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

**Danny Eytan,[1] Amir Minerbi,[2] Noam Ziv,[2] and Shimon Marom[1]**
[1]*Department of Physiology and Biophysics and* [2]*Department of Anatomy and Cell Biology, Faculty of Medicine, Technion, Haifa 31096, Israel*

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**Eytan, Danny, Amir Minerbi, Noam Ziv, and Shimon Marom.** Dopamine-induced dispersion of correlations between action potentials in networks of cortical neurons. *J Neurophysiol* 92: 1817–1824, 2004. First published April 14, 2004; 10.1152/jn.00202.2004. The involvement of dopamine in the process of learning, at the cellular and behavioral levels, has been studied extensively. Evidently, dopamine is released from midbrain nuclei neurons on 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 behaviorally 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 toward the identification of neural structures and mechanisms responsible for rewarding adaptive behaviors (Gisiger et al. 2000; Kalivas and Nakamura 1999; Schultz 1998; Schultz and Dickinson 2000; Spanagel and Weiss 1999; Tzschentke 2001). Underlying these endeavors is an attempt to map the behavioral 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 Dayan and Balleine 2002; Horvitz 2000; Redgrave et al. 1999; 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 (Calabresi et al. 1992; Cameron and Williams 1993; Collins et al. 1985; Gao et al. 2001, 2003; Gonzalez-Islas and Hablitz 2001, 2003; Gorelova and Yang 2000; Gorelova et al. 2002; Gulledge and Jaffe 1998, 2001; Gurden et al. 2000; Henze et al. 2000; Lavin and Grace 2001; Law-Tho et al. 1994, 1995; Picconi et al. 2003; Reynolds and Wickens 2002; Seamans et al. 2001a,b; Shi et al. 1997; Yang and Seamans 1996; Zhou and Hablitz 1999). The translation of the cellular level effects into behavioral 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 the following: 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 $D_1$- and $D_2$-like agonists, whereas selective $D_1$- and $D_2$-like antagonists block these effects.

## METHODS

### *Cell culture*

Primary cultures of rat cortical neurons were prepared as described previously (Eytan et al. 2003; Marom and Shahaf 2002; Shahaf and Marom 2001). Briefly, cortical neurons were obtained from newborn rats within 24 h 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 Fig. 1*A*). The cultures are

Address for reprint requests and other correspondence: S. Marom, Dept. of Physiology and Biophysics, Faculty of Medicine, Technion, Haifa 31096, Israel (E-mail: marom@tx.technion.ac.il).

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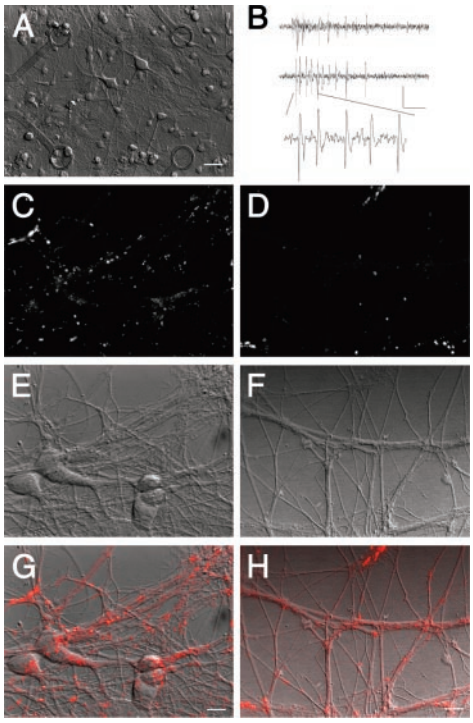



FIG. 1. *A*: network of cortical neurons grown on a multi-electrode array (15 days in vitro). For purposes of clarity, a transparent array was used in the generation of this image. Bar: 15 $\mu$m. *B*: 2 *top traces* show sample recordings from 2 adjacent electrodes during spontaneous activity. Horizontal bar: 10 ms. Vertical bar: 50 $\mu$V. Third trace is an enlargement of spikes shown in the *middle trace*. *C–H*: $D_1$ and $D_2$ receptor labeling of neonatal rat cortical neurons in culture (14 days in vitro): Fluorescent image after labeling 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* and *F*) and composite images (*G* and *H*). Bar: 7.5 $\mu$m.

grown in MEM supplemented with heat-inactivated horse-serum (5%), glutamine (0.5 mM), glucose (20 mM), and gentamycin (10 $\mu$g/ml), and maintained in an atmosphere of 37°C, 5% $CO_2$–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, 30 $\mu$m diam, spaced 200 $\mu$m from each other (MCS, Reutlingen, Germany). The insulation layer (silicon nitride) was pretreated with poly-L-lysine. A commercial 60-channel amplifier (B-MEA-1060, MCS) with frequency limits of 1–5,000 Hz and a gain of $\times$1,024 was used. The B-MEA-1060 was connected to *MCPPlus* variable gain filter amplifiers (Alpha-Omega, Nazareth, Israel) for further amplification. Data were digitized using two parallel 5200a/526 A/D boards (Microstar Laboratories). Each channel was sampled at a frequency of 24 ksample/s and prepared for analysis using the AlphaMap interface (Alpha Omegal). Thresholds ($\times$8 RMS units—typically in the range of 10–20 $\mu$V) 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).

### *Dopamine application*

Two methods of dopamine application were used. *1*) We applied 100 $\mu$l of tissue culture medium with dopamine (15–100 $\mu$M, 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 $<$2 min, 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. *2*) We locally applied 5–15 $\mu$l of tissue culture medium with dopamine (15–100 $\mu$M) 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 was rapidly diluted to a negligible level (the overall volume of medium in which the cells were bathed was ~200 times larger than the injected volume). Concentration of dopamine lower than 15 $\mu$M 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 (Kiyatkin and Rebec 1998; Sutor and ten Bruggencate 1990). 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 5 min in the same conditions as during the experiments (37°, 5% $CO_2$), longer than required for it to diffuse over the entire network.

### *Agonists and antagonists*

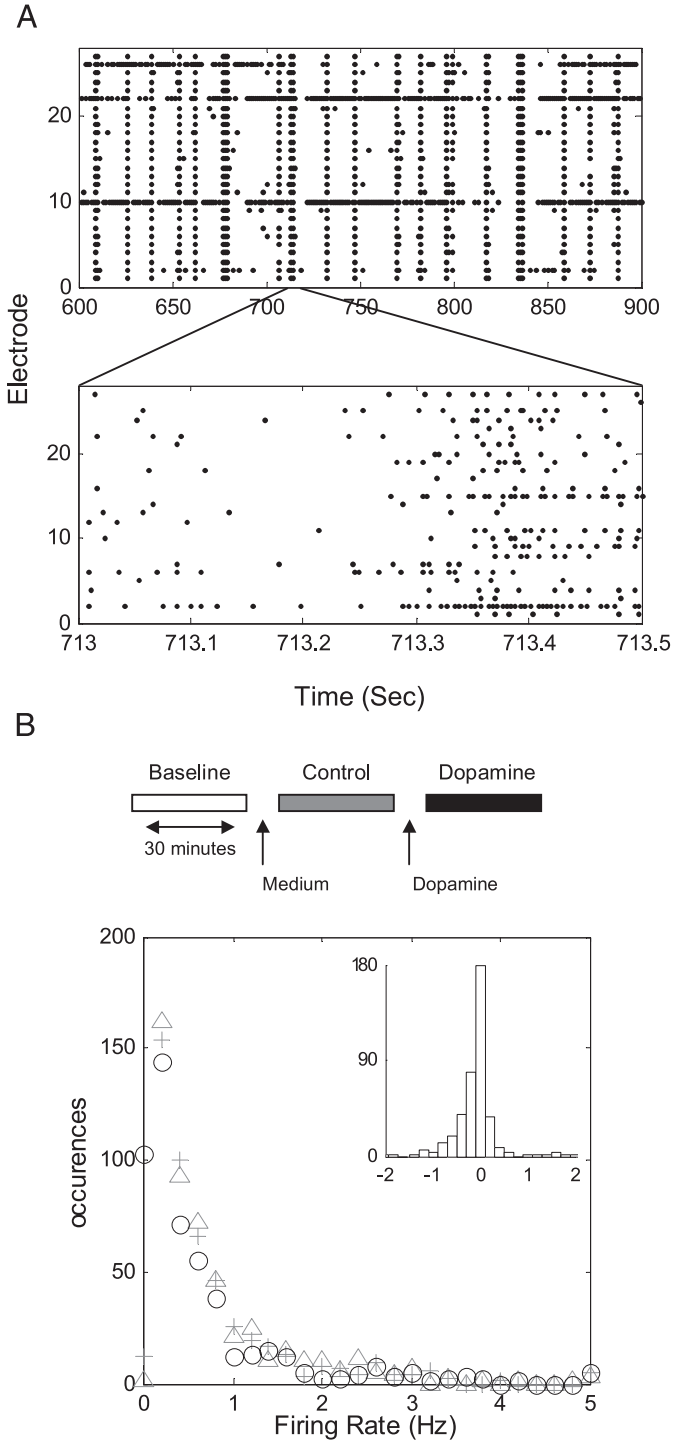
$D_1$-specific dopamine receptor agonist, SKF 38393 (15–25 $\mu$M), and $D_2$-specific agonist, quinpirole (15–30 $\mu$M), were applied using the same protocols as outlined for dopamine. $D_1$-specific antagonist, SCH 23390 (15–30 $\mu$M), and $D_2$-specific antagonist, remoxipride (15–30 $\mu$M), were dissolved in tissue culture medium and applied globally 30 min prior to dopamine application.

### *Dopamine receptor labeling*

$D_1$ receptors were labeled using the fluorescent $D_1$ antagonist Bodipy FL SCH 23390 (Molecular Probes). Labeling specificity was assessed by preapplying an excess of nonfluorescent SCH 23390, resulting in a 44% decrease in the mean fluorescence, indicating that labeling was, at least in part, specific. $D_2$ receptors were labeled using the fluorescent $D_2$ agonist Bodipy FL PPHT (Molecular Probes). The specificity of the label was assessed by postapplying an excess of the nonfluorescent $D_2$ 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 labeling 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 min of recordings without manipulation; *control phase*—30 min of recording after addition 5–100 $\mu$l of tissue culture medium (control solution); and *dopamine phase*—30 min of recording following addition of 5–100 $\mu$l of dopamine or other pharmacologically related compounds. Of the 30 min of each phase, analyses (see *Definition of correlation*) were confined to 25 min only; the first 5 min after addition of dopamine, control medium, or dopamine-related compounds were discarded from analysis 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*

Population data are 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 ($0 < \tau < 150$ ms; $\Delta\tau = 2.5$ ms); thus defined, this temporal binning yields a total of 30 activity pairs (each of which has a different $\tau \pm \Delta\tau$) for a given $A \rightarrow B$. Note that $A$ and $B$ may be action potentials recorded from the same or from different electrodes. For each $A \rightarrow B$ 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 $A$. $A \rightarrow B$ entailment strength may be affected by the activity of $A$, $B$, 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 $A \times B$ 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 ms), 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 $C(\tau)$ 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 were obtained. Changing either the resolution ($\Delta\tau$) or the maximal time delay for calculation of activity pairs (5–500 ms) did not qualitatively affect the results.

Since there is a stochastic element in the neuronal activity, a measure such as $C(\tau)$ is sensitive in cases of a small number of trials (occurrences of $A$ in the $A \rightarrow B$ activity pair). To circumvent this problem when comparing $C(\tau)$s between recording phases, the following criteria for inclusion of an activity pair in the analysis were used. *1*) $A$ was active $\geq$150 times during the two compared recording

FIG. 2. *A*: raster plot of network activity where each dot indicates a single spike. *Top*: raster plot of 27 electrodes over 5 min of spontaneous activity from 1 example network. *Bottom*: enlargement of 500 ms of activity of the same network. *B*: *top*: each experiment consists of 3 phases: *1*) *baseline phase*—during which the spontaneous activity was recorded for 30 min without any manipulations, *2*) *control phase*—30 min of recording after addition 5–100 $\mu$l of culture medium, and *3*) *dopamine phase*—30 min of recording following addition of 5–100 $\mu$l of dopamine (or other pharmacologically related compounds). *Bottom*: Firing rate histograms for all the electrodes that in the 1st recording phases showed an average firing rate of $>0.1$ spikes/s; *y*-axis depicts number of electrodes. 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 3 distributions yield characteristic firing rates of 0.99, 0.97, and 0.98 for baseline, control, and dopamine recording phases, respectively (95% CI $< 0.2$; 16 networks, $n = 506$ active electrodes). *Inset*: 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 Hertz, while the *y*-axis denotes number of occurrences. Note that while the distribution is slightly skewed toward a decrease in firing rates, the majority of electrodes did not change their firing rate, and many electrodes even showed an increase in rates.

phases (i.e., average firing rate of 0.1 spikes/s). *2*) $A \rightarrow B$ appeared more than five times during each of two compared recording phases. These criteria left us with ≥360,000 pairs for analysis in each of the reported experimental conditions (actual numbers are reported in RESULTS).

### *Number of experiments*

Sixteen experiments of dopamine application in 16 different networks were conducted; in addition, in 9 experiments, specific $D_1$ or $D_2$ agonists were used, and in 3 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 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 1*A* shows an ex vivo network of cortical neurons grown on a multi-electrode array. To use such preparations for characterizing network responses to dopamine, it was necessary to show 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. 1, *C–H*. The specificity of these labels was verified by preapplying or postapplying an excess of nonfluorescent competitive antagonists of $D_1$ and $D_2$ receptors, as described in METHODS. Pre- or postapplication 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 (Hoyt 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 with complex temporal structure (Beggs and Plenz 2003) and sparse low-frequency uncorrelated single spike activity (Figs. 1*B* and 2*A*), 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 16 networks used for this study; this number ranged from 8 to 48 (of 60).

To examine the effects of dopamine on spontaneous activity in our preparation, we performed the following experiment (Fig. 2*B*, *top*). Spontaneous activity in each network was recorded during three phases: *1*) *baseline phase*, *2*) *control phase*, and *3*) *dopamine phase*. The distributions of firing rates recorded from individual electrodes during all phases are shown in Fig. 2*B* (*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 (Fig. 2*B*, *inset*) suggests that, while most neurons did not change their firing rates (peak centered around 0 change), there is some tendency for a decrease in the firing rates.

### *Effects of dopamine application on correlations between neuronal activities*

To determine the effects of dopamine application on the correlations between individual neuronal activities, we calcu-

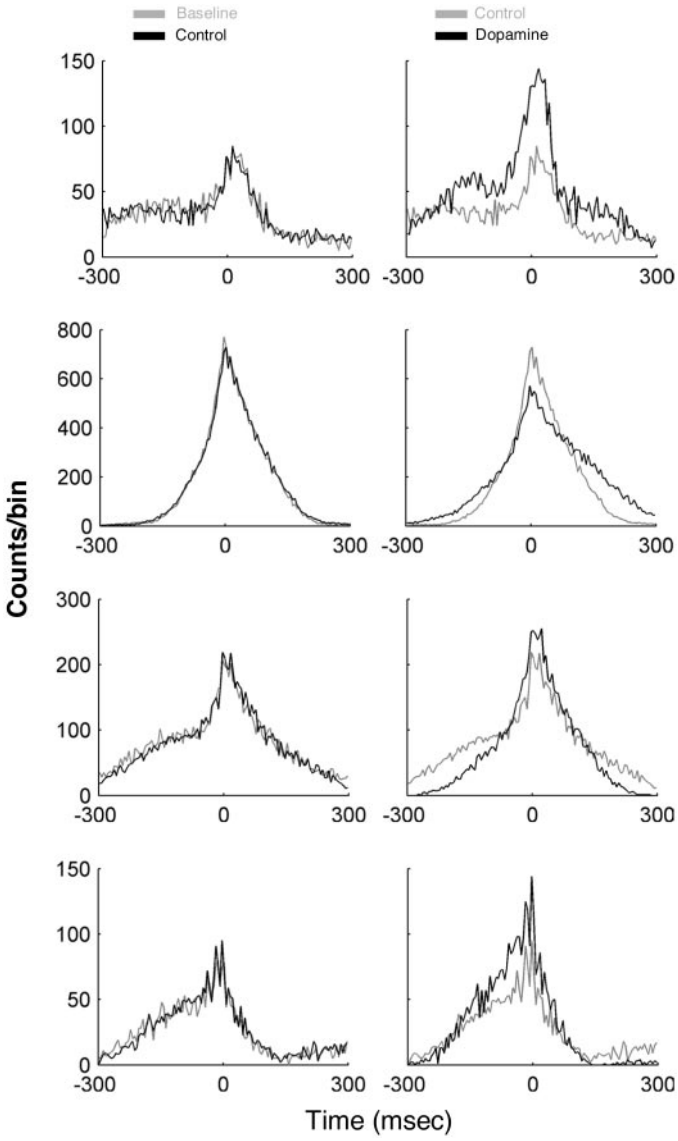



FIG. 3. Cross-correlograms of 4 different pairs of electrodes are shown. *Left*: cross-correlograms in the baseline (gray) and control (black) phases. *Right*: for the same electrode pairs, cross-correlograms in the control (gray, same correlogram as in the *left*) and dopamine phase (black). Bin size is 5 ms. Electrode pairs were chosen from 3 different experiments.

lated cross-correlograms of the activity recorded from all pairs of electrodes in each phase in each network and examined the effects of dopamine on these correlograms. The *left column* of Fig. 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* ($A \cap B$) fired an action potential with a precise time delay represented by the abscissa. Each of the panels in the *left column* of Fig. 3 contains two correlograms; one obtained from the baseline phase and 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 column* of Fig. 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 Fig. 3. To quantify the effects of dopamine on pairwise correlativity of the entire population of pairs, we did the following. First, we assumed that a given $A \cap B$ activity pair with a given time delay ($\tau$) 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 $A \cap B$ correlograms into discrete *activity pairs* according to their respective time delays ($\tau$). Depending on the temporal order, we depicted these as $A \rightarrow B$ (if *A* fired before *B*) and $B \rightarrow A$ (if *B* fired before *A*). Therefore we ended up with a set of 60

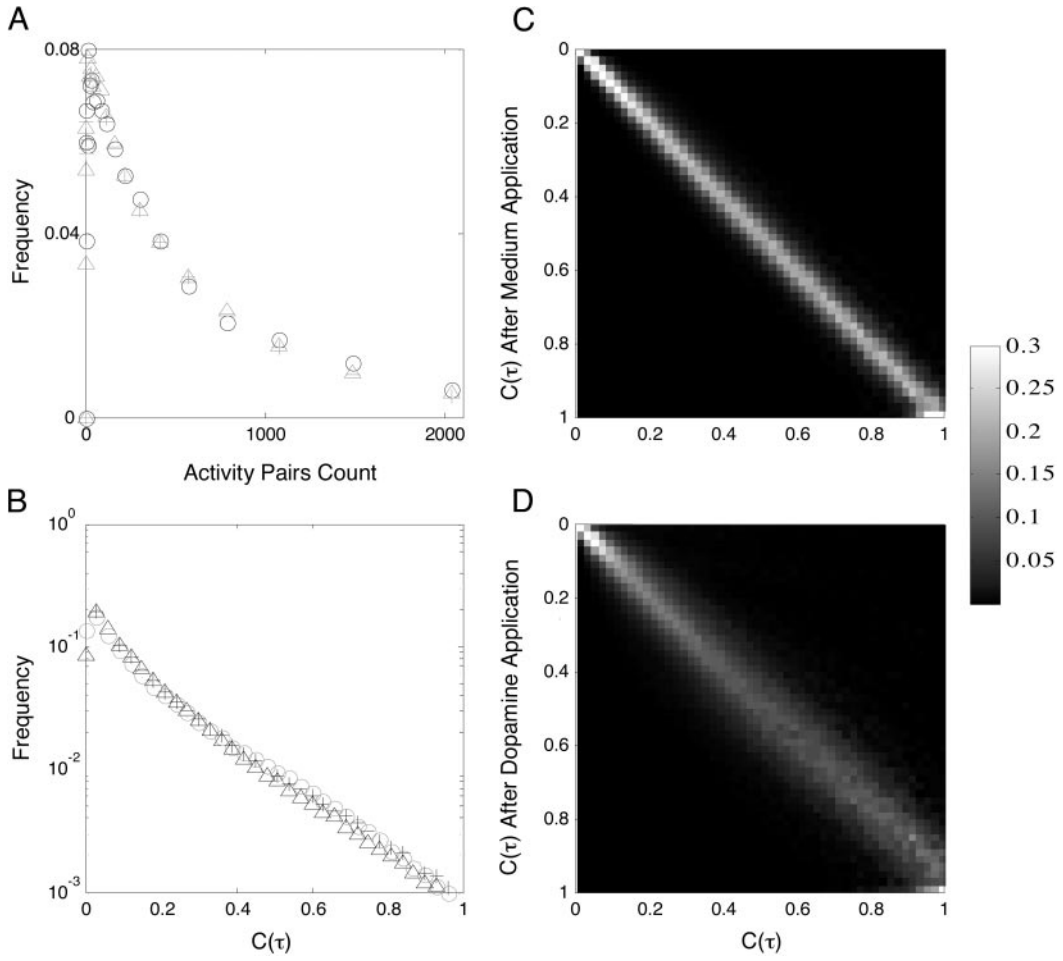



FIG. 4. *A*: histograms depicting the distribution of counts of activity pairs observed in baseline (triangles), control (plusses), and dopamine (circles) phases. Poisson distribution functions fitted to the 3 distributions yield characteristic λ values (which is both the mean and the variance) of 163.8, 160.1, and 166.9 for baseline, control, and dopamine recording phases, respectively (95% CI $<$ 0.1; 16 networks; included are all pairs that appeared $>$5 times within a recording phase: $n = 585{,}119$ activity pairs for baseline phase, $n = 582{,}401$ for control phase, and $n = 460{,}972$ in the dopamine phases). *B*: distribution of $C(\tau)$, calculated for all activity pairs observed in 16 networks. 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 3 distributions yield characteristic $C(\tau)$s of 0.19, 0.19, and 0.20 for baseline, control, and dopamine recording phases, respectively (95% CI $<$ 0.002; 16 networks; included are all pairs that appeared $>$5 times within a recording phase: $n = 585{,}119$ activity pairs for baseline phase, $n = 582{,}401$ for control phase, and $n = 460{,}972$ in the dopamine phases). *C*: 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 $C(\tau)$ in the control phase. Distribution of $P[C(\tau)_{\text{control}} | C(\tau)_{\text{base}}]$, calculated for 477,500 pairs, is depicted using grayscale (cut-off at 0.3). *D*: similar representation as in *C* for $P[C(\tau)_{\text{dopamine}} | C(\tau)_{\text{control}}]$; 361,943 activity pairs. The latter distribution is more dispersed across the entire range of $C(\tau)$.

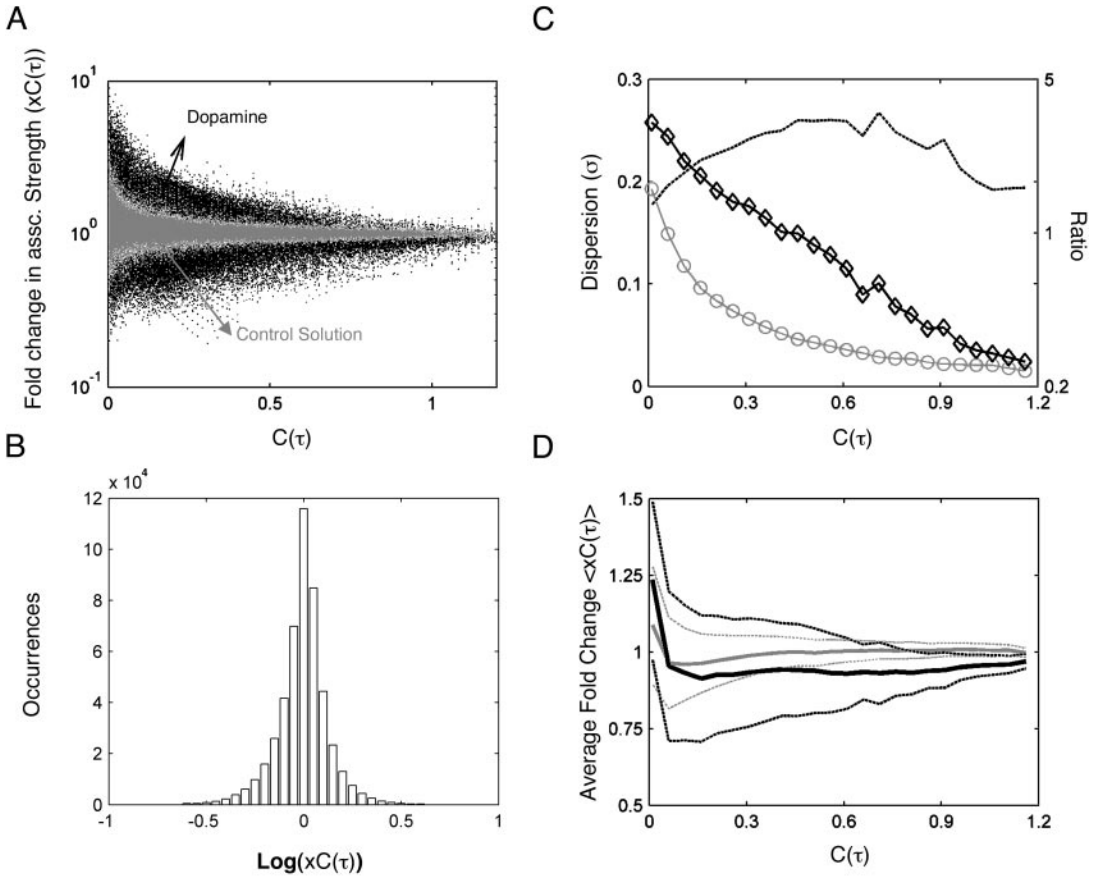



FIG. 5. Dopamine application enhances changes in correlations. *A*: fold change in correlation [$xC(\tau)$] after application of control solution (gray) and 30 $\mu$M dopamine (black) in 1 representative network. Approximately 70,000 activity pairs are included. Value at the abscissa is $C(\tau)$ obtained from baseline phase (for control solution application) or control phase (for dopamine application). Dispersion caused by dopamine application is much larger compared with control solution application. *B*: histogram of distribution of changes in correlation, log($xC\tau$), due to application of control solution, is shown for all activity pairs in all 16 networks ($n$ = 477,500, bin size for calculation is 0.05). *C*: dispersion, $\sigma$[the SD of log($xC(\tau)$] distribution), due to dopamine application (black continuous line, $n$ = 361,943) and control solution application (gray continuous line, $n$ = 477,500) as a function of $C(\tau)$ 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*: average fold change in $C(\tau)$ as a function of $C(\tau)$ obtained from the baseline phase (for control solution application) and control phase (for dopamine application). All activity pairs ($n$ = 361,943 for dopamine application, $n$ = 477,500 for control solution application) from all 16 networks are included. Areas between dashed lines depict the extent of dispersion ($\pm\sigma$); dispersion due to control solution application is narrower, over all $C(\tau)$, compared with dopamine application.

discrete values for each pair of neurons in each phase ($\tau$ ranging from –150 to +150 ms, with a bin size of 5 ms). Figure 4*A* 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 $A \cap B$ activity pair to the spike counts of *A*, *B*, or $A \times B$, thus obtaining a correlation coefficient $C(\tau)$ (see METHODS). The data presented in the remaining of this study is normalized to *A* because 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 $A \times B$ does not produce qualitatively different results (see Supplementary Fig. 1)[1].

### *Effects of dopamine application on the correlation coefficients C(τ)*

Figure 4*B* shows distributions of $C(\tau)$ in the three recording phases; This analysis revealed that the *population* distribution of $C(\tau)$ was largely stable, i.e., 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 (Fig. 4, *C* and *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? Simi-

[1] The Supplementary Material for this article (a figure) is available online at http://jn.physiology.org/cgi/content/full/00202.2004/DC1.

larly, 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?

Figure 4, *C* and *D*, shows these conditional probabilities coded as grayscale intensities. The distribution of $P[C(\tau)_{control}|C(\tau)_{base}]$, calculated for 477,500 pairs (Fig. 4*C*) is considerably different from that of $P[C(\tau)_{dopamine}|C(\tau)_{control}]$ (Fig. 4*D*; 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 Fig. 5 in terms of fold change of association strength [$xC(\tau)$]: note in Fig. 5*A* (data from 1 representative network) that the dispersion of $C(\tau)$ due to dopamine application (black) is large compared with control conditions (gray) throughout the range of measured $C(\tau)$. To obtain a single variable that reflects the extent of dopamine-induced dispersion, we use the distribution of fold change in $C(\tau)$. Figure 5*B* shows this distribution, obtained from changes between baseline and control recording phases, in the entire set of experiments (477,500 pairs). We use the SD of the distribution ($\sigma$) 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 Fig. 5*C*, $\sigma$ 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. Figure 5*D* 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 on $C(\tau)$ before dopamine is applied. Note that the above-mentioned observations are insensitive to the methods of normalization as explained before (see Supplementary Fig. 1).

### *Effects of dopamine agonists and antagonists*

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 with the $D_1$ agonist SKF-38393 (15–25 $\mu$M) or $D_2$ agonist quinpirole (15–30 $\mu$M) 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 (15–30 $\mu$M), respectively, blocked the dispersion effects.

## DISCUSSION

This 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.

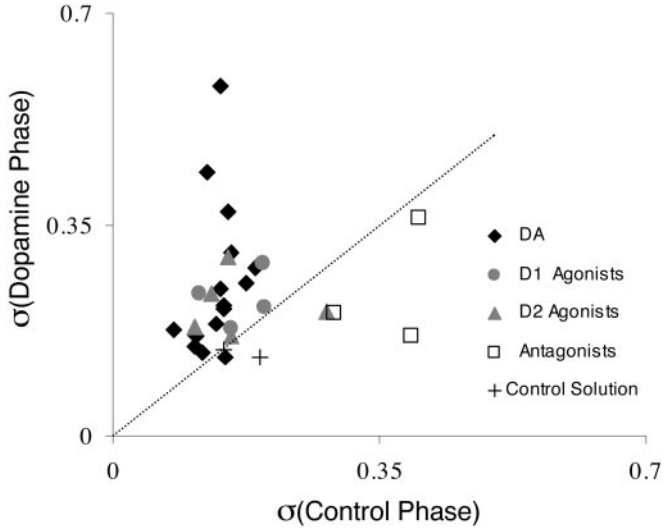



FIG. 6. Effect of dopamine and selective agonists/antagonists application is shown for all networks. For each network, the dispersion ($\sigma$) is represented by the SD of distribution of fold changes in $C(\tau)$ as in Fig. 5*B*. Abscissa value is dispersion due to control solution application, and ordinate value is 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 2 phases of control solution after the initial baseline phase, are also shown.

Dopamine is believed to act, during the learning process, as a modulator of neuronal connections (example reviews in Dayan and Balleine 2002; Dehaene and Changeux 2000; Joel et al. 2002; Schultz 2002; Spanagel and Weiss 1999; Tzschentke 2001). 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 behavior goes through changes in ensemble behavior. 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 (e.g., 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 behavioral 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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