

# "Gravitational clustering" software manual

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**This manual is intended to aid use of the 'gravity suite' of programs enhanced or developed at the University of South Florida (USF). Source code for the original 'core' gravity algorithms was provided by George L. Gerstein, University of Pennsylvania.**

This suite, written in FORTRAN 77/FORTRAN90 originally ran on Hewlett-Packard computers under the HP-UX 10.2 or 11.0 operating system. Graphics routines in the core gravity calculation and display programs make calls to HP Starbase libraries. A port by Russell O'Connor allows operation in the Linux environment. Other utility programs included in the distribution package, such as "scope" and the "surrogate spike train generator program", are open source programs written in C; they also run under Linux.

## **Acknowledgements**

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## Introduction

The "gravity" method is a tool for the detection and analysis of dynamic associations among groups of simultaneously monitored neurons. Various references to the literature are provided at the end of this manual. For a concise introduction to the method, see Gerstein, GL, "Gravitational Clustering", Chapter 8 of *Analysis of Parallel Spike Trains*, S. Grün, S. Rotter (eds.), Springer Series in Computational Neuroscience 7, DOI 10.1007/978-1-4419-5675-0\_8, © Springer Science+Business Media, LLC 2010.

The gravity programs in this package currently have parameters set to support up to 64 simultaneously recorded spike trains. Projections from N-space to a plane are currently limited to 16 neurons. Up to 10,000 spikes from each neuron can be processed. (The **scope** program can be used to edit spike files to pare down the data).

The suite includes:

- A new graphical user interface for the suite developed by Dale Shuman in 2017
- A program to calculate gravitational representations of the data with various options
- A program to calculate sets of gravity representations of surrogate data files for significance testing
- A variety of display programs for viewing gravity results from various perspectives.
- Utility programs, including **scope** to "view" and edit spike train data files and a **surrogate spike train generation** program for significance testing of gravity results

## Running gravity

The programs can be run individually from the command line prompt or with a "new" (2017) graphical user interface... type 'gravity\_gui' at the prompt

### The input files

The main gravity program "gbatch\*" requires a \*.gdt input file

[With the tuning options, an "offsets.gnew" file must also be available at run time (see Lindsey and Gerstein, 2006). This formatted file (I5, I5, I5) is currently created and viewed with a text editor, so the process is a bit tedious, but can be accomplished by examination of the corresponding cross-correlograms from, say, the xanalysis program. Each line has the gravity particle pair and the offset value in msec (integer) for the PRIMARY significant cross-correlogram feature, e.g., an offset peak with a positive 5 ms lag in a 1-to-2 (reference-target) cross would be (2, 1, -5)].

The data read in by **gbatch** needs to have a particular format and a \*.gdt extension. Use a utility program such as scope or a text editor to create a \*.gdt file from a \*.bdt file. The bdt file is in a text or ASCII format and is a continuous "time chunk of data" in a list format '(I5, I8)' where I5 is the spike code and I8 is the time of that particular event with 0.5 ms resolution. This format can represent a maximum time of 13.8 hours, i.e., the largest positive value for I8: 99,999,999. However, the single precision float (the default in FORTRAN) used in old programs will not work for recordings longer than 2.3 hours - clearly a problem with longer recordings. The \*.gdt file format is like the bdt format except that it has a single markb code (21) at the start and a marke code (22) at the end, so obviously 21 and 22 are reserved and cannot be used as spike codes, which typically have values between 100 and 999.

A program similar to gbatch is called "**gsig\***", which reads in a set of "surrogate "**\*.rdt**" files (with the same format as the gdt file) for a Monte Carlo significance test.

The user needs to generate the set of **rdt files** with a **surrogate generator** program. This program generates a specified number of files (say 1000) using an algorithm that maintains a similar firing rate modulation but alters the detail spike timings.

The current surrogate generator is based on *An Accurate Measure of the Instantaneous Discharge Probability, with Application to Unitary Joint-Event Analysis* by Quentin Pauluis, Stuart N. Baker, *Neural Computation* 2000 12:647-666, 2000. The surrogates have gamma-distributed inter-spike intervals with the shape parameter of the gamma distribution for each spike train estimated from the data as in *Estimating Spiking Irregularities under Changing Environments* by Keiji Miura, Masato Okada, Shun-ichi Amari, *Neural Computation* 18: 2359-2386, 2006.

The current surrogate program can have "bdt" or "edt" format files as input (edt files have a 0.1ms time resolution).

edt\_surrogate

surrogate 1.0.6

usage: edt\_surrogate whatever.edt [OPTIONS]

Generates N files (in the current directory) named whatever\_01.edt, whatever\_02.edt, etc., with surrogate spike trains. Will overwrite any existing files by those names.

OPTIONS:

include N ... : channels N ... from whatever.edt will be included in the output. All others will be excluded.

exclude N ... : channels N ... from whatever.edt will be excluded from the output. All others will be included.

keep N ... : included digital channels N ... from whatever.edt will be included as-is in the output file. All other included digital channels will be replaced with surrogate spike trains.

replace N ... : included digital channels N ... from whatever.edt will be replaced with surrogate spike trains. All other included channels will be included as-is in the output file.

count N : N is the number of output files. Default is 1.

seed N : N is used as the seed of the random number generator.

\* If neither "include" nor "exclude" is specified, all channels will be included. Don't use both.

\* If neither "keep" nor "replace" is specified, all included digital channels will be replaced. Don't use both.

\* To specify an analog channel, add 1000 to the analog ID.

\* Included analog channels are always included as-is in the output files.

\* The input file can be .bdt or .edt. Output files will be the same type.

**If the input is .gdt, the outputs files will be shN.rdt**

EXAMPLE:

**Simple example with options used for gravity analysis...**

edt\_surrogate whatever.gdt count 100 seed 37

## Running gravity – the parameter file (param)

Create (or edit) the file with an editor in the following format (typical values). Note that the first line is ignored by gbatch\* but used by gsig\* to indicate how many surrogate files to read in. **The red text (for an option not developed) must be included for correct program behavior.**

```

1000      ! number of shift control sets to generate - currently MUST = 100, 1000, or 10000!!
filename.gout ! position file - position of particles at time t (Input file for projection display program)
4         ! number of particles (N) (= number of spike trains; up to 64)
2.0       ! time step in ms
2.5e-5    ! slide value - inverse of viscosity
10        ! normalization factor
-1        ! acceptor forward (1) or backward (-1) For central peak features, start with both set to +1.
1         ! effector forward (1) or backward (-1)
1.        ! force sign - +1. excitation, -1. inhibition
3         ! no rate normalization: 3 is the only choice now
5.0       ! fwd Tau value (The tau values are usually between say 1.5 and 5.5,given correlation feature duration)
5.0       ! bck Tau values, N lines (Default: same as fwd Tau values for each spike train)
100.      ! fwd charge increment
100.      ! bck charge increment
10.       ! force well diameter - repelling distance
?         ! sets some gravity 'enhancement' must select one of four options (N, O, P, or Q)- see below
50        ! channel numbers - N lines
49        ?
77        ?
73        ?
y         ! Is this correct ?
filename.gdt ! input file
1000.     ! time span of each blocks INCLUDING 4*tau gap - between markb, marke
filename.hdt ! HDT file name for intracellular rec. data;
N         ? Enter "N" on this line to run without intracellular data
2         ! number of channels to read in hdt file
2         ! which channels in HDT file to read from
1         ! Enter 1 to use EPSP's; 0 to use IPSP's
1         ! width of action potential in number of msec
1         ! signal processing option
          ? 1= calc mean from raw data
          ? 2= top half wave rectify, then calc mean
          ? 3= bottom half wave rectify, then calc mean
          ? 4= full wave rectify
1         ! 1= call subroutine ap_del
2         ! number of records to offset to avoid calibrating
          ? signal, etc.
1000     ! window size for local averaging; MUST be even integer
          ? number indicates number of array elements to use
9        ! channel showing spikes for intracellular trace
filename.pos ! for trydis - plots distance between particles
filename.dir ! for direct3d - plot -> direction of interaction
e         ! exit

```

---

gbatch options	Charge decay option:	AF-EF	AB-EF	AF-EB	Output files
OPTION ('N') - Standard gravity, N-Dimensions		OK	OK	OK	*.pos (distance between pairs), *.gout (n-space projections), and *.dir files.
OPTION ('O') - Tuned gravity, N-Dimensions		X	X	X	*.pos (distance between pairs), *.gout (n-space projections), and *.dir files.
OPTION ('P') - Standard gravity 1-Dimension		X	NA	NA	*.pos (distance between pairs)
OPTION ('Q') - Tuned gravity, 1-Dimensions		X	NA	NA	*.pos (distance between pairs)

The \*.pos output file can be viewed with the **xtrydis** or **xslope** programs.

The \*.gout output file can be viewed with the **xproitm** (2-D projection).

The \*.dir output file is viewed with **direct3d** programs for the 3-D" gravity option described in Lindsey and Gerstein 2006. For correct behavior, these files should ONLY be used when acceptor charge is backward (-1) and effector charge is forward (1). A=acceptor charge on particles; E=effector charge on particles; F=forward, B=backward; see Gerstein and Aertsen 1985 - references at end of this manual):

### ***A typical initial gravity run***

1. After creating the gdt file, use a shell script "run.sh" to run the gbatch\* program. "run.sh" contains something like the following command line (path may be included): **gbatch\_05 < param** At the prompt: **./run.sh** to "feed" the param file to gbatch.
2. Recommendation.. First "play" with the parameters and use xtrydis to see how the particle pairs are aggregating. If too much, back off on the slide (viscosity) parameter value; if too little, increase it.
3. Note that if the particles get closer than 10 distance units, aggregation stops and the distance line will be flat in the PDFT plot seen with xtrydis.
4. Between trials and until you want to keep the pos file, be sure to remove the \*.pos \*.gout \*.dir", before running the script again.
5. Start with the N option and P options. For the P (on-dimension) option, only use xtrydis and xslope.
6. Once you are satisfied with the parameters, create the surrogates. Modern computers can generate a 1000 in a few minutes. Use a shell script to run the gsig\* program, e.g., **gsig\_05 < param**, making sure to use the same parameters as the original calculation.
7. Now you are ready to use xslope to (1) see if there is significant aggregation and (2) to look for regions with high aggregation velocities.

## Displays of Gravity Results

Program	Display	Input file	Comment	Uses surrogate "control" data sets
<b>xtrydis</b>	1. Particle distance v. time plots in 2-D or 3-D. 2. Spike rate plot.	*.pos	Shows PDFT plots	
<b>xprojt</b>	Various 2-D animated projections of groups.	*.gout	N-space trajectories projected to a plane	
<b>xslope</b>	XSLOPE REQUIRES THAT INTERPARTICLE DISTANCES ALWAYS > 10 DISTANCE UNITS	*.pos and *.sh controls	Can show single PDFT plot and significance "cone"	Uses surrogate "control" data sets
<b>direct3d_bl</b> <b>direct3d_bl_mp</b> <b>direct3d_bl_sig_01</b> <b>direct3d_bl_sig_05</b> <b>direct3d_bl_sub</b>	3-D plot of particle pair properties - used ONLY with acceptor charge decay BACKWARD and effector charge decay FORWARD to infer direction of interaction. Different versions show single trajectory, multiple trajectories, with/without surrogate projections – as dots	*.dir	For plotting 3-D enhancement (see Lindsey & Gerstein 2006)	Uses surrogate "control" data sets (for "sig" versions)
<b>Other display options</b>				
<b>spkpat6bg</b>	text list of results; *.spk is 1 spk series *.txt is details- can go to spread sheet. *.fwk is times of all spk series for 1 criterion	idl.out	file. <b>pat</b> is main file; also opt.: *. <b>spk</b> *. <b>txt</b> *. <b>fwk</b>	Uses xslope output files; <b>Generates null hypothesis surrogates internally</b>
<b>spkpat6bgr</b> (formerly <b>spkpat6bgsig</b> )				Compares shuffled template with other shuffled sets
<b>3djmpbg</b>	3-D projection of idl*.out data: SPARK plots, inc. side wall analog & back wall phase plane – up to 3 sets	<b>idl.out</b> from <b>xslope</b> & *. <b>spk</b> from <b>spkpat6bg</b>		Uses xslope output files
<b>fireworks2</b>	Rows of all spark patterns & bdt analog	*.fwk		
<b>hires2kv32</b>	3-D spark at single time step & dynamic planes-see source code	Idl.out and offsets.gnew (from gbatch2kv11 with tune option selected		Uses xslope output files



### Displays of gravity clustering: xslope

After using **xtrydis** for initial views of the \*.pos (pair distances vs. time) output file from gravity, and maybe looking at the animated projection from n-space to a plane with **xprojetm** (the \*gout file as input) it is time for **xslope**.

The program gives the following plot options:

1. Particle distance as a function of time (like xtrydis), but with a Monte-Carlo significance band generated from the surrogate spike trains
2. Graph of when all pairs are "significantly" close. You MUST select this option to generate the data needed to use the MASK option in spkpat6bg.
3. Color scaled histogram of particle velocity for all pairs at all steps. You MUST select this option. The output file from this choice is the file normally used by spkpat6bg.

NOTE: You need to adjust parameters in the **param** file so that INTERPARTICLE DISTANCES ALWAYS > 10 DISTANCE UNITS... so movements can occur. (Gravity limits movements when particles close (due to the math) - see source code for gbatch\* and early papers by G. L. Gerstein *et al.*, 1985)

The program xslope generates 2D data files that can be plotted. Each row has elements that contain the velocity of aggregation for a pair of particles at each "plotted" step (a range of computed gravity steps). The files can be displayed in dynamic (through time) "spark plots" using the program **3djmp**, as described subsequently.

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### spkpat6bg

Repeated patterns of synchronous spiking can be identified by comparing the aggregation velocities of particle pairs over successive plotted time steps in the gravity calculation. Gravity particle condensation rates portrayed in each column of a two-dimensional matrix (in which each row corresponds to a cell pair and each column to a time step) serve in turn as a template that is compared with all other columns for a match. Velocities of at least two particle pairs have to be represented in a column for it to be used as a template; pairs that do not aggregate can be excluded. Velocity values less than 3.25% of the maximum velocity detected in the data set can be nulled to filter out small fluctuations in aggregation velocity. Values in each column of a matching set that occurred more frequently than in any of the surrogate trials can be displayed as a set of vectors with a common origin. The length of the vector indicates the aggregation velocity of the corresponding particles in that pair; the direction of each vector in a plane identifies the neuron pair represented. Because of the appearance of the vectors in animations of successive planes through time, these graphs are called spark plots.

**Spkpat6bg** accepts as input the output files from **xslope**. The algorithm determines whether particular combinations of neurons exhibit recurring moments of impulse synchrony or near synchrony more frequently than would be expected by chance. Unlike other measures used to detect "favored" spatiotemporal patterns of activity, the new approach screens higher order measures derived from particle aggregation in the gravity method rather than interspike intervals. Each "column" in the data file **xslope** is compared to all other *columns* for "matching" velocities of aggregation.

Match criteria based on velocity thresholds were used in each pattern search and tested for significance. Seven different "match" criteria are tested concurrently ... the most stringent match criterion being a "near perfect duplication".

A Monte-Carlo significance test compares the frequency of occurrence of each detected pattern with the number of repetitions found in each of 1000 surrogate data sets. **For significance testing, only the most "stringent" set of criteria (option 7) should be used:** More than 99% of the locations of non-nulled elements in the template column had to match with corresponding locations of non-nulled velocities in the target column; there could be no extra non-nulled velocities in the target column, and target velocities for more than 75% of the matched pairs had to be within 25% of the corresponding template velocity values.

By "preselecting one set of criteria, the user can **avoid the consequences of multiple comparisons when the original set of templates is compared with data sets in which each row of pair velocity values has been shuffled to give a Monte Carlo significance test.**

A pattern is recorded if the *number of matches* detected for each template column in the original data set *is greater than* the maximum number of matches found between the same template and the surrogate data. Results are written to the user named output file (typically \*.pat).

The *original statistical significance* test was based on a comparison between the number of occurrences of repeating patterns in the original data with the maximum number found when the "original" column in array "aryin" was compared with all remaining columns in all 100 (or 1000) control data sets (each iteration of "aryin2"). In this case, the null hypothesis is that the number of repeating patterns found when a particular template

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column of velocity values is compared with all subsequent columns is NOT greater than expected by chance. IF the maximum number of matches for a particular column is greater than the number found when the same column is instead compared to all subsequent columns in all shifted control data sets, THEN the null hypothesis is rejected.

**NOTE:** by default spkpat6bg and spkpat6kbg behave exactly as they used to back in 1997. **In order to use the new improved surrogate\***, put "newsur=1" on the command line before the command, like this:

**newsur=1 spkpat6bg**

\* New algorithm (by Russell O'Connor, 2017): Each surrogate data set is produced by first randomly rotating the rows of the centered original data set. The rotated data is premultiplied by the transpose of the eigenvector matrix of the sample covariance matrix of the rotated data to get uncorrelated data, which is then scaled to make the variances the same as the eigenvalues of the original data. The scaled, uncorrelated data is then premultiplied by the eigenvectors of the original data and uncentered. The resulting surrogate data rows have the same means, variances, and covariances as those of the original spike data. This new algorithm replaces the "MOVE each pair row in aryin TO aryin2 IN RANDOM ORDER" loop with **code to generate the new surrogate** in aryin2 from the data in aryin, and makes no other changes. This won't do FDR (false discovery rate), but you can estimate the FDR after the fact by dividing the number of columns by the surrogate count and then dividing that by the number of positives. (This information is in a text file generated by the programs)

**Confirm availability of this program...** [spkpat6bgr (formerly spkpat6bgsig) is a special version used solely to check for matches using AN ALREADY SHUFFLED ARRAY AS THE FIRST INPUT ARRAY. The prediction is that there will be few sig matches as compared to the number found when the real original data is used to generate templates as with spkpat6bg]

*Options for spkpat6bg*

1. **One option allows particle velocities less than a defined percentage ("thrnl" variable) of the variable "tmpmax" to be nulled to 0.0;** "tmpmax" = is the maximum velocity found in the file from xslopebgv2, AFTER scaled by sclfac. The default value for "thrnl" is .03125 or 1/32; this value is the same as the nulling threshold in xslopebgv2 to null small velocities. All control files are scaled and nulled by the same values.
2. Another allows *inc* values >1 to calculate aggregation velocity over multiple successive plotted time steps.
3. The **"MASK"** option limits the pattern search to data from pairs of particles that aggregate significantly as shown by option 2 in xslope.

After the program runs and outputs the primary output text file (file.pat), **spkpat6bg** prompts you to write out optional output file sets – for any ONE criterion OR ALL CRITERIA. **Specificall, spkpat6bg allows specific patterns detected by that program to be written in a form that can be read and displayed by another program - 3djmp.** Up to 3 different sets of spark patterns can be displayed in 1 plot. These files are automatically named **fxxx-y.spk** where *xxx* is the template column and *y* is the criterion matched.

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Corresponding text files (**fxxx-y.txt**) are also written out when this option is selected. The text files are comma delimited. Each one consists of a list of column numbers and the pair velocity in the column. It can be imported into excel. The following files are written to the current directory and will over-write previous files with the same name.

Note: IT IS YOUR RESPONSIBILITY TO DELETE OR MOVE \*.spk, \*.txt, and \*.fwk files BEFORE running the program. TO view the subdirectory you can open a second terminal and 'cat' the file.pat file to get the information needed to run the option.

### 3djmp

*Spark plots - moments of synchrony in neuronal assemblies*

**3djmp** accepts files generated by **xslope** that have the \*.out format (options 3,4, and 5), specifically **idlmov.out**. Jumps in gravity particle condensation rates displayed as a set of vectors with a common origin. The direction of each vector in a plane identified the neuron pair represented; vector length indicated the aggregation velocity of corresponding particles. Because of the appearance of the vectors in animations of successive planes 'through time', these graphs are called 'spark' plots.

The user can also define a \*.bdt file with at least one 'bdt' type analog signal that can be plotted on the side wall of the 3djmp spark display. Up to two analog signals can be plotted. (The one defined as the second signal is a) plotted on the 'bottom' of the side wall **AND** plotted as a *phase plane* on the 'back wall'. **The time base in the "depth" axis of the spark plot is taken from the \*.bdt file with the analog signal(s).**

Finally, from 1 to 7 **\*.fwk (fireworks2 input files) are written out with the automatically generated file name(s): f-\*.fwk** where \* is 1-7.

Gravity Spark Program Development Update and Manual Supplement

Russ O'Connor November, 2016

Running spkpat6bg

**Example**

spkpat6bg

Input file: idlmov.out

Output file: 2013-8-1-pc4L.pat

Mask or no mask option: 1, yes

Enter 1 criterion: 7

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[3djmp](#)

How many spark sets from xslope.out? 1

Input data file from [xslope:](#) idlmov.out

BDT file for analog signal: 2013-8-1-pc4L.gdt

Analog plots change color with sparks? Y or N

"Faint" phase plan at non spark times? Y or N

1 or 2 analog channels?

Enter chan # (1 or 2 times)

Spark will be displayed at 25 frames/sec Enter animation speed in frames per spark step:

(Note a value of 25 is very slow; try a value of 1.. or maybe .1?)

Save movie to avi file?

Title:

---

## fireworks2

The program **fireworks2** is a utility program that allows the user to view times of all spark sequences for a particular criterion for 1 xslope/spkpat6bg "run". Two analog channels from the corresponding (i.e., EXACTLY the same duration) \*bdt file can be optionally plotted at the bottom of each page displayed.

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Appendix

**MATCH CRITERIA for spkpat6bg**The percentage of non-nulled velocities in the TEMPLATE column matched in the TARGET column is greater than value of *threshold*

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1. The percentage of non-nulled velocities in the TEMPLATE column matched in the TARGET column is greater than value of *threshold 1* **AND** the percentage of these "hits" that fall within the range (+/-) of *threshold 3* is greater than *threshold 4*.
2. The percentage of non-nulled velocities in the TEMPLATE column matched in the TARGET column is greater than value of *threshold 1* **AND** the percentage of "extra" non-nulled velocities in the TARGET column is less than *threshold 2*.
3. The percentage of non-nulled velocities in the TEMPLATE column matched in the TARGET column is greater than value of *threshold 1* **AND** the percentage of "extra" non-nulled velocities in the TARGET column is less than *threshold 2* **AND** the percentage of these "hits" that fall within the range (+/-) of *threshold 3* is greater than *threshold 4*.
4. "All" (.gt.0.99) of the non-nulled velocities in the TEMPLATE column are matched in the TARGET column **AND** the percentage of "extra" non-nulled velocities in the TARGET column is less than *threshold 2*.
5. "All" (.gt.0.99) of the non-nulled velocities in the TEMPLATE column are matched in the TARGET column **AND** there are *no* "extra" non-nulled velocities in the TARGET column.
6. "All" (.gt.0.99) of the non-nulled velocities in the TEMPLATE column are matched in TARGET column **AND** there are *no* "extra" non-nulled velocities in the TARGET column **AND** the percentage of these "hits" that fall within the range (+/-) of *threshold 3* is greater than *threshold 4*.

As indicated above, values are compared to user defined *thresholds*.

Threshold	Value with which compared	Larger value is
threshold 1 def = <b>0.90</b>	percentage of non-nulled template elements matched in target column	more stringent criterion
threshold 2 def = <b>0.05</b>	percentage ('perxt') of "extra" non-nulled elements in target column; = $\text{ixtra}/\text{float}(\text{jmtal})^1$	less stringent criterion
threshold 3 def = <b>0.25</b>	maximum % magnitude difference between template and target velocity	less stringent criterion
threshold def = <b>0.75</b>	percentage of matching non-nulled elements that must meet threshold 3 criterion	more stringent criterion

**Notes:**

1. (perxt = non-nulled velocities in a target column/total pairs in sample) see spkpat6bg source code for details.

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***A summary of the gravity method and enhancements through 2010 is given in:***

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