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Dopamine-induced Dispersion of Correlations Between Action Potentials in Networks of Cortical Neurons

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ABSTRACT

The involvement of dopamine in the process of learning, at the cellular and behavioural levels, has been studied extensively. Evidently, dopamine is released from midbrain nuclei neurons upon exposure to salient unpredicted stimuli, and binds to neurons of cortical and subcortical structures, where its neuromodulatory effects are exerted. The neuromodulatory effects of dopamine at the synaptic and cellular levels are very rich, but it is difficult to extrapolate from these elementary levels what their effect might be at the behaviourally relevant level of neuronal ensembles. Using multi-site recordings from networks of cortical neurons developing *ex-vivo*, we studied the effects of dopamine on connectivity within neuronal ensembles. We found that dopamine disperses correlations between individual neuronal activities, while preserving the global distribution of correlations at the network level. Using selective D_1 and D_2 modulators we show that both receptor types are contributing to dopamine-induced dispersion. Our results indicate that at the neuronal ensemble level, dopamine acts to enhance changes in network connectivity rather than stabilize such connections.

INTRODUCTION

In recent years, considerable effort has been directed towards the identification of neural structures and mechanisms responsible for rewarding adaptive behaviours (Schultz, 1998; Kalivas and Nakamura, 1999; Spanagel and Weiss, 1999; Schultz and Dickinson, 2000; Gisiger et al., 2000; Tzschentke, 2001). Underlying these endeavours is an attempt to map the behavioural concept of reward to neural processes that change the functionality of a subset of neurons, based on past performance of the system. Within this context, the role of dopaminergic neurons, residing in the ventro-anterior midbrain and projecting to the striatum and the neocortex, is considered central. These neurons are reported to be transiently activated in response to surprising events such as novel stimuli, salient sensory stimuli, unexpected primary rewards and arbitrary stimuli that are associated with primary rewards, thus reporting an error in the prediction of the stimulus (Reviewed in Redgrave et al., 1999; Horvitz, 2000; Dayan and Balleine, 2002; Schultz, 2002). The activation of dopaminergic neurons is correlated with the learning process, suggesting that dopamine modulates the function of its target tissues.

Cellular-level experiments indicate that dopamine has a wide range of (often contradictory) effects on synaptic plasticity and cellular excitability (Collins et al., 1985; Calabresi et al., 1992; Cameron and Williams, 1993; Law-Tho et al., 1994; Law-Tho et al., 1995; Yang and Seamans, 1996; Shi et al., 1997; Gulledge and Jaffe, 1998; Zhou and Hablitz, 1999; Gurden et al., 2000; Henze et al., 2000; Gorelova and Yang, 2000; Gulledge and Jaffe, 2001; Gonzalez-Islas and Hablitz, 2001; Gao et al., 2001; Lavin and Grace, 2001; Seamans et al., 2001a; Seamans et al., 2001b; Reynolds and Wickens, 2002; Gorelova et al., 2002;

Gonzalez-Islas and Hablitz, 2003; Gao et al., 2003; Picconi et al., 2003). The translation of the cellular-level effects into behavioural effects passes through an intermediate level of integration – i.e., the level of neuronal ensembles. In this study, we addressed this intermediate level of organization, exploring the effects of dopamine on the correlations between the activities of neurons separated by many synapses (Marom and Shahaf, 2002). We asked: how does dopamine affect the correlations between the activities of two such neurons?

We approached this question using multi-site recordings from networks of cortical neurons developing *ex-vivo*. The functional characteristics of these cortical networks are similar to those observed *in-vivo* in terms of connectivity, inhibition-excitation ratio, electrophysiological measures of activity, plasticity and responses to pharmacological and electrical stimuli (reviewed in Corner et al., 2002; Marom and Shahaf, 2002). Furthermore, the *ex-vivo* arrangement allows for simultaneous measurements of thousands of neuronal correlations, to perfuse the system with known concentrations of dopamine, and to follow the stability of these neuronal correlations over long periods of time.

Using this system we found that, at the polysynaptic level, dopamine enhances changes (i.e. disperses) in correlations between individual neuronal activities while preserving the global distribution of these correlations within the network. These effects could be mimicked by selective D_1-like and D_2-like agonists, whereas selective D_1 and D_2-like antagonists block these effects.

MATERIALS AND METHODS

Cell Culture. Primary cultures of rat cortical neurons were prepared as described previously (Shahaf and Marom, 2001; Marom and Shahaf, 2002; Eytan et al, 2003). Briefly, cortical neurons were obtained from newborn rats within 24 hours of birth. The cortex tissue was digested enzymatically and dissociated mechanically and the neurons were plated directly onto substrate-integrated multi-electrode array (MEA) dishes (Gross, 1979; Stenger and McKenna, 1994) (see Figure 1a). The cultures are grown in MEM supplemented with heat-inactivated horse-serum (5%), Glutamine (0.5 mM), Glucose (20 mM), and Gentamycin (10 µg/ml), and maintained in an atmosphere of 37°C, 5% CO_2 and 95% air in a tissue culture incubator and during the recording phases. Experiments were performed during the third week after plating, following the period of functional and structural network maturation.

Electrophysiological methods. We used commercial arrays of 60 Ti/Au/TiN electrodes, 30µm in diameter, spaced 200µm from each other (MCS, Reutlingen, Germany). The insulation layer (silicon nitride) was pre-treated with poly-L-lysine. A commercial 60-channel amplifier (B-MEA-1060, MCS, Reutlingen, Germany) with frequency limits of 1-5000Hz and a gain of x1024 was used. The B-MEA-1060 was connected to *MCPPlus* variable gain filter amplifiers (Alpha-Omega, Nazareth, Israel) for further amplification. Data were digitised using two parallel 5200a/526 A/D boards (Microstar Laboratories, WA, USA). Each channel is sampled at a frequency of 24 ksample/second and prepared for analysis using the AlphaMap interface (Alpha Omega, Nazareth, Israel). Thresholds (x8 RMS units- typically in the range of 10-20 µV) were defined

separately for each recording channel prior to the beginning of the experiment. All data presented in this manuscript were obtained from threshold crossing events. Analysis of sample experiments revealed that the results were not qualitatively affected by passing the data through a spike-sorting procedure (principal component methodology; AlphaSort software, Alpha Omega, Nazareth, Israel).

Dopamine application. Two methods of dopamine application were used: (i) Applying 100µl of tissue culture medium with dopamine (15µM to 100µM, final concentration) onto the surface of the solution surrounding the network (2 ml), thus allowing the dopamine to reach the cells by diffusion. A relatively homogeneous concentration of dopamine in the tissue culture medium surrounding the networks was reached in less than two minutes, as verified using methylene blue distribution in a control application. Because the medium, supplemented with serum, is slightly basic (thus promoting oxidation of the dopamine), and due to possible residual activity of serum amine-oxidase, the nominal concentration in this method reflects the upper limit of effective concentration to which the neurons are exposed. (ii) Local application of 5-15µl of tissue culture medium with dopamine (15µM to 100µM) directly onto the recording area within the neuronal network using a micropipette and a picoinjector (World Precision Instruments), creating a local, transient increase of dopamine concentration in the immediate vicinity of the neurons. Dopamine concentration is rapidly diluted to a negligible level (the overall volume of medium in which the cells were bathed was ca. 200 times larger than the injected volume). Concentration of dopamine lower than 15µM did not cause a consistent effect in networks tested. The overall observed effects of dopamine

were the same for both methods, although slightly less pronounced for the local application method. Although ideally one would like to wash the cells with media containing known concentrations of dopamine, complete media changes severely impact the long-term vitality of these preparations and were thus avoided. Therefore, the absolute dopamine concentrations in the vicinity of the neurons were somewhat variable, and thus the dependence of the effects of dopamine on the amounts of dopamine added to the media did not reach statistical significance ($p>0.3$).

A note concerning oxidation. Ascorbic Acid, a common antioxidant used to protect dopamine in *in-vitro* experiments was not added because of reports that this compound has direct effects on the neuronal excitability (Sutor and ten Bruggencate,1990; Kiyatkin and Rebec,1998). However, the tissue culture medium in which the networks were grown and maintained during the experiments contained several potent antioxidants such as Thiamine, Riboflavin, Nicotinamide, D-Ca Pantothenate and choline. Moreover, using HPLC we verified that dopamine levels in the media remained stable for at least five minutes in the same conditions as during the experiments (37^0, 5%CO_2), longer than required for it to diffuse over the entire network.

Agonists and antagonists. D_1-specific dopamine receptor agonist (SKF 38393, 15-25μM) and D_2-specific agonist (Quinpirole, 15-30μM) were applied using the same protocols as outlined for dopamine. D_1-specific antagonists (SCH 23390, 15-30μM) and D_2-specific antagonists (Remoxipride, 15-30μM) were dissolved in tissue culture medium and applied globally 30 minutes prior to dopamine application.

Dopamine receptor labelling. D_1 receptors were labelled using the fluorescent D_1 antagonist Bodipy FL SCH 23390 (Molecular Probes, OR, USA). Labeling specificity was assessed by pre-applying an excess of non-fluorescent SCH 23390, resulting in a 44% decrease in the mean fluorescence, indicating that labelling was at least in part specific. D_2 receptors were labelled using the fluorescent D_2 agonist Bodipy FL PPHT (Molecular Probes, OR, USA). The specificity of the label was assessed by post-applying an excess of the non-fluorescent D_2 antagonist spiperone, and thereby comparing the number of fluorescent puncta and their mean fluorescence. We observed a 30% decrease in the number of fluorescent puncta, indicating that labelling was at least in part specific.

Basic experimental design and analysis. The experiments were designed in such a way as to allow internal controls. Each network was exposed to three recording phases: *Baseline Phase* – 30 minutes of recordings without manipulation, *Control Phase* – 30 minutes of recording after addition 5-100 µL of tissue culture medium (control solution), and *Dopamine Phase* – 30 minutes of recording following addition of 5-100 µL of dopamine or other pharmacologically-related compounds. Of the 30 minutes of each phase, analyses (see below) were confined to 25 minutes only; the first five minutes after addition of dopamine, control medium, or dopamine related compounds were discarded from analysis in order to allow complete diffusion. The changes in correlations that occurred between baseline and control phases, provided a measure of the baseline drift (since only tissue culture medium was applied to the network) as well as an internal control for the application of dopamine. The changes in

correlations between the control and dopamine phases served to define the effect of dopamine application.

Definition of correlation. While example data are presented using cross-correlograms, population data is presented in terms of pair-wise correlations between diachronically (i.e. over-time) related spikes, denoted activity pairs. We define an activity pair as an action potential *A* that is followed by another action potential *B* with a given time delay of $\tau \pm \Delta\tau$ milliseconds between the two ($0<\tau<150$msec; $\Delta\tau$=2.5 msec); thus defined, this temporal binning yields a total of 30 activity pairs (each of which with a different $\tau \pm \Delta\tau$) for a given *A*®*B*. Note that *A* and *B* may be action potentials recorded from the same or from different electrodes. For each *A*®*B* activity pair we define a correlation measure, $C(\tau)$, as the number of occurrences of the pair within a given recording phase, divided by the number of occurrences of *A* in the same recording phase. Thus defined the correlation measure is physiologically interpretable as the strength of entailment of *B* by *A*. *A*®*B* entailment strength may be affected by the activity of *A* or *B* or both; therefore, the supplementary data shows comparisons between results analyzed by the measure as defined above (*A* in the denominator), and other normalization methods (*B* or *AXB* in the denominator), suggesting that the main results reported in this manuscript are qualitatively similar for all three normalization methods.

Note that $C(\tau)$ is always >0; however, the upper limit of $C(\tau)$ depends on the $\Delta\tau$ chosen: if $\Delta\tau$ is wide enough to allow more than one spike to occur, $C(\tau)$ is greater than unity. For the $\Delta\tau$ used here (2.5 msec) the largest $C(\tau)$ value obtained was 1.38 (the $C(\tau)$ of only 0.002%, from the total number of pairs in the

reported experiments, was > 1). Changes in C(τ) for each two consecutive recording phases defined above (*baseline* – *control* – *dopamine*) were calculated from the number of occurrences of all possible activity pairs in those phases, and the set of all the correlations and their changes was obtained. Changing either the resolution (Δτ) or the maximal time delay for calculation of activity pairs (from 5 to 500 msecs) did not qualitatively affect the results described below.

Since there is a stochastic element in the neuronal activity, a measure such as C(τ) is sensitive in cases of a small number of trials (occurrences of *A* in the *A*®*B* activity pair). In order to circumvent this problem when comparing C(τ)'s between recording phases, the following criteria for inclusion of an activity pair in the analysis were used: (I) *A* was active at least 150 times during the two compared recording phases (i.e. average firing rate of 0.1 spikes/sec), and (II) *A*®*B* appeared more than five times during each of two compared recording phases. These criteria left us with at least 360,000 pairs for analysis in each of the reported experimental conditions (actual numbers are reported in the Results section and in figure captions).

Number of experiments. Sixteen experiments of dopamine application in sixteen different networks were conducted; in addition, in nine experiments, specific D_1 or D_2 agonists were used, and in three control experiments, the effects of application of dopamine in the presence of antagonists were tested.

RESULTS.

The basic experimental question asked here is how dopamine affects correlations between individual neuronal activities within a large network. In what follows we show that there are dopamine receptors in our preparations of cortical neuronal networks, and characterize the measure of neuronal correlations. We then proceed to examine the effects of dopamine (and its various pharmacological derivatives) on neuronal correlations within these networks.

Ex vivo networks of cortical neurons express dopamine receptors

Figure 1a shows an *ex vivo* network of cortical neurons grown on a multi-electrode array. In order to use such preparations for characterizing network responses to dopamine, it was necessary to demonstrate that these cultured neurons express dopamine receptors. To that end we labeled cultured cortical neurons with fluorescent derivatives of the D_1 antagonist SCH 23390 and the D_2 agonist PPHT. A punctate labeling pattern was observed as shown in Fig 1c-h. The specificity of these labels was verified by pre-applying or post-applying an excess of non-fluorescent competitive antagonists of D_1 and D_2 receptors, as described in Materials and Methods. Pre or post application of such competitive antagonists significantly reduced the labeling intensity and number of fluorescent puncta whereas application of carrier solution alone had no such effects (data not shown). Interestingly, within minutes of application we observed rapid uptake and transport of these fluorescent antagonists within neuronal processes as previously described (Kari and Reynolds, 1996) (Data not shown). Taken

together, these experiments indicate that D_1 and D_2 dopamine receptors are expressed by the cultured cortical neurons used here.

Effects of dopamine application on spontaneous firing rates

The spontaneous activity in networks of cultured cortical neurons is composed of high-frequency bursts having complex temporal structure (Beggs and Plenz, 2003) and sparse low frequency uncorrelated single spikes activity (Figures 1b, 2a) whose nature and statistical properties are reviewed in Marom and Shahaf (2002). The number of active electrodes (i.e. electrodes that detect spikes) varies between different networks; in the sixteen networks used for the present study, this number ranged from 8 to 48 (out of 60).

To examine the effects of dopamine on spontaneous activity in our preparation we performed the following experiment as illustrated in Figure 2b (top): Spontaneous activity in each network was recorded during three phases: 1) *Baseline phase* – during which the spontaneous activity was recorded for 30 minutes without any manipulations, 2) *Control phase* – 30 minutes of recording after addition 5-100 μL of culture medium, and 3) *Dopamine phase* – 30 minutes of recording following addition of 5-100 μL of dopamine (or other pharmacologically-related compounds).

The distributions of firing rates recorded from individual electrodes during all phases are shown in figure 2b (bottom). This figure indicates that the firing rate distributions were quite similar in all phases although they were not entirely identical. A closer examination of the changes in firing rates that followed dopamine application (inset of Figure 2b) suggests that while most

neurons did not change their firing rates (peak centered around zero change), there is some tendency for a decrease in the firing rates.

Effects of dopamine application on correlations between neuronal activities

In order to determine the effects of dopamine application on the correlations between individual neuronal activities, we calculated cross correlograms of the activity recorded from all pairs of electrodes in each phase in each network, and then examined the effects of dopamine on these correlograms. The left column of Figure 3 shows correlograms obtained from four different pairs of neurons. For each of the pairs (neurons *A* and *B*), these correlograms depict the counts, during each experimental phase, in which both neuron A and neuron B (*AÇB)* fired an action potential with a precise time delay represented by the abscissa. Each of the panels in the left column of Figure 3 contains two correlograms; one obtained from the baseline phase, while the other obtained from the control (medium applied) phase. Note the similarity of these correlograms over time and their indifference to the control solution application. In contrast, as shown in the right hand column of Figure 3, application of dopamine has a marked effect on the correlograms from the same pairs; while the direction and extent of dopamine effect on the correlograms is variable in these example pairs, the fact that dopamine does make a difference is evident.

Quantifying correlations at the entire network level

In each network there are hundreds of pairs such as those shown in Figure 3. In order to quantify the effects of dopamine on pair wise correlativity of the entire population of pairs, we did the following: First, we assumed that a given *AÇB* activity-pair with a given time delay (τ) represents, by definition, a set of activation paths that are distinctive from those represented by a different time delay. Therefore, we broke each of the *AÇB* correlograms into discrete *activity-pairs* according to their respective time delays (τ). Depending on the temporal order we depicted these as *A®B* (if A fired before B) and *B®A* (if B fired before A). Therefore we ended up with a set of 60 discrete values for each pair of neurons in each phase (τ ranging from –150 msec to +150 msec, with a bin size of 5 msec). Figure 4a shows that the counts of such activity pairs followed a Poisson distribution; the average pair appeared ~160 times in a recording phase. The second step we took was to normalize the counts of each *AÇB* activity pair to the spike counts of *A*, *B*, or *AxB*, thus obtaining a correlation coefficient C(τ) (see Methods). The data presented in the remaining of this study is normalized to *A*, as it is the most natural normalization from a functional point of view; i.e. the resulting correlation provides an answer to the question *How successful is A in entailing B?* In practice, the normalization to A, B or *AxB* does not produce qualitatively different results (supplementary Figure 1).

Effects of dopamine application on the correlation coefficients C(t)

Figure 4b shows distributions of C(τ) in the three recording phases; This analysis revealed that the *population* distribution of C(τ) was largely stable, that is – it was not strongly affected by the addition of dopamine (or control media).

Whereas the *population* distributions of $C(\tau)$ were stable, at the level of individual activity pairs dopamine exerted a marked effect. In other words, we noted that for a particular activity pair, the likelihood of it changing its $C(\tau)$ following dopamine application was much greater than the likelihood of changing its $C(\tau)$ after application of control media (Figure 4c,d). This effect was quantified by calculating the conditional probabilities of changes in $C(\tau)$ from the entire population of activity pairs in all experiments. To obtain these probabilities we asked: If $C(\tau)$ of a given pair in the baseline recording phase is $C(\tau)_{base}$ what is the probability of finding $C(\tau)_{control}$ in the control recording phase for that pair? Similarly, if $C(\tau)$ of a given pair in the control recording phase is $C(\tau)_{control}$ what is the probability of finding $C(\tau)_{dopamine}$ in the dopamine recording phase?

Figures 4c and 4d show these conditional probabilities coded as grayscale intensities. The distribution of $P(C(\tau)_{control}| C(\tau)_{base})$, calculated for 477,500 pairs (panel 4c) is considerably different from that of $P(C(\tau)_{dopamine}| C(\tau)_{control})$, (panel 4d; 361,943 activity pairs). The latter distribution is more dispersed, suggesting that dopamine enhances changes in correlation of activity pairs, $C(\tau)$.

The extent of change is quantified in Figure 5 in terms of fold change of association strength (x $C(\tau)$): Note from panel 5a (data from one representative network) that the dispersion of $C(\tau)$ due to dopamine application (black) is large compared to control conditions (gray) throughout the range of measured $C(\tau)$. In order to obtain a single variable that reflects the extent of dopamine-induced dispersion, we use the distribution of fold change in $C(\tau)$. Figure 5b shows this distribution, obtained from changes between baseline and control recording

phases in the entire set of experiments (477,500 pairs). We use the standard deviation of the distribution (σ) as a measure for the dispersion; the wider the distribution of changes, the greater the dispersion and the tendency of pairs to change their correlation. In Figure 5c, σ is plotted as a function of $C(\tau)$, suggesting that dopamine-induced dispersion is not a simple scaling up of the dispersion observed under control conditions. Panel 5d shows the average fold-change in $C(\tau)$ as a function of initial $C(\tau)$ for control solution (gray) and dopamine (black) application. Our findings clearly show that (1) dopamine induces dispersion of correlations between individual activity pairs, and (2) the extent of dispersion is dependent upon $C(\tau)$ before dopamine is applied. Note that the above-mentioned observations are insensitive to the methods of normalization as explained before (supplementary Figure 1).

Effects of dopamine agonists and antagonists

In order to verify that the effects of dopamine described above were mediated by dopamine receptors we determined if the dopamine-induced dispersions in $C(\tau)$ can be mimicked by dopamine agonists and blocked by dopamine antagonists

Figure 6 shows the effects of dopamine, agonists and antagonists, on the dispersion of correlations in a series of individual networks. Selective agonists were applied in the same manner as dopamine. On average, application of dopamine caused a more pronounced dispersion compared to the D_1 agonist SKF-38393 (15-25μM) or D_2 agonist Quinpirole (15-30μM) alone, suggesting that the effect of dopamine is the result of an additive effect of the two receptors subtype families. In contrast, application of dopamine in

the presence of the selective D_1-like and D_2-like antagonists SCH 23390 and Remoxipride (15-30μM) blocked the dispersion effects described above.

DISCUSSION

The present study shows that the exposure of *ex-vivo* cortical networks to dopamine enhances changes in correlations between the activities of individual neurons, while preserving the overall distribution of such correlations. Both D_1 and D_2 related receptors are involved in the dispersing effect of dopamine.

Dopamine is believed to act, during the learning process, as a modulator of neuronal connections (example reviews in Spanagel and Weiss, 1999; Dehaene and Changeux, 2000; Tzschentke, 2001; Joel et al., 2002; Schultz, 2002; Dayan and Balleine, 2002). Its effects at the cellular level are state-dependent, and vary between different types of neurons and synapses. The path from modulation of activity at the cellular level to alteration of observed behaviour goes through changes in ensemble behaviour. Here we report that the modulatory effect of dopamine, observed at the level of ensembles of neurons and synapses, is that of a catalyst of change.

As reviewed in (Schultz, 2002), see also (Horvitz, 2000), dopamine is released when an animal experiences unpredicted stimuli. The observation that dopamine changes neuronal associations seems reasonable in that context, if one considers the unpredictability of a stimulus as an indication for the inadequacy of an existing association. While such extrapolations are inherently limited because of the *ex-vivo* unnatural context in which the networks are kept (for example, since there are no dopaminergic neurons in these cultures, hypersensitivity to dopamine cannot be excluded), many

similarities between features of the *ex-vivo* and *in-vivo* networks in terms of structure, biochemistry, physiology and pharmacology indicate that the results reported here may be very relevant to intact neuronal networks *in vivo*.

Extrapolating the results reported here to whole-animal behavioural and electrophysiological experiments, we predict that during instrumental conditioning, dopamine release from mid-brain neurons causes a *change* of connectivity rather than a *stabilization* of connectivity in target brain tissues. These opposite effects are equivalent from a functional point of view; learning may be obtained either by strengthening appropriate responses or disrupting inappropriate ones. While the formal consequences of these two possible learning processes are beyond the scope of the present experimental report, it is tempting to speculate that to the extent that no definitive directional effects of dopamine were described at the single neuron level, our *in-vitro* observation and interpretation of dopamine as a “disperser” is plausible.

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Figure Legends

Figure 1 (a) A network of cortical neurons grown on a multi electrode array (15 days *in-vitro*). For purposes of clarity a transparent array was used in the generation of this image. Bar: 15μm. (b) Two upper traces show sample recordings from two adjacent electrodes during spontaneous activity. Horizontal bar: 10 msec. Vertical bar: 50μV. Third trace is an enlargement of the first spikes shown in the middle trace. (c-h) D_1 and D_2 receptor labelling of neonatal rat cortical neurons in culture (14 days *in-vitro*): Fluorescent image after labelling with D_1 receptor antagonist Bodipy FL SCH23390 (c), and D_2 receptor agonist Bodipy FL PPHT (d), Differential interference contrast images of the same fields of view (e,f), Composite images (g,h). Bar: 7.5μm.

Figure 2 (a) Raster plot of network activity where each dot indicates a single spike. Shown in the upper panel is a raster plot of 27 electrodes over five minutes of spontaneous activity from one example network. Lower panel depicts an enlargement of 500 milliseconds of activity of the same network. (b) Top: Each experiment consists of three phases: 1) *Baseline phase* – during which the spontaneous activity was recorded for 30 minutes without any manipulations, 2) *Control phase* – 30 minutes of recording after addition 5-100 μL of culture medium, and 3) *Dopamine phase* – 30 minutes of recording following addition of 5-100 μL of dopamine (or other pharmacologically-related compounds). Bottom: Firing rate histograms for all the electrodes that in the first recording phases demonstrated an average firing rate of >0.1 spikes/sec. Y-axis depicts number of electrodes. The

distributions of firing rates observed in baseline (triangles), control (plusses) and dopamine (circles) phases are similar: exponential functions fitted to the declining section of the three distributions yield characteristic firing rates of 0.99, 0.97, and 0.98 for baseline, control and dopamine recording phases respectively (95% confidence intervals are <0.2; 16 networks, n=506 active electrodes). Inset: a histogram of changes in firing rate between control and dopamine phases, for all electrodes that fulfilled the inclusion criteria (see Methods, 16 networks, n=400 active electrodes). X-axis denotes the change in firing rate in Hz while the Y-axis denotes the number of occurrences. Note that while the distribution is slightly skewed towards a decrease in firing rates, the majority of electrodes did not change their firing rate and many electrodes even showed an increase in rates.

Figure 3 Cross-correlograms of four different pairs of electrodes are shown. The *left* column compares the cross-correlograms in the baseline (grey) and control (black) phases; the *right* column shows, for the same electrode pairs the cross-correlograms in the control (grey, same correlogram as in the left column) and dopamine phase (black). Bin size is 5 msec. Electrode pairs were chosen from three different experiments.

Figure 4 (a) Histograms depicting the distribution of counts of activity pairs observed in baseline (triangles), control (plusses) and dopamine (circles) phases. Poisson distribution functions fitted to the three distributions yield characteristic λ values (which is both the mean and the variance) of 163.8, 160.1, 166.9 for baseline, control and dopamine recording phases respectively

(95% confidence intervals are <0.1; 16 networks; included are all pairs that appeared >5 times within a recording phase: n=585119 activity pairs for baseline phase, n=582401 for control phase, and n=460972 in the dopamine phases). (b) Distribution of $C(\tau)$, calculated for all activity pairs observed in sixteen networks. The distributions of $C(\tau)$ observed for baseline (triangles), control (plusses) and dopamine (circles) phases are very similar: exponential functions fitted to the declining section of the three distributions yield characteristic $C(\tau)$s of 0.19, 0.19, and 0.20 for baseline, control and dopamine recording phases respectively (95% confidence intervals are <0.002; 16 networks; included are all pairs that appeared >5 times within a recording phase: n=585119 activity pairs for baseline phase, n=582401 for control phase, and n=460972 in the dopamine phases). (c) The conditional probability of $C(\tau)$ after addition of tissue culture medium. The X-axis depicts $C(\tau)$ during the baseline phase; the Y-axis depicts the $C(\tau)$ in the control phase. The distribution of $P(C(\tau)_{control}|C(\tau)_{base})$, calculated for 477,500 pairs is depicted using gray scale color code (cut off at 0.3). (d) A similar representation as in (c) for $P(C(\tau)_{dopamine}|C(\tau)_{control})$,; 361,943 activity pairs. The latter distribution is more dispersed across the entire range of $C(\tau)$.

Figure 5. Dopamine application enhances changes in correlations. (a) Fold change in correlation ($xC(\tau)$) after application of control solution (grey) and 30 μM dopamine (black) in one representative network. ~70,000 activity pairs are included. The value at the abscissa is the $C(\tau)$ obtained from the baseline phase (for control solution application), or control phase (for dopamine

application). The dispersion caused by dopamine application is much larger compared to control solution application. (b) The histogram of the distribution of changes in correlation, log(xCτ), due to application of control solution is shown for all activity pairs in all 16 networks (n=477500, bin size for calculation is 0.05). (c) Dispersion, σ (the standard deviation of log(xC(τ)) distribution), due to dopamine application (black continuous line, n=361943) and control solution application (gray continuous line, n=477500) as a function of C(τ) obtained from the baseline phase (for control solution application), or control phase (for dopamine application). Black dashed line depicts the ratio of dispersion due to dopamine application and control solution; this ratio is shown on the right Y-axis using a logarithmic scale. (d) The average fold change in C(τ) as a function of C(τ) obtained from the baseline phase (for control solution application), and control phase (for dopamine application). All activity pairs (n=361943 for dopamine application, n=477500 for control solution application) from all 16 networks are included. Areas between dashed lines depict the extent of dispersion ($\pm\sigma$); the dispersion due to control solution application is narrower, over all C(τ), compared to dopamine application.

Figure 6. The effect of dopamine and selective agonists/antagonists application is shown for all networks. For each network, the dispersion (σ) is represented by the standard deviation of the distribution of fold changes in C(τ) as in Figure 5b. The abscissa value is the dispersion due to control solution application, and the ordinate value is the dispersion due to dopamine (filled diamonds; n=16 networks), D_1-agonist (skf-38393; gray circles; n=4 networks), D2-agonist (Quinpirole; gray triangles; n=5 networks), and selective D_1 and D_2

antagonists (SCH 23390 and Remoxipride; applied with control solution, prior to dopamine application; open squares; n=3 networks) applications. Additional control experiments (black plusses, n=2 networks), which consist of two phases of control solution after the initial baseline phase, are also shown.

Supplementary figure:

Comparison between different statistical normalization methods. Each column shows the resulting analyses using different activity source for the normalization of $C(\tau)$. Rows compare calculation of change of correlation (upper two rows) and dispersion (lower two rows) in terms of difference-($\Delta C(\tau)$), or fold- $\log(xC(\tau))$. The main effect, i.e. enhanced dispersion of $C(\tau)$ due to dopamine is conserved, regardless of statistical representation used.

Figure 1

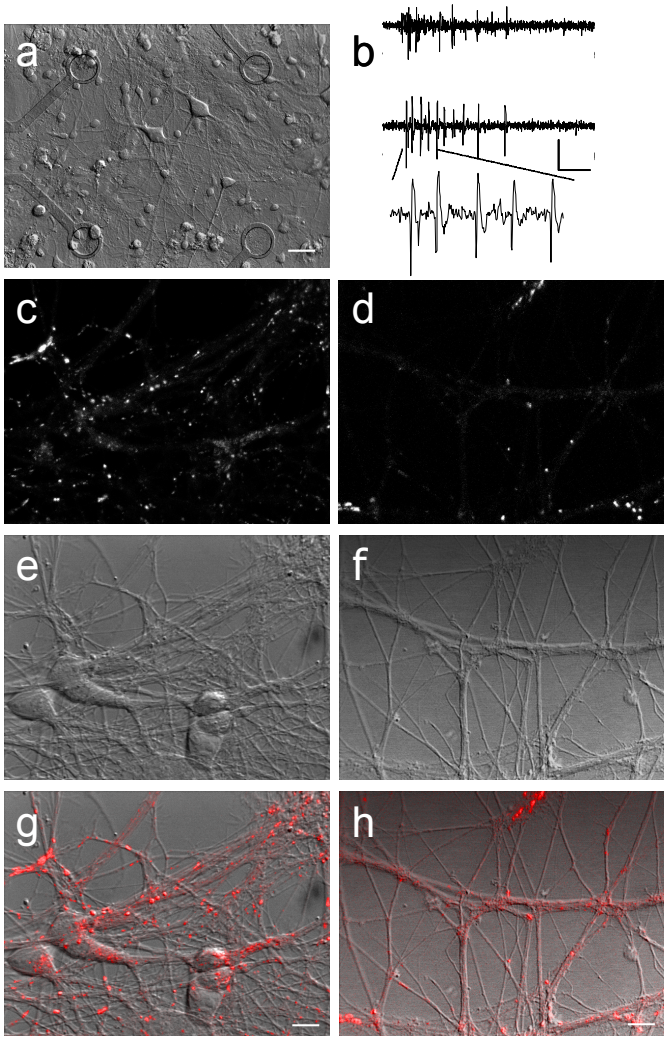
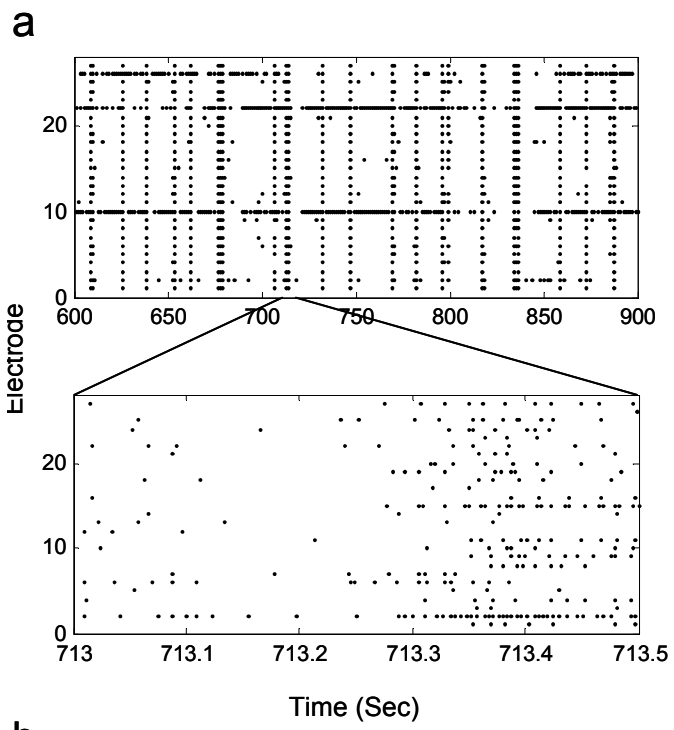


Figure 2


a
Electrode
20
10
0
600
650
700
750
800
850
900
20
10
0
713
713.1
713.2
713.3
713.4
713.5
Time (Sec)

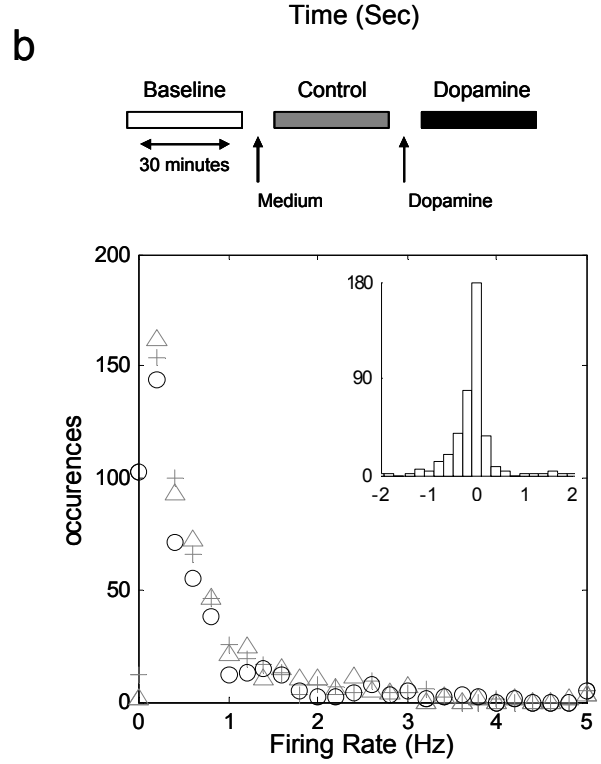

b
Baseline
Control
Dopamine
30 minutes
Medium
Dopamine
200
150
100
50
0
occurences
180
90
0
-2
-1
0
1
2
0
1
2
3
4
5
Firing Rate (Hz)

Figure 3

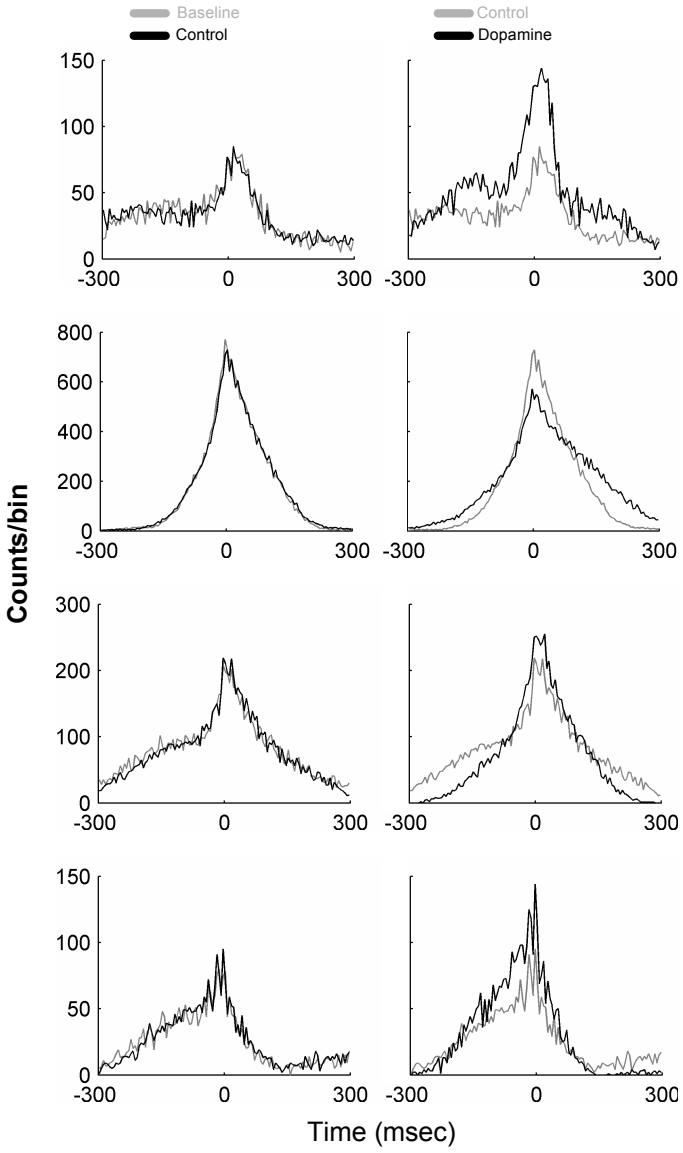

Baseline
Control
Control
Dopamine
Counts/bin
Time (msec)
150
100
50
0
800
600
400
200
300
-300
0
300

Figure 4

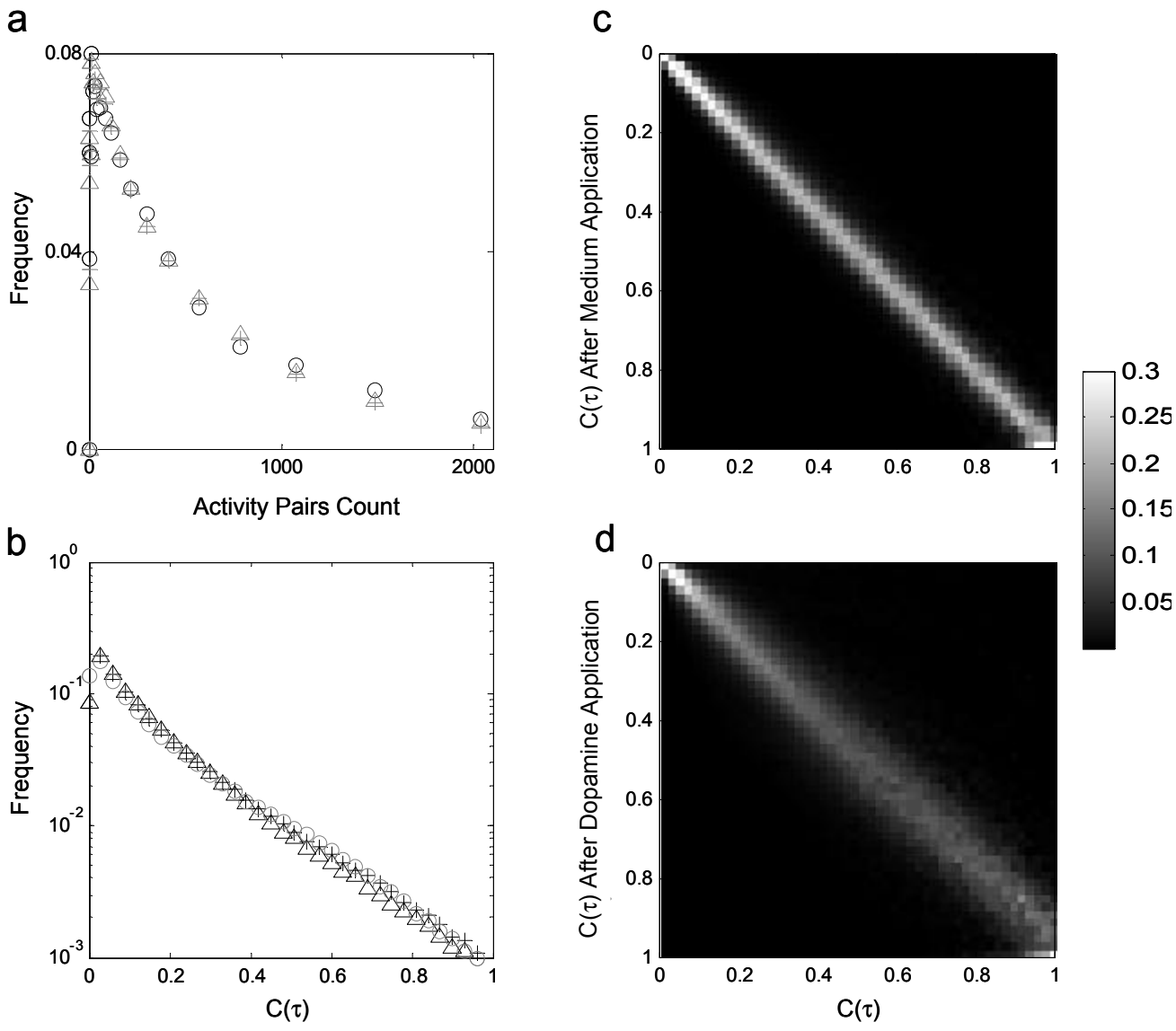

a
0.08
0.04
0
Frequency
0
1000
2000
Activity Pairs Count
b
10^0
10^{-1}
10^{-2}
10^{-3}
Frequency
0
0.2
0.4
0.6
0.8
1
C(τ)
c
C(τ) After Medium Application
d
C(τ) After Dopamine Application
0.3
0.25
0.2
0.15
0.1
0.05

Figure 5

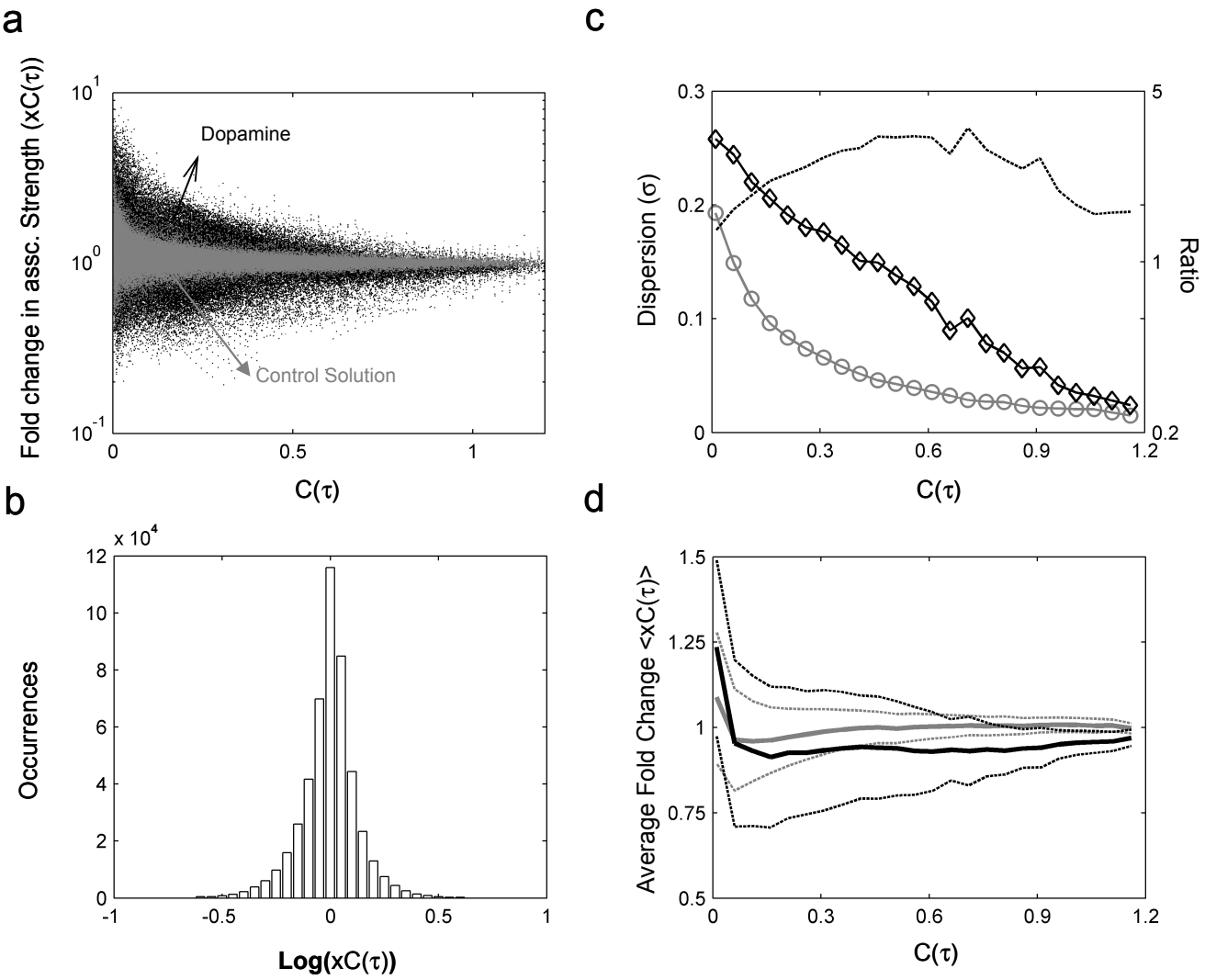
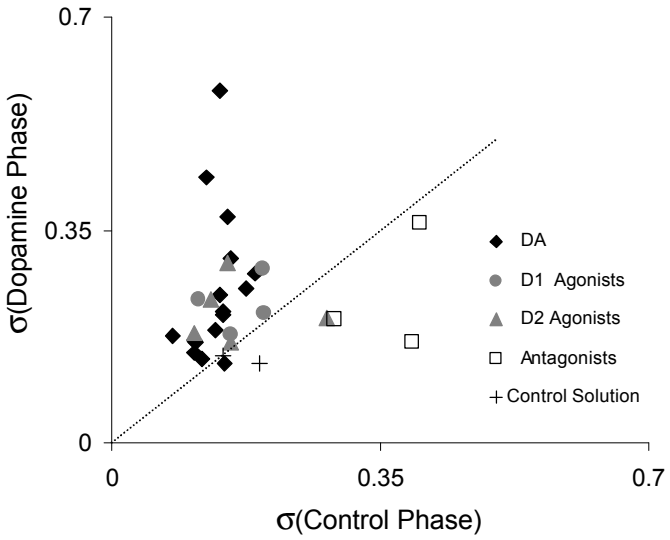

a
c
b
d
Fold change in assc. Strength (xC(τ))
Dopamine
Control Solution
C(τ)
Dispersion (σ)
Ratio
x 10^4
Occurrences
Log(xC(τ))
Average Fold Change <xC(τ)>

Figure 6


0.7
0.35
0
σ(Dopamine Phase)
0
0.35
0.7
σ(Control Phase)
DA
D1 Agonists
D2 Agonists
Antagonists
Control Solution

Supplementary Figure