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DISTINCT MODIFICATIONS OF CONVERGENT EXCITATORY AND INHIBITORY INPUTS IN DEVELOPING OLFACTORY CIRCUITS

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Abstract—The interaction between excitatory and inhibitory inputs is critical to neuronal signal processing. However, little is known about this fundamental property, largely due to the inability to clearly isolate the respective inputs. Here we took advantage of the characteristic stereotypical architecture of synaptic connections in the main olfactory bulb, which enabled us to entirely separate excitatory and inhibitory inputs. Using paired stimulation of two glomeruli located apart at different intensities, we separately elicited excitatory and inhibitory inputs and mimicked stimulation of competing mitral cells (MCs) with different odorants. We performed dual whole-cell patch recording of evoked excitatory postsynaptic responses (EPSPs) and inhibitory postsynaptic responses (IPSPs) in current-clamp mode from two competitive MCs that are connected to the two stimulated glomeruli in slices of the main olfactory bulb in 2–3-week-old rats. We deliberately held the recorded cells at a relative hyperpolarized potential. This manipulation not only suppressed action potential generation but also excluded the possible contamination of inhibitory components in excitatory inputs. We found that in weakly activated MCs repetitive EPSP–IPSP interactions (5 Hz, 180 times) induced long-term potentiation (LTP) and long-term depression (LTD) in convergent excitatory and inhibitory inputs, respectively. Unexpectedly, these forms of plasticity depend on activity of somatic (mainly non-synaptic) NMDA receptors (NMDARs). In contrast, the same repetitive stimulation induced the LTP of excitatory inputs in strongly activated MCs (MC2) that require activity of synaptic NMDARs. These distinct forms of plasticity in the developing olfactory circuit may represent a novel rule of modification in convergent inputs that leads to decorrelation of inputs and facilitates odor discrimination.

Key words: main olfactory bulb, LTP, LTD, EPSPs, modification, mitral cells.

INTRODUCTION

Activity-dependent modifications of neurotransmission, such as long-term potentiation (LTP) and long-term depression (LTD), are proposed as cellular substrates for learning and memory (Malenka and Bear, 2004). These forms of modifications may occur in both excitatory and inhibitory inputs. Because information processing in the nervous system occurs when incoming excitatory and inhibitory synaptic signals are integrated, the interaction between these two forms of inputs exerts a significant influence in signal transmission and thus forms a fundamental aspect of neuronal computation. However, the study on the interaction between excitatory and inhibitory inputs has been hindered by the difficulty in clearly isolating these two inputs, while still keeping both inputs intact. The excitatory inputs are often intermingled and interact with inhibitory inputs at dendritic spines, dendrites and/or soma. Fortunately, the characteristic stereotyped architecture of the main olfactory bulb provides us with the opportunity to undertake this task. In this first station of central odor processing in mammals, odorants are represented by ensembles of activated glomeruli (Buck, 1996; Uchida et al., 2000; Murthy, 2011; Spors et al., 2012). The principal mitral/tufted (M/T) cells receive excitatory glutamatergic and inhibitory GABAergic inputs from olfactory sensory neurons and inhibitory interneurons, respectively. The GC inhibitory inputs to the soma via lateral dendrites are entirely separated from their excitatory olfactory sensory inputs and thus provide an attractive model for examining the regulation of excitatory responses by synaptic inhibition.

One fundamental task for the olfactory system is to decorrelate similar stimuli via competitive interaction between activated neurons (Friedrich et al., 2009). Odor discrimination can be facilitated by GABAergic lateral inhibition (Lledo et al., 2005). When different components in odor mixtures are simultaneously detected by olfactory sensory neurons (OSNs) and subsequently processed in the olfactory bulb, inhibitory inputs from granular cells

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Abbreviations: ACSF, artificial cerebrospinal fluid; D,L-AP5, D,L-(−)-2-amino-5-phosphonopentanoic acid; EPL, external plexiform layer; EPSPs, excitatory postsynaptic responses; GCs, granular cells; GL, glomerular layer; IPSPs, inhibitory postsynaptic responses; LLD, long-lasting depolarization; LTD, long-term depression; LTP, long-term potentiation; M/T, mitral/tufted; MCs, mitral cells; MOB, main olfactory bulb; NMDAR, NMDA receptor; ONL, olfactory nerve layer; OSNs, olfactory sensory neurons; PGs, periglomerular interneurons; PSP, postsynaptic potential; STDP, spike timing-dependent plasticity.

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(GCs) driven by dominant mitral cells (MCs) need to travel two more synapses to the target MCs (Fig. 1). This will cause a 5–8-ms delay of onset in inhibitory inputs. This time delay happens to be within the critical window for the induction of a form of synaptic plasticity termed as spike timing-dependent plasticity (STDP) (Caporale and Dan, 2008). STDP is typically induced by initiation of action potentials (i.e. spikes), and most STDP of excitatory inputs typically is induced under the blockade of GABAergic inhibition, and glutamergic inputs are typically blocked when studying inhibitory STDP (Nishiyama et al., 2000; Xu et al., 2008). Could repetitive interaction between these subthreshold excitatory inputs and inhibitory inputs still induce modification of these inputs? How do STDP rules function when excitation and inhibition are both functional, and how do inhibitory inputs of MCs cooperate or compete with excitatory inputs to optimize patterns of olfactory bulb outputs?

In the present study, we took advantage of the characteristic stereotype architecture of the neural circuit in the main olfactory bulb (MOB) to investigate the concurrent long-lasting modifications in excitatory and inhibitory inputs. By simultaneous dual whole-cell patch recordings from both weakly and strongly activated MCs, we detected persistent modifications in both inputs with opposite directions and different underlying mechanisms. Our results revealed a novel spike-independent form of plasticity induced by excitatory–inhibitory interaction that may lead to decorrelation of inputs and facilitate odor discrimination.

EXPERIMENTAL PROCEDURES

Animals

In this study, adult female rats with infants were housed in a room maintained at 25 ± 1 °C with a 12-h light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were available *ad libitum*. All animal procedures were made to minimize any suffering of animals used in the experiments. The protocols for animal care and use were approved by the Experimental Animal Ethics Committee at the Nanjing Medical University.

Olfactory bulb brain slice preparation. Acute olfactory bulb slices were prepared from P14-21 Sprague–Dawley rats. Rats were deeply anesthetized with ketamine (140 mg/kg ip), decapitated, and the brain was quickly placed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.23 NaH_2PO_4, 1.2 $MgSO_4$, 26 $NaHCO_3$, 10 dextrose and 2.5 $CaCl_2$.

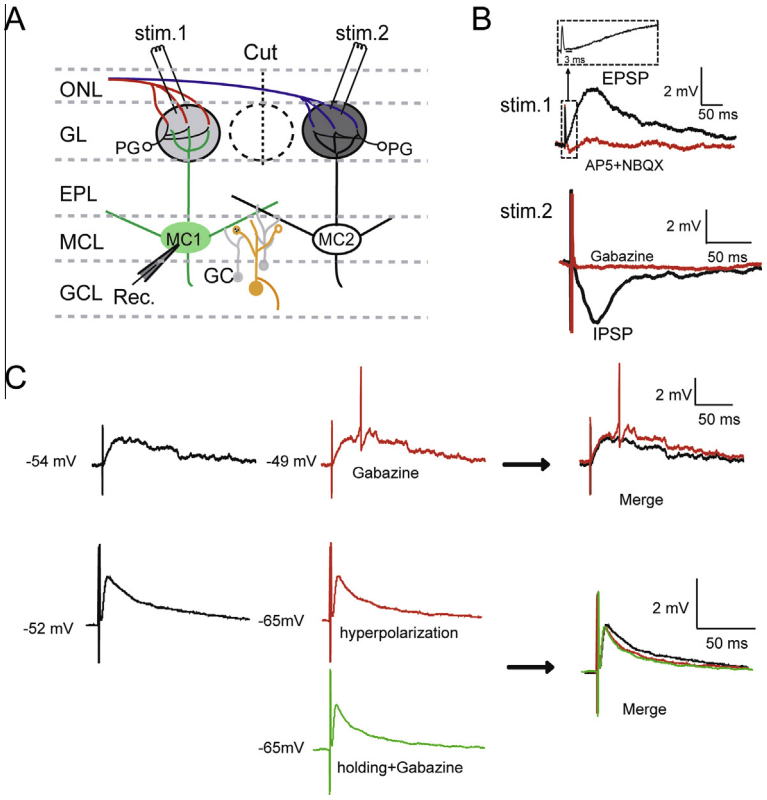

Fig. 1. Identification of excitatory and inhibitory inputs. (A) Schematic representation of the experimental strategy. The arrangement of recording (Rec.) and stimulating (Stim.) electrodes is shown. The MCs receiving weak and strong stimulations were termed as MC1 and MC2, respectively. (B) Identification of EPSP and IPSP in MC1. The evoked EPSPs or IPSPs could be totally abolished by selective antagonists of glutamatergic receptor (AP5 + NBQX) or antagonist of $GABA_A$ receptor (gabazine), respectively. (C) Sample traces showing EPSPs under various conditions. Top panel, gabazine treatment increased the excitability of recorded cells by potentiating the amplitude, shifting the membrane potential and in some cases initiating the spike. Traces before and after gabazine treatment were overlaid (right panel). Bottom panel, holding the recorded cells at a relative hyperpolarized potential shortened EPSPs and excluded the possible involvement of spike. Further gabazine treatment failed to display any obvious effect on EPSPs.