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Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus

(long-term potentiation/synaptic plasticity/catecholamines)

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ABSTRACT **The study presented here indicates that norepinephrine (NE) selectively induces long-lasting modifications of synaptically mediated responses in the dentate gyrus of the rat hippocampal slice. A low concentration of NE (1.0 μM; in the presence of 50 μM phentolamine, an α-adrenergic antagonist) or a 1.0 μM concentration of the specific β-adrenergic agonist isoproterenol induced long-lasting pathway-specific alterations of granule cell electrophysiological responses. Excitatory postsynaptic potentials and population spikes evoked by stimulation of the medial perforant pathway (PP) were potentiated for more than 45 min. In contrast, responses to lateral PP stimulation were depressed for the same period. Both potentiation and depression were blocked by the β-adrenergic antagonist propranolol (1.0 μM). These results indicate that NE can act differentially on projections to the dentate gyrus arising in the entorhinal cortex. Such selective persistent modifications of cortical circuits may be involved in processes in the mammalian brain underlying attention, learning, and memory.**

Mnemonic processes are thought to be initiated as persistent modifications of neuronal responses (1). The hippocampal formation, a cortical structure reputed to be involved in memory (2), is exemplary in this regard. Long-term potentiation (LTP) of synaptically mediated responses in the hippocampus and dentate gyrus has been induced by high-frequency stimulation of afferents (3) and intrahippocampal pathways (4). A persistent potentiation of dentate gyrus granule cell activation not requiring high-frequency afferent stimulation is seen after exposure to norepinephrine (NE). This effect is known as NE-induced long-lasting potentiation (NELLP) (5).

LTP and NELLP in the dentate gyrus are seen as increments in amplitude of granule cell excitatory postsynaptic potentials (EPSPs) and population spikes evoked by stimulation of the perforant pathway (PP). This pathway, the major input to the hippocampus via the dentate gyrus, consists of axons whose cell bodies are located in the lateral and medial entorhinal cortices, respectively (6, 7). Medial and lateral PP terminals differ histochemically, and activation is differentially affected by naloxone and excitatory and inhibitory amino acid ligands (8–10). Although high-frequency stimulation of either the medial or the lateral PPs can produce LTP (11, 12), when LTP is induced in one pathway there is a concurrent long-lasting depression of responses evoked by activation of the other pathway (13).

Relationships between NELLP and LTP are not yet well understood, but NE is apparently necessary for a complete expression of LTP in the dentate gyrus (14, 15) and, when paired with high-frequency stimulation, NE may facilitate LTP induction in hippocampal field CA3 (16). Furthermore, D-(−)-2-amino-5-phosphonovaleric acid, an *N*-methyl-D-aspartate receptor antagonist that blocks LTP in the dentate gyrus (17), also blocks the induction of NELLP (18). In this report, we present results from electrophysiological experiments on hippocampal slices in which the medial and lateral PPs were selectively stimulated and the long-term effects of NE and other adrenergic ligands on the activation of the respective pathways were studied.

MATERIALS AND METHODS

Transverse slices (400 μm thick) of dorsal hippocampi from adult male rats (Sprague-Dawley, Taconic Farms; 200–250 g) were maintained in a modified Andersen interface chamber. Standard techniques of electrical stimulation, electrophysiological recording, and tissue maintenance were used (19). Artificial cerebrospinal fluid (ACSF) consisting of 125 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 2.4 mM $CaCl_2$, 1.25 mM $NaHPO_4$, 1.3 mM $MgSO_4$, and 10 mM dextrose was continuously bubbled with 95% O_2/5% CO_2 and maintained at 35°C. Field potential recordings were made with glass micropipette electrodes filled with 2 M NaCl (resistance, 5–15 MΩ). Monopolar stimulating electrodes were single strands of Teflon-insulated stainless steel wire 100 μm in diameter, with the return path in contact with bath ACSF. Stimulation current varied with the preparation (0.05–0.15 mA) but was kept to <50% of that which elicited a maximum response to preclude confounding the responses by current spread to both pathways. Stimulation frequency was 0.2 Hz. Fig. 1*A* illustrates a hippocampal slice and the positions of the stimulating and recording electrodes in the molecular and granule cell layers of the dentate gyrus. Stimulation of the lateral or medial PP (Fig. 1*A*) evoked maximal EPSPs in the outer or middle third of the molecular layer of the dentate gyrus, corresponding to terminal zones of these projections on granule cell dendrites. These responses are illustrated in Fig. 1*B*. A distinguishing characteristic of the respective response profiles is that, at the level of the maximum medial PP-evoked EPSP (the midmolecular layer), the lateral PP-evoked EPSP is reversed in polarity (Fig. 1*B*, recording site 2). The profiles indicate that for the lateral PP, the current sink is in the distal third of the dendrites, whereas the current sink for the medial PP is in the middle third (20).

The drugs used in these experiments were (−)-NE [1.0 μM; (−)-arterenol hydrochloride or bitartrate; Sigma], (−)-isoproterenol hydrochloride (1.0 μM; a specific β-adrenergic agonist; Sigma), phentolamine (50 μM; an α-adrenergic antagonist; CIBA–Geigy), and DL-propranolol hydrochloride (1.0 μM; a β-adrenergic antagonist; Sigma). All drugs were readily soluble in ACSF and were applied by perfusion.

Field potential responses to stimulation of the medial and lateral PPs were recorded in the molecular and cell layers of the dentate gyrus at 15-min intervals. Stimulation current was kept constant, but pulse duration varied between 20 and 200

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Abbreviations: LTP, long-term potentiation; NE, norepinephrine; NELLP, NE-induced long-lasting potentiation; PP, perforant pathway; EPSP, excitatory postsynaptic potential; ACSF, artificial cerebrospinal fluid.

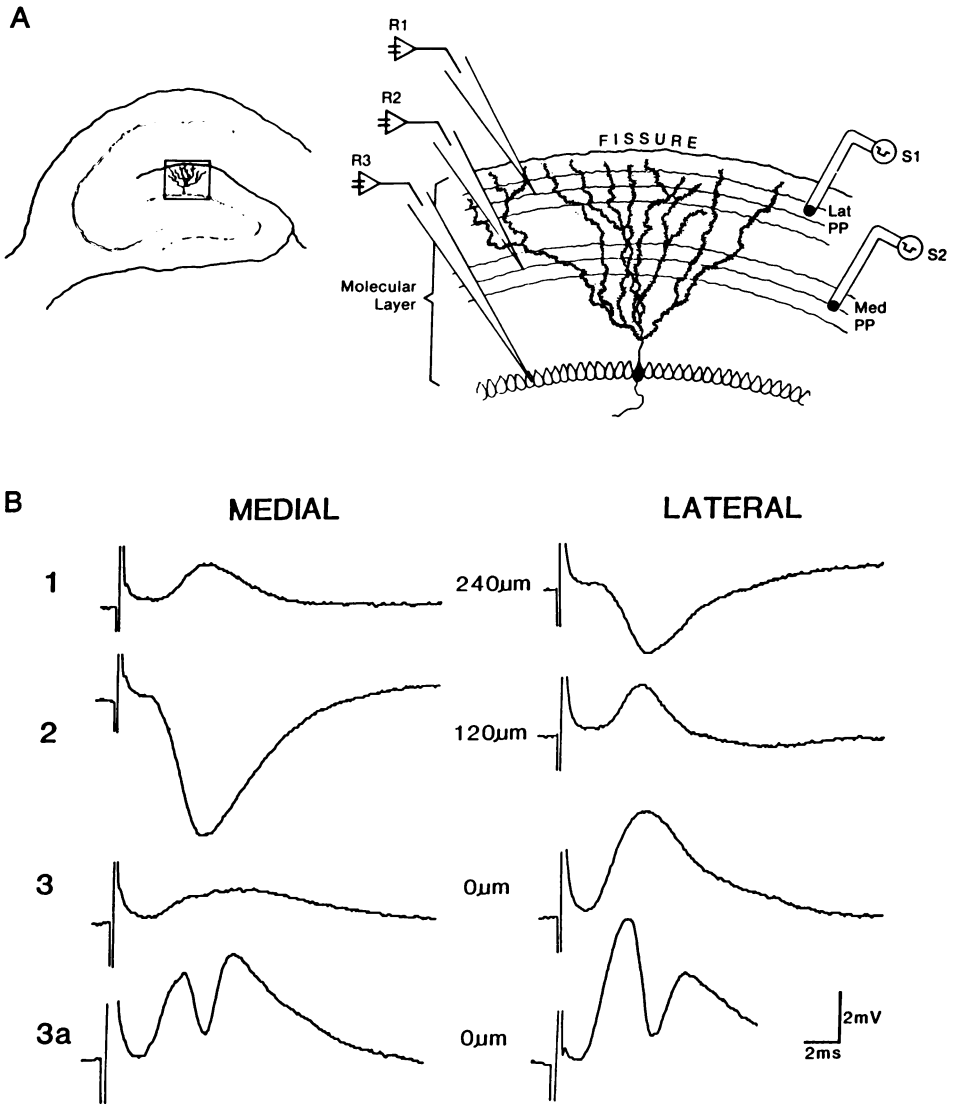

FIG. 1. (*A*) Scheme of a hippocampal slice showing the dentate gyrus and projections of the lateral and medial PPs, the respective stimulating electrodes (S1 and S2) and recording electrodes (R1, R2, and R3) in the outer molecular (R1), midmolecular (R2), and granule cell (R3) layers. The cells of origin of the medial and lateral PPs are in their respective entorhinal cortex areas (not shown, but to the right of the figure) and project to the mid- and outer molecular layers, thus forming laminae in the dentate gyrus. (*B*) Stimulation of the medial PP evokes a maximum negative EPSP (positivity is in an upward direction in these illustrations) at position 2 (the midmolecular layer), whereas stimulation of the lateral PP evokes a maximum negative EPSP at position 1 (recording site 1 in *A*, near the hippocampal fissure). At position 2 (recording site 2 in *A*), the lateral PP-evoked EPSP has reversed in polarity to a positive response. In the granule cell layer at position 3, responses to stimulation of either pathway are of the same polarity. Distance (in μm, center of figure) is measured from the cell body layer. Recordings taken at positions 1–3 (both medial and lateral PPs) were evoked with 40-μsec, 100-μA pulses. Responses at site 3a indicate that sufficiently intense stimulation (100 μsec, 100 μA) of either medial or lateral PP evokes population spikes.

μsec to elicit a range of four response amplitudes at each time point. Each experiment was terminated after a 45-min period of wash with drug-free ACSF. Long-term effects are from data taken during the ACSF wash and 30 min after termination of all drug applications. All response measurements used standard procedures (21). The maximum initial negative slope of EPSPs recorded in the outer or middle third of the molecular layer of the dentate gyrus was measured as an index of synaptic excitation and population spikes in the cell layer taken as measures of granule cell activation. Mean differences were evaluated by the paired *t* test and were considered significant if $P < 0.05$.

RESULTS

A 30-min perfusion with 1.0 μM NE plus 50 μM phentolamine produced long-lasting potentiation of responses to medial PP stimulation and a persistent decrease in amplitude of responses to activation of the lateral PP. These selective effects are illustrated in Fig. 2. Only population spikes (Fig. 2*A*) evoked by stimulation of the medial PP were potentiated (203% ± 19% of baseline; mean ± SEM; $n = 6$; $P < 0.05$), while there was a simultaneous depression (78% ± 9% of baseline; $n = 6$; $P < 0.05$) of population spikes to stimulation of the lateral PP. EPSPs recorded in the mid- and outer molecular layers were also selectively affected by a 30-min application of NE and phentolamine; the medial PP-evoked EPSP increased to 143% ± 7% of baseline ($n = 6$; $P < 0.05$) and the lateral PP-evoked EPSP decreased to 83% ± 6% of baseline ($n = 6$; $P < 0.05$). These effects are illustrated in Fig. 2*B*. Potentiation and depression of population spikes and EPSPs persisted for at least 45 min after the removal of drugs, as shown in Fig. 2.

To verify that the β-adrenergic receptor subtype was involved, the specific β-adrenergic agonist isoproterenol was

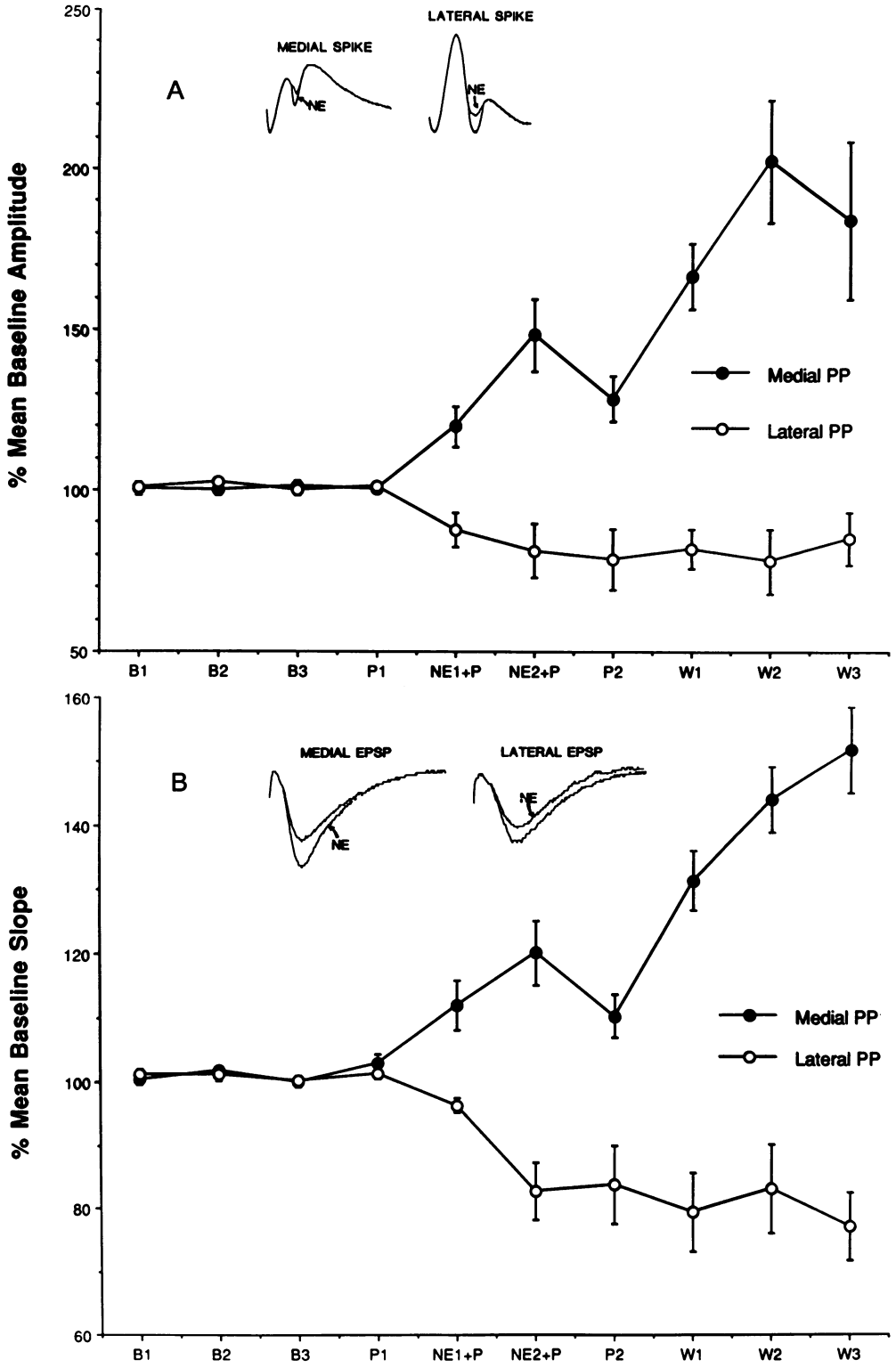

FIG. 2. Time course of effects of NE and phentolamine on medial and lateral PP-evoked responses. (*A*) Population spikes (mean and SEM; $n = 6$ slices) recorded in the cell body layer (R3 in Fig. 1*A*) of the dentate gyrus with 30-min concurrent administration of 1.0 μM NE and 50.0 μM phentolamine (NE1+P and NE2+P). (*B*) EPSPs [from another set of slices (mean ± SEM; $n = 6$)] from the outer molecular (R1 in Fig. 1*A*) and midmolecular (R2 in Fig. 1*A*) layers to stimulation of the lateral and medial PPs, respectively, with 30-min concurrent administration of 1.0 μM NE and 50 μM phentolamine. Data are from another set of slices ($n = 6$). Time points on the abscissa in *A* and *B* are 15 min apart and indicate the sequence in which data were taken and drugs administered. Baseline values were recorded for 30 min prior to administration of drugs (B1, B2, and B3). The 30-min NE and phentolamine superfusion (NE1+P and NE2+P) was bracketed by preceding and subsequent 15-min superfusions with phentolamine alone (P1 and P2). Experiments were terminated after a 45-min period of wash with drug-free artificial cerebrospinal fluid (W1, W2, and W3). Mean baseline population spike amplitudes were 1.8 ± 0.12 mV ($n = 6$) to medial PP stimulation and 2.0 ± 0.10 mV ($n = 6$) to the lateral PP; mean baseline EPSP slopes were 2.7 ± 0.09 mV/msec ($n = 6$) to medial PP activation and 2.5 ± 0.08 mV/msec ($n = 6$) to the lateral PP. These responses were ≈20% of maximum amplitudes and slopes. (*Insets*) Superimposed responses from baseline (B1) and after 30-min washout of drugs (W2), indicated by arrow and NE.

used. Persistent selective effects on PP activation were also obtained by 30 min of superfusion with isoproterenol (1.0 μM). Long-lasting potentiation of the medial PP-evoked population spike was 148% ± 11% of baseline ($n = 7$; $P < 0.05$), confirming that NELLP is a β-adrenergic receptor-mediated effect (5, 15, 21, 22). Isoproterenol also reduced the amplitude of the lateral PP-evoked population spike to 77% ± 7% of baseline ($n = 7$; $P < 0.05$) of baseline. Isoproterenol selectively potentiated medial PP-evoked EPSPs (125% ± 5% of baseline; $n = 6$; $P < 0.05$) and depressed lateral PP-evoked EPSPs (85% ± 4% of baseline; $n = 6$; $P < 0.05$). Consistent with the activation of a β-adrenergic receptor, propranolol (1.0 μM), a β-antagonist, completely blocked induction of long-lasting potentiation and depression of EPSPs and population spikes by NE and phentolamine ($n = 2$ EPSP and 2 spike) or isoproterenol ($n = 2$ EPSP and 2 spike). Neither propranolol nor phentolamine alone affected the population spikes or EPSPs evoked by stimulation of either the medial or lateral PP. Phentolamine alone, however, transiently reduced acute NE-induced potentiation of medial PP-evoked population spikes to 83% ± 4% ($n = 6$; $P < 0.05$) and EPSPs to 89% ± 2% ($n = 6$; $P < 0.05$) of the responses in the presence of the NE and phentolamine combination (Fig. 2*B*). These effects occurred during the second period of exposure to phentolamine; upon wash with drug-free ACSF, the responses recovered to, and exceeded, their preceding potentiated levels (Fig. 2).

These results indicate that activation of β-adrenergic receptors selectively modifies synaptically mediated responses in the dentate gyrus evoked by stimulation of the medial and lateral PPs. A low concentration of NE (in the presence of an α-adrenergic antagonist) or isoproterenol selectively elicited a long-lasting potentiation of EPSPs and population spikes to stimulation of the medial PP from the medial entorhinal cortex. This potentiation was accompanied by a long-lasting depression of responses evoked by stimulation of the lateral PP from the lateral entorhinal cortex. Potentiation and depression were blocked by the β-adrenergic antagonist propranolol.

DISCUSSION

Differential NE-induced effects on medial and lateral PP–granule cell synaptic responses may be important for selective information processing in anatomically and physiologically distinct pathways. Central olfactory and subcortical structures, such as the amygdala, project to the lateral entorhinal cortex, which, in turn, projects to the outer third of the granule cell dendrites. The medial entorhinal cortex receives projections from nonolfactory cortical sensory areas and projects to the middle third of the granule cell dendrites (23, 24). Furthermore, electrical stimulation of the olfactory tract or lateral entorhinal cortex evokes EPSPs limited to the apical one-third of the molecular layer (the termination of the lateral PP), whereas auditory-evoked potentials are recorded only in the middle one-third of the molecular layer (the termination of the medial PP), ≈150 μm dorsal to the cell layer (25–27). These results are consistent with the laminar array of medial and lateral PP termini in the dentate gyrus molecular layer (28). Previous work indicated that LTP of synaptically mediated granule cell responses can be induced by high-frequency stimulation of either the medial or lateral PP. When LTP is induced in one of the pathways, however, there is a concurrent depression of responses evoked by activation of the other. NELLP and LTP in either pathway may, therefore, share a common step at some level of induction. Indeed, LTP induction in field CA3 of the hippocampus is facilitated by NE and blocked by the β-antagonist propranolol as it is in the dentate gyrus (15, 16).

Noradrenergic innervation of the hippocampal formation originates in the locus coeruleus, with NE-containing terminals found throughout the dentate gyrus and concentrated in the hilar region (29). Binding studies have indicated distribution of β-adrenergic receptors throughout the dentate gyrus, but have not suggested a laminar organization that would correspond to the terminal zones of the medial and lateral PPs (30). Nevertheless, the present results suggest that the outer and middle thirds of the dentate gyrus molecular layer respond differently to NE. Perhaps future studies of the ultrastructure of noradrenergic innervation of the dentate gyrus will reveal differences, such as axoaxonic versus axodendritic synapses. Single unit recordings from the locus coeruleus of freely behaving rats have indicated an increased frequency of action potentials when the rat is in a different environment (31). This presumably results in enhanced release of NE; in fact, prestimulation of the locus coeruleus was found to potentiate population spike responses evoked by stimulation of the medial PP (32). An NE-induced enhancement of medial PP activation would presumably strengthen multimodal input to granule cells over the concurrently depressed olfactory and subcortical input via the lateral PP.

Gating of neuronal transmission by the dentate gyrus to the hippocampus, which varies with the behavioral state of the animal (33), requires noradrenergic innervation from the locus coeruleus via the dorsal noradrenergic bundle (34). Although only the medial PP was examined in those studies (33, 34), our findings suggest that, under certain conditions, pathway-selective gating may be converted by NE to an enduring modification of transmission from the entorhinal cortex. In another well studied model of neuronal plasticity, the visual cortex of monocularly deprived kittens, NE as well as other factors appear to be required for the selective strengthening of projections from the open eye and a relative decrease in responsiveness to stimulation of the deprived eye (35–37).

The physiological relevance of bath-applied NE is difficult to interpret. Nevertheless, depletion of brain NE is known to reduce the expression of LTP in the dentate gyrus. Depletion of brain NE also impairs acquisition of learning in spatial discrimination tasks, particularly when auditory or visual cues were involved; administration of NE, however, enhances acquisition of learning (38–41).

The present results suggest that NE may induce a long-lasting, pathway-specific gating and differentiation of input to the dentate gyrus from the entorhinal cortex. Selective, persistent NE-induced effects upon neocortical input to the hippocampal formation via the entorhinal cortex may underlie mechanisms of attention, learning, and memory (42). Our results are consistent with the suggestion (43, 44) that, with respect to learning and memory, NE may selectively enhance specific intracortical circuits against a background of NE-induced suppression of neuronal activity.

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