Facilitating Neuron-Specific Genetic Manipulations in *Drosophila melanogaster* Using a Split-GAL4 Repressor

Michael-John Dolan, Haojiang Luan, William C. Shropshire, Ben Sutcliffe, Benjamin Cocanougher, Robert L. Scott, Shahar Frechter, Marta Zlatic, Gregory S. X. E. Jefferis, Benjamin H. White

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Abstract

Efforts to map neural circuits have been galvanized by the development of genetic technologies that permit the manipulation of targeted sets of neurons in the brains of freely behaving animals. The success of these efforts relies on the experimenter's ability to target arbitrarily small subsets of neurons for manipulation, but such specificity of targeting cannot routinely be achieved using existing methods. In *Drosophila melanogaster*, a widely-used technique for refined cell type-specific manipulation is the Split GAL4 system, which augments the targeting speci city of the binary GAL4-UAS (Upstream Activating Sequence) system by making GAL4 transcriptional activity contingent upon two enhancers, rather than one. To permit more refined targeting, we introduce here the "Killer Zipper" (KZip+), a suppressor that makes Split GAL4 targeting contingent upon a third enhancer. KZip+ acts by disrupting both the formation and activity of Split GAL4 heterodimers, and we show how this added layer of control can be used to selectively remove unwanted cells from a Split GAL4 expression pattern or to subtract neurons of interest from a pattern to determine their requirement in generating a given phenotype. To facilitate application of the KZip+ technology, we have developed a versatile set of LexAop-KZip+ y lines that can be used directly with the large number of LexA driver lines with known expression patterns. KZip+ signi cantly sharpens the precision of neuronal genetic control available in Drosophila and may be extended to other organisms where Split GAL4-like systems are used.

Key Terms

- *Drosophila melanogaster*
- Transgene expression
- Neural circuits
- Gal4-UAS system and Split-Gal4
- LexA-LexAop system

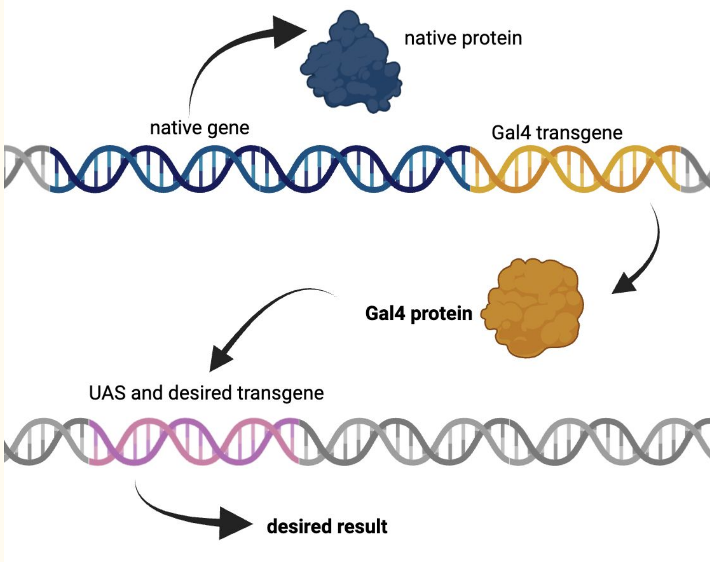
***Drosophila melanogaster*:** a species of fruit/vinegar flies used for genetic research

transgene expression: transgenes are genes artificially inserted into model organisms (like *Drosophila*); transgene expression refers to the location of cells where those genes are active, and the creation of the gene product occurs (usually a protein). This expression could be, for example, every cell in the organism; the expression could also be limited to certain kinds of cells (like neurons), or to cells where another specific "linked" native gene is expressed.

neural circuits: a population of neurons connected by synapses, with an assigned role carrying out a certain function.

Gal4-UAS system:

used for limiting transgene expression. Gal4 is a transcription factor that activates the expression of the gene next to the UAS (Upstream Activating Sequence) in a chromosome. Most organisms, including *Drosophila*, do not naturally have a UAS region. If the UAS region, along with the neighboring transgene, is inserted into the organism's genome, the Gal4 transcription factor will find that the UAS and that transgene and activate it.



LexA-LexAop system: similar to the Gal4-UAS system, where LexA activates a transgene inserted in the LexA-operator (LexAop). These two systems don't affect each other, so they can be used simultaneously with no reaction.

Split-Gal4:

The Split-Gal4 approach modifies the Gal4-UAS system. Most organisms do not normally express Gal4. Normally, Gal4's expression can be linked to a single native gene by inserting it next to that gene. When that native gene gets expressed, so does Gal4, and subsequently the transgene of choice.

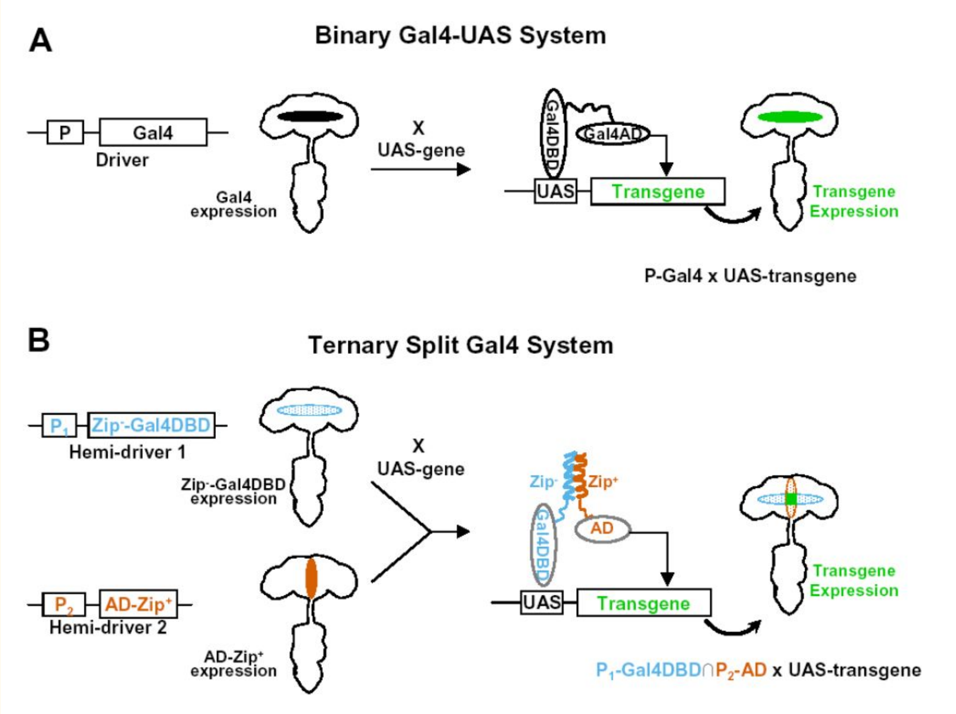
Split-Gal4 allows Gal4 to be broken into two domains (AD and DBD), each of which are linked to separate native genes; when both native genes are active, both halves are transcribed and created, they join to form Gal4 and express the transgene.

The two domains are: AD (Activation Domain) and DBD (DNA-Binding Domain)

The Split-Gal4 system was first described in this study:

https://pubmed.ncbi.nlm.nih.gov/17088209/

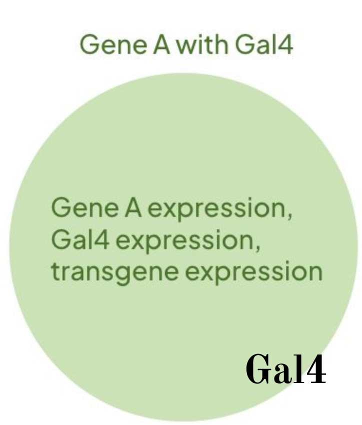
Refined Spatial Manipulation of Neuronal Function by Combinatorial Restriction of Transgene Expression, Luan et al, 2006.

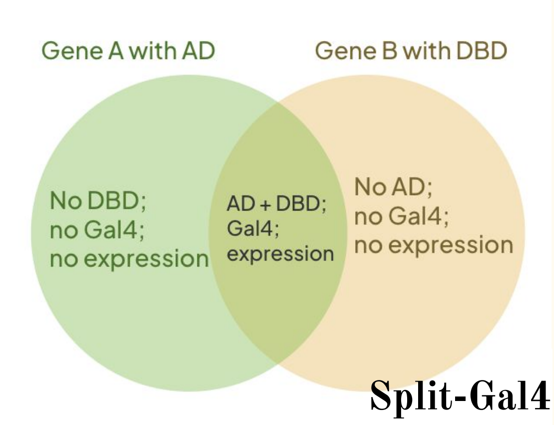


Luan et al's work is the precursor to this study. This diagram from the earlier study shows how the Split-Gal4 system works.

Objective

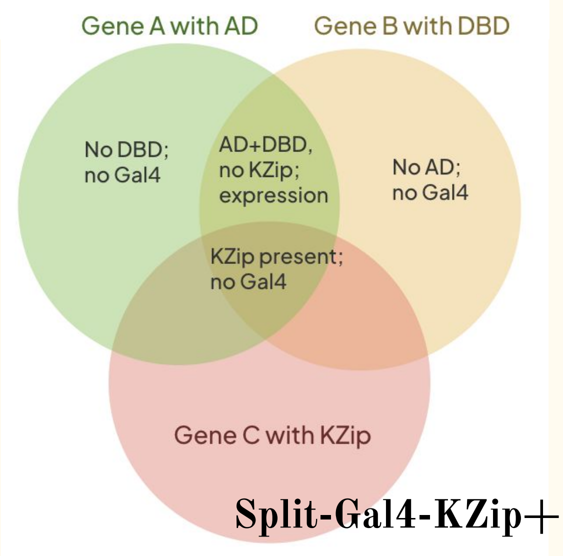
The Gal4-UAS system allows a transgene to be expressed in cells where one specific native gene is also being expressed; this is "one dimensional" expression.





Split-Gal4 allows the transgene expression to be linked to the activity of two native genes; this is 2D expression.

This study creates a third dimension to transgene expression by creating the KZip, or Killer Zipper construct.



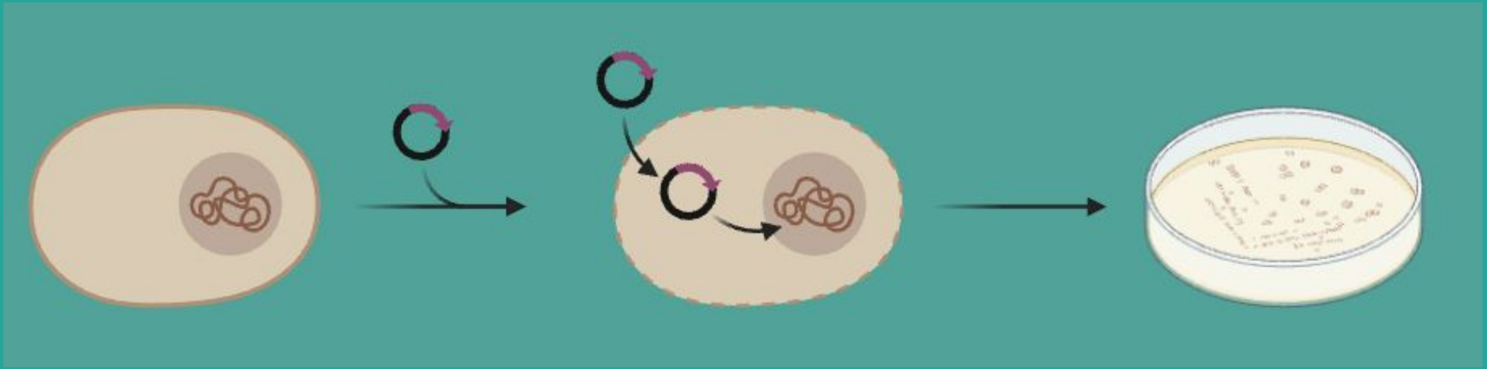
Introduction

Studying gene expression is a wide field in biology, and being able to identify which cells express what genes is something of interest. Even better, being able to tie transgene expression to certain cell types or certain genes allows studying of certain specific things, applicable in many kinds of genetics. For example, this Split-Gal4 system is widely used for studying neural circuits, and synapse formation.

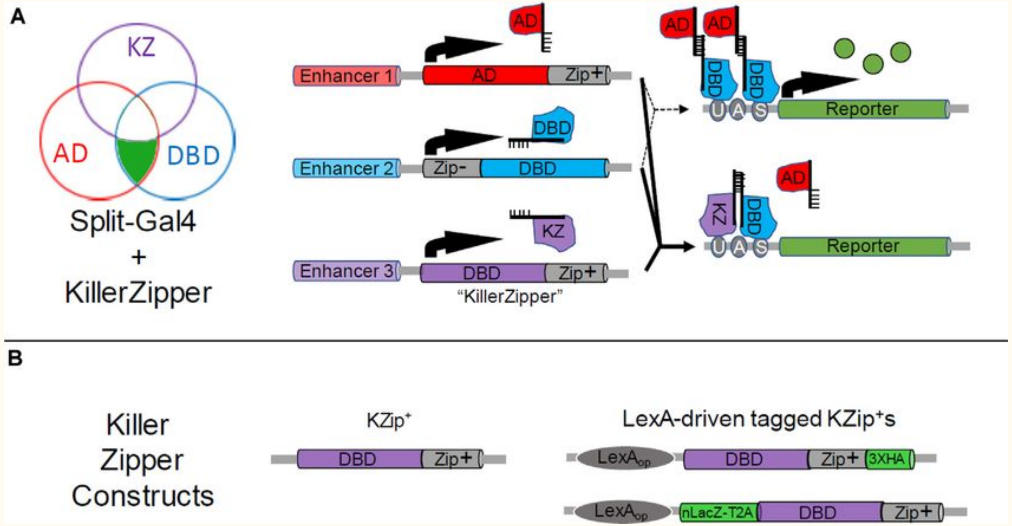
It is of interest to be able to introduce a mutation into a cell through a transgene, and limit the activity of that transgene to certain types of neurons.

The current Split-Gal4 method lets us target cells using the overlap in expression of up to two different genes, but a new method created in this study will expand this to three.

Methodology



A Killer Zipper (KZip) construct was made from piecing together existing DNA domains. The result is a KZip+ bound to a DBD, which is similar to the Gal4 transcription factor, but is inactive.

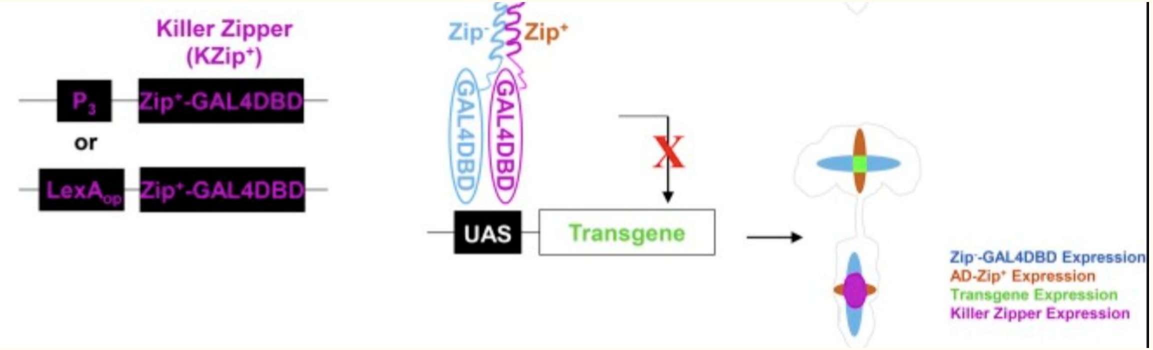


This inactive version competes with the normal AD-DBD by binding to the UAS region but not actually activating the gene's transcription, not allowing the actual Gal4 construct to do its work. It is effectively a Gal4 repressor.

This KZip construct was tested multiple times and modified to maximize its ability to disrupt Gal4 activity. Multiple different versions were created, using different domains or different KZip+ constructs.

To test the effectiveness, *Drosophila* fly stocks were created with these mutations, in order to see if the KZip construct accurately repressed Gal4 in the cells where it was present. A final KZip+ construct was designed, for best performance.

All of these were done using the LexA-LexAop system, so that it wouldn't interfere with the Split-Gal4 system.



Fly transformation takes multiple steps.

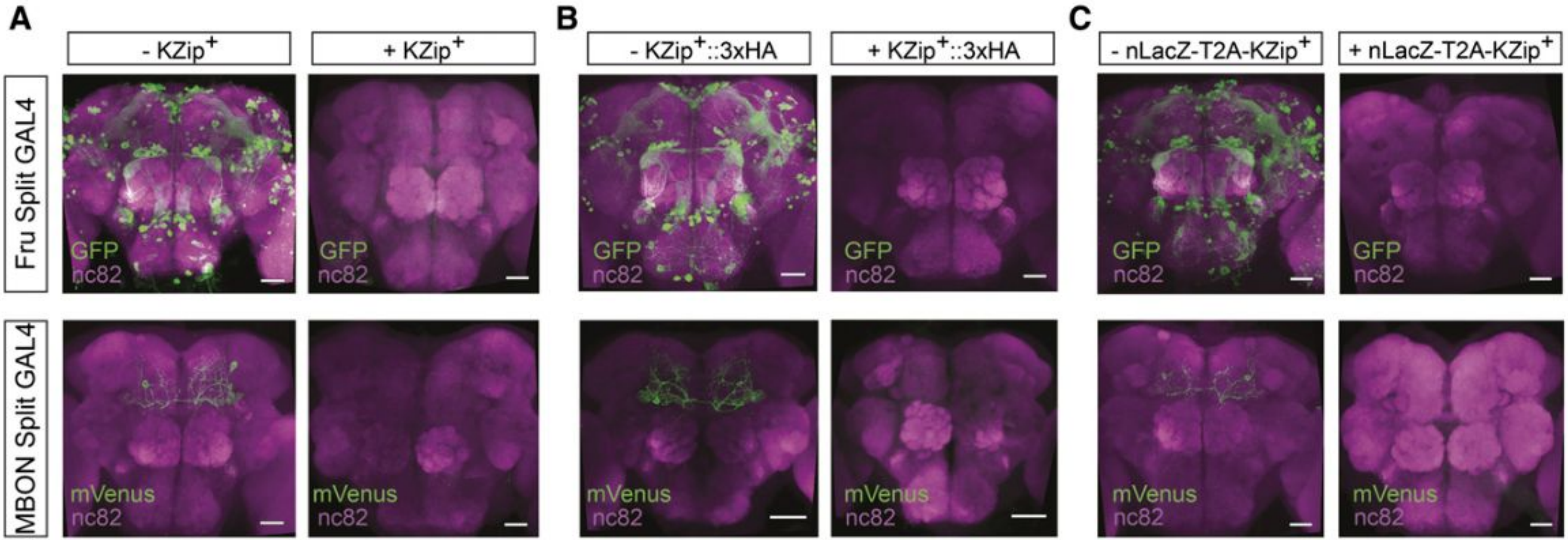
There are four mutations necessary to create and use a KZip stock:

1. The AD must be inserted into a native gene of choice, whose expression we want to include
2. The DBD must be inserted into a second native gene, whose expression we want to include
3. The KZip must be inserted into a third native gene, whose expression we want to exclude
4. The UAS and desired transgene must be introduced in the genome.

Then, stocks, or populations of flies, can be created, all with the same modifications. These can reproduce and be studied.

Results

In every case where the KZip+ construct was used to repress the SplitGal4, there was no remaining mVenus expression (in green); this shows the high affinity of KZip+ to the UAS, and shows how well it blocks expression of the transgene.



Dolan et al. also created stable fly lines (a population/genotype in which all flies have the same mutation) that contain this mutation, using the LexA-LexAop system. This allows the expression of KZip to be linked to almost any desired native gene, to block SplitGal4 expression in those cells.

These fly lines also are available for purchase, allowing other researchers to start where Dolan et al. stopped, instead of trying to create the KZip+ construct mutations from scratch. This allows the results to actually be utilized for other researchers, which was the entire goal.

Overall, this study created a successful method for repressing Gal4 activity, limiting the expression of UAS transgenes by a third dimension.

Discussion

Use of the KZip+ construct adds a third dimension to the specificity of transgene expression, allowing desired inserted genes to only be expressed in very few, select cells. This lets researchers more accurately note the effects, as well as conduct many other kinds of research, especially concerning neurons and neural circuit formation.

This method, developed in 2017, has become a common way of specifying transgene expression. It has been used for neural circuit mapping and research, and has use in many other genetic fields. It adds a layer of specificity to the Split-Gal4 system (developed in 2006), that allows researchers in the field to study gene expression to precise levels, more than previously thought.

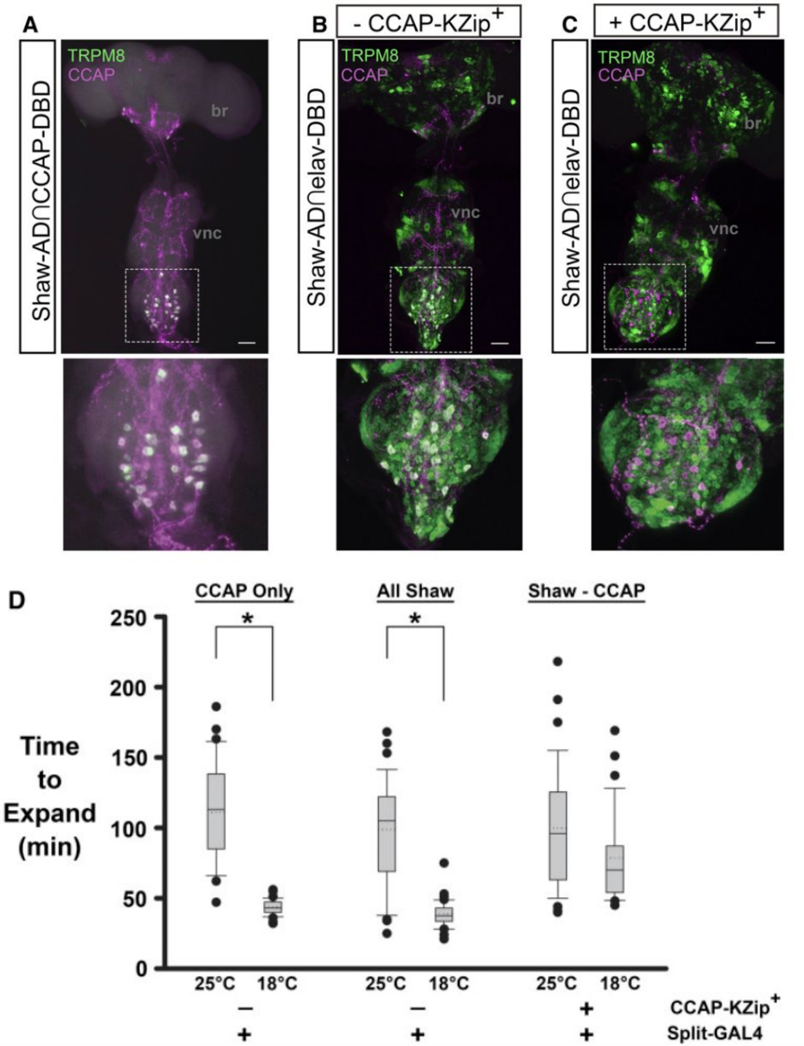
Analysis

This study created a new, effective way to repress the Split-Gal4 construct.

The study presents the results in a thorough and detailed way, especially the methodology. Since this is an experimental study, the results are designed to be as replicable as possible, and the methodology is very specific to aid in that. Also, their “results” (The LexA-LexAop KZip+ fly lines) are available for purchase, allowing other researchers to either verify their results or conduct their own research using the tools this study created.

The study didn't talk about specific statistics associated with each version of the KZip+ construct; no detailed quantitative analysis was performed per version to see which was the best, because the results are qualitative and visually apparent: it is easy to see which neural cords don't have certain genes expressed (green and purple), but harder to quantify that into percentages.

The study did, however, perform statistical analysis on measured outcomes that they created using this system (secondary/consequential statistics).



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