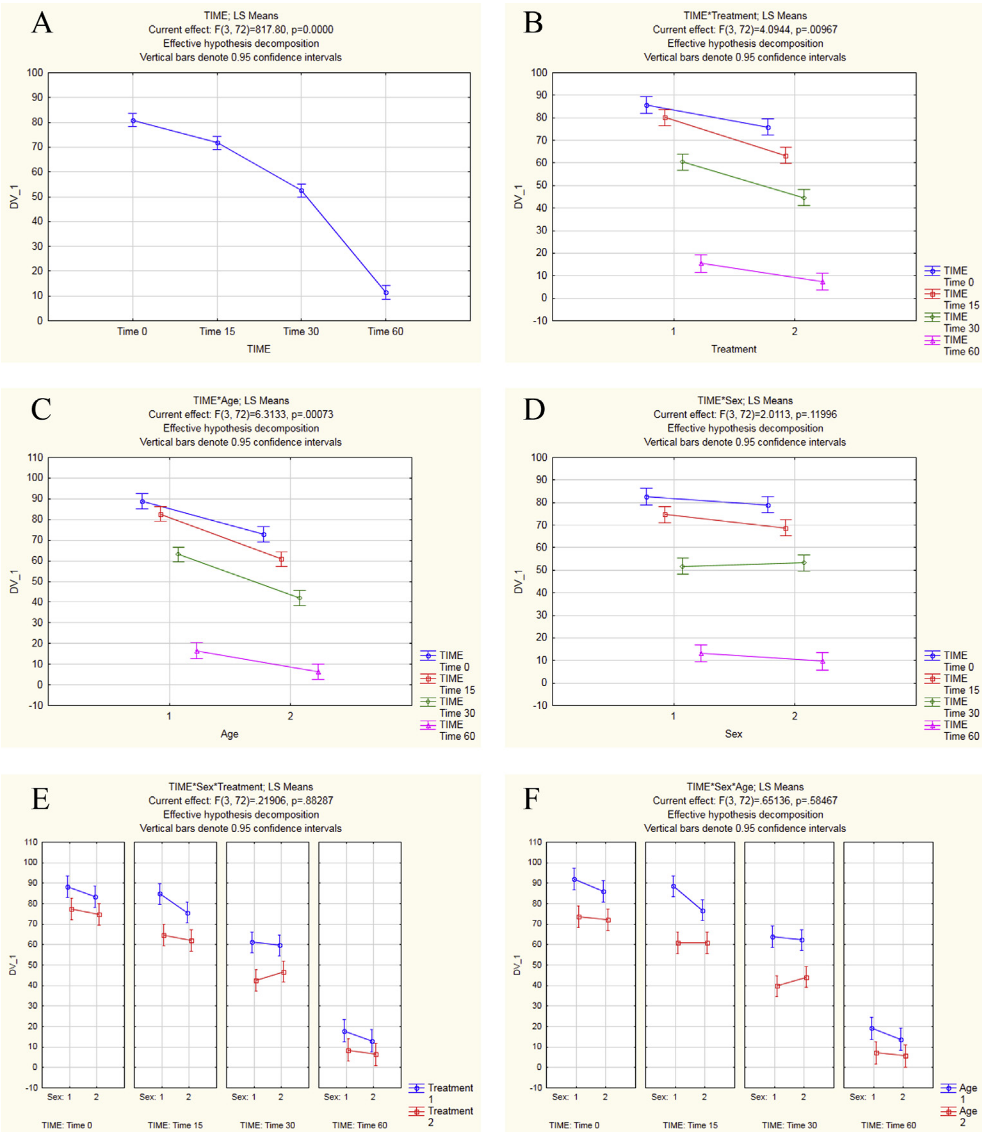



**Fig. 4.** Statistica data within subject part of the analysis - 2 treatment (control/$Cu^{2+}$) x 2 sex (male/female) x 2 age (4-days-old/11 days-old) x 4 times (O, 15, 30 and 60 min after shock). Repeated measures analysis demonstrates a significant main effect of (A) time [F (3,72) = 817.8; p < 0,0001], (B) time *versus* treatment [F (3,72) = 4.09; p < 0,01] and (C) time *versus* age [F (3,72) = 6,31; p < 0,001] interaction. No significant effect comparing (D) time *versus* sex, (E) time *versus* sex *versus* treatment, (F) time *versus* sex *versus* age, (G) time *versus* treatment *versus* age and (H–K) time *versus* sex *versus* treatment *versus* age at all time points.

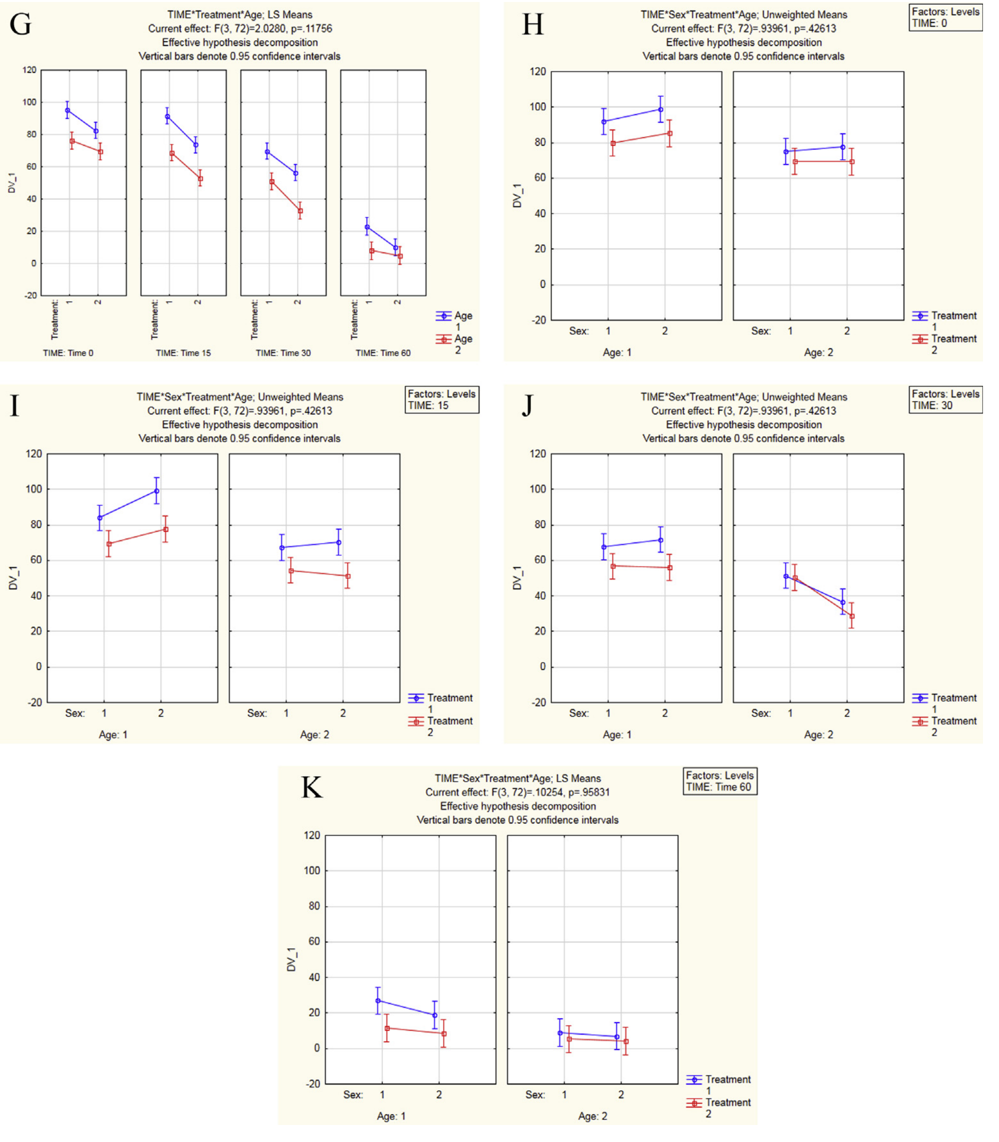



**Fig. 4.** (*continued*).

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## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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