Tokai J Exp Clin Med., Vol. 24, No. 2, pp.41-51, 1999

Developmental Changes that Regulate the Activity of Locus Coeruleus Neurons

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(Received December 7, 1998; Accepted February 19, 1999)

Keywords : Brain stem, Noradrenaline, Development, Electrical coupling, Synchronized activity, Sleep-wake cycle

Locus coeruleus (LC) neurons are among the first neurons to differentiate in the brain. As a consequence, noradrenergic innervation of target areas also occurs at a relative early developmental stage. This feature has supported the idea that the LC plays an important neurotrophic role during development. In adults, the extensive projections of this pontine nucleus have been implicated in generalized behaviors sueh as sleep-wake cycle, vigilance and attentiveness to novel stimuli. So, it appears that the functional role of the LC is dependent on the stage of development.

Interestingly, LC neurons display distinctive characteristics that are also dependent on the developmental stage of the animal. In brain slice preparations from neonatal rats, the rate of spontaneous activity varies from 0.2 to 1 Hz with subthreshold oscillations of the membrane potential. These rhythmic oscillations are dependent on the spontaneous activity of the entire nucleus and their frequency is similar to the intrinsic firing rate. This activity results from electrotonic coupling between LC cells. The combination of extensive electrotonic coupling between LC neurons and the spontaneous activity of individual neurons produces synchronized electrical activity and therefore coordinated noradrenaline release in the developing central nervous system.

The frequency of spontaneous action potentials is higher and the subthreshold oscillations of membrane potential are less frequent in slices from adult animals. This developmental change suggests that the factors that drive LC cells change with age. This article will review the membrane and synaptic properties of LC neurons during different stages of development and discuss their significance on the functional role of LC.

Anatonry

The LC is a tightly packed cluster of cells in the rostral rhomboencephalic tegmentum, along the ventrolateral edge of the fourth ventricle. About 1400 to 1800 neurons have been estimated per unilateral LC in rat (Descarries and Saucier, 1972; Swanson, 1976; Goldman and Coleman, 1981; McBride *et al*., 1985). Although this is a small nucleus, it is the major source of noradrenaline in the brain and gives rise to a divergent and complex network of projections through the neuroaxis (Foote *et al*., 1983).

An interesting anatomical feature of the LC is that the processes of these neurons extend for a few hundred micrometers outside the nuclear core of LC (Cintra *et al*.; 1982; Grzanna and Molliver,1980; Shiizu and Imamoto 1970; Swanson, 1976). The vast majority of these processes ramify into two distinct, focal pericoerulear zones: (1) the pontine tegmentum medial and rostral to loeus coeruleus, termed the rostromedial pericoerulear region; and (2) a narrow region adjacent to the fourth ventricle caudomedial to locus coeruleus, designated as the caudal juxtaependymal pericoerulear region (Figure 1).

Both EM and light microscopy studies revealed that the pericoerulear processes are

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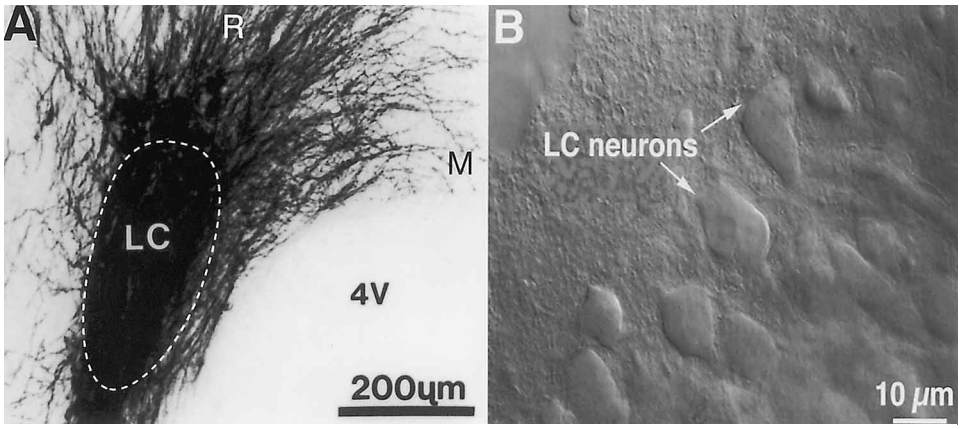

Fig. 1 ***A***: Locus coeruleus neurons stained using antisera to tyrosine hydroxylase, the rate-limiting enzyme for the catecholamine synthesis. The picture corresponds to a horizontal section from adult rat brainstem where the dash-line circle delimits the core region of the LC. Notice the dendritic processes of LC neurons extending rostromedially within the pericoerulear region (R, rostral; M, medial; 4V, fourth ventricule) [From Johnson *et al*., 1996, with permission].
B: LC neurons visualized under infrared illumination in a horizontal brainstem slice from a 7 days-old rat. With this technique, *in vitro* electrophysiological studies can be achieved on morphologically selected cell types.

dendrites, which are heavily targeted by non-catecholaminergic afferent synapses (Shipley *et al*. 1996). Thus, LC neurons have an appreciable postsynaptic surface that lies a considerable distance outside the nuclear core of LC and it is the target of dense inputs that could strongly regulate the LC activity.

Although the widespread projections of the LC have been known for almost three decades, the afferent inputs are still being studied. With the development of limited-diffusion tracers, afferents to the cell body region were found to be restricted to two major structures, the nucleus paragigantocellularis (PGi) and the nueleus prepositus hypoglossi (PrH), both located in the rostral medulla (Aston-Jones *et al*., 1986). Minor afferents to LC were found in the dorsal cap of the paraventricular hypothalamus and spinal lamina X, the caudal midbrain periaqueductal gray (PAG) and the ventromedial pericoerulear region, which may provide a local circuit (Aston-Jones *et al*., 1986; Aston-Jones *et al*., 1990).

Physiology

Action Potentials

LC neurons exhibit spontaneous activity that ranges from 0.2 to 3 Hz, depending on the age of the animal (Williams *et al*., 1984). Under voltage-clamp, a persistent inward current is recorded when LC neurons are held at the resting membrane potential (-60 to -50 mV). In the presence of tetrodotoxin, most of this inward current is blocked however spontaneous calcium dependent activity persists (Williams *et al*., 1984). After blockade of both potassium and sodium currents, high threshold calcium currents can be observed (Ingram *et al*., 1997). Thus, tetrodotoxin-sensitive sodium channels, together with calcium channels, mediate the inward current, which is presumably responsible for depolarizing the membrane potential toward threshold. As threshold for action potential generation is about -55 mV, the non-inactivating inward current could be critically important for the regulation of the spontaneous activity of LC cells.

Both sodium and calcium currents are also responsible for the depolarizing phase of the action potential that peaks at +30 mV. Figure 2A shows an action potential recorded from a LC neuron in control conditions and during the superfusion of tetrodotoxin to reveal the calcium component of the action potential. Thus, tetrodotoxin-sensitive sodium currents, together with high-threshold calcium currents are involved in the rais-

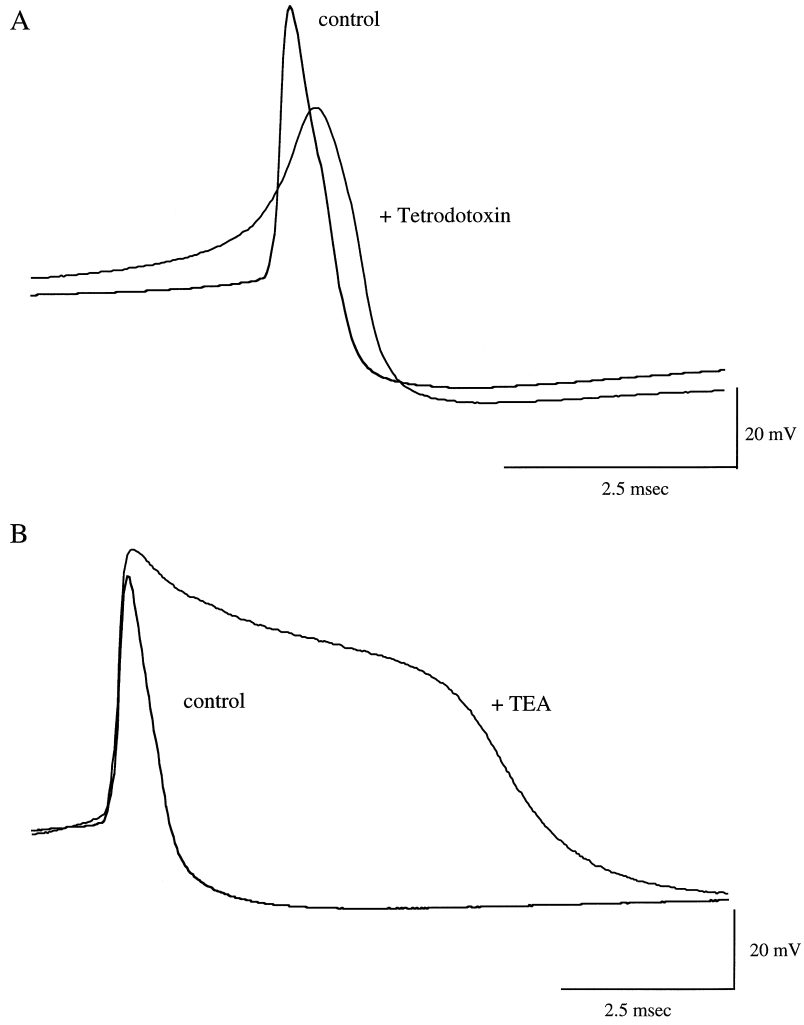

Fig. 2 Waveform of a typical action potential recorded from a LC neuron and the pharmacological sensitivity of the currents involved in it.
A: Control action potential superimposed to calcium spike recorded in the presence of tetrodotoxin (300nM).
B: Control action potential superimposed to another one recorded in the presence of the tetraethylamonium (TEA) (10mM). Notice how by inhibiting the repolarization phase, the potassium channel blocker prolongs the action potential. Intracellular recordings from a horizontal brainstem slice from a 5 days old rat. All traces are average of 10-15 episodes.

ing phase of the action potential (Williams *et al.*, 1984; Illes and Regenold, 1989). Several potassium conductances participate in the repolarization phase of the action potential (Figure 2B). Tetraethylamonium, barium and 4-aminopyridine affect these currents causing an increase in amplitude and duration of the action potential (Osmanovic and Shefner, 1993).

Following an action potential there is a large afterhyperpolarization (AHP) that peaks at −75 mV. The decay of AHP current brings the membrane potential back towards the threshold for the next action potential. Two calcium-activated potassium conductances are involved in the AHP; one of them is blocked by apamin (Osmanovic and Shefner, 1993). Increasing the duration of the action potential has a potent effect on the AHP suggesting that calcium entry dur-

ing the action potential activates the conductances involved in AHP (Osmanovic and Shefner, 1993; Aghajanian *et al.*, 1983).

Noradrenergic system in neonatal nervous system

In the developing brain, the LC appears to play a different, but not less important, role than that in the adult brain. The noradrenergic-LC system exhibits an early developmental pattern, so that it has been implicated in the control of morphologieal and functional characteristics of other CNS areas.

In the rat, LC cells undergo final differentiation at about 12 days of gestation and, by using the formaldehyde-induced fluorescence technique for catecholamines, noradrenaline was demonstrated in the LC by embryonic day 14 (Maeda and Dresse, 1969). Catecholamine fibers are observed as early as E18 in neocortex and hippocampus (Levitt and Moore, 1979; Loy and Moore, 1979) and at birth in the cerebellum (Loren *et al.*, 1976; Satoh *et al.*, 1977). Thus, noradrenergic nerve terminals are present in target areas before the formation of most synapses.

Noradrenaline has been proposed to exert neurotrophic influences, in particular during restricted developmental stages like synaptogenesis (Blue and Parnavelas, 1982; Gordon *et al.*, 1988). Morphological abnor-

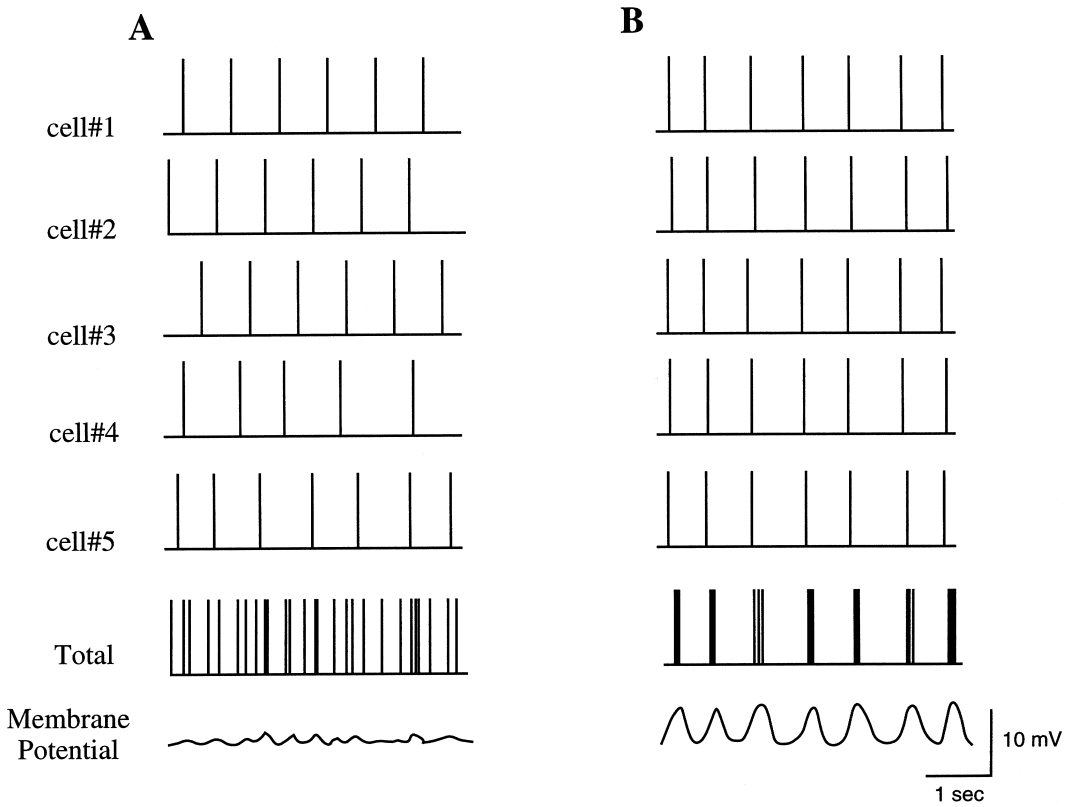

Fig. 3 A model showing how electrotonic coupling synchronizes the spontaneous activity of locus coeruleus neurons and generates subthreshold membrane potential oscillations. Schematic representation of a group of five LC neurons which displayed spontaneous activity in a non-synchronous way (***A***) and in a synchronized fashion (***B***). Strong electrotonic coupling between the cells synchronizes the spontaneous activity moving the system from condition *A*, non-synchronized, to condition *B*, synchronized activity. As a consequence of the coupling and the synchronized activity, oscillations of the membrane potential (bottom traces) are recorded only in condition *B*. Total, represents the summed activity of all five cells. This is only a model to illustrate the behavior of LC cells in neonatal animals. Calibration bars are used only as a reference.

malities (diminish in length and branching) have been reported in pyramidal cell dendrites in the cerebral cortex after neonatal treatment of rats with 6-OHDA, a drug that induces noradrenaline depletion (Felten *et al.*, 1982)

Spontaneous activity in neonates

LC neurons are spontaneously active and, in animals from birth to about 20 days old, the firing of action potentials is synchronized throughout the nucleus. In neonatal rats, LC neurons are electrically coupled forming an extensive network of interconnected cells. As a consequence of the spontaneous activity and the electrotonic coupling, rhythmic membrane potential oscillations are recorded from LC neurons in young brain slices.

Figure 3 describes schematically how the electrotonic coupling between a group of cells synchronizes the spontaneous activity and generates subthreshold oscillations of membrane potential. In both conditions (A and B), each neuron fires spontaneous action potentials due to intrinsic membrane properties. However in A, the activity of the neurons is not synchronized because of weak or null coupling between the cells. Conversely, the strong coupling displayed by the cells in condition B synchronizes the activity of the group. Action potentials produce transfer of current to each coupled cell of the network. The transferred current generates a subthreshold change in the membrane potential (the rising phase of an oscillation) which indeed increases the probability of firing an action potential at the top of each oscillation. Thus, the coupling produces synchronized action potentials that generate the inward current responsible for the depolarizing phase of the membrane oscillations. The positive feedback makes condition B very stable and causes a gradual recruitment of cells throughout the nucleus.

As the model can predict, action potentials arise almost exclusively from the peak of the subthreshold oscillations. Thus, oscillations display a frequency similar to that of spontaneous action potentials. This frequency progressively increases during the first three weeks (from < 1 to 3 Hz) (Williams and Marshall, 1987). The amplitude of the oscillations is 7 to 20 mV in young neonatal rats (one week old) and decreases in older animals (3-8 mV in 3 week old rats).

Neither the amplitude nor the frequency of the oscillations are affected by changing the membrane potential of only one cell (voltage-clamp) or by injecting current into the soma of one neuron (current clamp) suggesting that the oscillations are generated in electrically distal compartments as dendrites. Ishimatsu and Williams (1996) confirmed this assumption by showing that sectioning the brain slices rostral and caudally to the cell body region reduced or abolished the rhythmic oscillations.

Paired recordings from neonatal LC neurons and extracellular field potential recordings showed that the spontaneous oscillations are synchronous among all neurons in the nucleus (Figure 4A) (Christie *et al.*, 1989; Ishimatsu and Williams, 1996). The synchronous activity is not generated by coordinated synaptic afferents to the LC because it is not blocked by tetrodotoxin (Christie *et al.*, 1989) or the combination of neurotransmitter blockers (Ishimatsu and Williams, 1996).

Although changing the membrane potential of one single cell had no effect on the spontaneous oscillations, bath-applied drugs did. Phenylephrine and muscarine increased the frequency, while noradrenaline (through α 2-adrenoreceptors) and μ-opioid agonists reduced oscillations at low concentration and completely abolished the oscillations at high concentrations. This suggests that rhythmic synchronous oscillations are consequence of the spontaneous activity of individual neurons that were synchronized throughout the whole nucleus.

Evidence for coupling

Taking together all this evidence, it was proposed that electrotonic coupling between the LC neurons may occur at dendritic areas in neonatal rats (Christie *et al.*, 1989). As a consequence of coupling, the spontaneous activity of the entire nucleus was synchronized and indeed the noradrenergic release along their widespread projections in the CNS would also be synchronous.

Electrotonic coupling between neonatal LC neurons was demonstrated by both dye and electrical coupling (Christie *et al.*, 1989; Christie *et al.*, 1993). Using an intracellular electrode to load the cytoplasm of a single LC neuron with a low molecular weight marker, such as biocytin, multiple neurons

were stained in brain slices from rats less than one week old (Christie *et al.*, 1993). While recording simultaneously from two cells, evoking a large electrotonic potential in one cell (>30 mV) caused a smaller and slower electrotonic potential in the other (0.1 -2 mV) (Figure 4B). This experiment shows the ability to pass current from one cell to another and unequivocally establishes direct connections between the cytoplasmic compartment of LC neurons.

Treatment of the slices with the gap junction blocker carbenoxolone disrupted electrical coupling and synchronous oscillations, with little effect on other membrane properties of the cells (Travagli *et al.*, 1995; Ishimatsu and Williams, 1996). Finally, the most critical evidence for the coupling is the identification of gap junction proteins, connexins, at membrane appositions between LC dendrites (Veznedaroglu *et al.*, 1998). These electron microscopic studies constitute the final evidence that an extensive connective network of neurons form the neonatal

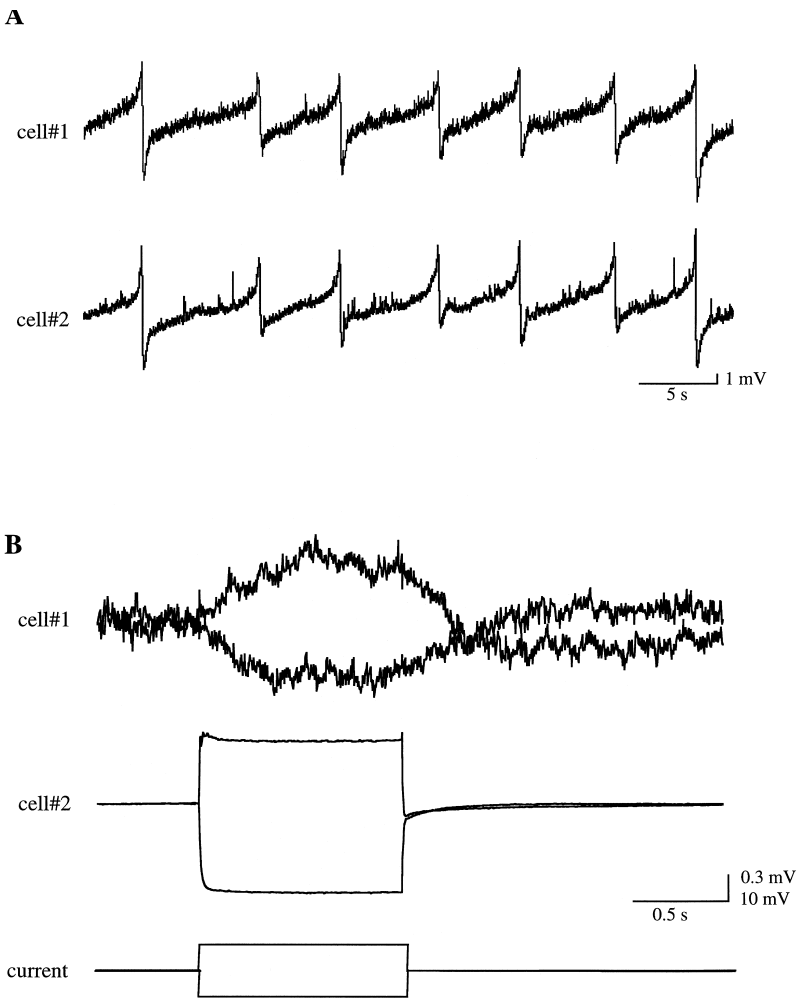

Fig. 4 Electrotonic, coupling between locus coeruleus neurons in neonates.
A: Paired intracellular recordings from LC neurons in a horizontal brain slice from a 7-days old rat. The subthreshold oscillations are synchronous among the LC cells.
B: Current transfer between LC neurons during intracellular paired recordings in the presence of sodium and calcium channel blockers to minimize participation of active currents. The lower trace shows the time course of the current (I) injection in cell #2. The other two traces are the electrotonic membrane potential change (average of 10 episodes) produced in each cell by current injection in cell #2.

LC.

Similar to what has been described in other brain regions, the degree of coupling between LC neurons declines during early postnatal periods, affecting the properties of each individual LC neuron as well as the output of the whole nucleus. The idea that the strength of electrical coupling declines with the age is supported by the reduced ability to pass current from one cell to another and by the inability to observe dye-coupling in slices from adult animals (Christie *et al*., 1989; 1993).

In adult rats, oscillations of the membrane potential are rarely observed in control solutions, however, they can be induced with bath-application of barium or tetraethylamonium (Ishimatsu and Williams, 1996). These drugs increase the input resistance of cells and also prolong the duration of action potentials (by inhibiting K currents involved in repolarization). The combination of theses two actions facilitates the appearance of synchronous oscillations in membrane potential even in slices from adult animals.

Synaptic potentials

Important information about the nature of the synaptic inputs to the LC has come from *in vivo* physiological studies in adult animals. Two major afferents to the cell body region of the LC, the nucleus paragigantocellularis (PGi) and the nucleus prepositus hypoglosi (PrH), potently influence LC activity through glutamate (Ennis and Aston-Jones, 1986a; Aston-Jones and Ennis, 1988) and GABA release (Ennis and Aston-Jones, 1989a; 1989b), respectively. Thus, PGi activation predominately excites LC neurons through non-NMDA receptors, while PrH potently inhibits LC neurons through activation of $GABA_A$ receptors in the LC.

The LC also receives inputs from a variety of neurotransmitter systems, indicating that its afferent organization is complex. For example, within the PGi there are not only glutamatergic projections to the LC, but also prominent adrenergic inputs. When glutamate receptors were blocked pharmacologically, stimulation of PGi caused a long lasting inhibition of the LC neurons through these adrenergic projections. Under these conditions, an underlying inhibition from PGi was observed in 88% of LC cells (Ennis and Aston-Jones, 1988). This inhibition was blocked by systemic administration of α_2-antagonists (Astier *et al*., 1990).

The PGi is not the only source of glutamatergie inputs to the LC. Electrical stimulation of the sciatic nerve and a variety of sensory stimuli result in a short-latency excitation that is blocked by local injection of glutamate receptor antagonists into the LC (Ennis and Aston-Jones, 1988; Chiang and Aston-Jones, 1993). There are two major aspects of the glutamate excitation: first, it is short lived and any single cell fires not more than 2 to 4 action potentials following a single stimulus, and second, it appears that many if not all LC cells respond to a given stimulus.

The dendritic arbor of LC neurons that extends out the LC core constitutes an important target for projections (Shipley *et al*., 1996). Many studies have demonstrated dense terminal fields from several brain regions to the pericoerulear region (Aston-Jones *et al*. 1990; Shipley *et al*., 1996; Wallace *et al*., 1989). The influence of these afferents to the pericoerulear region remains to be determined. Future studies on these circuits are critical for the understanding of the regulation of LC activity.

In brain slice preparations, electrical stimulation in the area of the LC evokes a series of synaptic potentials. There are three short-latency synaptic responses mediated by glutamate, GABA and glycine, followed by a slow inhibitory postsynaptic potential (IPSP) mediated by noradrenaline (Cherubini *et al*., 1988; Williams *et al*., 1991; Osmanovic and Shefner, 1990) (Figure 5). The slow IPSPs arise not only from PGi projections but also from noradrenaline release from LC cells dendrites themselves (Egan *et al*., 1983; Eunis and Aston-Jones, 1986b). The local noradrenaline mediated synaptic potential peaks after about 400 msec, has a total duration of 2 sec and is blocked by α_2-antagonists. These characteristics parallel those of the inhibition evoked by PGi stimulation *in vivo*.

In addition to these established afferents, LC neurons are strongly influenced by a wide range of neurotransmitters; modulators and peptides. Although information about the endogenous sources of these substances is still missing, studies using brain slice preparations have been very useful in deter-

mining the potential physiological actions. Vasopressin, adrenocorticotropin hormone, corticotropin releasing factor, substance P, ATP, VIP and acetylcholine increased the firing rate of LC neurons. Conversely, somatostatin, adenosine, angiotensin, glycine, galanin, NPY, nociceptin and enkephalin inhibit LC neurons (Williams, 1998).

LC spontaneous activity is potently suppressed by enkephalin through activation of μ-opioid receptors (Williams and North, 1984). Enkephalin-positive fibers are especially dense at the rostral and ventromedial pericoerulear region suggesting that distal dendrites are the target of opioid innervation (Drolet *et al.*, 1992). Retrograde tracing studies propose that at least part of the opioid inputs arise from LPGi and PrH (Drolet *et al.*, 1992).

Unfortunately, there are very few studies of LC synaptic potentials in neonatal rats. Intracellular recordings from LC neurons in slices from young rats (postnatal day 8 to 26) have shown a transient α_1-adrenoceptor responsiveness (Williams and Marshall, 1987). Bath-application of a α_1-agonist, phenylephrine, caused a membrane depolarization and increased the firing rate in about 800% of LC cells. Conversely, in LC neurons from adult rats, phenylephrine had no effect or only an inhibitory action.

It was proposed by Williams and Marshall (1987) that if α_1-adrenoceptors excitation is more pronounced in early postnatal stages, an increased activity and/or responsiveness of LC neurons would be expected during this period. This statement seems to be confirmed by one study where sensory responses of LC in fetal and neonatal rats were examined. Sensory stimuli were very effective in producing excitation of LC neurons in immature animals (Nakamura and Sakaguchi, 1990). Even in fetal LC neurons, non-noxious sensory stimuli such as a puff of air to the skin caused a vigorous long-lasting excitation. This was different from what

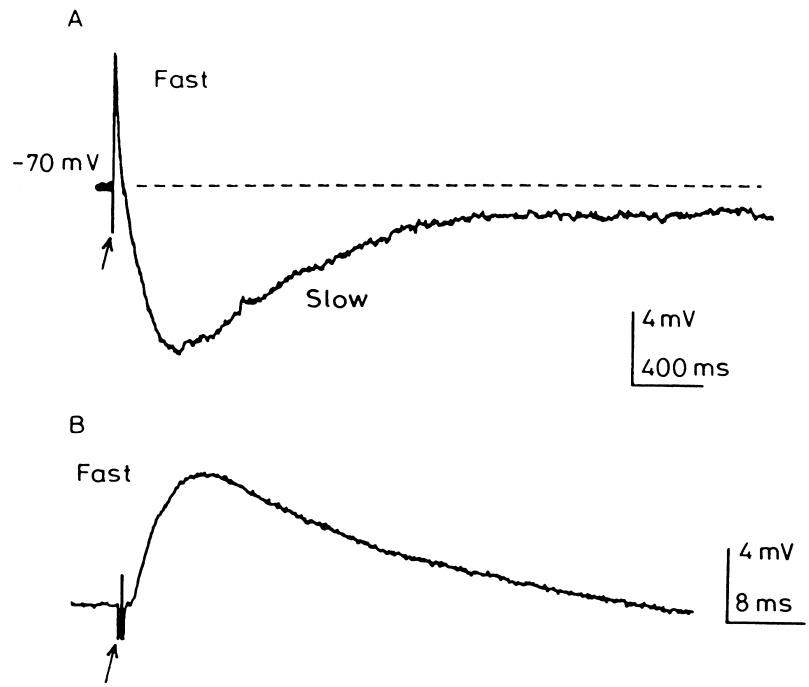

Fig. 5 Fast and slow synaptic potentials.
A: Intracellular recordings showing the relative time course of fast depolarizing and slow hyperpolarizing synaptic potentials.
B: Expanded time scale that shows the time course of the fast synaptic potential. Traces are average of 4 synaptic potentials evoked with electrical stimulation (at the arrow) at 0.1 Hz in horizontal brainstem slice from an adult rat. The membrane potential was held at -70 mV. (From Williams *et al.*, 1991).

was observed in adult rats, where sensory responses were characterized by a transient excitation followed by an inhibition.

The developmentally regulated expression of α_1-adrenoceptors could explain the change in responsiveness to adrenergic agents that have been described during early life (Reinstein and Isaacson, 1977). As previously mentioned, an important part of the noradrenaline release in the LC comes from recurrent axons and dendritic release from LC neurons themselves. This opens the possibility that the developmental regulation affects the way LC neurons autoregulate activity within the nucleus.

Functional role of the LC

In the mature LC, the two major afferents can modify the spontaneous intrinsic activity. Thus, functions previously ascribed to these medullary areas provide important insight into the function of the noradrenaline-LC system. For example, PrH has been extensively studied as a preoculomotor nucleus, important in control of eye movements (Baker, 1979; McCrea *et al*., 1979) and it also has connections to pinnae motor areas (Henkel, 1981) and to vestibular nuclei that influence head movement (Cazin *et al*., 1982; 1984). These implicate the LC with attention to environmental stimuli and orienting responses that accompany increased attentiveness to external stimuli (Foote *et al*., 1991).

The PGi area has been linked to cardiovascular, nociceptive and respiratory functions (Milner *et al*., 1988; Ross *et al*., 1984). In particulary, activation of PGi broadly increases activity in the peripheral sympathetic nervous system. These observations are important to understand why sympathetic stimuli are so effective in activating the LC (Elam *et al*., 1984; 1985) and support the idea that the noradrenergic-LC system serves as the cognitive limb of the sympathetic nervous system.

A more general idea about the function of LC in the adult brain is that this nucleus responds to a broad range of sensory stimuli (auditory, visual, somatosensory and olfactory). Interestingly, the response is larger when the stimulus is particularly meaningful and it works as an orienting response of the animal toward the stimulus (Foote *et al*., 1980; Foote *et al*., 1991).

An increased activity in the LC also correlates with the onset of cortical desynchronization, further suggesting a role of LC in cortical activation (Foote *et al*., 1991). Finally, an important role on the sleep-waking cycle has been proposed for the mature LC (Aston-Jones and Bloom, 1981; Jones, 1991). The LC activity and the noradrenergic tone in the brain are low during REM and slow-wave sleep and, during the transition between sleep and waking, sensory stimuli increase noradrenergic tone and induce cortical desynchronizations to foster waking.

The membrane properties and synaptic inputs change during development to influence the role of LC. Extensive coupling displayed by neonatal LC neurons entrains the excitability and thus the spontaneous activity of the entire nucleus. As action potentials occur at the peak of the synchronous oscillations, the frequency of noradrenaline release is similar to the frequency of oscillations throughout the nucleus. This produces highly synchronized release of noradrenaline throughout the CNS that could be critical for the trophic role of LC during development.

Although the coupling persists into adulthood, the activity of adult LC neurons is strongly influenced by the dense afferent innervation. At the mature stage, the synchronous activation of several LC neurons seems to be driven by the fact that afferent inputs are highly convergent before projecting to the LC and so a small response in a single cell is amplified because the stimulus affects a large number of cells in the nucleus.

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