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Input-Specific Effects of Acetylcholine on Sensory and Intracortical Evoked

Responses in the "Barrel Cortex" *in vivo*

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Abstract

The somatosensory neocortex processes extrinsic information from the thalamus and intrinsic information from local circuits. We compared the effects of acetylcholine (Ach) on neocortical field potential responses evoked by stimulation of the whiskers and by local electrical stimulation in the upper layers of the neocortex vibrissae representation ("barrel cortex") of adult rats anesthetized with urethane. The two responses differed in terms of their frequency-dependent dynamics. In particular, the whisker response depressed when stimulated repetitively, whereas the intracortical response did not. The cholinergic system was manipulated in the barrel cortex by application of exogenous Ach using microdialysis, by increasing the endogenous levels of Ach with physostigmine and by applying specific cholinergic agonists. The results revealed that Ach selectively enhances the sensory response relative to the intracortical response via a nicotinic effect. Thus, pathways in the barrel cortex are differentially regulated by cholinergic inputs.

Abrégé

Le néocortex somatosensoriel traite de l'information extrinsèque du thalamus et de l'information intrinsèque de circuits locaux. Nous avons comparé les effets de l'acétylcholine (Ach) sur les réponses potentielles de zone néocorticale évoquées par stimulation des moustaches et par stimulation électrique locale dans les couches supérieures de la représentation de vibrissae du néocortex ("cortex baril") de rats adultes anesthésiés avec uréthane. Les deux réponses ont différé quant à leur dynamique fréquence-dépendante. En particulier, la réponse de la moustache fut réduite lorsque stimulée répétitivement, alors que ceci n'était pas le cas avec la réponse intra-corticale. Le système cholinergique a été manipulé dans le cortex baril en utilisant la microdialyse par application exogène de l'Ach, en augmentant les niveaux endogènes de l'Ach avec physostigmine et en appliquant des agonistes cholinergiques spécifiques. Les résultats ont indiqué que l'Ach augmente sélectivement la réponse sensorielle relativement à la réponse intra-cortical via un effet nicotinique. Ainsi, des voies dans le cortex baril sont réglées différemment par des entrées cholinergiques.

INTRODUCTION

The somatosensory cortex processes extrinsic information originating from the peripheral environment as well as intrinsic information from within the cortex. Extrinsic information reaches the cortex via thalamocortical synapses, whereas intrinsic information is locally distributed via intracortical synapses. The purpose of the current project is twofold: 1) to examine the short-term dynamics of these two pathways *in vivo* and 2) to determine how they are modulated by the presence of acetylcholine (Ach). Ach is an important neuromodulator that is known to produce slow and long lasting effects in many brain regions. It is projected diffusely to the neocortex from the basal forebrain and is implicated in many important brain functions. Various behavioral states modulate basal forebrain activity, which in turn modulate cortical levels of Ach. Our long-term goal will be to understand the functional role of the differential regulation of neocortical inputs by Ach.

The thalamocortical pathway we have chosen to study is that arising from the mystacial vibrissae. Rats utilize their whiskers to actively explore their environment. A behavior called whisking (Welker, 1964; Wineski, 1983) is characterized by movement of the posterior vibrissae at a frequency of approximately 8 Hz (Carvell & Simons, 1990). The rhythmicity of the tactile behavior increases the temporal sampling rate of an object and thereby facilitates object recognition (Carvell & Simons, 1990). Remarkably, rats using their whiskers have similar discrimination abilities as primates using their fingertips.

Information concerning a rats somatosensory environment is transmitted from external tactile receptors to the neocortex via three synapses, located sequentially in the ipsilateral trigeminal brainstem, the contralateral thalamus and the contralateral somatosensory cortex. The thalamus serves as the main gateway into the cortex and is therefore important in controlling and modulating the amount of information reaching the cortex. Although some subcortical fibers have direct cortical terminations, such as fibers from the basal forebrain (Divac, 1975; Kievit & Kuypers, 1975), most reach the cortex via nuclei in the dorsal thalamus. All projecting thalamocortical nuclei of the dorsal thalamus can be divided into three groups based on their layers of termination: specific, intralaminar and paralaminar (Herkenham, 1986). These groups differ in the specificity and location of terminating fibers. Of particular interest to the somatosensory system is the specific group, which transmits primary somatosensory information. In particular, tactile information from the whiskers is projected via the ventroposterior medial (VPM) nucleus of the thalamus to the posteriomedial barrel subfield (PMBSF) of the primary somatosensory cortex (S1). These fibers terminate densely in layer IV and lower layer III, and less densely in upper layer VI. In addition to the specific thalamic nucleus, a parallel thalamocortical pathway projects from the paralaminar group. The medial division of the posterior complex (Pom) projects fibers which terminate in layers I and V, and the interbarrel septa of layer IV (Lu & Lin, 1993). This pathway provides secondary somatosensory information that is complementary to the primary information projected from VPM. In contrast to VPM, the activity of Pom is strongly regulated by descending corticothalamic influences (Diamond, 1995). The intralaminar nuclei are non-specific

nuclei carrying information from multiple sensory modalities. They have widespread projections, terminating mainly in layers V and VI (Herkenham, 1986).

Thalamocortical afferents from VPM terminate in layer IV in discrete cell clusters, which visually resemble barrels (Woolsey & Van de Loos, 1970). The cell-rich "barrels" are separated by cell-poor interbarrel "septa". These cytoarchitecturally distinct domains receive afferents from VPM and Pom, respectively (Lu & Lin, 1993). Both anatomical (Killackey, 1973) and electrophysiological (Simon, 1978) techniques have demonstrated a one to one correspondence between whiskers on the rats face and "barrels" in the contralateral somatosensory cortex, such that neurons in a particular barrel respond maximally (shortest latency and largest amplitude) to stimulation of its principle whisker (Armstrong-James & Fox, 1987).

All cell types within layer IV, including pyramidal cells, spiny stellate and inhibitory interneurons receive direct thalamocortical contact. In addition, apical dendrites of infragranular neurons and basal dendrites of supragranular neurons make synaptic contact with thalamocortical fibers (White, 1986). This neuronal arrangement within individual barrels gives rise to a larger structure called a "barrel column", which extends vertically throughout layers I to VI. Cells throughout the depth of a column respond preferentially to stimulation of a single whisker (Simons, 1978; Woolsey & Van der Loos, 1970). This enables incoming information to be distributed throughout the layers of a column (Mountcastle, 1957; Chmielowska et al., 1986), with a preference towards supragranular layers (Kim & Ebner, 1999). Despite the variety of cells receiving thalamocortical contacts, specificity is evidenced by the number and proportion of thalamocortical synapses with each neuronal type (White, 1989).

In order for normal processing to occur tactile information from neighboring whiskers must be integrated. The horizontal flow of information is primarily achieved through horizontal connections in upper layers II/III and lower layer V, which connect neighboring barrel columns (Hoeflinger et al., 1995; Johnson & Alloway, 1996; Kim & Ebner, 1999). Connections between barrels occur predominately between those of the same row (Bernardo et al., 1990; Hoeflinger et al., 1995). In addition, a dense network of fibers exists linking interbarrel septa (Kim & Ebner, 1999). These two distinct horizontal pathways appear to be differentially modulated by VPM and Pom respectively, and therefore possibly code different features of whisker stimulation. Due to these intracortical networks, longer latency responses following whisker stimulation likely result from a combination of horizontal disynaptic connections (Kim & Ebner, 1999) and direct monosynaptic thalamocortical connections (Petersen & Diamond, 2000) from non-principle whiskers.

CORTICAL NEURONS

Neocortical neurons can be divided using a broad range of criteria including morphological, electrophysiological and molecular characteristics. Broadly speaking, cortical neurons are either pyramidal or nonpyramidal. In addition, nonpyramidal cells can be further distinguished as spiny or non-spiny (including, sparsely spiny). These different cell types can be further classified by their different intrinsic membrane properties (Connors & Gutnick, 1990). Pyramidal cells make up the majority of cortical neurons and include excitatory regular spiking (RS) and intrinsically bursting (IB) neurons (McCormick et al., 1985). Nonpyramidal cells include inhibitory neurons such

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as non-spiny fast spiking cells (FS), and low-threshold spiking (LTS) cells (Kawaguchi, 1993). Furthermore, nonpyramidal cells also include excitatory neurons such as regular spiking spiny stellate cells found in layer IV of sensory areas.

Pyramidal Neurons

RS cells are characterized by broad action potentials due to a slow rate of repolarization. A suprathreshold current injection results in a train of action potentials that display different degrees of frequency adaptation depending on the neuron (Chen et al., 1996; McCormick et al., 1985). The majority of RS cells are pyramidal in shape and are densely located throughout layers II to VI (McCormick et al., 1985). The axon collaterals of RS cells generally ascend and branch in the superficial layers (Chagnac-Amitai et al., 1990).

In contrast, IB cells are characterized by their ability to generate bursts of 3-5 action potentials upon depolarization from hyperpolarized resting potentials (McCormick et al., 1985). The bursts are generated by a low-threshold Ca^{2+} conductance (Chen et al., 1996). IB cells are located predominately within layers V and IV (Chagnac-Amitai et al., 1990; McCormick et al., 1985), and their axons project horizontally within layers V and VI (Chagnac-Amitai et al., 1990). Both IB and RS pyramidal cells are believed to utilize glutamate as a their main excitatory neurotransmitter (Dori et al., 1989).

Nonpyramidal Neurons

The majority of nonpyramidal neurons in the neocortex are inhibitory as indicated by the presence of γ-aminobutyric acid (GABA) (Houser et al., 1983; Keller & White,

1986, 1987). FS and LTS cells are both included in this group, however they differ in their intrinsic membrane properties, molecular markers, distribution and axonal morphology. FS cells stain for the enzyme parvalbumin and are characterized by a rapid action potential due to a fast rate of repolarization (McCormick et al., 1985; Kawaguchi and Kubota, 1997). A suprathreshold depolarizing current pulse results in a train of spikes with little frequency adaptation. In contrast, LTS cells stain for calbindin and somatostatin and display a low-threshold spike in response to a small depolarizing current pulse from a negative potential (Kawaguchi, 1993; Kawaguchi and Kubota, 1997). FS cells are found throughout the layers of the neocortex (McCormick et al., 1985) and their axons extend primarily in the horizontal direction. In contrast, in the frontal cortex LTS cells have been localized primarily within layers V and VI (Kawaguchi, 1993) with a smaller population in other layers (Kawaguchi, 1995). Their axons extend preferentially in the vertical domain. In somatosensory cortex, both FS and LTS cells are also found in layer IV (Gibson et al., 1999).

In addition to inhibitory neurons, there is a group of excitatory nonpyramidal neurons referred to as spiny stellate cells. These cells are glutamatergic (Kaneko & Mizuno, 1996; White, 1989) and display RS properties. Spiny stellate cells are the main recipients of thalamocortical synapses and therefore are located primarily within layer IV (White, 1989), but are also found in layer VI (Kaneko & Mizuno, 1996) and layers II-III (Kawaguchi, 1995). Their axons and dendrites are largely confined to their parent barrel, thus strengthening the one to one correspondence between whiskers and barrels (Harris & Woolsey, 1983).

Thalamocortical Input

In the somatosensory cortex, layer IV contains inhibitory FS cells, LTS cells and excitatory spiny stellate cells, as well as the apical dendrites of infragranular pyramidal neurons and the basal dendrites of supragranular pyramidal neurons. Based on morphological (White, 1989) and electrophysiological (Gibson et al., 1999) studies, thalamic input is strong for spiny stellate RS cells and especially for FS cells. In contrast, LTS cells seem to be devoid of thalamic input. Moreover, spiny stellate RS cells strongly activate FS cells but not LTS cells. RS cells robustly activate LTS cells only at high frequencies due to facilitation. Thus, a thalamocortical input initially activates a network consisting of interconnected RS spiny stellate cells and FS cells which in turn disynaptically excite and inhibit each other. In contrast, LTS cells and IB cells are absent from this network until activity spreads to them through intracortical fibers.

SHORT-TERM PLASTICITY IN THE SOMATOSENSORY SYSTEM

Neocortical activity is influenced by stimulus repetition whereby stimulation of nuclei within the dorsal thalamus produces frequency-dependent responses in the neocortex (Dempsey & Morison, 1942a, 1942b, 1943; Morison & Dempsey, 1942, 1943). Morison & Dempsey described three characteristic response patterns following stimulation of thalamic nuclei: the primary response, the augmenting response and the recruiting response. The activity displayed by a neocortical region depends upon the origin of the projecting fibers, such that converging thalamocortical fibers may exhibit distinct spatiotemporal response characteristics (Castro-Alamancos & Connors, 1996b). Of particular interest to the somatosensory system is the primary response. This is a short

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latency biphasic response (surface positive-negative) resulting from monosynaptic thalamocortical projections from specific thalamic nuclei. Current source density (CSD) analysis following stimulation of ventrobasal thalamus (VB) demonstrate two current generators located in layer IV and upper layer VI (Castro-Alamancos & Connors, 1996b).

The Decremental Response

Repetitive stimulation of VB (>5 Hz) causes the primary response in S1 to exhibit strong depression both *in vivo* (Castro-Alamancos & Connors, 1996b) and *in vitro* (Castro-Alamancos, 1997). This depression has been named the decremental response (Castro-Alamancos & Connors, 1996b). Although the mechanisms causing frequency-dependent depression are unknown, several possibilities have been proposed (Castro-Alamancos, 1997).

First, excitatory postsynaptic potentials (EPSPs) may be reduced by a disynaptic inhibitory GABAergic pathway that is activated monosynaptically by thalamocortical fibers (Simon & Carvell, 1989; Staiger et al., 1996; Swadlow, 1995). In other words, subsequent excitatory input may be shunted. In fact, intracellular recordings following stimulation of specific thalamic nuclei have demonstrated inhibitory postsynaptic potentials (IPSPs) (<200 msec), which correspond in time to the early phase of depression (Castro-Alamancos & Connors, 1996a,c). Further, application of a $GABA_A$ antagonist (bicuculline methiodide; BMI) *in vivo*, abolishes the frequency-dependent depression at short inter-stimulus intervals (Castro-Alamancos, 1997).

Second, frequency-dependent depression may be a consequence of a high probability of neurotransmitter release from synaptic terminals. In such cases, a single

stimulus would reduce the pool of available releasable neurotransmitter. In general, synapses with a high probability of release display frequency-dependent depression due to a transient depletion in available neurotransmitter (Stevens & Wang, 1995). In fact, it has been recently demonstrated in a slice preparation that thalamocortical synapses have a greater probability of release, relative to intracortical synapses (Gil et al., 1999). The short-term dynamics of these two pathways correspond to what would be expected given differences in release probability. In particular, the thalamocortical synapses display depression whereas the intracortical synapses display either slight facilitation or modest depression (Gil et al., 1997).

Third, a modulatory substance may also act on thalamocortical nerve terminals causing a decrease in neurotransmitter release probability and resulting in a decremental response. In slice preparations, GABA and other neurotransmitters have been shown to act presynaptically to produce frequency-dependent depression of IPSPs (Deisz & Prince, 1989) and EPSPs (Kang, 1995). Thus, depression observed at longer inter-stimulus intervals may be due to substances acting presynaptically to modulate the probability of release (Castro-Alamancos, 1997).

The frequency-dependent depression observed at specific thalamocortical pathways may result from a combination of presynaptic, postsynaptic and circuit properties. The mechanisms described above are not mutually exclusive and it is likely that they all contribute to the decremental response.

Like VB stimulation, repetitive sensory stimulation produces frequency-dependent depression of evoked responses in S1. Repetitive stimulation of individual whiskers is characterized by a decrease in responsiveness in the neocortex. S1 neurons are generally only responsive to low frequency whisker stimulation and often respond exclusively to the first stimulus presented (Ahissar, 2000; Simons, 1978; Swadlow, 1989). Field potential responses evoked in S1 by whisker stimulation depress at frequencies above 5 Hz (Sheth et al., 1998). Intracellular recordings indicate that the period of depression corresponds roughly to the duration of cortical IPSPs (Carvell & Simons, 1988). Similar to decremental responses evoked by electrical stimulation of VB, whisker stimulation also evokes a short latency EPSP followed by a longer lasting IPSP. Interestingly, stimulation of an adjacent whisker can also elicit IPSPs in the barrel of the principal whisker (Carvell & Simons, 1988), and this reduces unit discharges to a subsequent deflection of the principal whisker in a time-dependent fashion (Simons, 1985; Simons & Carvell, 1989). However, it is important to note that cross-whisker interactions produce effects that differ from those observed by stimulating the same whisker repetitively.

Frequency-dependent depression in response to sensory stimulation will be referred to as sensory suppression. Sensory suppression has been observed in the visual, auditory and somatosensory systems of rodents, cats and monkeys (Kilgard & Merzenich, 1998; Laskin & Spencer, 1979; Nelson, 1991; Recanzone et al., 1992). One of the main consequences of sensory suppression (Sheth et al, 1998), and also of decremental responses evoked by VB stimulation (Castro-Alamancos and Connors, 1996b) is a

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reduction in the cortical representation of the stimulus. Sheth et al. (1998) used optical imaging *in vivo* to examine the spatial spread of activation following repetitive stimulation of whiskers at frequencies of 1, 5 and 10 Hz. Although stimulation at 1 Hz produced diffuse cortical activation, 5 and 10 Hz stimulation reduced the area activated by individual whiskers. Field potential recordings made from the center and periphery of the representation demonstrated that stimulation above 5 Hz virtually eliminated the response in the periphery, while the center of the representation produced a consistent response. Thus, high frequency whisker stimulation seems to both decrease sensitivity and increase specificity of incoming input, allowing for a greater relative signal from primary vibrissa (Moore et al., 1999).

The mechanisms contributing to sensory suppression are likely to involve both cortical and subcortical processes. At the level of the cortex, the cellular mechanisms are probably similar to those mediating decremental responses in primary sensory pathways. As described above, thalamocortical-activated disynaptic IPSPs, stimulus-induced release of neuromodulatory substances and/or excitatory synapses with high probability of transmitter release, likely contribute to the sensory suppression produced by high frequency whisker stimulation.

Additionally, sensory suppression may result from a reduction of thalamocortical activity, due to whisker evoked depression of thalamic responses. In fact, both VPM and Pom neurons depress when stimulated at frequencies above 5 Hz (Ahissar, 2000; Diamond et al., 1992). Depression of VPM neurons to repetitive whisker stimulation will have a great impact on cortical sensory suppression because they carry the input producing the primary response in S1. In addition, the even stronger frequency-

dependent depression found in Pom neurons (Ahissar, 2000; Diamond et al., 1992) could also contribute to cortical sensory suppression, but only for long latency cortical responses because Pom neurons discharge much later than the primary cortical response. This longer latency suppression may contribute to sensory suppression of the inter-barrel septa areas, which are the primary targets of Pom neurons (Lu & Lin, 1993).

In conclusion, the whisker evoked sensory pathway displays sensory suppression, such that the magnitude of the response to the second stimulus in a pair varies with inter-stimulus interval. These results have been documented in anesthetized animals. Work done in behaving animals however, illustrate that the thalamocortical response properties are not static, but can be modulated with behavioral state.

Modulation of Short-term Plasticity

Short-term plasticity of thalamocortical pathways can be dynamically modulated with behavioral state (Castro-Alamancos & Connors, 1996a; Fanselow & Nicolelis, 1999). For example, stimulating the ventrolateral nucleus of the thalamus at frequencies ranging from 7-14 Hz causes an enhanced response for each successive stimulus, relative to the first (Castro-Alamancos & Connors, 1996a, b, c). This augmenting response is robustly modulated with behavioral state, such that it is maximal when the animal is resting, but strongly suppressed when the animal is active (Castro-Alamancos & Connors, 1996a).

More recently, Fanselow & Nicolelis (1999) examined behavioral modulation of tactile responses. Both the primary response and the frequency-dependent depression were modulated with behavioral state. The primary response evoked in S1 following a

single stimulation was reduced during periods of vibrissal movements (whisking and whisker-twitching), as opposed to quiet or anesthetized states. Interestingly however, during these active states, the frequency-dependent depression was almost abolished (Fanselow & Nicolelis, 1999). This suggests that the somatosensory system is able to modulate the effectiveness of tactile information in order to optimize the transfer of information depending upon the behavioral state of the animal.

It seems that behavioral state strongly influences both primary responses and short-term plasticity of thalamocortical connections. The goal of the current project is to examine whether Ach modulates these response properties as well.

ACETYLCHOLINE

Neuromodulators influence the strength of synapses and produce effects on the firing properties and excitability of neurons. Ach is an important neuromodulator in the cerebral cortex. It has been implicated in many important brain functions, such as arousal (Celesia & Jasper, 1966), attention (Muir et al., 1994; for a review see Sarter & Bruno, 2000) and sensory discrimination (Jacobs & Juliano, 1995). It is projected from the nucleus basalis of the basal forebrain (Eckenstein et al., 1988; Mesulam et al., 1983; Mesulan & Van Hoesen, 1976) and terminates diffusely throughout the cerebral cortex. Examining the distribution of the catabolic enzyme, acetylcholinesterase (AchE), and the synthesizing enzyme, choline acetyltransferase (ChAT), provide an index of Ach availability. Both staining procedures have illustrated similar laminar patterns of Ach distribution in S1, such that high densities of both markers have been observed in layers

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V and I, whereas lower densities were observed in layers II, III and VI (Houser et al., 1985; Kristt, 1979a, b).

Ach acts on both muscarinic and nicotinic receptors. Muscarinic receptors are distributed profusely throughout the cerebral cortex and are generally present in both superficial and deep cortical layers (Lidow et al., 1989; Sahin et al., 1992). In general, the receptors are localized postsynaptically on cortical neurons (Houser et al., 1985; McCormick & Prince, 1985). However, evidence exists for presynaptic localization on intrinsic (Sahin et al., 1992), thalamocortical (Vogt, 1984), and basal forebrain-cholinergic (Mash & Potter, 1986) fibers in the cortex.

Nicotinic receptors have been localized to the outer part of layer I, layers III and IV and upper layer VI (Clarke et al., 1985; Fuchs, 1989; Sahin et al., 1992). They are found presynaptically on thalamocortical fibers (Sahin et al., 1992). In fact, electrophysiological studies have shown nicotine to be important in modulating neurotransmitter release at thalamocortical synapses (Gil et al., 1997; Gioanni, 1999), as well as at intracortical synapses (Vidal & Changeux, 1993). Additionally, they have been localized to inhibitory interneurons in the neocortex (McCormick & Prince, 1985; Xiang et al., 1998).

ACETYLCHOLINE ACTIONS IN THE SOMATOSENSORY CORTEX

Application of Ach to cortical neurons produces excitation to spontaneously active and silent neurons in the infragranular (Bessant et al., 1990; Lamour et al., 1983) and supragranular layers of the neocortex (Donoghue & Carroll, 1987). Nonetheless, some inhibition has also been reported in layer IV (Donoghue & Carrell, 1987; Sillito &

Kemp, 1983). These changes in excitability are often accompanied by an increase in responsiveness of neurons to sensory stimulation (Donoghue & Carroll, 1987; Lamour et al., 1988; Metherate et al., 1987, 1988; Sato et al., 1987) decreasing the stimulation necessary to produce a response. Ach has also been shown to increase receptive field size and to uncover hidden peripheral receptive fields (Metherate et al., 1988).

Pyramidal Neurons

Ach enhances neuronal activity in the neocortex by depolarizing pyramidal neurons (for a review see McCormick 1992). The excitatory effect of Ach has been attributed to muscarinic receptor activation, resulting in a reduction of potassium conductance and an increase in input resistance (Krnjevic et al., 1971; McCormick & Prince, 1985; McCormick & Williamson, 1989). Ach produces numerous effects on excitatory neurons throughout the cortex. However, not all of the effects of Ach have been revealed. A diverse population of pyramidal neurons exists and many differ on the basis of their electrophysiological properties. Therefore, various neuronal types may react differently to the presence of Ach.

Several effects of Ach have been described in pyramidal neurons of the neocortex. Muscarinic receptor activation causes the blockage of a voltage-dependent potassium current (I_M) and a relatively voltage-independent Ca^{2+}-activated K^+ afterhyperpolarizing current, (I_{APH}) (McCormick 1992; McCormick & Prince, 1986; 1987; McCormick & Williamson, 1989). Blockage of I_M and I_{APH} results in excitation by enhancing the response of neurons to depolarization and by reducing frequency adaptation (McCormick & Prince, 1987; McCormick & Williamson, 1989). As well,

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muscarinic blockade of a Na^+-activated K^+ current reduces spike frequency adaptation (Schwindt et al., 1989). Muscarine has also been linked to a slow afterdepolarization of neurons, which enhances excitability by extending membrane depolarization (McCormick & Prince, 1986; Schwindt et al., 1988). In IB neurons, muscarinic receptor activation has been shown to produce depolarization by suppressing both a voltage-independent and a voltage-dependent K^+ current (Wang and McCormick, 1993). Thus, in general pyramidal neurons of the neocortex are excited by Ach via the suppression of one or more types of potassium currents.

Nonpyramidal Neurons

Ach has been reported to provoke rapid excitation of FS GABAergic interneurons located in layers II-III (McCormick & Prince, 1985). Application of Ach to FS cells results in a short onset latency excitation that appears to be primarily muscarinic in nature. Excitation of FS cells results from mechanisms other than those mediating pyramidal excitation, as it has a short onset latency, is not voltage-dependent and is associated with a decrease in membrane resistance. McCormick & Prince (1985) reported that this short latency excitation produced a corresponding short latency inhibition in recipient pyramidal cells, in addition to a longer latency postsynaptic excitation mediated by the direct action of Ach.

More recently, Xiang et al. (1998) found the effects of Ach to differ depending upon the subtype of inhibitory interneuron in layer V. In contrast to McCormick & Prince (1985), they found Ach to elicit hyperpolarizing responses in FS cells through activation of muscarinic receptors, and depolarizing responses in LTS cells via activation

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of nicotinic receptors. In both cell types the effects were associated with an increase in membrane conductance. Interestingly, hyperpolarization of FS cells by Ach, which are the main recipients of thalamocortical input, would result in disinhibition of the thalamocortical pathway. Thus, Ach can produce complicated effects based on selective excitation and inhibition of inhibitory interneurons.

Cortical Activation

Ach influences populations of cortical neurons which can be visualized globally by using electroencephalographic (EEG) tools. Basal forebrain-projecting cholinergic neurons play an essential role in cortical EEG activation (Jones & Muhlethaler, 1999). Electrical stimulation of the basal forebrain increases cortical levels of Ach and induces cortical activation characterized by muscarinic induced depolarization of neurons (Metherate et al. 1992; Rasmusson et al., 1992). Selective modulation of cholinergic cells by naturally occurring neurotransmitters alters cortical activation levels as well (Cape & Jones, 1998). Additionally, Ach release and cortical activation are dynamically modulated with behavioral state. In particular, behaviors characterized by strong cortical activation (ie. arousal and REM sleep) are associated by the highest levels of Ach release (Jasper & Tessier, 1971; Marrosu et al., 1995). Therefore, it seems that Ach is an important regulator of neocortical activation levels.

Presynaptic Modulation

As described above, cholinergic receptors are located presynaptically on thalamocortical and intracortical fibers. Thus, Ach may act by regulating the

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neurotransmitter released from axon terminals. Because cholinergic receptors exhibit specificity in distribution, Ach may differentially modulate thalamocortical and intracortical information. In fact, cholinergic suppression specific to intrinsic fibers has been reported in the olfactory cortex (Hasselmo & Bower, 1992) and hippocampus (Hasselmo, & Schnell, 1994). These effects were observed in the absence of any postsynaptic changes, suggesting that cholinergic agonists modulate synaptic transmission via presynaptic mechanisms.

Gil et al. (1997) examined *in vitro* how cholinergic agonists differentially regulate thalamocortical and intracortical synapses in S1. They examined the primary response and short-term plasticity of these two pathways. In comparison to the depression exhibited by the thalamocortical pathway, the intracortical pathway exhibited slight facilitation at short intervals (10-25 msec) and modest depression at longer intervals (<2000 msec). Muscarine suppressed EPSPs for both synapses, although intracortical depression was stronger than thalamocortical depression. This caused an increase in the paired-pulse ratio (second-response amplitude/first-response amplitude) for both pathways. In contrast, application of both nicotine and Ach selectively enhanced the thalamocortical EPSP and reduced the paired-pulse ratio, while having no effect upon the intracortical pathway. Nicotinic enhancement of thalamocortical inputs has been observed *in vivo* in the prefrontal cortex as well, enhancing thalamocortical transmission and extracellular concentrations of glutamate (Gioanni, 1999).

More recently, Hsieh et al. (2000) examined the influence of cholinergic agonists on thalamocortical and intracortical synapses in the auditory cortex *in vitro*. Stimulation of both pathways resulted in fast EPSPs. At higher stimulus intensities, these EPSPs

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were often followed by slow long-lasting depolarizations. A cholinergic agonist, carbachol, reduced both fast and slow components for both pathways in a dose-dependent manner. In particular, low doses of carbachol reduced only slow polysynaptic potentials, whereas higher doses reduced both fast and slow components. Comparing pathways, carbachol reduced intracortical fast potentials more strongly than the thalamocortical fast potentials. Although they found no nicotinic enhancement, they proposed that the preferential suppression of the intracortical pathway is equivalent to a relative enhancement of the thalamocortical pathway. Therefore, the auditory cortex may prefer extrinsic information rather than intrinsic information during times of processing.

GOAL OF CURRENT STUDY

The goal of the current project was to decipher *in vivo* how the presence of Ach modulated two pathways of interest: a sensory pathway and an intracortical pathway. We studied a sensory response by mechanically deflecting whiskers on the animals face and recording the evoked response in layer IV of the somatosensory barrel cortex. This is an elegant method of accessing thalamocortical fibers, because it isolates a pure pathway from VPM to barrel cortex. Previous studies that have used electrical stimulation of the thalamus, *in vitro* (Gil et al, 1997; Hsieh et al, 2000) and *in vivo* (Gioanni et al, 1999), to investigate the modulation of thalamocortical and intracortical pathways, are plagued by the criticism that stimulation of the thalamus produces antidromic activation of corticothalamic neurons. Since corticothalamic neurons produce intracortical connections in the main thalamocortical-recipient layers (i.e. layers IV and VI), it is not possible to decipher which synapses are being tested. In contrast, the use of sensory

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stimulation in the current study eliminates the possibility of antidromic activation of corticothalamic fibers. In addition, by activating intracortical fibers with a stimulating electrode placed in layers II/III of the neocortex, we also studied an independent intracortical pathway and compared the intracortical responses with the sensory (i.e. thalamocortical) responses. Thus, by infusing drugs into the neocortex using microdialysis we examined the effects of specific drugs on these two pathways.

The immediate aim of our study was to answer the following questions:

1) *How are the dynamic response properties of the sensory and intracortical pathways affected by Ach?* Previous studies performed *in vitro* have suggested that the two pathways differ in their basic response properties, such as their short-term plasticity. We examined the short-term plasticity of these two pathways *in vivo* and described how Ach modulates them. Our hypothesis was that Ach would differentially regulate the response properties of the sensory and intracortical pathways. Thus, we monitored these properties before, during and after the application of Ach, using field potential recordings and current source density analysis techniques.

2) *Via which cholinergic receptors does Ach exert its effects on the sensory and intracortical pathways?* We used specific receptor agonists and antagonists (nicotinic and muscarinic) to determine which receptors mediated the effects of Ach on these pathways.

3) *Does endogenously released Ach produce the same effects on the sensory and intracortical pathways as exogenously applied Ach?* We enhanced endogenous Ach levels via application of physostigmine, an acetylcholinesterase inhibitor.

METHODS

Experimental Setup

Sprague Dawley rats (250-300g) were used for the current study. They were anesthetized with urethane and placed in a stereotaxic frame. All skin incisions and pressure points were injected with lidocane (2%) to prevent any discomfort. A small unilateral craniotomy was made over the whisker representation of the somatosensory cortex. Small incisions were made in the dura in order to accommodate the insertion of the necessary probes and electrodes. The surface of the brain was covered with artificial CSF (ACSF) which consisted of (in mM): NaCl 126, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_4$ 7 H_2O 1.3, dextrose 10, $CaCl_2$ 2, and H_2O 2.5. Body temperature was monitored and maintained at 36-37 °C with the use of a rectal thermometer and a heating pad. All surgical procedures were reviewed and approved by the Animal Care Committee of McGill University.

Electrophysiological Recordings

Recordings were made from Teflon-insulated tungsten electrodes and linear 16-channel silicon microelectrodes with 100 μm intersite spacing (Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI). To reduce and equalize the impedance (500 KΩ) of the recording sites on the silicon probes, they were oxidized before use. Electrophysiological recordings were sampled at 5 kHz for single channel recordings and 40 kHz for 16 channel recordings. Data was stored and analyzed on a computer using Experimenter's Workbench (Data Wave Technologies, Longmont, CO) and origin (Microcal software, Northampton, MA) software. CSDs were calculated

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from the voltage recordings made by the 16-channel probes. Bandpass filter settings were set at 1 Hz to 3 kHz.

Microdialysis

Drug infusions were made using a microdialysis probe; model CMA11 (CMA Microdialysis, Solna, Sweden). The probe was 2 mm in length and 200 μm in diameter. It was placed in the cortex adjacent to the recording electrode at a depth of 2 mm from the surface of the dura. ACSF was continuously infused through the probe during the experiment at a constant rate of 4 μl/min. Drugs were dissolved into oxygenated (95% O_2 and 5% CO_2) ACSF at specific times and concentrations throughout the experiment.

Probe Location

Microdialysis probes and electrodes were placed stereotactically in the somatosensory cortex (Paxinos & Watson, 1982). The single-site recording electrode was placed at the following coordinates from bregma; rostrocaudal, -2.75 mm, mediolateral, 5.5 mm, ventral, of 900 μm. Minor adjustments in position and depth were made to locate the response with the largest amplitude and the shortest onset to whisker stimulation. This was accomplished by mapping the cortical region with repeated penetrations. On occasions when the 16-channel electrode was used, it was placed at an angle of 45° so that it was perpendicular to the layers of the cortex.

Stimulation

A thalamocortical pathway was accessed by mechanically deflecting 2-4 posterior whiskers approximately 400 μm using a whisker stimulator. The whisker stimulator consisted of a hollow glass tube mounted onto a mini-speaker. The whiskers selected (usually D1, E1, E2 and E3) were inserted into the glass tube. Intracortical fibers were stimulated using a twisted bipolar stainless steel electrode placed less than 1 mm adjacent to the recording electrode at a depth of approximately 500 μm. This depth was chosen to minimize the activation of thalamocortical fibers. Stimulation intensity was generally 100 μA or less. Whisker stimulation had a duration of 1 msec and intracortical stimulation had a duration of 0.2 msec. The two pathways were stimulated alternatively every 6 s. In order to study frequency dependent plasticity, each stimulation consisted of two pulses presented at various inter-stimulus intervals (ISIs). ISIs were (in msec); 25, 75, 125, 175, 225, 275, 325 and 375.

Statistical Analysis

Statistical analyses were performed using Excel (Microsoft, Redmond, Seattle) and Datasim (Bates College, Lewiston, ME). The statistical tests conducted evaluated differences between baseline and drug conditions on several criteria. 1) Paired t-tests were calculated to determine whether response amplitude evoked from a single stimulus varied as a function of drug application. 2) A repeated measures analysis of variance (ANOVA) with two factors (baseline/drug X ISI) was conducted for each individual drug to evaluate changes in paired-pulse ratio. Calculated paired-pulse ratio was the dependent variable analyzed. Simple effects were used to decompose significant main

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effects and interactions. 3) A second two factor repeated measures ANOVA (baseline/drug X interval) was conducted for each individual drug to evaluate how the form of the evoked response differed at 1 msec intervals post-stimulus. The dependent variable was amplitude of evoked response. Simple effects were used to decompose significant main effects and interactions.

RESULTS

Properties of Field Potential Responses

Extracellular field potential recordings were made in the barrel cortex *in vivo*. A schematic representation of the experimental setup is illustrated in Figure 1. Two independent pathways were stimulated consecutively, a sensory pathway using whisker stimulation and an intracortical pathway using electrical stimulation. All measures of deviation from the mean are shown as standard error. Whisker stimulation produced a large amplitude negative field potential (1.2 ± 0.1 mV) that had an onset latency of 5.5 ± 0.2 msec and peaked in amplitude at 10.6 ± 0.4 msec (n = 13 experiments). Longer latency responses evoked by whisker stimulation were discarded because they were believed to contain an initial intracortical component. Intracortical stimulation evoked a negative field potential response that had an amplitude comparable to the whisker evoked response (1.8 ± 0.1 mV) but showed an onset latency of 1.9 ± 0.1 msec and peaked in amplitude at 5.9 ± 0.2 msec (n = 13 experiments). Figure 2A shows an example of a sensory (left) and intracortical (right) evoked response when one stimulus is given each pathway.

The sensory and intracortical pathways were activated with pairs of pulses presented at various ISIs to examine the nature of their frequency-dependent plasticity. The frequency-dependent responses for each pathway were very distinct. Figure 2B shows a representative example when two stimuli are presented to the sensory (left) and intracortical (right) pathways at an ISI of 125 msec. Figure 2D shows averaged paired-pulse (PP) ratios for all ISIs. The PP ratio consists of the peak amplitude of the second-response divided by the peak amplitude of the first-response, multiplied by 100. The sensory pathway (filled circles) exhibited strong depression at short ISIs. The depression was strongest at the 25 msec interval, at which time successive stimuli were usually unable to elicit a response. The depression attenuated at longer ISIs. One factor, influencing our measure of PP depression for the sensory pathway was the whisker evoked oscillation. Specifically, whisker stimulation triggered an oscillation in the neocortex which is illustrated in Figure 2C, *left*. This oscillation interfered with the response to successive stimuli. An example is represented by 75 msec ISI, at which time the PP ratio was characterized by partially recovery from depression. However at this interval, the second-response occurred at roughly the same time as the onset of the whisker evoked oscillation, enabling the two to summate and produce an enhanced response. Therefore, the measured response amplitude at various intervals may reflect factors other than frequency-dependent dynamics of the sensory pathway.

In contrast, the intracortical pathway exhibited less frequency-dependent modulation (Figure 2D, *hollow circles*). Specifically, each stimulus produced a response of similar amplitude, regardless of ISI. In general, modest depression was observed at long intervals (225-375 msec), however this depression was never as strong as that

observed in the sensory pathway. In addition, intracortical stimulation produced only a small and inconsistent oscillation (Figure 2C, *right*) which therefore contributed minimally to the amplitude of the evoked response.

Effects of Exogenous Acetylcholine

The majority of neocortical Ach originates extrinsically from the basal forebrain (Mesulam et al., 1983). Previous results have indicated that it has selective effects on different pathways (Gil et al., 1997; Hsieh et al., 2000). In the current study, Ach was applied via microdialysis to the neocortex to examine its effects on the sensory and intracortical pathways. Ach was applied at different doses that we termed low-Ach (1-10 mM), medium-Ach (100 mM) and high-Ach (200 mM).

A typical example of a low dose application is illustrated in Figure 3. Low-Ach depressed both pathways. The response amplitude for the sensory and the intracortical pathways were reduced to an average of 82% ± 4% (n = 6; t-test, $p < 0.05$) and 83% ± 4% (n = 6; t-test, $p < 0.01$) of baseline amplitude, respectively. Low-Ach depressed the sensory and intracortical responses without altering their duration. Depression of the sensory response started 9 msec post-stimulus (n = 6; ANOVA, $p < 0.05$), which corresponds to the short latency response component. Likewise, depression of the intracortical response began 5 msec post-stimulus (n = 6; ANOVA, $p < 0.0001$). This corresponds to the short latency response component for the intracortical pathway. The depression of the evoked responses induced by low-Ach were accompanied by changes in frequency-dependent plasticity for the intracortical pathway, but not for the sensory pathway. There was a global increase in the PP ratio (i.e. frequency-dependent

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depression was reduced) for the intracortical pathway (n = 6; ANOVA, $p < 0.05$). This increase was found to be significant at the 25, 125, 225 and 375 msec ISIs. In contrast, the PP ratio for the sensory pathway did not change significantly with drug application. Fast Fourier transforms (FFT) were calculated from every 2 sec of data during the experiment to evaluate changes in spontaneous cortical activity in the barrel cortex before and during application of the drug. Slow-wave activity of approximately 2-4 Hz dominated the spontaneous neocortical activity during baseline conditions. In general, application of low-Ach activated the neocortex, reducing slow oscillatory activity. The changes in spontaneous activity were correlated with depression of evoked responses and activity returned to baseline levels following the removal of the drug. Figure 4 shows spontaneous field potential activity recorded before and during application of low-Ach.

A typical example of a medium dose application of Ach is illustrated in Figure 5. Medium-Ach depressed both pathways. The response amplitude for the sensory and the intracortical pathways were reduced to an average of 83% ± 4% (n = 5; t-test, $p < 0.05$) and 82% ± 2% (n = 5; t-test, $p < 0.005$) of baseline amplitude, respectively. Depression of the sensory response started 10 msec post-stimulus (n = 5; ANOVA, $p < 0.05$), indicating that the depression was of the short latency response component. The intracortical response depressed 4 msec post-stimulus (n = 5; ANOVA, $p < 0.001$) indicating that depression was of the short latency response component for the intracortical pathway as well. The depression of the intracortical and sensory responses were not associated with any significant change in frequency-dependent plasticity. FFT analysis indicates that medium-Ach activated the neocortex, resulting in a reduction of slow oscillatory activity. These changes in spontaneous activity were correlated with

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depression and completely recovered following removal of the drug. Figure 6 shows spontaneous activity recorded in the neocortex before and during drug application.

A typical example of a high dose application of Ach is illustrated in Figure 7. High-Ach depressed the intracortical pathway but enhanced the sensory pathway. The response amplitude for the intracortical pathway was reduced to an average of 72% ± 6% (n = 11; t-test, $p < 0.0001$) of baseline level whereas the response amplitude of the sensory pathway was enhanced to an average of 136% ± 9% (n = 11; t-test, $p < 0.005$) of baseline level. Depression of the intracortical response began 4 msec post-stimulus (n = 7; ANOVA, $p < 0.0001$) indicating that it was of the short latency response component. The depression was not associated with a significant change in PP ratio. Enhancement of the sensory response began 11 msec post-stimulus (n = 7, ANOVA, $p < 0.02$) indicating that the enhancement was of the long latency response component. As well, the duration of the evoked response increased. The enhancement of the sensory response was accompanied by a significant global reduction in the PP ratio (n = 8, ANOVA, $p < 0.005$). This effect was significant at all ISIs except the 25, 225 and 375 msec intervals. FFT analysis indicates that high-Ach activated the neocortex causing a reduction in low frequency oscillatory activity. Also, high-Ach was characterized by the appearance of large amplitude spontaneous spikes in the neocortical activity. A typical example of this activity is shown in Figure 8. This activity corresponded in time to when the drug was maximally effective. In addition, the normal spontaneous activity returned following removal of the drug.

These results indicate that application of Ach has highly specific and dose-dependent effects. The next experiments explored whether specific cholinergic receptor agonists mimicked these effects.

Effects of Cholinergic Agonists

In order to decipher through which receptors the effects of Ach were being mediated, we tested the selectivity of the cholinergic agonists, muscarine and nicotine. Application of muscarine depressed both the sensory and intracortical responses. A representative example of muscarine application is illustrated in Figure 9. The intracortical pathway was reduced to an average of 70% ± 4% (n = 7; t-test, $p < 0.001$) of baseline amplitude, while the sensory pathway was reduced to an average of 85% ± 5% (n = 7; t-test, $p < 0.001$) of baseline amplitude. The intracortical depression started 4 msec post-stimulus (n = 6, ANOVA, $p < 0.0001$), indicating that depression was of the short latency response component. Likewise, depression of the sensory response started 10 msec post-stimulus (n = 5, ANOVA, $p < 0.05$), indicating that it was of the short latency response component, as well. The depression of the evoked responses were accompanied by changes in frequency-dependent plasticity for the intracortical pathway, but not the sensory pathway. There was a global increase in the PP ratio for the intracortical pathway, (n = 6 ANOVA, $p < 0.05$) which was locally significant at the 25, 75, 325 and 375 msec ISIs. In contrast, there was no change in the PP ratio for the sensory pathway. FFT analysis shows that application of muscarine activated the neocortex, reducing slow oscillatory activity. The changes in spontaneous activity were correlated with depression of evoked responses and activity returned to baseline levels

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following removal of the drug. Figure 10 shows spontaneous field potential activity recorded before and during application of muscarine.

A typical example of nicotine application is illustrated in Figure 11. Nicotine was found to selectively enhance the sensory pathway, while having no effect on the intracortical pathway. The amplitude of the sensory response increased to $167\% \pm 10\%$ (n = 7; t-test, $p < 0.0001$) relative to baseline. The enhancement began 9 msec post-stimulus (n = 7; ANOVA, $p < 0.01$), indicating that the enhancement was of the short latency response component. As well, the response was not characterized by enhancement of any longer latency components. The enhancement was accompanied by a global reduction in the PP ratio for the sensory pathway (n = 7; ANOVA, $p < 0.05$), which was locally significant at the 75, and 175 msec ISIs. FFT analysis indicates that application of nicotine did not produce consistent observable changes in spontaneous field potential activity. Figure 12 shows spontaneous activity recorded in the cortex before and during nicotine application.

Effects of Endogenous Acetylcholine

Acetylcholinesterase (AchE) is an endogenous enzyme responsible for the degradation of Ach released into the synaptic clef. Inhibiting this enzyme through application of physostigmine results in the gradual accumulation of synaptically located Ach. This method of Ach application is ideal because it permits the study of endogenous Ach localized to its physiological sites of release. Physostigmine was applied at different doses that we termed low-physo (0.5 mM), medium-physo (1-2 mM) and high-physo (5 mM).

Low doses of physostigmine did not produce consistent effects on the evoked responses for the sensory and intracortical pathways. On some occasions, drug application was accompanied by depression of the evoked responses for both pathways. However, neither the sensory, nor the intracortical depression was found to be significant ($p > 0.05$ for both).

A representative example of a medium dose application is illustrated in Figure 13. Application of medium-physo enhanced the sensory pathway to 144% ± 5%, (n = 7; t-test, $p < 0.005$) of baseline amplitude. The enhancement started 10 msec post-stimulus (n = 7; ANOVA, $p < 0.005$), which corresponds to the short latency response component. In addition, the response was not characterized by enhancement of any longer latency response components. The sensory enhancement was accompanied by a global decrease in the PP ratio (n = 7; ANOVA, $p < 0.005$) which was significant at the 75, 125, 175, 275, 375 msec ISIs. In contrast, the intracortical response was not significantly effected by application of medium-physo. As well, there was no significant change in the frequency-dependent plasticity for the intracortical pathway. FFT analysis indicates that medium-physo had minimal effects on neocortical spontaneous activity. Figure 14 shows spontaneous activity in the neocortex before and during the application of medium-physo.

A representative example of a high dose application of physostigmine is illustrated in Figure 15. High-physo enhanced the sensory pathway and depressed the intracortical pathway. The sensory pathway was enhanced to an average of 234% ± 21% (n = 10; t-test, $p < 0.0001$) relative to baseline, whereas the intracortical pathway was reduced to an average of 80% ± 7% (n = 10; t-test, p<0.05) relative to baseline. The enhancement of the sensory response started 10 msec post-stimulus (n = 9; ANOVA, $p <$

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0.01), indicating that the enhancement started with the short latency response component. In addition, the response was characterized by enhancement of longer latency response components, such that the duration of the response increased. The enhancement was accompanied by a global reduction in the PP ratio (n = 9; ANOVA, $p < 0.0001$) for the sensory pathway. This reduction was significant at all ISIs, except the 25 msec interval. Depression of the intracortical pathway started 3 msec post-stimulus (n = 9; ANOVA, $p < 0.05$), indicating that the depression was of the short latency response component. The depression of this pathway was accompanied by a global increase in the PP ratio (n = 9; ANOVA, $p < 0.05$), which was locally significant at the 25 msec interval. FFT analysis indicates that application of high-physo activated the neocortex, reducing slow oscillatory activity. In addition, high-physo was characterized by the appearance of large and small amplitude spontaneous spikes in the neocortical activity. A typical example of this activity is shown in Figure 16. This activity corresponded in time to when the drug was maximally effective. Normal spontaneous activity returned following removal of high-physo.

A summary of the effects of all drugs is illustrated in Figure 17. Figure 17A shows the effect of drug application of evoked response amplitude. The short latency component of the sensory response (<11 msec) was depressed following application of exogenous Ach and muscarine and enhanced following application of medium and high doses of endogenous Ach and nicotine. The long latency component of the sensory response (>11 msec) was enhanced following application of high doses of endogenous and exogenous Ach. The short latency component of the intracortical response (<6 msec) was depressed following application of exogenous Ach, muscarine and high doses of

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endogenous Ach. Figure 17B illustrates the corresponding changes in PP ratio at the 125 msec ISI. At this interval, the PP ratio for the sensory pathway was significantly reduced following application of high doses of exogenous and endogenous Ach and nicotine. The PP ratio for the intracortical pathway was significantly enhanced following application of low doses of exogenous Ach and muscarine.

Current Source Density Analysis

In order to understand how drug application influences laminar current flow, we conducted a CSD analysis, using 16 channel silicon microelectrodes. These electrodes record voltage concurrently from 16 channels, enabling the calculation of CSDs, which are displayed as colour-contour plots. Sinks are represented by white, sources are represented by black and neutral is represented by gray. Figure 18A (*middle*) illustrates a CSD derived following whisker stimulation. Stimulation of the whiskers produced short latency sinks in layers IV and VI. The largest, located in layer IV had a corresponding current source located in layers II-III, whereas the smaller layer VI sink had a corresponding source in lower layer V. In addition, a small longer latency current sink was located in upper layers II-III. Figure 18B (*middle*) illustrates a CSD derived following intracortical stimulation. Intracortical stimulation produced two short latency sinks. The largest was located in upper layers II-III with a corresponding current source in the superficial layers. The smaller sink was located in layer IV and it had a current source located in layers II-III. Both sinks are seen to be propagating downwards. In addition, a longer latency sink was observed in layer VI.

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Figure 18 shows the effects of high-Ach on laminar current flow evoked by sensory and intracortical stimulation. High-Ach reduced the intensity of the short latency sensory evoked current sinks. In particular, the current sinks in layers IV and VI became less intense, indicating that there was a reduction of current flow. In contrast, high-Ach strongly enhanced the long latency sink located in layers II-III producing a strong increase in the whisker evoked current flow in the upper layers. The intracortical evoked sinks all became less intense following application of high-Ach. This indicates that the drug reduced the current flow evoked by intracortical stimulation.

Figure 19 shows the effects of nicotine on laminar current flow evoked by sensory and intracortical stimulation. Nicotine enhanced the intensity of the sensory evoked current sinks in layers IV and VI. The enhancement corresponded to the short latency response component, indicating that there was an increase in current flow for the initial part of the response. There was no change of any longer latency response components. In contrast, the intracortical evoked current sinks were not altered by nicotine application.

Figure 20 shows the effects of high-physo on laminar current flow evoked by sensory and intracortical stimulation. High-physo produced a strong enhancement of all sensory evoked current sinks. The enhancement was characterized by an increase in the intensity and size of the sinks, indicating that there was a strong increase in current flow. As well, high-physo enhanced the longer latency sink located in the upper layers. In contrast, high-physo reduced the intensity of the intracortical evoked current sinks. This indicates that the drug reduced the current flow evoked from intracortical stimulation. As well, the duration of the sinks increased, suggesting that some longer latency components may have been slightly enhanced for that pathway.

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DISCUSSION

In the current project, a thalamocortical and an intracortical pathway were independently stimulated *in vivo*. The first series of experiments evaluated the response properties of both pathways. Our results indicate that the thalamocortical pathway and intracortical pathway differ in their short-term plasticity. In particular, the former exhibits strong frequency-dependent depression, whereas the latter does not. The second series of experiments evaluated how the response properties of these pathways are modulated by the presence of Ach. These experiments demonstrated that Ach has input-specific and dose-dependent effects in the neocortex. These effects can be segregated based on activation of muscarinic vs. nicotinic and synaptic vs. extrasynaptic receptors. In general, application of Ach resulted in a selective enhancement of the sensory pathway, relative to the intracortical pathway. Thus, pathways in the somatosensory cortex are differentially regulated by cholinergic inputs.

SHORT-TERM PLASTICITY

A thalamocortical pathway from VPM to barrel cortex was activated by mechanically deflecting whiskers at various ISIs. The whisker evoked response depressed when stimulated repetitively. Sensory suppression was strongest at the shortest ISI but attenuated at longer intervals. Frequency-dependent depression of this thalamocortical pathway has been previously described following electrical stimulation of VB *in vivo* (Castro-Alamancos & Connors, 1996b) and *in vitro* (Castro-Alamancos, 1997). Three mechanisms have been proposed which describe the thalamocortical

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decremental response (Castro-Alamancos, 1997). In particular, the decremental response may result from thalamocortical-activated disynaptic IPSPs, stimulus-induced release of neuromodulatory substances and/or excitatory synapses with high probability of transmitter release.

Although the mechanisms of sensory suppression were not investigated in the current study, it seems reasonable to speculate that those described in relation to the decremental response are important. However, in addition to these cortical mechanisms, sensory suppression may also reflect a loss of subcortical input. In particular, whisker stimulation may produce frequency-dependent changes at the relay stations along the sensory pathway. In fact, both VPM and Pom thalamic neurons depress when stimulated above 5 Hz (Ahissar, 2000; Diamond et al., 1992). VPM neurons project primary sensory information to the barrels, whereas Pom neurons project secondary sensory information to the inter-barrel septa (Lu & Lin, 1993). Therefore, depression of these thalamic neurons could result in a reduction of both center and surround vibrissa representations. In contrast, at the level of the brainstem, primary sensory information seems to be relayed with high fidelity, independent of frequency (Ahissar, 2000; Hartings & Simons, 1998).

An additional factor interfering with our measure of sensory suppression was the whisker evoked oscillation. In particular, whisker stimulation triggered an oscillation in the neocortex which likely influenced the evoked response to subsequent stimuli. Therefore, the interference produced by the oscillation prevented an accurate measure of frequency-dependent depression of the sensory pathway. In order to minimize the influence of this in the future, it may be useful to analyze the last response when a train

of stimuli are presented. Nonetheless, it is important to consider this as a potential source of contamination when interpreting the frequency-dependent results.

The intracortical pathway was accessed by electrically stimulating the upper layers of the cortex. This pathway was characterized by much less frequency-dependent modulation, such that pairs of pulses generally evoked responses of comparable amplitude. In general, the intracortical pathway responded with more fidelity during high frequency stimulation. These results strengthen the hypothesis that activity dynamics displayed in a cortical area, depend largely upon the origin of the projecting fibers (Castro-Alamancos et al., 1996b; Stratford et al., 1996). However, the pattern of termination for these two pathways is largely unknown and it is possible that thalamocortical and intracortical fibers activate independent local circuits. Analysis of laminar current flow illustrates that both pathways activated a similar pattern of current sinks, which differed in size and shape. These differences could reflect different populations of cells being activated or the same population of cells possessing input-specific distribution of synapses. It seems reasonable to speculate that evoked responses reflect a combination of both, due to the complexity of the neocortex.

EFFECTS OF ACETYLCHOLINE

Previous studies have shown that Ach differentially modulates input-specific pathways in the piriform cortex (Hasselmo & Bower, 1992), the hippocampus (Kahl & Cotman, 1989) and the neocortex (Gil et al., 1997; Hsieh et al., 2000). Our results indicate that the sensory and intracortical pathways are differentially modulated by Ach. In fact, there appears to be four main effects of Ach in the neocortex: 1) intracortical

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muscarinic depression, 2) sensory muscarinic depression, 3) sensory nicotinic enhancement and 4) long latency sensory enhancement. The first three effects involve modulation of the short latency response component, whereas the forth effect involves modulation of the long latency response component.

Intracortical Muscarinic Depression

Depression of the intracortical response was observed following application of exogenous Ach, high doses of endogenous Ach and muscarine. For each drug the depression started with the short latency response component and occurred throughout all cortical lamina. Depression of intracortical evoked responses following application of Ach or muscarinic agonists has been previously described in the neocortex (Hsieh et al., 2000; Gil et al., 1997; Vidal & Changeux, 1989, 1992). Although all doses of exogenous Ach depressed the intracortical response, only high doses of endogenous Ach were sufficient to produce depression. This discrepancy between endogenous and exogenous Ach application indicates that methodological differences may produce distinct effects due to differential activation of synaptic and extrasynaptic cholinergic receptors.

In fact, the cortex contains both synaptic and extrasynaptic cholinergic receptors. The former refers to receptors located in close spatial proximity with cholinergic fibers whereas the latter refers to those not associated with cholinergic fibers (Lidow et al., 1989; Spencer et al., 1986). During normal Ach release, synaptic receptors are preferentially activated. In contrast, the function of extrasynaptic receptors is unknown as they are generally unaffected under these same conditions. In the current study we applied Ach endogenously by enhancing internal levels and exogenously via

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microdialysis. Endogenous Ach application mimics physiological conditions, as rising Ach levels are confined to sites of release and therefore selectively activate synaptic receptors. In contrast, exogenous Ach application acts upon both synaptic and extrasynaptic receptors due to diffuse infusion of the drug (Wakade & Wakade, 1983).

The finding that only high doses of endogenous Ach could effectively mimic intracortical muscarinic depression suggests that this may not be a sensitive physiological effect. The strong depression observed following exogenous application of Ach and muscarine may be due to activation of extrasynaptic Ach receptors, or a combination of extrasynaptic and synaptic receptors. This is interesting when considering that intracortical muscarinic depression has been described as a sensitive cholinergic effect by researchers using exogenous application procedures. However these findings may not necessarily reflect the physiological consequence of Ach release.

The depressive effects of these drugs may be due to presynaptic or postsynaptic mechanisms. Evidence suggests that muscarinic receptors are localized presynaptically on intrinsic cortical fibers (Sahin et al., 1992) and postsynaptically on cortical neurons (Houser et al., 1985; McCormick & Prince, 1985). Depression of the intracortical response produced by low doses of exogenous Ach, high doses of endogenous Ach and muscarine were all accompanied by an enhancement in PP ratio. Specifically, there was a reduction of frequency-dependent depression, increasing the fidelity of high frequency information. The change in PP ratio suggests that the depression may be explained in part by a presynaptic mechanism. In contrast, a purely postsynaptic mechanism would effect responses to both pulses equally, producing no changes in frequency-dependent plasticity (Harris & Cotman, 1985).

In contrast, the depression produced by medium and high doses of exogenous Ach were not accompanied by any changes in PP ratio. This suggests that the depression produced by these doses resulted primarily from postsynaptic mechanisms. Thus, high concentrations of exogenous Ach may sufficiently depolarize neurons (Krnjevic & Phillis, 1963; Krnjevic et al., 1971; McCormick, 1992) resulting in a depolarization block and thereby reducing their responsiveness to input.

Sensory Muscarinic Depression

Depression of the sensory evoked response was observed following application of exogenous Ach and muscarine. The depression began with the short latency response component. Analysis of laminar current flow indicates that both drugs depressed current flow in layers IV and VI. Depression of the sensory pathway by muscarine and exogenous Ach were generally not as strong as that produced in the intracortical pathway. This corresponds with previous findings (Gil et al., 1997; Hasselmo & Bower, 1992; Hsieh et al., 2000) indicating that the suppressive effects of Ach are more selective for intrinsic, rather than afferent fibers in various cortical regions. This has also been described as a relative enhancement of afferent pathways. The fact that the sensory muscarinic depression could not be mimicked by any dose of endogenous Ach suggests that the depression is due to activation of extrasynaptic muscarinic receptors. Therefore, sensory muscarinic depression may be an unlikely consequence of physiological Ach release.

The sensory muscarinic depression may be mediated through presynaptic or postsynaptic mechanisms. It was never accompanied by any changes in frequency-

dependent plasticity however, suggesting that postsynaptic mechanisms were probably involved. Moreover, sensory muscarinic depression always occurred with intracortical muscarinic depression. This indicates that both effects may have resulted from general changes in postsynaptic cortical excitability. The only exception was high doses of Ach which produced a selective enhancement of the sensory response. However, this sensory enhancement corresponded to the long latency response component and therefore possibly co-existed with the short latency sensory depression. Under these conditions, the sensory depression would be sufficiently masked by the enhancement and therefore not easily seen. In fact, analysis of laminar current flow illustrates the co-existence of both, such that high-Ach produced a depression of the short latency response component and an enhancement of the long latency response component.

Sensory Nicotinic Enhancement

Sensory nicotinic enhancement was observed following application of nicotine and endogenous Ach. The enhancement started with the short latency response component in layers IV and VI and was not characterized by enhancement of any longer latency components. This is in contrast to the enhancement of the long latency response component observed following application of high doses of exogenous Ach. The ability of endogenous Ach to mimick the nicotinic enhancement suggests that it results from the activation of synaptically located cholinergic receptors. Therefore, this Ach induced nicotinic enhancement appears to be a sensitive physiological effect and is the likely consequence of Ach release during various behavioral states.

Our results indicate that methodological issues play an important role in differential effects of Ach reported on thalamocortical pathways. Specifically, we found that exogenous and endogenous application procedures produce effects which are opposite in nature. Most studies *in vitro* have reported sensory mucarinic depression of afferent fibers following bath application of Ach and cholinergic agonists (Gil et al., 1997; Hasselmo & Bower, 1992; Hsieh et al., 2000). Again, this may result from preferential activation of extrasynaptic receptors due to nonspecific application procedures. Vidal & Changeux (1989) demonstrated a similar finding on intracortical evoked responses *in vitro*. They reported that Ach consistently depressed the intracortical evoked response unless it was co-applied with eserine. During these conditions, the depression was reversed to a nicotinic enhancement. They interpreted the eserine-induced enhancement as evidence that high concentrations of Ach are necessary to activate nicotinic receptors. However, the results may also reflect the need to enhance synaptically-located Ach.

The increase in evoked responses produced by nicotine and endogenous Ach may involve presynaptic or postsynaptic mechanisms resulting in increased postsynaptic excitability. Evidence suggests that nicotinic receptors are located presynaptically on thalamocortical fibers (Sahin et al., 1992), and thereby serve as modulators of cellular excitability. As well, electrophysiological evidence indicates that nicotine modulates glutamatergic transmission at thalamocortical synapses (Gil et al., 1997; Gioanni et al., 1999). Nicotine probably increases the probability of transmitter release by increasing presynaptic Ca2+ concentrations (McGehee & Role, 1996). In the current study, enhancement of the sensory response was always accompanied by a reduction in the PP

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ratio, meaning that frequency-dependent depression was intensified. This supports a presynaptic mechanism of action (Stevens & Wang, 1995).

Long Latency Enhancement

Application of high doses of endogenous and exogenous Ach both produced an enhancement of the sensory response. In contrast to the sensory nicotinic enhancement, this enhancement was of the long latency response component, such that the duration of the response increased. Analysis of laminar current flow indicates that the enhancement occurred primarily in the upper layers of the cortex. In addition, it was generally accompanied by the appearance of large amplitude spikes in the spontaneous field potential activity.

The enhancement produced by high doses of endogenous and exogenous Ach were not identical. As previously described, exogenous Ach enhanced the long latency response component while simultaneously depressing the short latency response component. This indicates that the enhancement is due to the drug acting in the upper layers to facilitate the long latency response component. In contrast, endogenous Ach produced an enhancement which included both short and long latency response components. Specifically, it appeared to be a combination of sensory nicotinic enhancement and the long latency sensory enhancement. This indicates that the drug may be facilitating both the short and long latency response components or simply the short latency response component. In the case of the latter, strong facilitation of the short latency response component could resultantly enhance the longer latency sinks.

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Specifically, increasing the excitability of layer IV cells would in turn increase the excitability of target cells upon which these neurons project.

Alternatively, the change in time course of the response suggests that the enhancement may be due to a reduction of inhibitory circuits. As previously described, thalamocortical fibers recruit strong feed forward inhibition (Porter et al., 2001; Swadlow, 1995) which can be observed intracellularly as a long latency IPSP following a brisk EPSP (Carvell & Simons, 1988; Zhu & Connors, 1999). Disruption of this inhibition could partially or fully account for enhancement of the long latency response component produced by high doses of Ach. Diminishing inhibitory influences could result in a net enhancement of excitation. In fact, Ach has been reported to hyperpolarize FS inhibitory interneurons through activation of muscarinic receptors (Xiang et al., 1998). In the current study, the long latency enhancements were generally accompanied by large amplitude spontaneous spikes in the cortex. This epileptogenic activity may be indicative of a loss of GABAergic inhibition. In fact, application of a $GABA_A$ antagonist (BMI) *in vivo*, enhances VB evoked thalamocortical responses (Castro-Alamancos, 1997), which are characterized by the appearance of long latency response components.

The enhanced sensory response was always accompanied by a strong reduction in the PP ratio, such that frequency-dependent depression was strongly intensified. This may be a consequence of the synchronous firing of a large population of cells resulting in a transient refractory period. Alternatively, the enhanced depression may result from intensified feed forward inhibition (Porter et al., 2001; Swadlow, 1995).

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FUNCTIONAL CONSIDERATIONS

Ach seems to activate mechanisms within the cortex which adjust the relative strength of input-specific pathways. Differential effects on input-specific pathways likely reflect the distinct distribution of cholinergic receptors. In particular, the preferential distribution of nicotinic receptors in the vicinity of synapses relaying somatic information would modulate thalamic inputs while not affecting others. In fact, the nicotinic enhancement of the sensory response was the most sensitive effect produced by endogenous Ach. This suggests that the most likely consequence of Ach release during behavior is the selective enhancement of the sensory pathway through activation of nicotinic receptors.

What is the importance of modulating the relative strength of thalamocortical and intracortical inputs? Perhaps the importance may be elucidated when considering that the majority of synapses in the neocortex originate from cortical neurons. In particular, only a minority of synapses on layer IV thalamocortical-recipient cells are thalamic in origin (ie. approximately 5-10%), whereas the remaining are intracortical in origin (Douglas et al., 1995; White & Keller, 1989). This suggests that under normal conditions, intracortical pathways probably dominate cortical processing. Therefore, Ach release during various behaviors may serve to facilitate the numerically inferior thalamocortical pathways in order to maximize transfer of information from the periphery to the cortex, while simultaneously suppressing the dominate intracortical activity.

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FIGURE CAPTIONS

Figure 1. Schematic representation of experimental setup. A recording electrode was placed in layer IV. A stimulating electrode was placed as shown to activate horizontal fibers in layer II/III. A microdialysis probe was placed adjacent to the recording electrode. A sensory pathway was activated by mechanically deflecting 2-4 whiskers on the contralateral side of face using a whisker stimulator.

Figure 2. Frequency-dependent plasticity of the sensory and intracortical pathways. *A, C,* Averaged traces of sensory (left) and intracortical (right) evoked field potentials when 1 stimulus (arrow) was delivered to each pathway. *B,* Averaged traces when two stimuli were delivered to each pathways at an ISI of 125 msec. *D,* Averaged data from 18 experiments showing the calculated PP ratios for the sensory (solid circles) and intracortical (hollow circles) evoked responses. Each point is the mean ± SEM.

Figure 3. A typical experiment illustrating the effects of low-Ach on the sensory and intracortical pathways in the barrel cortex. *A,* Superimposed traces of sensory evoked (left) and intracortical evoked (right) field potentials, before (thick trace) and during (thin trace) application of low-Ach. *B,* Power spectrum derived from every 2 sec of spontaneous field potential activity recorded from the neocortex and displayed as a colour contour plot. Power of each frequency is colour-coded such that an increase in the power is displayed as black and zero is displayed as gray. *C,* Amplitude of evoked responses for both pathways as a function of time. Each point represents the response amplitude to a single stimulus and each pathway was stimulated once every 12 sec. The horizontal bar

represents the time the drug was applied via microdialysis. *D*, Calculated PP ratios for the sensory (left) and intracortical (right) responses before (solid circles) and during (hollow circles) application of the drug.

Figure 4. Spontaneous neocortical field potential activity before (left) and during (right) application of low-Ach. Each trace represents 12 sec of spontaneous activity recorded from layer IV. The traces for each condition occurred continuously in time.

Figure 5. A typical experiment illustrating the effects of medium-Ach on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 6. Spontaneous neocortical field potential activity before (left) and during (right) application of medium-Ach.

Figure 7. A typical experiment illustrating the effects of high-Ach on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 8. Spontaneous neocortical field potential activity before (left) and during (right) application of high-Ach.

Figure 9. A typical experiment illustrating the effects of muscarine on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 10. Spontaneous neocortical field potential activity before (left) and during (right) application of muscarine.

Figure 11. A typical experiment illustrating the effects of nicotine on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 12. Spontaneous neocortical field potential activity before (left) and during (right) application of nicotine.

Figure 13. A typical experiment illustrating the effects of medium-physo on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 14. Spontaneous neocortical field potential activity before (left) and during (right) application of medium-physo.

Figure 15. A typical experiment illustrating the effects of high-physo on the sensory and intracortical pathways in the barrel cortex. Refer to Figure 3 for explanation of figure.

Figure 16. Spontaneous neocortical field potential activity before (left) and during (right) application of high-physo.

Figure 17. Summary graph showing effects of different drugs on sensory and intracortical evoked responses. *A,* Change in amplitude (as a percentage) of sensory evoked (black bars) and intracortical evoked (gray bars) responses to a single stimulus following drug application. Bars greater than 100% indicate an increase in evoked response and bars less than 100% indicate a decrease in evoked response. *B,* Change in PP ratio (as a percentage) for the sensory and intracortical evoked responses. Bars deflected upwards indicate an increase in PP ratio and bars deflected downwards indicate a decrease in PP ratio. Each bar is the mean ± SEM.

Figure 18. CSDs illustrating typical effects of high-Ach on sensory (*A*) and intracortical (*B*) evoked responses in the barrel cortex. Field potentials (top) recorded in the neocortex at a depth of 900 µm before (thick trace) and during (thin trace) drug application. CSD analyses are displayed as colour contour plots corresponding to evoked responses before (middle) and during (bottom) application of high-Ach. White represents current sinks, black represent current sources, and grays are around zero.

Figure 19. CSDs illustrating typical effects of nicotine on sensory (*A*) and intracortical (*B*) evoked responses in the barrel cortex. Refer to Figure 18 for explanation of figure.

Figure 20. CSDs illustrating typical effects of high-physo on sensory (*A*) and intracortical (*B*) evoked responses in the barrel cortex. Refer to Figure 18 for explanation of figure.

Cortex

VPM Thalamus

Trigeminal Nucleus

Whisker

Stimulator
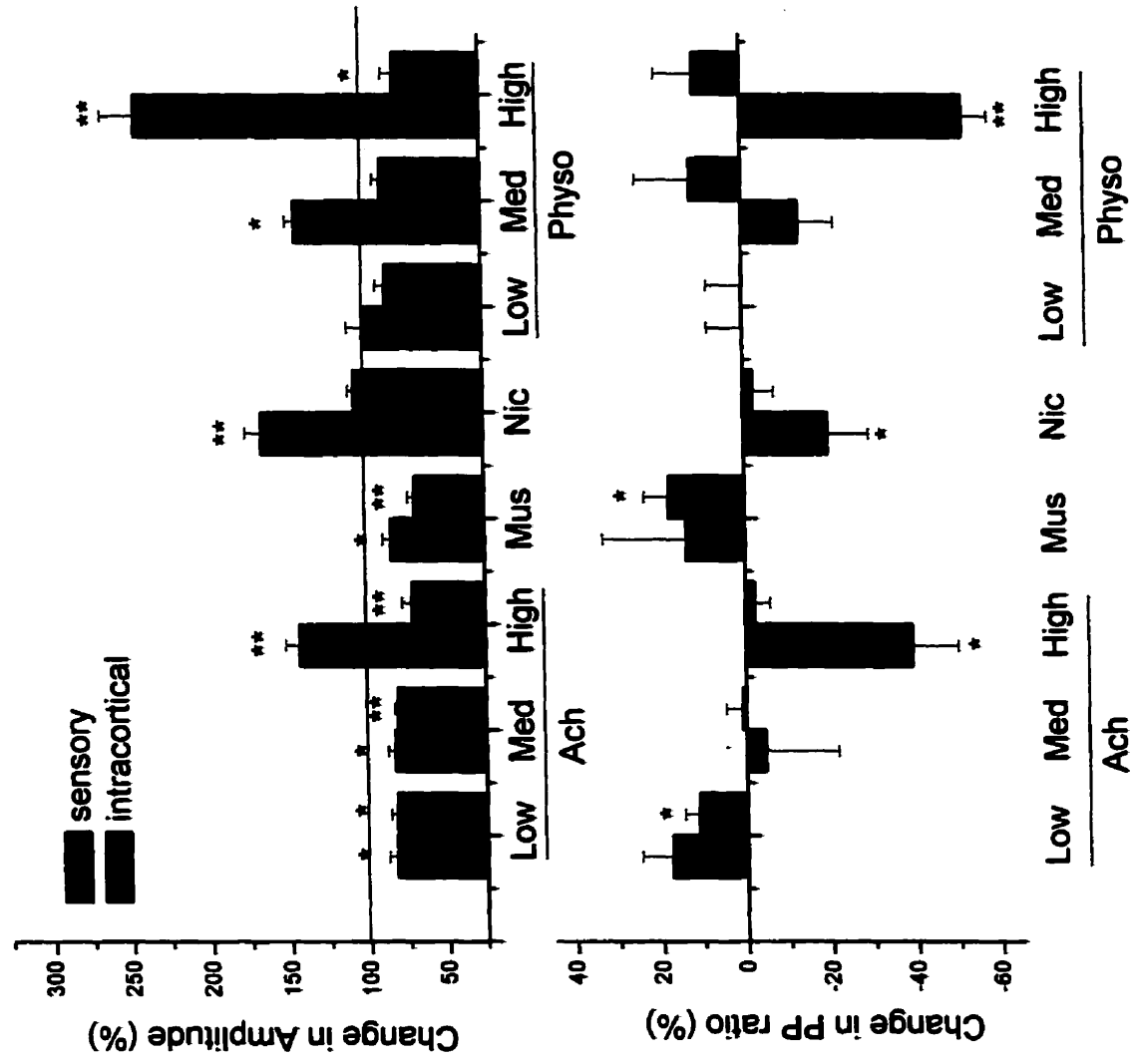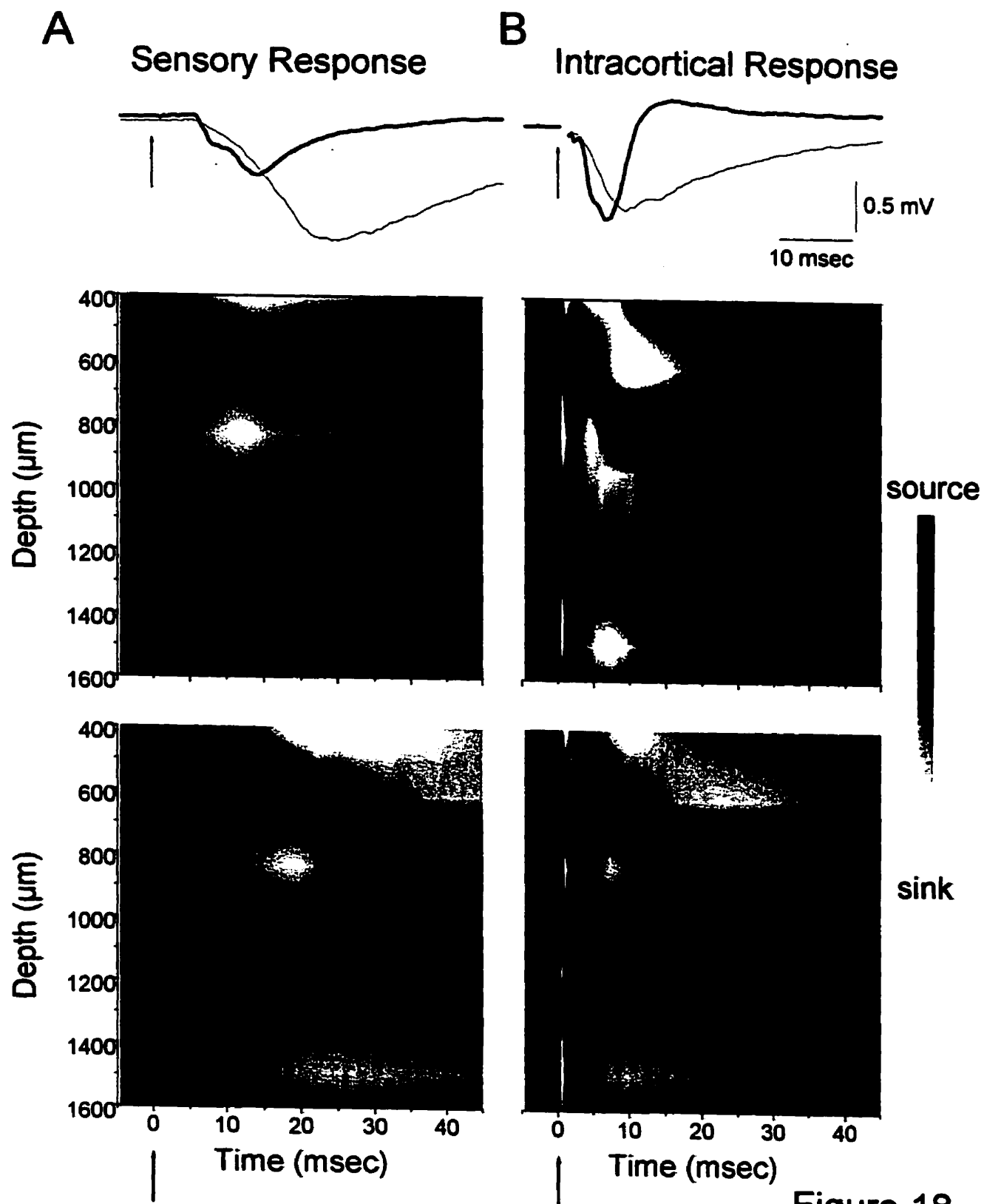
Figure 1

Figure 2

Sensory Response **Intracortical Response**

A

1.0 mV

10 msec
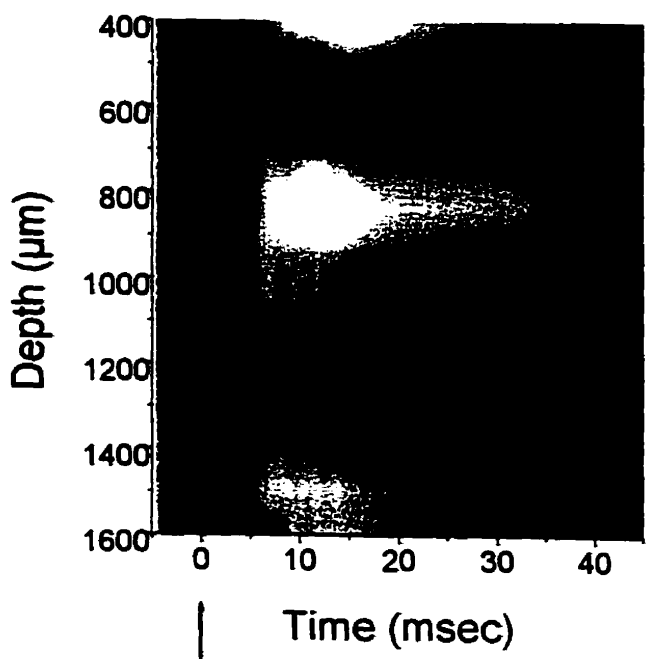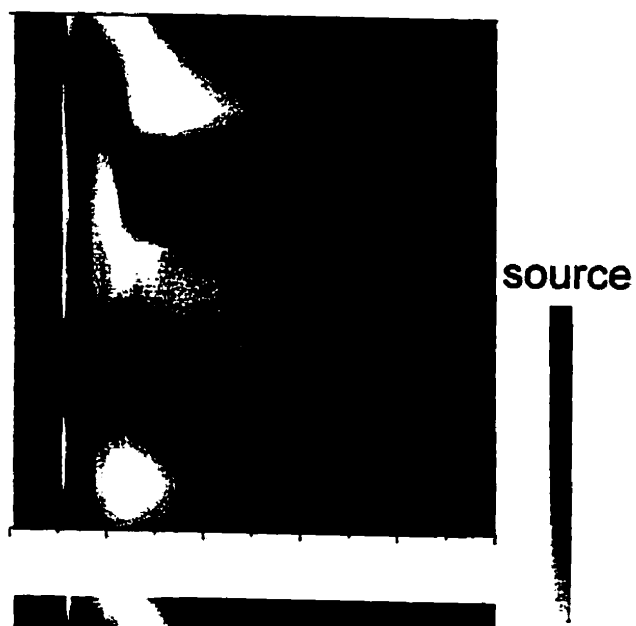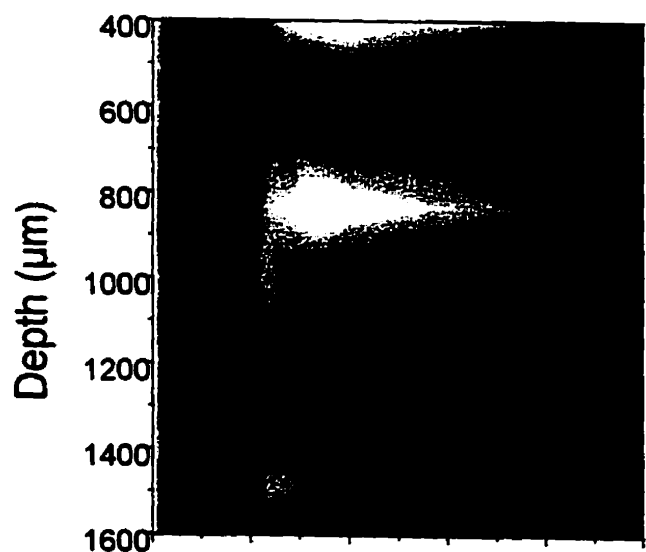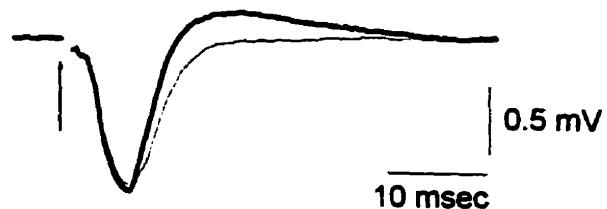
B

FFT (Hz)

C

Amplitude (mV)

Low-Ach

Sensory Response

Intracortical Response

Amplitude (mV)

Time (min)

D

PP ratio (%)

Sensory Response **Intracortical Response**

ISI (msec) ISI (msec)

Figure 3

Low-Ach

Control

0.2 mV

2 sec

Figure 4

Figure 5

Control   Med-Ach

0.5 mV

2 sec

Figure 6

Figure 7

High-Ach

Control

1.0 mV

2 sec

Figure 8

Figure 9

Control

Muscarine

0.2 mV

2 sec

Figure 10

Figure 11

Nicotine

Control

0.2 mV

2 sec

Figure 12

Figure 13

Med-Physo

Control

0.5 mV

2 sec

Figure 14

Figure 15

High-Physo

Control

1.0 mV

2 sec

Figure 16

Figure 17

A Sensory Response

B Intracortical Response

0.5 mV

10 msec

source

sink

Depth (µm)

Time (msec)

Time (msec)
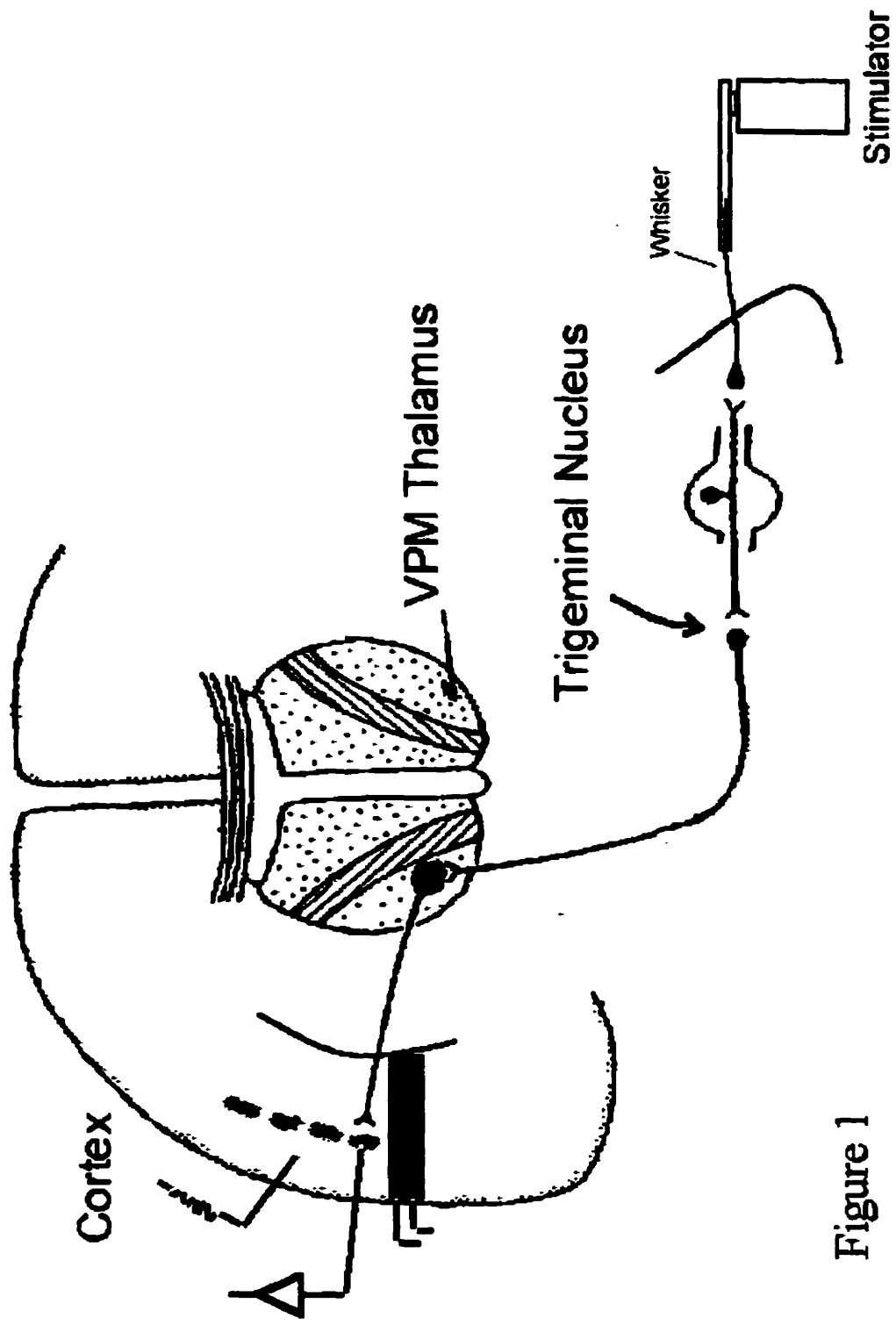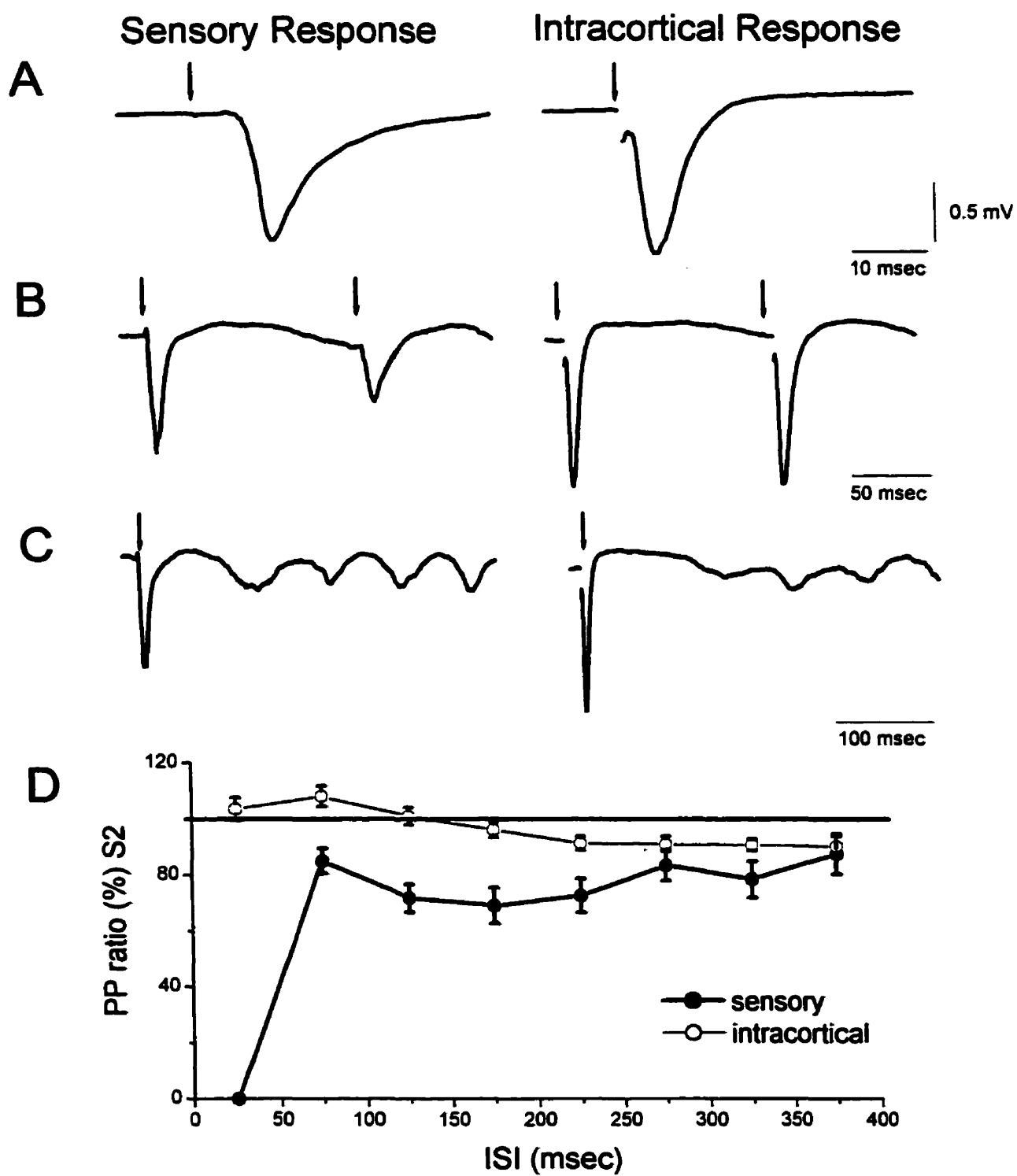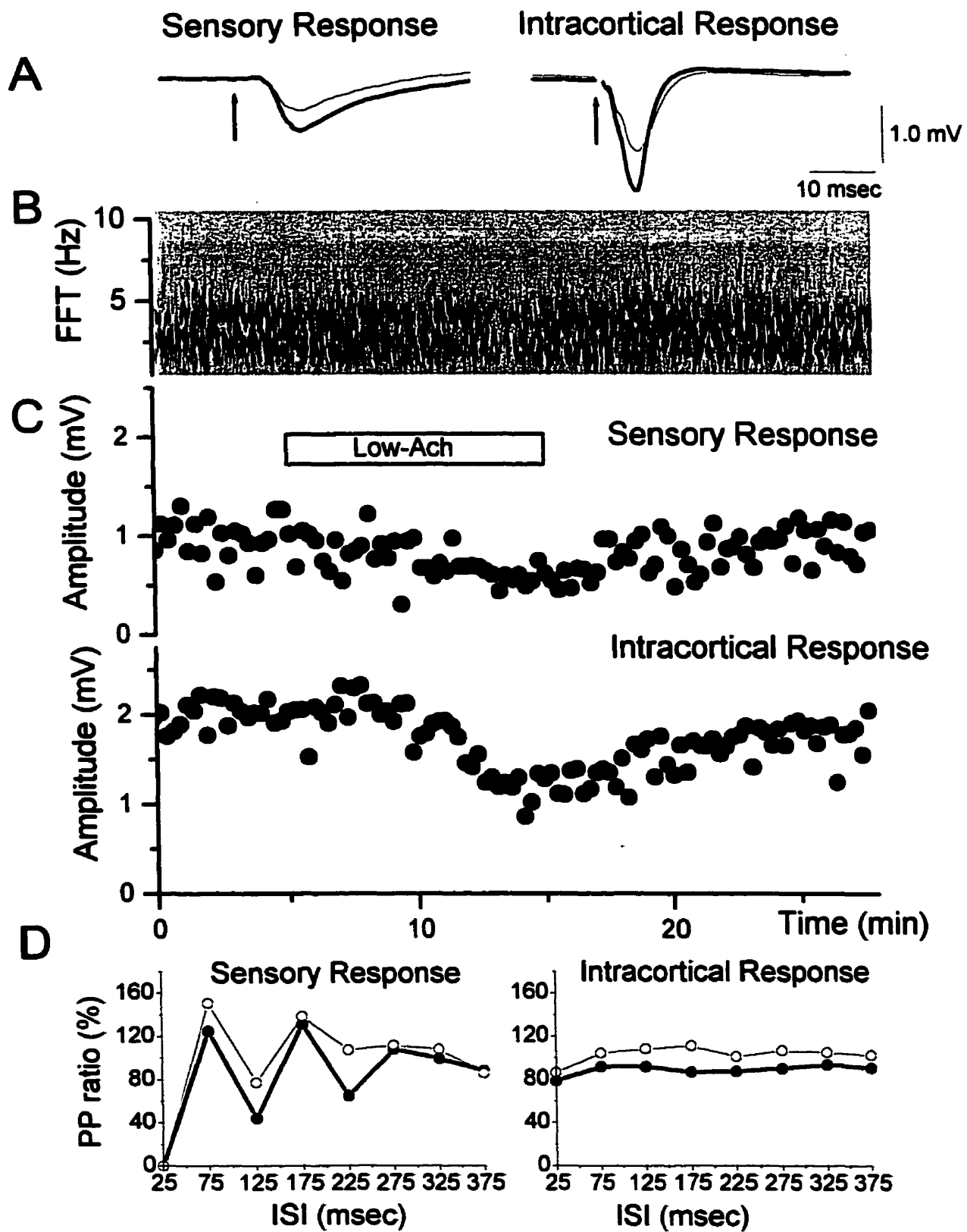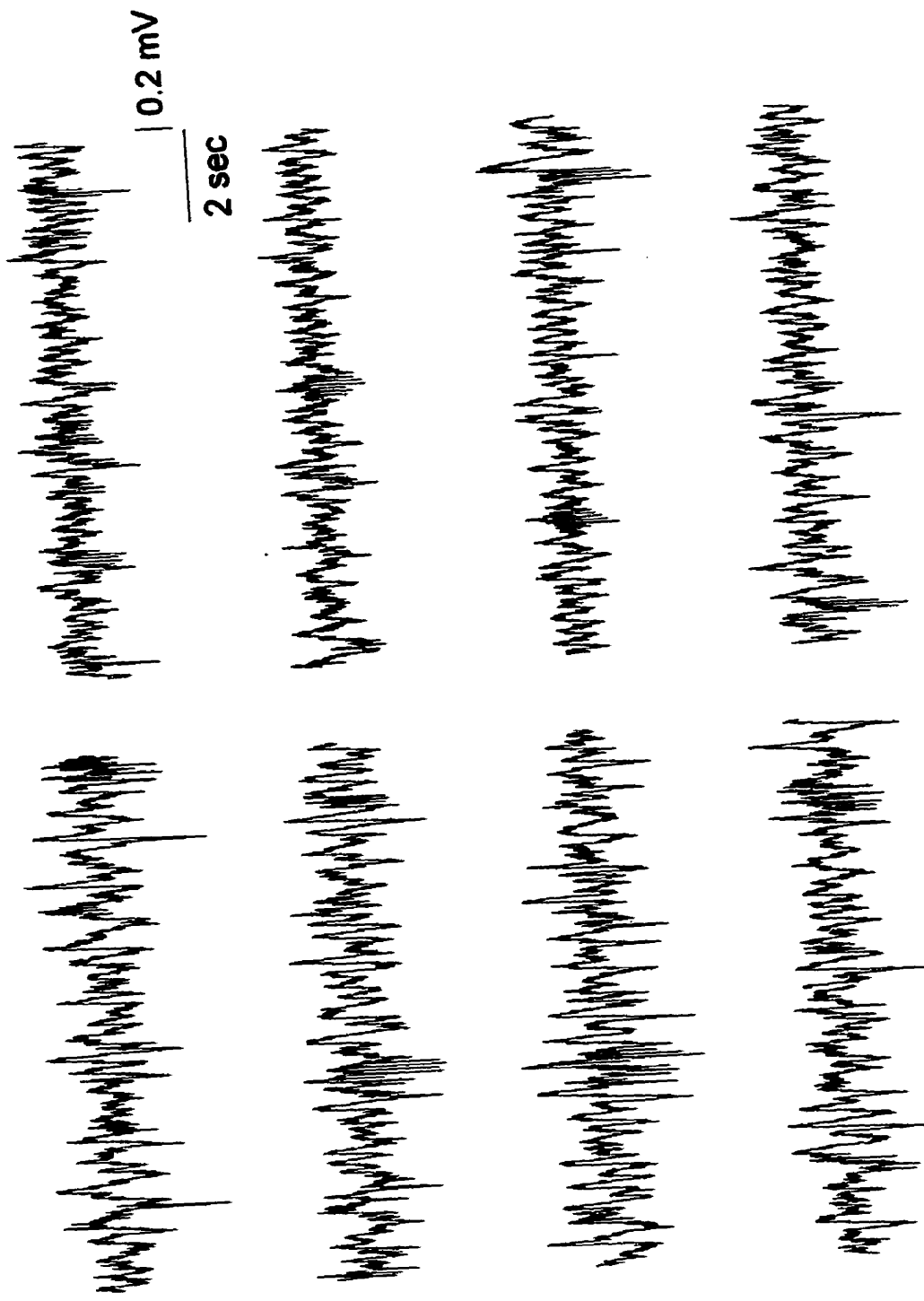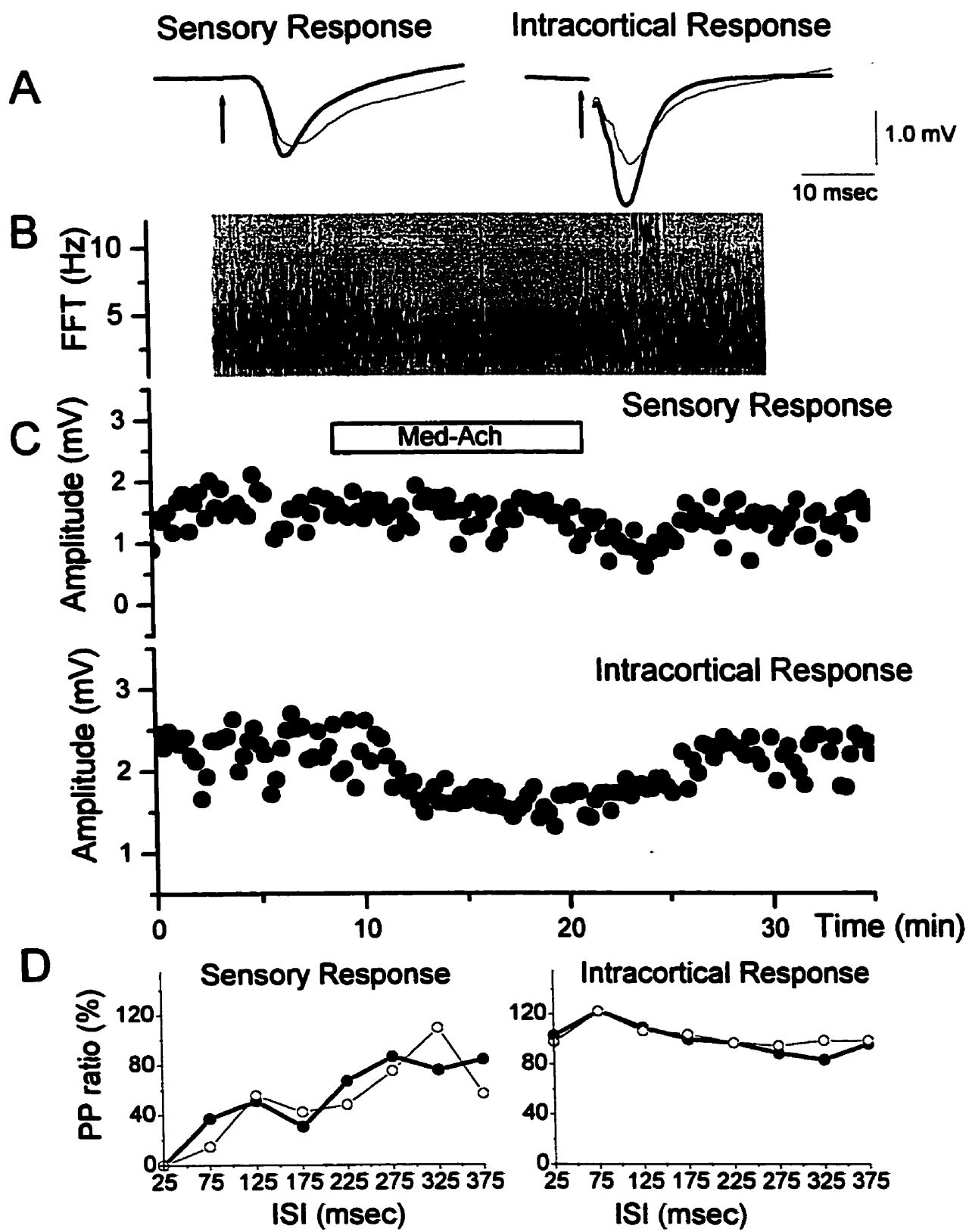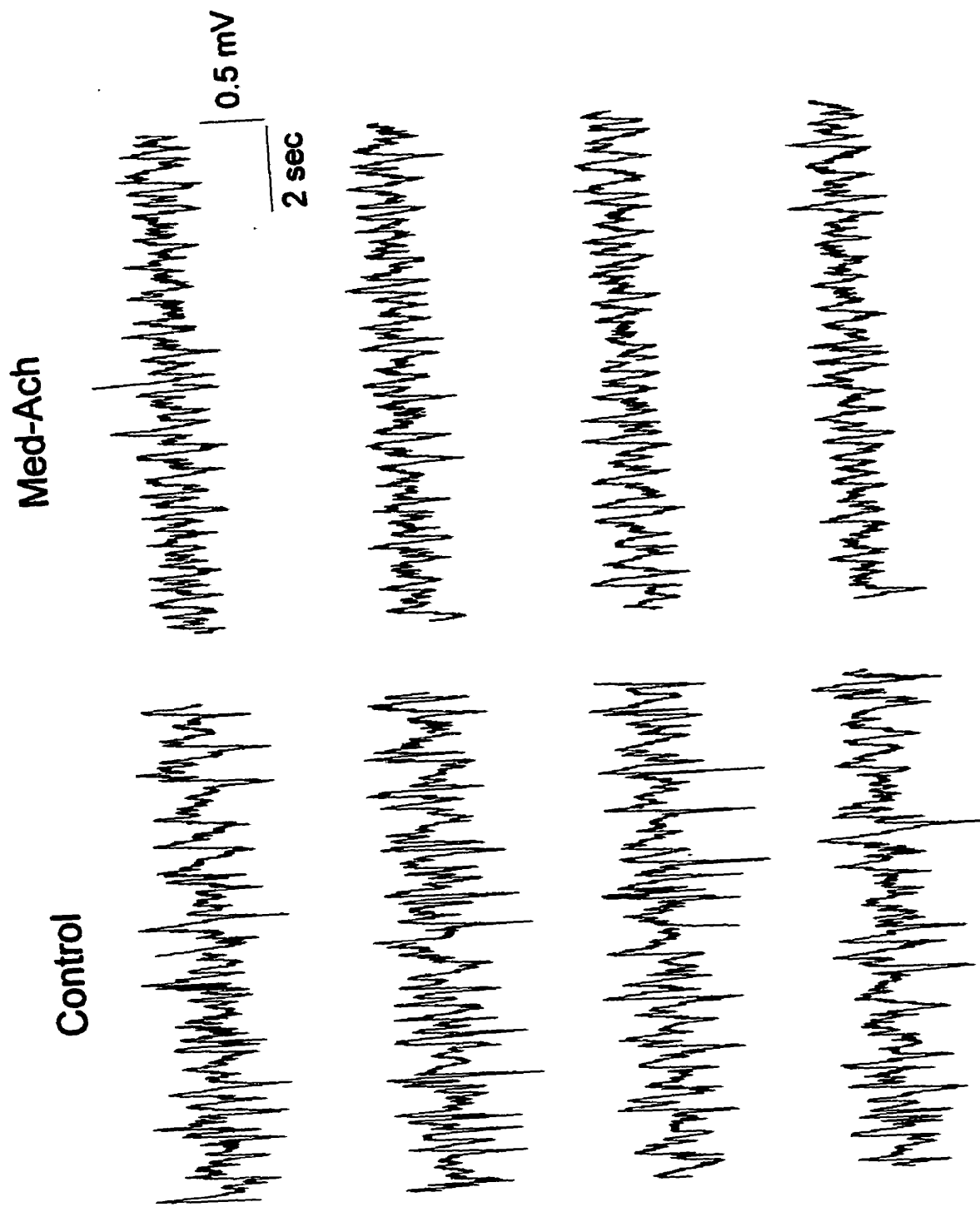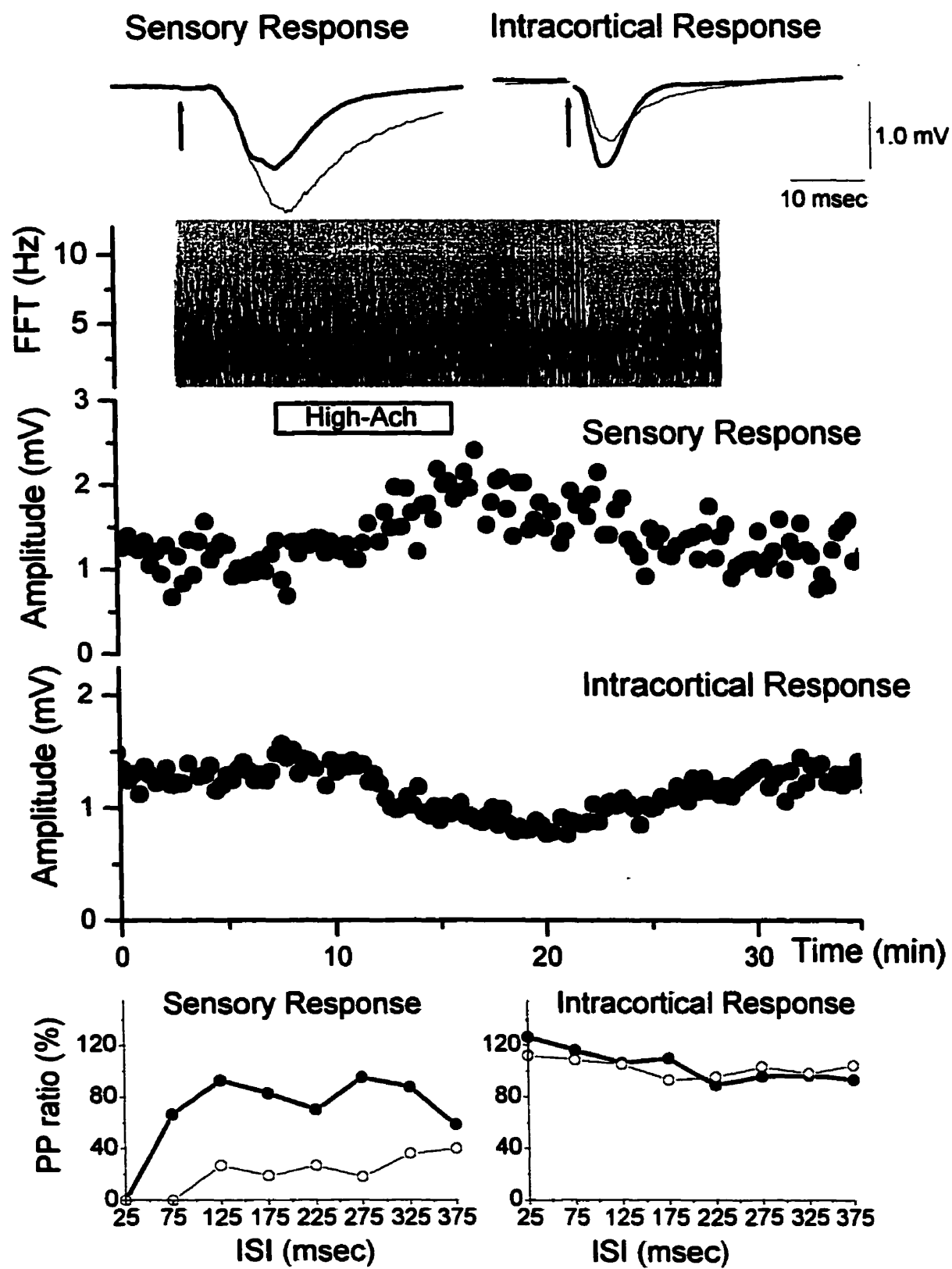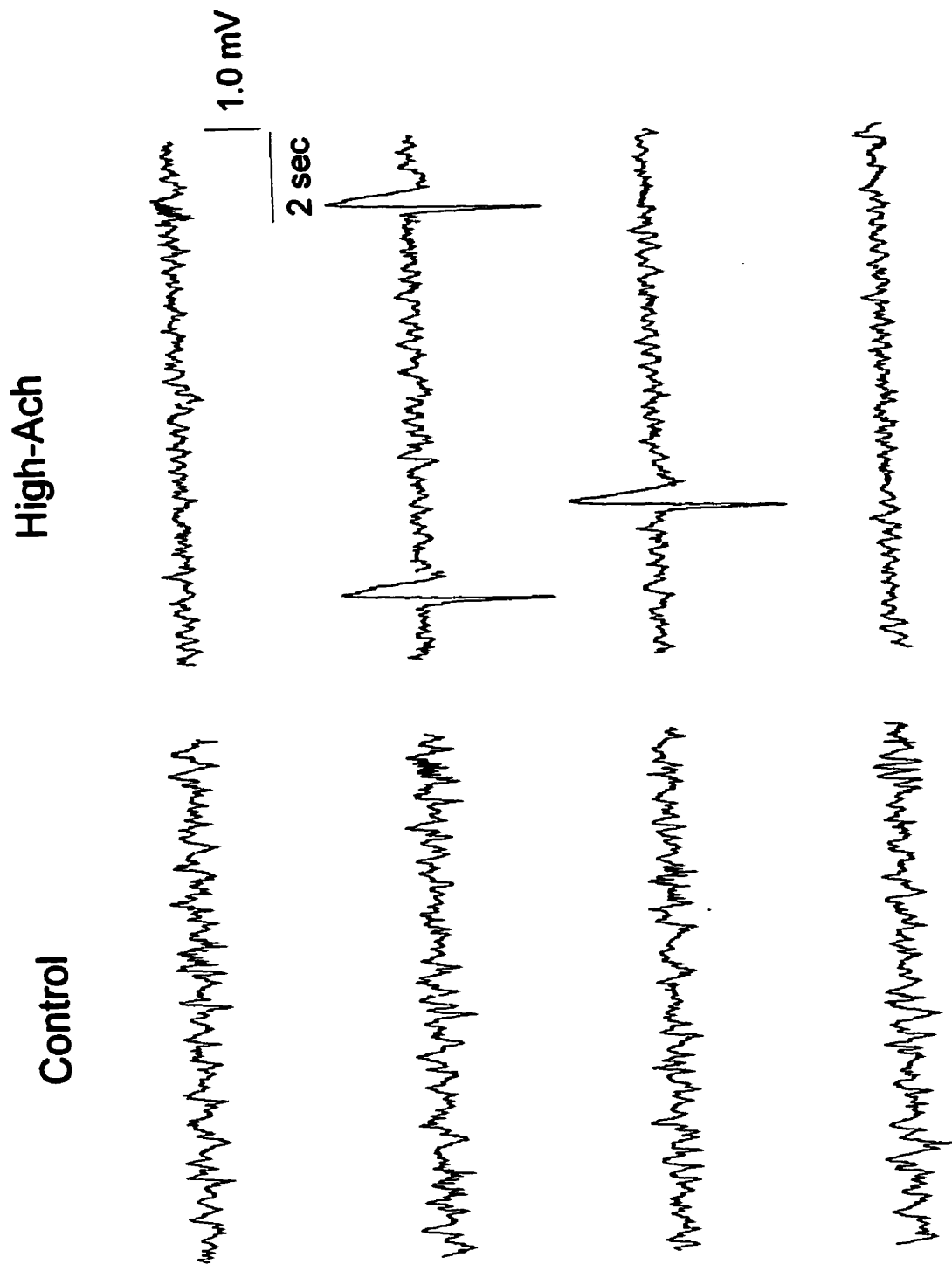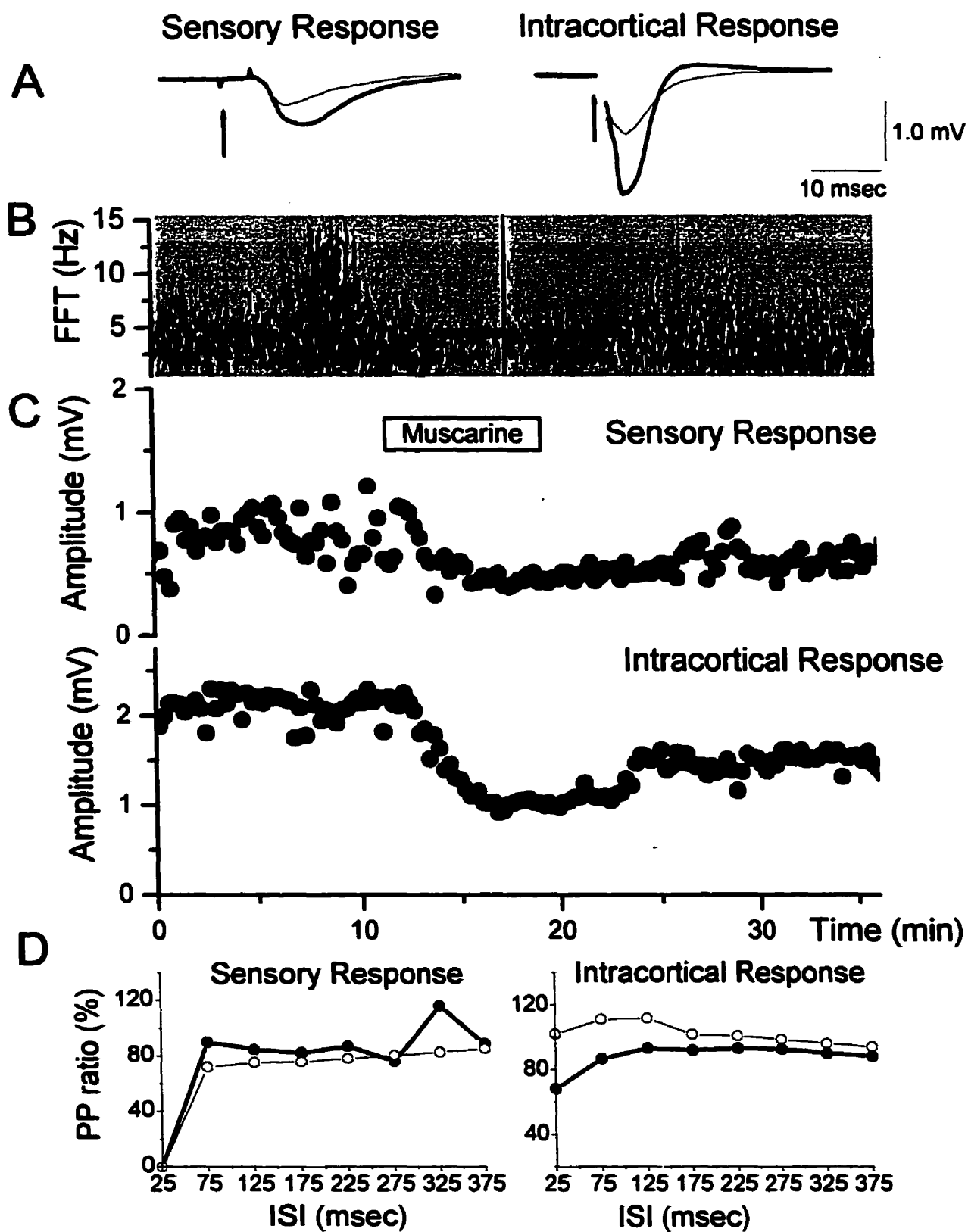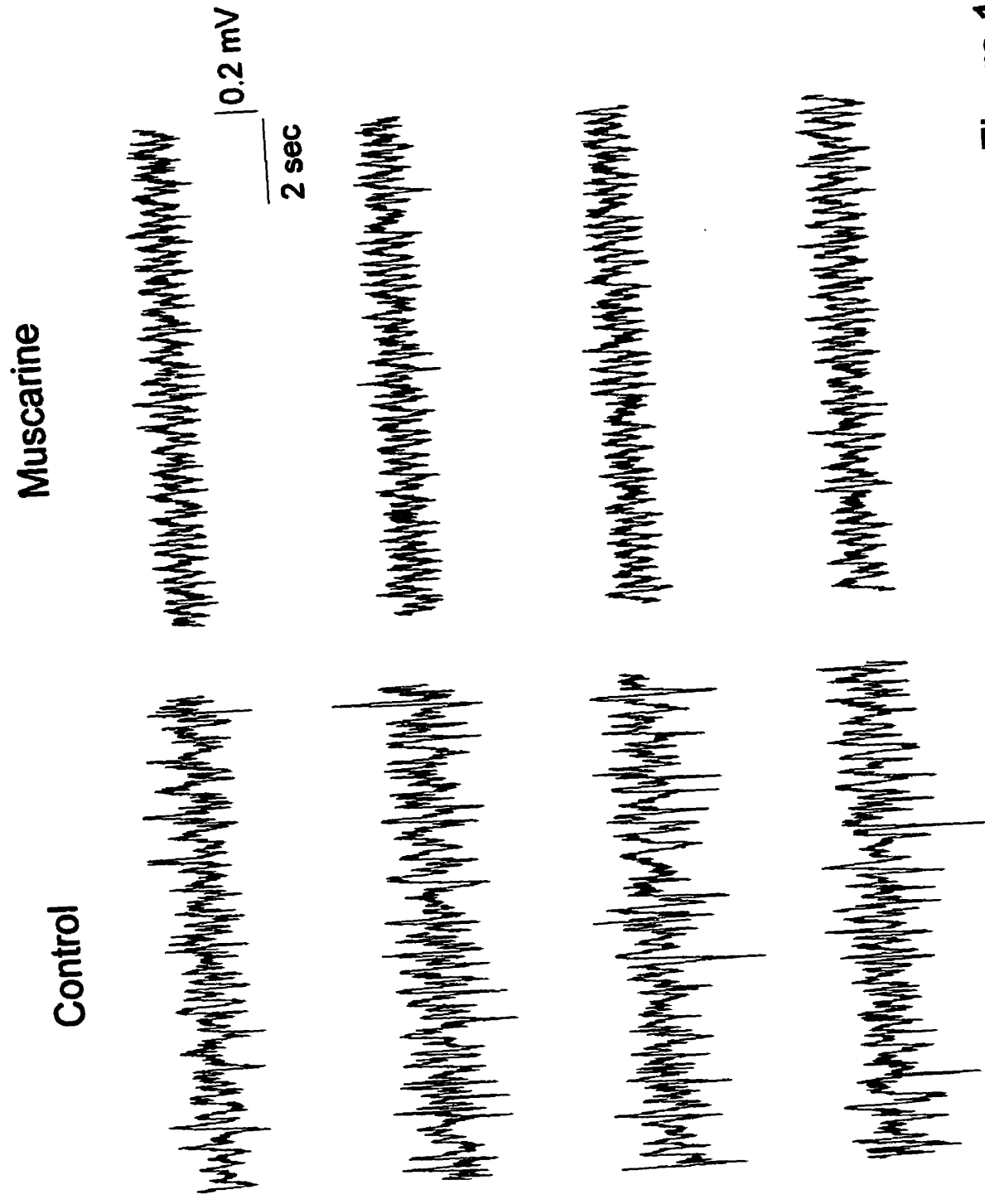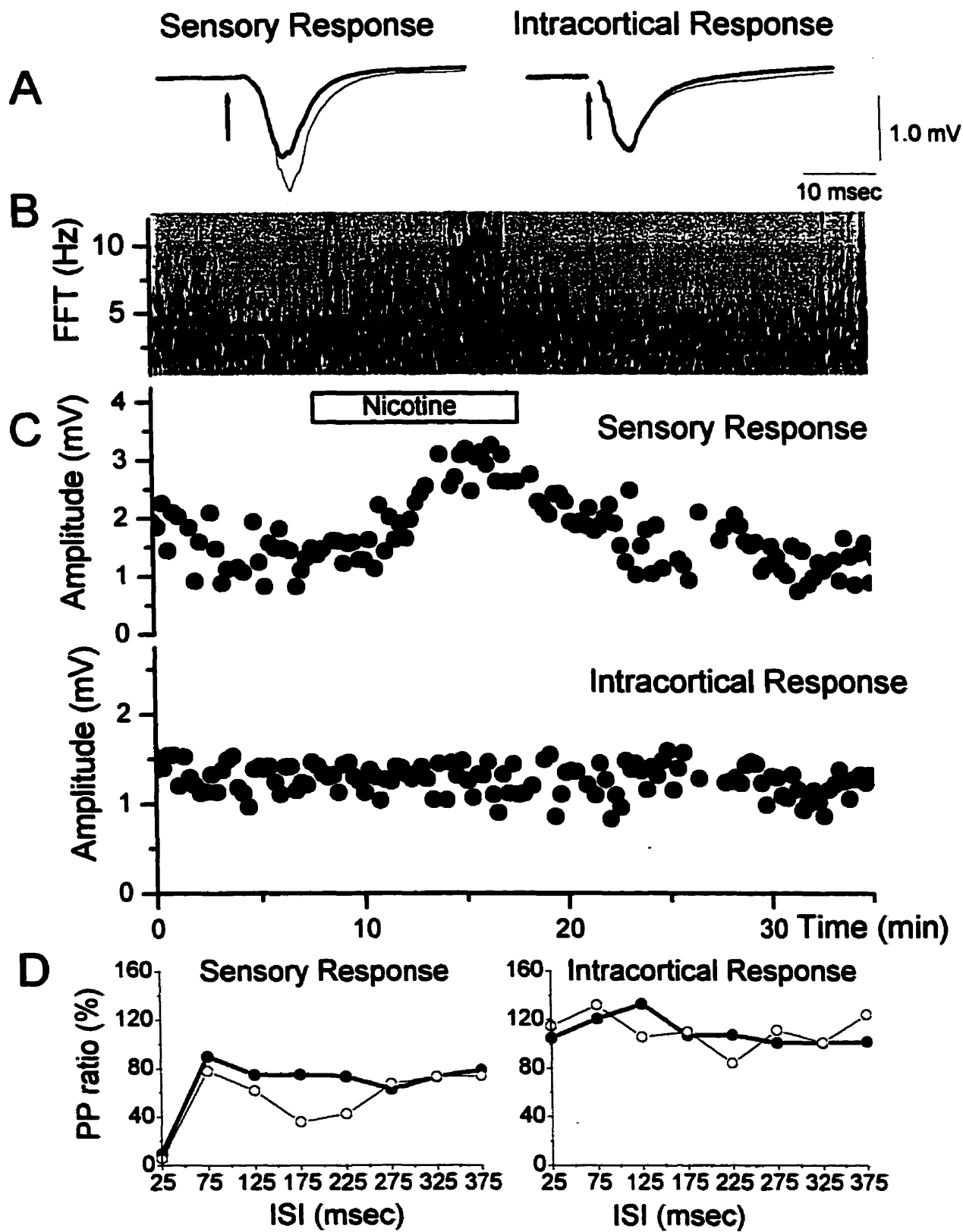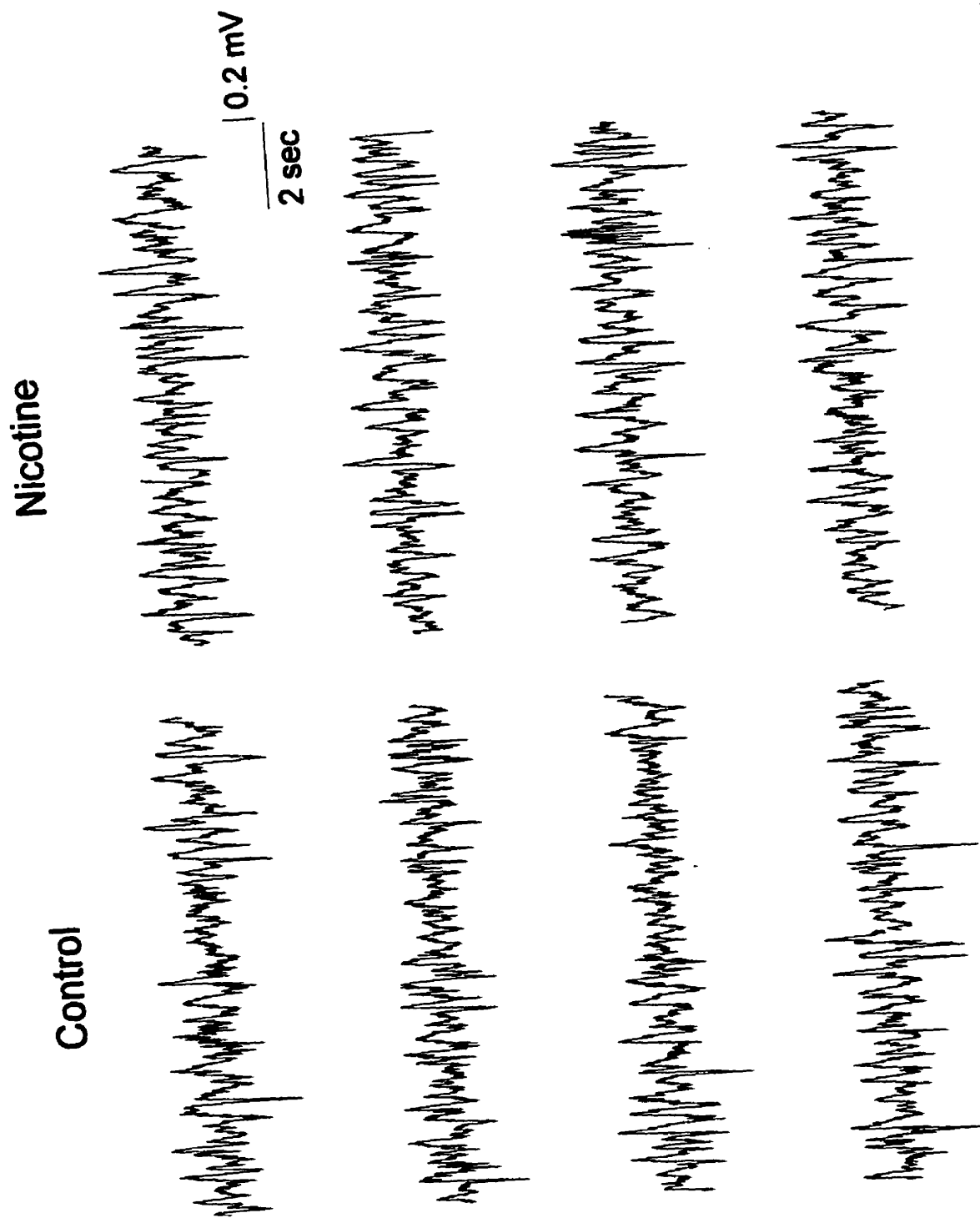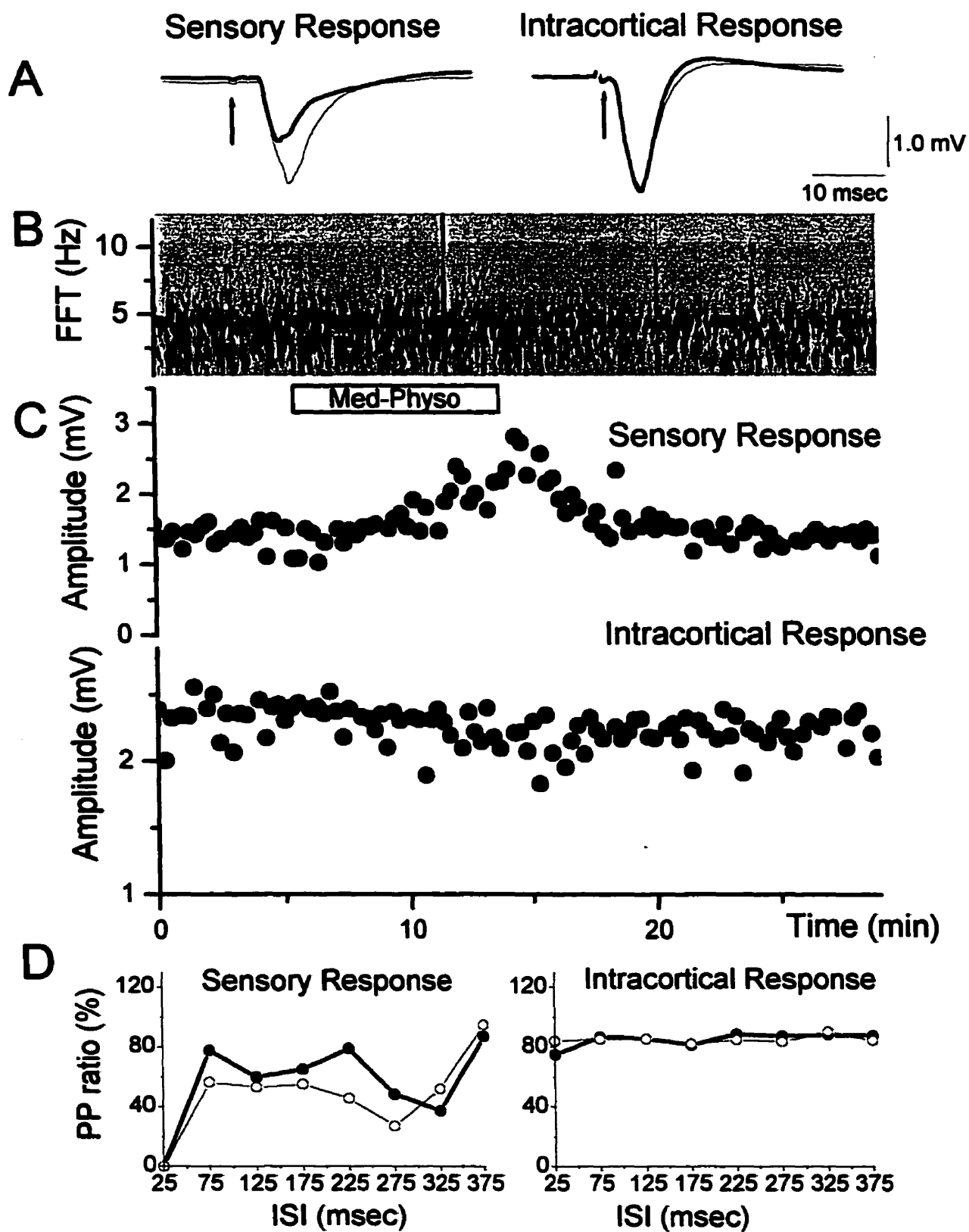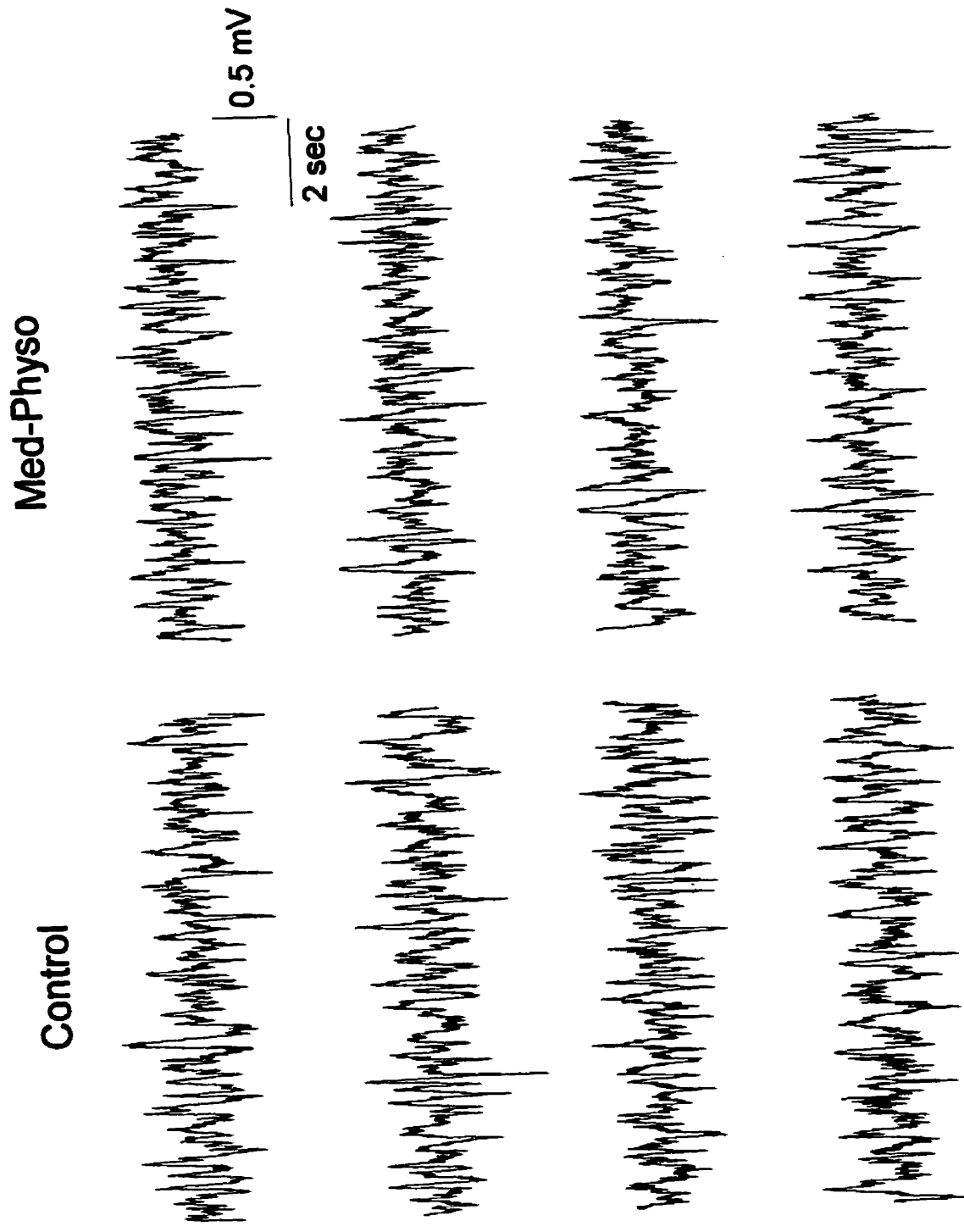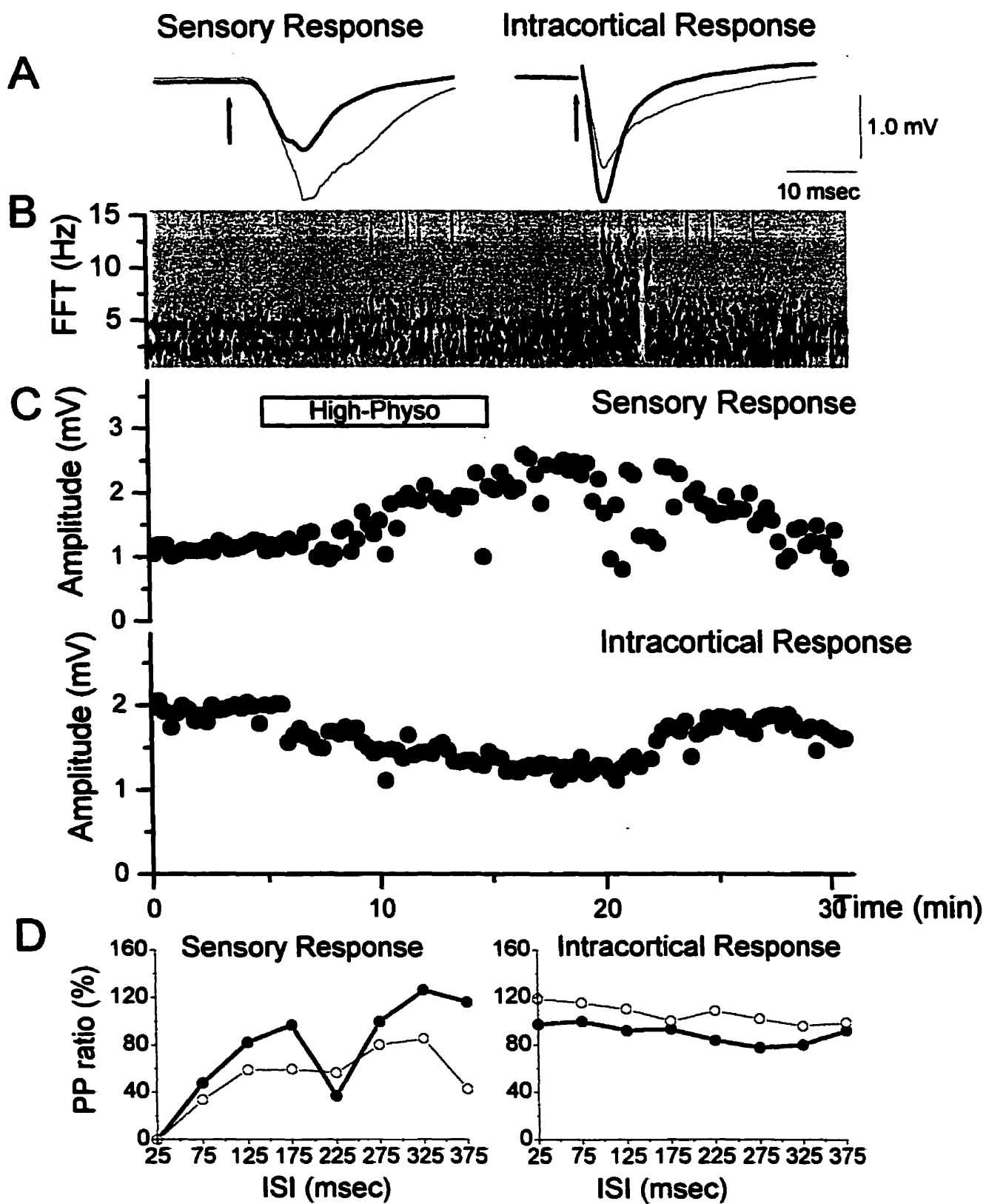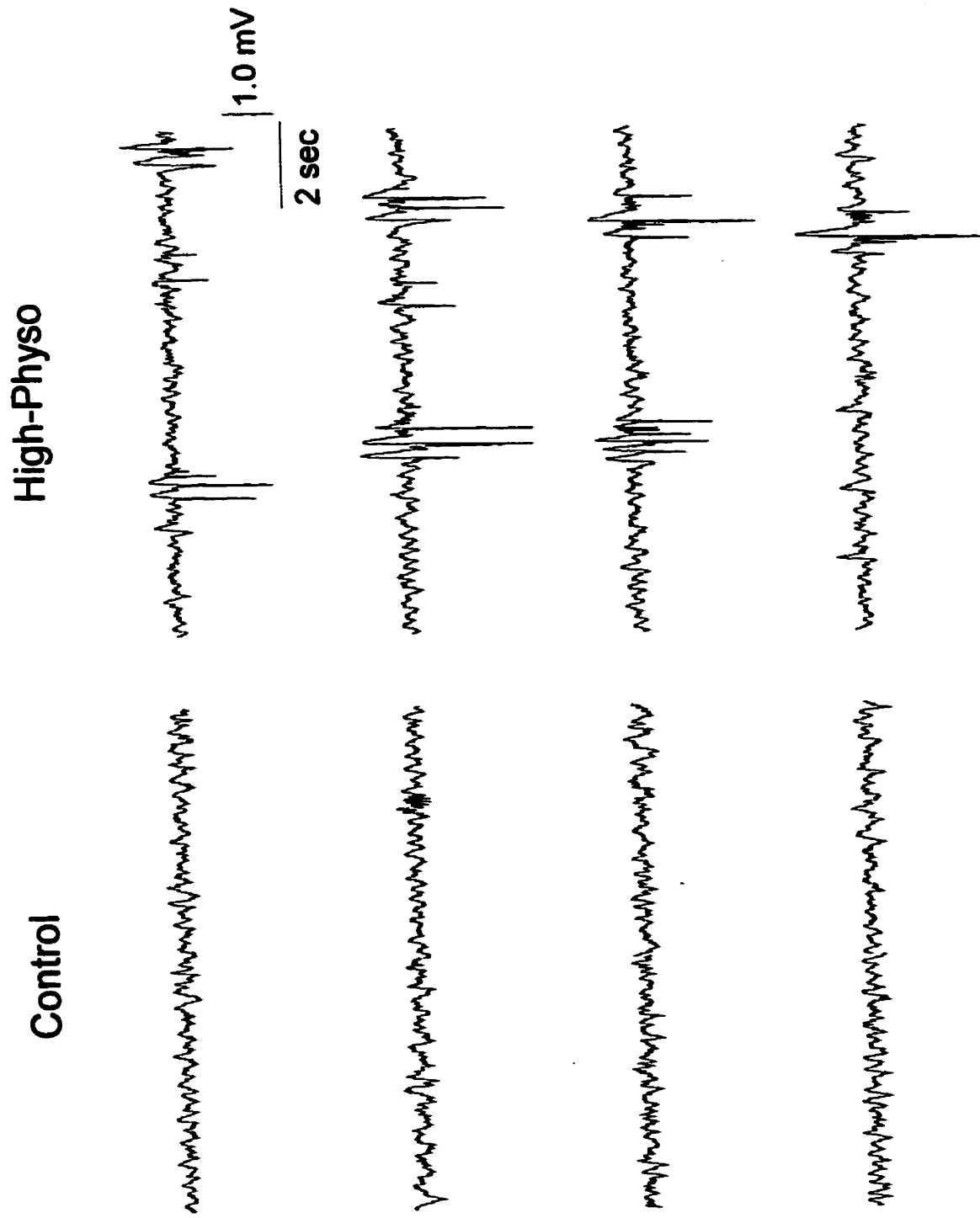
Figure 18

A
Sensory Response

B
Intracortical Response

0.5 mV

10 msec

Depth (μm)

source

sink

Time (msec)

Time (msec)
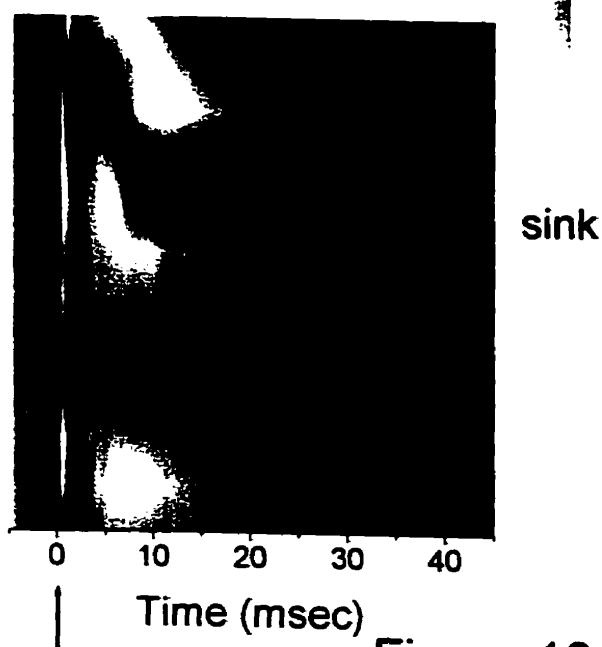
Figure 19

A
Sensory Response

B
Intracortical Response

0.5 mV

10 msec

Depth (μm)

source

sink

Time (msec)

Time (msec)

Figure 20