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Distinct presynaptic control of dopamine release in striosomal and matrix areas of the cat caudate nucleus

(striosome/acetylcholine/*in vitro* microsuperfusion method)

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ABSTRACT **By use of a sensitive *in vitro* microsuperfusion method, the cholinergic presynaptic control of dopamine release was investigated in a prominent striosome (areas poor in acetylcholinesterase activity) located within the core of cat caudate nucleus and also in adjacent matrix area. The spontaneous release of [^{3}H]dopamine continuously synthesized from [^{3}H]tyrosine in the matrix area was found to be twice that in the striosomal area; the spontaneous and potassium-evoked releases of [^{3}H]dopamine were calcium-dependent in both compartments. With 10^{-6} M tetrodotoxin, 5×10^{-5} M acetylcholine stimulated [^{3}H]dopamine release in both striosomal and matrix areas, effects completely antagonized by atropine (10^{-6} M), thus showing the involvement of muscarinic receptors located on dopaminergic nerve terminals. Experiments without tetrodotoxin revealed a more complex regulation of dopamine release in the matrix: (*i*) In contrast to results seen in the striosome, acetylcholine induced only a transient stimulatory effect on matrix dopamine release. (*ii*) Although 10^{-6} M atropine completely abolished the cholinergic stimulatory effect on [^{3}H]dopamine release in striosomal area, delayed and prolonged stimulation of [^{3}H]dopamine release was seen with atropine in the matrix. The latter effect was completely abolished by the nicotinic antagonist pempidine (10^{-5} M). Therefore, in the matrix, in addition to its direct (tetrodotoxin-insensitive) facilitatory action on [^{3}H]dopamine release, acetylcholine exerts two indirect (tetrodotoxin-sensitive) opposing effects: an inhibition and a stimulation of [^{3}H]dopamine release mediated by muscarinic and nicotinic receptors, respectively.**

Since the discovery of a heterogeneous localization of acetylcholinesterase (AChE) activity in cat striatum (1), several studies have verified the anatomical compartmentation of this structure. Two compartments—the striosomes and the matrix—have been distinguished in striatum of adult cat and other mammalian species, including human. The striosomes are poor in AChE activity, whereas the matrix is rich in AChE activity (1); these compartments also differ in their content of neuropeptide (2–4), biosynthetic enzymes of neurotransmitters (5, 6) or receptors (3, 7–9), and their afferent (7, 10–16) or efferent projections (2, 10, 17). Interestingly, the striosomes receive projections from the prefrontal cortex (10, 13, 14) and contain neurons that innervate the substantia nigra pars compacta (10, 15), whereas the matrix receives projections from the sensory–motor cortex (10, 12, 14) and innervates the substantia nigra pars reticulata (2, 10, 17). In addition, distinct populations of mesencephalic dopaminergic cells have been reported to innervate the striosomes and/or the matrix (15, 16).

The release of dopamine in the striatum not only depends on firing rate of mesencephalic dopamine cells but also on presynaptic regulation (18). Acetylcholine (ACh) was the first neurotransmitter shown to presynaptically stimulate dopamine release in rat striatum—its effect being mediated through muscarinic and nicotinic receptors (19). Since this observation, other neurotransmitters, including amino acids, amines, or neuropeptides, were found to modulate the spontaneous or evoked release of dopamine (18). These effects are either direct or indirect and are mediated, respectively, by receptors located either on dopamine nerve terminals or on striatal neurons or afferents. Because striosomes are rich in muscarinic receptors (8), we attempted to determine whether or not ACh exerts a distinct presynaptic control over dopamine release in the striosomes and the matrix of the cat caudate nucleus. By developing and using a sensitive *in vitro* method allowing dopamine release to be estimated in discrete zones of the caudate nucleus, we show that marked differences do exist in the presynaptic cholinergic control of dopamine release in two adjacent striosomal and matrix areas.

MATERIAL AND METHODS

Recently, using AChE activity as a marker, we made a tridimensional reconstruction of the organization of the striosomal and matrix compartments in cat caudate nucleus (17). Fig. 1*a* shows, particularly on successive frontal sections, that most striosomes, poor in AChE activity and located either in the medial anterior part of the structure or in its central core, were reproducibly localized from one animal to another. Therefore, a prominent striosomal area in the core of the caudate nucleus and a matrix area in the same anterior plane, but more lateral and dorsal, were selected for local superfusion experiments.

Male or female cats (2 to 2.5 kg) were sacrificed under halothane anesthesia. Their brains were immediately removed and cooled; two adjacent frontal slices (1 mm thick), located between anterior planes 17.7–16.7 and 16.7–15.7 [according to the atlas of Snider and Niemer (20)] were cut with a vibroslice instrument. These slices were rapidly placed in a specially designed superfusion chamber where they were continuously superfused by using a peristaltic pump (80 ml/hr) with an oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (19, 21) kept at 33°C. Microsuperfusion devices were vertically applied under light pressure on the selected striosomal and matrix areas with the aid of micromanipulators and a dissecting microscope. These microsuperfusion devices, constructed on the principle of push–pull cannulae, consisted of a guide (i.d. 1.4 mm) placed at the surface of the tissue and two inner tubes (i.d. 0.3 mm), one penetrating slightly into the slice (200 μm) to deliver the superfusion fluid and the other situated 5 mm above the tissue to collect superfusates. An oxygenated artificial cerebrospinal fluid enriched in L-[*ring*-3,5-^{3}H]tyrosine (50 Ci mmol^{-1},

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; TTX, tetrodotoxin.

New England Nuclear, 60 μCi ml^{-1}; 1 Ci = 37 GBq) kept at 33°C was delivered at 50 $\mu l \cdot min^{-1}$ to each microsuperfusion device. After a 40-min period, [^{3}H]dopamine, having been separated from [^{3}H]tyrosine and [^{3}H]metabolites by ion-exchange chromatography and alumina adsorption (21), was estimated in successive 5-min superfusate fractions.

ACh (5×10^{-5} M) was applied for 25 min, 65 min after onset of labeling. Calcium-free medium, 10^{-6} M tetrodotoxin (TTX), 10^{-6} M atropine, or 10^{-5} M pempidine were applied with [^{3}H]tyrosine during all 90-min superfusions.

At end of each experiment, identity of the superfused areas was verified by comparing the position of the inner delivering tubes (with appropriate drawings) to the mean localization of the selected striosome and matrix areas visualized by AChE staining, as determined in sections obtained at the same anteriority in six animals. Finally, in some experiments after superfusions, slices were washed with artificial cerebrospinal fluid, dried at room temperature, and then exposed for various times to LKB Ultrofilm ^{3}H.

Results obtained in a few experiments in which the tip of the inner cannula was not precisely located in the selected striosomal and matrix areas were eliminated from the analysis. At a given plane (as illustrated in Fig. 1), due to the complex shape of the striosome, the probability of a precise localization of the tip of the inner cannula within the striosomal tissue has been determined to be 65%. However, this probability is much higher when both the variability of the shape of the striosome from one plane to another and the volume of the labeled area are taken into account.

RESULTS

Spontaneous and Potassium-Evoked Release of [^{3}H]Dopamine in the Striosomal and Matrix Compartments. The spontaneous release of newly synthesized [^{3}H]dopamine could be easily estimated in both compartments; [^{3}H]dopamine released in matrix was about twice that found in striosomal area (440 ± 40 and 190 ± 15 pCi, 15 min, n = 20, corresponding to 16 and 7 times blank values, respectively).

Spontaneous release of [^{3}H]dopamine was reduced in both striosomal and matrix areas when tissues were superfused either with calcium-free medium (−70%) or with 10^{-6} M TTX (−30%). In addition, depolarization with 30 mM potassium markedly stimulated [^{3}H]dopamine release (+300%) in a calcium-dependent manner in both compartments (data not shown).

In some experiments, after superfusions the extent of diffusion of radioactive material from the superfused areas to the surrounding tissue was determined by autoradiography. Fig. 1 shows that the labeling was restricted to the superfused areas. Additionally, a vertical section shows that the intense labeling was restricted to a limited volume of tissue (≈0.6 mm^3) surrounding the inner tube of the microsuperfusion device.

Direct Presynaptic Facilitation of [^{3}H]Dopamine Release by ACh Through Muscarinic Receptors in the Striosomal Area. When applied into the striosomal area, 5×10^{-5} M ACh induced a prolonged stimulation of the [^{3}H]dopamine release (Fig. 2). This effect was antagonized by the muscarinic antagonist atropine (10^{-6} M) but remained unaffected when the nicotinic antagonist pempidine (10^{-5} M) was present (Fig. 2); both antagonists were without significant effect on the spontaneous release of [^{3}H]dopamine. Therefore, muscarinic receptors must be involved in the presynaptic facilitatory control of dopamine release in the striosomal area. These muscarinic receptors seem to be located on dopamine nerve terminals because a similar atropine-sensitive stimulatory effect of ACh on [^{3}H]dopamine release occurred with TTX (10^{-6} M) (Fig. 2). Due to the complexity of the approach no attempt was made to characterize pharmacologically the subtype(s) of muscarinic receptors involved.

Direct Presynaptic Facilitation of [^{3}H]Dopamine Release by ACh Through Muscarinic Receptors in the Matrix Area. In the matrix, 5×10^{-5} M ACh also stimulated the release of [^{3}H]dopamine, but in contrast to what had been seen in the

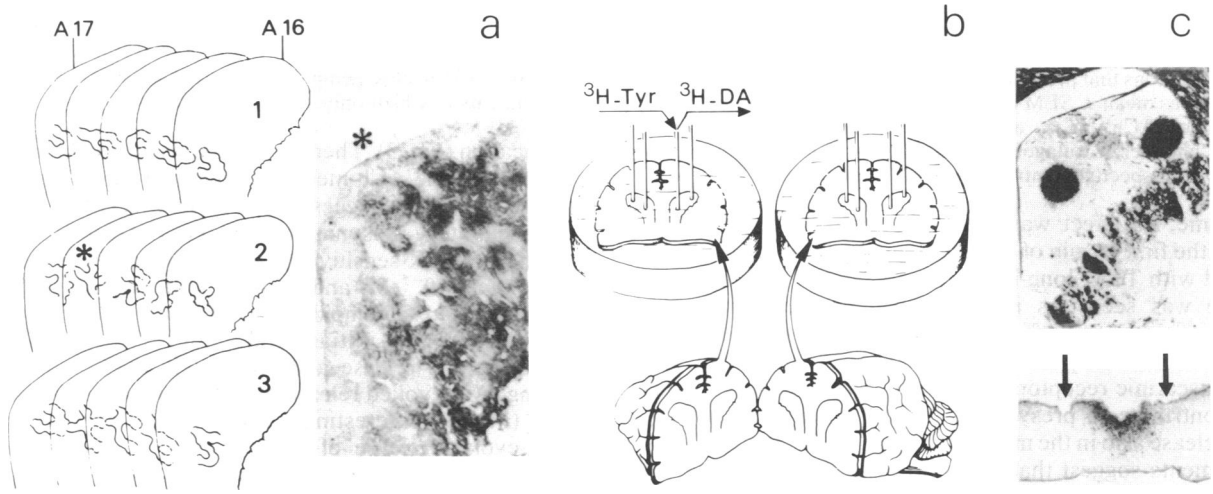

FIG. 1. Reproducibility of localization of a striosomal area and description of the *in vitro* method used to study [^{3}H]dopamine release in adjacent striosomal and matrix areas of cat caudate nucleus. (*a*) AChE staining (dark) of a frontal section (*) and schema showing the reproducibility of localization of a prominent striosomal area (AChE-poor zone) in the caudate nucleus core; these drawings were from frontal sections of three cats (nos. 1, 2, and 3) at anterior planes A17–A16, according to the Snider and Niemer atlas (20). Histochemical visualization of AChE activity was done as described (17). (*b*) Procedure used to study *in vitro* release of [^{3}H]dopamine in the prominent striosomal and adjacent matrix areas of caudate nucleus. As described, two adjacent frontal slices (1-mm thick) cut with a vibroslice instrument were superfused in a specially designed chamber. Two microsuperfusion devices were applied vertically with light pressure onto the selected striosomal and matrix areas of each caudate nucleus, and the release of [^{3}H]dopamine synthesized continuously from [^{3}H]tyrosine was estimated. (*c*) At end of experiments, extent of diffusion of radioactive material from superfused areas to surrounding tissue was determined by autoradiography (LKB Ultrofilm ^{3}H). (*Upper*) View revealing that labeling was restricted to superfused areas. (*Lower*) Intense labeling seen from a vertical section was restricted to a limited tissue volume (≈0.6 mm^3) surrounding the inner tube of the microsuperfusion device. Localization of external guide of the microsuperfusion device is represented by arrows.

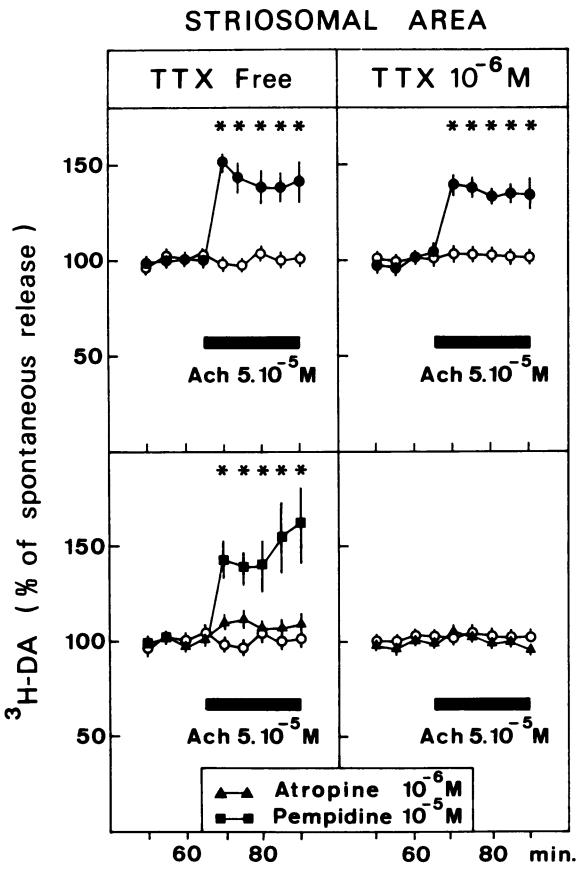

FIG. 2. ACh-evoked release of [^{3}H]dopamine (^{3}H-DA) with or without TTX in the striosomal area and the effects of cholinergic antagonists. Superfusion experiments were done as described. ACh (5×10^{-5} M) was applied for the last 25 min of the experiment, 65 min after onset of [^{3}H]tyrosine superfusion. When added, TTX (10^{-6} M), atropine (10^{-6} M), or pempidine (10^{-5} M) were included in the superfusion medium from the onset of [^{3}H]tyrosine superfusion. In each experiment, [^{3}H]dopamine recovered in successive 5-min fractions was expressed as a percentage of mean spontaneous release of [^{3}H]dopamine, determined from estimations made in the four successive fractions that preceded onset of ACh application. Results are expressed as mean ± SEM of data obtained in 8–14 experiments. ○, Control (no ACh); ●, ■, and ▲, ACh experiments done with or without cholinergic antagonists and TTX. *, $P < 0.01$ when compared with respective control values (8–10 experiments).

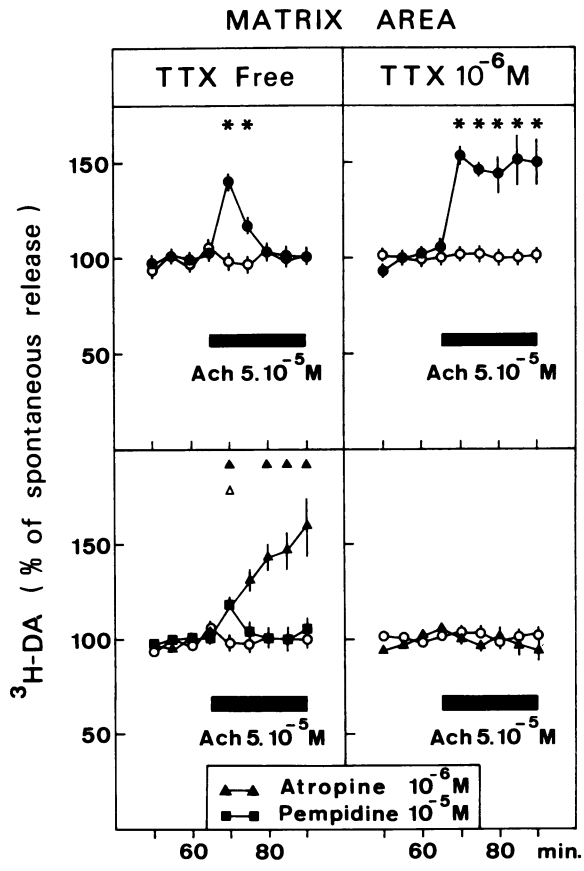

FIG. 3. ACh-evoked release of [^{3}H]dopamine (^{3}H-DA) with or without TTX in matrix area and the effects of cholinergic antagonists. Superfusion experiments, drug treatments, and expression of data are as described (*Material and Methods* and Fig. 2). (*Left lower*) For clarity of the figure, data from the experiment with ACh, 10^{-6} M atropine and 10^{-5} M pempidine are not shown because, under this condition, ACh had no effect—[^{3}H]dopamine release being identical to control (○). Results are expressed as mean ± SEM of data obtained in 7–11 experiments. *, $P < 0.01$ when compared with data obtained in 8–10 control experiments (○); ▲, $P < 0.02$ (ACh plus atropine); △, $P < 0.01$ (ACh plus pempidine) when compared with data from experiments in which only ACh was applied.

striosome, its effect was of short duration, occurring only during the first 10 min of application (Fig. 3). When ACh was applied with TTX, long-lasting stimulation of [^{3}H]dopamine release was seen. As in the striosomal area, this TTX-resistant stimulatory effect of ACh on [^{3}H]dopamine release disappeared in the presence of atropine (10^{-6} M), indicating that muscarinic receptors located on dopamine nerve terminals contribute to presynaptic facilitatory control of dopamine release also in the matrix area (Fig. 3). In addition, these experiments suggest that the short-lasting response evoked by ACh without TTX is linked to an additional indirect inhibitory effect of ACh.

Indirect Presynaptic Regulation of [^{3}H]Dopamine Release by ACh Through Muscarinic and Nicotinic Cholinergic Receptors in the Matrix. Further experiments were done in the matrix area to characterize the type of cholinergic receptor responsible for the TTX-sensitive ACh-mediated inhibitory response. Lacking TTX, atropine (10^{-6} M) decreased significantly (but not completely) the initial stimulatory ACh effect on [^{3}H]dopamine release, as well as preventing the ACh-mediated inhibitory response; under this condition, stimulation of [^{3}H]dopamine release could be seen to the end of ACh application (Fig. 3). Therefore, muscarinic receptors must be involved in the ACh-mediated inhibitory response. In addition, these results suggest that nicotinic receptors contribute to a facilitatory presynaptic control of [^{3}H]dopamine release through a TTX-sensitive process. Experiments done with pempidine (10^{-5} M) and without TTX confirmed this conclusion because pempidine significantly reduced (but not completely) the initial stimulatory effect of ACh on [^{3}H]dopamine release and completely abolished the long-lasting ACh-evoked release of [^{3}H]dopamine seen with atropine (Fig. 3). Interestingly enough, the summation of the ACh-evoked release of [^{3}H]dopamine in the presence of atropine or of pempidine during the initial superfusate fraction was equal to that found in the absence of antagonists.

DISCUSSION

An *in vitro* method has been developed to investigate local processes intervening either directly or indirectly in the presynaptic control of dopamine release in striosomal and matrix areas of cat caudate nucleus. In agreement with histochemical studies that have revealed the existence of higher levels of tyrosine hydroxylase immunoreactivity in the matrix (5), we first observed that the spontaneous release of newly synthesized [^{3}H]dopamine in the matrix was about

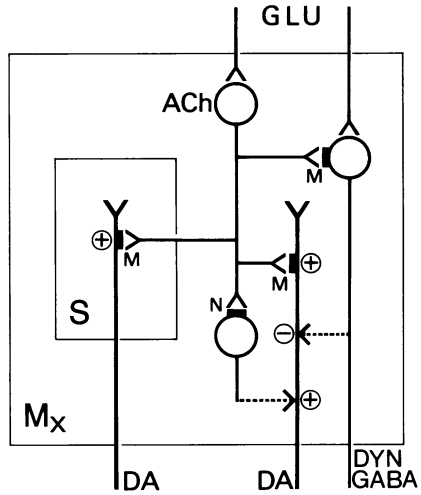

FIG. 4. Schematic role of cholinergic interneurons in presynaptic regulation of dopamine (DA) release in the striosomal and matrix compartments of cat caudate nucleus. Cholinergic interneurons are localized in the matrix, but they innervate both matrix (M_x) and striosomes (S) (6). Furthermore they receive a glutamatergic (GLU) input like efferent inhibitory neurons rich in γ-aminobutyric acid (GABA) and/or dynorphin (DYN) (22, 23). Our results show that ACh presynaptically facilitates (+) dopamine release through muscarinic (M) receptors localized on dopamine fibers in striosome and in matrix areas. In addition, in the matrix, ACh indirectly exerts, through local circuits, both facilitation (+) and inhibition (−) of dopamine release, mediated, respectively, by nicotinic (N) and muscarinic receptors localized on striatal neurons.

twice that found in the striosomal area. Confirming the validity of the approach, the spontaneous and potassium-evoked releases of [^{3}H]dopamine were found to be calcium-dependent in both compartments. In addition, as previously seen in rat striatal slices (19), TTX (10^{-6} M), a neurotoxin that prevents most indirect presynaptic regulations by blocking voltage-dependent sodium channels, slightly reduced spontaneous [^{3}H]dopamine release.

As suggested by anatomical studies, striatal cholinergic interneurons, the cell bodies of which are located in the matrix (6) and which are innervated by corticostriatal glutamatergic neurons (22, 23), seem to operate in transferring information between the matrix and striosomal compartments (4, 6) (Fig. 4). Supporting this conjecture, our results suggest that these striatal cholinergic interneurons presynaptically control dopamine release from nerve terminals of the distinct populations of dopamine cells that innervate the striosomes and the matrix.

Although the density of muscarinic receptors seems greater in striosomes than in matrix (5), ACh exerts a direct presynaptic facilitation of dopamine release in both compartments by action on muscarinic receptors located on dopamine nerve terminals (Fig. 4). Indeed, in the presence of TTX, an ACh-evoked release of [^{3}H]dopamine, selectively and completely antagonized by atropine, was seen in selected striosomal and matrix areas. These results differ slightly from those found on rat whole striatal slices, in which ACh stimulated dopamine release through a TTX-insensitive process by acting on both muscarinic and nicotinic receptors (19). It could be argued that the discrepancy is linked to the difference in the superfusion procedure used; however, more likely a species difference could be involved. In addition, we cannot completely exclude that nicotinic receptors intervene in direct control of dopamine release in other cat caudate nucleus areas.

However, cholinergic presynaptic control of dopamine release is more complex in the matrix area than in the adjacent striosome. This fact was particularly evident in experiments done without TTX. In contrast to results seen in the striosomal area, ACh alone induced only a transient stimulatory effect on [^{3}H]dopamine release in matrix, suggesting intervention of a delayed inhibitory process. In fact, in addition to the TTX-insensitive muscarinic effect of ACh, data obtained in the presence of atropine and/or pempidine alone indicate that ACh also exerts two indirect TTX-sensitive opposing effects: (*i*) inhibition of dopamine release mediated by muscarinic receptors and (*ii*) stimulation of dopamine release mediated by nicotinic receptors. Indeed, atropine antagonized the TTX-sensitive delayed inhibitory component of the ACh effect. Furthermore, the long-term stimulation of [^{3}H]dopamine release seen with the muscarinic antagonist was abolished by pempidine.

Further experiments are necessary to identify which neurons are involved in the indirect cholinergic presynaptic regulations of dopamine release in the matrix. That ACh stimulates the activity of numerous cells and the release of γ-aminobutyric acid (GABA) in cat caudate nucleus has already been reported (24, 25). In addition, several neurotransmitters other than ACh stimulate or inhibit dopamine release in striatum (18). Preliminary results suggest that the indirect inhibitory action of ACh on [^{3}H]dopamine release in matrix is mediated by the collaterals (26, 27) of the GABAergic (28) and/or dynorphin (29, 30) inhibitory neurons.

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