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The Effects of Amphetamine and Pilocarpine on the Release of Ascorbic and Uric Acid in Several Rat Brain Areas

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MUELLER, K. AND P. M. KUNKO. *The effects of amphetamine and pilocarpine on the release of ascorbic and uric acid in several rat brain areas*. PHARMACOL BIOCHEM BEHAV **35**(4) 871–876, 1990.—Linear sweep voltammetry was used to investigate the effects of amphetamine (which enhances the release of dopamine) and/or pilocarpine (a cholinergic agonist) on the release of ascorbic acid and uric acid in brain areas differing in dopamine and acetylcholine concentrations. In caudate, nucleus accumbens, and hippocampus, the magnitude of the amphetamine-induced increase in ascorbic acid was roughly correlated with dopamine content of the brain area tested. Cingulate cortex was a notable exception; the increase in ascorbic acid was greater than that in nucleus accumbens. Pilocarpine produced the greatest increase in ascorbic acid in cingulate cortex, even though cingulate cortex has the lowest acetylcholine concentration of the brain areas tested. Except for cingulate cortex, the ascorbic acid data were consistent with the hypothesis that amphetamine and pilocarpine release different pools of ascorbic acid. The uric acid data were consistent with the hypothesis that amphetamine and pilocarpine release the same pool of uric acid. The unexpected findings in cingulate cortex may point to an important role of ascorbic acid in this brain area.

Amphetamine Pilocarpine Ascorbic acid Uric acid Voltammetry Caudate Nucleus accumbens Hippocampus Cingulate cortex

ASCORBIC acid (AA) and uric acid (UA) are both present in mammalian brain, but their function (if any) is not yet understood. A variety of neuroactive substances increases the release of AA and UA into the extracellular space of the brain. This apparent lack of stimulus specificity is disturbing. The research described below reports the effects of amphetamine (an indirect dopamine agonist) and pilocarpine (an acetylcholine agonist) on the release of AA and UA in brain areas with differing concentrations of dopamine and acetylcholine. If drug-induced increases in AA and UA are truly nonspecific, then amphetamine and pilocarpine should have similar effects to each other and should have similar effects in each brain area examined.

High concentrations of AA are actively transported into brain by the choroid plexus (25,26). Ascorbic acid levels in the brain are carefully regulated (22), suggesting that AA might be important for proper function of the brain. Ascorbic acid is released from synaptosomes (5) and enhances the release of some neurotransmitters from synaptosomes (9). It also affects the binding of wide variety of neurotransmitters (7, 23, 27) (although whether this is a physiological or pharmacological effect is unclear). Therefore, some have suggested that AA might be a neuromodulator in brain.

Amphetamine (21) and pergolide (a dopamine agonist) (2) both increase extracellular AA; therefore, AA initially appeared to be intimately involved with dopaminergic neurotransmission. However, extracellular AA is also increased by a wide variety of nondopaminergic stimuli. For example, pilocarpine dramatically increases striatal AA (12). Excitatory amino acid transmitters also increase striatal AA (17) and higher doses of diazepam decrease striatal AA (3). This apparent lack of stimulus specificity is inconsistent with the hypothesis that AA might be a neuromodulator. On the other hand, if these various pharmacological agents release different pools of AA, the hypothesis that AA is a neuromodulator might be viable.

Brain UA has received much less attention than AA. Striatal UA is increased by amphetamine (14) and scopolamine (an acetylcholine antagonist) (12). The amphetamine-induced increase in UA is blocked by the dopamine antagonist haloperidol and the scopolamine-induced increase in UA is blocked by pilocarpine. Since UA is the end product of purine metabolism, it is unlikely to have any functional role in brain. However, extracellular UA levels may provide interesting information. One possibility is that extracellular UA may arise from the release of adenosine. There are high concentrations of adenosine receptors in striatum (4) and adenosine has been suggested to modulate dopamine activity. Therefore, extracellular UA levels may provide a marker for "purinergic" activity (16). On the other hand, extracellular UA

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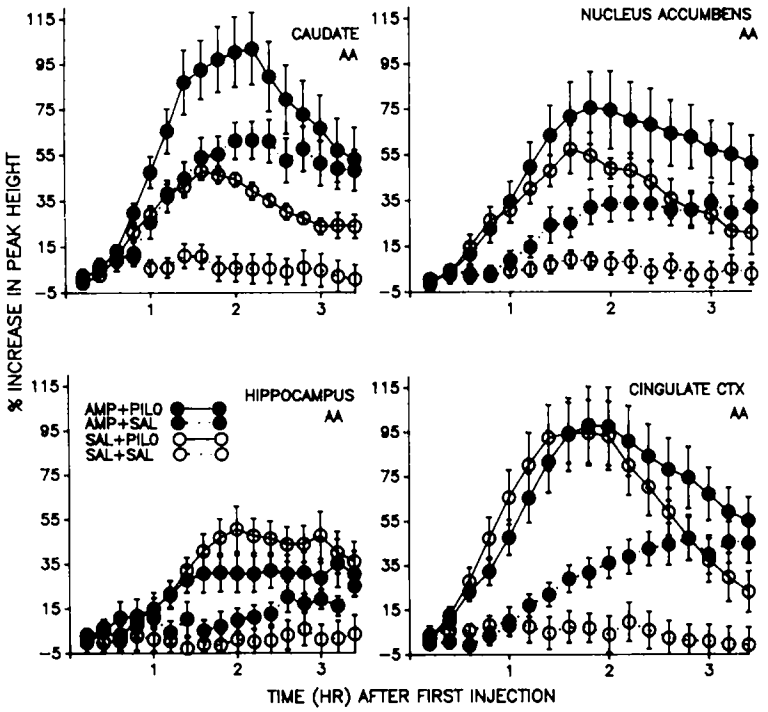

FIG. 1. The effects of amphetamine and pilocarpine on extracellular AA levels in various brain areas. AA = ascorbic acid; AMP = amphetamine; PILO = pilocarpine; SAL = saline.

may simply correlate with the amount of metabolic activity in the vicinity. ATP, GTP and other energy-related purines are all eventually metabolized to UA. Therefore, increased energy use would be expected to increase local extracellular UA levels.

The majority of the research discussed above has been limited to a few brain areas. Perhaps because AA was initially associated with dopamine, the vast majority of research has been limited to striatum. One way to address the problem of the possible function and/or meaning of extracellular AA and UA is to examine the effect of the same pharmacological agent in different brain areas. If AA and UA are neuromodulators (or markers of neuromodulators) there should be both some pharmacological and anatomical specificity in their release.

In the research described below, voltammetric electrodes are placed in four brain areas with differing concentrations of dopamine and acetylcholine. The AA and UA response to both amphetamine and pilocarpine is presented. The hypothesis is that the amphetamine-induced increase in AA and UA will be directly correlated with the concentration of dopamine while the pilocarpine-induced changes in AA and UA will be correlated with the concentration of acetylcholine. In addition, the effects of amphetamine + pilocarpine are presented. These data may indicate whether amphetamine and pilocarpine release the same "pool" of AA.

METHOD

Animals

Male Wistar rats (bred at the departmental vivaria) were housed individually on a 12-hr light/dark cycle. Testing was always conducted at 1.5 hr after lights-on. Preoperative body weights ranged from 350 to 470 grams.

Procedure

After pretreatment with atropine rats were anesthetized with 50 mg/kg Nembutal. Carbon paste working electrodes were fabricated as described previously (15) except that smaller stainless steel wire was used (i.d. = 200 μ). Electrodes were implanted in anterior caudate [2.8 mm anterior to bregma, 2.7 mm lateral, 5.0 mm beneath the cortex (19); n = 10], nucleus accumbens [3.6 mm anterior to bregma, 1.5 mm lateral, 6.7 mm beneath the cortex (19); n = 9], dorsal hippocampus [3.3 mm posterior to bregma, 1.5 mm lateral, and 2.7 mm below the cortex (18); n = 8], and cingulate cortex [3.5 mm anterior to bregma, 1.6 mm lateral, 3.1 mm below the cortex at 15° (18); n = 10]. An Ag/AgCl reference electrode was also implanted and a silver wire attached to a skull screw provided an auxiliary electrode.

A DCV-5 voltammetry controller (BAS) remotely controlled by a personal computer conducted linear sweep (10 mV/sec) semidifferential voltammetry. Electrodes were scanned (−100 to 500 mV) every 12 min. This technique produces voltammograms with two prominent peaks. Previous research from this lab has identified the first peak as AA and the second peak as UA (11,15). (However, there is a slight possibility that indoles might contribute to the second peak.) Animals were connected to the apparatus via a slip ring to provide freedom of movement.

Forty-eight hr after surgery animals were connected to the voltammetry apparatus, but no drugs were administered. Four, 6, 8, and 10 days after surgery animals were connected to the voltammetry apparatus and after a 2-hr recording period (to establish a stable baseline) were injected (IP) with either 3 mg/kg pilocarpine (Sigma) or saline. Either 3 mg/kg d-amphetamine sulfate (Sigma) or saline was injected (SC) 30 min later. Thus, for each brain area, there were four treatments: saline + amphetamine, pilocarpine + amphetamine, saline + saline, and pilocarpine + saline. Treatments were separated by 48 hr; each rat was injected with each combination of drugs, but the treatment order was counterbalanced. Recording continued for 3 hr after the second injection. The percent increase in peak heights (over the mean of the 3 scans prior to the first injection) was the

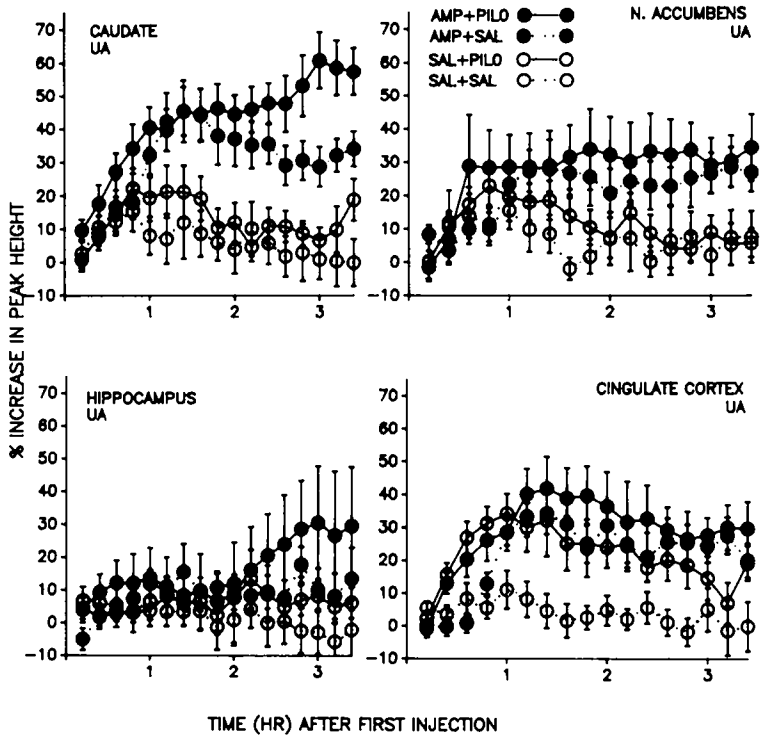

FIG. 2. The effects of amphetamine and pilocarpine on extracellular UA levels in various brain areas. UA = uric acid; AMP = amphetamine; PILO = pilocarpine; SAL = saline.

dependent variable.

Electrode placement was verified postmortem. After cardiac perfusion with saline and formalin, brains were sectioned at 20 μ. Data from rats with misplaced electrodes were discarded.

The data from each brain area were analyzed with a three-way repeated measures ANOVA (amphetamine × pilocarpine × time after injection). AA and UA data were analyzed separately. The Huynh Feldt probabilities (HFP) are presented for assessment of F values involving the effect of time. The HFP is a more conservative probability estimate that reduces the problems caused by correlations between repeated measures data when time is a factor (6).

RESULTS

Ascorbic Acid

As expected, amphetamine produced a dramatic increase in AA in caudate, $F(1,9) = 55.45$, $p<0.001$; the maximal increase produced by amphetamine was approximately 61% (see Fig. 1). Pilocarpine also produced a dramatic increase in AA in caudate, $F(1,9) = 12.13$, $p<0.01$; the maximal increase produced by pilocarpine was approximately 48%. Note that pilocarpine + amphetamine increased AA by up to 102%. Thus, the effects of pilocarpine and amphetamine are almost exactly additive in caudate. There was no interaction between pilocarpine and amphetamine, $F(1,9) = 0.00$, $p>0.97$. The main effect of time and the interactions between time and amphetamine and between time and pilocarpine are all significant but are not important for the purposes of this discussion. The three-way interaction is not significant, $F(16,144) = 0.94$, $p>0.53$.

In nucleus accumbens, both amphetamine, $F(1,8) = 7.01$, $p<0.05$, and pilocarpine, $F(1,8) = 38.28$, $p<0.001$, increased extracellular AA (see Fig. 1). The maximal increases produced by amphetamine and pilocarpine were 33% and 57%, respectively. The maximal increase produced by amphetamine + pilocarpine was 75%; thus, in nucleus accumbens, amphetamine + pilocarpine produced a slightly less than additive effect. Again, the interaction between pilocarpine and amphetamine, $F(1,8) = 0.00$, $p>0.97$, and the three-way interaction, $F(16,128) = 0.23$, $p>0.99$, are not significant. Again, the main effect of time and the interactions between time and amphetamine and between time and pilocarpine are significant but uninteresting for the present.

In the dorsal hippocampus, pilocarpine significantly increased AA, $F(1,7) = 79.42$, $p<0.001$, but amphetamine had no effect on AA, $F(1,7) = 0.04$, $p>0.84$. The combination of amphetamine + pilocarpine seemed to actually reduce the effect of pilocarpine on AA (see Fig. 1), but the interaction between amphetamine and pilocarpine was not significant, $F(1,7) = 3.38$, $p<0.11$. However, the three-way interaction is significant, $F(16,112) = 3.31$, HFP< 0.03, and this may indicate that at some times amphetamine reduced the effects of pilocarpine but at other times did not.

In cingulate cortex, amphetamine increased AA but in a somewhat delayed fashion. Although the main effect of amphetamine is not significant, $F(1,9) = 3.12$, $p<0.12$, the interaction between time and amphetamine is significant, $F(16,144) = 12.20$, HFP<0.001. Pilocarpine produced a dramatic increase in AA, $F(1,9) = 48.12$, $p<0.001$, that was virtually unaffected by the presence of amphetamine. Thus, in cingulate cortex, as in hippocampus, amphetamine and pilocarpine produced clearly nonadditive effects. The three-way interaction is not significant, $F(16,144) = 2.19$, HFP>0.08.

Uric Acid

In caudate amphetamine increased UA, $F(1,9) = 41.23$,

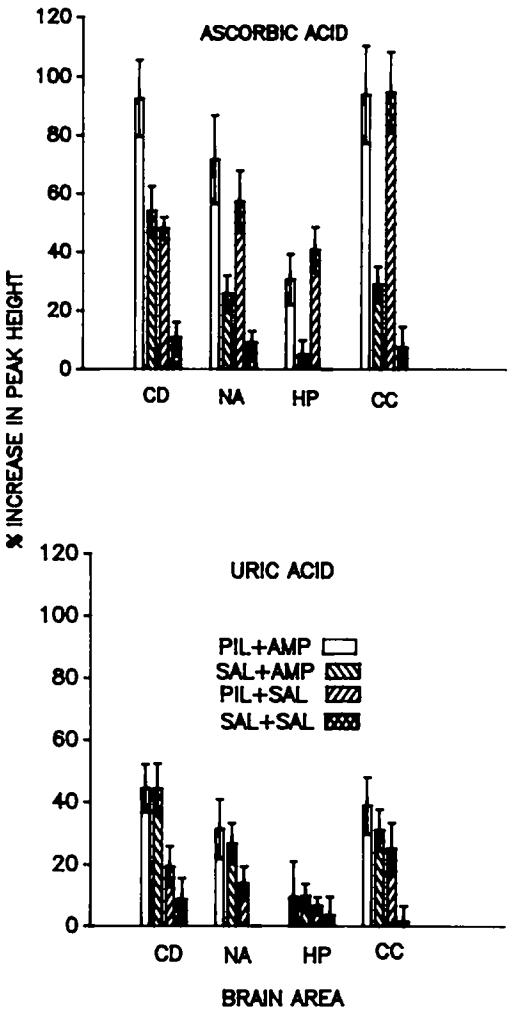

FIG. 3. Comparison between brain areas 1.6 hr after injection of pilocarpine and/or amphetamine. CD = caudate; NA = nucleus accumbens; HP = hippocampus; CC = cingulate cortex; PIL = pilocarpine; AMP = amphetamine; SAL = saline.

$p<0.001$, with a maximum increase of about 45%. Pilocarpine did not significantly affect UA; neither the main effect, $F(1,9)=3.35$, $p<0.11$, nor the interaction between pilocarpine and time, $F(16,144)=1.63$, HFP>0.15, is statistically significant. The three-way interaction is significant, $F(16,144)=2.25$, HFP<0.04. This may be due to the last third of the testing period in which pilocarpine seems to potentiate the effect of amphetamine, even though pilocarpine itself has no effect on UA (see Fig. 2).

In nucleus accumbens amphetamine produced the only significant effects on UA. Although the main effect of amphetamine is not significant, $F(1,8)=4.52$, $p<0.07$, the interaction between amphetamine and time is significant, $F(16,128)=4.44$, HFP< 0.001. Neither the main effect of pilocarpine, nor any of the interactions involving pilocarpine, were statistically significant.

Amphetamine also produced the only significant change in UA in hippocampus. Again, the main effect of amphetamine was not significant, $F(1,7)=2.19$, $p>0.18$; but the interaction between amphetamine and time was significant, $F(6,112)=4.65$, HFP< 0.001. Neither the main effect of pilocarpine, nor any of the interactions involving pilocarpine were statistically significant.

In cingulate cortex all drug treatments except saline + saline produced virtually identical changes in UA (see Fig. 2). Thus, the main effect of pilocarpine, the main effect of time, and the interaction between amphetamine and time are all significant, $F(1,9)=16.37$, $p<0.003$; $F(16,144)=12.54$, HFP<0.001; $F(16,144)=4.95$, HFP<0.001; respectively.

Comparisons Between Brain Areas

The analyses presented above do not permit direct comparisons between brain areas. Therefore, the AA and UA data in all brain areas were compared with ANOVA at a single time point—1.6 hr after the first injection. These data are shown in Fig. 3. In the AA data, the main effect of area of the brain is statistically significant, $F(3,33)=7.49$, $p<0.001$, as are the interactions between amphetamine and brain area, $F(3,33)=6.05$, $p<0.002$, and the interaction between pilocarpine and brain area, $F(3,33)=5.02$, $p<0.005$. There is no interaction between amphetamine and pilocarpine, $F(1,33)=1.19$, $p>0.28$, and there is no three-way interaction, $F(3,33)=0.43$, $p>0.72$. The results of these analyses simply mean that amphetamine and pilocarpine increase AA in some brain areas but not others.

In the UA data the main effect of brain area is statistically significant, $F(3,33)=7.8$, $p<0.001$, as is the main effect of amphetamine, $F(1,33)=21.32$, $p<0.001$, and the main effect of pilocarpine, $F(1,33)=6.57$, $p<0.02$. The interactions are not statistically significant. This can be interpreted to mean that, regardless of drug treatment, the increase in UA is lower in some brain areas (probably hippocampus) than others.

DISCUSSION

The hypothesis was that the amphetamine-induced increase in AA and UA would be correlated with the concentration of dopamine while the pilocarpine-induced changes in AA and UA would be correlated with the concentration of acetylcholine. With the notable exception of cingulate cortex, the amphetamine data supported the hypothesis. However, the pilocarpine data were clearly inconsistent with the hypothesis. The AA data were consistent with the hypothesis that pilocarpine and amphetamine release ascorbic acid from separate pools, but the UA data were more consistent with the hypothesis that both pilocarpine and amphetamine release UA from a common pool.

The brain areas tested were selected because of their differing concentrations of dopamine and acetylcholine. Caudate contains by far the greatest concentration of dopamine, followed by nucleus accumbens. Dorsal hippocampus and cingulate cortex both contain far less dopamine with cingulate cortex containing equal or slightly greater amounts than hippocampus (8,10).

Amphetamine produced the greatest increase in AA in caudate, followed by cingulate cortex, nucleus accumbens, and hippocampus. This confirms previous reports that amphetamine-induced AA release is greater in caudate than in nucleus accumbens (13). Except for cingulate cortex, this is consistent with the concentrations of dopamine in these brain areas.

The magnitude of the amphetamine-induced increase in AA in cingulate cortex was unexpected, and the time-course of the amphetamine-induced increase in AA in cingulate cortex was clearly different from that in caudate and, to a lesser extent, in nucleus accumbens. To our knowledge, cingulate cortex has no known role in the behavioral effects of amphetamine in rats.

Caudate also contains by far the greatest concentration of acetylcholine. We have been unable to locate estimates of the acetylcholine concentration of nucleus accumbens. Both hippocampus and cingulate cortex contain far less acetylcholine than caudate, and hippocampus appears to contain slightly more ace-

tylcholine than cingulate cortex (1, 20, 24, 29). Pilocarpine produced the greatest increase in AA in cingulate cortex, followed by nucleus accumbens. Pilocarpine produced nearly equal increases in AA in dorsal hippocampus and striatum. These data are clearly inconsistent with acetylcholine concentrations in these brain areas.

In each brain area tested, the AA data were consistent with the hypothesis that amphetamine and pilocarpine release separate pools of AA. In caudate the effects of amphetamine and pilocarpine were almost exactly additive. In nucleus accumbens, the effect of amphetamine and pilocarpine was not exactly additive, but was well within the margin of error. In dorsal hippocampus, amphetamine had no effect on AA even though pilocarpine increased AA.

Although AA can be released by diverse pharmacological agents, at least some of these agents appear to release different pools of AA. In addition, there are obvious differences in the AA response to the same pharmacological agent in different brain areas (13). However, if the release of AA produced by a particular pharmacological agent is unrelated to the neuronal activity produced by that agent, how can the release of AA be explained? Consideration of the anomalous response in cingulate cortex may provide important clues to the answer to this question.

Apparently, amphetamine and pilocarpine share some indirect effect that results in an increase in AA in cingulate cortex which is disproportionate to the tissue content of dopamine and acetylcholine. Pilocarpine produces dramatic autonomic effects which are likely to be aversive. Amphetamine also produces autonomic effects and is well known for its mood-altering properties. Amphetamine can be both aversive (as measured by conditioned taste aversion paradigms) or highly motivating (as measured by place preference or self-administration paradigms). Cingulate cortex, of course, is classically associated with the limbic system. It receives afferents from several thalamic nuclei. It is considered by some to be an integral portion of the "medial pain system" and it appears to be involved in the affective response to pain [cf. (28)]. Cingulate cortex is anatomically well-situated to respond to aversive effects of drugs. Perhaps this is why, in cingulate cortex, amphetamine and pilocarpine appear to release the same pool of AA. Our hypothesis is that the amphetamine- and pilocarpine-induced increase in AA in cingulate cortex is the result of neuronal activity which conveys information about the aversive effects of both drugs. This hypothesis is consistent with the original hypothesis; in the case of cingulate cortex another step has simply been added.

In general, both amphetamine- and pilocarpine-induced increases in extracellular UA were much less than increases in extracellular AA. The greatest amphetamine-induced increase in extracellular UA occurred in caudate, followed by nucleus accumbens and cingulate cortex (both were similar to each other), and hippocampus. Except for cingulate cortex (which contains much less dopamine than nucleus accumbens) these data are consistent with the dopamine concentrations of the brain areas tested.

The increase in UA produced by pilocarpine was greatest in cingulate cortex followed by caudate and nucleus accumbens (both of which were similar to each other), and hippocampus. Again, the response in cingulate cortex was anomalous considering the concentration of acetylcholine in cingulate cortex.

In general, the UA data were consistent with the hypothesis that pilocarpine and amphetamine release the same pool of UA. In nucleus accumbens, hippocampus, and cingulate cortex, the effects of pilocarpine + amphetamine were barely greater than the effects of either drug administered alone. In caudate, the effects of amphetamine were identical to the effects of amphetamine + pilocarpine until the latter portion of testing. At that time pilocarpine seemed to potentiate the effects of amphetamine, even though pilocarpine had little or no effect on its own.

The UA data are not consistent with the hypothesis that extracellular UA levels provide and index of "purinergic" activity. Rather, the increase in extracellular UA seems to be some sort of nonspecific response to changes in neuronal activity.

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