Erratum

Erratum: Jayakumar et al., “A Multicomponent Neuronal Response Encodes the Larval Decision to Pupariate upon Amino Acid Starvation”

In the article “A Multicomponent Neuronal Response Encodes the Larval Decision to Pupariate upon Amino Acid Starvation,” by Siddharth Jayakumar, Shlesha Richhariya, Bipan Kumar Deb, and Gaiti Hasan, which appeared on pages 10202–10219 of the November 21, 2018 issue, panels in Figures 7 and 8 were inadvertently duplicated, which also affected statistical data in Table 2 and Fig. 1-1. The authors regret the error and provided the following explanation:

“In Figure 7k, the last two panels for *Hk* and *eag* were duplicated as the data in the analysis file were inadvertently duplicated. We have now reanalyzed these data and present them in the corrected figure and in Table 2. Both genes remain downregulated, but the significance is less ($p < 0.1$) than reported previously ($p < 0.05$). A corrected Figure 7 legend is included and an Excel file with all statistics is also included (Fig. 1-1, available at <https://doi.org/10.1523/JNEUROSCI.1163-18.2018.f1-1>). While this does weaken our conclusion as to the extent of change in RNA levels of *Hk* and *eag* in VGN6341 neurons by dStim knockdown, our main conclusion, that calcium signaling in larval neurons regulates RNA levels of several ion channel genes, remains unchanged.”

“In Figure 8, panels *e* and *f* represent the area under the curve for the initial and later phase of the calcium response, respectively. These were inadvertently shown by the same boxplot. We have now rectified this error, and the corrected Figure 8e represents the area under the curve for calcium response in the initial phase of amino acid withdrawal, which is no different between control VGN6341 neurons and those expressing toxins. Corrected Figure 8f represents the area under the curve for calcium responses in the later phase of amino acid withdrawal, where the response in VGN6341 neurons expressing toxins is significantly lower than that in controls. Upon reanalysis of these data, the number of cells and the statistics changed numerically, and this has been indicated in the corrected legend for Figure 8 and in Fig. 1-1.”

This correction does not affect the main conclusion of the article. Figures 7 and 8, Table 2, and Figure 1-1 have been corrected in the online version, and corrected versions of Figures 7 and 8 and Table 2 are displayed below.

Figure 7. Intracellular calcium signaling through *IP₃R*/SOCE in glutamatergic neurons regulates the expression of genes encoding ion channels. **a**, Venn diagrams representing the number of genes identified, as differentially expressed by the three independent methods. *IP₃R* knockdown leads to downregulation of a set of genes. For a list of genes, refer to Figure 7-1 (available at <https://doi.org/10.1523/JNEUROSCI.1163-18.2018.f1-1>). **b**, Bars represent the number of genes upregulated or downregulated upon knockdown of the *IP₃R* in the CNS, as measured by RNA-seq. **c**, Bars represent the expression levels of *IP₃R* in the indicated conditions, *q* value refers to the corrected *p* value obtained from CuffDiff. **d**, Bars represent the fold enrichment in the number of genes of the indicated GO molecular function categories in the set of genes downregulated upon *IP₃R* knockdown, compared with all genes in *Drosophila*. Numbers on top of the bars indicate FDR-corrected *p* values. This analysis was performed using the Panther GO Slim Molecular Function option. **e**, Heatmap indicates the fragments per kilobase per million (FPKM) values as a proxy for expression level of the indicated cation channel genes in control (*UAS-IP₃R* IR/+; *UAS-dicer2*/+) and *IP₃R* knock-down (*elav^{C155}-GAL4>UAS-IP₃R* IR; *UAS-dicer2*) conditions. Red labels indicate genes whose expression is significantly altered by *IP₃R* knockdown identified by all three methods (CuffDiff, DESeq, and edgeR), and pink labels indicate differential gene expression significant by any two methods. Gray labels indicate differential expression of genes that are not significant. **f**, Bars indicate the fold change in expression levels of the indicated genes normalized to *rp49* measured by qRT-PCR from CNSs of second instar larvae to that of third instar: *p* = 0.019984 for *cac*, two-tailed Student's *t* test. **g**, Diagram representation of the procedure used to sort glutamatergic neurons of interest. **h**, Representative dot plots of flow cytometric analysis of cell suspensions made from the indicated genotypes. The x-axis indicates the extent of fluorescence; the y-axis indicates a measure of granularity based on the side-scatter. Threshold was set using the nonfluorescent wild-type cells, and pink dots were collected as GFP-positive cells. **i**, Bars represent the percentage of GFP-positive glutamatergic cells obtained by FACS from the indicated genotypes. One-way ANOVA: $F_{(2,9)} = 24.3$, $p < 0.05$; with *post hoc* Tukey's MCT. Bars with the same alphabet represent statistically indistinguishable groups. **j**, Comparison of the levels of *VGlut* in whole CNS vs sorted glutamatergic neurons compared with the housekeeping gene *rp49*. *VGlut* expression is enriched in the sorted glutamatergic neurons. **k**, Bars indicate the fold change in expression levels of the indicated genes normalized to *Act5c* measured by qRT-PCR from sorted glutamatergic cells of the control (*VGN6341-GAL4>UAS-eGFP*) and *dStim* KD (*VGN6341-GAL4>UAS-eGFP*; *UAS-dStim* IR; *dcr2*) genotypes: *p* = 0.012 (*dStim*), *p* = 0.003 (*mAChR*), *p* = 0.023 (*NaCP60E*), *p* = 0.068 (*Hk*), *p* = 0.087 (*eag*), *p* = 0.077 (*cac*), *p* = 0.008 (*Ca-α1D*). RNA was isolated from ~1200 sorted neurons and amplified using the SMART-seq method before performing qRT-PCR. **p* < 0.1, ***p* < 0.05; ****p* < 0.01; two-tailed *t* test. Exact *p* values are provided in Figure 1-1 (available at <https://doi.org/10.1523/JNEUROSCI.1163-18.2018.f1-1>).

Figure 8. IP₃R/SOCE in glutamatergic neurons regulates neuronal excitability. **a**, Line plots represent calcium transients observed upon depolarization by KCl in *VGN6341-GAL4* marked glutamatergic neurons from CNS of the indicated genotypes. Gray box represents the window of addition of KCl. Responses from all cells have been plotted. **b**, Box plots indicate the peak change in fluorescence from traces in **a**. One-way ANOVA: $F_{(3,80)} = 13.44943$, $p < 0.05$; with *post hoc* Tukey's MCT. **c**, Line plots indicate calcium transients observed upon withdrawal of EAAs in control CNSs and in CNSs with expression of the indicated toxins in *VGN6341-GAL4*-marked glutamatergic neurons. Red dot indicates the point of withdrawal of EAA. The transients are from cells that responded above an arbitrary threshold of $\Delta F/F \geq 1.5$ after withdrawal, as described in Materials and Methods. Data are from a minimum of five CNSs of the individual genotypes. Control trace is the same as in Figure 2b. **d**, Percentage of responders from the trace in **c**: one-way ANOVA: $F_{(3,23)} = 30.58001$, $p < 0.05$; with *post hoc* Tukey's MCT. **e**, **f**, Area under the curve quantified from the trace in **c** for the initial phase from 60 to 300 s; one-way ANOVA: $F_{(3,281)} = 0.29025$, $p < 0.05$; with *post hoc* Tukey's MCT (**e**) and the later phase from 300 to 600 s; one-way ANOVA: $F_{(3,281)} = 6.25773$, $p < 0.05$; with *post hoc* Tukey's MCT (**f**). **g**, Bar graphs indicate the percentage of pupariation of the indicated genotypes on an amino acid-deficient diet; one-way ANOVA: $F_{(3,12)} = 120.0924$, $p < 0.05$; with *post hoc* Tukey's MCT. Boxes and bars with the same alphabet represent statistically indistinguishable groups. **h**, Schematic summarizing cholinergic activation (mAChR) and peptidergic modulation (FMRFaR, CCHa2R, and AstAR) of glutamatergic neurons required for pupariation on a PDD. GPCRs stimulate calcium release through the IP₃R followed by SOCE in glutamatergic neurons. The intracellular calcium signaling regulates the expression of genes encoding several ion channels as well as the mAChR. Activation of intracellular calcium signaling mechanisms and ion channels stimulates a complex calcium response across glutamatergic neurons upon amino acid withdrawal. The neuronal response is necessary for pupariation on PDD. Exact *p* values are provided in Figure 1-1 (available at <https://doi.org/10.1523/JNEUROSCI.1163-18.2018.f1-1>).

Table 2. Fold changes in mRNA upon *dStim* knockdown in VGN6341 neurons

Gene	Normalized to <i>rp49</i>					Normalized to <i>tubulin</i>				
	Control		<i>dStim</i> KD		<i>p</i> value	Control		<i>dStim</i> KD		<i>p</i> value
	Mean FC	SEM	Mean FC	SEM		Mean FC	SEM	Mean FC	SEM	
<i>Act5c</i>	1.01	0.07	1.01	0.05	0.93	1.01	0.08	0.92	0.13	0.58
<i>itpr</i>	1.01	0.07	0.79	0.07	0.11	1.00	0.03	0.83	0.19	0.43
<i>Stim</i>	1.00	0.07	0.58	0.02	0.00	1.02	0.15	0.54	0.10	0.05
<i>mAChR</i>	1.04	0.20	0.41	0.03	0.04	1.02	0.16	0.42	0.09	0.03
<i>NaCP6OE</i>	1.04	0.23	0.52	0.06	0.09	1.03	0.18	0.55	0.14	0.10
<i>Hk</i>	1.07	0.26	0.47	0.03	0.08	1.08	0.29	0.45	0.10	0.11
<i>eag</i>	1.04	0.21	0.55	0.07	0.09	1.06	0.26	0.53	0.14	0.14
<i>cac</i>	1.00	0.06	0.63	0.15	0.08	1.01	0.13	0.61	0.23	0.20
<i>ca-alpha1D</i>	1.00	0.06	0.49	0.04	0.00	1.02	0.14	0.45	0.10	0.03
<i>VGlut</i>	1.01	0.12	0.58	0.07	0.03	1.01	0.10	0.58	0.07	0.02

Fold changes normalized to two housekeeping genes: *rp49* and *tubulin*. *p* Values obtained from a two-tailed *t* test. FC, Fold change; KD, knockdown. *n* = 3.

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