Synaptic Effects of Norepinephrine in Piriform Cortex

Michael C. Vanier*
Department of Computation and Neural Systems
California Institute of Technology
Pasadena, CA 91125
Email: *mvanier@bbb.caltech.edu*
Phone: (626) 395-6818
FAX: (626) 795-2088
URL: http://www.bbb.caltech.edu/USERS/mvanier

James M. Bower
Department of Computation and Neural Systems
California Institute of Technology
Pasadena, CA 91125
Email: *jbower@bbb.caltech.edu*
Phone: (626) 395-6817

Abstract

We recorded from a brain slice preparation of rat piriform (olfactory) cortex using extracellular techniques in order to characterize the effects of norepinephrine (NE) on synaptic transmission in this brain region. 25 μM NE was shown to cause a large decrease in synaptic field potentials in the association fiber layer of piriform cortex (layer 1b), whereas the same dose of NE caused a significant increase in field potential heights in the afferent fiber layer (layer 1a). The concentration-dependence of the NE effects were determined in each case. Pharmacological studies indicated that the NE effects in layer 1b are mediated primarily through a presynaptic effect dependent on α-2 adrenergic receptors, while layer 1a effects are dependent primarily on postsynaptic α-1 adrenergic receptors. NE was also shown to cause an increase in paired-pulse facilitation in layer 1b but not in layer 1a. We consider possible reasons for differences between these results and other investigations in the literature, and discuss the possible functional significance of these modulatory effects.

* To whom all correspondence should be addressed.

Theme: sensory systems
Topic: olfactory senses
Keywords: piriform cortex, norepinephrine, synaptic transmission
Acknowledgements: We would like to thank Karim Elaagouby for expert advice on experimental technique and slice chamber construction.
Contents: 23 pages, 6 figures, 0 tables

1 Introduction

Norepinephrine (NE) is a monoaminergic neuromodulator/neurotransmitter with a wide distribution throughout the CNS [13, 15]. NE can have a wide range of effects on cortical neurons, including changing neuronal excitability [37], increasing the strength and duration of LTP in the hippocampus [30, 31] and modulating ionic channels [17]. However, its computational roles in the brain remain unclear. There is evidence for involvement of NE in a variety of brain functions including memory [1, 12, 41, 46, 45, 55, 60], arousal [2, 40], and possibly modulation of neuronal signal-to-noise ratios [29, 48].

For the last several years our laboratory has been studying the possible functional effects of neuromodulators within the circuitry of the piriform (primary olfactory) cortex using a combination of physiological [27] and modeling [24] techniques. Piriform cortex has a characteristic three-layered structure that can be investigated directly using brain slice techniques [18, 19, 20, 51] (figure 1). Layer 1 consists primarily of afferent fibers to pyramidal neurons coming from olfactory bulb mitral cells via the lateral olfactory tract (layer 1a) and association fibers connecting pyramidal neurons with each other (layer 1b). Superficial pyramidal cells are located in layer 2 while deep pyramidal and excitatory and inhibitory multipolar cells are located in layer 3. This laminar arrangement makes it relatively easy to independently investigate the synaptic properties of the different fiber systems, since stimulating electrodes placed in layer 1a or 1b selectively activate afferent and association fiber synapses, respectively [27] (figure 1).

Both the circuitry of the olfactory cortex and the probable computational requirements of olfactory processing suggest that this structure may implement some form of associative memory [7, 18, 20, 24, 61]. We have previously reported [27] that the neuromodulator acetylcholine (ACh) can transiently decrease the strength of association fiber synapses while having no effect on afferent fiber synapses. In abstract models of piriform cortex [24], we found that this effect could reduce interference between newly-stored and old memories during an associative learning process, thus preserving the fidelity of previously-stored memories. Since NE is also strongly implicated in at least some memory processes (*e.g.* [10, 41, 53, 55]) and since piriform cortex receives a significant noradrenergic projection from forebrain neurons in the locus coeruleus (LC) [51, 52], we decided to characterize the effects of NE on synaptic transmission in piriform cortex.

In these studies we demonstrate a differential effect of NE on afferent and association fiber synapses. In agreement with a previous report [29], NE transiently and substantially decreased the size of field potentials in layer 1b, presumably reflecting a decrease in synaptic transmission. In contrast to previously published results, we found that NE caused a transient and significant increase in field potential amplitudes in layer 1a. Pharmacological studies indicated that the effects on association fiber synapses in layer 1b were primarily mediated by α-2 adrenergic receptors while the effects on afferent fiber synapses in layer 1a were primarily mediated by α-1 adrenergic receptors. In addition, application of NE increased the amplitude of paired-pulse facilitation (PPF) in layer 1b but had no effect on PPF in

layer 1a, similar to the effects we previously observed for ACh [27]. In the discussion we suggest possible reasons for the different results obtained here and in a previous investigation [29], and suggest possible computational roles for these effects of NE. A preliminary report on these results has appeared in abstract form [58].

2 Materials and Methods

2.1 Preparation of brain slices

All experiments were performed on brain slices prepared from female albino Sprague-Dawley rats as described in previous publications [26, 27]. Slices with a thickness of 400 μm were cut perpendicular to the laminar organization of piriform cortex in the coronal plane, using an oscillating tissue slicer (Vibratome). The location of the piriform cortex was determined visually using a rat brain atlas as a reference [42]. Slices for experiments in layer 1a were taken from the most rostral part of piriform cortex where layer 1a is the thickest. Slices for experiments in layer 1b were usually taken from rostral piriform cortex but occasionally from more caudal regions of piriform cortex. 1b results from rostral and caudal piriform cortex were essentially identical. Slices were maintained in an artificial cerebrospinal fluid (ACSF) solution ($NaHCO_3$ 26 mM; NaCl 124 mM; KCl 5 mM; KH_2PO_4 1.2 mM; $CaCl_2$ 2.4 mM; $MgSO_4$ 1.3 mM; glucose 10 mM) at room temperature for approximately two hours before beginning the experiments. Albumin (0.125 g/L) and kynurenic acid (0.66 mM) were added to the solution during this time but were not used in the solution bathing the slices during experiments.

Slices were placed in a submersion-type slice chamber on top of a small nylon mesh which kept both sides of the slice exposed to ACSF. ACSF was kept oxygenated with a 95% O_2/5% CO_2 mixture bubbled through the solution. The flow rate was 4 ml/min. The slice chamber included a heating element which kept ACSF maintained at a temperature of 33-35^o C. Slices were transilluminated, allowing visually-guided placement of stimulating and recording electrodes. Slices were left in the chamber for at least 15 minutes before commencing recording in order to wash off all traces of kynurenic acid, which would otherwise have interfered with the recordings due to its effects as a glutamate antagonist.

2.2 Preparation and application of pharmaceuticals

All pharmaceuticals were obtained from Sigma Chemical Co. and were freshly prepared before each experiment. Since norepinephrine and some other pharmaceuticals used are light-sensitive, recordings were done in darkness and drug solutions were kept in bottles covered with aluminum foil to block exposure to stray light. In addition, NE oxidizes rapidly when exposed to air, so 25 μM ascorbate was added to the solutions as an antioxidant. Ascorbate was also added to the regular ACSF (without added pharmaceuticals) as a control. Ascorbate by itself had no noticeable effect on the slices except that they stayed healthy for longer periods than slices without ascorbate.

2.3 Electrophysiology

Figure 1 shows the arrangement of stimulating and recording electrodes. On any given experiment, stimulating electrodes were placed in either or both of layers 1a and 1b. Monopolar or bipolar tungsten stimulating electrodes (Micro Probe Inc.)

with an impedance of 1-2 $M\Omega$ were used. Stimuli were low-amplitude (2-10 V) short duration (0.1 $msec$) voltage shocks, with voltages greater than 5 V being used only in layer 1a. Extracellular field potential recordings were obtained with glass electrodes filled with 3 M NaCl with impedances of $\sim$ 5 $M\Omega$. At the start of each experiment stimulating electrodes were placed either in layer 1a or layer 1b or both as shown in figure 1.

For recordings in layer 1a, slices were taken from the rostral piriform cortex exclusively, where layer 1a is the thickest. Stimulating electrodes were placed close to the lateral olfactory tract (LOT) and high in layer 1a (among the myelinated fibers of layer 1α [21]) to reduce the chance of inadvertently stimulating layer 1b fibers. Recording electrodes were placed some distance away from the stimulating electrode to minimize the possibility of inadvertently recording from the layer 1b region adjacent to the stimulating electrode, which, due to the relatively high stimulation voltages necessary to elicit 1a field potentials (typically 5-10 V), could respond to some extent to stimulation in layer 1a. Field potentials in layer 1b decrement rapidly with distance (data not shown) so placing the recording electrode some distance from the stimulating electrode in layer 1a minimizes contamination of the layer 1a field potential with layer 1b field potentials. This arrangement is feasible since the myelinated fibers at the surface of layer 1a conduct the stimulus for relatively long distances. In addition, this arrangement reduces the size of stimulation artifacts, which is helpful since layer 1a field potentials are typically somewhat smaller than those in layer 1b. For recordings in layer 1b, stimulating and recording electrodes were placed in the deepest part of layer 1b to reduce the possibility of inadvertently stimulating and recording from layer 1a as well as layer 1b. Stimulating electrodes were placed very close to the recording electrodes in layer 1b to give the largest signal. Typical field potential heights were 1 mV for layer 1b and 0.5 mV for layer 1a. The intertrial interval was 15 seconds.

Occasionally, simultaneous layer 1a/1b recordings were made. In order to ensure that the recordings in one layer were not contaminated by artifacts from stimulation in the other layer, stimulations were staggered in time by 7.5 seconds with respect to each other. These recordings gave identical results to recordings done only in layers 1a or 1b.

All field potentials were allowed to stabilize for at least 15 minutes before recording began. Once field potentials had stabilized, a baseline of 10 minutes (40 trials) was recorded. NE or NE agonists were then applied for 10 or 20 minutes and washed out for 30 minutes to assess recovery from the effects of the treatment. For experiments involving NE antagonists the sequence was: baseline, antagonist only (20 minutes), antagonist + NE (20 minutes). This sequence was done to ensure that the antagonists alone had no effect on field potential amplitudes, which was the case for all antagonists used. Occasionally at the end of an experiment the chamber was perfused with a low-calcium solution (100 μM $CaCl_2$, 8 mM $MgSO_4$) to eliminate synaptic potentials. This verified that the field potential was in fact due to synaptic transmission.

2.4 Data analysis

All data analysis was done using custom-written software. Field potential amplitude was measured in terms of both peak height and initial slope. Since these gave essentially identical results, peak heights were used exclusively in the data analysis. Results of a pharmacological treatment were expressed as the ratio of the average of the final ten trials during the treatment versus the average of the baseline trials. All pharmacological and dose-response treatments were done on at least four slices from at least three different rats (usually considerably more). All results are expressed as the mean ± the standard error of the mean (SEM).

The dose-response curves were fitted to theoretical curves of the following form:

$$f_{1a}(c) = (1+x) - \frac{x}{1+c/K_d} \quad (1)$$

$$f_{1b}(c) = x + \frac{(1-x)}{1+c/K_d} \quad (2)$$

where c represents the concentration of NE and x represents either the maximal (asymptotic) effect of NE expressed as a proportion of the total response (equation 1) or the proportion of the response insensitive to NE (equation 2). Equation 2 represents a single antagonist binding equation with a Hill coefficient of 1.0 [27], and was fit to the data from layer 1b. Equation 1 is identical to equation 2 except that x has been replaced with $1+x$ to give the proper limiting behavior; this equation was used to fit the layer 1a data. Curves were fit to the data using a nonlinear Levenburg-Marquardt algorithm [43].

3 Results

3.1 Synaptic effects of norepinephrine

Figure 2 shows the effects of 25 μM NE on the height of field potentials recorded in layers 1a and 1b of rat piriform cortex. NE causes a substantial decrease in the height of field potentials in layer 1b ($41.05 \pm 1.88\%$ of baseline, $n = 27$, $p < 0.001$). NE also causes a somewhat smaller increase in the height of field potentials in layer 1a ($124.52 \pm 4.89\%$, $n = 10$, $p < 0.001$).

Figure 3 shows the time course of the effects for each layer. The NE-induced decrease in synaptic transmission in layer 1b occurs very rapidly after NE is added to the superfusion medium (Figure 3A). The slight delay seen (less then 3 minutes) is due to the time required for the NE-containing ACSF to travel to the chamber. The NE effects in layer 1a (Figure 3B) have a somewhat longer latency (up to 4 minutes) and the field potentials rise more slowly to their maximum value. This difference may reflect a slower diffusion of NE to synapses in the myelin-rich region in layer 1a.

3.2 Concentration-dependence of effects

The effects of norepinephrine on field potentials in layers 1a and 1b were tested at a wide range of concentrations ranging from 0.2 μM to 500 μM. The effects of NE were tested on from 4-27 slices from at least three different rats per concentration. The results of these experiments are summarized in figure 4. The upper curve represents the effects of NE in layer 1a and the lower curve the effects of NE in layer 1b. The curves represent the optimal fits to the binding equation described in the Methods section, as determined by a nonlinear regression procedure. The asymptotically maximum increase in field potential height in layer 1a due to NE was 23.47% with a dissociation constant K_d of 8.82 μM. The concentration giving 50% of the maximum response according to this curve was 8.8 μM. The asymptotically maximum decrease in field potential height in layer 1b due to NE was 34.02% (this is the component of the response resistant to NE) with a dissociation constant K_d of 4.66 μM. The concentration giving 50% of the maximum response was 4.37 μM.

3.3 Pharmacology of effects

We examined the effects of a number of noradrenergic agonists and antagonists in order to determine the likely receptor type(s) responsible for the NE effects. The results, together with the effects of NE alone, are shown in figure 5.

In layer 1a, the β-agonist isoproterenol at 25 μM caused a small but significant rise in field potential heights ($105.22 \pm 1.42\%$ of baseline, $n = 6$, $p < 0.05$). The α-1 agonist phenylephrine (50 μM) reproduced the NE effect on field potentials ($122.83 \pm$ 5.31% of baseline, $n = 7$, $p < 0.01$). The α-2 agonist clonidine (25 μM) also caused a smaller but significant increase in layer 1a field potential heights ($113.04 \pm 1.28\%$ of baseline, $n = 5$, $p < 0.001$). However, the α-1 antagonist prazosin (2 μM) completely blocked the effect of 25 μM NE in layer 1a, while the α-2 blocker yohimbine (5 μM)

had virtually no effect, suggesting that the NE effect in layer 1a is primarily mediated by α-1 and not α-2 receptors.

In layer 1b, isoproterenol at 25 μM caused a significant *increase* in field potential heights ($113.14 \pm 3.65\%$ of baseline, $n = 10$, $p < 0.01$), in sharp contrast to the effects of NE. The α-2 agonist clonidine (25 μM) caused a decrease in layer 1b field potential heights to $77.85 \pm 2.38\%$ of baseline ($n = 6$, $p < 0.001$). In addition, the α-2 antagonist yohimbine (5 μM) almost completely blocked the effects of 25 μM NE. Interestingly, the α-1 agonist phenylephrine (50 μM) also caused a slight decrease in layer 1b field potentials to $84.49 \pm 2.49\%$ of baseline ($n = 5$, $p < 0.001$). However, the α-1 receptor antagonist prazosin (2 μM) was not able to block the NE effect in layer 1b at all. This suggests that the NE effect on layer 1b field potentials is primarily mediated by α-2 receptors.

3.4 Effects of norepinephrine on paired-pulse facilitation

Figure 6 presents the effects of NE on paired-pulse facilitation (PPF) in piriform cortex. Layer 1a field potentials typically exhibit a large degree of paired-pulse facilitation, whereas layer 1b field potentials show much less PPF [9]. The effects of 25 μM NE on PPF are shown in figure 6 for an interpulse interval of 50 μsec. NE had virtually no effect on PPF in layer 1a (PPF without NE: 1.487 ± 0.109; PPF with NE: 1.487 ± 0.055; $n = 4$), but caused a significant increase in PPF in layer 1b (PPF without NE: 1.197 ± 0.055; PPF with NE: 1.444 ± 0.042; $n = 10$, $p < 0.05$). As a result, the depression of field potential height with NE application is considerably reduced for the second pulse. Since PPF is a presynaptic effect [22, 35] this strongly suggests that the effect of NE on association fiber synapses in layer 1b is primarily presynaptic, since if NE was acting postsynaptically both pulses should have been affected equally [22]. The lack of effect of NE on PPF in layer 1a is completely consistent with the α-1 receptor-dependence of the effect, since α-1 receptors are generally located postsynaptically [6, 15].

4 Discussion

4.1 Differential Effects of NE on layer 1a and 1b field potentials

Our results show that norepinephrine causes a pronounced decrease in the height of synaptic field potentials in layer 1b of piriform cortex (figures 2 and 3). NE decreases the height of field potentials to about one-third of their original height at high doses with a half-maximal effect at 4.4 μM (figure 4). This effect is consistent with previous results we [58, 59] and others [29] have obtained. In contrast, norepinephrine causes a significant increase of about 25% in field potential heights in layer 1a of piriform cortex (figures 2 and 3). This effect is concentration-dependent with a half-maximal effect at 8.8 μM (figure 4), and has not been previously reported in the literature.

4.2 Pharmacological basis for the NE effects in layer 1a and 1b

The results in Figure 5 indicate that the effect of NE on layer 1b field potentials is most likely due to NE acting on an α-2 adrenergic receptor subtype. The α-2 agonist clonidine (25 μM) caused a decrease in field potential height to 77% of baseline. This is considerably less than the full NE effect; however, clonidine is very weakly water-soluble and thus it was difficult to precisely control the concentration of drug delivered to the chamber. Therefore, we may be overestimating the amount of clonidine that actually was in contact with the slices. In addition, clonidine is known to be a partial agonist for α-2 receptors [14, 44]. More significantly, the α-2 antagonist yohimbine (5 μM) almost completely blocked the effects of 25 μM NE. It is possible that some of the 1b effect can also be attributed to NE acting on α-1 receptors since the α-1 agonist phenylephrine (50 μM) also caused a slight decrease in 1b field potentials. However, in contrast to the α-2 receptor results, the α-1 receptor antagonist prazosin (2 μM) was not able to block the NE effect at all. α-2 receptors generally have a presynaptic inhibitory effect [6, 15, 32, 56, 57] which suggests that the effects of NE on synapses in layer 1b are also presynaptic (see below).

In contrast to layer 1b, the effect of NE on layer 1a field potentials appears to be due to NE acting on an α-1 receptor subtype. The α-1 agonist phenylephrine (50 μM) fully reproduced the NE effect (122% of baseline). Clonidine (25 μM) also caused a smaller but significant increase in layer 1a field potential heights (113% of baseline). However, the α-1 antagonist prazosin (2 μM) completely blocked the effect of 25 μM NE in layer 1a, while the α-2 blocker yohimbine (5 μM) had virtually no effect, suggesting that the NE effect is primarily mediated by α-1 and not α-2 receptors. α-1 receptors are generally located postsynaptically [6, 15] suggesting that the effects of NE in layer 1a are also postsynaptic (see below).

There is no compelling evidence that the NE effects we observed in either layer 1a or layer 1b are mediated to any significant degree by β adrenergic receptors. The β agonist isoproterenol caused a significant *increase* in field potential heights in layer 1b, which is the exact opposite of the effects of NE. However, the size of the increase was fairly small (only about 13% of baseline). It is thus quite possible that the α-2

receptor-mediated suppression of field potentials we postulate are actually stronger than we observed, being masked to some extent by the β receptor-mediated increase in field potential heights. β agonists also cause a small but significant increase in layer 1a field potentials; thus, we cannot rule out the possibility that some of the NE effects on layer 1a field potentials may be mediated by NE acting on β receptors.

From the pharmacological data we also conclude that the NE effects on layer 1a neurons are likely to be mediated postsynaptically, while the effects in layer 1b are most likely to be presynaptic. The evidence we present here for this conclusion is circumstantial, but plausible. First, α-1 receptors, of the sort implicated in the layer 1a responses, are generally found postsynaptically [47, 54], whereas the α-2 receptors mediating the layer 1b effects are usually found in the presynaptic terminal [6, 15, 32, 56, 57]. These assumptions are also consistent with our experimental results from paired-pulse facilation (PPF). PPF is generally believed to rely on a presynaptic mechanism [22, 35], and NE has no effect on PPF in layer 1a (proposed postsynaptic receptors) but does affect PPF in layer 1b (proposed presynaptic receptors). Thus the pharmacology and the PPF data both suggest a second fundamental difference between the effects of NE in these two populations of synapses.

4.3 Differences from previously-reported results

In contrast to the results reported here, Hasselmo et. al. [29] reported that application of NE in layer 1a caused no effect on layer 1a field potentials for low concentrations and a slight decrease for higher concentrations. Collins et. al. [11] using transverse slices also suggested a concentration-dependent effect of NE, but in their case low concentrations were reported to result in an increase in evoked potentials, while higher concentrations produced a decrease. It is difficult to directly relate the results from different slice preparations; we suspect that the recordings of Collins et. al. combined both 1a and 1b field potentials to varying extents, making a direct comparison with our results impossible. The concentrations used in the experiments described here bracketed those used in both of the previous studies.

In our data it is quite clear that the effects of NE on field potentials in layer 1a are more variable than in layer 1b, ranging from a very slight increase to increases of over 200% in some slices. Nevertheless, our data show that the only effect of NE on layer 1a field potentials is an increase in field potential amplitude regardless of the concentration. We found, however, that extreme care must be taken to assure that layer 1a recording conditions are consistent and optimal. For example, because the size of layer 1a decreases substantially in caudal piriform cortex it is almost impossible to correctly place stimulating and recording electrodes in this region. Improper placement can easily lead to confusion between layer 1a (enhanced) and layer 1b (suppressed) responses. Additionally, even with optimal placement stimulation may spread from layer 1a to layer 1b, making it very difficult to interpret the resulting field potential recordings. For this reason, in the current experiments, all the layer 1a data reported was obtained from rostral slices where layer 1a is much thicker. In addition, care was taken to place the stimulating and recording electrodes as far apart as possible but still within layer 1a. Restriction to layer 1a

was also verified using paired-pulse facilitation which has previously been shown to be strong in this layer [9]. Typically, when any of these recording conditions were not met, the effects of NE on layer 1a field potentials were greatly diminished or absent.

4.4 Functional significance

Piriform cortex is a popular area for computational modeling and is considered by many to be a good candidate for a biological model of associative memory [20, 27, 61]. Neuromodulators such as NE and acetylcholine have long been linked to learning and memory effects (*e.g.* [5, 12, 41, 45, 55, 60]) although it is still far from clear how these effects are mediated at the level of single cell biophysics or network learning mechanisms. We and others have demonstrated, however, that these modulators can have strong effects on the behavior of synapses and cells in piriform cortex [3, 4, 16, 27, 28, 23, 25, 29, 36, 38, 39, 49, 50].

Taken in the context of our efforts to build realistic models of the olfactory cortex [8, 33, 34, 61], the differential biophysical effects of both ACh and now NE on the two principle sources of excitatory inputs on the apical dendrites of pyramidal cells can be proposed to have a direct effect on the way in which this network processes incoming sensory data [7]. Specifically, we have proposed that ACh, which suppresses layer 1b association fiber synapses, may serve to make the piriform cortical network more responsive to olfactory afferent sensory inputs than to internal dynamics. We show here that NE both suppresses association fiber synaptic transmission and enhances afferent fiber synaptic transmission. Thus it would appear that NE provides a more extreme form of regulation than ACh although with effects in the same direction. Realistic modeling efforts currently underway in our laboratory will use the data presented in this paper as well as previously published results on ACh and NE to more directly contrast the consequences of these two important neuromodulators on cortical function.

References

[1] A. F. T. Arnsten and J. D. Jentsch, The alpha-1-adrenergic agonist, cirazoline, impairs spatial working-memory performance in aged monkeys, *Pharmacology Biochemistry and Behavior*, 58 (1997), 55–59.

[2] G. Aston-Jones, C. Chiang, and T. Alexinsky, Discharge of noradrenergic locus-ceruleus neurons in behaving rats and monkeys suggests a role in vigilance, *Progress in Brain Research*, 88 (1991), 501–520.

[3] E. Barkai, R. E. Bergman, G. Horwitz, and M. E. Hasselmo, Modulation of associative memory function in a biophysical simulation of rat piriform cortex, *Journal of Neurophysiology*, 72 (1994), 659–677.

[4] E. Barkai and M. E. Hasselmo, Modulation of the input/output function of rat piriform cortex pyramidal cells, *Journal of Neurophysiology*, 72 (1994), 644–658.

[5] A. Blokland, Acetylcholine: a neurotransmitter for learning and memory?, *Brain Research Reviews*, 21 (1995), 285–300.

[6] A. Borsodi and M. Wollemann, α-adrenergic receptors, in A. Lajtha, ed., Handbook of Neurochemistry, 2nd. Ed., vol. 6, Plenum Press, New York, 1982, 27–38.

[7] J. M. Bower, Reverse engineering the nervous system: an in vito, in vitro, and in computo approach to understanding the mammalian olfactory system, in C. L. S. F. Zornetzer, T. M. McKenna, ed., An Introduction to Neural and Electronic Networks, 2nd. Ed., Academic Press, San Diego, 1994, 27–38.

[8] J. M. Bower and D. Beeman, The Book of Genesis, Springer Verlag, New York, 1995.

[9] J. M. Bower and L. B. Haberly, Facilitating and nonfacilitating synapses on pyramidal cells - a correlation between physiology and morphology, *Proceedings of The National Academy of Sciences of The United States of America*, 83 (1986), 1115–1119.

[10] F. Camacho, C. P. Smith, H. M. Vargas, and J. T. Winslow, Alpha(2)-adrenoceptor antagonists potentiate acetylcholinesterase inhibitor effects on passive-avoidance learning in the rat, *Psychopharmacology*, 124 (1996), 347–354.

[11] G. G. S. Collins, G. A. Probett, J. Anson, and N. J. Mclaughlin, Excitatory and inhibitory effects of noradrenaline on synaptic transmission in the rat olfactory cortex slice, *Brain Research*, 294 (1984), 211–223.

[12] D. M. Compton, K. L. Dietrich, J. S. Smith, and B. K. Davis, Spatial and nonspatial learning in the rat following lesions to the nucleus locus-coeruleus, *Neuroreport*, 7 (1995), 177–182.

[13] J. R. Cooper, F. E. Bloom, and R. H. Roth, The Biochemical Basis of Neuropharmacology, Oxford University Press, New York, 1996.

[14] T. J. Feuerstein, W. Sauermann, C. Allgaier, E. Agneter, and E. A. Singer, New insights into receptor theory, as provided by an artificial partial agonist made-to-measure, *Naunyn Schmiedebergs Archives of Pharmacology*, 350 (1994), 1–9.

[15] M. Fillenz, Noradrenergic neurons, Cambridge University Press, Cambridge, 1990.

[16] R. L. Gellman and G. K. Aghajanian, Pyramidal cells in piriform cortex receive a convergence of inputs from monoamine activated GABAergic interneurons, *Brain Research*, 600 (1993), 63–73.

[17] R. Gray and D. Johnston, Noradrenaline and beta-adrenoceptor agonists increase activity of voltage-dependent calcium channels in hippocampal-neurons, *Nature*, 327 (1987), 620–622.

[18] L. B. Haberly, Neuronal circuitry in olfactory cortex - anatomy and functional implications, *Chemical Senses*, 10 (1985), 219–238.

[19] L. B. Haberly and J. M. Bower, Analysis of association fiber system in piriform cortex with intracellular-recording and staining techniques, *Journal of Neurophysiology*, 51 (1984), 90–112.

[20] L. B. Haberly and J. M. Bower., Olfactory cortex - model circuit for study of associative memory?, *Trends In Neurosciences*, 12 (1989), 258–264.

[21] L. B. Haberly and S. L. Feig, Structure of the piriform cortex of the opossum. 2. Fine structure of cell bodies and neuropil, *Journal of Comparative Neurology*, 216 (1983), 69–88.

[22] E. W. Harris and C. W. Cotman, Effects of synaptic antagonists on perforant path paired-pulse plasticity - differentiation of presynaptic and postsynaptic antagonism, *Brain Research*, 334 (1985), 348–353.

[23] M. E. Hasselmo, Acetylcholine and learning in a cortical associative memory, *Neural Computation*, 5 (1993), 32–44.

[24] M. E. Hasselmo, B. P. Anderson, and J. M. Bower, Cholinergic modulation of cortical associative memory function, *Journal of Neurophysiology*, 67 (1992), 1230–1246.

[25] M. E. Hasselmo and E. Barkai, Cholinergic modulation of activity-dependent synaptic plasticity in the piriform cortex and associative memory function in a network biophysical simulation, *Journal of Neuroscience*, 15 (1995), 6592–6604.

[26] M. E. Hasselmo and J. M. Bower, Afferent and association fiber differences in short-term potentiation in piriform (olfactory) cortex of the rat, *Journal of Neurophysiology*, 64 (1990), 179–190.

[27] M. E. Hasselmo and J. M. Bower, Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex, *Journal of Neurophysiology*, 67 (1992), 1222–1229.

[28] M. E. Hasselmo and J. M. Bower, Acetylcholine and memory, *Trends In Neurosciences*, 16 (1993), 218–222.

[29] M. E. Hasselmo, C. Linster, M. Patil, D. Ma, and M. Cekic, Noradrenergic suppression of synaptic transmission may influence cortical signal-to-noise ratio, *Journal of Neurophysiology*, 77 (1997), 3326–3339.

[30] W. F. Hopkins and D. Johnston, Frequency-dependent noradrenergic modulation of long-term potentiation in the hippocampus, *Science*, 226 (1984), 350–352.

[31] W. F. Hopkins and D. Johnston, Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus, *Journal of Neurophysiology*, 59 (1988), 667–687.

[32] Y. Kamisaki, T. Hamahashi, C. M. Okada, and T. Itoh, Clonidine inhibition of potassium-evoked release of glutamate and aspartate from rat cortical synaptosomes, *Brain Research*, 568 (1991), 193–198.

[33] C. Koch and I. Segev, Methods in Neuronal Modeling, MIT Press, Cambridge, Massachusetts, 1989.

[34] C. Koch and I. Segev, Methods in Neuronal Modeling, 2nd. Ed., MIT Press, Cambridge, Massachusetts, 1997.

[35] S. W. Kuffler, J. G. Nicholls, and A. R. Martin, From Neuron to Brain: A Cellular Approach to the Function of the Nervous System, Sinauer Associates, Sunderland, Massachusetts, 1984.

[36] H. Liljenstrom and M. E. Hasselmo, Cholinergic modulation of cortical oscillatory dynamics, *Journal of Neurophysiology*, 74 (1995), 288–297.

[37] D. V. Madison and R. A. Nicoll, Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus, *Nature*, 299 (1982), 636–638.

[38] G. J. Marek and G. K. Aghajanian, Excitation of interneurons in piriform cortex by 5-hydroxytryptamine - blockade by MDL-100,907, a highly selective 5-HT2a receptor antagonist, *European Journal of Pharmacology*, 259 (1994), 137–141.

[39] G. J. Marek and G. K. Aghajanian, Alpha(1B)-adrenoceptor-mediated excitation of piriform cortical interneurons, *European Journal of Pharmacology*, 305 (1996), 95–100.

[40] D. A. McCormick, H. C. Pape, and A. Williamson, Actions of norepinephrine in the cerebral-cortex and thalamus - implications for function of the central noradrenergic system, *Progress in Brain Research*, 88 (1991), 293–305.

[41] M. Ohno, M. Kobayashi, A. Kishi, and S. Watanabe, Working-memory failure by combined blockade of muscarinic and beta-adrenergic transmission in the rat hippocampus, *Neuroreport*, 8 (1997), 1571–1575.

[42] G. Paxinos and C. Watson, The rat brain in stereotaxic coordinates, Academic Press, San Diego, 1997.

[43] W. H. Press, S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery, Numerical Recipes in C, 2nd. Ed., Cambridge University Press, New York, 1992.

[44] K. Rasmussen, M. Brodsky, and C. E. Inturrisi, NMDA antagonists and clonidine block C-FOS expression during morphine withdrawal, *Synapse*, 20 (1995), 68–74.

[45] S. J. Sara, C. Dyonlaurent, and A. Herve, Novelty seeking behavior in the rat is dependent upon the integrity of the noradrenergic system, *Cognitive Brain Research*, 2 (1995), 181–187.

[46] S. J. Sara, A. Vankov, and A. Herve, Locus coeruleus-evoked responses in behaving rats - a clue to the role of noradrenaline in memory, *Brain Research Bulletin*, 35 (1994), 457–465.

[47] M. Segal, H. Markram, and G. Richterlevin, Actions of norepinephrine in the rat hippocampus, *Progress In Brain Research*, 88 (1991), 323–330.

[48] D. Servan-Schreiber, H. Printz, and J. D. Cohen, A network model of catecholamine effects - gain, signal-to-noise ratio, and behavior, *Science*, 249 (1990), 892–895.

[49] P. W. Sheldon and G. K. Aghajanian, Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex - evidence for the involvement of a 5-HT2-activated interneuron, *Brain Research*, 506 (1990), 62–69.

[50] P. W. Sheldon and G. K. Aghajanian, Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex - evidence for mediation by 5-HT1c receptors in pyramidal cells and 5-HT2 receptors in interneurons, *Synapse*, 9 (1991), 208–218.

[51] G. R. Shepherd, ed., The Synaptic Organization of the Brain, Oxford University Press, New York, 1990.

[52] M. T. Shipley and M. Ennis, Functional organization of olfactory system, *Journal of neurobiology*, 30 (1996), 123–176.

[53] L. R. Squire, Memory and Brain, Oxford University Press, New York, 1987.

[54] I. Takayanagi and K. Koike, Effects of aging on postsynaptic alpha-1-adrenoceptor mechanisms in rat aorta, *General Pharmacology*, 22 (1991), 211–218.

[55] S. A. Thomas and R. D. Palmiter, Disruption of the dopamine-beta-hydroxylase gene in mice suggests roles for norepinephrine in motor function, learning, and memory, *Behavioral Neuroscience*, 111 (1997), 579–589.

[56] A. U. Trendelenburg, K. Starke, and N. Limberger, Presynaptic alpha(2a)-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices, *Naunyn Schmiedebergs Archives of Pharmacology*, 350 (1994), 473–481.

[57] P. Q. Trombley, Noradrenergic modulation of synaptic transmission between olfactory-bulb neurons in culture - implications to olfactory learning, *Brain Research Bulletin*, 35 (1994), 473–484.

[58] M. Vanier and J. M. Bower, Differential effects of norepinephrine on field potentials in layers 1a and 1b of rat olfactory cortex, *Soc. Neuroscience Abstracts*, 18 (1992), 1353.

[59] M. Vanier and J. M. Bower, Differential effects of norepinephrine on synaptic transmission in layers 1a and 1b of rat olfactory cortex, in Computation and Neural Systems, Kluwer Academic Publishers, Boston, 1993, 267–271.

[60] D. A. Wilson, T. C. Pham, and R. M. Sullivan, Norepinephrine and posttraining memory consolidation in neonatal rats, *Behavioral Neuroscience*, 108 (1994), 1053–1058.

[61] M. Wilson and J. M. Bower, Cortical oscillations and temporal interactions in a computer simulation of piriform cortex, *Journal of Neurophysiology*, 67 (1992), 981–995.

Figure legends

Fig. 1: A schematic diagram of the piriform cortex and the setup of the recording and stimulating electrodes. Pyramidal cells are shaded gray. Note that the stimulating electrode in layer 1a is relatively far from the recording electrode in layer 1a, while the stimulating electrode in layer 1b is close to the recording electrode in layer 1b. Abbreviations: LOT, lateral olfactory tract. See text for details.

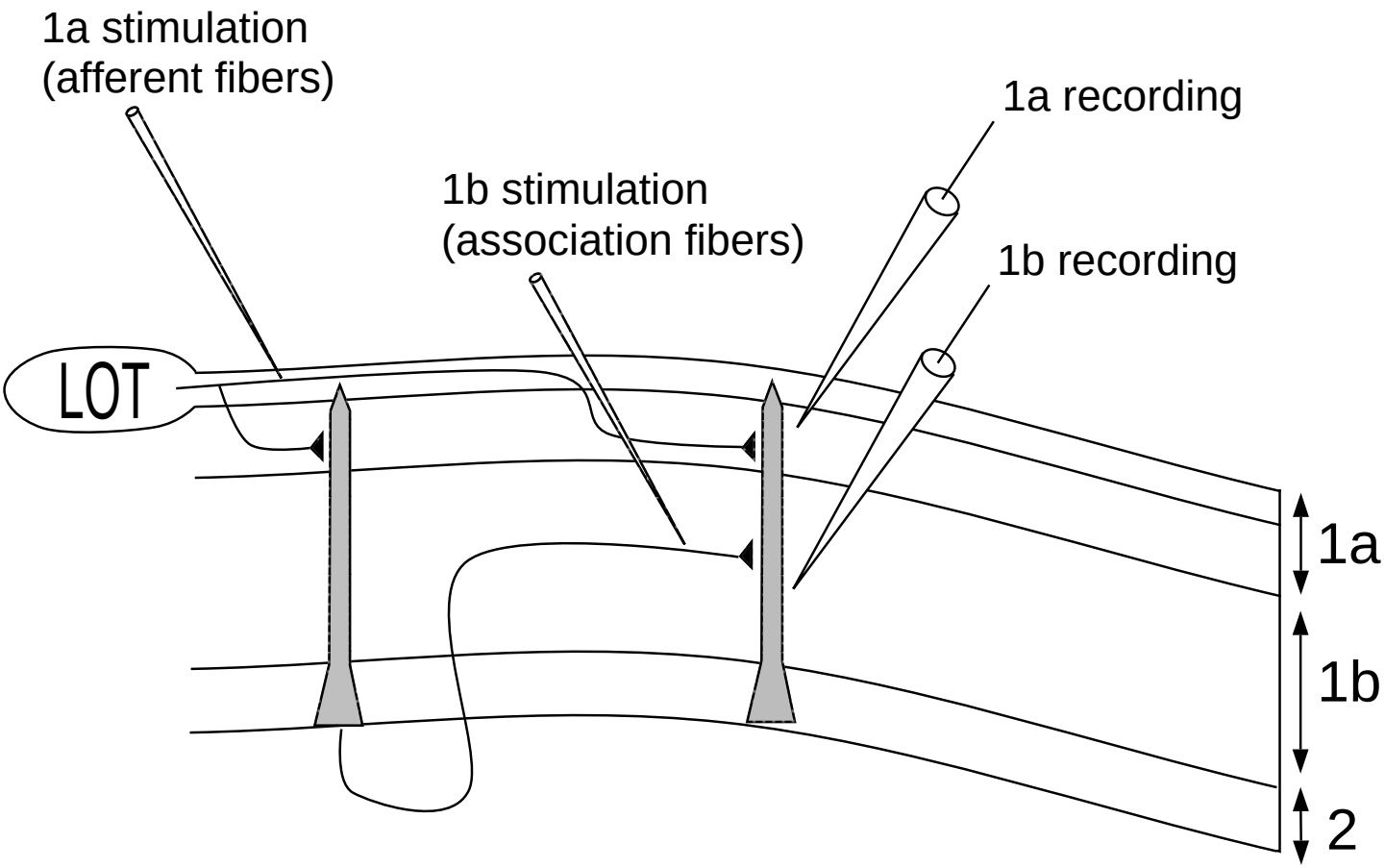
Fig. 2: The effect of 25 μM NE on extracellular field potentials in layers 1a and 1b of piriform cortex. Stimulation artifacts have been removed. NE causes a pronounced depression in the magnitude of the field potential in layer 1b, and a smaller but significant increase in the magnitude of the field potential in layer 1a. Both effects are reversible. Horizontal bar: 5 $msec$. Vertical bar: 0.2 mV.

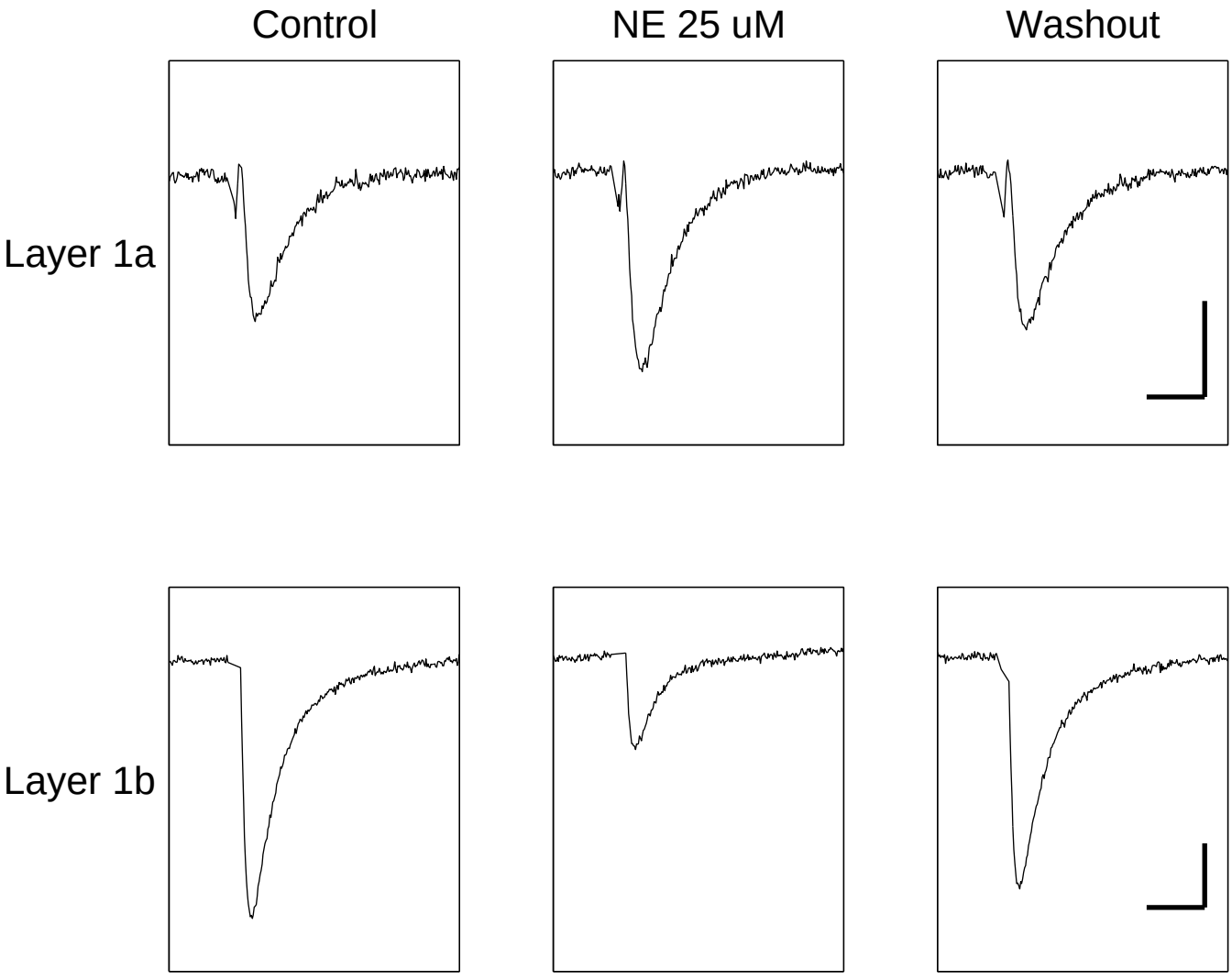
Fig. 3: The time-course of the NE effect in layers 1a and 1b, relative to baseline. Layer 1a data are displayed with open circles while layer 1b data are displayed with filled circles. The dark bar shows the duration of NE application (10 minutes at 25 μM).

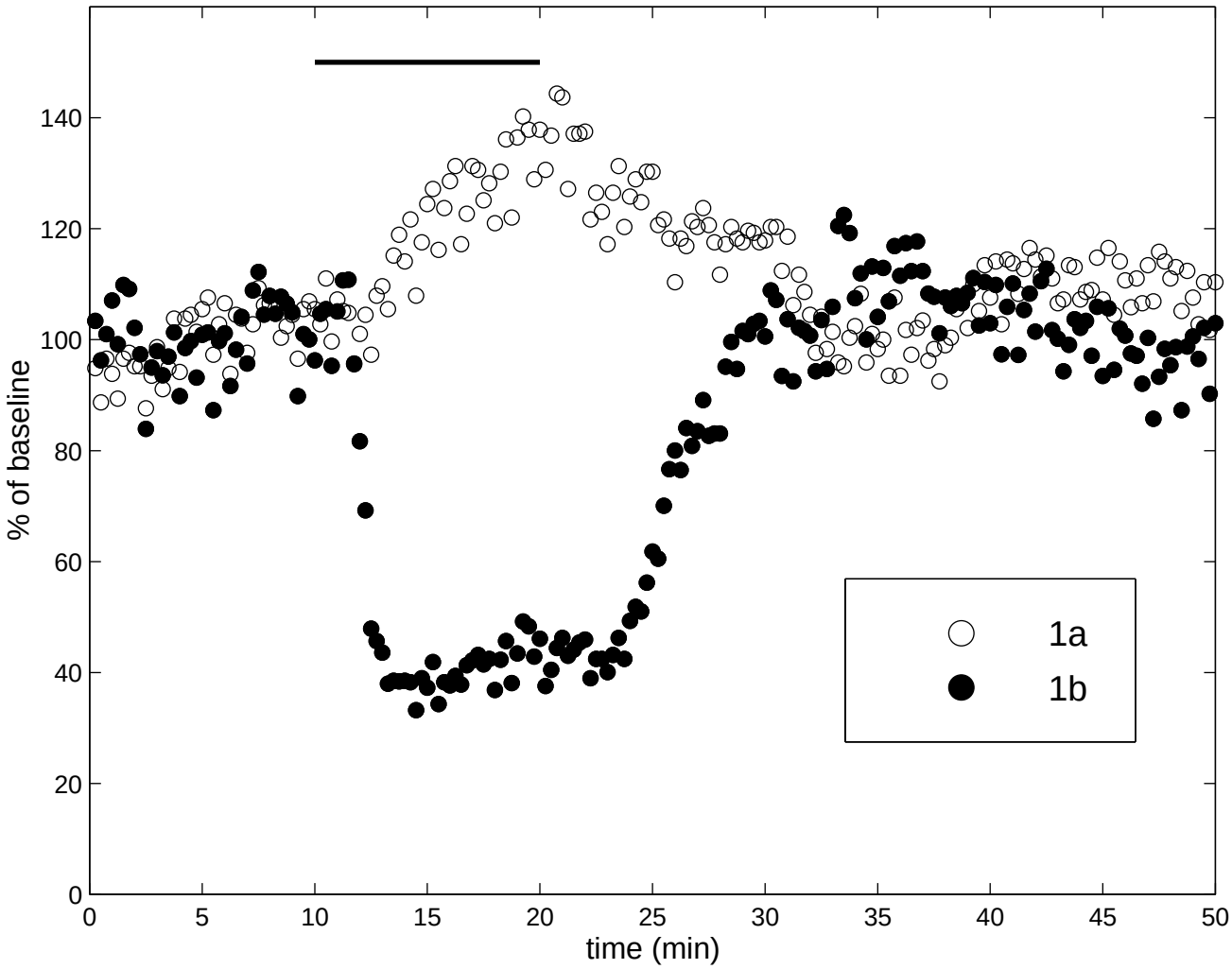
Fig. 4: Dose-response curve of NE effect in layers 1a and 1b, relative to baseline. The curves were calculated using a nonlinear regression procedure described in the Methods section. Error bars represent standard errors of the mean (SEM). 1a data are displayed with open circles while 1b data are displayed with filled circles.

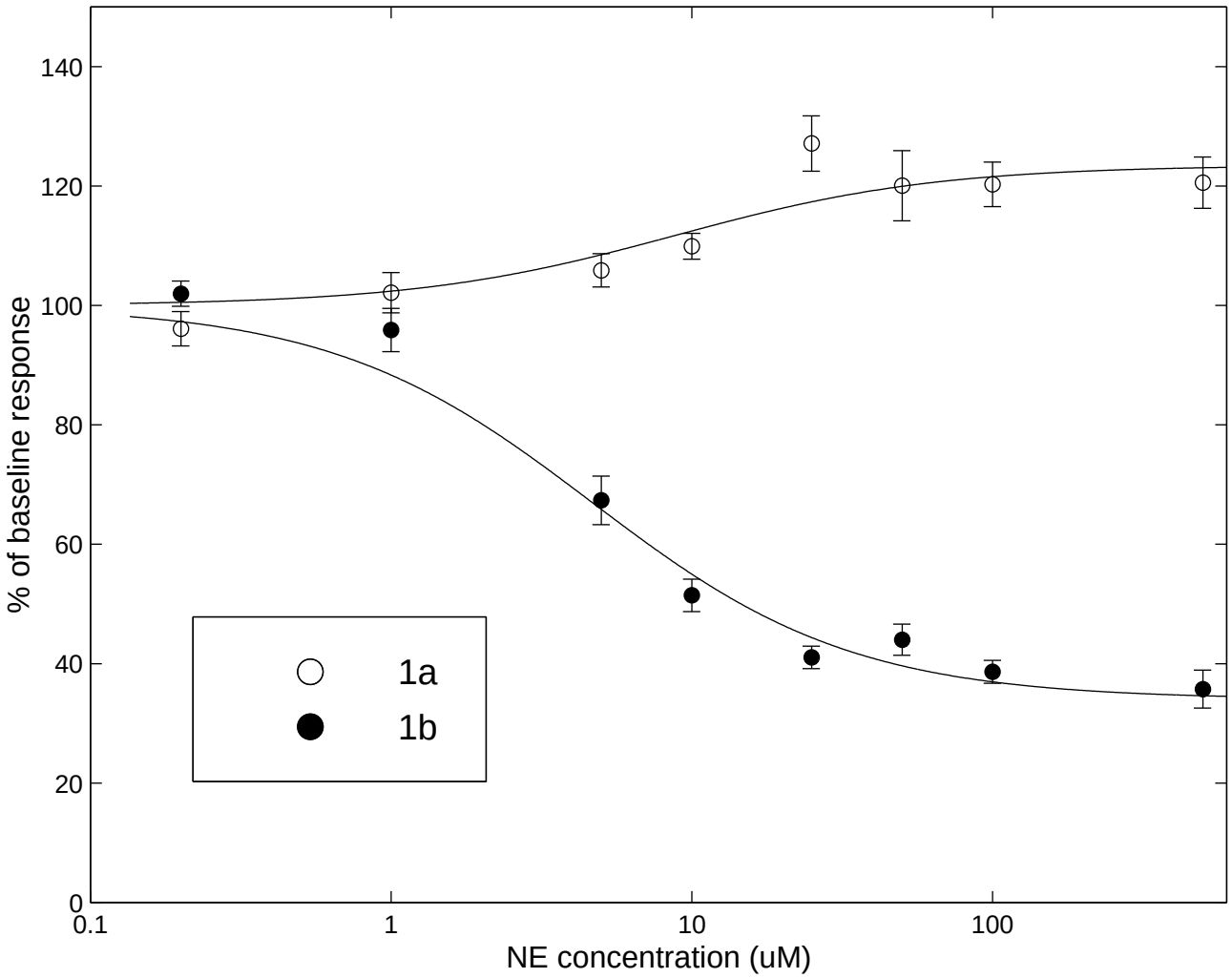
Fig. 5: Effects of various pharmacological agents on 1a and 1b field potential heights relative to baseline. Error bars represent standard errors of the mean (SEM). Abbreviations: NE, norepinephrine 25 μM; iso, isoproterenol 25 μM; phen, phenylephrine 50 μM; clo, clonidine 25 μM; NE/praz, norepinephrine 25 μM and prazosin 2 μM; NE/yoh, norepinephrine 25 μM and yohimbine 5 μM.

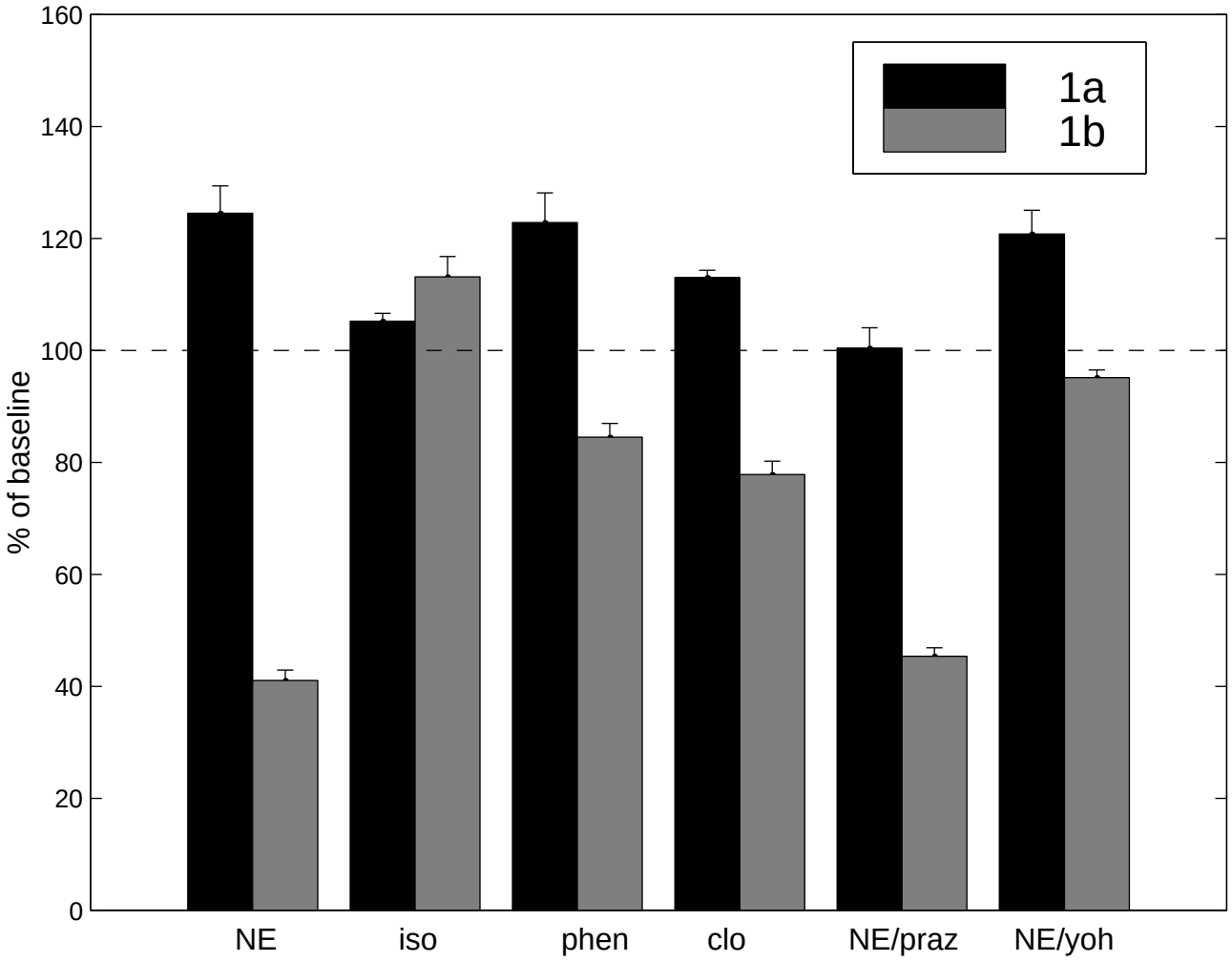
Fig. 6: Effects of NE on paired-pulse facilitation (PPF) in layers 1a and 1b. The traces have been normalized so that the first pulse is of a constant height for comparison. The horizontal scale bar represents 20 $msec$ while the vertical scale bar represents 200 μV. Pulses were 50 $msec$ apart. Note that NE increases PPF in layer 1b but not in layer 1a.

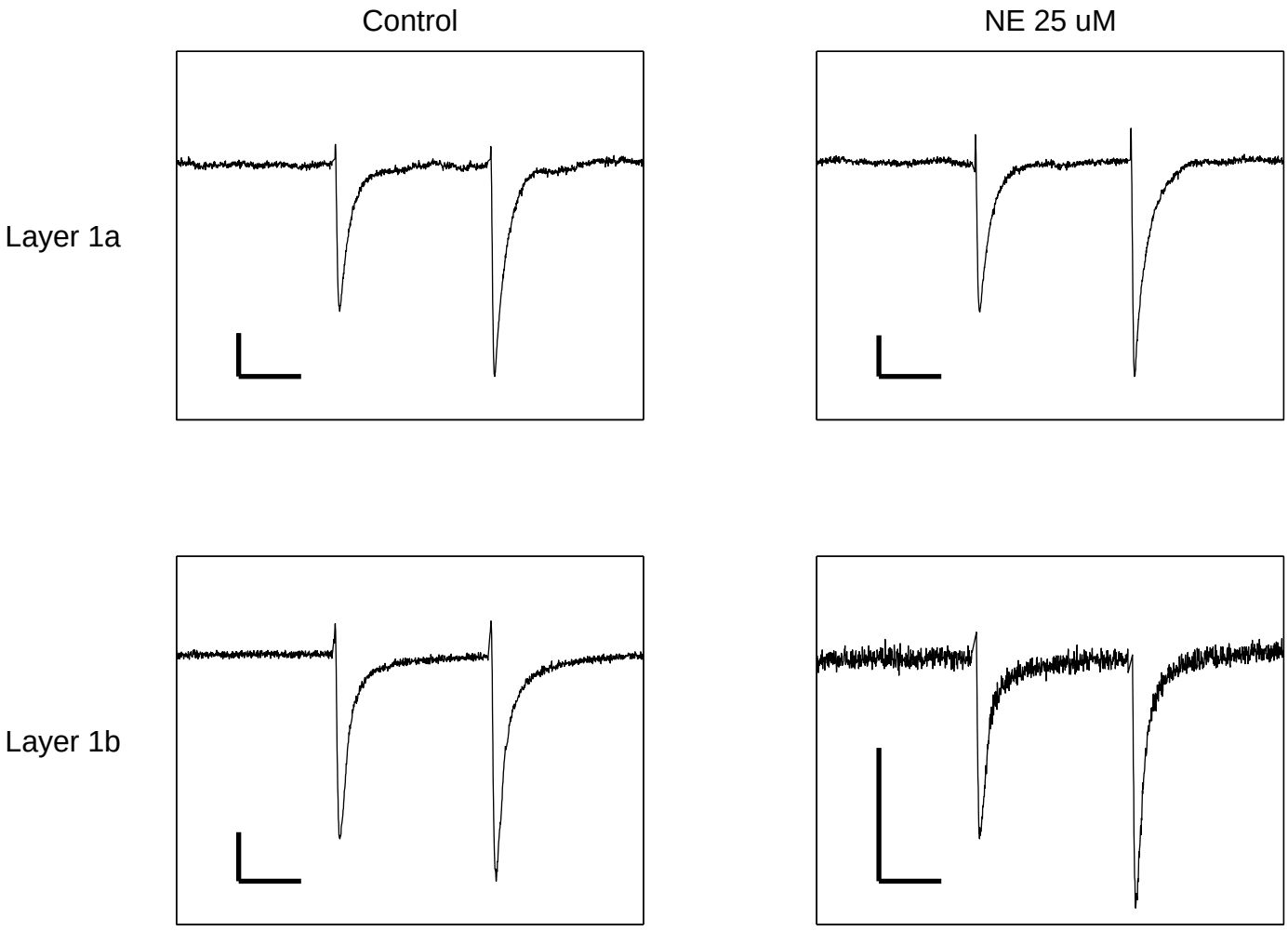

1a stimulation
(afferent fibers)
1a recording
1b stimulation
(association fibers)
1b recording
LOT
1a
1b
2


Control
NE 25 uM
Washout
Layer 1a
Layer 1b


% of baseline
time (min)
0
20
40
60
80
100
120
140
0
5
10
15
20
25
30
35
40
45
50
1a
1b


% of baseline response
NE concentration (uM)
0
20
40
60
80
100
120
140
0.1
1
10
100
1a
1b


160
140
120
100
80
60
40
20
0
% of baseline
1a
1b
NE
iso
phen
clo
NE/praz
NE/yoh


Control
NE 25 uM
Layer 1a
Layer 1b