Short Communications

Dopamine induces sign reversal at mixed chemical–electrical synapses

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A mixed chemical/electrical synapse can generate variable output when the strength of each synaptic component is modulated. At mixed synapses of the lobster pyloric network, the chemical component is inhibitory. Without neuromodulation, the chemical component is weak or absent and the electrical component often dominates. Dopamine reverses the sign of these mixed synaptic interactions by a reduction in the strength of electrical coupling and an enhancement of chemical inhibition, including activation of silent chemical synapses. Sign reversal at mixed synapses by neuromodulators may contribute to functional rewiring of neural networks.

Mixed synapses, with anatomical specializations for both chemical and electrical synaptic transmission, are widespread in nervous systems, including higher cortical areas of mammals. The presence of both types of interactions at a synaptic junction creates the possibility of more subtle and complex neuronal computations than might occur with only one component alone. When an electrical synapse is found together with chemical synaptic inhibition, the resulting mixed synaptic interaction is the summation of the opposing excitatory electrical and inhibitory chemical components. Neuromodulators are known to alter the strength of both electrical coupling and chemical transmission. Theoretical studies suggest that differential modulation of electrical and inhibitory chemical components at a mixed synapse could dramatically change the synaptic interaction, and could even reverse the sign of the net synaptic interaction from inhibitory to excitatory or vice versa. Here, we show that the monoamine dopamine (DA) reverses the sign of mixed synapses in the crustacean stomatogastric ganglion (STG).

Our experiments were performed on mixed synapses from the pyloric network in the STG of the spiny lobster Panulirus interruptus. This tiny network is one of the best understood neural circuits, containing neurons in six major classes. All the synaptic interactions between these neurons are either electrical or inhibitory chemical. Chemical synaptic inhibition is primarily graded in the STG; the presynaptic neuron releases transmitter as a continuous function of voltage from a threshold near the resting potential. Two pairs of neurons in the pyloric network communicate via mixed synapses: the anterior burster (AB) – ventricular dilator (VD) pair and the lateral pyloric (LP) – pyloric (PY) pair. Both neuron pairs show rectifying electrical coupling and chemical inhibition mediated by glutamate. We studied the effects of DA on graded synaptic transmission between each neuronal pair under conditions where all other known synaptic inputs to these neurons were eliminated.

The stomatogastric nervous system was dissected from spiny lobsters and the STG was constantly perfused at 5 ml/min with oxygenated lobster saline (in mM: NaCl 47.9, KCl 12.8, CaCl2 13.7, Na2SO4 3.9, MgSO4 1.0, glucose 2.0, Tris base 11.1, maleic acid 5.1; pH 7.4) at 19–21°C. Standard intracellular techniques were used for current injection and voltage recording using KCl-filled (3 M, 10–20 MΩ) microelectrodes. The cell bodies of pyloric neurons were identified during rhythmic pyloric activity by: (1) matching action potentials recorded extracellularly from an ap-
propriate motor nerve root and intracellularly from the soma; (2) the timing of spike activity within the pyloric rhythm; (3) the characteristic shape of membrane potential oscillations and action potential amplitudes; and (4) the synaptic connectivity.

Synaptic isolation of each mixed synaptic pair of neurons was accomplished by: (1) blockade of inputs from other ganglia via a tetrodotoxin block on the sole input nerve to the STG; and (2) selective photoinactivation of other pyloric neurons that are presynaptic to the neurons of interest. Synaptic transmission was measured with two electrodes in each neuron to inject current and to record the membrane potential. We use arrows to define each cell: for example, AB → VD refers to an experiment in which current was injected into the presynaptic AB cell and the mixed chemical/electrical response was recorded in the postsynaptic VD cell. The postsynaptic cell was typically held at -55 mV by constant current injection. This eliminated changes in driving force for the chemical synapse caused by DA's direct effects on pyloric neuron membrane potential. Tetrodotoxin (10^{-7} M, in lobster saline) was bath applied to eliminate action potentials and allow measurements of graded synaptic transmission during 1-s steps in presynaptic membrane potential. DA (10^{-4} M, in lobster saline) was bath applied for 5 min and its effects on the strength of mixed synaptic interactions recorded at 20°C. We measured three different postsynaptic responses: net responses (calculated as the integrated area of the postsynaptic response, relative to its resting potential, during the presynaptic voltage step) peak chemical responses and peak electrical responses. Oscillatory electrical activity induced by DA in the AB neuron was blocked by low Na lobster saline (50% NaCl, replaced by N-methyl glucamine), which does not appear to affect synaptic transmission. The effects of DA were reversible within 5-10 min. Mean data are ± S.E.M.

Fig. 1 shows the effects of DA on the AB → VD mixed synapse. These two neurons are coupled via a rectifying electrical connection, with preferential negative current flow in the direction AB → VD. Thus,

![Diagram](Image)

**Fig. 1.** DA induces sign reversal at the AB → VD mixed synapse. The diode symbol indicates, by convention, the preferred direction of depolarizing current flow (VD → AB) at the rectifying electrical synapse. As shown in the figure, substantial depolarizing current does, however, flow in the opposite direction. A1: moderate AB depolarization in TTX-saline (control) causes a weak VD electrotetic response while the same AB depolarization in DA causes only VD chemical inhibition. B: integrated VD response plotted against AB depolarizing steps in control (open circles) and DA conditions (closed circles). C: peak VD electrotetic response plotted against AB depolarization in control (open circles) and DA conditions (closed circles). D: DA eliminates the VD electrotetic response to AB depolarization in saline containing 5×10^{-5} M picrotoxin to block AB chemical inhibition of VD. E: peak VD chemical inhibitory response plotted against AB polarization in control (open circles) and DA conditions (closed circles). Dashed lines in VD traces indicate the level of postsynaptic resting potential. Lines connecting the points in B, C and E were fit by eye.
the electrical coupling is greatest during hyperpolarization of AB and weaker during AB depolarization (Fig. 1C). In addition, the AB neuron chemically inhibits VD by a picROTOxin-sensitive glutamatergic synapse. The two synaptic components can be partially separated, based on differences in their rates of activation and voltage dependence. With relatively weak AB depolarization (below the threshold for detectable glutamate release), only the weak electrical component is observed in the absence of neuromodulators (Fig. 1A1, control). Stronger depolarizations evoke release of glutamate by AB, and the VD responds with a biphasic potential, composed of an initial electrical component and a delayed chemical component (Fig. 1A2, control).

In Fig. 1B, the open circles represent the net (integrated) synaptic interaction under control conditions. The net synaptic interaction is > 0 whenever the electrical component of the synapse is stronger than the chemical component during the 1-s presynaptic voltage step. Note that, under these unmodulated conditions, the electrical component of the synapse dominates over the chemical component for all AB depolarizations. The relative temporal separation of these two components allows us to plot their amplitudes independently. The amplitude of the initial peak of electrical coupling is shown in Fig. 1C (open circles) while the difference between this initial peak and the subsequent minimum represents the peak of the inhibitory chemical component (Fig. 1E, open circles).

DA (10^{-4} M) has dramatic effects on the sign of the net synaptic interaction at this mixed synapse (n = 4). During moderate AB depolarization, the depolarizing response of VD is converted to a hyperpolarizing response (Fig. 1A1, DA) and, during stronger AB depolarization, strong- and short-latency hyperpolarization of VD replaces the biphasic control response (Fig. 1A2, DA). Thus, after DA application, the VD neuron responds with hyperpolarization to all AB depolarizations (Fig. 1B, filled circles). In addition, DA weakens the electrotectic hyperpolarization of VD during AB hyperpolarization (Fig. 1B, filled circles). As a consequence of these changes, the AB → VD synaptic interaction becomes a full-wave rectifier.

We took advantage of the temporal distinction between the peak electrotectic and chemical components of the synapse to measure the effects of DA on each component. Electrical coupling between these neurons is weakened during DA application (Fig. 1C). DA reduces the slope of the VD hyperpolarizing response during AB hyperpolarization by 48 ± 9%, and the rectification of the synapse becomes extreme, with no detectable initial electrotectic response in the VD cell during AB depolarization (Fig. 1C). Similar results are observed when the electrical component of this synapse is isolated using 5 × 10^{-4} M picROTOxin to eliminate the glutamatergic chemical component. DA can eliminate isolated electrotectic responses in VD during AB depolarization (Fig. 1C,D). In addition, DA enhances the glutamatergic inhibition of VD by AB, primarily by a 14 ± 3 mV hyperpolarizing shift in the apparent voltage dependence of release (Fig. 1E). Thus, DA appears to alter the AB → VD synapse by both reducing electrical coupling and enhancing chemical inhibition. The AB neuron oscillates between ~60 and ~30 mV. In the isolated, unmodulated AB → VD synapse, electrical coupling predominates over this entire range. However, in the presence of DA, chemical inhibition now predominates for all AB voltages above ~30 mV (Fig. 1B). Thus, DA reverses the sign of the synaptic interaction during AB depolarization.

Fig. 2 illustrates the effects of DA on the mixed synapse LP → PY. These neurons are coupled by rectifying electrotectic junctions, with preferential flow of positive current from LP to PY. In addition, the two neurons inhibit each other via picROTOxin-sensitive glutamatergic synapses. In the absence of neuromodula-
tors, however, this chemical inhibition is weak or undetectable. In Fig. 2A, during LP depolarization, the PY also depolarizes, and the delayed chemical inhibition that would generate a biphasic response (as in Fig. 1A) is not observed. Weaker electrical coupling is observed during hyperpolarization of the LP, reflecting the rectifying nature of the electrical coupling (Fig. 2B). Thus, rectifying electrical coupling eliminates this synapse in the absence of neuromodulators.

DA evokes a dramatic sign reversal in these mixed synapses (n = 3). Now depolarization of the presynaptic LP cell causes a hyperpolarization in the PY cell, with no detectable initial depolarizing component over the entire range of voltages tested (Fig. 2A,B). In addition, hyperpolarization of the LP cell no longer evokes any detectable change in membrane potential in the postsynaptic PY cell (Fig. 2B). The elimination of any PY response upon hyperpolarization of the LP neuron strongly suggests that electrical coupling has been reduced or eliminated by DA. This result was verified directly by removing the glutamatergic chemical component of the mixed synapse with picrotoxin15. DA strongly reduces or eliminates (Fig. 2C) the isolated component of electrical coupling between these cells (a change reduction 75.5 ± 11%, n = 615). The appearance of a strong inhibitory synapse during LP depolarization is not simply due to the elimination of electrical coupling, however, but to the appearance of the chemical inhibition which was not detectable before addition of DA (Fig. 2A). Thus, DA converts this synapse from primarily electrotonic coupling to primarily chemical inhibition, reversing the sign of the synaptic interaction.

Fig. 3 shows similar effects of DA on the opposite synaptic interaction, PY → LP, where the PY neuron is the presynaptic neuron. Under control conditions, this isolated mixed synapse is again dominated by rectifying electrical coupling, manifested in this direction as preferential flow of hyperpolarizing current from PY to LP (Fig. 3B); little or no depolarization of LP is seen upon depolarization of PY (Fig. 3A). The chemical component of this mixed synapse is very small or entirely absent. DA activates this silent chemical synapse (n = 5); depolarization of PY now evokes release of transmitter to hyperpolarize the LP cell (Fig. 3A,B). Again, the electrical component of the mixed synapse is weakened or eliminated under these conditions, as seen by the loss of LP response during PY hyperpolarization.

These results show that the sign of the mixed chemical/electrical synaptic interaction between two neurons can be reversed by the neuromodulator DA. The sign is determined by the relative strength of the opposing electronic and chemical inhibitory synaptic components. In the three synaptic interactions we studied, DA weakened electrical coupling and strengthened chemical inhibition. In two cases (LP → PY and PY → LP), the chemical component of the synapse was silent before DA, so the activation of this synapse resulted in a true reversal of the interaction. In the case of the AB → VD, DA shifted the apparent voltage dependence of the synaptic interaction such that for all physiologically relevant AB depolarizations, the chemical component outweighed the electrotonic component, again resulting in reversal of the synaptic interaction.

When pairs of neurons in the STG are isolated from all detectable synaptic input, the graded chemical synapses appear weak (Fig. 1A) and in some cases nonexistent (Figs. 2A,3A). Application of neuromodulators, such as DA, restores this chemical component. In the intact lobster, the STG receives modulatory input from neurons in other ganglia via 100–120 axons in the stomatogastric nerve15. There are many different modulatory compounds present in these input fibers, including DA15. It is likely that these modulatory inputs to the STG are constantly active, although in varying mixtures which can enhance, diminish and even eliminate these chemical synapses15. Thus, in addition
to their sign, the very existence of synaptic interactions within the pyrlic network depends on the appropriate modulatory milieu.

The electrotonic interactions dominating the pyrlic mixed synapses before DA modulation are reminiscent of those vertebrate mixed synapses which have anatomical, electrophysiological, and biochemical synaptic specializations but physiologically express only the electrotonic component. When there is no chemical transmission, the vesicles present at mixed synapses have been suggested to play trophic or neurosecretory roles or to be involved in membrane recycling, regulation of gap junctional permeability and endo- and/or exocytosis of gap junction channels. Perhaps some of these vesicles do mediate chemical transmission but need the proper modulatory environment for their action to be expressed. There are also anatomically defined chemical synapses in vertebrate spinal cord preparations that do not appear functional. The silence of chemical synapses in these preparations may be an artifact of anesthetics that block intrinsic activity of modulatory inputs or an artifact of experimental conditions, especially in cell culture, where the normal modulatory controls on the functionality and strength of the synapse are removed. Physiological results under control conditions would thus indicate only a fraction of the computational potential of the neural networks being examined.

The synaptic plasticity we have demonstrated for the mixed pyrlic synapses may have general significance for understanding how anatomically defined networks can be modulated to produce many different neural outputs. Synaptic sign reversal and the creation of chemical synapses by neuromodulators would functionally rewire anatomical circuits, leading to very different neuronal computations than occurred before. Such dramatic changes in synaptic interactions may contribute to the re specification of neural networks which lead to changes in behavior. DA has a wide distribution in the vertebrate central nervous system and has been implicated in motor control and behavioral arousal. Recently, it was shown that DA increases the gain at a mixed vertebrate central synapse by independently enhancing electrical and chemical transmission. This demonstrates that DA can alter the synaptic output of a vertebrate mixed synapse. Perhaps the kinds of synaptic rewiring that DA induces in the pyrlic network contribute to DA's actions in vertebrate nervous systems.

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