

**Properties of network synaptic transmission in the rat spinal cord dorsal horn: actions of excitatory and inhibitory amino acids and their receptors**

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**Aim of the study:** The spinal cord dorsal horn (scDH) plays key roles in transmission, coding and processing of nociceptive primary afferent input in the pain signaling pathway through which perception of pain and nocifensive behavioral reflexes are produced. Although the knowledge associated with molecular and cellular functions of the scDH have been greatly gained through studies at the single cell level, so far the properties of network functions are poorly understood. The aim of the present study is to establish a novel spinal cord slice model to which multi-electrode array technique (Med 64, Alpha-Med Sciences Co., Ltd., Japan) was successfully applied and the properties of network synaptic transmission in the scDH were analyzed in both spacial and temporal domains.

**Materials and methods:** Acute coronal spinal slice (500  $\mu\text{m}$  thick) was obtained from urethane-anesthetized postnatal rats (day 10-11). To observe synaptic connections between lamina II and deep layers, electrical stimulation was applied locally to the superficial layers (Rexed laminae I-II) via bipolar rectangular pulse (intensity: 10-150  $\mu\text{A}$ , duration: 10-150  $\mu\text{s}$ ) to evoke field excitatory postsynaptic potential (fEPSP) in both superficial and deep layers. Electrophysiological parameters including the peak amplitude, the slope and the duration of the fEPSP were used to see whether excitatory or inhibitory amino acids (EAAs or IAAs) and their membrane receptors are involved in generation of network fEPSP. For this purpose, CNQX (10  $\mu\text{M}$ ) or AP-5 (50  $\mu\text{M}$ ) were used to antagonize glutamate non-NMDA or NMDA receptor subtypes, while bicuculline (10  $\mu\text{M}$ ) or strychnine (1  $\mu\text{M}$ ) were used to antagonize  $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) or glycine receptor. Meanwhile, exogenous GABA (2 mM) and glycine (1 mM) were also used to seek whether endogenous and exogenous GABA or glycine have the same actions.

**Results:** Multi-electrode simultaneous recordings were obtained from a total of 41 spinal cord slices. Stable recordings could be obtained from slices maintained *in vitro* for more than 12 h. The electrically-evoked fEPSPs were seen in diverse spatial regions of the dorsal horn, including both the adjacent superficial region and deep laminae, suggesting a widespread synaptic connections between intra-lamina II neurons as well as between lamina II and the deep layer neurons. All evoked fEPSPs were pulse intensity- or pulse duration-dependent in terms of the peak amplitude, the slope and the duration of the fEPSP, but the peak latency was unchanged significantly suggesting the synaptic connections are monosynaptic. Bath application of CNQX produced a dramatic depression of the evoked fEPSP in diverse regions of the spinal dorsal horn in both peak amplitude and duration in a reversible manner (Fig.1). In sharp contrast with the effect of CNQX, the evoked fEPSPs were not significantly inhibited by AP-5 implicating that the network field potentials in the scDH evoked by local lamina II stimulation were excitatory and mediated mainly by non-NMDA, but not NMDA, receptor subtype. To see whether endogenous IAAs including GABA and glycine and their receptors have modulatory actions on the evoked fEPSPs, bath applications of bicuculline or strychnine were also performed. However, unlike the effects of antagonist of non-NMDA receptor (CNQX), bicuculline or strychnine had bi-phasic effects on the fEPSPs. Among the slices being tested with CNQX or APV, network fEPSPs were enhanced or reduced by bicuculline in 73.3% (11/15) and 13.3% (2/15) slices respectively (with 15.38% unaffected), meanwhile, the fEPSPs were enhanced or reduced by strychnine in 57.14% (8/14) and 7.14% (1/14) slices respectively (with 35.71% unaffected) (Fig.2). These results suggest that predominant endogenous GABA and glycine might tonically inhibit network fEPSPs via mediation of GABA<sub>A</sub> or glycine receptors and antagonism of the two receptors should result in release of such intra-segmental inhibition. To prove

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this presumption, effects of exogenous GABA or glycine were also tested in 17 or 13 slices, respectively. Bath application of GABA produced reduction in network fEPSPs in predominant number of slices (64.70%), while enhancement was only observed in 29.40% slices (with 5.80% no effect) (Fig.3B and D). Similar results were also obtained from slices treated by bath glycine application (decrease: 61.5%, increase: 30.7%, on effect: 7.69% 1/13) (Fig.3C and E).

**Conclusions:** (1) focal stimulation of lamina II can evoke widespread monosynaptic transmission in a network of the scDH including both intra-lamina II and deep layers, suggesting that activation of lamina II by nociceptive primary afferent fibers might cause widespread transmission of the pain signaling in the scDH; (2) EAAs glutamate non-NMDA, but not NMDA, receptor subtype is involved in mediation of the nociceptive information between neurons in lamina II and deep layers of the scDH; (3) endogenous IAAs (GABA and glycine) and their ionic receptors (eg., GABA<sub>A</sub> and glycine receptors) have dual modulatory actions, but the tonic inhibition of the non-NMDA receptor-mediated fEPSPs is likely to be predominant.

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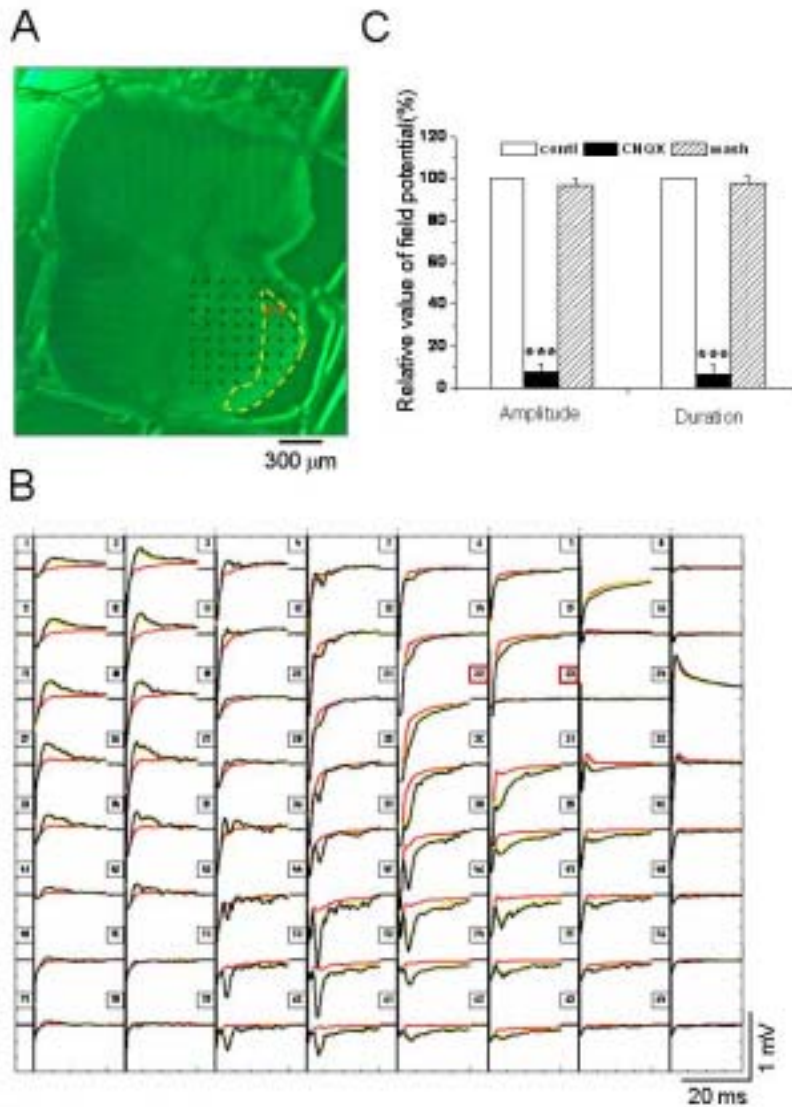


Fig.1 Multi-electrode array recordings of the network synaptic transmission in the rat spinal cord dorsal horn. A, focal electrical stimulation sites (double red dots on #22 and #23 electrodes) in the Rexed lamina II and 8 x 8 array (64 channels) recording sites located in the dorsal horn. B, window show of the network field excitatory postsynaptic potentials (fEPSPs) in both lamina II and deep layers and the effects of CNQX (10  $\mu$ M), an antagonist of glutamate non-NMDA receptor (control: black, CNQX: red, and wash: yellow). C, column graphs showing % relative value of fEPSP averaged from the three groups.

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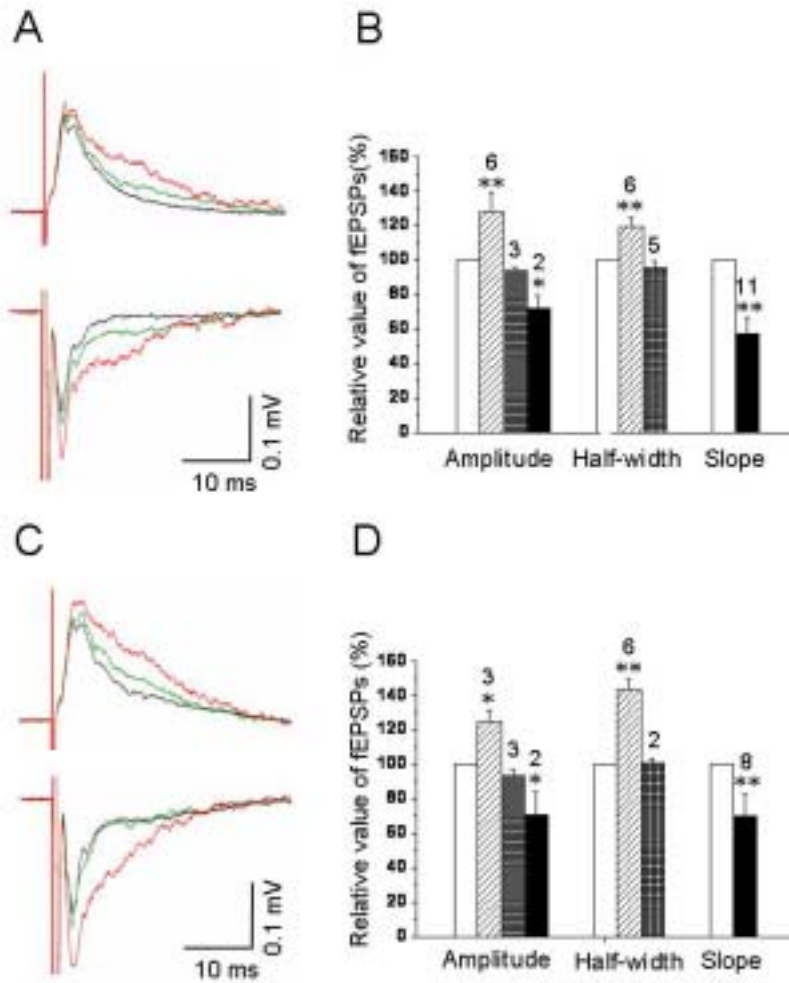


Fig.2 Enhancing effects of bicuculline (10  $\mu$ M, A and B) and strychnine (1  $\mu$ M, C and D) on the network field excitatory postsynaptic potentials (fEPSPs) in both deep layer (upper: recording site #3) and lamina II (lower: recording site #39) in another slice (control: black, drugs: red, and wash: green). The numerical above the column in B and D indicates number of slices in which the fEPSPs were enhanced (hatched), unaffected (crossed) or reduced (dark filled).

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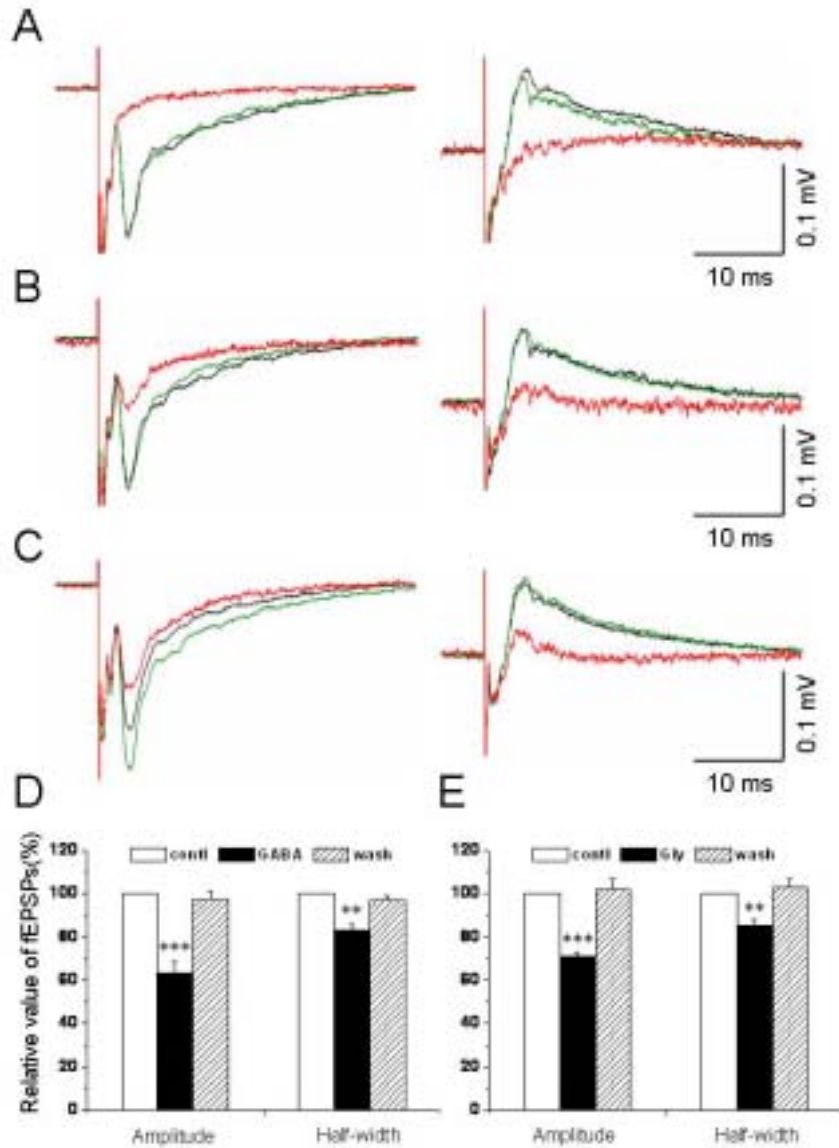


Fig.3 Predominantly inhibitory effects of exogenous GABA (B and D) and glycine (C and E) on the network field excitatory postsynaptic potentials (fEPSPs) in the deep layer (left: recording site #48; right: recording site #59) in another slice evoked by focal electrical stimulation in lamina II (site #12 and #13) (control: black, drugs: red, and wash: green). A, the same inhibitory effect of CNQX on the fEPSPs on sites #48 and #59 was observed in the same slice.