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Contextualizing experience: plasticity of subcortical and cortical systems for controlling auditory perception

Doctoral Thesis in the area of Biosciences, specialty of Neuroscience, supervised by Dr. Robert Froemke and Dr. Carlos Duarte, and presented to the Department of Life Sciences of the School of Science and Technology, University of Coimbra.

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Contextualizing experience:

plasticity of subcortical and cortical systems for controlling auditory perception

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(Cover illustration by Shari Ross)

This work was conducted entirely in New York, at the Skirball Institute of Biomolecular Medicine, part of New York University School of Medicine, in the laboratory of Dr Robert C. Froemke. The work was fully was supervised by Dr Robert C. Froemke at New York University. Dr Carlos B. Duarte was the project supervisor at University of Coimbra.

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"In any field, find the strangest thing and then explore it."
- John A. Wheeler

Published work

Martins AR and Froemke RC (*under revisions for Nature Neuroscience*) Long-term modification of Noradrenergic circuitry prolongs cortical synaptic receptive field plasticity.

Froemke RC, Carcea I, Barker AJ, Yuan K, Seybold BA, Martins AR, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE (2013). Long term modification of cortical synapses improves sensory perception. **Nature Neuroscience**, 16, 79-88.

Froemke RC, Martins AR (2011). Spectrotemporal dynamics of auditory cortical synaptic receptive field plasticity. **Hearing Research, 279,** 149-61

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Abstract

The cerebral cortex is plastic and represents the world according to the significance of sensory stimuli. However, cortical networks are embedded within complex neural circuits that include neuromodulatory systems such as the noradrenergic locus coeruleus, which contextualize incoming stimuli taking into account internal state and behavioral relevance. While noradrenalin and other neuromodulators are important for cortical plasticity, it is unknown how subcortical neuromodulatory neurons themselves might respond and adapt to changes in sensory input providing differential neuromodulatory control based on past experience.

Here we examine how noradrenergic the noradrenergic locus coeruleus interacts with cortical circuits and enables plasticity.

In vivo whole-cell recordings were made from primary auditory cortical neurons, and pure tones of varying frequencies were presented to the animal to characterize auditory responses. Pairing tones with locus coeruleus activation greatly increased synaptic and spiking responses, elevating and flattening tuning curves across frequencies. After tens of minutes, tuning curve structure returned, leaving the paired frequency selectively

enhanced. Multiple recordings after a single episode of pairing demonstrated that tuning changes stabilized after 3-6 hours and persisted for the duration of our recordings, for 11+ hours. Pairing noradrenalin iontophoresis in cortex with a tone, increased synaptic and spiking responses, but was not enough for the long term duration of tuning changes to be apparent. Moreover, blocking noradrenalin receptors only during pairing temporarily blocked cortical effects, but keeping receptors blocked continuously after pairing prevented expression of long-term changes. This suggested that release of noradrenalin from locus coeruleus neurons might persist for hours post-pairing. To determine how locus coeruleus neurons might be modified by experience, in vivo whole-cell recordings were obtained from neurons within the locus itself. These recordings showed that pairing induced long-term changes in the local noradrenergic circuitry. Although previously unresponsive to sounds, after pairing, locus coeruleus neurons developed and maintained synaptic and spiking auditory responses to paired stimuli with short latency (~30-50 msec). These changes were prevented by infusion of APV (NMDA receptor blocker) in locus coeruleus, suggesting the occurrence of NMDA receptor-dependent plasticity. Preventing plasticity in the locus coeruleus reduced the duration of cortical plasticity (from 11+ hours to ~4 hours). Furthermore, to investigate the perceptual consequences of

locus coeruleus stimulation in this way, rats were implanted with locus coeruleus stimulation electrodes and trained to respond to a target tone, for a food reward. Notably locus coeruleus pairing increased detection of quiet tones, after a single pairing episode, lasting for days or even months in some animals.

Our results demonstrate that synapses within locus coeruleus are highly plastic and that plasticity of subcortical neuromodulation has a major impact on the dynamics of cortical plasticity, creating persistent changes in sensory perception.

Keywords: Neuromodulation, Locus coeruleus, Noradrenaline, primary auditory cortex

Resumo

O cortex cerebral tem a capacidade de mudar, exibindo plasticidade que lhe permite representar o mundo de acordo com a significancia dos estimulos sensoriais que o rodeiam. Contudo, as redes neuronais corticais fazem parte de circuitos que tambem incluem sistemas neuromodulatorios subcorticais, como o Locus Coeuruleus noradrenergico, que contextualizam os estimulos tendo em conta a importancia do mesmo e o estado interno do cerebro que inclui experiencias passadas. Enquanto a noradrenalina e outros nueromoduladores sao importantes para plasticidade cortical, e ainda desconhecido como os neuronios dos sistemas neuromoduladores podem tambem exibir plasticidade e mudar e adapatar-se ao ambiente, fornecendo contexto diferencial, baseando-se me experiencias passadas.

Neste trabalho examinam-se os mecanismos pelos quais o Locus Coeruleus noradrenergico interage com os circuitos corticais e permite a occurencia de plasticidade.

Gravacoes electrofisiologicas intracellulares in vivo foram feitas em neuronios do cortex auditivo primario, e "tons puros" de frequencia variada foram apresentados em ordem pseudo-

aleatoria de modo a caraterizar repostas neuronais auditivas. Apresentacao simultanea de tons com estimulacao do Locus Coeruleus aumentou substancialmente as respostas sinapticas e potenciais de accao em neuronios do cortex auditivo. Inicialmente observou-se uma robusta elevacao das curvas de resposta a estimulos auditivos, com um aumento de resposta a toda as frequencias e uma perda da preferencia de frequencia (o maximo da curva de resposta) que caracteriza estes neuronios. Apos umas dezenas de minutos, a estrutura da curva de resposta neuronal regressa, e uma preferencia de frequencia e visivel, mas o maximo da curva e agora e diferente do inicial, correspondendo agora a frequencia apresentada durante estimulacao do Locus Coeruleus. Multiplas gravacoes, apos um unico episodio de estimulacao, demonstrou que as curvas estabilizam em 3-6h e o novo maximo persiste durante a duracao da experiencia, por 11+h. Apresentar uma frequencia com iontoforese de noradrenalina no cortex, aumenta as respostas sinapticas e os potenciais de accao, mas nao e suficiente para a notavel longa duracao das mudancas nas respostas auditivas neuronais, vistas com estimulacao directa do Locus Coeruleus.

Alem disto, bloquear receptores noradrenergicos no cortex durante a estimulacao do Locus Coeruleus, apenas bloqueia os efeitos corticais temporariamente observando-se apos algumas

horas mudanca nas curvas de reposta neuronais semelhantes as observadas com estimulacao do Locus na ausencia de bloqueador. No entanto, manter os receptores continuamente bloqueados, atraves da aplicacao constante de bloqueador, apos estimulacao do Locus Coeurleus, previne totalmente a expressao das mudancas de longa duracao no cortex. Isto sugere que o Locus Coeruleus pode libertar nradrenalina constantemente durante horas apos estimulacao. Para determinar se os neuronios do Locus Coeruleus podem de facto ser modificados apos estimulacao de forma a permitir alteracoes na libertacao de noradrenalina em respota ao tom apresentado durante a estimulacao, gravacoes intracellulares neuronais foram efectuadas no proprio Locus. estas gravcoes mostraram que estimulacao e apresentacao de tom de facto induz mudancas de longa duracao no circuito noradrenergico local. Inicialmente nao respondendo a apresentaco passiva de tons, apos estimulacao os neuronios do Locus Coeruleus desenvolvem e mantem uma resposta a tons de curta latencia (~30-50 mseg). Esta mudanca foi eficazmente prevenida pela aplicacao da droga APV (bloqueador de receptores NMDA) no Locus Coeruleus antes da estimulacao, sugerindo que a emergencia de respostas no Locus e dependente de receptores NMDA. Prevenir a ocorrencia de plasticidade no Locus Coeruleus deste modo reduziu a duracao dos efeitos corticais (de 11h para ~4h).

Alem disto para investigar as consequencias comportamentais e perceptivas da estimulacao do Locus Coeruleus desta maneira, ratos foram implantados com electrodos de estimulacao no Locus, e treinados para responder a um tom alvo por uma recompensa. Estimulacao do Locus Coeruleus nestas condicoes aumentou a deteccao de tons silenciosos, tendo este efeito sido mantido, apos um unico episodio de estimulacao, por dias e ate alguns meses em certos animais.

Os nossos resultados demonstram que as sinapses no Locus Coeurleus sao altamente plasticas e que a plasticidade de sistemas neuromoduladores subcorticais tem um impacto notavel na plasticidade cortical e percepcao sensorial.

Palavras chave: Neuromodulacao, Locus coeruleus, Noradrenalina, cortex auditivo primario

Chapter I. Introduction: An Overview of Synaptic and Functional Plasticity in the Auditory Cortex

Preface

The nervous system must dynamically represent sensory information in order for animals to perceive and navigate a complex, dynamic external environment. Plasticity of neural circuits involves interaction of cortical networks with subcortical neuromodulatory centers, necessary for appropriate contextualization of incoming stimuli. This interaction of cortical and subcortical elements is essential for a dynamic and appropriate sensory representation and subsequent behavioral response. However how it is that cortical networks and subcortical neuromodulatory systems interact to create these successful sensory representations is still a subject of active study.

This introductory chapter describes the current status of the literature on how receptive field plasticity in the auditory cortex allows cortical networks to organize around salient features of the sensory environment, and how the release of

neuromodulators, focusing on noradrenaline, contributes to those changes.

Portions of this Chapter have been published in Hearing Research (Froemke R.C. and Martins A.R.O., 2011).

Auditory cortex: Primary Auditory Cortex as a Model for Changes in Cortical Networks

In the auditory system, neurons are tuned to various acoustic properties and parameters such as sound frequency and intensity. The receptive fields and tuning preferences of auditory cells to these variables can adapt and change depending on the forms of sensory experience during neonatal development and throughout life.

Primary auditory cortical neurons are generally tuned to sound frequency (Fig. I-1 A). While many neurons have a clear preference for pure tones of a specific frequency (the 'best frequency'), the tuning curve width, and overall response rates depend strongly on sound level. Neurons in adult rat primary auditory cortex, for example, exhibit broad sub- and suprathreshold tuning at high intensities, potentially spanning much of the total cochlear frequency range (Sally and Kelly,

1988; Zhang et al., 2003; Metherate et al., 2005). Regardless of bandwidth, the spiking tuning curve of a neuron (Fig. I-1 A, left) is necessarily a subset of synaptic tuning (Fig. I-1 A, right).

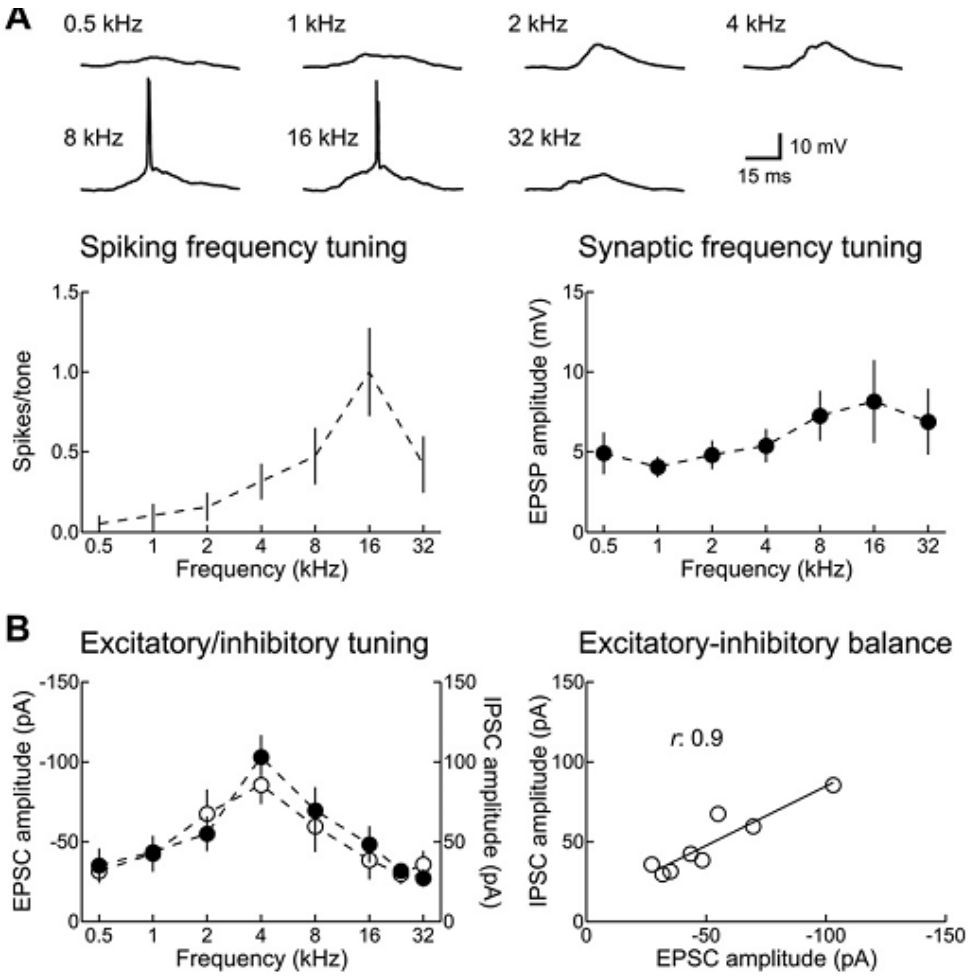

Fig I-1. Spiking and synaptic frequency tuning curves of adult rat primary auditory cortex. A, Example current-clamp recording of spikes and excitatory postsynaptic potentials (EPSPs) from an AI neuron. Top, representative tone-evoked responses. Bottom left, spiking tuning curve of this neuron. Bottom right, excitatory synaptic tuning curve of this cell. B, Example voltage-clamp recording of excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) from a different adult rat AI neuron. Left, synaptic frequency tuning. Right, correlation between peak excitatory and inhibitory responses across tone frequencies *Adapted from Froemke and Martins (2011).*

As the entire receptive field of a particular neuron can be difficult or impossible to completely characterize in high detail, here frequency tuning preference in the rodent primary auditory cortex will be used as a model for investigating the phenomenology, mechanisms, and functional consequences of synaptic receptive field plasticity in general. While these changes can be registered at the suprathreshold spiking level, using extracellular recording techniques (Bakin and Weinberger, 1996; Rasmusson D.D. and Dykes R.W., 1988; Kilgard MP. and Merzenich M.M., 1998), this plasticity is ultimately due to the adjustment of the subthreshold events that lead to spike generation and collectively determine the tuning preferences and therefore tuning curves of cortical neurons (Losonczy A. et al., 2008; Feldman D.E., 2009; Dorrn et al., 2010). To a first approximation, the organization of suprathreshold spiking tuning curves is governed by the strengths and kinetics of excitatory and inhibitory synapses, (Monier C. et al., 2003; Wehr M. and Zador A.M., 2003; Zhang L.I. et al., 2003; Dorrn A.L. et al., 2010). For this reason, data discussed throughout this thesis refer to the synaptic basis of receptive field plasticity, although bearing in mind that other factors that influence postsynaptic integration- directly or indirectly- also play important roles in shaping the tuning properties of cortical neurons (Häusser M. and Mel B., 2003).

Receptive Field Plasticity in Primary Auditory Cortex: Inhibition Regulation and the Case of Nucleus Basalis

Changes in the patterns of acoustic input, including sensory deprivation and repetitive tonal presentation, can lead to enduring changes in neuronal tonal preferences. However, the strength and endurance of cortical changes through simple manipulation of external acoustic environment is highly dependent on developmental period (Chang E.F. et al., 2005; de Villers-Sidani E. et al., 2007; Razak K.A. and Fuzessery Z.M., 2007; Insanally M.N. et al., 2009; Sanes D.H. and Bao S., 2009; Popescu M.V. and Polley,D.B., 2010). After a certain developmental stage, and especially in adult life, long-term cortical plasticity seems to depend more on stimulus history and internal state factors like arousal state and motivation. This behavioral context is conveyed by co-activation of subcortical neuromodulatory nuclei such as the cholinergic nucleus basalis (Rasmusson D.D., 2000; Weinberger N.M., 2007).and encoded as stimulus salience level in cortical networks. In primary auditory cortex, co-release of neuromodulators with tonal presentation translates into a change in tonal response preference. Regardless of developmental stage, the state of the literature to the present indicates that regulation of the

relationship between inhibitory and excitatory inputs appears to be a general mechanism by which changes in sensory experience and neuromodulatory state can remodel cortical receptive fields (Letzkus J.J. et al., 2011; Kuhlman S.J. et al., 2013; Froemke R.C. et al., 2007, Fig. I-2 ; Dorrn A.L. et al., 2010, Fig. I-3). Extracellular recording studies in vivo have shown that pairing pure tones of a specific frequency with electrical stimulation of nucleus basalis induce large, long-lasting enhancements of spontaneous and tone-evoked spiking (Bakin and Weinberger, 1996; Rasmusson and Dykes, 1988; Kilgard and Merzenich, 1998). Whole-cell recordings in vivo have revealed the mechanisms by which stimulation of the nucleus basalis neuromodulatory system activates cortical networks and enables receptive field plasticity (Froemke R.C. et al., 2007; Letzkus et al., 2011). In these, in vivo whole-cell voltage-clamp recordings were performed from neurons in adult rat primary auditory cortex (Fig. I-2). Excitatory and inhibitory synaptic frequency tuning profiles were initially measured, followed by pairing of tones of a specific non-preferred frequency with electrical stimulation of nucleus basalis. After the start of pairing, there was a large suppression of inhibitory events evoked by the paired tone, followed by a gradual enhancement of tone-evoked excitation. These changes persisted 20 minutes or more after the end of the pairing procedure.

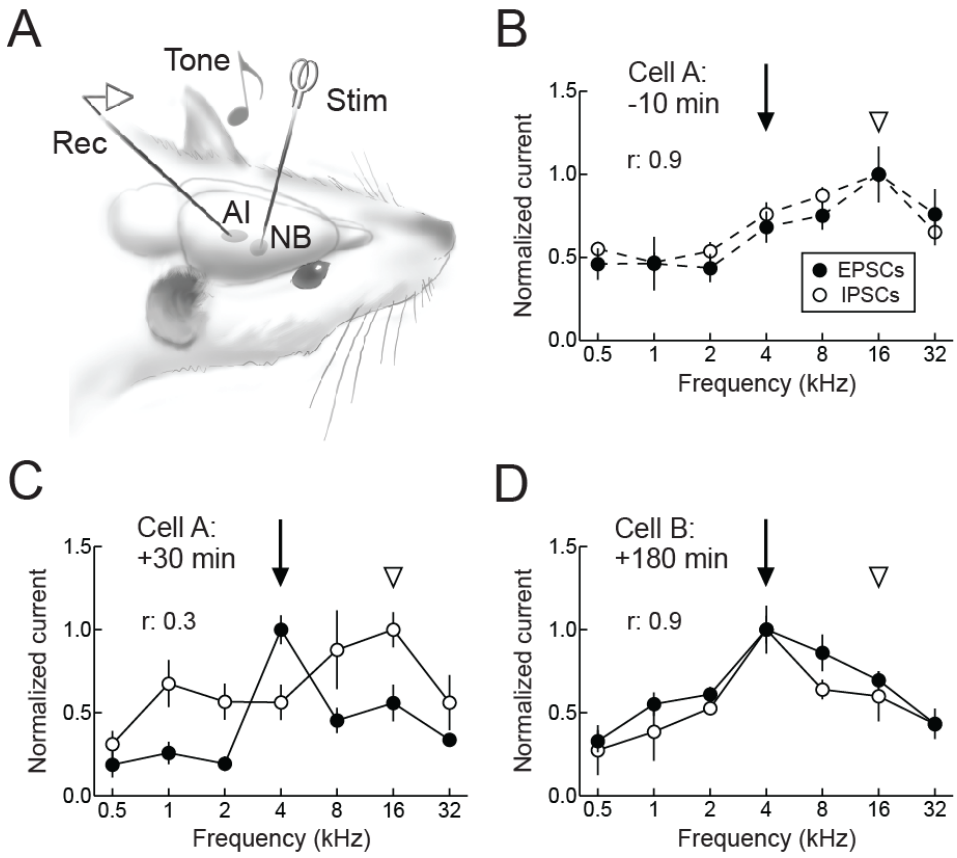

Figure I-2. Temporal dynamics of synaptic receptive field plasticity in adult rat AI. A, Experimental setup. A stimulation electrode was acutely implanted in nucleus basalis and whole-cell recordings were obtained from AI neurons. Synaptic responses to pure tones were recorded in voltage-clamp. B, Synaptic frequency tuning of excitation (filled) and inhibition (open) for the first cell 10 minutes prior to pairing 4 kHz tones (arrow) with nucleus basalis stimulation. Note the initial balance (*r*: 0.9) and co-tuning of excitation and inhibition (original best frequencies of both are 16 kHz, arrowhead). C, Frequency tuning of the same cell in B, recorded 30 minutes after NB pairing. Excitability at 4 kHz was increased due to the enhancement of excitation and suppression of inhibition after pairing, while overall excitatory-inhibitory balance was reduced (*r*: 0.3). D, Another cell from same region of AI, recorded 180 minutes after pairing. The paired frequency was the best frequency for both excitation and inhibition, and the excitatory-inhibitory balance was restored (r: 0.9). *Adapted from Froemke R.C. et al. (2007).*

Furthermore, during development, changes to inhibitory and excitatory responses seem to be underlying previous findings observed in young animals from manipulating acoustic environment, by using patterned stimulation (Fig. I-3).

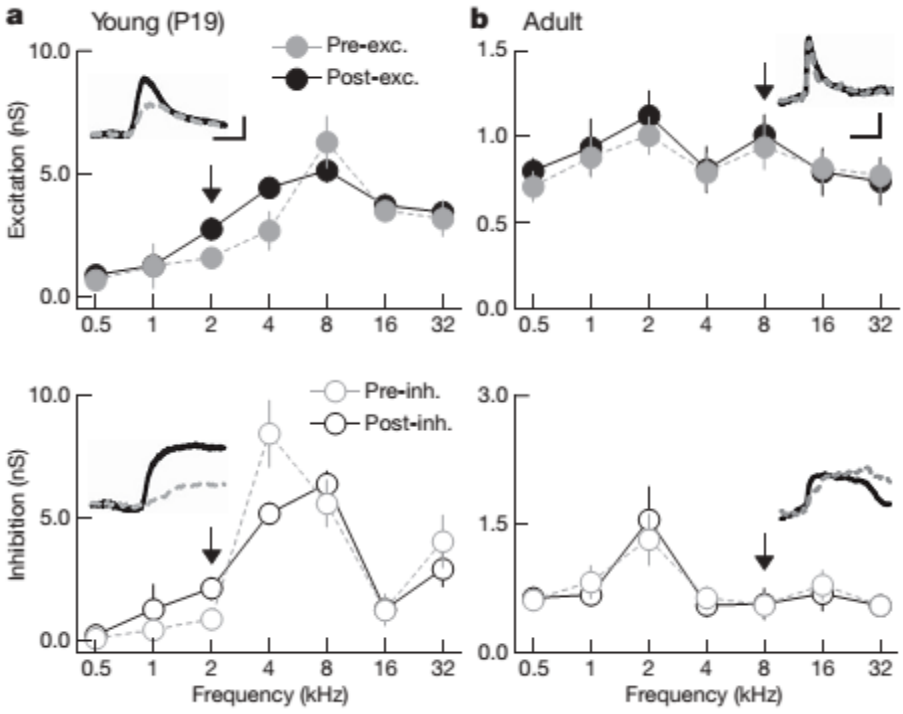

Figure I-3. Patterned stimulation improved excitatory–inhibitory coupling by coordinated synaptic modifications across multiple inputs. a, Synaptic modifications at the presented tone frequency spread to other inputs within one octave (excitation one octave from presented frequency: 21.6±6.7%, n=12,P<0.01; inhibition: 36.0±12.5%,P<0.02), but not two or more octaves away (P>0.3).**P<0.01;*P<0.05.b, After patterned stimulation, responses at original best frequency were reduced (excitation: -34.8±6.4%,n=12,P<0.0003; inhibition:-22.7±6.1%,P<0.004). *Adapted from Dorrn A.L. et al. (2010).*

Eventually, these dynamic changes in inhibitory and excitatory balance lead to wide-scale changes to many synapses throughout cortical networks. These changes are coordinated to enhance the representations of newly-significant stimuli, possibly for improved signal processing (Froemke R.C. and Carcea I. et al., 2013).

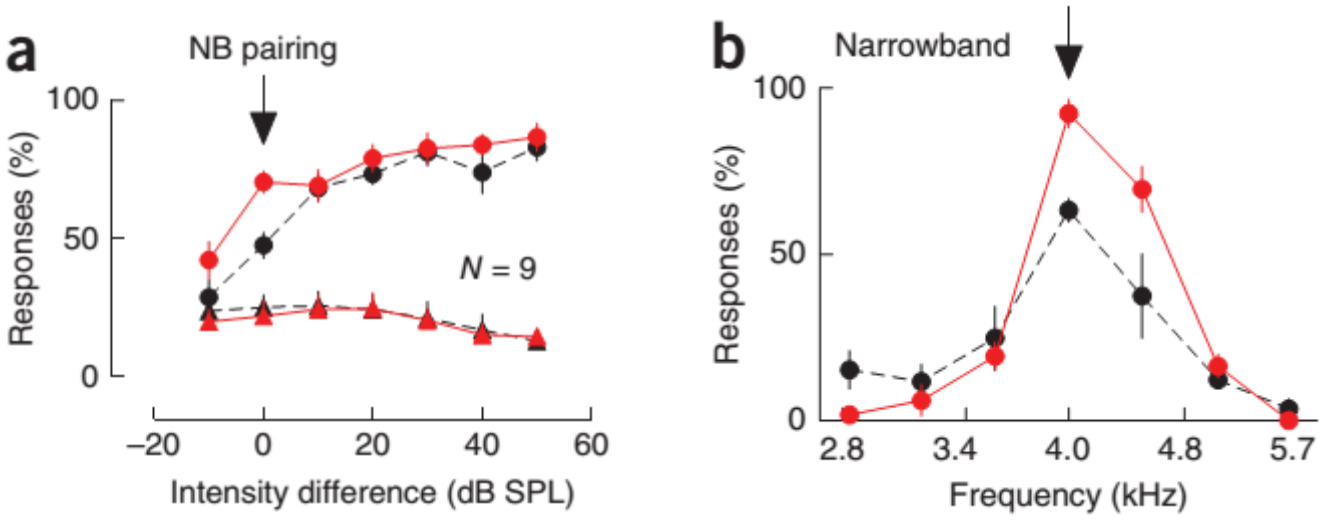

Fig. I-4. Nucleus basalis pairing improves detection and recognition. a, Changes to mean response rate across animals. Response rate increased after pairing at the paired intensity level (hits before pairing, 47.7 ± 4.7%; after, 70.6 ± 4.0%; N = 9, P < 0.002) and −10 dB SPL from paired level (before, 28.9 ± 6.4%; after, 42.4 ± 6.7%; P < 0.03) but not at higher intensities (P > 0.1). False alarms were unchanged (before, 25.2 ± 4.6%; after, 22.1 ± 5.5%; P > 0.3). b, Responses from another animal. Pairing improved narrowband performance (d' before, 1.3; after, 2.3). *Adapted from Froemke R.C. and Carcea I. et al., 2013.*

Receptive Field Plasticity in Primary Auditory Cortex: The Unsolved Case of Noradrenaline and Noradrenergic Locus Coeruleus

The behavioral context of an incoming stimulus is conveyed by activation of subcortical neuromodulatory systems, like the cholinergic nucleus basalis or the noradrenergic locus coeruleus.

Noradrenergic and cholinergic systems while different functionally, seem to have a similar developmental trajectory/origin, with Locus coeruleus and Nucleus basalis axons growing and developing closely in the brain during development and both systems taking part in mechanisms important for plasticity (Bear M.F. & Singer W, 1986).

The mechanisms underlying Nucleus basalis stimulation -induced auditory cortical plasticity have been addressed in the previous chapter, so the question arises of how it is that noradrenergic Locus coeruleus interacts with and refines auditory cortical networks.

The noradrenergic Locus coeruleus is a small nucleus of electrotonically-coupled noradrenergic neurons in the brainstem, with an extensive axonal network reaching all areas of the brain

with the notable exception of basal ganglia. Locus coeruleus dendrites group in a spatially distinct area in the brainstem, the sub coeruleus area, right bellow the cell bodies. The sub coeruleus area is a quaint place with a little studied local inhibitory network, and receives most of the top-down inputs from higher brain areas. The Locus coeruleus cell bodies receive inputs mostly from local brainstem centers (including Raphe Nucleus). The excitatory or inhibitory nature of most of the inputs onto the locus coeruleus and subcoeruleus area remains mostly unknown. Interestingly, Locus coeruleus axons have direct synapses on astrocytic endfeet, and the role of noradrenaline in the regulation of blood brain barrier and astrocytic activity is an active field of study (Foote et al., 1983a; Foote et al., 1983b; Loughlin S.E., et al, 1986; Morrison J.H. and Foote S.L., 1986; Simpson K.L. et al, 1997; Waterhouse B.D. and Berridge C.W. , 2003; Sara S.J., 2009).

The locus coeruleus is the sole source of noradrenaline in the central nervous system, but similar to other neuromodulatory systems, it also releases small amounts of other factors, like vasopressin, somatostatin, neuropeptide Y, enkephalin, neurotensin, CRH and galanin. Galanin, a hyperpolarizing neuropeptide, is of particular interest since it has been estimated that 80% of Locus coeruleus neurons colocalize galanin

and noradrenaline. However only half of the Locus coeruleus neurons that project to either cortical or sub-cortical somatosensory circuits contain galanin (Waterhouse B.D. and Berridge C.W. , 2003)

Noradrenaline is important for learning, synaptic plasticity, and modification of sensory representations and is released throughout the brain, including the auditory cortex, by locus coeruleus during periods of arousal or anxiety (Bear, M.F. & Singer, W, 1986; Berridge, C.W., 2008; Sara S.J., 2009; Carter, M.E. et al, 2009; Bush, D.E. et al., 2010; Constantinople, C.M. and Bruno, R.M, 2011). Locus coeruleus has two modes of firing: tonic (range between 0-5Hz, in mammals; full silence of 0Hz seems to happen specifically during REM sleep) and phasic (bursts in the range of 5-20Hz have been reported, in mammals). The tonic mode controls general states of arousal and sleep-wake cycle, while the phasic mode is elicited by behaviorally-relevant salient stimuli, as well as top-down decision- and response-related signals from prefrontal cortical regions (Berridge and Waterhouse 2003; Aston-Jones and Bloom 1981b, 2005a,b; Devilbiss D.M and Waterhousse B.D., 2010). It is hypothesized that locus coeruleus plays a major role in adjusting gains of cortical synapses (Aston-Jones G et al., 1994; Usher M., et al., 1999, Yu A.J. and Dayan P., 2005, Kuo,

S.P. and Trussel L.O., 2011). This occurs when locus coeruleus neurons are activated by behaviorally significant stimuli (Aston-Jones G. and Cohen J.D., 2005; Berridge, C.W., 2008; Sara S.J., 2009; Carter, M.E. et al, 2009). However, it is unknown how locus coeruleus neurons and noradrenaline interact with and refine cortical circuits.

Work done by the Edeline and Manunta (Edeline J.M. et al., 2011) has showed that stimulation of Locus coeruleus changes auditory cortical receptive fields in a very heterogeneous way (Fig. I-5). In their study, specific increases and decreases in the paired tone frequency were observed, and also increases or decreases across all tones. A general rule is drawn that the distance between the initial preferred frequency and the paired frequency is critical for the occurrence of frequency specific effects. These types of effects would happen when the paired frequency and the initial preferred frequency were spectrally very close (<1/4 of an octave).

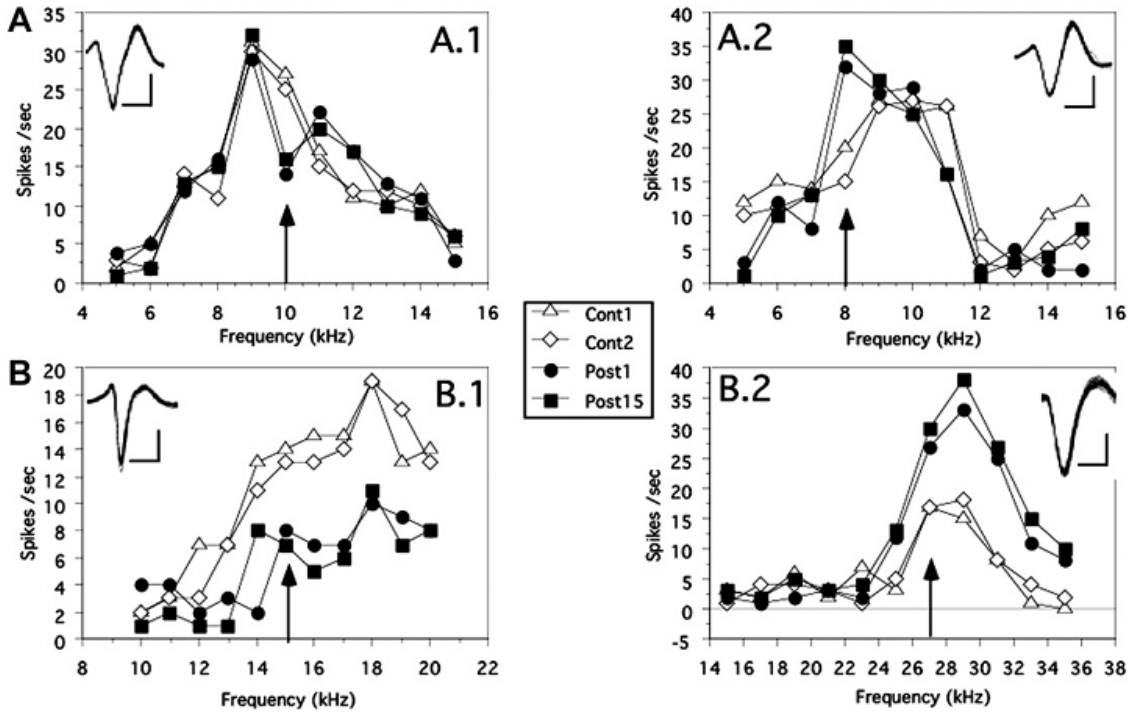

Figure I-5. Locus coeruleus pairing-induced changes in primary auditory cortical receptive fields in rat. Individual examples of frequency-specific (FS) changes and of general changes observed in the auditory cortex after pairing with locus coeruleus stimulation. Each tuning curve was tested by 20 repetitions of each frequency at 20 dB above threshold. On each graph, the arrow indicates the frequency which was paired with LC stimulation for 100 trials. Each cell was tested immediately (Post 1) and 15 min (Post 15) after the pairing protocol. For each cell, the two control tuning curves (Cont 1 and Cont 2) were quite similar. The effects presented in A correspond to a case of FS decrease (A1) and to a case of FS increase (A2) with the maximal change occurring at the paired frequency. The effects presented in B correspond to a case of general decrease (B1) and to a case of general increase (B2) with large changes occurring at almost all frequencies. *Adapted from Edeline J.M. et al, 2011.*

In this work the parameter space of stimulation is complex and the octave space of the stimulus presented is narrow. These two factors might act as confounds to extract a general rule from

the results- the octave spacing might be too narrow to see consistency, and stimulation parameters might sit at an ambiguous level, where the Locus is not robustly "tipping the scale" one way or another.

This created the need to explore unequivocal, high frequency, behaviorally-relevant LC firing in order to clarify the effects of Locus coeruleus stimulation in cortical networks.

Conclusion

This chapter described the current state of the literature in primary auditory cortex plasticity, and the contribution of neuromodulator release for synaptic cortical changes and adaptation. In the rest of this thesis, I focus on studying the noradrenergic Locus coeruleus and the neuromodulator noradrenaline in the context of auditory cortical plasticity and auditory perception in adult rats. I chose to study locus coeruleus due to the importance of noradrenergic neuromodulation in normal arousal states and behavior output, and the relatively limited body of literature on the contribution of noradrenalin to plasticity of neural networks. Despite all the work done to understand how noradrenaline and the noradrenergic locus coeruleus interact with and change auditory cortical networks,

consistency is lacking and a general rule for this interaction is still unable to be drawn.

Furthermore, it remains unexplored how neuromodulator systems, and in particular the noradrenergic Locus coeruleus, can be an active element of the circuit, adapting and providing differential modulatory control based on past experience and the needs of the current environmental context.

Chapter II. Locus Coeruleus Activation-Induced Primary Auditory Cortex Plasticity

Preface

Sensory cortical networks are plastic, their receptive fields dynamically adapting in response to changes activity or sensory experience. Receptive field plasticity in the auditory cortex, driven by environmental statistics combined with neuromodulation, allows cortical networks to re-organize around salient acoustic features of the external environment. Here I describe how stimulation of Locus coeruleus paired with a passive tonal presentation re-organizes tonal response receptive fields in primary auditory cortex.

Most of this chapter has been submitted for publication in Nature Neuroscience. The Nucleus basalis experiment, depicted in Fig. II-5 was entirely performed by Dr Robert C. Froemke.

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Introduction

Receptive fields of sensory cortical neurons are highly structured. The spatial arrangement and strength of synaptic

inputs contribute to the organization of receptive fields, which relay the perception of the external world (Hubel D.H. and Wiesel T.N., 1962; Hirsch and Martinez, 2006; Huberman et al., 2008; Ye et al., 2010). Receptive field plasticity in the auditory cortex allows cortical re-organization around salient features of the sensory environment, and depends on the patterns of electrical activity (Fregnac Y., et al, 1988; Talwar S.K., et al, 2001; Meliza C.D. and Dan Y., 2006; Jacob V., et al, 2007), sensory experience (katz L.C. and Shatz C.J., 1996; Buonomano D.V. and Merzenich M.M, 1998; Fritz J.S. egt al,2003; Feldman D.E. and Brecht M., 2005; Dan Y. and Poo M.M., 2006; de Villers-Sidani, E., et al, 2007; Li Y., et al, 2008; Dahmen J.C., et al, 2008; Dorrn A.L., et al, 201) and engagement of neuromodulatory systems such as the cholinergic Nucleus basalis and the noradrenergic Locus Coeruleus.

Previous work in vivo extracellular recording work done on cholinergic Nucleus basalis modulation of primary auditory cortical receptive fields found that the have shown that pairing pure tones of a specific frequency with electrical stimulation of nucleus basalis induces large, long-lasting enhancements of spontaneous and tone-evoked spiking (Bakin and Weinberger, 1996; Rasmusson and Dykes, 1988;Kilgard and Merzenich, 1998). But the mechanism underlying this change is spiking remianed unknown

until postdoctoral work done by my mentor, Dr Robert C. Froemke, shed light on the synaptic mechanism of this. In Froemke et al., 2007, the synaptic mechanism by which stimulation of the nucleus basalis neuromodulatory system activates cortical networks, enabling receptive field plasticity is revealed. By performing in vivo whole-cell voltage clamp recordings from layer 5 pyramidal neurons in primary auditory cortex, this work showed that Nucleus basalis stimulation and pairing with a tone, leads to a disinhibition of the response to the paired tone, by temporarily de-coupling excitatory and inhibitory tuning profiles specifically at the paired tone frequency, creating this "synaptic memory trace" of disinhibition to the tone paired (Fig. I-2, Froemke R.C. et al., 2007) .

However, how do other neuromodulatory systems interact with and change cortical networks? Is there a general rule to be drawn? Do they each have their own set of different rules to contextualize stimuli? We decided to address these questions by looking at the noradrenergic Locus coeruleus system, given the parallelisms with the cholinergic basalis, in its general role on attention and arousal, and the lack of coherent literature on the cortical effects of Locus coeruleus stimulation.

As a step towards understanding how noradrenergic Locus coeruleus stimulation affects receptive field plasticity, in

this chapter I perform *in vivo* intracellular recordings from 100+ cortical neurons in Layer 5 of primary auditory cortex, looking at early and long-term changes brought about by pairing stimulation of the Locus with passive tonal presentation.

Methods: Electrophysiological Recordings of Layer 5 Pyramidal Neurons of the Rat Primary Auditory Cortex *In Vivo*

Here I will describe the methods used for performing my electrophysiological studies *in vivo*. All procedures were approved under NYU IACUC protocols. Experiments were carried out in a sound-attenuating chamber. Female Sprague-Dawley rats 3-5 months old were anesthetized with ketamine (1.2 ml/kg) and dexmedetomidine (1.0 ml/kg). A bipolar stimulation electrode was implanted in the right locus coeruleus using stereotaxic coordinates (from lambda, in mm: 3.6 posterior, 1.2 lateral, 5.6-6 ventral). Location was verified during procedures by measuring responses to noxious stimuli (tail pinch, Fig. II-1a) and other electrophysiological criteria (spontaneous rates, Fig. II-1d), and afterwards using histological methods (Fig. II-1b,c). To determine the effective activation radius of locus coeruleus stimulation in Figure II-1e, local field potentials (LFPs) were recorded with a tungsten electrode (0.5-1 MΩ)

lowered to 5500-6000 µm below the cerebellar surface. Several penetrations were made at different distances from the stimulation electrode (100-2000 µm). LFPs were digitized at 20 kHz and bandpass filtered between 1-100 Hz.

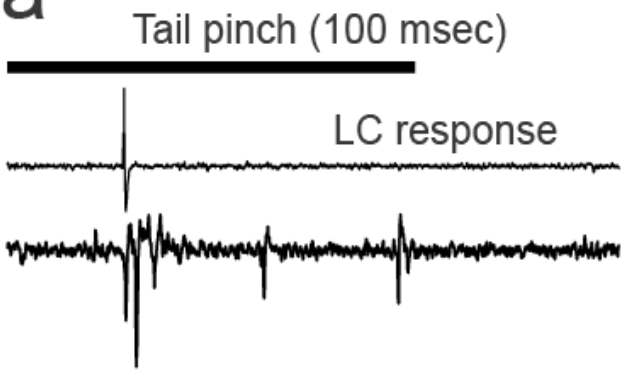
A craniotomy was performed over the right temporal lobe and the right auditory cortex was exposed. Pure tones (10-80 dB SPL, 0.5-32 kHz, 50 msec, 3 msec cosine on/offramps) were delivered in pseudo-random sequence at 0.5-1 Hz. AI location was determined by mapping multiunit responses 500-700 µm below the surface using tungsten electrodes. I*n vivo* whole-cell recordings from AI neurons were made with a Multiclamp 700B amplifier (Molecular Devices). For current-clamp recordings, patch pipettes (5-9 MΩ) contained (in mM): 135 K-Gluconate, 5 NaCl, 5 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, pH 7.3. For voltage-clamp recordings, pipettes contained: 125 Cs-gluconate, 5 TEACl, 4 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, 0.5 EGTA, 3.5 QX-314, 2 CsCl, pH 7.2. In some cases, 1% biocytin (Sigma) was added to the internal solution for post-hoc recovery of recorded neurons (Fig II-1d). Recordings from AI neurons were obtained from cells located 400-1200 µm below the pial surface. Resting potential of AI neurons: −62.6±11.7 mV; Rs: 21.9±12.2 M· ; Ri: 106.0±55.3 MΩ. Data were excluded if Rs changed >30% or Ri changed >50% from values measured during baseline (as locus

coeruleus stimulation modestly but significantly increased Ri, in AI current-clamp recordings from 126.6±68.4 MΩto 135.6±75.1 MΩ, n=21, $p<0.03$). Data were filtered at 5 kHz, digitized at 20 kHz, and analyzed with Clampfit 10 (Molecular Devices).

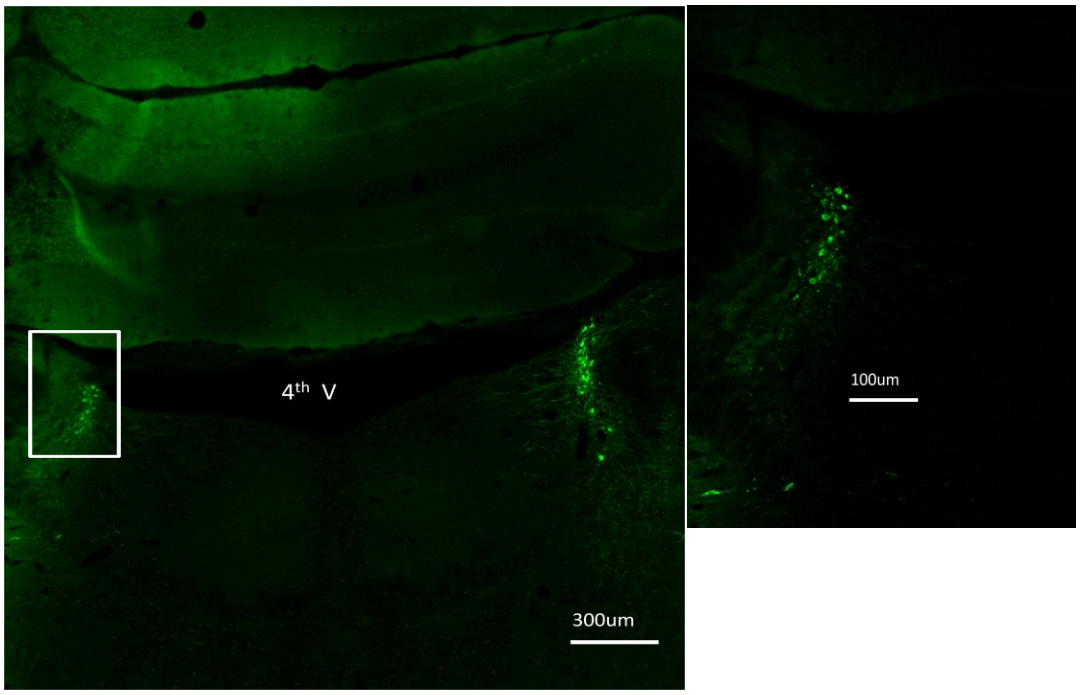
For noradrenaline iontophoresis in Fig. II-7a, double-barreled iontophoresis pipettes (30 MΩ) contained noradrenaline (1 mM) and were placed ~700 µm below the pial surface, ~250-300 µ m from the recording pipette. For experiments of Fig. II-76b,c, phentolamine (1 mM in saline) was topically applied to AI.

Animals were perfused with 4% paraformaldehyde, brains recovered, and embedded in Optimal Cutting Temperature compound prior to freezing at −80°C. Afterwards, 40 µm thick slices were cut from the brainstem and stained using standard immunohistochemistry histological methods. Staining for tyrosine hydroxylase (primary antibody 1:1000, Aves Labs; secondary antibody, DYL488 anti-chicken, 1:500, Life Technologies Labs) was co-localized with biocytin staining revealed with Alexa Fluor 555 conjugated Streptavidin (1:100, Life Technologies Labs).

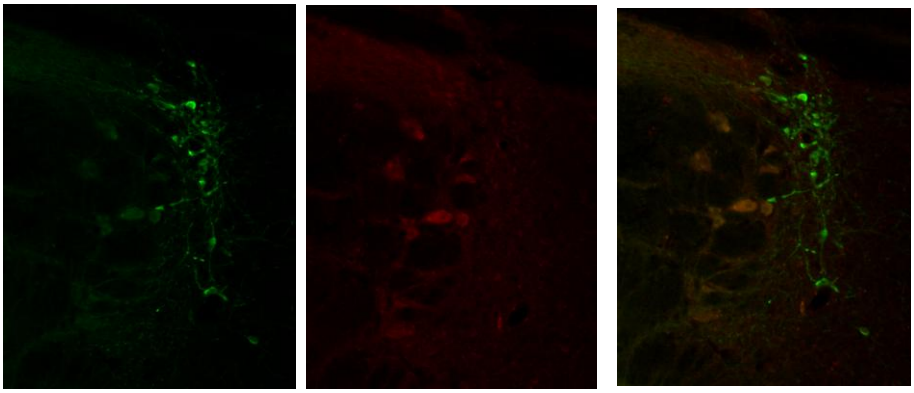
a



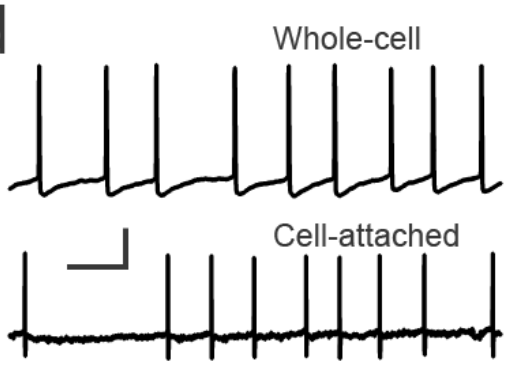
b



c



d


Whole-cell
Cell-attached

e

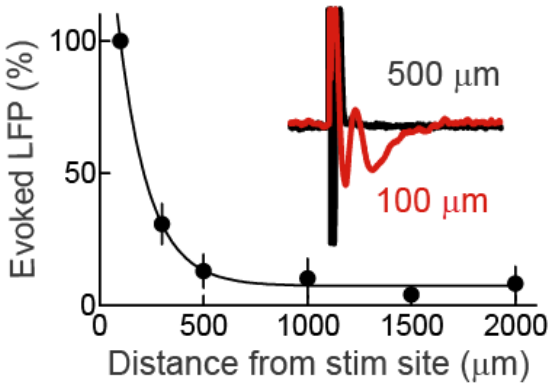

Evoked LFP (%)
100
50
0
0
500
1000
1500
2000
Distance from stim site (μm)
500 μm
100 μm

Figure II-1. Recording from locus coeruleus. a, Noxious stimuli evoke phasic spike bursts in rat locus coeruleus. Top, brief tail pinch (100 msec duration) evoked phasic spiking in locus coeruleus, as measured through the stimulation electrode. b, example post hoc histological recovery of electrode placement in Locus coeruleus. Boxed area ex exemplifying correct electrode placement in the Locus amplified on the side. c, example locus coeruleus neurons filled with biocytin during whole-cell recording in vivo and stained for tyrosine hydroxylase. d, Whole-cell current-clamp (top) and cell-attached (bottom) recordings from single neurons in locus coeruleus in vivo. Shown are traces of spontaneous spiking activity. e, Local field potentials (LFPs) measured at distances from locus coeruleus stimulation electrode. LFPs were only observed <500 µm.

Primary Auditory Cortex Plasticity induced by Activation of Locus Coeruleus

A major output of locus coeruleus is the cerebral cortex, where noradrenergic modulation controls sensory processing and behavior (Aston-Jones G and Cohen J.D., 2005; Berridge C.W., 2008; Sara S.J., 2009). To determine how locus coeruleus pairing affected cortical auditory representations, recordings were made in vivo, whole-cell from 91 (51 current-clamp, 40 voltage-clamp) and cell-attached from 50 primary auditory cortex cells in 49 adult rats implanted with stimulation electrodes in locus coeruleus(Fig. II-2a). Baseline responses to pure tones were recorded from primary auditory cortical neurons, locus coeruleus

pairing was performed, and responses measured as long as recordings remained stable. When the first recording ended, 1-7 more recordings were sequentially made from that cortical location to document the dynamics of post-pairing modification over 12 hours.

One set of recordings demonstrating the cortical effects of locus coeruleus pairing is shown in Fig. II- 2b,c. Recordings from five neurons from the same region of AI initially tuned to 4 kHz, for 11 hours after pairing. The first cell had a peak in the synaptic tuning curve at 4 kHz. The paired frequency was 16 kHz; after pairing, large increases were observed in responses to all frequencies (Fig. 2b) and the peak shifted to the paired frequency (Fig. II- 2c, upper left, black line). This recording then re-sealed and cell-attached spiking responses were measured. It was observed that the paired 16 kHz tone was peak of the spiking tuning curve (Fig. II-2c, lower left). Over the next 10 hours, four more recordings were obtained (Fig. II-2c middle, right). Tuning width recovered but 16 kHz remained best frequency.

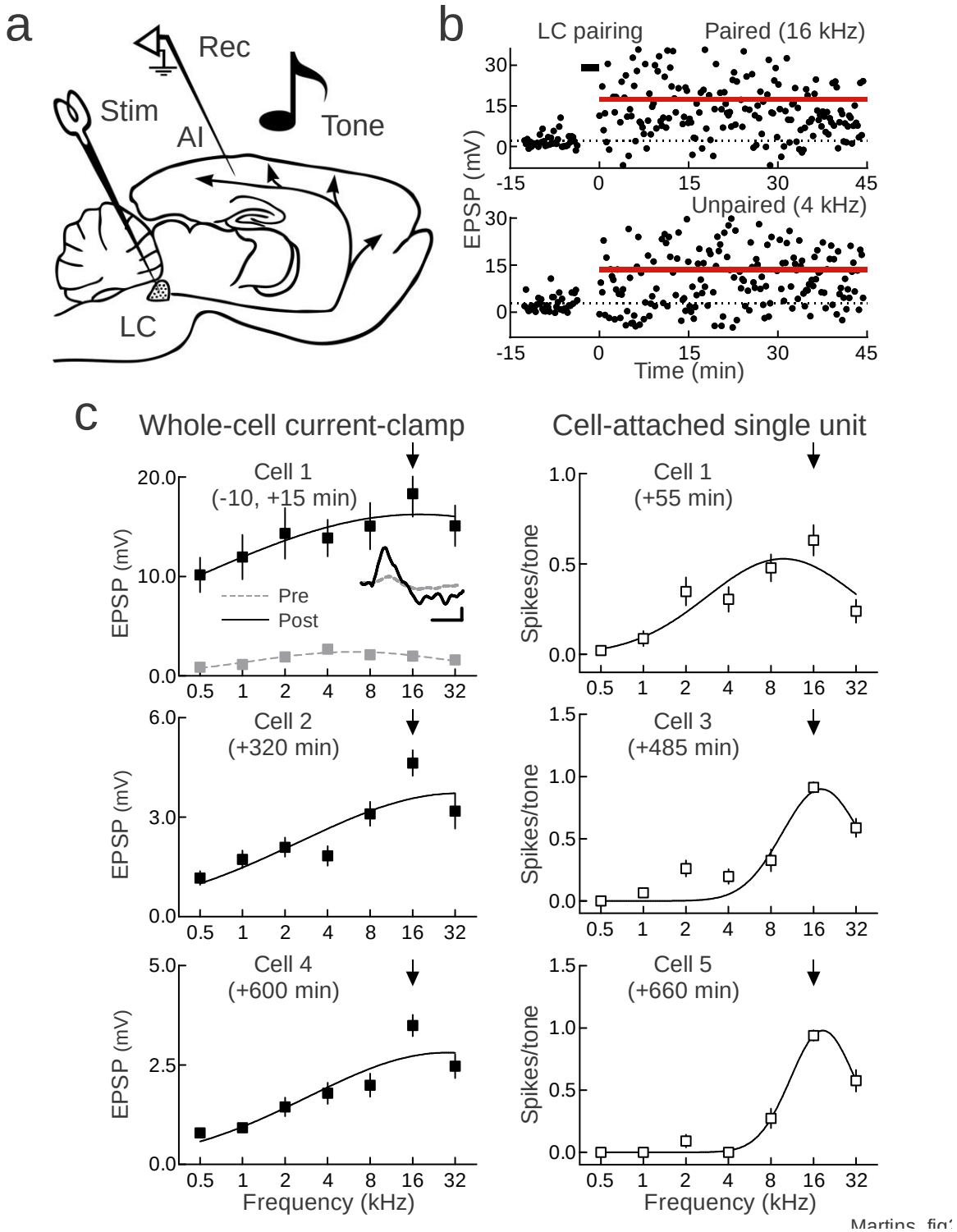


Figure II-2. Primary auditory cortex plasticity induced by locus coeruleus pairing. a, Setup: stimulation electrode ("Stim") in locus coeruleus("LC") and recordings ("Rec") from AI neurons. b, Current-clamp recording of

responses to paired 16 kHz and unpaired 4 kHz tones. c, Synaptic (top) and spiking (bottom) tuning curves from five neurons before and 0-11 hours post-pairing from current-clamp (filled) or cell-attached recordings (open). Each recording from same AI location. Upper left, first recording ten minutes before (gray) and fifteen minutes after (black) pairing with 16 kHz. After pairing, peak shifted to 16 kHz (100% shift) and tuning width increased from 2.4 octaves to 5.3 octaves (221% width). EPSPs increased across frequencies (paired 16 kHz EPSPs: 2.0±0.4 mV pre-pairing, 18.3±2.3 mV post-pairing, $p<10^{-8}$; unpaired EPSPs across other frequencies: 1.7±0.3 mV pre-pairing, 13.4±0.8 mV post-pairing, $p<10^{-5}$). Same cell as b. Inset, 16 kHz EPSPs before (gray) and after (black) pairing; scale: 6 mV, 25 msec. Lower left, cell-attached recording 55 minutes post-pairing (best frequency shift: 100%, tuning curve width: 1.8 octaves). Upper middle, second recording 320 minutes post-pairing (shift: 100%, width: 3.8 octaves). Lower middle, third recording 485 minutes post-pairing (shift: 100%, width: 0.9 octaves). Upper right, fourth recording 600 minutes post-pairing (shift: 100%, width: 3.3 octaves). Lower right, fifth recording 660 minutes post-pairing (shift: 100%, width: 0.8 octaves). d, Changes to paired (filled symbols) and unpaired (open symbols) synaptic responses from individual recordings (paired frequencies after 5-10 minutes: 335.9±74.8%, n=37, p<0.0004; paired after 45-60 minutes: 261.2±62.8%, n=17, p<0.02; unpaired after 5-10 minutes: 231.1±38.3%, n=37, p<0.04; unpaired after 45-60 minutes: 151.0±49.4%, n=17, p>0.2). e, Best frequency shifts of synaptic (filled symbols) and spiking tuning curves (open symbols) over multiple recordings (synaptic: 87 recordings, 37 animals, shift after 5-30 minutes: 90.6±10.7%, n=37 cells, 37 animals, $p<10^{-9}$; shift after 7-12 hours: 65.6±12.5%, n=27 cells, 13 animals, p<0.0003; spiking: 72 recordings, 34 animals; shift after 5-30 minutes: 89.7±11.4%, n=29 cells, 29 animals, $p<10^{-5}$; shift after 7-12 hours: 66.1±11.5%, n=21 cells, 11 animals, p<0.003).

Over 141 primary auditory cortical recordings, three general features of cortical plasticity induced by locus coeruleus pairing were apparent: 1) large increases in tone-evoked responses to all stimuli; 2) shifts in peak towards paired inputs; and 3) return of average tuning width over several hours, with maintained peak preference at paired inputs (Fig.II-3). In individual neurons, responses at paired inputs were substantially larger 45-60 minutes after pairing (Fig. II-2d, filled; Fig.II-3a). Responses to unpaired inputs were also enhanced after pairing, but returned towards original levels 45-60 minutes post-pairing (Fig. II-2d, open; Fig. II-32b). Similar changes were also observed for spiking responses (Fig. II-2e, open; Fig.II-3e,f) and intensity profiles (FigII-4).

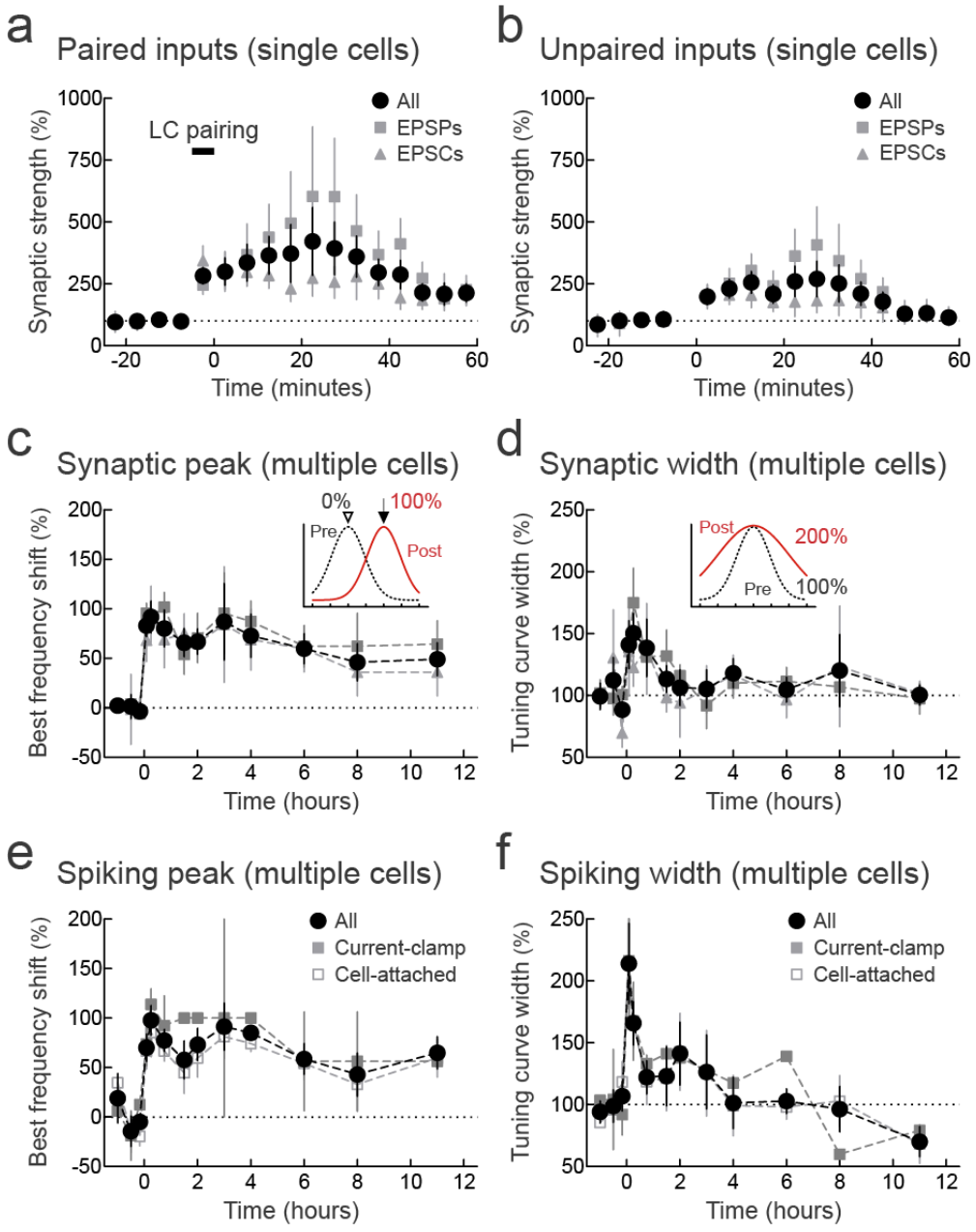


Figure II-3. Changes to synaptic and spiking tuning curves after locus coeruleus pairing. **a**, Changes to paired inputs from individual recordings. Squares, current-clamp (EPSP amplitude 5-10 minutes post-pairing: 367.9±124.7%, n=21, p<0.05; 45-60 minutes: 341.3±80.8%, n=7, p<0.03). Triangles, voltage-clamp (5-10 minutes: 296.9±56.4%, n=16, p<0.004; 45-60 minutes: 197.1±53.8%, n=10, p<0.06). Circles, all recordings (5-10 minutes:

335.9±74.8%, n=37, p<0.0004; 45-60 minutes: 261.2±62.8%, n=17, p<0.02). **b**, Changes to unpaired inputs from individual recordings for current-clamp (5-10 minutes: 253.3±59.4%, n=21, p<0.02; 45-60 minutes: 168±40.3%, n=7, p>0.1), voltage-clamp (5-10 minutes: 205.8±39.1%, n=16, p<0.03; 45-60 minutes: 139.1±47.4%, n=10, p>0.3) and all recordings (5-10 minutes: 231.1±38.3%, n=37, p<0.04; 45-60 minutes: 151.0±49.4%, n=17, p>0.2). Same recordings as **a**. **c**, Best frequency shift of synaptic tuning curves over multiple recordings. Circles, current-clamp (total: 47 cells, 21 animals; best frequency shift 5-30 minutes post-pairing: 96.6±10.4%, n=21 cells, 21 animals, $p<10^{-7}$; shift 7-12 hours post-pairing: 74.4±15.5%, n=15 cells, 7 animals, p<0.003). Triangles, voltage-clamp (total: 40 cells, 16 animals; 5-30 minutes: 82.8±20.8%, n=16 cells, 16 animals, p<0.002; 7-12 hours: 55.5±20.9%, n=12 cells, 6 animals, p<0.05). Circles, all recordings (total: 87 recordings, 37 animals; 5-30 minutes: 90.6±10.7%, n=37 cells, 37 animals, $p<10^{-9}$; 7-12 hours: 65.6±12.5%, n=27 cells, 13 animals, p<0.0003). **d**, Synaptic tuning curve width over multiple recordings. Values normalized to baseline width. Squares, current-clamp (total: 47 cells, 21 animals, baseline width: 3.7 octaves; width 5-30 minutes post-pairing: 154.3±17.6%, n=21 cells, 21 animals, p<0.02; width 7-12 hours post-pairing: 106.1±9.7%, n=15 cells, 7 animals, p>0.5). Triangles, voltage-clamp (total: 40 recordings, 16 animals, average baseline width: 4.0 octaves; 5-30 minutes: 129.0±23.8%, n=16 cells, 16 animals, p<0.004; 7-12 hours: 106.6±14.6%, n=12 cells, 6 animals, p>0.2). Circles, all recordings (total: 87 recordings, 37 animals; 5-30 minutes: 146.6±14.8%, n=37 cells, 37 animals, p<0.0002; 7-12 hours: 103.3±7.8%, n=27 cells, 13 animals, p>0.1). Same recordings as **c**. **e**, Best frequency shifts of spiking tuning curves over multiple recordings. Filled squares, current-clamp (total: 22 cells, 13 animals; shift in best frequency 5-30 minutes after pairing: 92.0±16.0%, n=13 cells, 13 animals, p<0.003; shift 7-12 hours after pairing: 37.5±23.9%, n=4 cells, 2 animals). Open squares, cell-attached recordings

(total: 50 recordings, 22 animals; 5-30 minutes: 85.4±16.3%, n=16 cells, 16 animals, $p<0.0003$; 7-12 hours: 65.2±10.9%, n=17 cells, 10 animals, $p<10^{-4}$). Circles, all recordings (total: 72 recordings, 34 animals; 5-30 minutes: 89.7±11.4%, n=29 cells, 29 animals, $p<10^{-5}$; 7-12 hours: 66.1±11.5%, n=21 cells, 11 animals, $p<0.003$). **f**, Widening of spiking tuning curves over multiple recordings. Filled squares, current-clamp (total: 22 cells, 13 animals, baseline width: 2.3 octaves; width 5-30 minutes post-pairing: 190.4±26.0%, n=13 cells, 13 animals, $p<0.006$; width 7-12 hours post-pairing: 88.4±5.3%, n=4 cells, 2 animals, $p>0.1$). Open squares, cell-attached recordings (total: 50 recordings, 22 animals, baseline width: 2.3 octaves; 5-30 minutes: 200.3±41.4%, n=16 cells, 16 animals, $p<0.04$; 7-12 hours: 107.0±16.7%, n=17 cells, 10 animals, $p>0.6$). Circles, all recordings (total: 72 recordings, 34 animals; 5-30 minutes: 188.5±15.1%, n=29 cells, 29 animals, $p<0.002$; 7-12 hours: 93.6±11.5%, n=21 cells, 11 animals, $p>0.7$). Same recordings as **e**. For analysis of tuning curve shifts in **Figures 2** and **5**, and **Supplementary Figures 2** and **6**, best frequency shift was computed as the normalized difference in octaves between the paired frequency and the original best frequency, such that if the peak of the tuning curve became the best frequency, this was a 100% shift, whereas if the peak stayed the same, this was a 0% shift. To determine tuning curve width, Gaussians were fit to tuning profiles, and the changes in standard deviation measured in terms of number of octaves

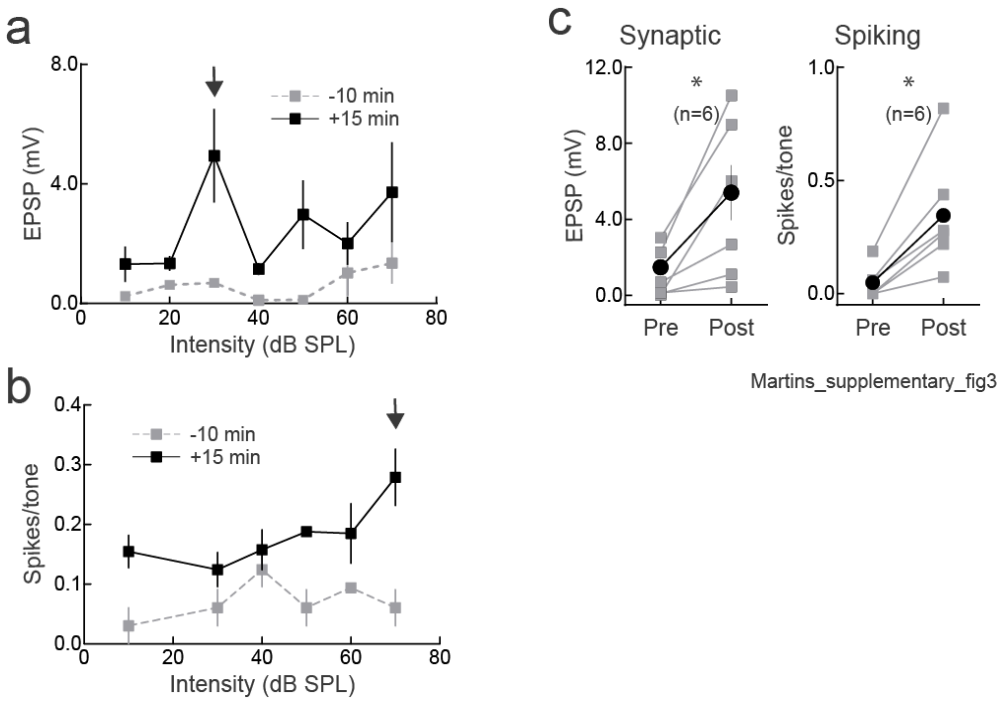


Figure II-4. Locus coeruleus pairing modifies intensity tuning of AI neurons. **a,** Example current-clamp recording showing synaptic intensity tuning curves (measured at 8 kHz) before and after locus coeruleus pairing at a lower intensity (50 dB SPL). **b,** Example current-clamp recording showing spiking intensity tuning curves (at 8 kHz) before and after locus coeruleus pairing at a higher intensity (70 dB SPL). **c,** Summary of changes to evoked synaptic and spiking responses after pairing. Left, EPSPs evoked by the paired intensity before and after pairing (pre: 1.0±0.5 mV, post: 5.0±1.7 mV; n=6, $p<0.04$). Right, tone-evoked spikes before and after pairing (pre: 0.05±0.03 spikes/tone, post: 0.35±0.11 spikes/tone; n=6, $p<0.02$).

Importantly, these data revealed that the duration of cortical frequency preference changes induced by Locus coeruleus pairing were incredibly long lasting. In fact, one single episode of pairing, lasting only 1-2min, seemed to allow an unusually enduring maintenance of the new frequency preference for the duration of our recordings (~11h+). Noteworthy is the dissimilarity between this finding and the maintenance of cortical effects observed for Nucleus basalis Pairing (Fig. II-5), where the new frequency preference is only maintained for a period of ~5h, before a return to baseline.

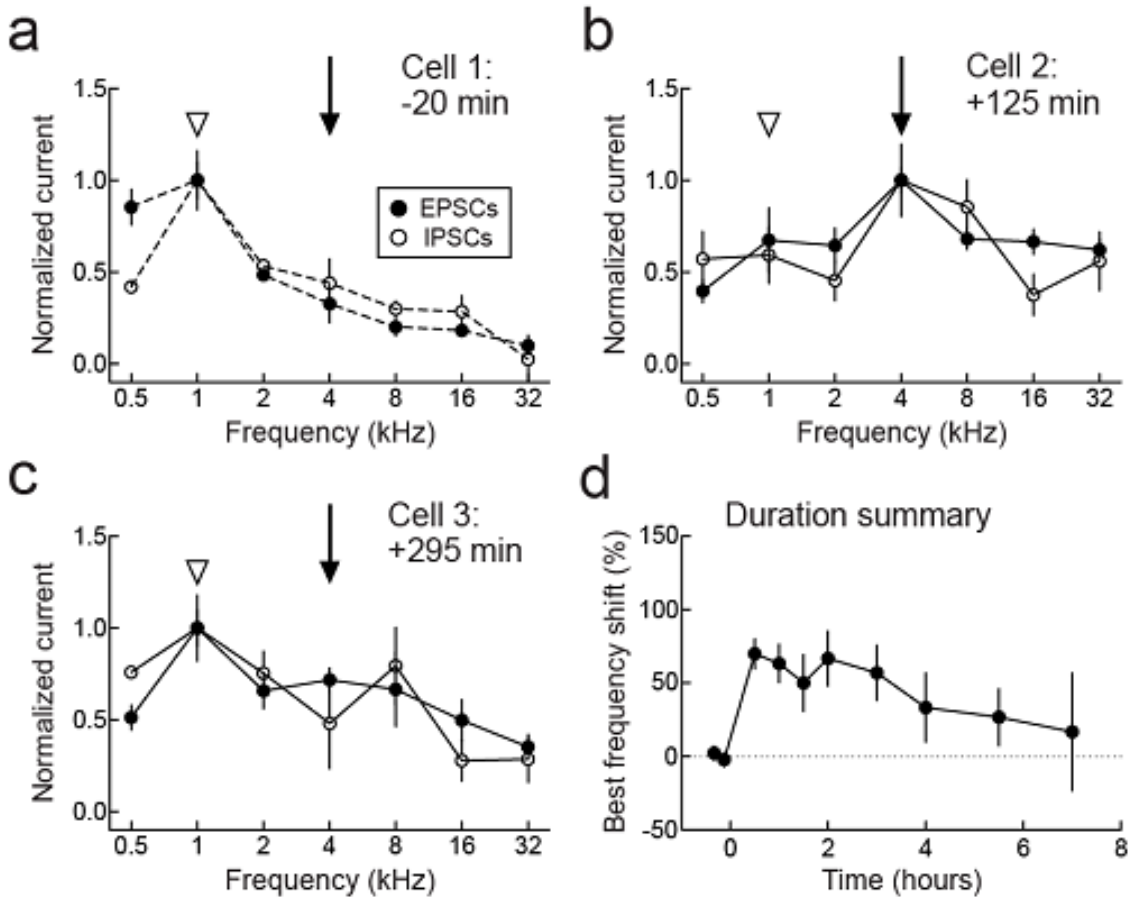
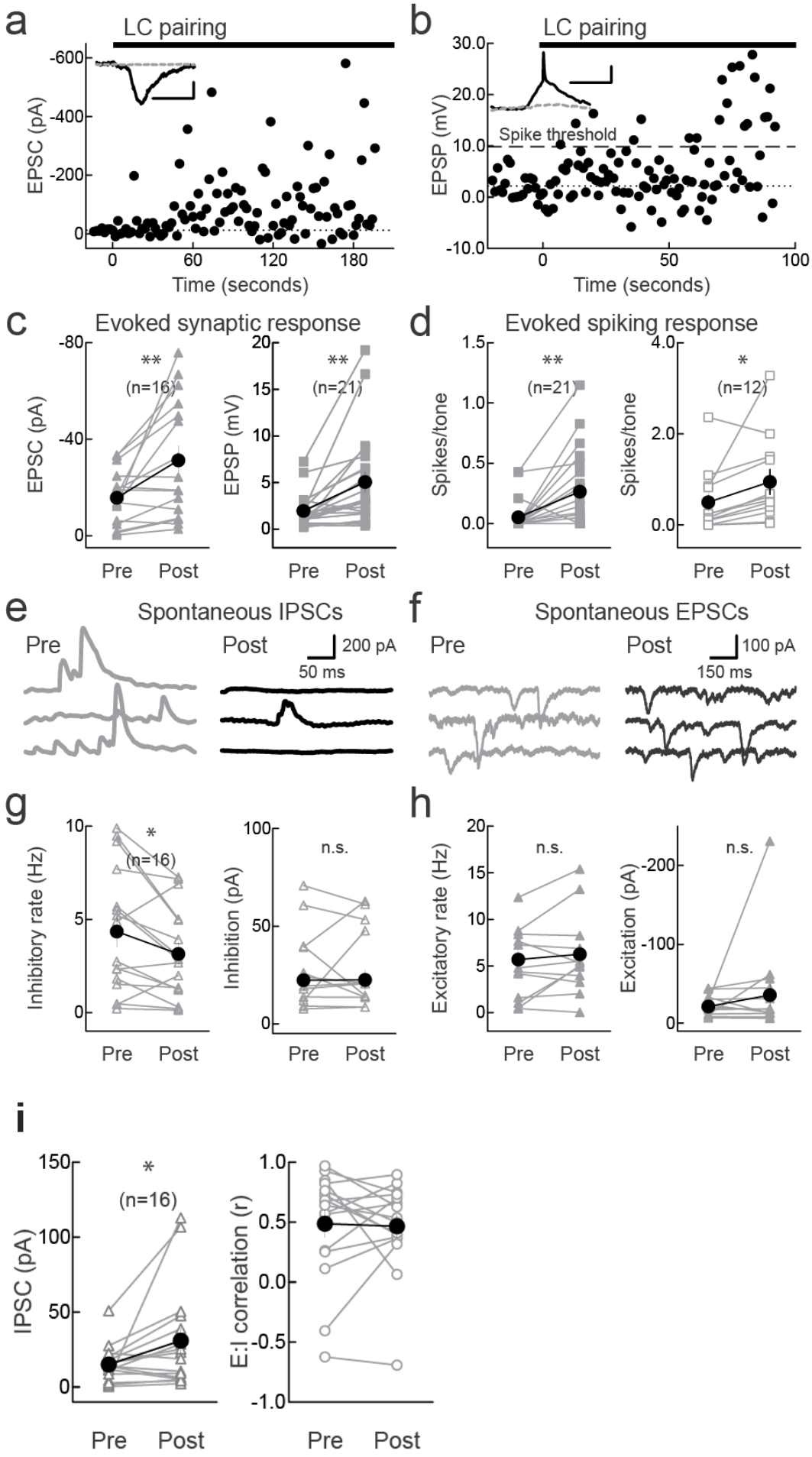


Figure II-5. effects of one single episode of Nucleus basalis pairing on the maintenance of new frequency preference (best frequency) in primary auditory cortical neurons duration of cortical effects. Data collected and analyzed by Robert C. Froemke.

In the remainder of this chapter, the question is asked about what circuit mechanisms increase tone-evoked responses to this large degree after pairing (Figs. II-6), how locus coeruleus plasticity and AI plasticity are related (Fig.II-7). We found that locus coeruleus pairing could induce tone-evoked responses in previously-silent AI cells and converted subthreshold (non-

spiking) responses into suprathreshold (spiking) responses (Fig. II-6a-d). Interestingly, tone-evoked responses were not detected in 9/37 AI neurons before pairing. In 6/9 non-responsive cells, pairing rapidly induced responses that were maintained for the recording duration. Moreover, paired tones initially evoked spikes only in 3/21 current-clamp and 9/12 cell-attached recordings. After pairing, spikes were evoked in 13/21 current-clamp and 12/12 cell-attached recordings. These results indicate that pairing increases the size of the cell assembly encoding paired tones, amplifying AI output to environmental stimuli linked with Locus coeruleus activation.


a
LC pairing
EPSC (pA)
Time (seconds)
b
LC pairing
Spike threshold
EPSP (mV)
Time (seconds)
c
Evoked synaptic response
EPSC (pA)
EPSP (mV)
**
(n=16)
(n=21)
Pre
Post
d
Evoked spiking response
Spikes/tone
**
(n=21)
*
(n=12)
e
Spontaneous IPSCs
Pre
Post
200 pA
50 ms
f
Spontaneous EPSCs
100 pA
150 ms
g
Inhibitory rate (Hz)
Inhibition (pA)
*
(n=16)
n.s.
h
Excitatory rate (Hz)
Excitation (pA)
i
IPSC (pA)
E:I correlation (r)

FigureII-6. Cortical circuit mechanisms of enhanced AI responses after locus coeruleus pairing. a, Voltage-clamp recording showing new tone-evoked responses during pairing. Inset, responses before (gray) and during pairing (black); scale: 150 pA, 10 msec. b, Current-clamp recording showing sub- to suprathreshold tone-evoked responses. Dashed line, spike threshold; responses above this evoked 1+ action potentials. Inset, responses before (gray) and during pairing (black); scale: 5 mV, 10 msec. c, Synaptic changes. Left, voltage-clamp recordings pre/post pairing (pre: −15.7±2.8 pA, post: −31.3±6.2 pA; n=16, $p<0.007$). 10/16 recordings had significant tone-evoked responses before pairing, increased to 14/16 post-pairing ($p<0.05$). Right, current-clamp recordings (pre: 2.0±0.4 mV, post: 5.1±1.1 mV; n=21, $p<0.003$). 18/21 recordings had significant tone-evoked responses before pairing, increased to 20/21 post-pairing. d, Spiking changes. Left, current-clamp recordings before and after pairing (pre:0.05±0.03 spikes/tone, post: 0.25±0.07 spikes/tone; n=21, $p<0.005$). 4/21 recordings had 1+ tone-evoked spikes before pairing, increased to 13/21 after pairing. Right, cell-attached recordings (pre: 0.5±0.2 spikes/tone, post: 0.9±0.3 spikes/tone; n=12, $p<0.05$). 9/12 recordings had 1+ tone-evoked spikes before pairing and 12/12 post-pairing. e, Pairing decreased spontaneous IPSC rate (pre: 9.2 Hz, post: 5.0 Hz) but not amplitude (pre: 70.8 pA, post: 61.3 pA). f, Pairing did not affect spontaneous EPSC rate (pre: 12.4 Hz, post: 15.4 Hz) or amplitude (pre: −44.3 pA, post: −56.7 pA). Same recording as e. g, Spontaneous IPSCs. Left, IPSC rate pre/post-pairing (pre: 4.3±0.8 Hz, post: 3.1±0.6 Hz; n=16, $p<0.02$). Right, amplitude (pre: 24.6±4.7 pA, post: 24.7±4.9 pA; n=16, $p>0.9$). 'n.s.', non-significant. h, Spontaneous EPSCs. Left, EPSC rate pre/post-pairing (pre: 5.7±1.0 Hz, post: 6.3±1.1 Hz; n=16, $p>0.3$). Right, amplitude (pre: −21.1±3.0 pA, post: −35.7±13.7 pA; n=16, $p>0.2$). i, Summary of changes to tone-evoked IPSCs and excitatory-inhibitory correlation after pairing. Left, voltage-clamp recordings of IPSCs evoked by paired tones before and after pairing

(pre: 15.1±3.1 pA, post: 31.0±8.6 pA; n=16, $p<0.05$, Student's paired two-tailed t-test). Right, linear correlation coefficient *r* of excitation and inhibition for all presented tones before and after pairing was unchanged (pre: 0.5±0.1, post: 0.5±0.1 mV; n=21, $p>0.8$).

The neuromodulator acetylcholine reduces evoked inhibition to shift cortical excitatory-inhibitory balance in favor of excitation Thus we next asked if locus coeruleus stimulation had a similar effect, making voltage-clamp recordings from AI neurons to assess changes to inhibitory postsynaptic currents (IPSCs). Pairing greatly increased tone-evoked EPSCs and IPSCs (Fig. II-6) but decreased tonic inhibition by reducing spontaneous IPSC rate while spontaneous IPSC amplitudes and spontaneous EPSCs (Fig. II-6f-h) were unaffected. These findings show that locus coeruleus activation seems to specifically decrease tonic (spontaneous) inhibition rather than phasic (stimulus-evoked) inhibition. This provides a basic gain control mechanism by which responses to any incoming stimuli would be transiently enhanced after noradrenergic modulation, as reduction of spontaneous inhibition would affect all subsequent inputs, paired and unpaired. In this manner, locus coeruleus may increase broadband sensory processing in novel or hazardous environments, where one or more of many environmental cues are important for behavioral performance. Furthermore, these results

also suggest that spontaneous and tone-evoked inhibition are under distinct forms of neuromodulatory control.

Next, the pharmacology of pairing-induced AI changes was examined, to connect these forms of subcortical and cortical plasticity. First, tones were paired with noradrenaline iontophoresis locally in AI instead of locus coeruleus stimulation (**Fig. II-7a**). While 'noradrenaline pairing' increased responses and shifted tuning curves, these changes lasted <1 hour (**Fig. II-7d**). Noradrenaline, paired with sensory input, is not by itself sufficient for the long-lasting changes to AI responses observed with locus coeruleus pairing.

The need for noradrenegic receptor activation during pairing was examined, by topically applying the alpha-adrenergic receptor antagonist phentolamine (1 mM). Phentolamine initially blocked effects of pairing, but minutes afterward AI tuning curves shifted towards the paired frequency, resulting in enduring changes similar to those induced by locus coeruleus pairing (**Fig. II-7b,d,** 'Phento during').

Surprisingly, when phentolamine was applied to AI starting ~30 minutes post-pairing, these changes were diminished within an hour and AI tuning curves shifted back to their original best frequency in the presence of phentolamine (**Fig. II-7c,d,** 'Phento after'). These findings reveal two important features of

neuromodulatory plasticity. First, alpha-noradrenergic receptor activation is required to maintain long-lasting cortical changes after locus coeruleus pairing, suggesting that locus coeruleus plasticity and enhanced noradrenaline release is required for long-term modification of AI tuning curves. Second, this modulatory control over cortical plasticity must occur within AI itself, despite potential for plasticity to be induced in other brain areas beyond AI. This is because local AI application of noradrenergic receptor antagonist prevented tuning curve shifts. Thus plasticity of locus coeruleus directly controls AI plasticity: each time paired tones are presented after pairing, newly-responsive locus coeruleus neurons release noradrenaline into AI, maintaining changes to cortical representations in a selective and powerful manner.

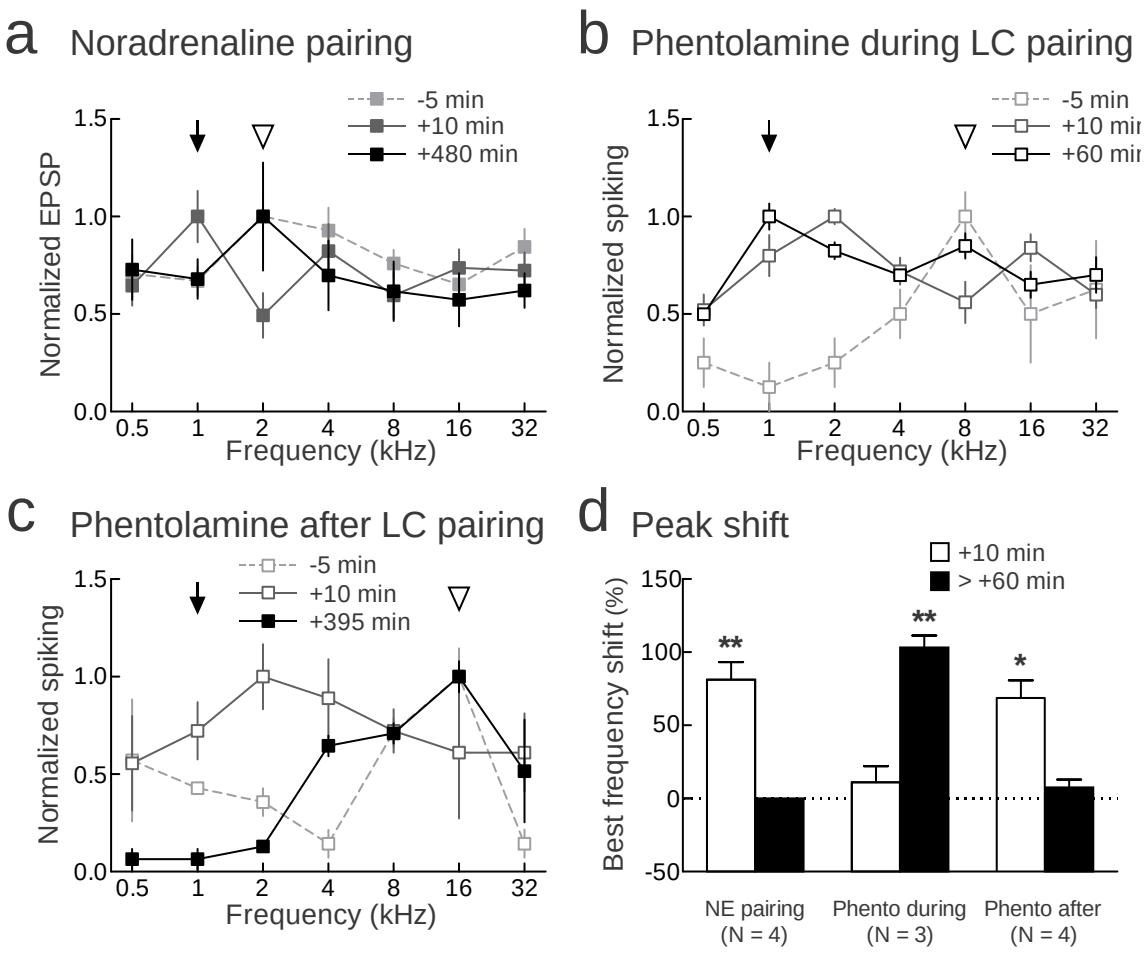


Figure II-7. Noradrenergic receptor activation is required for AI plasticity during and after pairing. **a,** Noradrenaline pairing does not induce long-term changes to AI tuning curves. Example synaptic tuning curves from two neurons recorded in current-clamp from the same AI before and after pairing 1 kHz tones (arrow) with AI noradrenaline iontophoresis (1 mM) instead of locus coeruleus pairing. In the first cell, the best frequency (open arrowhead) shifted from 2 kHz (light gray dashed line) to the paired 1 kHz input for minutes after cortical noradrenaline pairing (dark gray solid line). However, the best frequency had returned to 2 kHz as measured in a second cell 6 hours after noradrenaline pairing (black solid line). **b,** Phentolamine during pairing does not prevent long-term changes to AI tuning curves. Spiking tuning curves from one cell-attached recording before and after locus

coeruleus pairing. Phentolamine (1 mM) was topically applied just before pairing. The best frequency was 8 kHz (light gray dashed line), partially shifted towards the paired input (1 kHz) 10 minutes after pairing (dark gray solid line), and fully shifted to 1 kHz 60 minutes after pairing (black line). **c**, Phentolamine applied after pairing for hours shortens the duration of AI tuning curve shifts. Spiking tuning curves from a cell-attached recording (open symbols) and a current-clamp recording (filled symbols) before and after locus coeruleus pairing with 1 kHz tones. Phentolamine was not applied during pairing, but applied continuously thereafter. In the first recording, pairing led to a shift in best frequency towards the paired input 10 minutes later. In the second recording, tuning had returned to the original best frequency (16 kHz). **d**, Summary of shorter-term (~10 minutes post-pairing; open bars) and longer-term (>60 minutes post-pairing; filled bars) changes to AI tuning curves after noradrenaline (NE) pairing (total of 7 cells from 4 animals; shift in best frequency 10 minutes after pairing: 81.3±12.0%, n=4 cells from 4 animals, $p<0.007$; shift 60+ minutes after pairing: 0%, n=3 cells from 3 animals), phentolamine application during pairing (total of 8 cells from 3 animals; shift in best frequency 10 minutes after pairing: 11.0±11.0%, n=3 cells from 3 animals, $p>0.4$; shift 60+ minutes after pairing: 103.2±8.1%, n=5 cells from 3 animals, $p<0.0003$), and phentolamine application only after pairing (total of 14 cells from 4 animals; shift in best frequency 10 minutes after pairing: 68.8±12.0%, n=4 cells from 4 animals, $p<0.02$; shift 60+ minutes after pairing: 7.5±5.3%, n=10 cells from 4 animals, $p<0.004$).

Conclusion

The findings in this chapter demonstrate that noradrenergic Locus coeruleus stimulation changes receptive fields in a similar but also very different way than the cholinergic Nucleus basalis. The first detectable effect of Locus coeruleus pairing on cortical responses is to first greatly increase responses to all sensory stimuli, regardless of context. Afterwards eventually tuning is re-gained, but the tuning preference has changed- the peak is now the paired frequency. This is comparable to the clean shift in the tuning curve peak towards the paired frequency seen with Nucleus basalis pairing. However, looking at the duration of the specificity for the paired input, there is a notable difference: while Nucleus basalis stimulation allowed new specificity maintenance for only a few hours (~5h), Locus coeruleus pairing allows the new preference to be maintained for the duration of our recordings, at least twice the time (~11h+).

Furthermore, the pharmacology data presented here suggests that Locus coeruleus effects on cortex are mostly driven by the effects of noradrenaline on cortical alpha-1 noradrenergic receptors in primary auditory cortex. This data further exposes a possible two phase process for Locus coeruleus modulation. The

initial release of noradrenaline in cortex from the stimulation, represented by iontophoretic pairing experiment, is enough for the initial stage of increased non-specific responses across all frequencies, but not sufficient for the longer-term maintenance of paired frequency preference. Moreover the noradrenergic receptor blocker experiments show that there is a need for a Locus coeruleus near-continual release of noradrenalin for maintenance of cortical effect, since near-continual blocking of noradrenergic cortical receptors is needed for muting the long-term cortical changes.

These findings sparked an interest regarding differences that might occur at the level of the neuromodulatory nuclei themselves after activation, that might account for the interesting observations of the two-phase long-duration of cortical effects in the case of Locus Coeruleus, but not Nucleus basalis pairing.

Chapter III. Locus coeruleus plasticity

Preface

Cortical networks are embedded within complex neural circuits including neuromodulatory systems such as the noradrenergic locus coeruleus, providing context to environmental stimuli. While neuromodulators are important for cortical plasticity and adaptation, it is unknown how subcortical neuromodulatory neurons themselves might respond and adapt to changes in sensory input.

In this chapter I will explore this question, by using the same experimental procedure and protocol as before, but instead of recording from primary auditory cortical neurons, I will directly record from Locus coeruleus neurons, while under the same experimental conditions and pairing protocol, in an effort to tie observations in cortical networks with subcortical findings.

Most of this chapter is in resubmission for publication in Nature Neuroscience.

Introduction

In the last chapter, the results showed an unusual and unexpected observation: a need for a possible near-continual release of noradrenaline for maintenance of longer-term cortical effects.

Locus coeruleus neurons are activated by noxious, surprising stimuli, and also respond directly to previously-innocuous stimuli that have been linked to behaviorally-significant episodes in the past (Aston-Jones G., et al, 1994; Usher J.D. et al., 1999; Yu A.J. and Dayan P., 2005; Sugyama D. et al., 2012). However, it is unknown how locus coeruleus neurons are affected by experience and come to respond to sensory inputs, or how modifications to noradrenergic and cortical circuits interact and are coordinated. In this chapter I set out to explore this question, and attempt to further explain the findings of the previous chapter, by recording directly from the Locus coeruleus neurons in rat brainstem *in vivo*, under the same experimental conditions recordings in primary auditory cortex were made in the previous chapter, in order to tie the findings in both areas, to the higher level changes in circuitry that might be underlying the unusually long-term maintenance of

cortical effects. The goal was to understand the basic mechanisms by which subcortical neuromodulatory centers might acquire sensitivity to behaviorally-important stimuli, that would contribute to the way future related (and unrelated) stimuli would be contextualized and processed in the brain.

Electrophysiological Recording of Locus Coeruleus Neurons in the Rat Brainstem

General surgical preparation of animals and patch clamping internal solutions and recording equipment were the same as in primary auditory cortex recordings.

Whole-cell recordings from locus coeruleus neurons were obtained using two different methods, depending on the manner of postsynaptic stimulation during pairing. For single cell stimulation, recordings were obtained 5500-6000 □m from the pial surface. During pairing, cells were depolarized through the patch pipette (20 Hz for 500 msec). For extracellular stimulation, the cerebellum was aspirated with light suction and recordings were obtained ~300 µm below the surface of the pons (similar to Sugyama et al., 2012). For locus coeruleus pairing, after recording baseline responses to the pseudo-random tone sequence for each cell for 5-20 minutes, a non-preferred tone of

a given intensity level and frequency was repetitively presented for 1-5 min, concurrent with locus coeruleus stimulation (500 msec, 20 Hz) starting at tone onset. Afterwards, locus coeruleus stimulation was ceased and pseudo-random tone sequences were resumed. Resting potential of locus coeruleus neurons: −61.5±13.0 mV (s.d.); series resistance (Rs): 30.5±11.1 MΩ; input resistance (Ri): 219.0±141.7 MΩ.

For analysis of tonal responses in locus coeruleus neurons in Figure III-1f, we computed z-scores from mean peak EPSPs 20-50 msec after tone onset post-pairing, compared to the mean and standard deviation of responses during this same period before pairing. For analysis of tuning curve shifts, best frequency shift was computed as before for cortical tuning curves (see Fig. II-3). For APV infusions in Figures 1 and 3, in some experiments a custom hybrid cannula/stimulation electrode was used, implanted on locus coeruleus, and APV was infused (1 mM in saline, 1 µl total volume at 0.2µl/min). In other experiments in which a hemi-cerebellectomy was performed, APV (1 mM) was topically applied to locus coeruleus. For foot shock experiments of Figure III-1a,b, a silver wire was connected to the hindlimb footpad. Foot shock (20-100 Hz, 500 msec duration,

40-150 V) was applied for 2-5 minutes. Unless otherwise noted, all statistics and error bars are reported as means±SEM.

Locus Coeruleus Plasticity

As a first approach to answer the question "can Locus coeruleus exhibit adaptation to sensory stimuli after activation?", extracellular recordings were made from Locus coeruleus neurons from five anesthetized adult rats (Fig. III-1). Intense stimuli such as foot shock was shown to be able to produce a phasic, high-frequency spiking (Fig. III-1a), similar to the patterned electrical stimulation of the nucleus used in the earlier chapter. Innocuous stimuli (pure tones) did not evoke detectable responses (Fig.II-1b, 'Pre'). However, after tones were repetitively paired with foot shock for 1-5 minutes, paired tones could evoke locus coeruleus spikes for 1+ hours (Fig. III-1b, 'Post').

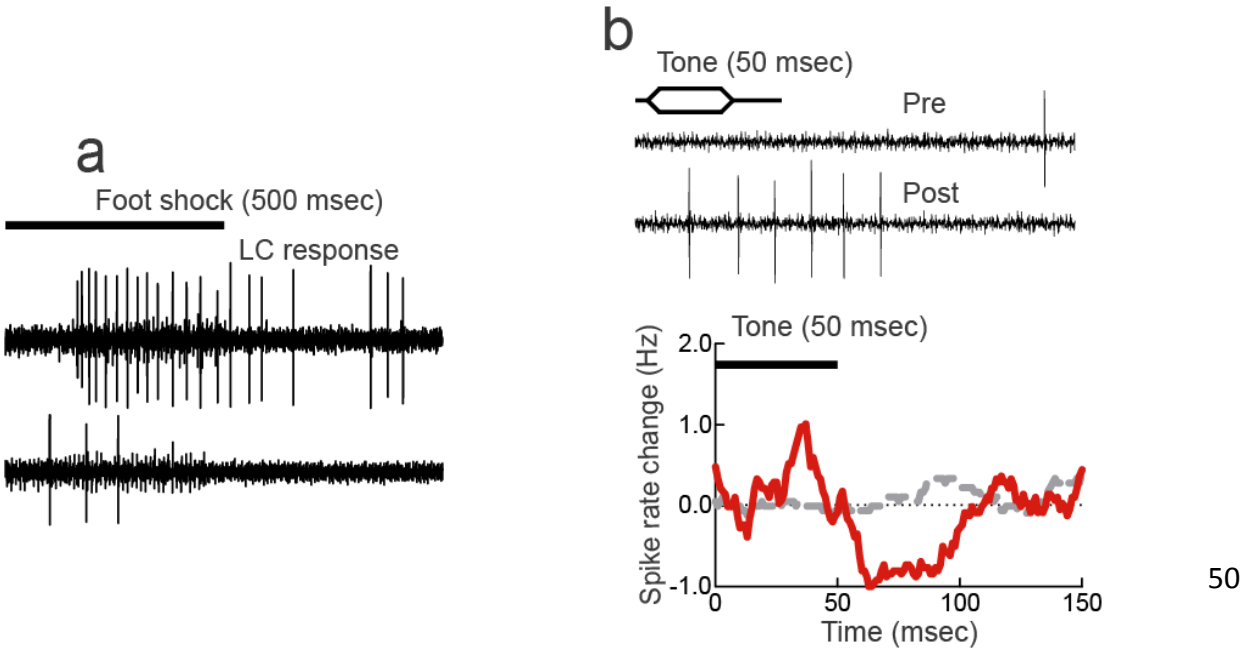


Figure III-1. Recording from locus coeruleus. **a**, Noxious stimuli evoke phasic spike bursts in rat locus coeruleus. Top, brief tail pinch (100 msec duration) evoked phasic spiking in locus coeruleus, as measured through the stimulation electrode. Bottom, foot shock (100 Hz for 500 msec, 50 V) also evoked high frequency bursts of activity as measured with unit recordings. Traces shown are from separate animals. **b**, Pairing a pure tone with foot shock leads to a long-lasting increase in tone-evoked spiking in locus coeruleus. Top, example traces showing that before foot shock pairing ('Pre'), presentation of a pure tone (8 kHz, 80 dB SPL, 50 msec duration) did not evoke significant spiking in locus coeruleus. Ten minutes after pairing foot shock (as in **a**) with 8 kHz tones, the paired tone by itself evoked spiking responses in locus coeruleus. Bottom, post-stimulus time histogram of multiunit recording from locus coeruleus showing change in firing rate after tonal presentation just before (gray) and one hour after (red) pairing. Pairing induced a large (~1 Hz) increase in firing rate with ~30 msec latency

We then examined if pairing auditory stimuli with locus coeruleus activity ('locus coeruleus pairing') was sufficient to modify neuronal responses. Pairing was performed either by depolarization through the recording electrode or extracellular stimulation. For single-cell depolarization, we made current-clamp recordings from locus coeruleus neurons, and measured responses to pure tones 5-20 minutes before and 5+ minutes after pairing a specific tone with postsynaptic spiking. After the

baseline period, neurons were phasically depolarized at 20 Hz for 500 msec, repeated at 0.5-1 Hz for 1-5 minutes (Fig. III-1b), similar to firing patterns observed in locus coeruleus neurons during foot shock (Fig. III-1a). This procedure mimics what occurs when sounds are linked to arousing situations (Aston-Jones G., et al, 1994; Usher J.D. et al., 1999; Yu A.J. and Dayan P., 2005; Sugiyama D. et al., 2012).

Pairing tones with postsynaptic depolarization induced long-lasting responses to auditory stimuli in previously-unresponsive locus coeruleus neurons. An example in vivo whole-cell recording is shown in Figure III-2c. Initially, this cell did not respond to sounds. After pairing, paired 16 kHz tones evoked sizable EPSPs at ~30 msec latency for the recording duration. This locus coeruleus plasticity required NMDA receptors, as local infusion of APV (1 mM) prevented emergence of tone-evoked responses after pairing (Fig. III-2d). These results are reminiscent of 'silent synapses' activated after induction of long-term potentiation, although here it appeared that auditory responses had developed in formerly 'silent cells'.

We tested how long these auditory responses would last after pairing. To simultaneously induce plasticity in multiple locus coeruleus neurons, we paired tones with extracellular

locus coeruleus stimulation (20 Hz, 500 msec at 0.5-1 Hz, 1-5 minutes) confined to <500 □m around the electrode (FigII-1f). When the first recording ended, we recorded from up to four more neurons in the same animals to assess changes to other neurons throughout locus coeruleus after a single pairing episode.

Three recordings from the same animal are shown in Figure III-2e, a current-clamp recording before and after pairing (Fig. III-2e, left; gray, red), a cell-attached recording 5+ hours after pairing (Fig. III-2e, right), and a final current-clamp recording 6+ hours afterward (Fig. III-2e, left; black). Locus coeruleus neurons responded to tones for hours after pairing. Additionally, locus coeruleus plasticity seemed specific to paired tones, as responses to unpaired tones were sometimes initially enhanced but not persistently modified after pairing (Fig. III-2e, left).

The duration of locus coeruleus plasticity is shown in Figure III-2f for 27 neurons from nine animals (filled symbols), and 18 neurons from five animals after APV infusion (open symbols). Significant increases in synaptic strength and spike generation (as measured by z-scored responses relative to baseline noise) lasted for at least several hours after pairing, but were prevented by APV infusion. These findings demonstrate that locus coeruleus neurons can become activated by previously-

innocuous sounds (i.e., stimuli that did not evoke any detectable subthreshold response).

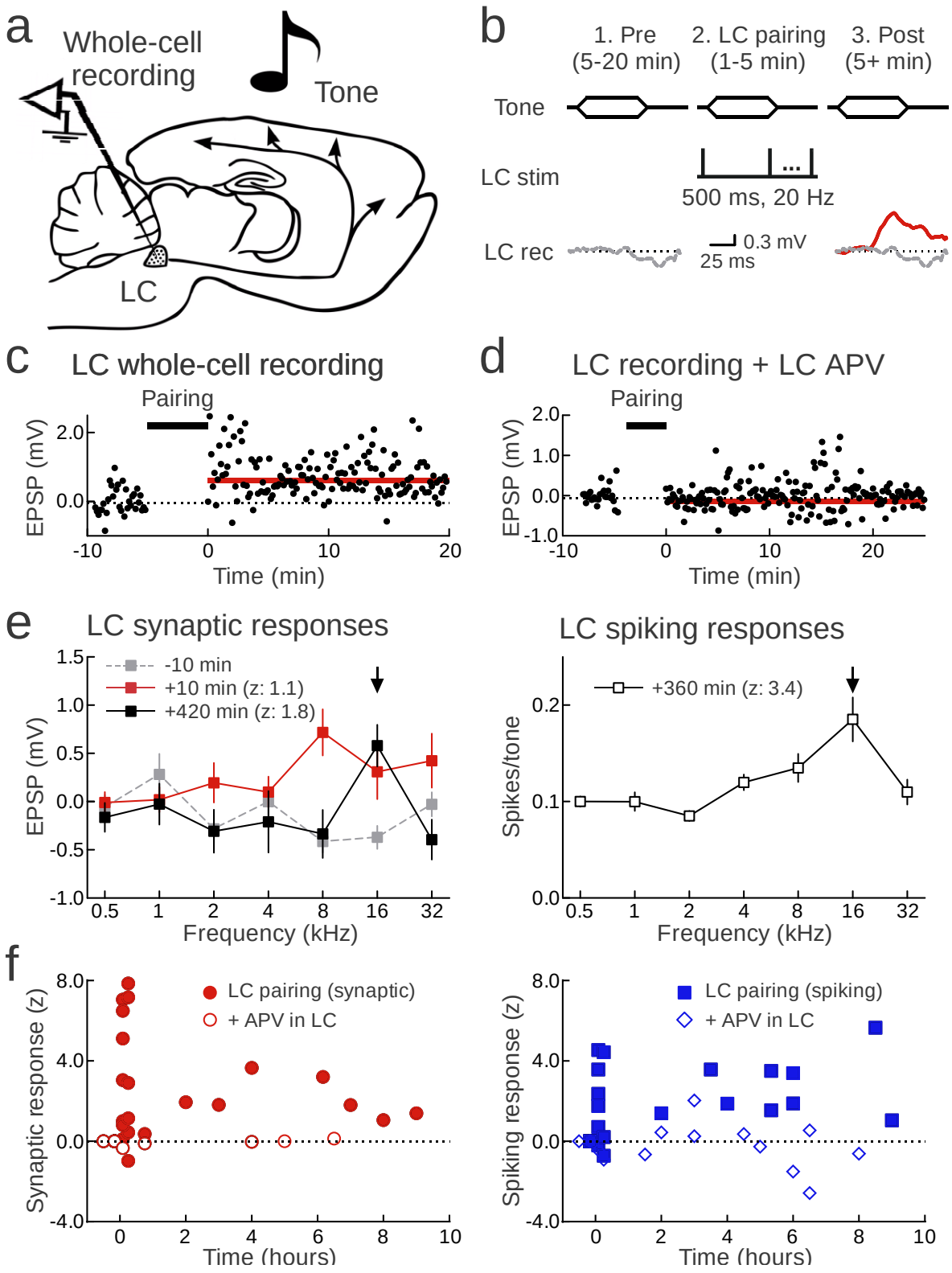

a
Whole-cell recording
Tone
LC
b
1. Pre (5-20 min)
2. LC pairing (1-5 min)
3. Post (5+ min)
Tone
LC stim
500 ms, 20 Hz
LC rec
0.3 mV
25 ms
c
LC whole-cell recording
Pairing
EPSP (mV)
Time (min)
d
LC recording + LC APV
Pairing
EPSP (mV)
Time (min)
e
LC synaptic responses
-10 min
+10 min (z: 1.1)
+420 min (z: 1.8)
EPSP (mV)
Frequency (kHz)
LC spiking responses
+360 min (z: 3.4)
Spikes/tone
Frequency (kHz)
f
LC pairing (synaptic)
+ APV in LC
Synaptic response (z)
Time (hours)
LC pairing (spiking)
+ APV in LC
Spiking response (z)
Time (hours)

Figure III-2. Locus coeruleus responses are plastic. a, Setup for in vivo whole-cell or cell-attached recording from locus coeruleus("LC") neurons. b, Locus coeruleus pairing procedure. Scale:0.3 mV, 25 msec. c, Current-clamp recording from locus coeruleus neuron. Dotted line, baseline tone-evoked EPSP amplitude (0.0±0.1 mV). Red line, tone-evoked EPSP amplitude after pairing (0.7±0.1 mV, $p<10^{-8}$; z-score: 3.0). 16 kHz responses before (gray) and after pairing (red) shown in b. d, Recording with APV infusion (1 mM); no response to tones before (0.0±0.1 mV) or after pairing (−0.1±0.1 mV, p>0.3; z-score: −0.3). e, Three locus coeruleus neurons for 7+ hours before and after pairing. Left, first recording ten minutes before (gray) and ten minutes post-pairing (red); third recording 420 minutes post-pairing (black). Before pairing, tone-evoked EPSPs were not detected (−0.4±0.1 mV; gray dashed line); post-pairing, responses were observed (0.3±0.3 mV, p<0.005, z-score: 1.1; black) and maintained seven hours later in another cell (0.6±0.2 mV, z-score: 1.8). Right, second cell-attached recording 360 minutes post-pairing (0.2±0.02 spikes/tone; z-score: 3.4). f, Summary of new tonal responses in locus coeruleus neurons after pairing. Left, synaptic responses (29 measurements, 13 neurons, seven animals; z-score 5-15 minutes post-pairing: 3.2±1.2, p<0.03; z-score 4-10 hours post-pairing: 2.2±0.5, p<0.02). Open symbols, APV in locus coeruleus(four neurons, three animals; z-score: −0.2±0.1, p>0.2). Right, spiking responses (24 measurements, 15 neurons, seven animals; z-score 5-15 minutes post-pairing: 1.9±0.8, p<0.04; z-score 4-10 hours post-pairing: 2.7±0.6, p<0.03; APV, 11 neurons, four animals; z-score 5-15 minutes post-pairing: −0.7±0.2, p>0.2; z-score 4-10 hours post-pairing: −1.0±0.7, p>0.2).

This might lead to a different more prolonged noradrenergic modulation of target projection areas in response to tonal presentation alone Therefore, in our last physiological experiment, we asked whether modifications to locus coeruleus neurons were required for cortical plasticity observed. As APV prevented locus coeruleus plasticity, we used this manipulation to selectively block subcortical changes, measuring the effects of pairing on AI in absence of locus coeruleus plasticity. Infusing APV into locus coeruleus during pairing greatly reduced the duration of AI tuning curve shifts. Three cells recorded over eight hours from the 4 kHz region of AI are shown in Figure III-3a. The first recording showed that 4 kHz was the best frequency (**Fig. III-3a**, left, gray). 1 kHz was paired with locus coeruleus stimulation, and three hours after pairing, a second AI neuron had new preference for 1 kHz (**Fig. III-3a**, right). But eight hours after pairing, tuning reverted to 4 kHz (**Fig. III-3a**, left, black). We conclude that locus coeruleus plasticity is required for long-term maintenance of AI plasticity. These effects are summarized in **Figure III-3b** for nine whole-cell and nine cell-attached recordings, showing that APV infused into locus coeruleus dramatically reduced duration of cortical modifications.

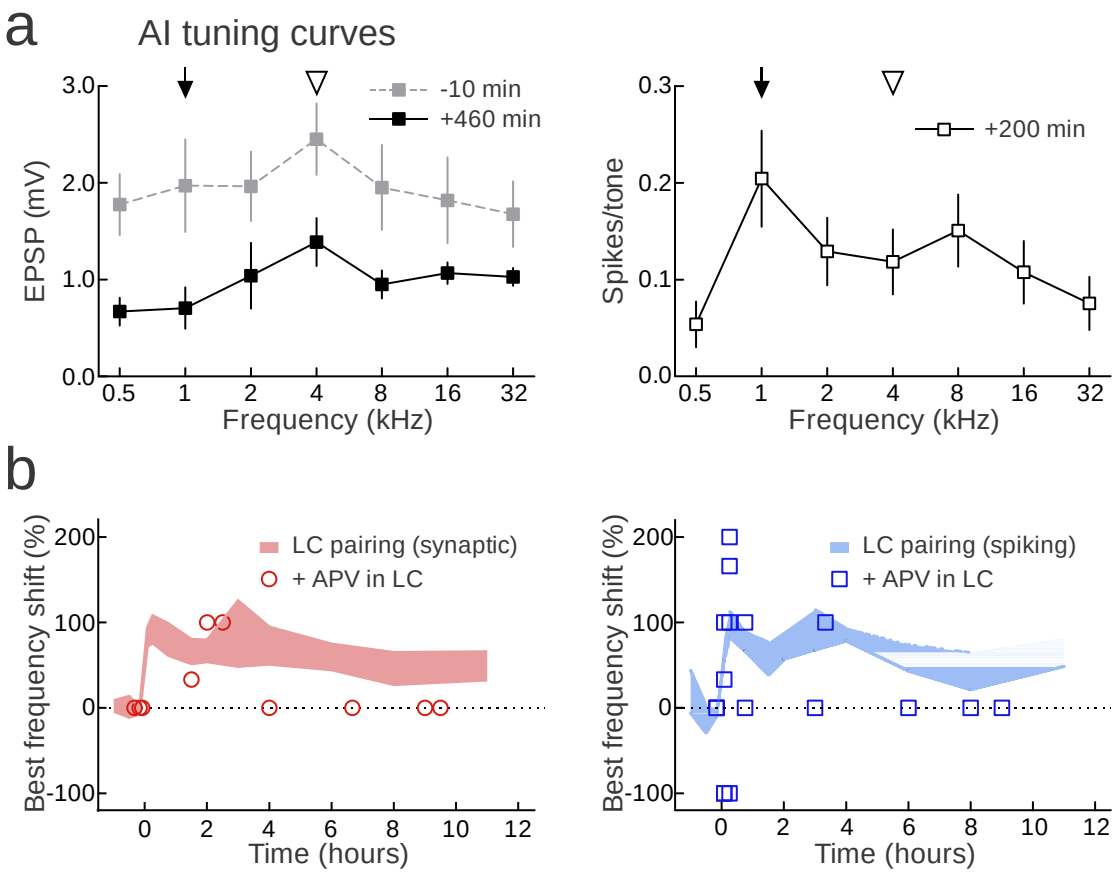


Figure III-3. Locus coeruleus plasticity controls the duration of cortical plasticity. **a**, Three AI recordings for 7+ hours pre/post-pairing with APV in locus coeruleus. Left, first current-clamp recording ten minutes before (gray) pairing; third current-clamp recording 460 minutes post-pairing (black). 4 kHz was original best frequency (arrowhead); 1 kHz was paired frequency (arrow). Right, second cell-attached recording 200 minutes post-pairing. **b**, Summary of AI best frequency shift of synaptic (left) and spiking tuning curves (right) with APV in locus coeruleus. Shaded area, mean±SEM of shifts from figure II-3, e .

Conclusion

These findings demonstrate that after pairing, Locus coeruleus neurons became part of the overall circuit activated by once-innocuous stimuli. Importantly, these data suggest that after Locus coeruleus sensitization, noradrenergic modulation in target projection areas might happen in response to tonal presentation alone. This might be a causal mechanism supporting the long duration of cortical effects: once sensitized, the Locus will release a certain amount of noradrenaline in response to passive presentation of the paired tone, maintaining the cortical noradrenergic receptors active in the presence of this tone, thereby keeping relevance of the new frequency preference for long periods of time. Although it will be important in future studies to examine which specific inputs to locus coeruleus are activated and modified by pairing, we focused our next experiments on the functional consequences of neuromodulatory plasticity on cortical responses and perceptual learning, described in the next chapter.

Chapter IV. Perceptual consequences of Locus Coeruleus Pairing

Preface

Until now this thesis has explored how noradrenergic locus coeruleus stimulation paired with tonal presentation, re-organizes primary auditory cortical receptive fields, and how these changes relate to plasticity in neurons in Locus coeruleus itself.

In this chapter I examine how locus coeruleus neurons experience, and the consequences of this plasticity on cortical representations and sensory perception in behaving animals.

Most of this chapter is in review at Nature Neuroscience.

Introduction

Synapses and receptive fields of the cerebral cortex are plastic. However, changes to specific inputs must be coordinated within neural networks to ensure that excitability and feature selectivity are appropriately configured for perception of the sensory environment.

Locus coeruleus neurons are activated by noxious and surprising stimuli, and also respond directly to previously-innocuous stimuli that have been linked to behaviorally-significant episodes in the past (Aston-Jones G., et al, 1994; Usher J.D. et al., 1999; Yu A.J. and Dayan P., 2005; Sugyama D. et al., 2012). It is hypothesized that locus coeruleus plays a major role in adjusting gains of cortical synapses in a task-dependent manner in particular, higher-frequency phasic activity of noradrenergic neurons may facilitate the formation of task-specific behavioral patterns, to optimize perceptual abilities and motor outputs (Aston-Jones G et al., 1994; Usher M., et al., 1999, Yu A.J. and Dayan P., 2005, Kuo, S.P. and Trussel L.O., 2011).

Previous work done in Froemke R.C. and Carcea I. et al., 2013, has studied the perceptual consequences of activation of Nucleus basalis. That work shows that Nucleus basalis stimulation and pairing with a tone can both increase the recognition of a paired frequency, and increase detection of a paired low decibel level (see Fig I-4).

While in previous chapters, recordings were made directly from brain areas in anesthetized animals, for the mechanical stability required by the technique, in this chapter I set out to explore the perceptual consequences of the network changes

measured. To this point and given the interesting functional similarities and disparities between the cholinergic Nucleus basalis and the noradrenergic Locus Coeruleus, the question was asked: what will we see using the a similar operant task as with Nucleus basalis, but stimulating Locus coeruleus instead.

In this chapter the question is posed: what do the interesting cortical and subcortical changes recorded, by activating Locus coeruleus in this high frequency phasic manner, mean for auditory perception.

Behavior Training and Chronic Implantation of Adult Rats

Rats were lightly food-deprived and pre-trained for 2-6 weeks on a frequency recognition go/no-go task. Animals were rewarded with food for nose-poking within three seconds of presentation of a target tone (4 kHz, any intensity), and given a short (~5 s) time out if they incorrectly responded to non-target tones. After learning to nosepoke to 4 kHz tones, spectrally-wideband foils were also presented (0.5, 1, 2, 8, 16, 32 kHz). Animals that achieved hit rates >80% for targets were then anesthetized with ketamine/dexmedetomidine, had stimulation electrodes or

hybrid cannula/stimulation electrodes chronically implanted in right locus coeruleus, and were allowed to recover for a week.

Each implanted animal was first tested on the 'wideband' recognition task or the detection task for at least 1-2 days. On the wideband and narrowband recognition tasks, tones (wideband target: 4 kHz, foils: 0.5, 1, 2, 8, 16, 32 kHz; narrowband target: 4 kHz, foils: 2.8, 3.2, 3.6, 4.5, 5.1, 5.7 kHz) were presented at 70 dB SPL. For the detection task, tones were presented at 20-90 dB SPL. On the first day at each task, tones were presented for 30-60 minutes to assess performance at baseline; 4 kHz tones (at 70 dB SPL for the wideband recognition task; 30-45 dB SPL for the detection task; hits binned over 20-40 dB SPL) were then paired with locus coeruleus stimulation in the training box for two to three minutes, and behavior performance assessed and quantified one, three, twelve and/or twenty four hours after one single episode of stimulation. In some cases, animal performance was monitored over the span of weeks, once a day, to examine the duration of behavioral changes induced by locus coeruleus pairing. For reversal learning, 17 animals had the rewarded tone switched from 4 kHz to 16 kHz on the wideband task after achieving d'>2.0. Six of those animals had stimulation electrodes implanted in locus coeruleus, and locus coeruleus stimulation was paired with 16 kHz tones for

five minutes just prior to testing behavioral performance on the first day that 16 kHz was rewarded. Performance was measured daily thereafter. Cannulated animals had APV (1 mM in saline, 0.4-1 µl at 0.2µl/min) or saline infused into the locus coeruleus immediately prior to stimulation for either task. d' values were computed as the difference in z-scores between hits and false positives: d' = z(hit rate) – z(false positive rate), using the responses between 20-40 dB SPL for detection and responses to 3.6-4.5 kHz for narrowband recognition. Power analysis was performed to determine the number of animals required for statistical significance.

Perceptual Consequences of Locus Coeruleus Pairing

To assess the functional consequences of cortical and neuromodulatory plasticity findings reported, I set out to determine how these changes might affect sensory perception. Here a behavioral task is used, sensitive to plasticity of AI tuning curves (Froemke R.C. et al., 2013). Three predictions suggested by our physiological results were examined: 1) behaviorally-important stimuli should be easier to detect and recognize after locus coeruleus pairing, as primary auditory cortical synaptic and spiking responses to tones of all

intensities are greatly enhanced; 2) recognition of specific stimuli may initially be impaired, as primary auditory cortical tuning curves first widen and responses to distinct stimuli become more similar for a few hours after locus coeruleus pairing; and 3) improvements to perceptual abilities should persist for hours or perhaps indefinitely even after a single brief pairing episode, given the prolonged maintenance of AI plasticity by locus coeruleus plasticity.

A total of 33 adult rats were operantly conditioned to nose-poke for a food reward in response to 4 kHz target stimuli, withholding responses to six foil tones of other frequencies irrespective of intensity (Fig. IV-1a). Animals had stimulation electrodes and drug delivery cannulas implanted into locus coeruleus. Psychophysical detection performance of a representative animal is shown in Figure IV-1b. At ≥50 dB sound pressure level (SPL), this animal had close-to-perfect performance detecting target 4 kHz tones ('Hits', circles) and a low false alarm rate ('F+', triangles). However, this animal had a low response rate for tones of 20-40 dB SPL, resulting in a poor d' of 0.5. After measuring behavior for 30-60 minutes, we paired 4 kHz, 30 dB SPL tones with locus coeruleus stimulation for 3 minutes in the training chamber. We then re-tested detection abilities 12 hours later and found that detection at

20-40 dB SPL was greatly enhanced (Fig. IV-1b, red). Similar improvements in detection were observed for 16 animals (Fig. IV-1c). Remarkably, enhanced detection abilities lasted up to 20 days post-pairing (Fig. IV-1d). Behavioral changes were prevented when APV (1 mM) was infused into locus coeruleus before pairing (Fig. IV-1c, open), indicating that locus coeruleus plasticity is required for behavioral improvement. Thus locus coeruleus stimulation facilitates detection of previously-imperceptible stimuli, and brief episodes of pairing optimize signal processing and formation of sensorimotor associations.

We then asked how pairing affected abilities to distinguish targets from foils. Initially, foil stimuli were spectrally dissimilar from the target, separated at one octave intervals. Animals performed well on this 'wideband' task before pairing. We then compressed the spectral range of foils to resemble the target. Before pairing, behavioral performance on this 'narrowband' task was low (Fig. IV-2a), and pairing initially reduced recognition even further for a few hours (Fig. IV-2a,b). However, performance improved above baseline levels 12 hours post-pairing (Fig. IV-2c). These changes in auditory perception seem similar to AI modifications: tuning profiles first broadened (leading to similar neural responses for different sensory stimuli) before tuning width recovered and many more AI

neurons responded strongly to paired stimuli (facilitating detection and recognition of target tones). Changes in performance required locus coeruleus plasticity, and were prevented by infusion of APV into locus coeruleus(Fig. IV-2b,c, open).

To document the time course of behavioral enhancement more thoroughly, in our last experiment we asked whether locus coeruleus pairing might also accelerate perceptual learning. After initial training, the rewarded tone was switched to 16 kHz, 4 kHz became a foil, and one cohort of animals received locus coeruleus stimulation paired with 16 kHz tones. We monitored animals for several weeks afterward and found that while unpaired animals required >20 days to recover initial performance levels (Fig. IV-1e,g, black), paired animals learned the new association in almost half the time (Fig. IV-1f,g, red).

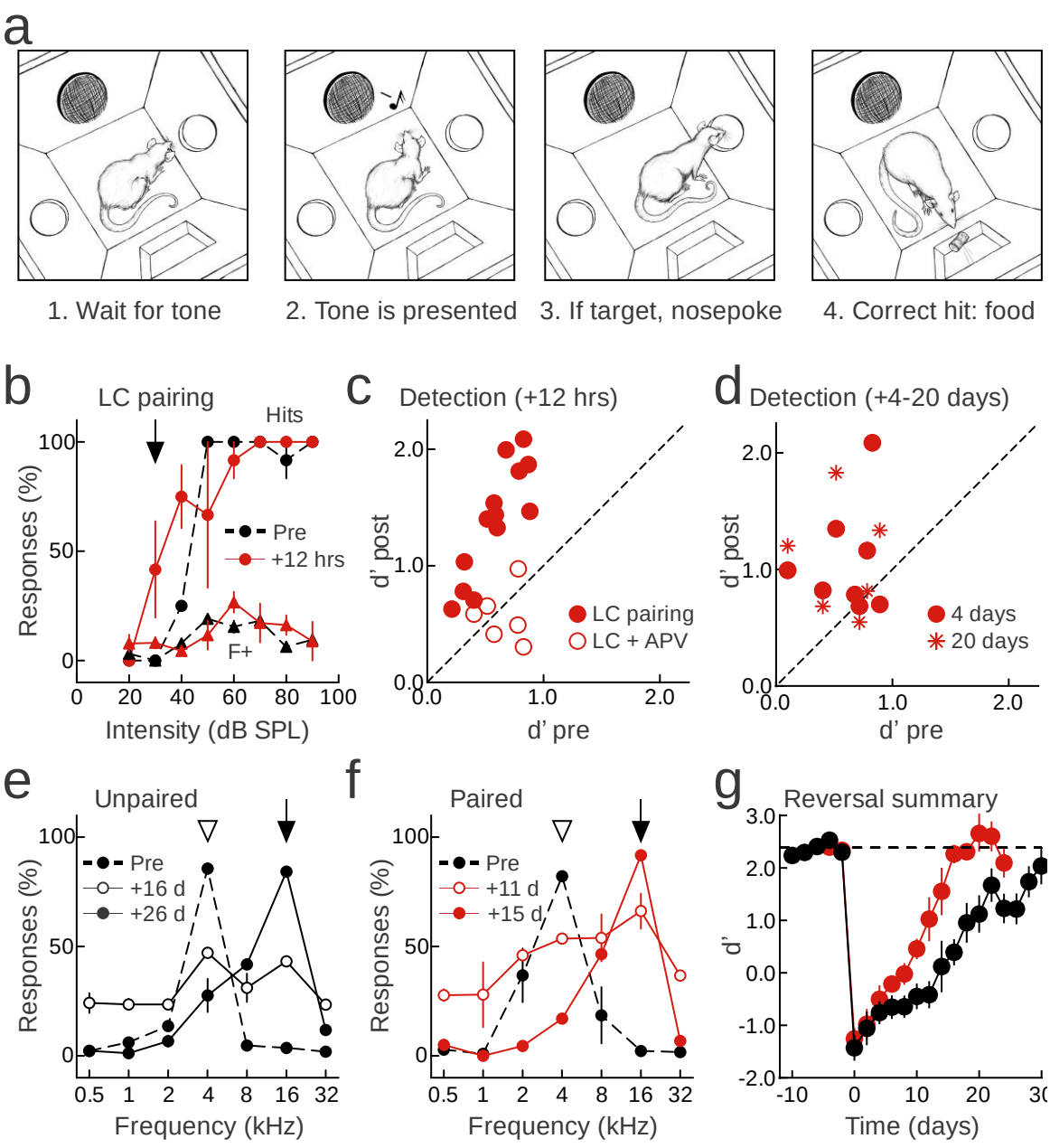


Figure IV-1. Locus coeruleus pairing improves sensory perception.

a, Behavioral task. b, Enhanced detection after pairing with 30 dB SPL, 4 kHz tone. Hits (circles) at 20-40 dB SPL were increased after 12 hours (pre-pairing, black: 8.3±5.3%, post-pairing, red: 43.7±14.0%, $p<0.05$); foil responses (triangles) were unchanged (false alarms at 20-40 dB SPL pre-pairing, black: 3.6±1.7%, post-pairing, red: 6.8±1.8%, $p>0.2$). 20-40 dB SPL

d' increased (0.5 to 1.4). c, d' values before and 12 hours after pairing (d' before: 0.57±0.06, after: 1.25±0.14, n=16 animals, $p<10^{-4}$). APV in locus coeruleus prevented improvement (open circles; d' before: 0.65±0.07, after: 0.57±0.10, n=6, p>0.5). d, Detection was enhanced four (circles; d' before: 0.61±0.09, after: 1.07±0.17, n=8, p<0.04) and 20 days after pairing (stars; d' before: 0.57±0.11, after: 1.04±0.19, n=6, p<0.05). e, Example of reversal learning without pairing. After learning to respond to 70 dB SPL, 4 kHz tones ('Pre', d': 2.67), rewarded frequency was changed to 16 kHz. After 16 days, hits increased, although false alarms were high (d': 0.39). After 26 days, performance recovered (d': 2.03). f, One pairing episode accelerated reversal learning. Before switching rewarded tone to 16 kHz, pairing with 16 kHz tones was performed for 3-5 minutes. After initial training ('Pre', d': 2.17), performance improved by day 11 (d': 0.64), and after 15 days, performance recovered (d': 2.50). g, Accelerated reversal learning after pairing. A sliding t-test (width: two days) was used to determine when performance recovered to baseline (black, control: 22 days, n=11; red, paired animals: 13 days, n=6).

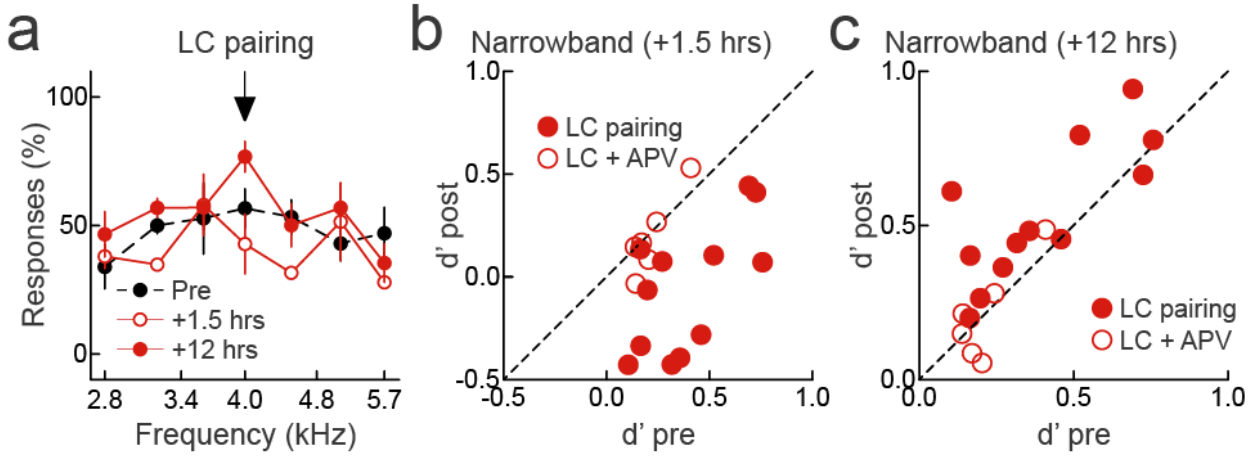


Figure IV-2. Frequency recognition abilities are first reduced before being enhanced by pairing. a, Pairing initially impaired and then improved 'narrowband' performance (d' pre: 0.52, 1-2 hours post: 0.11, 12 hours post: 0.79). b, Summary of impairment to recognition 1-2 hours after pairing (d' before: 0.39±0.07, after: −0.06±0.09, n=12, $p<10^{-4}$). APV infusion to locus coeruleus blocked effects of pairing (open circles; d' before: 0.22±0.04, after: 0.19±0.08, n=6, p>0.6). c, Summary of recognition enhancement 12 hours after pairing (d' after:0.53±0.07, p<0.02). Locus coeruleus infusion of APV prevented this (open circles; d' after:0.21±0.06, p>0.8).

Conclusion

In this chapter, the auditory perceptual consequences of Locus coeruleus pairing were studied. The findings presented suggest that the observations reported, in the recordings in the previous chapters, can indeed be tied to auditory perceptual changes. Recordings in primary auditory networks can be tied to we saw the initial tuning breakdown in tuning curves in primary auditory cortex. This may contribute to the interesting initial decrease in frequency discrimination performance observed in Figure IV-2a, post +1.5h. Furthermore at the 12h time point animal performance seems to increase from baseline, well in line with the recordings showing the 12h after Locus coeruleus stimulation, the new frequency preference is well established

and maintained both in cortex. and in the Locus Coeruleus. Furthermore the Locus coeruleus exhibits sensitization to the paired stimulus that remains for the duration of our recordings (~10h). This along with the fact that locus coeruleus plays may play a major role in adjusting gains of cortical synapses in a task-dependent manner in particular, higher-frequency phasic activity of noradrenegic neurons may facilitate the formation of task-specific behavioral patterns, to optimize perceptual abilities and motor outputs (Aston-Jones G et al., 1994; Usher M., et al., 1999, Yu A.J. and Dayan P., 2005, Kuo, S.P. and Trussel L.O., 2011), could explain the duration of the detection changes in Figure IV-1b. Furthermore, the powerful involvement of noradrenaline in learning and plasticity of cortical networks, allowing re-organization around behaviorally salient stimuli, could well explain the accelerated perceptual learning, in Figure IV-1g.

Interestingly comparing, again, these findings to the perceptual consequences of cholinergic Nucleus basalis stimulation, provides us with a nice multilevel relationship between synaptic, physiological, and functional roles of these two similar but very different neuromodulator systems.

In conclusion, the data presented in this chapter demonstrates that the Locus coeruleus improves perceptual abilities for at

least hours after pairing, and even longer-lasting gains in performance can emerge over days to weeks even after just a single pairing episode. These abilities can be enhanced beyond the gains induced by reward-based training alone, accelerated by neuromodulation and cortical plasticity.

Chapter V. Conclusion: Locus coeruleus Plasticity Controls Duration of Auditory Cortical Plasticity

Discussion

In the work presented in this thesis, I investigated the rules by which activation of neuromodulator systems, can interact with and change cortical networks. Furthermore I asked and explored the question of sensory sensitization of these nuclei themselves as an active part of a circuit engaged by a behaviorally relevant stimulus. Specifically I studied the noradrenergic Locus Coeruleus, and the cortical and subcortical synaptic effects of its stimulation at a high frequency, behaviorally-relevant phasic pattern, as well as the possible perceptual consequences.

The locus coeruleus is the principal source of noradrenergic modulation for the central nervous system, providing a fundamental mechanism for rapidly adapting cortical circuits to task demands. Here we showed that enduring contextual associations can be rapidly formed within the locus coeruleus, and neuromodulatory cells that were previously unresponsive to sounds can become sensitized and tonally responsive. The rapid

induction and prolonged duration of these changes to the central arousal system is reminiscent of changes to brain state and behavior in cases of one-trial learning or post-traumatic stress disorder, suggesting that locus coeruleus plasticity might be a fundamental determinant of these memory processes.

This plasticity of noradrenalin release exerts a profound effect on downstream neuromodulatory targets, controlling dynamics of cortical plasticity and auditory perceptual learning from induction to long-term maintenance. Noradrenergic modulation seems to provide a specific disinhibitory signal to cortical circuits, transiently reducing spontaneous inhibition in a manner distinct from that provided by cholinergic modulation from nucleus basalis, suggesting that separate neuromodulatory systems can differentially control neural circuits. Noradrenaline in particular seems to control overall gain of cortical neurons, transiently increasing the effective salience of incoming signals before more selective adjustments of cortical representations become manifest. Although these changes can initially impair some aspects of sensory perception, lasting behavioral enhancements can emerge over a period of hours to weeks, even after one pairing episode. While the full neural circuit representing learned associations between external stimuli and internal state is likely to be distributed

throughout much of the central nervous system, modifications to neuromodulator systems such as locus coeruleus provide a powerful mechanism for storing and restoring the most behaviorally-important memories.

Future Directions

While the work presented here was an extensive study of the question initially proposed, as with any interesting scientific finding, from these results many more avenues of exploration present themselves.

One major source variability might be the parameter space in which stimulation was performed. While the parameters used where robust, physiological and maintained constant throughout the work, one might wonder what would happen if parameters where changed: what if a different frequency of stimulation was used? How about changing the tone presentation latency relative to the stimulation?

The interesting question also comes about, of how it is that auditory responses emerge in the Locus Coeruleus? This nucleus has a notoriously expansive axonal network. What other auditory areas engaged by the behaviorally relevant stimulus, and receiving noradrenergic inputs from the Locus can change also? What are the inputs into the Locus that are changed and allow for auditory input to now reach and originate a response in these noradrenergic cells. Furthermore what is the role of the local inhibitory interneurons in this matter?

Moreover one can wonder, what is so special about the Locus Coeruleus? One quaint characteristic that comes to mind is the existence of gap junctions. What is their role in Locus coeruleus sensitization to sensory stimuli? Conversely, will we find any such type of sensitization if we record in other neuromodulatory nuclei.

Interestingly, Locus coeruleus axons synapse directly on astrocytic endfeet. What might be the contribution of astrocytes to the intensity or duration of the cortical effects observed?

Finally, behavior is a very rich field that can be expansively explored . In this work we used an operant task similar to the previously used with Nucleus basalis stimulation studies, in order to be able to compare the two systems. What if we tested our parameters with a different task. Or what if we tested different parameters with our task.

All these questions, important as they are, remain unanswered and will be the subject of future studies.

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