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Modulation of Synaptic Transmission by Psychostimulants and Dopamine in the Nucleus Accumbens

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For Maddy,

who keeps me sane

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Modulation of Synaptic Transmission by Psychostimulants and Dopamine in the Nucleus Accumbens

Saleem M. Nicola

The dopaminergic innervation of the nucleus accumbens (NAc) plays a pivotal role in drug abuse and other behavioral processes. Psychostimulants such as cocaine and amphetamine interact with the dopamine transporter to enhance dopaminergic synaptic transmission in the NAc. However, the effects of dopamine and psychostimulants on the physiology of NAc cells have not been extensively explored. Electrophysiological recording techniques (extracellular synaptic field potential recording and whole-cell current- and voltage-clamp of synaptic responses) were employed in a slice preparation of the NAc to examine the modulation of fast glutamatergic and GABAergic synaptic transmission by dopamine, amphetamine and cocaine. These studies revealed that dopamine and psychostimulants depress both excitatory and inhibitory synaptic transmission by activating a receptor with properties in common with D1-like dopamine receptors. The depression of synaptic transmission occurs through reduction of the release of glutamate and γ-aminobutyrate (GABA) from presynaptic terminals. For inhibitory transmission, this is accomplished by the reduction of Ca^{2+} influx into GABAergic terminals during the presynaptic action potential. For excitatory transmission, however, a direct interaction with the vesicular release mechanism occurring after the entry of Ca^{2+} into the presynaptic terminal is responsible for most or all of the dopamine-induced depression. Potential implications of these results for the means by which dopamine and psychostimulant drugs of abuse influence information processing by NAc cells are discussed.

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Chapter 1

General Introduction

Some of the most debilitating diseases of neurological and mental health result from disturbances of the midbrain-to-forebrain ascending dopamine system. Parkinson's disease and other movement disorders, schizophrenia, depression, bipolar disorder, psychostimulant abuse and opioid abuse are all at least partly the result of deficiencies in the proper functioning of this system (Groves, 1983; Swerdlow and Koob; 1987). Because of the large number of people directly or indirectly affected by one or more of these disorders, investigation of their biological mechanisms is of great importance.

The ascending dopamine system consists of dopaminergic cell bodies in the substantia nigra (SN) and ventral tegmental area (VTA) and the targets of their axons in the striatum, nucleus accumbens (NAc) and prefrontal cortex. The nigrostriatal portion of the ascending dopamine system is implicated in planning and initiating motor behavior (Graybiel et al., 1994), whereas the mesolimbic path (between the VTA and NAc) is thought to be part of one of the main mechanisms responsible for reward (Le Moal and Simon, 1991). The NAc and striatum receive excitatory inputs from the cortex, hippocampus and thalamus, and extend inhibitory fibers to the globus pallidus. The globus pallidus inhibits certain nuclei in the thalamus, which project excitatory fibers to the cortex. These connections have lead to the proposal that the behavioral importance of the ascending dopamine pathways derives mainly from the dopaminergic modulation, in the NAc and striatum, of a circuit involving these structures (Groves, 1983; Swerdlow and Koob, 1987; Pennartz et al., 1994). While there is some evidence in favor of the proposed circuit, the hypothesis that this particular circuit is responsible for the panoply of dopamine-related behaviors is currently speculative. However, it is clear that any reasonably complete model of the neural interactions that give rise to dopamine-related behaviors and disorders will require a thorough understanding of the contribution of dopaminergic synaptic transmission to information flow through the NAc and striatum.

While the study of psychiatric diseases such as schizophrenia and depression is difficult due to the lack of animal models of such diseases, the reductionist study of drug addiction has been greatly aided by the propensity of animals such as rats to self-

administer most, if not all, of the addictive drugs abused by humans. In these studies, rats are implanted with an intravenous catheter that delivers a dose of drug when the animal depresses a lever. Given free access to the psychostimulants cocaine or amphetamine, animals will self-administer the drug in a pattern that mirrors the binge/abstinence cycle of human abusers (Withers et al., 1995) and will often self-administer themselves to death (Bozarth and Wise, 1985; Deneau et al., 1969; Johanson et al., 1976; Yokel and Pickens, 1973). The behavioral similarities between psychostimulant self-administration in animals and psychostimulant addiction in humans are striking.

Humans report that cocaine and amphetamine produce feelings of intense euphoria and excitability followed by deep craving for the drug. Cheap, inhalable forms of these drugs, such as "crack" cocaine (the free base analog of cocaine hydrochloride) and "ice" (extremely pure D-methamphetamine hydrochloride, an N-methylated analog of amphetamine), both of which produce particularly intense highs and craving for the drug, have contributed to the current epidemic of psychostimulant abuse (Beebe and Walley, 1995; Cornish and O'Brien, 1996; Das, 1993; Derlet and Heischober, 1990). Initial use of psychostimulants results in enhanced alertness, heightened sexual pleasure, lower anxiety, a feeling of confidence and even increased productivity at work. After the first encounters with the drug, however, getting high becomes more and more important to a large proportion of users. The abuser neglects his or her family, work and other interests and spends an insupportable amount of time and money on the drug. The pattern of drug use becomes cyclical, with binges lasting several hours or days followed by a recovery period of similar length in which no drug is administered. As time progresses the abuser ignores impending personal disaster and often turns to criminal behavior to support the drug habit. Death from overdose, myocardial infarction or other complications is often the end result. Abusers who attempt to abstain from the drug invariably experience an intense craving for it, along with anxiety and irritability, all of which are temporarily relieved by another dose of the drug. Even after years of abstention, memories of drug-induced euphoria,

stimulated, for instance, by the sight of drug paraphernalia, can be enough to cause the abuser to relapse. Because of these psychological effects of psychostimulant abuse, treatment of psychostimulant addiction is extremely difficult, and no treatment method is commonly accepted as effective (Das, 1993). The development of effective treatments for cocaine and amphetamine abuse are likely to be aided by a more complete understanding of the physiological processes by which these drugs act.

The link between the abuse of psychostimulants and dopamine is clear: psychostimulants interact with monoamine uptake transporters to enhance monoamine (dopamine, norepinephrine and serotonin) synaptic transmission (Ritz et al., 1987; Seiden et al., 1993). Of the monoamine transmitters, dopamine has been most often implicated in psychostimulant abuse and in natural reward (Le Moal and Simon, 1991). There is a great deal of evidence in support of the involvement of the mesolimbic (as opposed to the mesocortical or nigrostriatal) dopamine system in psychostimulant abuse and reward (Gratton, 1996; Koob, 1992a; Le Moal and Simon, 1991). Degeneration of the nigrostriatal dopamine system, however, is clearly implicated in the severe motor disorder Parkinson's disease (Graybiel, 1994). Despite the obvious importance of dopaminergic synaptic transmission in the NAc and striatum and much research attempting to define its role, a clear picture of the mechanisms by which dopamine acts has yet to emerge.

Neuromodulators such as dopamine acting at their receptors can influence neuronal physiology in many ways, including modulation of ion conductances, of the function of neurotransmitter receptors, and of the release of neurotransmitter (Nicoll et al., 1990). Modulation of each of these components of neuronal physiology can be achieved by a number of different mechanisms; for instance, the release of neurotransmitter can be influenced by activation of any of several different second-messenger systems which can act at many steps during the complex process of action potential-triggered vesicular release. A thorough understanding of the mechanisms dopamine uses to influence the physiology of NAc and striatal cells is important not only for the development of biological models of addiction, reward, motor control and

psychiatric diseases, but also for the development of pharmacological tools to study these processes and treat their disorders. The work presented here attempts to contribute to a detailed analysis of the actions of dopamine and psychostimulants on NAc and striatal cell physiology by focusing on the effects of these drugs on synaptic transmission and particularly on the process of neurotransmitter release in these brain areas.

A. Role of the Ascending Dopamine System in Behavior

Psychostimulant self-administration. Over the past 25 years much evidence has been gathered in support of the hypothesis that psychostimulant self-administration by animals is a result of the increased effectiveness of dopaminergic synaptic transmission in the NAc. When given to an animal that is self-administering cocaine or amphetamine, broad-spectrum dopamine receptor antagonists (i.e., acting at both D1-like and D2-like receptors) such as butaclamol, haloperidol and flupenthixol change the rate at which the animal presses the lever for a drug reward. Lever pressing is increased by lower doses, while higher doses cause first an increase and then an extinction of lever-pressing (De Wit and Wise, 1977; Ettenberg et al., 1982; Risner and Jones, 1976; Roberts et al., 1989; Yokel and Wise, 1975, 1976). Control experiments demonstrate that the motor act of lever-pressing is not itself impaired by dopamine antagonists (Yokel and Wise, 1976). Antagonists specific for both D1-like and D2-like dopamine receptors also increase and then decrease lever pressing (Britton et al., 1991; Caine and Koob, 1994a; Chang et al., 1994; Corrigall and Coen, 1991; Koob et al., 1987). Similar "frustration"-induced increases in response followed by extinction are seen in animals conditioned to perform an arbitrary behavior to obtain other types of reward, such as food, when the operant response suddenly no longer results in a reward (Kimble, 1961, pp. 309 – 317). The interpretation of the dopamine antagonist results is therefore that partial blockade of dopamine receptors reduces the reward obtained by psychostimulant injection, thus making animals work harder to get the same reward;

full blockade of dopamine receptors, on the other hand, eliminates the reward entirely, so the animal ceases to work for it.

A number of further experiments support the idea that an increased effect of dopamine acting at dopamine receptors is responsible for psychostimulant self-administration. First, animals will self-administer the dopamine agonist apomorphine, and self-administration of this drug is disrupted by a dopamine antagonist (Baxter et al., 1974). Second, animals trained to respond for amphetamine or cocaine will continue to respond when dopamine agonists such as bromocriptine, apomorphine or piribedil are substituted for the psychostimulant, whereas substitution with saline (Wise et al., 1990; Yokel and Wise, 1978) or the noradrenergic agonist methoxamine (Risner and Jones, 1976) causes increased responding followed by extinction. Third, administration of adrenergic antagonists either does not affect or only somewhat reduces lever-pressing for psychostimulants (De Wit and Wise, 1977; Yokel and Wise, 1975, 1976), in contrast to the increase followed by extinction observed when dopamine antagonists are administered or psychostimulant is substituted with saline. Fourth, destruction of serotonergic neurons with intraventricular or intracerebral injections of 5,7-dihydroxytryptamine causes an increase in self-administration in animals that are then trained to self-administer amphetamine (Lyness et al., 1980) and an increase in the maximum number times an animal will press a lever to receive one injection of cocaine (Loh and Roberts, 1990); taken together, these results are consistent with the hypothesis that reduction of serotonergic synaptic transmission increases (rather than decreases) the reward value of self-administered psychostimulants. Thus, evidence from behavioral pharmacology supports the hypothesis that the rewarding properties of psychostimulants depend on their interaction with the dopamine transporter, but not the norepinephrine or serotonin transporter.

A further set of experiments suggests that the NAc is a critical locus of psychostimulant action during self-administration. First, kainic acid lesions of the NAc reduce cocaine self-administration, and the degree of reduction in self-administration

positively correlates with the extent of the NAc lesion (Zito et al., 1985). Second, selective destruction of NAc dopaminergic terminals with injections of 6-hydroxydopamine (6-OHDA) directly into the NAc (Caine and Koob, 1994b; Lyness et al., 1979; Pettit et al., 1984; Roberts et al., 1977, 1979) or VTA (Roberts and Koob, 1982) reduces psychostimulant self-administration. Similar lesions of noradrenergic fibers have no effect (Roberts et al., 1977). Furthermore, 6-OHDA lesions are thought not to interfere with the rats' motor ability to press the lever, since neither heroin self-administration (Pettit et al., 1984) nor lever-pressing for apomorphine or food (Caine and Koob, 1994b; Roberts et al., 1977) is disrupted by NAc 6-OHDA lesions. Third, direct injection of the D1 antagonist SCH23390 into the NAc increases cocaine self-administration (Caine et al., 1995; Maldonado et al., 1993; McGregor and Roberts, 1993). And fourth, studies utilizing *in vivo* microdialysis or voltammetry to measure extracellular dopamine levels in the NAc during self-administration have found that dopamine levels in the NAc are increased during psychostimulant self-administration (Gratton, 1996; Kiyatkin, 1995; Koob, 1992b; Wise et al., 1995).

While these studies provide convincing evidence of the importance of dopamine in the NAc for psychostimulant self-administration, more confusing results are obtained from studies in which animals are trained to lever-press for injections of psychostimulants directly into localized brain regions such as the NAc or prefrontal cortex. For instance, one study found that rats can be trained to self-administer amphetamine into the NAc (Hoebel et al., 1983), while another found that monkeys will self-administer amphetamine into the orbitofrontal cortex but not the NAc (Phillips et al., 1981). Another group has found that rats will self-administer cocaine into the prefrontal cortex, but not the NAc (Goeders and Smith, 1983, 1986; Goeders et al., 1986). However, this group did find that with the cocaine injection cannula implanted in the NAc, "moderate rates of responding were demonstrated, but after three experimental sessions the behavior underwent extinction" (Goeders and Smith, 1983). The interpretation of these studies is further complicated by findings that 6-OHDA lesions in the prefrontal cortex either do not affect or increase cocaine self-

administration (Martin-Iverson et al., 1986; Schenk et al., 1991). Thus, the preponderance of the evidence suggests that the main locus of the rewarding action of psychostimulants is the NAc, but other sites of action such as the prefrontal cortex (which also receives a dopaminergic projection from the VTA) cannot be ruled out.

In addition to their addictive properties, psychostimulants produce hyperactivity in humans and animals. This is manifested by both general enhanced locomotor activity and increased repetitive stereotyped behavioral sequences. 6-OHDA lesion studies have found that dopaminergic innervation of the NAc is required for the enhanced locomotor activity, while dopaminergic innervation of the striatum is required for the enhanced stereotypy (Kelly et al., 1975; Kelly and Iversen, 1976; Pennartz et al., 1994). These enhanced motor behaviors become more pronounced with successive doses of psychostimulant given days or hours after the preceding dose has worn off (Kalivas and Stewart, 1991). This process, known as sensitization, may play a role in the mechanism of psychostimulant addiction, particularly in the long-lasting susceptibility of recovering addicts to relapse (Robinson and Berridge, 1993). The processes by which psychostimulants cause sensitization are likely to be complex. There is substantial evidence that psychostimulants acting in the VTA and SN can induce sensitization (Kalivas and Stewart, 1991), but many studies involving measurements of amphetamine-induced or synaptic dopamine release in striatal or NAc slices from psychostimulant-sensitized animals support a role for long-lasting changes in dopamine release mechanisms in these nuclei (Castañeda et al., 1988; Kolta et al., 1985, 1989; Peris and Zahniser, 1989; Peris et al., 1990; Robinson and Becker, 1982; Wilcox et al., 1986; Yamada et al., 1988; but see Kalivas and Duffy, 1988). Whether the long-term changes responsible for sensitization occur in the midbrain or forebrain or both, the striatum and NAc almost certainly play some role in the development and expression of behavioral sensitization.

While dopaminergic transmission in the NAc appears to be required for the development of drug dependence, the nature of the role it plays is unknown. It has been suggested that dopaminergic transmission itself is the neural substrate of reward, but

this "dopamine hypothesis of reward" has been challenged by the results of several experiments. For instance, a major concern comes from self-stimulation experiments, in which animals are trained to press levers to elicit stimulation in brain areas such as the NAc, the VTA or the medial forebrain bundle (which carries fibers from the VTA to the NAc). While 6-OHDA lesions reduce self-stimulation in the VTA, self-stimulation in the NAc is much more resistant to 6-OHDA lesions (Phillips and Fibiger, 1978). Therefore, stimulation of the NAc is rewarding even without the presence of dopamine (Le Moal and Simon, 1991). Another problem with the dopamine hypothesis is that animals that have sustained 6-OHDA lesions in the NAc will cease to lever-press for cocaine, but responding for food and heroin is maintained (Roberts et al., 1977). Consistent with this finding, changes in the level of dopamine in the NAc are not found during lever-pressing for food (Fibiger et al., 1987), although dopamine increases are found during psychostimulant self-administration (Gratton, 1996) and electrical self-stimulation of the VTA (Fibiger et al., 1987). These results, and indeed the very fact that animals continue to eat, drink and live for long periods of time after bilateral 6-OHDA lesions of the ascending dopamine system suggest that this system is not essential for reward. Two conclusions may therefore be drawn: first, that other systems besides the ascending dopamine system are likely to be involved in reward, and second, that dopamine in the NAc does not necessarily carry rewarding information; instead, its role may be to modulate information processing in the NAc to facilitate or allow the conveyance of information that a particular stimulus is rewarding. A provocative recent theory of drug addiction proposes that mesolimbic dopamine is not necessary for the subjective feeling of reward, but rather for highlighting novel and potentially important (for the animal) stimuli as salient (Robinson and Berridge, 1993).

Psychiatric Diseases. Schizophrenia, depression and bipolar disorder are among the most debilitating and widespread psychiatric diseases. For instance, between one and 10 persons per thousand are diagnosable as schizophrenics (Jablensky, 1995). Symptoms of schizophrenia include bizarre delusions, hallucinations, inappropriate

affect, impaired interpersonal interaction and various disturbances of psychomotor behavior (American Psychiatric Association, 1987). While the biological causes of this disorder are clearly complex, there are indications that a disturbance of the ascending dopamine system is at least in part responsible. For instance, abuse of amphetamines or cocaine can result in psychosis that is similar in many respects to schizophrenia (Snyder, 1972). Furthermore, the ability of various antischizophrenic drugs to alleviate symptoms of the disease correlates with their ability to bind to (and presumably antagonize) dopamine receptors (Creese et al., 1976; Peroutka and Snyder, 1980). Further evidence includes elevated numbers of dopamine receptors, as well as elevated dopamine and dopamine metabolite levels, in the postmortem brains of schizophrenics (Swerdlow and Koob, 1987).

Mania, another serious psychiatric disease, may also involve the ascending dopamine system. During manic episodes, patients experience psychomotor agitation, grandiosity, distractibility and flights of ideas (American Psychiatric Association, 1987). Similar to their ability to cause excessive motor and stereotyped behaviors in laboratory animals, psychostimulants can precipitate manic episodes in humans (Snyder, 1972) and dopamine antagonists reduce symptoms of mania (Juhl et al., 1977; Murphy et al., 1971; Post et al., 1980). The "opposite" of mania is depression, in which the patient experiences loss of interest and pleasure, lethargy, inappropriate guilt and difficulty concentrating (American Psychiatric Association, 1987). There is evidence that depression also involves dopamine, since drugs that decrease dopaminergic synaptic transmission (such as dopamine synthesis inhibitors) can cause depression in humans, and antidepressant drugs usually promote dopaminergic synaptic transmission (Swerdlow and Koob, 1987).

It appears, therefore, that schizophrenia and mania involve elevated dopaminergic synaptic transmission, while depression involves reduced dopaminergic transmission. While other systems are likely to be involved in some or all of these diseases, understanding the actions of dopamine in the target nuclei of the ascending dopamine projections will be of great importance in the development of theories to explain and

treat these diseases.

Motor Control and Motor Disorders. The striatum receives excitatory afferents conveying both sensory and motor information from many areas of the cortex (Albin et al., 1989). The pathology of neurodegenerative disorders of movement such as Parkinson's and Huntington's disease has provided some of the most useful clues as to the function of the striatum. For example, patients with Parkinson's disease exhibit slowness of movement, tremor, difficulty in initiating voluntary movement and increased muscle tone (Albin et al., 1989). The major pathological change in parkinsonism is degeneration of nigrostriatal dopaminergic fibers (Albin et al., 1989; Jellinger, 1995). Parkinsonian symptoms can be alleviated by facilitating the dopamine path with the dopamine precursor L-DOPA, whereas dopamine receptor antagonists exacerbate or induce parkinsonian symptoms (Albin et al., 1989). In contrast, Huntington's disease, symptoms of which include chorea (rapid movements of the head, trunk and limbs that disrupt normal movement) and athetosis (slower, writhing disruptive movements) involves degeneration of striatal cells (Albin et al., 1989; Jellinger, 1995). The symptoms of this disease are alleviated by dopamine antagonists and exacerbated by dopamine agonists (Albin et al., 1989). Thus, the striatum appears to be required for coordinated motor behavior, for instance, by "specify[ing] the combination, direction and sequence of movements" of a particular set of muscles (Goldman-Rakic and Selemon, 1990) or for "motor planning or predictive control, motor sequencing, motor learning, and action repertoires involving motivational and cognitive drive" (Graybiel et al., 1994). The precise role played by dopaminergic input from the SN is a matter for speculation, but dopamine is clearly important for the proper functioning of the striatum.

B. Anatomy of the Nucleus Accumbens and Striatum

The cytoarchitecture, neurochemistry, afferent connections and efferent connections of the NAc and the much larger striatum are so similar that the NAc (along with the adjacent olfactory tubercle) is often referred to as the "ventral striatum" (Heimer et al., 1982). The major cell type that makes up the vast majority (99% by some accounts) of cells in both structures is the GABAergic medium spiny neuron (Chronister et al., 1981; Groves, 1983). Most of the remaining neurons are likely to be cholinergic interneurons (Fonnum and Walaas, 1981; Kawaguchi et al., 1995). Cells in the NAc and striatum can be further classified by neurochemical markers such as parvalbumin, calretinin and somatostatin (Kawaguchi et al., 1995), and these and other neurochemical identifiers can be used to divide the NAc and striatum into "patch" (also called "striosome") and "matrix" compartments (Gerfen, 1992; Graybiel, 1990). These compartments are also characterized by their different afferent and efferent connections. For instance, cortical afferents to the patch originate from deep layer V and layer VI, whereas those to the matrix originate from superficial layer V and supragranular layers; limbic areas such as the amygdala project preferentially to patches, whereas sensorimotor cortical areas project to the matrix; and the globus pallidus receives fibers mainly from the matrix (Gerfen, 1992; Graybiel, 1990). Projection cells of the matrix can be further subdivided based on whether they contain enkephalin and neurotensin or substance P and dynorphin (Graybiel, 1990). Yet another distinction, also based on neurochemical evidence, is between the "core" and "shell" regions of the NAc, which can be further subdivided not only into patch and matrix compartments, but also into at least five additional overlapping neurochemically-identifiable compartments (Pennartz et al., 1994). The functional significance of these classifications is unknown, but the correlation between extrinsic connections and neurochemical markers, particularly peptidergic transmitters, suggests that there are many parallel pathways of information processing in the striatum and NAc.

The major excitatory drive in both the NAc and striatum comes from

glutamatergic projections from the cortex (Groves, 1983; McGeorge and Faull, 1989; Parent, 1990; Pennartz et al., 1994). Most areas of the cortex project to the striatum, and do so in a topographical fashion; for instance, the sensorimotor cortex sends most of its projections to the putamen (as opposed to the caudate nucleus; the neostriatum is anatomically divided into the caudate and putamen), where a somatotopic representation of the body can be found (Parent, 1990). The NAc receives cortical innervation almost exclusively from allo- and mesocortical areas, including the prefrontal, cingulate, entorhinal and perirhinal cortices (Chronister et al., 1981; McGeorge and Faull, 1989; Parent, 1990; Pennartz et al., 1994; Sesack et al., 1989; Sesack and Pickel, 1992). Other areas that send excitatory fibers to the NAc and ventral parts of the striatum include the amygdala, thalamus and hippocampus (Christie et al., 1987; Chronister et al., 1981; DeFrance et al., 1985a, 1985b; Johnson et al., 1994; Parent, 1990; Pennartz et al., 1994; O'Donnell and Grace, 1995; Sesack and Pickel, 1990; Shinonaga et al., 1994; Swanson and Cowan, 1975; Yim and Mogenson, 1988). In contrast to the diversity of nuclei sending excitatory projections to the NAc and striatum, the major, if not the only source of GABAergic inhibition in these structures arises from NAc and striatal neurons themselves (Christie et al., 1987; Pickel et al., 1988; Smith and Bolam, 1990). GABAergic medium spiny neurons exhibit dense axonal arborization within the approximate area of their dendritic arbor (DeFrance et al., 1985a; Groves, 1983; O'Donnell and Grace, 1993; Pennartz et al., 1991; Wilson and Groves, 1980) and have been shown to be extensively reciprocally connected (Chang and Kitai, 1985; Groves, 1983; Kawaguchi et al., 1995; Pennartz and Kitai, 1991; Pickel et al., 1988; Smith and Bolam, 1990).

In addition to innervation by glutamatergic and GABAergic synapses, the NAc and striatum receive a dense projection from the midbrain dopaminergic cells in the SN and VTA (Andén et al., 1966; Dahlström and Fuxe, 1964; Ungerstedt, 1971b). In ultrastructural studies, dopaminergic axon terminals have been found to synapse onto the dendrites and cell bodies of GABAergic medium spiny neurons in both the striatum (Kubota et al., 1987) and NAc (Pickel et al., 1988). In both areas, small numbers of

axo-axonic synapses between dopaminergic and non-dopaminergic terminals have been observed as well (Bouyer et al., 1984a; Freund et al., 1984; Pickel et al., 1981), but the number of axo-axonic synapses may be greatly underestimated (Kornhuber and Kornhuber, 1983, 1986). In the striatum, dopaminergic terminals and terminals from cortical axons have been found to synapse onto the same dendrite, and evidence for axo-axonic synapses between cortical and dopaminergic terminals was also found (Bouyer et al., 1984b). In the NAc, dopaminergic and cortical terminals have been found to converge onto the same dendrite (Sesack and Pickel, 1992), as have dopaminergic terminals and the terminals of fibers from the hippocampus (Sesack and Pickel, 1990) and amygdala (Johnson et al., 1994). In addition to dopaminergic and cortical convergence onto the same neuron, a few axo-axonic connections between dopaminergic and cortical terminals have also been found in the NAc (Sesack and Pickel, 1990). In addition to finding specific synaptic connections between dopaminergic terminals and structures in the striatum, Descarries et al. (1996) argue that many dopaminergic terminals have no identifiable synaptic target, and may serve to elevate local extracellular concentrations of dopamine; these asynaptic terminals may provide a mechanism for "volume" or hormone-like transmission (Agnati et al., 1995; Herkenham, 1987) of dopamine to nonsynaptic targets in the striatum and NAc. Thus, there is anatomical evidence for dopaminergic modulation of NAc and striatal function at several sites, including the axon terminals of nondopaminergic afferents, the dendrites onto which these afferents synapse, and other nonsynaptic sites.

The NAc and striatum project to anatomically similar structures. The major target of striatal efferents is the dorsal part of the globus pallidus, while the NAc projects to the ventral part of this structure, known as the ventral pallidum (Heimer et al., 1982; Heimer and Wilson, 1975). Striato-pallidal projections can be further classified by whether they project to the internal or external segment of the globus pallidus. The projection to the external segment is regarded as an "indirect path" to the internal segment, since cells in the external segment inhibit the subthalamic nucleus, which excites cells of the internal segment (Alexander and Crutcher, 1990). Projections from

the NAc to the ventral pallidum, however, are not divided in this way. Another major projection from both the NAc and the striatum terminates in the substantia nigra, pars reticulata (Alexander and Crutcher, 1990; Swanson and Cowan, 1975) and thus presumably can influence the ascending dopamine system.

The dorsal globus pallidus projects to the VA and VL (*ventralis anterior* and *ventralis lateralis*) nuclei of the thalamus, while the ventral pallidum projects to the MD (mediodorsal) thalamic nucleus (Heimer et al., 1982). VA and VL project to motor and premotor cortical areas, whereas MD projects to the prefrontal and anterior cingulate cortex (Alexander and Crutcher, 1990; Heimer et al., 1982). Thus, the cortex, striatum (dorsal and NAc), globus pallidus (dorsal and ventral pallidum), thalamus (VA/VL and MD) are connected in a circuit, and the many different functions of these nuclei are likely to be subserved by multiple parallel loops involving subdivisions of these areas dedicated to specific tasks. For instance, the existence of "motor", "oculomotor" and "limbic" loops is widely accepted, and the modulation of the circuit by dopamine in the striatum and NAc is thought to be crucial for the proper functioning of the circuit (Alexander and Crutcher, 1990). Major hypotheses of motor disorders, psychiatric disorders, emotion and reward begin with these circuits and their modulation by dopamine (Albin et al., 1989; Pennartz et al., 1994; Swerdlow and Koob, 1987).

C. Dopamine Receptors

Pharmacological Classification and Cloning. Data from biochemical studies in the 1970s lead to the conclusion that there are two types of dopamine receptors: those that cause an increase in cAMP synthesis upon binding of dopamine, and those that either decrease or have no effect on cAMP levels. The former type were named "D1" receptors, and the latter "D2" receptors (Kebabian and Calne, 1979). Agonists and antagonists specific for each of the subtypes were subsequently developed. For instance, dopamine-stimulated cAMP production was blocked by SCH23390 and mimicked by SKF38393, while D2 effects were antagonized by sulpiride and mimicked by quinpirole (Andersen and Jansen, 1990; Sibley, 1995). Analysis of cloned dopamine receptors verified the distinction between D1 and D2 receptor types. Two receptors with D1-like properties have been cloned from rodent and human cDNA: the D1a receptor (Dearry et al., 1990; Gingrich et al., 1991; Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990) and the D1b receptor (Grandy et al., 1991; Jarvie et al., 1993; Sunahara et al., 1991; Tiberi et al., 1991; Weinshank et al., 1991); these are also known, respectively, as the D1 and D5 receptors. The pharmacological profile of D1a and D1b receptors is extremely similar: both cause activation of cAMP production, classical D1 agonists such as the benzazepine compounds (SKF38393, SKF81297, etc) are effective agonists of both, and both are well antagonized by D1 antagonists such as SCH23390 (Himmler et al.,1993; Jensen et al., 1996). Three D2 receptors (termed D2, D3 and D4) have also been cloned from mammals; the pharmacology of each D2 receptor is very similar to that of the other cloned D2 receptors, and matches that defined for D2 receptors prior to their cloning (Boundy et al., 1993; Bunzow et al., 1988; Chabert et al., 1994; Freedman et al., 1994; Javitch et al., 1994; Mills et al., 1993).

There is evidence, however, that there remain dopamine receptors yet to be cloned. Analysis of cellular subfractions from rat striatum reveals that [^{3}H]-SCH23390 binding sites are found in subfractions different from those in which dopamine-stimulated adenylate cyclase activity can be found (Mailman et al., 1986). Furthermore, despite

high numbers of [^{3}H]-SCH23390 binding sites in the amygdala, very little dopamine-stimulated adenylate cyclase activity can be found in the amygdala (Andersen et al., 1990; Mailman et al., 1986). These results suggest that there are dopamine receptors that bind agonists supposedly specific for D1 receptors, but which do not activate adenylate cyclase. A number of behavioral pharmacology studies provide further evidence for this hypothesis. The relative potency of various D1 agonists to promote D1 antagonist-sensitive behaviors such as vacuous chewing, circling behavior (after unilateral 6-OHDA lesions), grooming, rearing, and seizures does not correlate well with the ability of the agonists to stimulate adenylate cyclase activity *in vitro* (Arnt et al., 1992; Deveney and Waddington, 1995; Downes and Waddington, 1993; Gnanalingham et al., 1995; Murray and Waddington, 1989; Starr and Starr, 1993; Waddington and Deveney, 1996). In fact, one compound, SKF83959, actually inhibits dopamine-stimulated cAMP production *in vitro*, and yet is very capable of inducing typical D1 agonist-induced behaviors such as intense grooming (Deveney and Waddington, 1995; Downes and Waddington, 1993). In addition, an electrophysiological study of iontophoretic responses in the NAc of anesthetized rats found that D1 agonists were capable of facilitating D2 agonist-induced reductions in NAc cell firing, whereas the membrane permeable cAMP analogue 8-bromo-cAMP was incapable of such facilitation (Johansen et al., 1991). There is therefore suggestive evidence that there exist dopamine receptors with D1-like agonist and antagonist binding properties, but without the ability to activate adenylate cyclase.

One candidate second messenger system that may be responsible for some of the apparently cAMP-independent effects of D1 receptor activation involves the activation of phospholipase C. This enzyme catalyzes the production of diacylglycerol, which activates protein kinase C (PKC), and inositol phosphates, the classical effect of which is to cause the release of Ca^{2+} from intracellular stores (Conn et al., 1987, pp. 576 – 578). In cultured fish retinal horizontal cells, both D1 receptor activation and PKC activation cause neurite retraction, but introduction of nonhydrolyzable cAMP analogues has no effect (Rodrigues and Dowling, 1990). In rat striatal slices, dopamine

and D1 agonists at high concentrations can stimulate the production of inositol phosphates (Undie and Friedman, 1990, 1992; Undie et al., 1994; Wang et al., 1995) and dopamine- and D1 agonist-stimulated inositol phosphate production can be observed after mRNA from rat striatum is injected into *Xenopus* oocytes (Mahan et al., 1990). The receptor responsible for these effects has yet to be cloned, however, since expression of D1a or D1b receptors in cell lines (e.g., CHO, BHK or COS-7) does not result in expression of dopamine-stimulated phosphoinositide turnover (Jensen et al., 1996). Unique adenylate cyclase-coupled D1-like receptors have been cloned from *Xenopus* (Sugamori et al., 1994) and chicken (Demchyshyn et al., 1995), but neither of these is coupled to phosphoinositide turnover. Two additional adenylate cyclase-coupled dopamine receptors have been cloned from *Drosophila*; while dopamine-stimulated phosphoinositide turnover was not examined for these receptors, their pharmacology is different from that of other cloned D1 receptors in that typical D1 agonists do not bind them particularly well (Feng et al., 1996; Sugamori et al., 1995). The existence of this wide variety of D1-like receptors within and across species is further evidence for the heterogeneity of D1 receptors, and suggests that mammalian dopamine receptors have not all been cloned.

Subcellular Localization in the NAc and Striatum. Several ultrastructural studies utilizing antibodies specific for D1a or D2 receptors have examined the subcellular localization of these receptors in the striatum and NAc. With the exception of one study which examined only the NAc (Delle Donne et al., 1996), all of these either examined only the striatum or reported no difference between results from the striatum and NAc. The unanimous consensus of these studies is that the vast majority of labeling for both D1a and D2 receptors is localized to dendritic shafts and dendritic spine heads and necks of medium spiny neurons (Caillé et al., 1996; Delle Donne et al., 1996; Fisher et al., 1994; Hersch et al., 1995; Huang et al., 1992; Levey et al., 1993; Sesack et al., 1994; Yung et al., 1993). Varying degrees of labeling on axon terminals was reported. Of the studies that examined both D1a and D2 receptor localization, one found that if either of the receptors was expressed on a terminal, the terminal usually

made an asymmetrical synapse (Huang et al., 1992); another found that presynaptic D1a and D2 receptors are exclusively on terminals that form symmetrical synapses (Levey et al., 1993); another found that immunoreactivity for both receptors could be seen on terminals forming symmetrical synapses, but only D2 immunoreactivity was seen on terminals with asymmetrical synapses (Yung et al., 1995); and another found that D1-labeled terminals were very rare, while D2-labeled terminals mostly formed asymmetrical synapses (Hersch et al., 1995). One study that examined only D1 receptors reported that labeling of terminals of either type (with symmetrical or asymmetrical synapses) was extremely rare (Caillé et al., 1996). Studies examining only D2 receptors have all found some degree of labeling of terminals with either synapse type, with one reporting more labeling of asymmetrical than symmetrical synapses (Fisher et al., 1994) and two from another lab reporting the converse (Delle Donne et al., 1996; Sesack et al., 1994). The nearly absolute lack of agreement on what types of receptors, if any, label axon terminals in the striatum is striking given the unanimity of the findings that both D1a and D2 receptors are strongly expressed on dendritic structures. All of these studies agree, however, that labeling of terminals is not as common as labeling of dendrites; the disagreement on the specifics of terminal labeling is probably the result of the different sensitivities of the various electron microscopic techniques employed, as well as multiple opinions concerning what constitutes "rare" labeling. Thus, it is difficult to conclude that one receptor type as opposed to the other is preferentially found on inhibitory (symmetrical) or excitatory (asymmetrical) terminals, although most of the data is consistent with the presence of D2 (but not D1) receptors on dopaminergic terminals (which are on nonmyelinated axons, exhibit symmetrical synapses, and are tyrosine hydroxylase-positive) (Caillé et al., 1996; Delle Donne et al., 1996; Sesack et al., 1994).

D. Electrophysiological Effects of Dopamine Receptor Activation in the Brain

Nuclei other than the striatum and NAc. One well-characterized effect of dopamine is the inhibition of hormone (prolactin and others) secretion from pituitary cells. The pituitary receives no dopaminergic innervation. Instead, dopaminergic cells in the hypothalamus release dopamine into the portal blood vessels, which transport hypothalamic hormones, including dopamine, through the pituitary stalk to the pituitary gland. A dopamine receptor of the D2 class is responsible for the reduction in prolactin secretion (Ben-Jonathan, 1985). Recordings from cells in intact pituitaries found that dopamine caused the increase of a K^+ conductance which resulted in a hyperpolarization and reduced action potential frequency (MacVicar and Pittman, 1986; Williams et al., 1989), an effect which was confirmed and shown to inhibit prolactin release in primary pituitary cell cultures (Israel et al., 1987). Later analyses in cultured cells showed that similar effects in intermediate pituitary cells were dependent on D2 receptor activation (Stack and Surprenant, 1991) and that D2 activation in anterior pituitary cells enhances two voltage-dependent K^+ currents, a non-inactivating I_K current and a delayed I_A current (Lledo et al., 1990c). A D2-mediated reduction in L and T-type Ca^{2+} currents was also found (Lledo et al., 1990a,b; Stack and Surprenant, 1991); the effects on Ca^{2+} currents are reported to involve a G protein different from the G protein responsible for the enhancement of K^+ currents, despite the activation of presumably the same D2 receptor (Lledo et al., 1992). These studies have therefore delineated most, if not all, of the mechanisms by which dopamine reduces the release of hormones from pituitary cells.

Intracellular recordings in slices of the substantia nigra (SN) have also found D2 receptor-mediated hyperpolarizations and reductions in spike frequency, effects that could be attributed to the activation of a K^+ conductance (Lacey et al., 1987, 1988, 1989; Seutin et al., 1994). Release of endogenous dopamine by application of amphetamine causes a similar response (Nedergaard et al., 1988). A recent whole-cell voltage clamp study from cultured nigral cells has suggested that these effects are due

to an increase in an inwardly rectifying K^+ current (Kim et al., 1995).

A number of *in vivo* studies have found negative modulation of the firing frequency of cells in the prefrontal cortex by iontophoretically applied dopamine or agonists (Bunney and Aghajanian, 1976; Williams and Goldman-Rakic, 1995), systemically administered dopamine agonists (Mora et al., 1976) or VTA stimulation (Ferron et al., 1984; Jay et al., 1995; Peterson et al., 1990). *In vitro* studies in cortical slices have suggested that several different dopamine receptor-mediated mechanisms could be responsible for depression of firing, including inhibition of a slow depolarizing Na^+ current that contributes to membrane potential oscillations (Geijo-Barrientos and Pastore, 1995), reduction of excitatory synaptic transmission by a D1 receptor-dependent mechanism (Law-Tho et al., 1995; Pralong and Jones, 1993) and excitation of GABAergic neurons within the cortex (Penit-Soria et al., 1987). However, none of these mechanisms have been worked out in detail and most remain to be replicated.

In contrast to the generally inhibitory effects of dopamine in other parts of the brain, in the retina, dopamine appears to exert several excitatory actions. By a D1 receptor-dependent mechanism, dopamine depolarizes horizontal cells and reduces electrical coupling between them (Dowling, 1991; Pereda et al., 1992). A D1 receptor is also responsible for the enhancement of kainate-activated excitatory responses by modulation of glutamate receptors on horizontal cells (Knapp and Dowling, 1987; Knapp et al., 1990; Liman et al., 1989). All of these effects appear to be mediated by activation of adenylate cyclase and consequent increase in intracellular cAMP levels.

Striatum. Since the 1960s a great number of electrophysiological studies have attempted to elucidate the role of dopamine in the striatum and, more recently, the NAc. Unfortunately, results from these studies have often been confusing. *In vivo* intracellular and extracellular recordings from striatal neurons were initially paired with stimulation of the SN or medial forebrain bundle (MFB), which carries the axons of the ascending dopamine system. Some of these yielded the conclusion that synaptically released dopamine excites striatal cells, others found an inhibition, and

still others found complex patterns of excitation and inhibition (Dray, 1980). Siggins (1978) pointed out that the long response latencies expected from SN or MFB stimulation – which result partly from the low conduction velocity of the unmeyelinated, small diameter dopaminergic fibers and partly from the metabotropic nature of dopamine receptors – make it extremely difficult to demonstrate conclusively that responses recorded in the striatum are monosynaptic. Thus, the various patterns of excitation and inhibition were probably the result of activation of several sets of fibers, including unintentionally stimulated striatal afferent systems and antidromic activation of striato-nigral fibers.

Subsequent studies largely abandoned the fiber stimulation approach in favor of iontophoretic application of dopamine, and their results were somewhat less contentious. An initial intracellular recording study (Kitai et al., 1976) found that very short (less than 100 ms) iontophoretic pulses of dopamine depolarized striatal cells and could cause spiking, but this result was challenged on the basis of evidence that the initial response to the iontophoresis of any substance is likely to be an excitation that arises from electrical artifact (Siggins, 1978). Further extracellular single unit studies found that longer (i.e., several seconds) iontophoretic pulses of dopamine inhibit spontaneous striatal cell firing (Brown and Arbuthnott, 1983; Chiodo and Berger, 1986; Hu and Wang, 1988; Johnson et al., 1983; Nisenbaum et al., 1988; Rolls et al., 1984; Siggins, 1978). Some extracellular studies found that iontophoretic or pressure-ejected dopamine decreased spiking evoked by cortical stimulation or iontophoresis of glutamate (Brown and Arbuthnott, 1983; Johnson et al., 1983) but later it was observed that lower doses of dopamine facilitated glutamate-evoked spiking while higher doses inhibited it (Chiodo and Berger, 1986; Hu and Wang, 1988; Nisenbaum, 1988). Interestingly, those studies that compared the degree of dopamine-induced inhibition of spontaneous firing with the degree of inhibition of glutamate-evoked (Chiodo and Berger, 1986) or cortical stimulation-evoked (Johnson et al., 1983) firing found that spontaneous activity was routinely more attenuated than evoked activity. Thus, extracellular single unit studies suggest that dopamine facilitates the effects of strong

excitation by reducing spontaneous "noise" firing and either facilitating evoked firing or reducing it to a lesser degree than spontaneous firing.

Intracellular studies conducted *in vivo* cast some light on the mechanism by which dopamine inhibits striatal cell firing, although all of these were conducted before the discovery that lower doses of dopamine are excitatory, and therefore they do not address this issue. Bernardi et al. (1978) found that dopamine iontophoresis caused a slow depolarization of striatal cells, paradoxically accompanied by a decrease in the spontaneous firing rate. Later it was found that dopamine also reduced the amplitude of the EPSP-IPSP sequence evoked by stimulation of the cortex (Herrling and Hull, 1980; Mercuri et al., 1985) and reduced the amplitude of depolarizations and hyperpolarizations resulting from glutamate and GABA iontophoresis (Mercuri et al., 1985). Furthermore, dopamine elevated the threshold for action potentials generated by depolarization of the cell by injection of current through the recording electrode, suggesting that part of the inhibition of striatal cell firing by dopamine may be due to an effect on voltage-dependent conductances of the striatal cell membrane (Mercuri et al., 1985). However, a possible presynaptic effect of dopamine on voltage-dependent conductances in cortical axons or terminals was suggested by the finding that for an extracellular stimulating electrode placed in the striatum, the stimulus intensity required to observe an antidromic action potential in a cell recorded in the cortex was greater when amphetamine or dopamine agonists were locally applied to the striatum (Garcia-Munoz et al., 1991).

Two further sets of *in vivo* studies provided additional information concerning the effects of dopamine on striatal cells. One technique was to observe the effects of systemically administered amphetamine on striatal cells recorded in anesthetized animals. Consistent with an inhibitory effect of dopamine, increases in spike frequency in response to sciatic nerve stimulation were attenuated by amphetamine, and this effect was not present in animals that had sustained 6-OHDA lesions in the striatum (Abercrombie and Jacobs, 1985). However, an intracellular study (Schneider et al., 1984) found that systemic amphetamine increased the EPSP evoked by cortical

stimulation (while depolarizing the cell and reducing spontaneous firing frequency), but the localization of amphetamine's actions to the striatum was questionable since no experiments to determine its site of action were performed.

The second *in vivo* technique was to record from striatal cells in awake animals while locally administering dopamine or agonists. Rolls et al. (1984) found in monkey striatum that dopamine iontophoresis decreased both the spontaneous firing rate and the excitatory response that accompanied a particular behavioral situation, such as licking for a food reward. The behavior-associated responses were depressed to a lesser degree than the spontaneous firing rate, leading to the suggestion that dopamine increases the signal to noise ratio for relevant information (Rolls, 1984; Rolls et al., 1984). In resting (i.e., not moving) awake rats, dopamine iontophoresis or local infusion of amphetamine into the striatum increases the firing rate of striatal cells that tend to respond during movement, but decreases it for cells that are quiescent during movement (Pierce and Rebec, 1995; Wang and Rebec, 1993). In both the striatum and NAc, dopamine sometimes increased and sometimes decreased the responses of cells to glutamate iontophoresis, but the net result was an enhancement of the glutamate response relative to the baseline firing rate (Kiyatkin and Rebec, 1996). These studies in awake animals suggest, as do several studies in anesthetized preparations (see above) that the effects of dopamine may be to sharpen or facilitate stronger (and presumably therefore more relevant) excitatory inputs and weaken less effective "noise" inputs, but the physiological mechanism by which this is accomplished remains to be discovered.

In vitro studies have suggested a variety of possible mechanisms by which dopamine can influence striatal cell excitability. Using intracellular recording in striatal slices, Calabresi et al. (1987) found that dopamine reduced the size of EPSPs, increased the amount of positive current injection required to elicit a spike and left the membrane potential unchanged. They attributed these effects to a dopamine-induced reduction of a tetrodotoxin-sensitive Na^+ current that causes inward rectification at potentials between –80 mV and the spike threshold (–50 mV). Thus, the EPSP was not

reduced by dopamine at more negative potentials, where inward rectification is due to TEA-sensitive (and dopamine-insensitive) K^+ currents (Calabresi et al., 1987). The reduction of EPSPs and membrane rectification was found to be an effect of D1 receptor activation (Calabresi et al., 1988); consistent with this result, later whole-cell voltage clamp studies found that activation of D1 receptors on striatal cells reduces a voltage-dependent Na^+ current that contributes to action potential generation (Schiffmann et al., 1995; Surmeier et al., 1992). Further intracellular recordings confirmed that dopamine reduces the number of spikes elicited by intracellular positive current injection (Akaike et al., 1987; Rutherford et al., 1988), and found that this effect could not be caused by an increase in a long-lasting K^+-mediated afterhyperpolarization (slow AHP; Nicoll, 1988) since the slow AHP was reduced by dopamine (Rutherford et al., 1988). However, another group did not observe a slow AHP in striatal cells (Calabresi et al., 1987). A later study found that dopamine increases the fast AHP in striatal cells by a D1 receptor and L-type Ca^{2+} channel dependent mechanism, although the ability of the increased fast AHP to affect firing rates was not demonstrated (Hernández-López et al., 1996).

Other experiments conducted in striatal slices suggest a role for postsynaptic D2 receptors in the modulation of striatal cell excitability. Activation of D2 receptors appears to reduce EPSPs and the response to iontophoretically applied glutamate and AMPA (Cepeda et al., 1993; Hsu et al., 1995; Levine et al., 1996b). These results were challenged by Calabresi et al. (1995), who found no effect of dopamine, D1 or D2 agonists on the responses of striatal cells to iontophoresed AMPA or NMDA. This group has observed D2-mediated reduction of the EPSP, but only in slices taken from animals that had undergone 6-OHDA lesions of the striatum, pretreatment with the dopamine receptor antagonist haloperidol, pretreatment with the dopamine synthesis inhibitor α-methylparatyrosine, or pretreatment with reserpine, which depletes monoamines from synaptic vesicles (Calabresi et al., 1988, 1992, 1993).

Two groups have reported D2-mediated enhancements of K^+ currents. Preliminary studies from one group report that a D2 agonist caused an enhancement of a slowly-

recovering A_S current in a population of striatal cells, while a D1 agonist reduced this current (Kitai and Surmeier, 1993; Surmeier and Kitai, 1993). Another group using single-channel recording has found that D2 receptors activate an 85 pS K^+ channel (Freedman and Weight, 1988; Greif et al., 1995). However, the contributions of neither of these effects to cell excitability have been directly assessed. Altemus and Levine (1996) found that dopamine's modulation of the EPSP was not influenced by blockade of K^+ channels by cesium ions, which have been shown to block the dopamine-dependent 85 pS K^+ channel (Lin et al., 1996).

Several further electrophysiological studies have found dopamine effects with the potential for influencing striatal cell excitability. D1 receptor activation has been found to reduce NMDA receptor-dependent currents resulting from excitatory synaptic transmission or NMDA iontophoresis (Cepeda et al., 1993; Levine et al., 1996a, 1996b), although another group failed to observe an effect of D1 receptor activation on NMDA currents (Calabresi et al., 1995). Trulson and Arasteh (1986) found that spontaneous spiking of striatal cells in mouse slices was increased by dopamine; while this result is difficult to reconcile with the literature describing dopamine-induced depression of spontaneous firing *in vivo* (see above), none of the *in vivo* studies were conducted in mice. Cepeda et al. (1995) reported the dopamine-induced reduction of a persistent non-inactivating Na^+ conductance, and Surmeier et al. (1995) found dopaminergic modulation of several Ca^{2+} currents by D1 receptor activation, but the consequences of these effects for membrane excitability have not been analyzed. Lastly, one group (Calabresi et al., 1996) has found effects of dopamine on long-term synaptic plasticity in striatal slices.

Despite the large number of *in vitro* studies reporting effects of dopamine on striatal cells, discrepancies between the dopamine effects observed *in vivo* and *in vitro* remain. For instance, intracellular studies *in vivo* observed a depolarization of the cell membrane when dopamine was applied (Bernardi et al., 1978; Herrling and Hull, 1980; Mercuri et al., 1985; Schneider et al., 1984), but only one (Akaike et al., 1987) of many intracellular studies in slices confirmed this effect. Inhibition of IPSPs was also

observed *in vivo* (Herrling and Hull, 1980; Mercuri et al., 1985), but to date no studies have examined the effects of dopamine on striatal IPSPs. Dopamine applied locally to cells recorded *in vivo* has consistently been found either to potentiate glutamate-, stimulation- or movement-evoked responses, or to depress spontaneous firing more than evoked firing (Chiodo and Berger, 1986; Hu and Wang, 1988; Johnson et al., 1983; Nisenbaum, 1988; Rolls et al., 1984), but the mechanisms of such an effect have not been explored *in vitro*. Thus, while *in vivo* studies provide important clues pertaining to how dopamine influences information processing by striatal cells, *in vitro* studies have yet to provide mechanistic details of these processes.

Nucleus Accumbens. A much smaller body of literature addresses the effects of dopamine on NAc cell physiology. *In vivo* studies involving iontophoretic application of dopamine to extracellular single unit recordings have found that dopamine depresses NAc cell firing evoked by stimulation of the amygdala (Yim and Mogenson, 1982) or hippocampus (DeFrance et al., 1985b; Yang and Mogenson, 1984) or by iontophoretic application of glutamate (White and Wang, 1986). Stimulation of the VTA appears to have the same effect as dopamine application (Yang and Mogenson, 1984; Yim and Mogenson, 1982, 1986, 1988). An *in vivo* field recording experiment found dopamine-induced depression of the (putatively synaptic) "N" potential elicited by amygdala stimulation (DeFrance et al., 1985b), and an *in vivo* intracellular recording study revealed that iontophoretic application of dopamine depresses both EPSPs and IPSPs resulting from amygdala stimulation and is accompanied by a depolarization of the NAc cell (Yim and Mogenson, 1988). These results are similar to those observed in *in vivo* intracellular studies in the striatum, where dopamine depolarizes cells and reduces the magnitude of EPSP-IPSP sequences evoked by cortical stimulation (Bernardi et al., 1978; Herrling and Hull, 1980; Mercuri et al., 1985). Whereas several studies in the striatum of both anesthetized and behaving animals have reported dopamine-induced enhancement of evoked firing relative to spontaneous firing (Chiodo and Berger, 1986; Hu and Wang, 1988; Johnson et al., 1983; Nisenbaum et al., 1988; Rolls et al., 1984), some studies in anesthetized animals have reported that evoked firing in the NAc is

reduced *more* than spontaneous firing (Yang and Mogenson, 1984; Yim and Mogenson, 1982) whereas another has found in awake rats that glutamate-evoked firing is reduced less than spontaneous firing in both the NAc and striatum (Kiyatkin and Rebec, 1996).

Further indirect evidence suggests that dopamine does in fact reduce the signal-to-noise ratio for NAc cell firing. During lever-pressing for cocaine, a population of NAc cells fires specifically during the lever-press and during the subsequent light stimulus that is given in conjunction with the cocaine injection (Carelli et al., 1993, 1994, 1996b; Chang et al., 1994). These specific lever-press associated responses are not always observed during the "load-up" phase of the self-administration session (Carelli et al., 1993; Carelli and Deadwyler, 1996b). During load-up, the animal presses the lever rapidly, and only begins to respond at longer, more regular intervals when dopamine concentrations in the NAc reach an elevated plateau (Wise et al., 1995). Specific firing associated with lever-presses often emerges exactly as load-up is completed; when load-up is delayed by systemic SCH23390 administration, the transition to specific firing is delayed by an identical amount of time (Carelli and Deadwyler, 1996a). These experiments are suggestive that increased dopamine levels in the NAc are responsible for facilitating specific NAc cell firing, perhaps by increasing the signal-to-noise ratio.

In vitro studies using slices of the NAc initially found that dopamine and cocaine caused a D1 receptor-mediated hyperpolarization and a D2 receptor-mediated depolarization of the NAc cell membrane (Higashi et al., 1989; Uchimura et al., 1986; Uchimura and North, 1990). These responses were extremely small, no more than 6 mV on average, although another group found larger (18 mV) depolarizations in response to the broad-spectrum dopamine agonist apomorphine (O'Donnell and Grace, 1994). On the other hand, a third group failed to see any effect of dopamine on the membrane potential (Pennartz et al., 1992a). Attempts by these groups to elucidate the effects of dopamine on excitatory synaptic transmission provided results that were no less conflicting. Initially it was found that dopamine did not affect the EPSP evoked by

periaccumbens stimulation, but when the animals were chronically treated with amphetamine prior to the experiments, a D1 receptor-mediated inhibitory effect of dopamine was revealed (Higashi et al., 1989). A different group found that amphetamine pretreatment was not necessary to observe effects on synaptic transmission: Pennartz et al. (1992a) found that dopamine depressed EPSPs recorded in NAc slices from naïve animals by a D1 receptor-dependent mechanism. The effects were limited to the shell (as opposed to the core) region of the NAc (Pennartz et al., 1992b) and were thought to be presynaptic, since paired-pulse facilitation was increased by dopamine (Pennartz et al., 1992a). These results were challenged by another group, who found that a D2 receptor-dependent mechanism is responsible for dopamine-induced depression of the EPSP (O'Donnell and Grace, 1994). Complicating these results even further are data from *in vivo* iontophoresis experiments, which found that D1 receptor activation can result in inhibition of glutamate-evoked NAc cell firing, as well as facilitation of D2 receptor-mediated inhibition of glutamate-evoked firing (Johansen et al., 1991; White, 1987; White and Wang, 1986). The fact that the measured firing rates were evoked by glutamate application suggests that the effects observed by this group were due to activation of postsynaptic dopamine receptors, in disagreement with the paired-pulse facilitation data of Pennartz et al. (1992a). Thus, while *in vivo* and *in vitro* studies agree that excitatory synaptic transmission in the NAc is depressed by dopamine at least under some conditions, based on these studies it is difficult to draw conclusions concerning the dopamine receptor subtype(s) and the mechanism (e.g., pre- or postsynaptic) responsible for this depression.

The main aims of the present work derive from this confusion regarding the specifics of dopaminergic actions on synaptic transmission in the NAc as well as in the striatum. The slice preparation can be used to conduct a careful pharmacological analysis to determine the dopamine receptor subtypes involved, and to examine the mechanisms by which dopamine influences both excitatory and inhibitory synaptic transmission.

Chapter 2

Experimental Procedures

Slice preparation, electrophysiological recording and data collection. The methods used in this work are largely identical to those described (Kombian and Malenka, 1994; Nicola et al., 1996; Nicola and Malenka, 1997). Sprague-Dawley rats (13 – 40 days postnatal) were used for all experiments except those involving mutant mice or 6-OHDA lesioned rats. Animals were completely anesthetized with Halothane and sagittal NAc slices or coronal striatal slices (400 μm thick) were prepared from both hemispheres using a vibratome. Throughout the procedure, the tissue was maintained in ice-cold artificial cerebrospinal fluid (CSF) which was continuously bubbled with 95% O_2 and 5% CO_2. The composition of the CSF was (in mM): NaCl, 126; KCl, 1.6; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 18; glucose, 11. After at least 1 h incubation at room temperature, slices were transferred to a recording chamber and submerged beneath continuously flowing (at least 2 ml per min) CSF at a temperature of 21 – 29 °C. For experiments involving $CoCl_2$ or $CdCl_2$, NaH_2PO_4 was eliminated from the CSF to prevent precipitation of phosphates. Axons forming synapses onto NAc cells were activated at 0.1 Hz with a bipolar stainless steel microelectrode placed at the prelimbic cortex-NAc border, and recordings were made in the core region of the NAc using the anterior commissure and lateral ventricles as anatomical markers. In striatal slices, stimulating electrodes were placed at the border with the cortex.

All recordings were performed using either an Axoclamp 2A or Axopatch 2D amplifier (Axon Instruments). For field recordings, glass micropipettes were filled with 3 M NaCl or with CSF. For whole-cell current clamp recordings (Blanton et al., 1989), micropipettes (8 – 15 MΩ) were filled with one of several solutions:

1) Potassium methylsulphate solution consisted of (in mM): 134.5 potassium methylsulphate, 8 NaCl, 10 HEPES, 0.2 EGTA, 2.5 MgATP and .1 GTP; pH 7.2

2) Cesium gluconate solution consisted of (in mM): 117.5 cesium gluconate, 17.5 CsCl, 8 NaCl, 10 HEPES, .2 EGTA, 2.5 MgATP and .1 GTP; pH 7.2

3) Cesium chloride (CsCl) solution consisted of (in mM): 134.5 CsCl, 8 NaCl, 10 HEPES, .2 EGTA, 2.5 MgATP and .1 GTP; pH 7.2

4) Potassium methylsulphate with QX314 solution consisted of (in mM): 129.5 potassium methylsulphate, 8 NaCl, 10 HEPES, 0.2 EGTA, 5 QX314 chloride, 2.5 MgATP and .1 GTP; pH 7.2

5) Cesium gluconate with QX314 solution consisted of (in mM): 137.5 cesium gluconate, 10 HEPES, .2 EGTA, 5 QX314 chloride, 2.5 MgATP and .1 GTP; pH 7.2.

These solutions were made by dissolving all salts except for ATP and GTP and adjusting the pH to 7.2. The osmolarity was then adjusted to the desired range (276 – 287 mOsm) by addition of water; if the solution became too dilute, it was discarded and remade from the beginning. At this point the pH was adjusted to a value (usually 7.8 – 8.0) such that addition of the indicated amounts of ATP and GTP resulted in a final pH of 7.1 – 7.3. This solution, without the nucleotides, was divided into aliquots and kept for months or even years in the freezer, and fresh ATP and GTP were added to each aliquot after thawing.

In most cases, inhibitors of either inhibitory synaptic transmission (25 μM picrotoxin) or excitatory synaptic transmission (10 μM DNQX and 75 μM d,l-APV) were included in the CSF to isolate, respectively, excitatory or inhibitory responses. In experiments examining excitatory responses, cells were held near their resting potential (holding potential, V_h = –80 mV) during both current clamp and voltage clamp recordings. At this potential, the response was mediated mainly by non-NMDA receptors (Kombian and Malenka, 1994). For inhibitory responses, cells were held at 0 mV for the Cs gluconate pipette solution or at –80 mV for the CsCl solution. Outward IPSCs and hyperpolarizing IPSPs could be observed at 0 mV, and inward IPSCs were observed at –80 mV.

Input resistance was monitored continuously by applying a negative current step (usually –.03 nA) after synaptic stimulation in current clamp recordings, or a

hyperpolarizing voltage step (usually 5 – 10 mV) in voltage clamp recordings. Spontaneous miniature EPSCs (mEPSCs) and miniature IPSCs (mIPSCs) were collected under these same conditions, except that 1.5 μM tetrodotoxin (TTX) was present in the perfusion medium throughout the experiment.

Data were collected (3 – 10 KHz sampling rate) and analyzed online using custom written software. The amplitudes of IPSPs, IPSCs, EPSPs, EPSCs and synaptic field potentials were computed; the initial slope of EPSPs was also computed using a least square regression. Each point on the illustrated graphs is the mean of a one minute bin of successive responses. Each representative data trace is the mean of 9 – 11 successive responses. For experiments studying mEPSCs or mIPSCs, 0.1 Hz negative voltage pulses were used throughout the experiment to monitor input and access resistance. Data were continuously digitized at 10 kHz and stored on a computer hard disk. After the experiment, mEPSCs and mIPSCs were detected using software (generously provided by J. Steinbach, Washington University) that used the fast rise time of synaptic currents to determine the presence of each putative synaptic event. If the amplitude of the event fell within the limits expected (usually 3 – 70 pA), the event was counted and its amplitude computed. In most cases the final determination of whether an event was a mini was made by eye, although in some cases the event frequency was too high for manual selection to be feasible. In these cases, the ability of the program to detect genuine synaptic events was always checked by eye at several times during the experiment. A plot of input resistance and access resistance over time was always computed, and experiments were rejected if the results could be explained by artifacts arising from changes in these factors. Average mEPSC and mIPSC amplitude and frequency were computed in 1 minute bins.

Statistics. For all slice experiments, the experimental comparison was between the magnitude of the baseline responses and the magnitude of the responses in agonist. For each individual experiment, two data points were computed for use in the statistical analysis: the average of all points in the 10 min baseline, and the average of all points in a 3 min period (which was the same for all experiments in one set of comparisons) during agonist application. Thus, all experiments involved repeated measures of one factor,

Presence of Agonist. When this was the only experimental factor, a paired t-test was used to determine whether there was a significant effect of the agonist on the magnitude of the response compared with the baseline magnitude. In some experiments an additional comparison, such as the degree to which the agonist-induced change was affected by an antagonist, was also made. In these cases, the statistical test used was a two-factor repeated measures analysis of variance (ANOVA) with repeated measures on one factor (Presence of Agonist). The ANOVA revealed whether the agonist caused a change in the response, as well as whether the antagonist significantly altered the degree of change induced by the agonist. The ANOVA generally revealed no significant difference between the different levels of the non-repeated measures factor, Presence of Antagonist, and therefore these results are not reported.

All statistical calculations were based on unnormalized data; however, graphs of the averages of experiments, the calculated percent change, and the calculated standard error of the mean (SEM) of the percent change are all based on data normalized to a 10 min baseline. All statistical tests assumed that the underlying distribution of the data was normal unless the data were obviously skewed. ANOVAs were calculated using SigmaStat (Jandel Scientific), and $p \leq .05$ was considered statistically significant for all tests.

Drug application. All drugs were applied by bath perfusion with CSF containing the final concentration of the drug. Appropriate stock solutions of drugs were made and diluted with CSF just before application. APV, CNQX, DNQX and TTX stock solutions were frozen and thawed before use. Stocks of all other drugs were made daily and at least at 1000-fold higher concentrations than were applied to the slices. Antagonists were applied at least 10 min prior to the addition of agonist in the continued presence of antagonist. CGP35348 was dissolved directly in CSF. Dopamine HCl, (±)-norepinephrine HCl, (–)-quinpirole, (±)-2-amino-6,7-dihydro-1,2,3,4-tetrahydronaphthalene HBr (6,7-ADTN), dihydrexidine, SKF38393 and (±)-SKF81297 were prepared in a water stock solution containing sodium metabisulphite (50 mM in stock solutions, 50 μM in the final solution) to protect them from oxidation. S(–)-Sulpiride, 6,7-nitroquinoxaline-2,3-

dione (DNQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), picrotoxin at concentrations > 50 mM, forskolin, isobutylmethylxanthine (IBMX) and 8-cyclopentyltheophylline (8-CPT) were prepared in dimethyl sulfoxide (DMSO). Tetrodotoxin (TTX) was prepared in DMSO or ethanol. Cocaine, S(+)-amphetamine, R(+)-SCH23390, 2-amino-5-phosphonovaleric acid (APV), picrotoxin, S(–)-propranolol, picrotoxin at concentrations ≤ 50 mM, phentolamine mesylate, and serotonin HCl were all made up in water. Lidocaine N-ethyl chloride (QX314 chloride) was synthesized by Precision Biochemicals (Colton, CA). Other chemicals were from Sigma, Research Biochemicals or Tocris-Cookson.

6-OHDA lesion experiments. Female Wistar rats (160 – 175 g) were anesthetized with ketamine (60 mg/kg, i.p.) and acepromazine (.6 mg/kg, i.p.) and ventilated with a mixture of O_2, N_2O, and Halothane while stereotaxic injections of 6-OHDA were made into the left medial forebrain bundle (Ungerstedt, 1971b). Injection coordinates (König and Klippel, 1963; Paxinos and Watson, 1986) were 4.4 mm posterior to Bregma, 1.2 mm lateral to the midline, and 8.5 mm ventral to the top of the skull at Lambda. Freshly prepared 6-OHDA solution (8 μg/μl in .1% ascorbic acid) was placed in a glass micropipette (tip diameter 100 μm) and 4 μl was injected over 2 – 3 min. After 5 min, the pipette was removed and the animal allowed to recover. To assess the effectiveness of the lesion, animals were tested for rotation behavior 10 days after surgery by administering 1 mg/kg apomorphine (i.p.) and counting the number of rotations contralateral to the lesion made during a 5 min period beginning 15 min after the apomorphine injection (Hefti et al., 1979). Slices were cut 10 – 15 days after the rotation test.

Chapter 3

Actions of Dopamine and Psychostimulants on Synaptic Transmission in the Nucleus Accumbens and Striatum

Introduction

While there is general agreement that dopamine reduces excitatory synaptic transmission in the NAc, the mechanisms by which dopamine acts have not been clarified (Higashi et al., 1989; O'Donnell and Grace, 1994; Pennartz et al., 1992a,b; White and Wang, 1986). Furthermore, despite the extensive GABAergic inhibition between NAc cells (Chang and Kitai, 1985; Pennartz and Kitai, 1991) no studies have examined isolated inhibitory synaptic potentials or currents to determine whether they, too, are modulated by dopamine. The effects of psychostimulants on excitatory and inhibitory synaptic transmission have also not been extensively examined. And despite the results of several studies demonstrating modulation by dopamine of excitatory synaptic transmission in the NAc, attempts to demonstrate a similar effect in the striatum have yielded often confusing results (Calabresi et al., 1987, 1988, 1992, 1993, 1995; Cepeda et al., 1993; Hsu et al., 1995; Malenka and Kocsis, 1988). Because of the importance of dopamine in the NAc and striatum in many behaviors, as well as the importance of synaptic transmission as a target for the modulation of neural information processing, a thorough examination of the interaction of dopamine with synaptic transmission in the NAc and striatum is clearly necessary. The brain slice technique is perhaps the most appropriate method by which to conduct such a study, since slices maintain local circuitry while allowing relatively easy access of drugs to the cells of interest by application in the bathing medium.

Results

Identification of EPSPs and IPSPs. Extracellular field recording in the NAc in the presence of picrotoxin (25 μM) revealed that electrical stimulation at the border of the prelimbic cortex and the nucleus accumbens (NAc) resulted in a biphasic response (Figure 1) (Horne et al., 1990; Pennartz et al., 1992a). Bath application of the ionotropic glutamate receptor antagonist CNQX (10 μM) abolished the later of the two negative potentials (n=5), demonstrating that this component ("N2", Figure 1) resulted from

excitatory synaptic transmission. The earlier component ("N1", Figure 1) was not diminished by CNQX but was eliminated by the Na^+ channel blocker tetrodotoxin (TTX, 1 μM, n=5). These results demonstrate that the amplitude of N1 is a measure of the direct, non-synaptic generation of action potentials, while the amplitude of N2 is a measure of excitatory postsynaptic responses (Horne et al., 1990; Malenka and Kocsis, 1988). Similar field potentials were obtained in the striatum (e.g., Figure 15).

Monosynaptic inhibitory postsynaptic potentials (IPSPs) or currents (IPSCs) were recorded from cells in the striatum or the core region of the NAc by blocking excitatory synaptic transmission with the glutamate receptor antagonists DNQX (10 μM) and d,l-APV (75 μM) and directly stimulating within the nucleus. IPSPs or IPSCs were observed in all cells recorded under these conditions (n=147) and were found to have a reversal potential of about –58 mV (Figure 2; n=3), in close agreement with the calculated reversal potential for Cl^- under the recording conditions used (potassium methylsulphate-based pipette solution with the Na^+ channel blocker QX314). Picrotoxin (50 – 200 μM) completely abolished the IPSPs (Figure 3, n=7) confirming that they were mediated by activation of $GABA_A$ receptors. Because IPSPs were often small when cells were held near their resting membrane potentials, in most experiments cells were held at 0 mV using a cesium gluconate-based pipette solution. Similar IPSPs were obtained in the striatum (e.g., Figure 14).

Cocaine, amphetamine and dopamine attenuate excitatory and inhibitory synaptic transmission in the NAc. Cocaine has at least two pharmacological actions: it blocks the re-uptake of monoamines such as dopamine (Kuhar et al., 1991; Ritz et al., 1987) and exerts a local anesthetic effect on axons (Dunwiddie et al., 1988). In NAc field recordings, bath application of cocaine (30 μM) reversibly depressed the excitatory synaptic response but also decreased N1 amplitude (Figure 4). However, when the stimulus strength was increased such that the N1 amplitude in the presence of cocaine was identical to the baseline N1 amplitude, the synaptic response remained depressed (Figure 4), indicating that the synaptic depression induced by cocaine is not due to its local anesthetic actions alone. To confirm this result, the local anesthetic reduction in

the number of axons firing action potentials was mimicked by stimulating the slice with a range of stimulus intensities both before and after the application of cocaine (Figure 5). In 6 of 7 experiments, the plot of N2 amplitude *vs* N1 amplitude was shifted to the right by cocaine (30 μM). Such a shift is inconsistent with the superimposable curves that would be expected if cocaine had no effect on synaptic transmission other than to reduce action potential activation. In addition, cocaine (30 – 100 μM) reduced the size of evoked EPSPs recorded in whole-cell current clamp without affecting the cells' membrane potential (not shown) or input resistance (Figure 6, n=7). These results suggest that cocaine depresses excitatory synaptic transmission in the NAc by means of a specific mechanism that is independent of both local anesthetic effects and effects on postsynaptic conductances.

Similar results were obtained for amphetamine (Figure 7), which also increases extracellular dopamine levels (Seiden et al., 1993). Amphetamine (10 μM) reversibly depressed the NAc EPSP while having no effect on action potential generation (Figure 7A, n=8). Moreover, neither the membrane potential nor the input resistance of NAc cells was influenced by a concentration of amphetamine (10 μM) that caused a substantial decrease in the EPSP (Figure 7B, n=8). If, in fact, cocaine and amphetamine depress synaptic transmission by increasing extracellular dopamine (Kuhar et al., 1991; Ritz et al., 1987; Seiden et al., 1993) then dopamine should mimic the effects of these psychostimulants. Indeed, dopamine (75 μM) depressed excitatory synaptic transmission in NAc cells without affecting N1 amplitude (Figure 8A, n=21) and without affecting the membrane potential or input resistance of postsynaptic cells (Figure 8B, n=8). Cocaine, amphetamine, and dopamine therefore all affect excitatory synaptic transmission in a similar fashion in the NAc, with the exception that the reversal of the amphetamine effect after wash-out of the drug is prolonged (40 – 60 min) compared to the time course of dopamine recovery (10 – 20 min, Figure 7 and Figure 8). This prolonged time course may be a consequence of the reversal of the vesicular dopamine transporter caused by the intracellular action of amphetamine which is taken up into dopaminergic terminals (Sulzer et al., 1995).

Amphetamine also depressed IPSPs in the NAc recorded in APV and DNQX, again without changing the cells' input resistance (Figure 9). The reduction of the IPSP amplitude caused by amphetamine (10 μM) was 19±4% (n=10, p<.005). Dopamine (75 μM) reduced the IPSP amplitude as well (Figure 10), by 28±4% (n=28, p<.001). Thus, in the NAc, dopamine and psychostimulants reduce the magnitude of both excitatory and inhibitory synaptic transmission.

Dopamine and amphetamine have comparatively minimal effects on excitatory and inhibitory synaptic transmission in the striatum. The similar structure and dopaminergic innervation of the NAc and striatum suggests that dopamine and psychostimulants may exert a similar depression of synaptic transmission in the striatum. However, bath application of dopamine (100 μM) to EPSPs recorded in striatal cells resulted in a much smaller depression than observed in the NAc (Figure 11). The magnitude of the depression was only 11±3%; although this was a significant difference from baseline (p<.02, n=12), it is much smaller than the reduction of NAc EPSPs by approximately 40% routinely observed in response to lower concentrations of dopamine (e.g., Figure 8, Figure 17, Figure 20, Figure 22). One possible explanation is that dopamine reuptake is more effective in the striatum than in the NAc. To test this hypothesis, dopamine was applied in the presence of the dopamine uptake inhibitor GBR12935 (Figure 12). In the absence of GBR12935, dopamine (100 μM) caused a 3.7±2.8% depression of the EPSP, and in the same cells in the presence of GBR12935, the depression was 15.3±4.7%; the effect of GBR12935 was not significant (p>.19; n=4). If indeed dopamine uptake were more efficient in the striatum than in the NAc, one might expect the effects of amphetamine, which causes the release of dopamine by reversal of the dopamine transporter (Seiden et al., 1993), to be the same if not larger in the striatum as opposed to the NAc. However, consistent with the inability of GBR12935 to facilitate dopamine effects, amphetamine was without effect on the striatal EPSP (Figure 13). In amphetamine (10 μM) the EPSP was only 11±5% smaller than during the baseline, a nonsignificant reduction (p>.05, n=7).

IPSPs in the striatum were also not reduced by dopamine and amphetamine. In

dopamine (100 μM) the IPSP was depressed by .6±3.2% (Figure 14A,B,C), and in amphetamine the IPSP was depressed by .6±5.7% (Figure 14A,B,D). Neither of these differences were significant ($p>.5$, $n=5$ for dopamine and $n=4$ for amphetamine). Application of serotonin to striatal excitatory field potentials, however, reduced the synaptic response by 18±5% ($n=7$, $p<.02$, Figure 15), indicating that striatal slices cut and examined under these conditions are healthy and capable of exhibiting synaptic depression. Thus, dopamine does not appear to have the same depressant effects on synaptic transmission observed in the NAc, a difference which is most likely due neither to a difference in health of the slices nor to greater effectiveness of dopamine uptake in the striatum.

The lack of effect of dopamine on EPSPs in the striatum is surprising given the reported reduction by dopamine of Na^+ current-dependent inward rectification ("anomalous rectification") between –80 and –50 mV, an effect that has been shown to reduce the size of striatal EPSPs recorded with intracellular electrodes (Calabresi et al., 1987, 1988). To determine whether such a reduction of inward rectification can be observed under the present recording conditions, a Cs gluconate-based electrode solution was used to record the change in membrane potential in response to a series of positive and negative current injections (resting membrane potential was –70 mV). As shown in Figure 16, very little, if any, rectification was observed, and the plot of the change in membrane potential *vs* injected current was unaltered by dopamine (100 μM; $n=4$). The results were similar whether the amplitude of the voltage deflection was measured toward the end of the 500 ms current pulse (Figure 16B) or between 50 and 75 ms after the beginning of the current pulse (not shown).

Integrative consequences of dual negative modulation of EPSPs and IPSPs in the NAc. Cells in the NAc inhibit each other with extensive GABAergic axon collaterals. Therefore, if an excitation is strong in that it activates a large number of NAc cells, a response recorded in one NAc cell will be inhibited by the GABAergic synapses it receives from other excited NAc cells. On the other hand, a weak excitation of only a few NAc cells will not be as strongly inhibited. If dopamine acts in a diffuse manner,

inhibiting synaptic release at many excitatory and inhibitory terminals (see the Discussion in Chapter 5, p. 169), the effect of dopamine on excitation measured in a single NAc cell will be to reduce the magnitude of weak excitations more than the magnitude of strong excitations. This is because strong excitations are already attenuated by GABAergic inhibition from other NAc cells, and this inhibition will be reduced along with the glutamatergic excitation. Weak excitations that incur less GABAergic inhibition from surrounding NAc cells, on the other hand, will be more severely attenuated by dopamine. If *in vivo* these weak excitations contribute to the baseline "noise" firing that is not associated with specific external stimuli or behavioral events, then the effect of dopamine should be to increase the signal-to-noise ratio for events that activate many NAc cells.

A complete test of this hypothesis must be conducted *in vivo*, preferably in behaving animals. However, conditions of strong and weak excitation can be introduced to cells recorded in slices. An example of an attempt to determine whether dopamine differentially affects strong and weak inputs is shown in Figure 17. Slices were bathed in solution containing no antagonists of excitatory or inhibitory synaptic transmission, and a Cs gluconate-based electrode solution with the Na^+ channel blocker QX314 was used. The compound synaptic potential was recorded in current clamp at –70 mV; the slice was stimulated alternately (5 s between stimuli) at two different stimulus strengths. One stimulus strength was approximately 5 – 10 times larger than the other, but the magnitude of the response to the higher stimulus was usually no more than twice as large as the response to the lower stimulus strength (Figure 17A), and the time course of decay was often shorter for the larger response. As shown in Figure 17C, on average (n=7), the response to the higher stimulus was attenuated by dopamine (100 μM) less than the response to the lower stimulus (21±3% depression for the higher, 45±8% for the lower). After recovery of the dopamine effect, picrotoxin was applied (Figure 17B). Picrotoxin (200 μM) caused a much greater increase in the response for the higher stimulus than for the lower stimulus (150±12% of baseline for the higher, 102±8% for the lower), indicating that the larger response was more

attenuated by GABAergic inhibition than the smaller response. When dopamine was applied to the same cells in the presence of picrotoxin (with the same stimulus strengths used to elicit the responses in the absence of picrotoxin), the inhibition of the larger and smaller responses was approximately equal (31±6% depression for the larger, 38±9% for the smaller; Figure 17C). For the higher stimulus strength, picrotoxin caused an increase in the magnitude of the depression elicited by dopamine ($p<.003$), whereas for the lower stimulus strength, there was no difference in dopamine's effectiveness whether or not picrotoxin was present ($p>.7$). Thus, as a result of inhibition among NAc cells, dopamine depresses weak excitation more than stronger excitation.

One potential explanation for these results is that the large synaptic potentials recorded in picrotoxin consisted of both EPSPs and depolarizations resulting from the activation of voltage-dependent conductances, and that differences in the effects of dopamine were the result of bringing the membrane potential during the larger EPSP below the threshold for activation of such conductances. Fast Na^+-mediated action potentials were not observed due to the QX314 in the recording electrode. However, broad regenerative action potentials (presumably Ca^{2+} spikes) were frequently observed if the EPSP reached an amplitude of greater than 50 mV; these spikes were eliminated from the analysis. Nevertheless, EPSPs of 50 mV may have activated voltage-dependent conductances that did not lead to regenerative potentials, but still contributed to the calculated EPSP amplitude. To test whether this was the case, cells were first held at –70 mV and EPSPs of 45 – 50 mV in amplitude were obtained. The membrane potential was then elevated to +70 mV and the absolute values of EPSP amplitudes obtained at the two potentials were compared. Because of the linear current-voltage relationship for non-NMDA glutamate receptors from –80 to +80 mV (Ascher and Nowak, 1988), it was expected that if no other conductances are reflected in the EPSP amplitude at –70 mV, then providing the glutamate receptors with an equivalent but opposite ionic driving force should result in an EPSP of equivalent but opposite amplitude. However, as shown in Figure 18A, EPSPs were broader and about 5 mV greater in magnitude at +70 mV than at

–70 mV (n=2). This was due to the activation of NMDA receptors that were blocked by Mg^{2+} at the negative potential (Hestrin et al., 1990), since during blockade of NMDA receptors with D-APV (50 μM) the amplitude of the EPSP was 0 – 5 mV smaller at +70 mV than at –70 mV (n=2; Figure 18B). Therefore, because activation of voltage-dependent conductances is unlikely at positive membrane potentials, the similarities in the magnitudes of EPSPs recorded with identical positive and negative driving force indicates that very little, if any, of the amplitude of EPSPs recorded at –70 mV was due to activation of voltage-dependent conductances. The differences between the effects of dopamine on synaptic responses to large and small stimulus strengths observed in Figure 17 are therefore most likely not due to differential activation of voltage-dependent conductances.

Discussion

Electrical stimulation of NAc and striatal slices in the presence of antagonists of inhibitory synaptic transmission resulted in robust EPSPs that were sensitive to AMPA and NMDA receptor antagonists. The source of these EPSPs are likely to be excitatory terminals on axons from afferent nuclei, since the NAc and striatum do not contain glutamatergic cells (Chronister et al., 1981; Groves, 1983; Kawaguchi et al., 1995). The main source of excitation in the striatum is the cerebral cortex, while the NAc receives excitatory activation from the cortex, hippocampus, amygdala and thalamus (Pennartz et al., 1994). Thus, EPSPs recorded in the striatum are likely to arise from release of glutamate from cortical terminals; but in the NAc, the identity of the stimulated axons is less clear. An effort was made to place the stimulating electrode at the border between the cortex and the NAc, but one can by no means be certain that fibers other than cortical afferents do not contribute to the response.

GABAergic IPSPs, on the other hand, are likely to arise entirely from activation of the collaterals of axons within the NAc or striatum. Anatomical evidence suggests the lack of GABAergic afferents to the NAc (Christie et al., 1987) while at the same time

demonstrating extensive axonal arborization within the NAc and striatum (DeFrance et al., 1985a; Groves, 1983; O'Donnell and Grace, 1993; Pennartz et al., 1991; Wilson and Groves, 1980) as well as profuse GABA-containing terminals with synaptic specializations at the ultrastructural level (Pickel et al., 1988; Smith and Bolam, 1990). It is therefore not surprising that IPSPs have frequently been observed as a result of stimulation within the borders of the NAc and striatum (Chang and Kitai, 1985; Groves, 1983; Jaeger et al., 1994; Pennartz and Kitai, 1991; Pickel et al., 1988; Smith and Bolam, 1990). The IPSPs reported in this work are therefore likely to result from the direct stimulation of the axons or cell bodies of other NAc or striatal cells. IPSPs were recorded in the glutamate receptor antagonists DNQX and APV, and the latency between the stimulation artifact and the synaptic potential was constant over the course of the experiment for each cell. Therefore, since IPSPs were entirely blocked by the $GABA_A$ antagonist picrotoxin, the IPSPs observed here are monosynaptic $GABA_A$ receptor-mediated potentials.

The recorded cells themselves are likely to be GABAergic medium spiny neurons, the most numerous cell type (~96% of cells) in the NAc and striatum (Chronister et al., 1981; Groves, 1983; Kawaguchi et al., 1995). It is, of course, possible that some of the recorded cells were of another type, such as the large cholinergic cell (Groves, 1983; Kawaguchi et al., 1995). The "blind" whole cell recording technique used in this study may be biased towards larger cells (since larger cells will be encountered more frequently than smaller ones, and healthier and longer recordings can presumably be made from larger cells than from smaller ones); even so, given the relative paucity of the large cholinergic cells, it is unlikely that more than a few of the recorded cells were of this type.

A more difficult question is whether IPSPs recorded in NAc and striatal neurons result from the release of GABA from the axon terminals of interneurons or of projection cells which, in addition to the long projection axon, extend local axon collaterals coextensive with the cell's dendritic arbor (Groves, 1983). This issue is important for its consequences for theories concerning the circuits by which information is processed in the NAc and striatum: the question is whether cells that transmit information simultaneously

inhibit their neighbors (surround inhibition), possibly resulting in a "sharpened" output signal, or whether inhibition is of the more general feed-forward type. In as much as most medium spiny neurons are projection neurons it would seem that IPSPs recorded in a NAc or striatal cell probably result mostly from activation of projection neurons; however, an *in vivo* investigation using antidromic activation of striatal efferents with electrodes in the efferent target nuclei failed to find evidence for IPSPs in the striatum caused by such activation (Jaeger et al., 1994). Electrical stimulation of slices resulted in readily observable IPSPs, but intracellular recordings from pairs of projection neurons did not reveal evidence for reciprocal inhibition (Jaeger et al., 1994). The authors conclude that slice stimulation-evoked IPSPs arise mostly from interneurons (most likely of the non-medium spiny type); however, this result is questionable, since IPSPs were not pharmacologically isolated, the membrane potentials used were not ideal for the observation of IPSPs, and the IPSPs resulting from activation of only one other cell are likely to be small. This result is also difficult to reconcile with the vast numbers of synapses apparently formed locally by striatal projection cells (Groves, 1983), the small number of interneurons (Kawaguchi et al., 1995), and the findings of a similar study in which IPSPs resulting from antidromic activation of NAc cells were observed (Chang and Kitai, 1985). Thus, the IPSPs observed here are likely to be the result of activation of projection cells, particularly in the NAc, where activation of projection cells has been shown to result in IPSPs in other NAc cells (Chang and Kitai, 1985).

Early intracellular recording studies of the NAc conducted *in vitro* found that dopamine could cause a D1 receptor-dependent increase and a D2 receptor-dependent decrease in a K^+ conductance, which resulted in either hyperpolarization or depolarization of the cell (Uchimura et al., 1986; Higashi et al., 1989). No such changes in membrane potential were observed here, consistent with the findings of another group (Pennartz et al., 1992a). The changes in membrane potential reported by the Uchimura group (Uchimura et al., 1986; Higashi et al., 1989) were small (6 mV); in the present work, conditions for observation of the effects of K^+ conductances were not optimal, since most recordings were conducted with Cs^+ in the recording electrode.

Membrane potential changes in response to dopamine may therefore have been undetectable.

The present findings that dopamine depresses EPSPs in the NAc is consistent with earlier observations from studies conducted *in vivo* (DeFrance et al., 1985b; Yang and Mogenson, 1984; Yim and Mogenson, 1988) and *in vitro* (Higashi et al., 1989; O'Donnell and Grace, 1994; Pennartz et al., 1992a,b). The finding that NAc IPSPs are depressed by dopamine is also consistent with *in vivo* and *in vitro* studies that have found a negative modulation of the putatively GABAergic potentials recorded as parts of EPSP-IPSP sequences (Pennartz et al., 1992a; Yim and Mogenson, 1988). Thus, one of the ways by which dopamine may influence NAc function is by modulation of excitatory and inhibitory synaptic transmission. The psychostimulant drugs of abuse cocaine and amphetamine also depress synaptic transmission, most likely as a result of their interaction with the dopamine transporter (Ritz et al., 1987; Seiden et al., 1993).

Given the consistent and sizable effects of dopamine and amphetamine in the NAc, it is surprising that these drugs have little, if any, effect on synaptic transmission in the striatum. Previous studies using intracellular recording of striatal cells have found that dopamine acting at a D1 receptor reduces subthreshold inward rectification by reduction of a Na^+ current, and that this effect reduces the size of EPSPs recorded at potentials between spike threshold and –80 mV (Calabresi et al., 1987, 1988). In the present experiments the membrane potential was held at –80 mV, and yet no depression of the EPSP slope (Figure 11 – Figure 14) or amplitude (not shown) was observed. However, under the conditions employed for these studies, no inward rectification and no change in the rectification properties by dopamine was observed between –50 and –80 mV. It is possible that some intracellular component necessary for Na^+-dependent inward rectification and dopamine's effects on it could have "washed out" of the cell, although other whole-cell studies have observed effects of dopamine on Na^+ currents in cultured or acutely isolated striatal cells (Schiffmann et al., 1995; Surmeier et al., 1992). Another study has reported no effect of dopamine on striatal synaptic field potentials (Malenka and Kocsis, 1988), although this may be due to the very hyperpolarized resting membrane

potential of striatal cells (–90 mV), which might preclude the activation of the current responsible for rectification. Other reports demonstrate a D2 receptor-mediated depression of striatal EPSPs as well as of potentials resulting from iontophoretic application of glutamate (Cepeda et al., 1993; Hsu et al., 1995; Levine et al., 1996b), but this result is controversial (Calabresi et al., 1992, 1993, 1995). One possible explanation for the lack of effect of dopamine is that striatal slices cut and examined under the conditions used for the present work are unhealthy and are therefore unable to express synaptic depression. While this remains a possibility, it is unlikely given the robust effect of serotonin on striatal fields (Figure 15). Neither dopamine, amphetamine nor dopamine in the presence of GBR12935 caused a depression even in striatal field recordings (data not shown).

Another possible explanation for the difference in dopamine's effects between the NAc and the striatum may be that dopamine reuptake is more efficient in the striatum than in the NAc, for instance, as a result of a greater number of transporter proteins. However, two experiments ruled out this possibility: the dopamine uptake inhibitor GBR12935 had no effect on the magnitude of the depression of the EPSP induced by dopamine, and amphetamine, which reverses the dopamine transporter, had no effect on striatal EPSPs or IPSPs even at concentrations that have large and consistent effect on NAc EPSPs and IPSPs. These results suggest that another factor is involved. For instance, the density of the dopamine receptor responsible for the depression may be higher in the ventral striatum (NAc) than in its dorsal counterpart. In the case of EPSPs, cortical axon terminals may not express the dopamine receptor, and thus the depressant effect may only be observed in the NAc, which receives relatively large numbers of excitatory fibers from noncortical nuclei.

What role could inhibition by dopamine of both excitatory and inhibitory synaptic transmission play in the integration of information by NAc cells? The results of the experiment of Figure 17 suggest that, due to inhibition among NAc cells, excitations of many NAc cells will be attenuated by dopamine less than excitations of only a few NAc cells. One function of dopamine in the NAc may therefore be to increase the signal-to-noise ratio for stimuli in the animal's environment that provide stronger excitation to the NAc. *In vivo* recordings of NAc cells in rats lever-pressing for cocaine have suggested

that one effect of dopamine on NAc cells may be to reduce spontaneous firing rates compared with firing that is associated with "important" events (i.e., those that activate many NAc cells) (Carelli et al., 1993; Carelli and Deadwyler, 1996a). If weak glutamatergic excitation contributes to the baseline firing rate, as is likely given the hyperpolarized resting potential of NAc cells in the absence of excitation, then the reduction of both excitatory and inhibitory synaptic transmission described here may be part of the mechanism by which the signal-to-noise ratio is enhanced *in vivo*. Postsynaptic effects on the membrane conductance may also contribute to such an effect. Enhancement by dopamine of the signal-to-noise ratio for evoked versus spontaneous firing is in fact fairly well documented in the striatum (Chiodo and Berger, 1986; Hu and Wang, 1988; Johnson et al., 1983; Nisenbaum et al., 1988; Rolls et al., 1984), but the present results find only very minimal (if any) presynaptic effects of dopamine on synaptic transmission in the striatum. However, the previously reported modulation of membrane rectification (Calabresi et al., 1987, 1988) was also not observed, perhaps as a result of the whole-cell recording conditions employed in the present work. Thus, a dopamine-induced decrease in inward rectification may contribute to the enhancement of the signal-to-noise ratio observed *in vivo* in both the NAc and striatum. Further study of postsynaptic effects of dopamine, perhaps using intracellular recording or perforated patch techniques that are less susceptible to wash-out, may clarify the mechanisms by which dopamine influences information processing in the striatum.

Figure 1. Field potentials in the NAc are biphasic.

An extracellular recording of a typical biphasic potential, demonstrating that the later event ("N2") was sensitive to CNQX (10 μM), while the earlier event ("N1") was sensitive to TTX (1 μM, n=5). In this and most subsequent figures, sample data traces (averages of 9 – 11 consecutive sweeps) are presented above graphs representing measurements taken from the raw sweeps, in this case N2 amplitude (*top*) and N1 amplitude (*bottom*). The representative sweeps are taken at the times indicated by the numbers on the graphs.

NAc Field Potentials

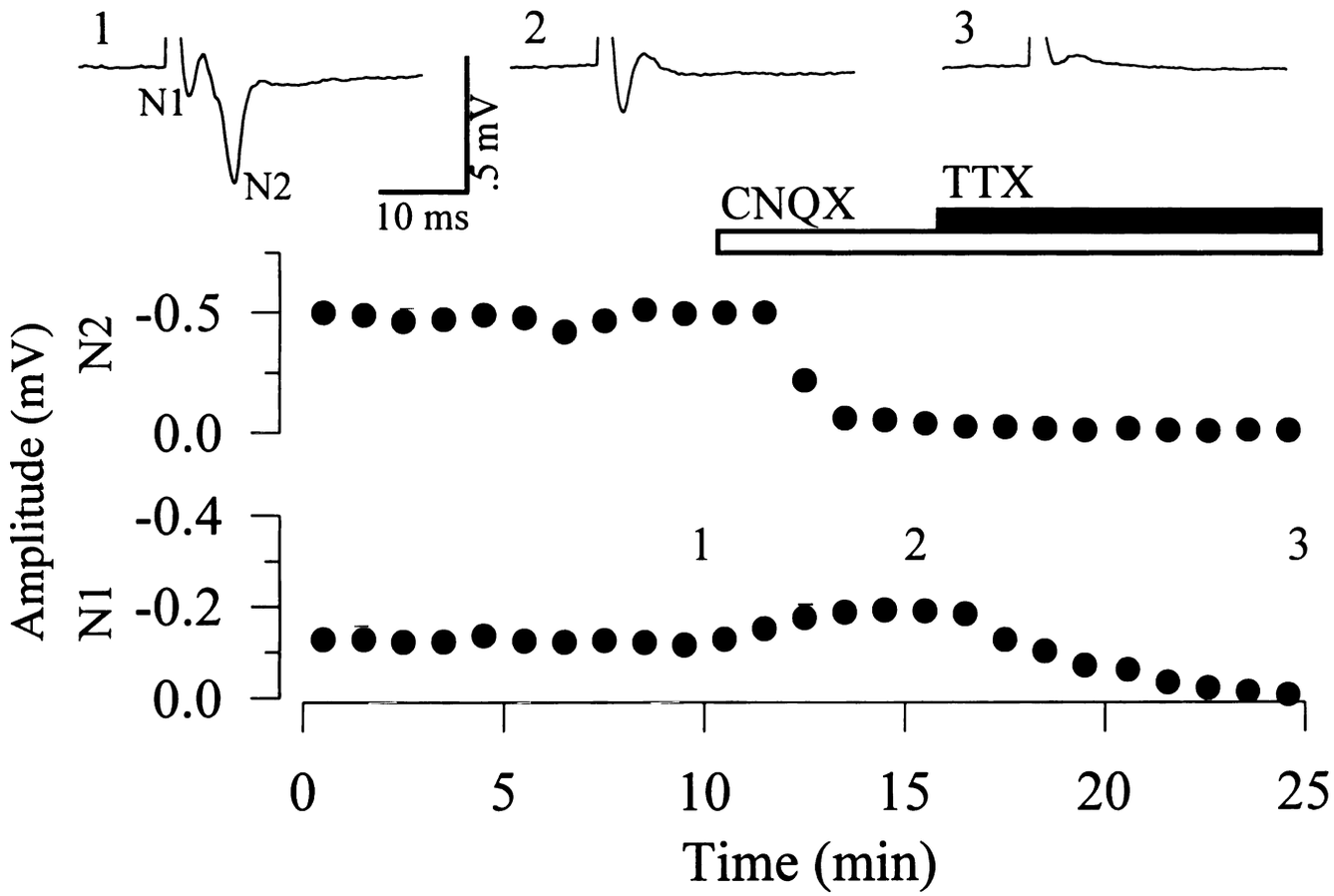


Figure 2. NAc IPSPs reverse at –58 mV.

As demonstrated by the traces (*left*) and a plot of IPSP amplitude versus membrane potential (*right*), IPSPs recorded with a potassium methylsulphate-based electrode solution containing QX314 exhibit a linear dependence on membrane potential and reverse at the estimated Cl^- reversal potential of –58 mV. Experiments were performed in DNQX (10 μM) and APV (75 μM).

NAc IPSPs

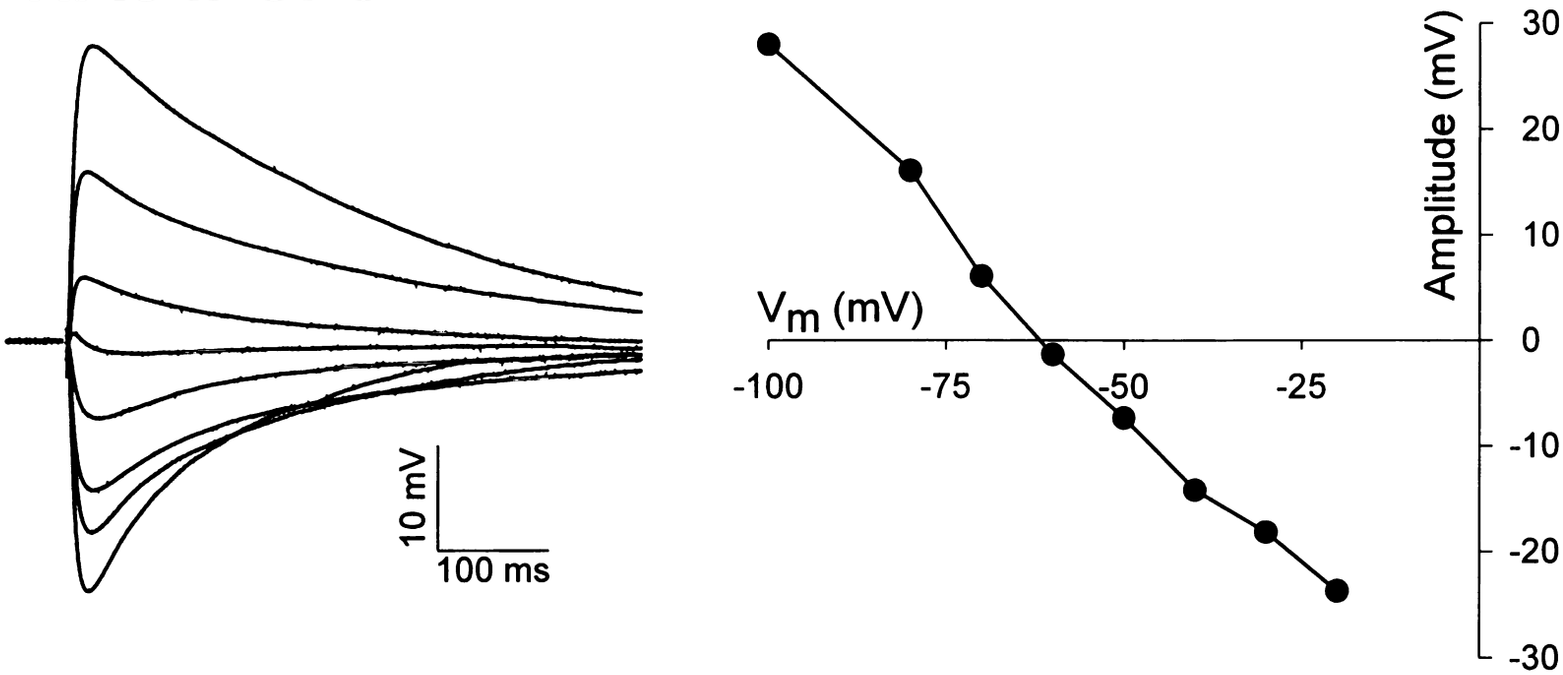


Figure 3. NAc IPSPs are sensitive to picrotoxin.

Application of picrotoxin (50 μM) completely abolished the IPSP. In this and subsequent figures (except where noted otherwise), the negative voltage deflection after the synaptic potential is the result of a –.03 nA current pulse given through the recording electrode.

NAc IPSPs

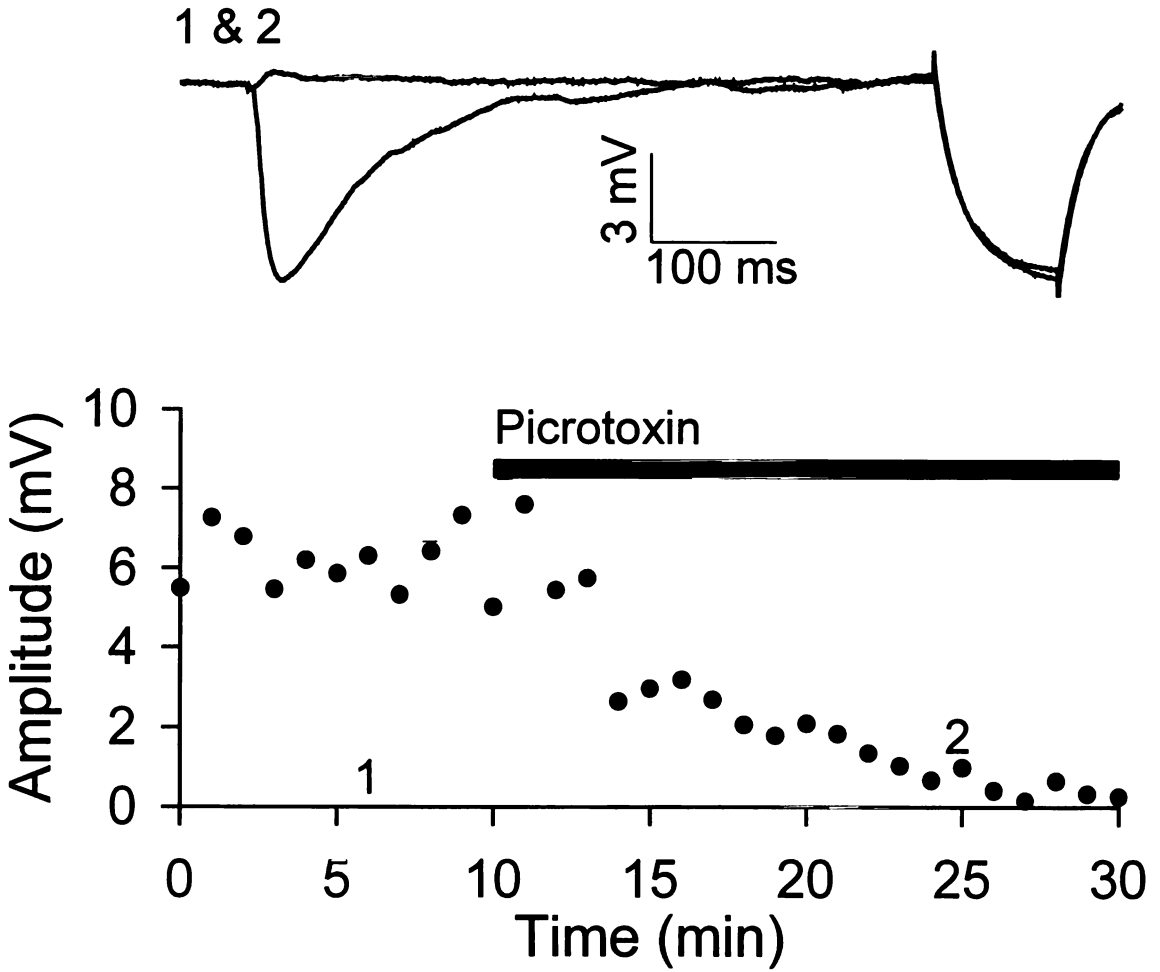

1 & 2
3 mV
100 ms
Picrotoxin
Amplitude (mV)
10
8
6
4
2
0
1
2
0
5
10
15
20
25
30
Time (min)

Figure 4. Cocaine reduces the nonsynaptic and synaptic field potential recorded in the NAc.

The *traces* illustrate the effects of cocaine on the field potential. N2 amplitude (*middle*) and N1 amplitude *(bottom*) are plotted across time. In the presence of 30 μM cocaine (*solid bar*), the stimulus strength was increased such that the N1 amplitude matched the baseline N1 amplitude (*open bar*).

NAc Field Potentials

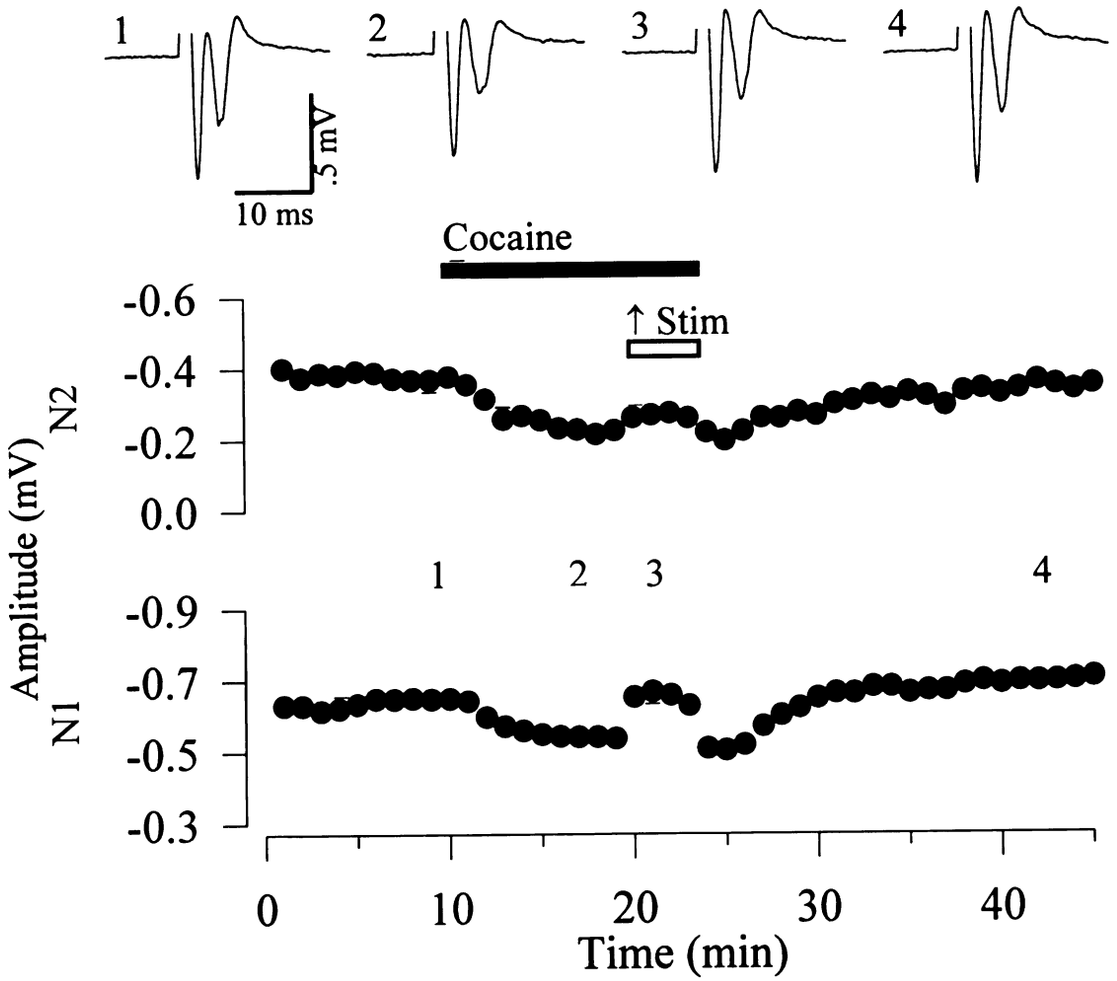


Figure 5. Cocaine shifts the input-output curve to the right for fields recorded in the NAc.

The graph is a representative input-output curve (n=7) for responses prior to the application of cocaine (*circles*) and in the presence of 30 μM cocaine (*squares*). *Error bars* represent standard deviations.

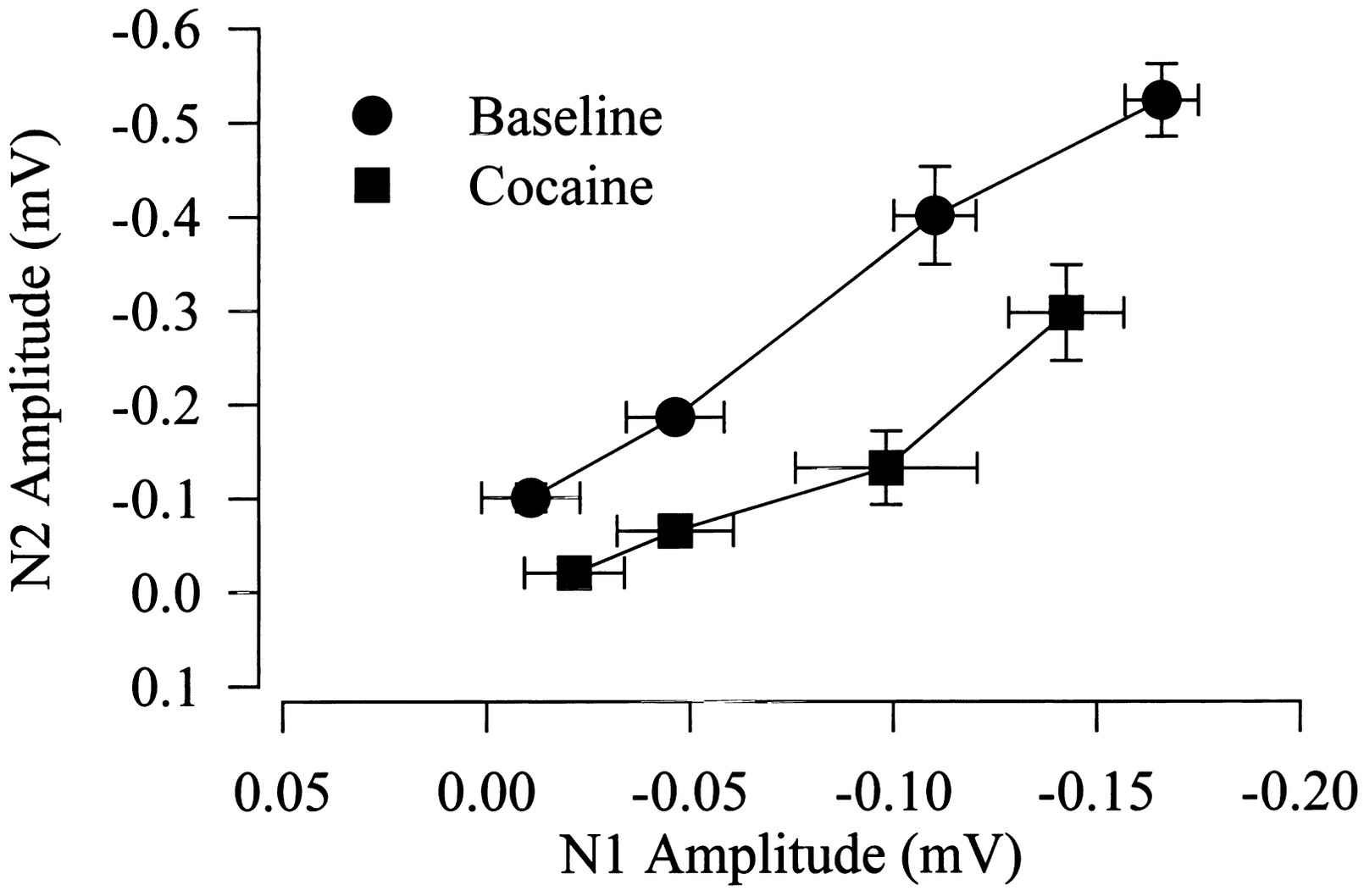


Figure 6. Cocaine depresses the evoked NAc EPSP without affecting the input resistance.

In a typical current-clamped cell, 30 μM cocaine depressed the initial slope of the EPSP (*middle*) but not the input resistance as measured by the amplitude of the voltage response to a –.1 nA current injection (*bottom*).

NAc EPSPs

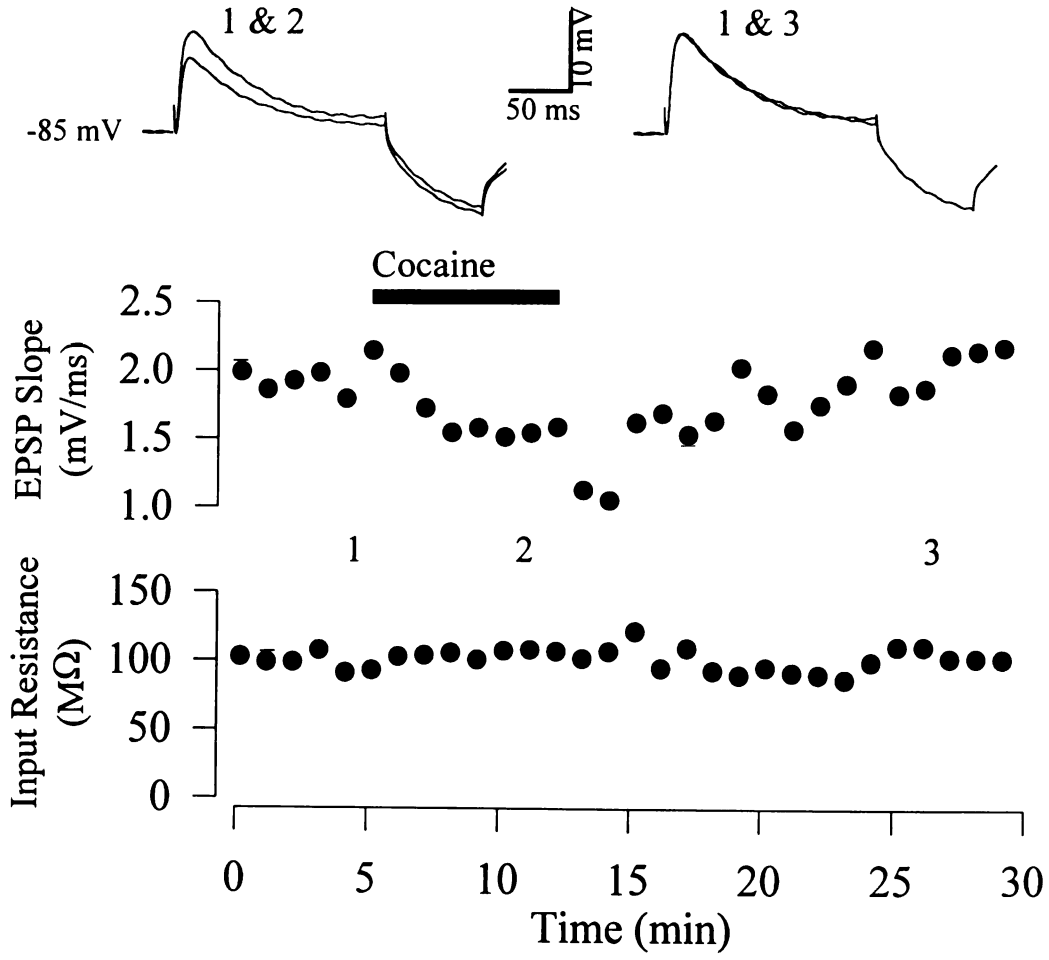

1 & 2
1 & 3
10 mV
50 ms
-85 mV
Cocaine
EPSP Slope (mV/ms)
2.5
2.0
1.5
1.0
1
2
3
Input Resistance (MΩ)
150
100
50
0
0
5
10
15
20
25
30
Time (min)

Figure 7. Amphetamine reduces evoked excitatory synaptic responses in the NAc. ***A***, A typical field recording in which 10 μM amphetamine reduced the synaptic response (N2) without affecting the non-synaptic potential (N1). ***B***, When 10 μM amphetamine was applied to a cell in whole-cell current clamp, the initial slope of the EPSP was reduced while the input resistance (–.03 nA current injection) was unchanged.

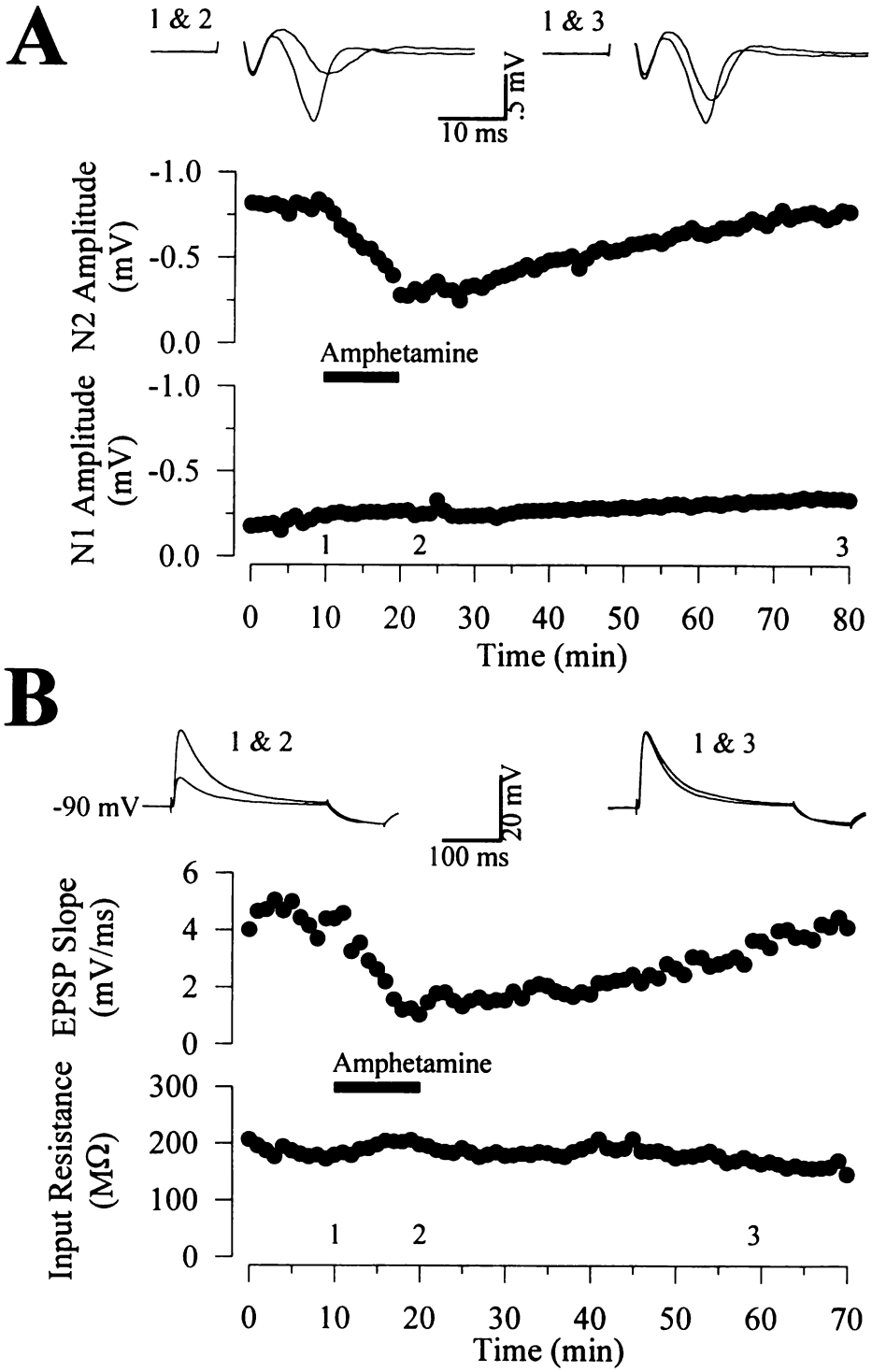

A
1 & 2
1 & 3
.5 mV
10 ms
N2 Amplitude (mV)
-1.0
-0.5
0.0
Amphetamine
N1 Amplitude (mV)
-1.0
-0.5
0.0
1
2
3
0
10
20
30
40
50
60
70
80
Time (min)
B
1 & 2
1 & 3
-90 mV
20 mV
100 ms
EPSP Slope (mV/ms)
6
4
2
0
Amphetamine
Input Resistance (MΩ)
300
200
100
0
1
2
3
0
10
20
30
40
50
60
70
Time (min)

Figure 8. Dopamine reduces evoked excitatory synaptic responses in the NAc. ***A***, A representative field experiment in which 75 μM dopamine reduced the synaptic potential (N2) but did not change the non-synaptic potential (N1). ***B***, Application of 75 μM dopamine to a current-clamped cell reduced the initial slope of the EPSP without affecting the input resistance (–.03 nA current injection).

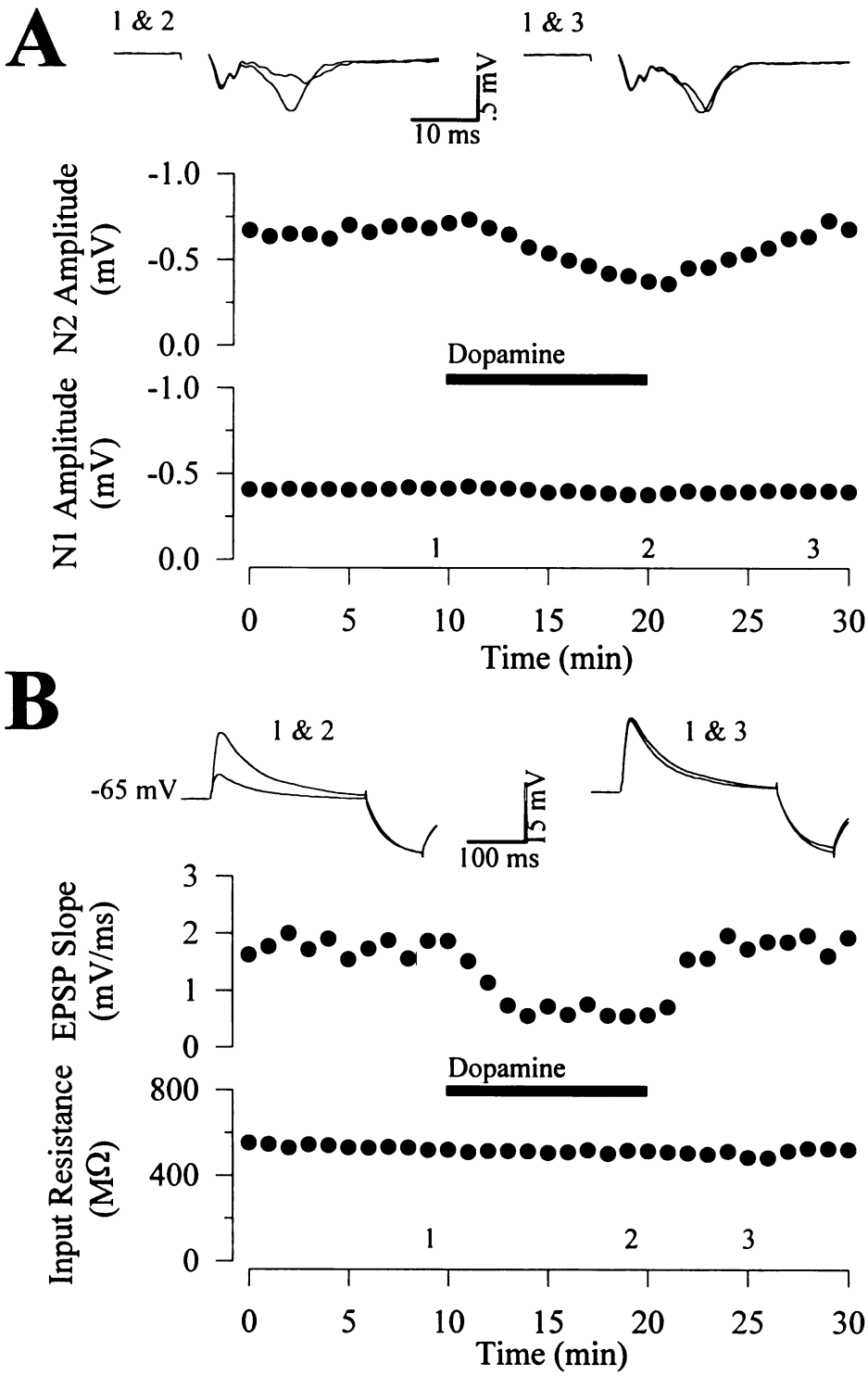

A
1 & 2
1 & 3
5 mV
10 ms
N2 Amplitude (mV)
-1.0
-0.5
0.0
Dopamine
N1 Amplitude (mV)
-1.0
-0.5
0.0
1
2
3
0
5
10
15
20
25
30
Time (min)
B
1 & 2
1 & 3
-65 mV
5 mV
100 ms
EPSP Slope (mV/ms)
3
2
1
0
Dopamine
Input Resistance (MΩ)
800
400
0
1
2
3
0
5
10
15
20
25
30
Time (min)

Figure 9. Amphetamine depresses evoked IPSPs in the NAc.

A, Representative IPSP traces taken from one NAc experiment before application of amphetamine, in the presence of bath-applied amphetamine (10 μM) and after recovery of the response. ***B***, The time course of the experiment illustrated in A. ***C***, A normalized average (n=10) of the time course of the effects of amphetamine on NAc IPSPs. *Error bars* in this and all subsequent graphs represent standard errors of the mean.

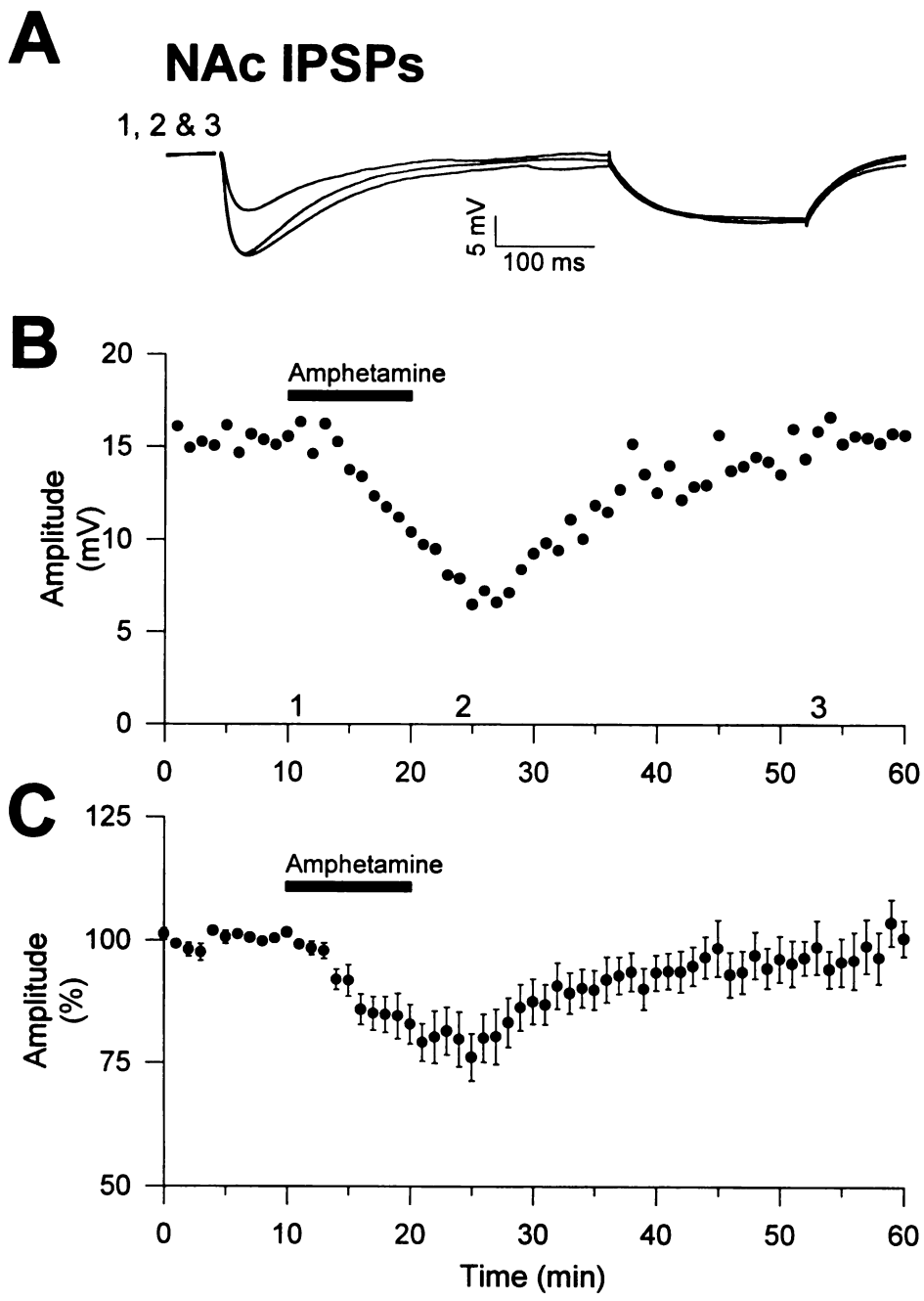

A
NAc IPSPs
1, 2 & 3
5 mV
100 ms
B
Amphetamine
Amplitude (mV)
20
15
10
5
0
1
2
3
0
10
20
30
40
50
60
C
Amphetamine
Amplitude (%)
125
100
75
50
0
10
20
30
40
50
60
Time (min)

Figure 10. Dopamine depresses evoked IPSPs in the NAc.

A, The *traces* are taken from one experiment during the baseline, before application of dopamine (75 μM) and during recovery. ***B***, The time course of the experiment illustrated in A. ***C***, A normalized average (n=28) demonstrating that dopamine reversibly depressed the IPSP.

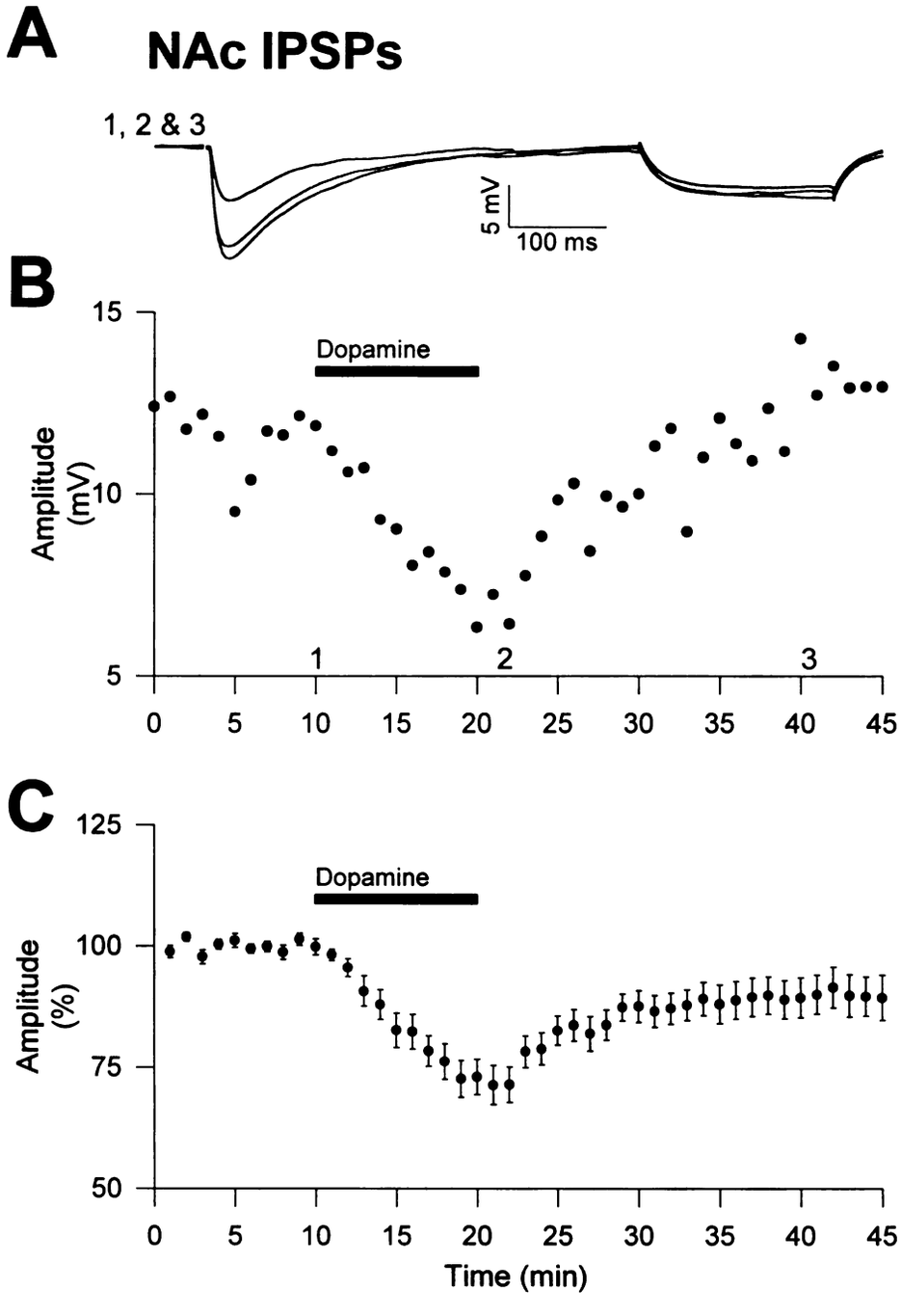

A
NAc IPSPs
1, 2 & 3
5 mV
100 ms
B
Dopamine
Amplitude (mV)
15
10
5
1
2
3
0
5
10
15
20
25
30
35
40
45
C
Dopamine
Amplitude (%)
125
100
75
50
Time (min)

Figure 11. Dopamine does not affect evoked EPSPs in the striatum.

A, Sweeps taken from the experiment shown in ***B***, in which dopamine (100 μM) was applied while recording striatal EPSPs. ***C***, A summary of experiments in which dopamine (100 μM) was applied (n=12), demonstrating only minimal effects of dopamine.

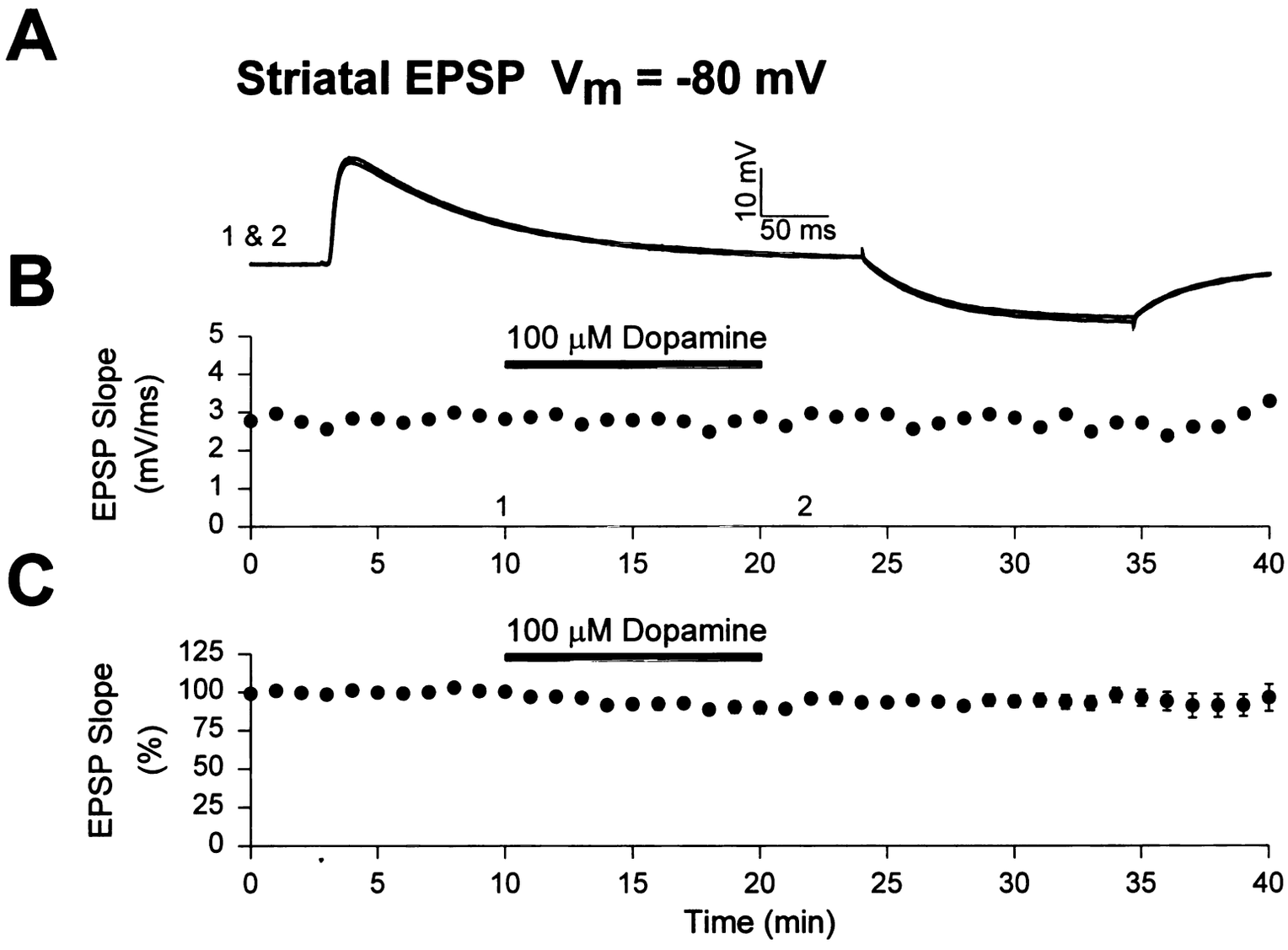


Figure 12. GBR12935 does not reveal an effect of dopamine on striatal EPSPs. ***A***, Sweeps taken from the experiment shown in ***B***, in which dopamine was applied to striatal EPSPs in the absence and then in the presence of the dopamine uptake inhibitor GBR12935. ***C***, A comparison of the effects of dopamine in the absence and in the presence of GBR12935 illustrates that there was no difference in dopamine's effectiveness on striatal EPSPs whether or not GBR12935 was present (n=4).

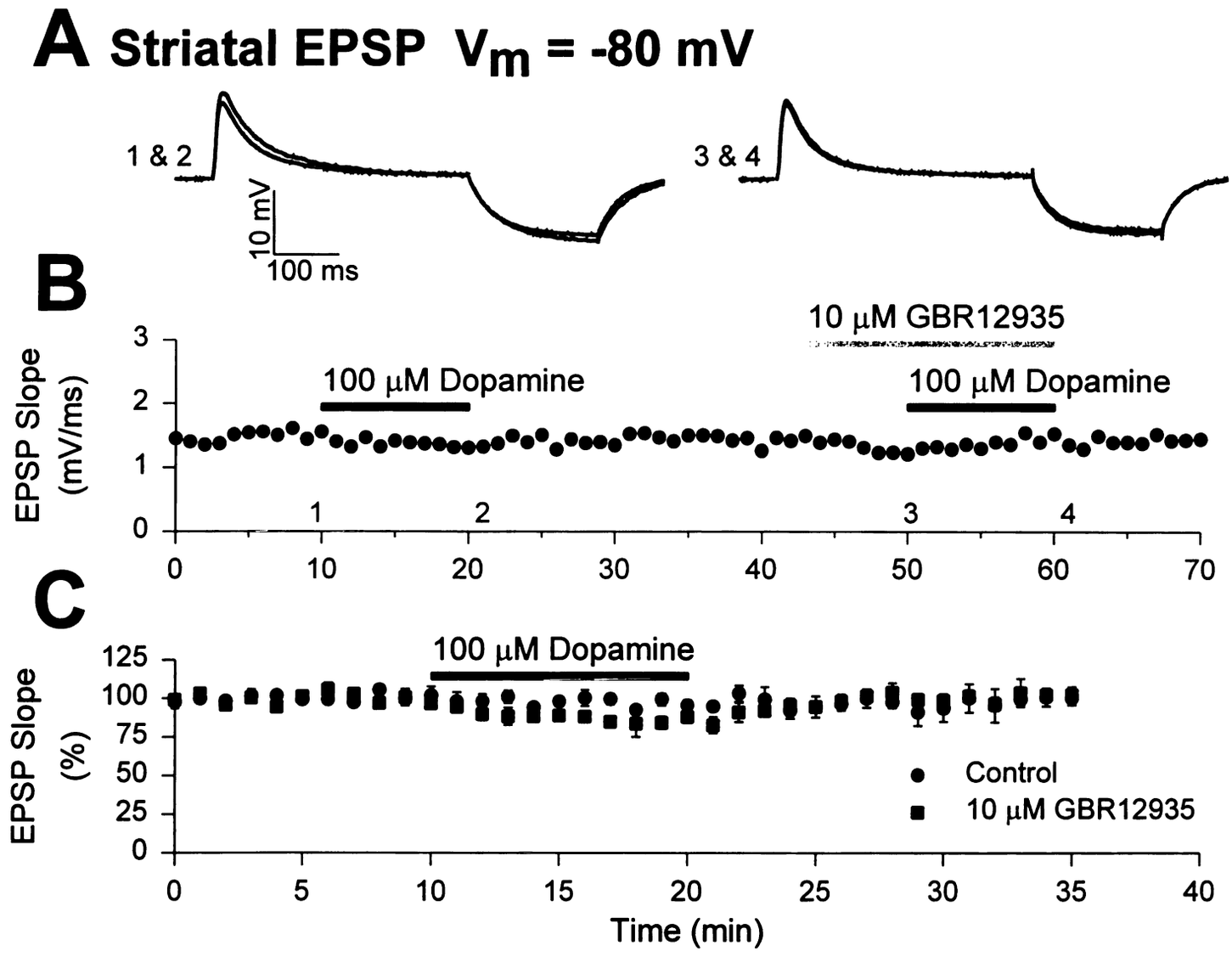


Figure 13. Amphetamine does not affect striatal EPSPs.

A, Sweeps taken from the experiment summarized in ***B***, in which amphetamine (10 μM) was applied to EPSPs recorded in the striatum. ***C***, An average of experiments like that in A and B demonstrating the very minimal effects of amphetamine (n=7).

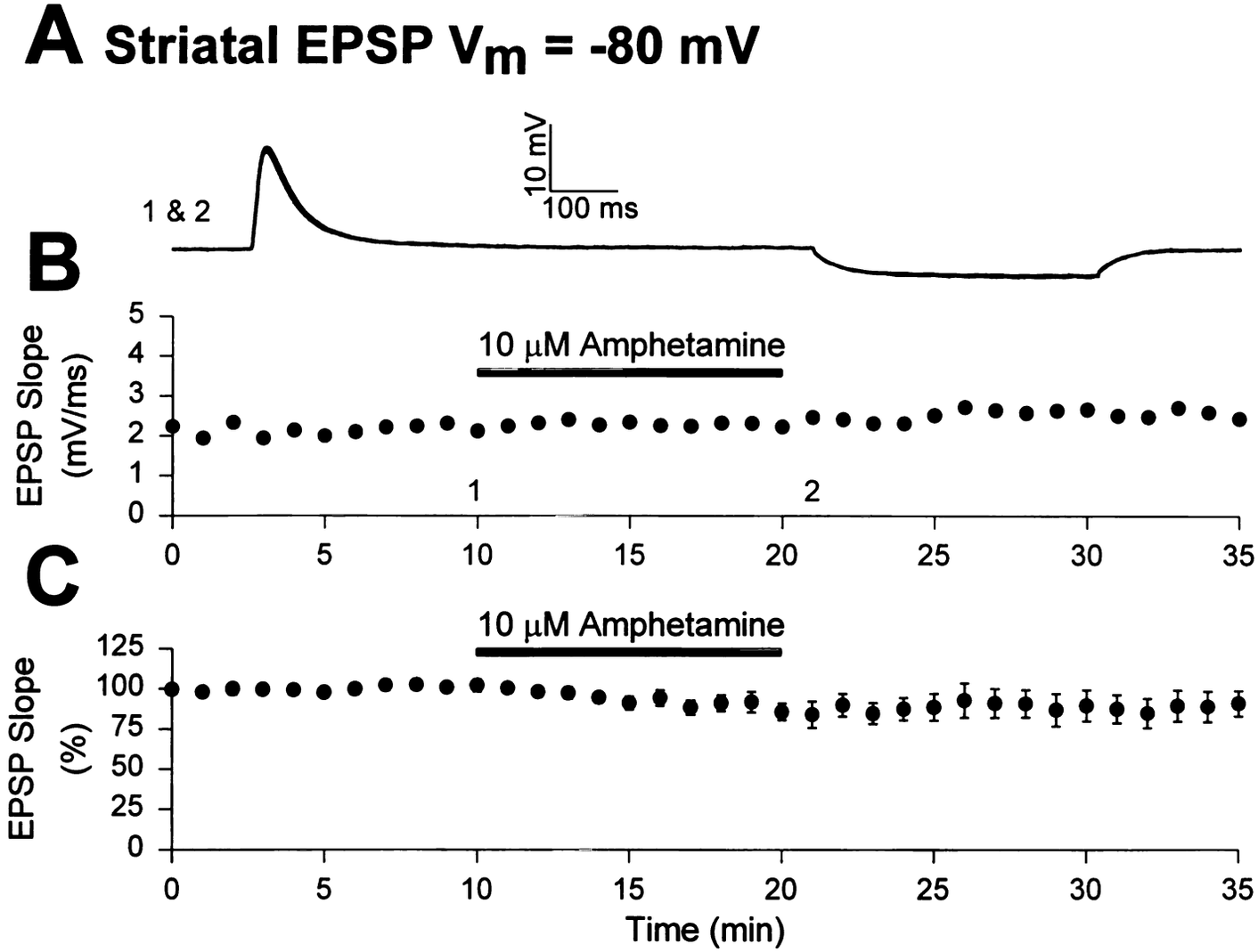


Figure 14. Amphetamine and dopamine do not affect striatal IPSPs.
A and ***B***, A recording from a striatal cell in which both dopamine and amphetamine were applied sequentially. ***C***, A summary of the lack of effect of dopamine (100 μM) on striatal IPSPs (n=5). ***D***, A summary of the lack of effect of amphetamine (10 μM) on striatal IPSPs (n=4).

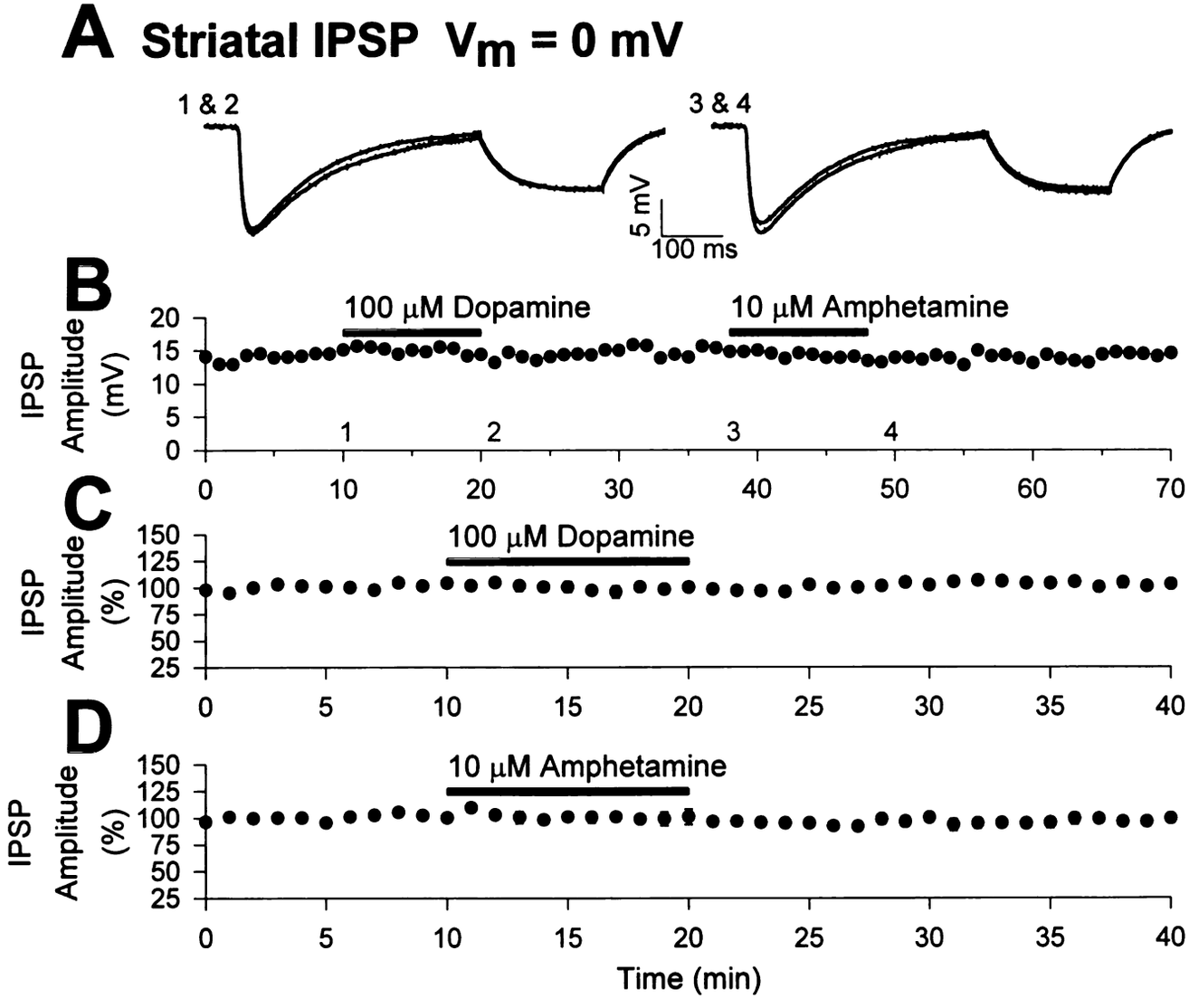


Figure 15. Serotonin depresses excitatory synaptic responses in the striatum. ***A***, Striatal synaptic fields taken from the experiment illustrated in ***B***, which shows the effects of serotonin on the synaptic response in one striatal field experiment. ***C***, A summary of experiments like that in B, demonstrating that serotonin depresses excitatory synaptic transmission in the striatum (n=7).

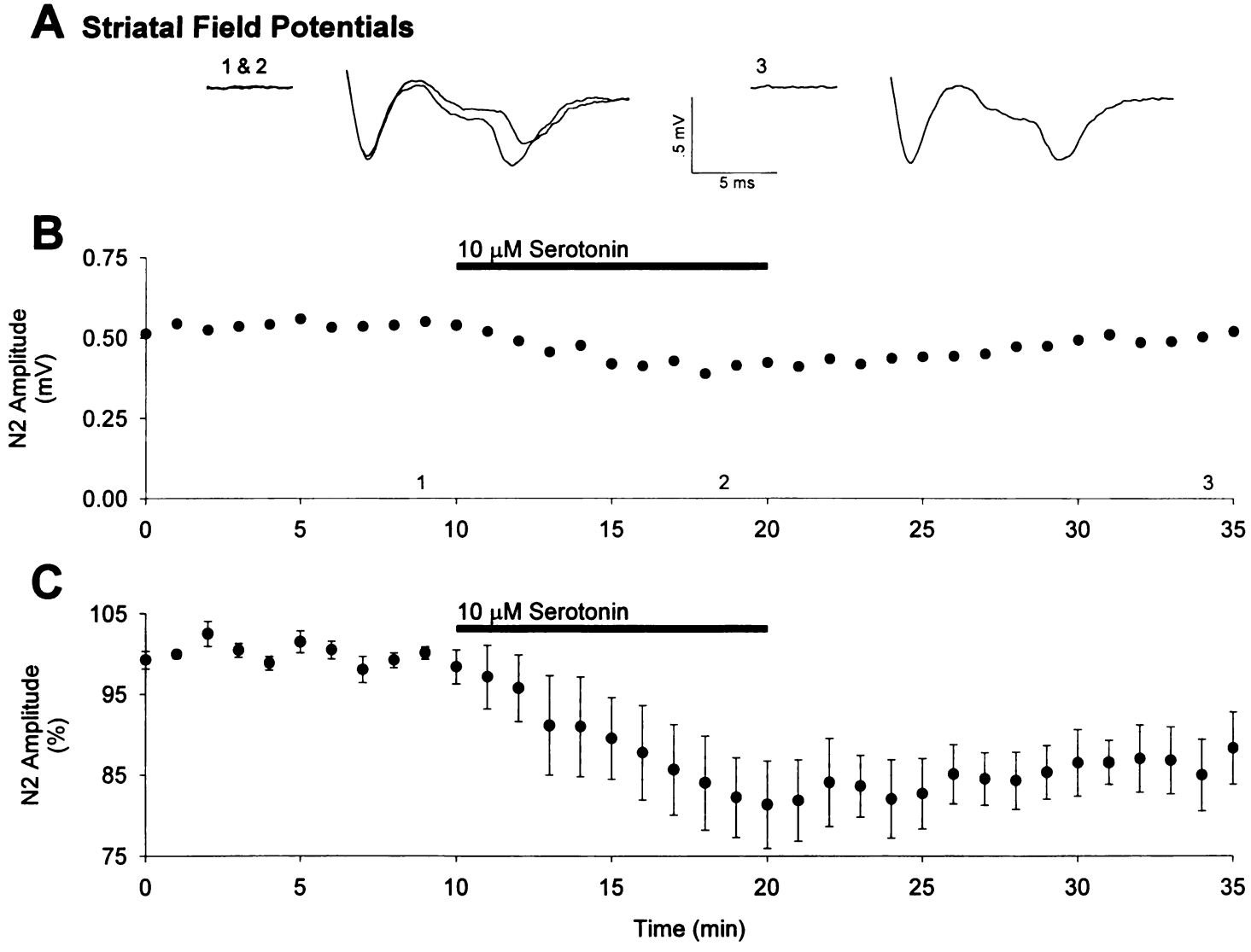
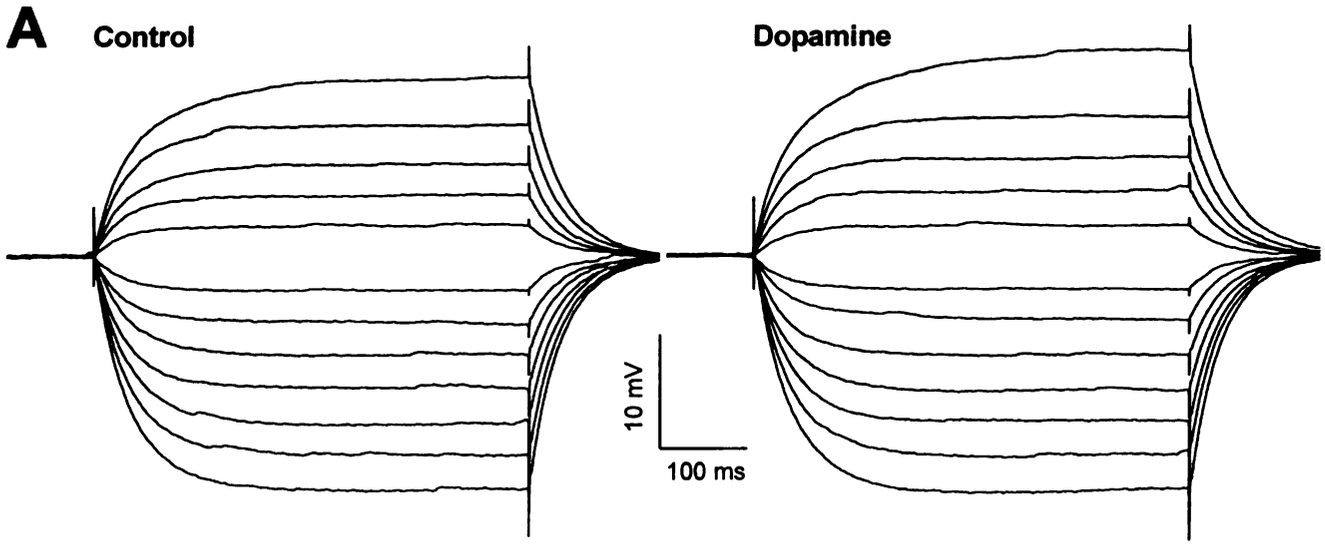


Figure 16. Dopamine does not change the rectification properties of striatal cells. ***A***, Several negative and positive current steps (500 ms) were given to a striatal cell held at –70 mV, both before application of dopamine (*left*) and in the presence of 100 μM dopamine (*right*). ***B***, A plot of the change in membrane potential as a function of the amplitude of the current step in the presence and absence of dopamine for the cell shown in A.



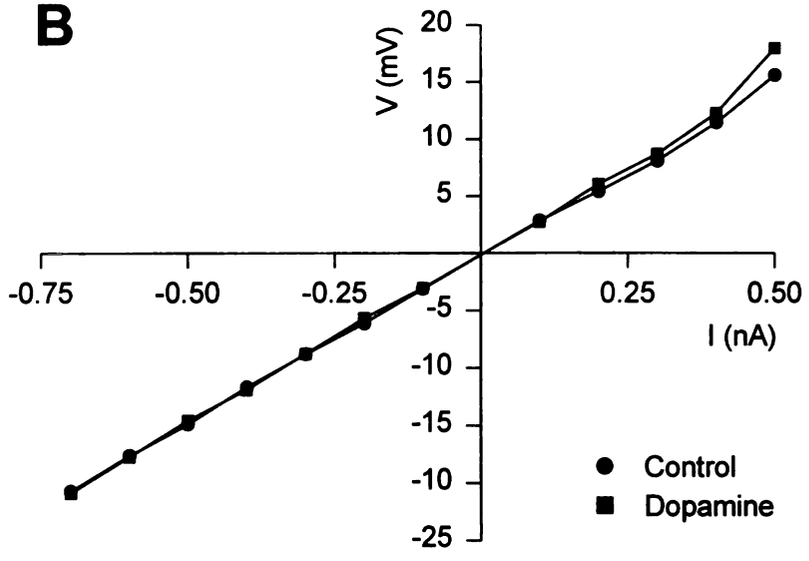
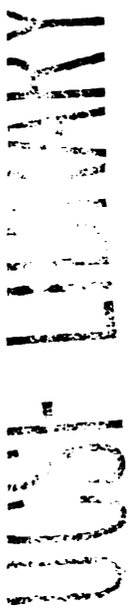


Figure 17. GABAergic inhibition reduces the magnitude of dopamine-induced depression of compound excitatory-inhibitory synaptic potentials in the NAc. ***A***, Slices were recorded in the absence of GABA and glutamate antagonists and stimulated alternately with high and low stimulus strengths. The *top* traces are the result of stimulation with the lower stimulus strength, whereas the *bottom* traces are the result of stimulation with the higher stimulus strength during the same time window. ***B***, In this cell, dopamine (100 μM) was first applied to the compound synaptic response. After its wash-out, picrotoxin (200 μM) was applied, and then dopamine (100 μM) was reapplied to the EPSP without adjusting the stimulus strength. The *top* graph illustrates the effects of these drugs on synaptic potentials evoked by the lower stimulus strength, and the *bottom* graph shows their effects on synaptic potentials evoked by the higher stimulus strength. ***C***, A summary of 7 similar experiments. The graph on the *left* compares the effects of dopamine on the compound synaptic potentials evoked at different stimulus strengths in the absence of picrotoxin, and the graph on the *right* makes the same comparison for EPSPs evoked at different stimulus strengths in the presence of picrotoxin.



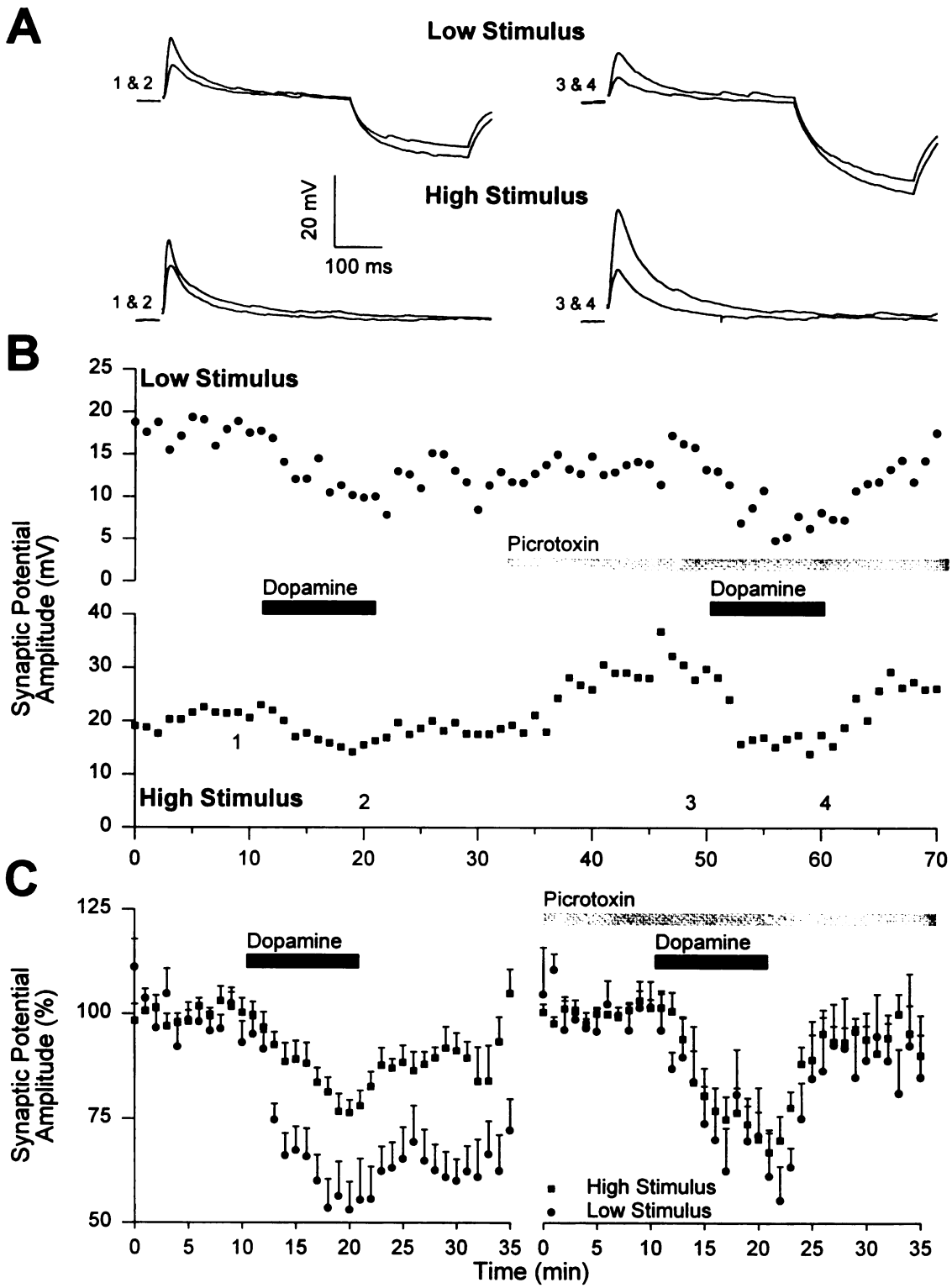
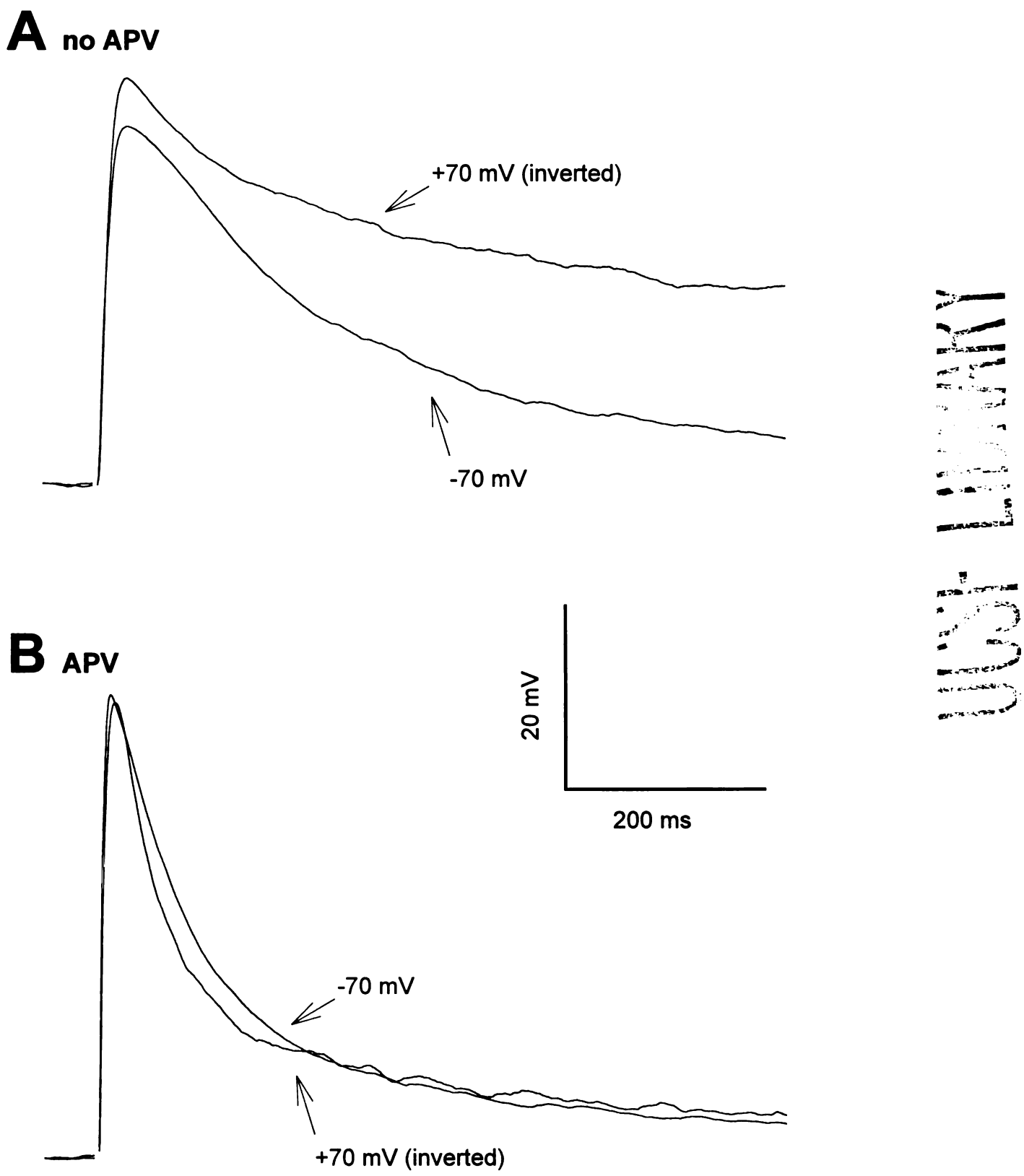

A
Low Stimulus
1 & 2
3 & 4
High Stimulus
20 mV
100 ms
1 & 2
3 & 4
B
Low Stimulus
Picrotoxin
Dopamine
Dopamine
Synaptic Potential Amplitude (mV)
1
High Stimulus
2
3
4
C
Picrotoxin
Dopamine
Dopamine
Synaptic Potential Amplitude (%)
High Stimulus
Low Stimulus
Time (min)

Figure 18. Voltage-dependent conductances do not contribute to the EPSP amplitude.

A, Averaged traces of EPSPs of approximately 50 mV in amplitude. Traces were taken in the absence of APV at membrane potentials of +70 and –70 mV; the inverse of the trace at +70 mV is shown. ***B***, A similar experiment, except that EPSPs at +70 and –70 mV were obtained in the presence of D-APV (50 μM).


A no APV
+70 mV (inverted)
-70 mV
B APV
20 mV
200 ms
-70 mV
+70 mV (inverted)

Chapter 4

Pharmacological Characterization of Modulation of Synaptic Transmission by Dopamine in the Nucleus Accumbens

Introduction

Attempts to identify the dopamine receptor subtype (D1 or D2) responsible for depression of EPSPs using specific agonists and antagonists in NAc slices have yielded confusing results. Originally it was reported that D1 receptors are responsible for the depression, although the depression could only be observed in slices taken from animals that had been chronically pretreated with amphetamine (Higashi et al., 1989). A later report found D1 receptor mediated depression of EPSPs in slices from naïve animals (Pennartz et al., 1992a) but only in cells recorded in the shell region (as opposed to the core) of the NAc (Pennartz et al., 1992b). Conflicting with both of these studies was a report that D2 receptors mediate depression of the EPSP in NAc slices (O'Donnell and Grace, 1994). The identity of the dopamine receptor responsible for depression of NAc IPSPs remains as much of a mystery as there have been no reports of attempts characterize it.

Ultrastructural studies using antibodies against the cloned dopamine receptors have consistently found both D1a and D2 receptors on the dendrites and spines of medium spiny neurons in the NAc and striatum (Caillé et al., 1996; Delle Donne et al., 1996; Fisher et al., 1994; Hersch et al., 1995; Huang et al., 1992; Levey et al., 1993; Sesack et al., 1994; Yung et al., 1993). Since labeling of both D1a and D2 receptors occurs on spines that form both symmetrical (putatively inhibitory) and asymmetrical (putatively excitatory) synapses, both of these receptors are candidates for the receptor underlying dopamine-induced modulation of synaptic transmission in the NAc. Because many fewer, if any, of these receptors are found on axon terminals, any modulation mediated by these receptors would most likely occur at a postsynaptic site. Another possibility that should not be discounted given the evidence for D1-like receptors not coupled to adenylate cyclase (Waddington and Deveney, 1996) is that a dopamine receptor that remains to be cloned is responsible for the observed dopaminergic depression of EPSPs and IPSPs in the NAc.

Results

A. Excitatory Synaptic Transmission

D1 and D2 receptor antagonists. To begin the pharmacological characterization of the receptor responsible for the psychostimulant- and dopamine-induced depression of synaptic transmission the effects of specific D2 and D1 receptor antagonists on excitatory responses were examined (Figure 19 – Figure 22). Sulpiride (10 μM), an antagonist specific for D2-like receptors (Civelli et al., 1993; Sibley, 1995), did not reduce the synaptic depression elicited by application of amphetamine (10 μM, $p>.9$, Figure 19). This lack of effect of sulpiride was observed in 8 slices that were exposed both to amphetamine alone (10 μM, 40±2% of baseline, $p<.0001$, Figure 19B) and to amphetamine in the presence of sulpiride (30±4% of baseline, Figure 19C). Sulpiride also did not antagonize the depressant effect of dopamine (75 μM, $p>.1$, Figure 20). In the absence of sulpiride, dopamine depressed the synaptic response to 58±8% of baseline ($n=8$, $p<.0001$, Figure 20B) whereas, in the presence of sulpiride, dopamine reduced the response to 65±5% of baseline ($n=12$, Figure 20C). Therefore, neither amphetamine nor dopamine appears to act through a D2-like receptor to produce depression of excitatory synaptic transmission.

In contrast, the D1 receptor antagonist SCH23390 (Civelli et al., 1993; Iorio et al., 1983; Sibley, 1995) blocked the depressant action of both dopamine and amphetamine (Figure 21 and Figure 22). Figure 21A shows an example of a cell in which amphetamine (10 μM) had very little effect on the EPSP in the presence of SCH23390 (2 μM), whereas it greatly reduced the EPSP after wash-out of the SCH23390. Similar results from a total of 10 cells are illustrated in Figure 21B and C. In the absence of SCH23390, amphetamine (10 μM) reduced EPSPs to 57±4% of baseline ($n=8$, $p<.0001$) whereas in the presence of 2 μM SCH23390, the EPSPs in amphetamine remained at 92±8% of baseline ($n=8$). The effect of amphetamine was therefore significantly reduced by SCH23390 ($p<.001$). As shown in the example in Figure 22A, SCH23390 antagonized the effects of dopamine as well. On average, dopamine (75 μM)

reduced the synaptic response to 51±4% of baseline when SCH23390 was not present (n=14, p<.0001, Figure 22B), while in the same slices it reduced the response to only 81±5% of baseline in the presence of SCH23390, a significant reduction in the degree of depression caused by dopamine (p<.002, Figure 22C).

D1 and D2 receptor agonists. If psychostimulants and dopamine act through a D1-like receptor to depress synaptic transmission, then D1 but not D2 agonists should mimic the depressant actions of amphetamine, cocaine, and dopamine. 6,7-ADTN (50 μM), a dopamine agonist that activates both D1-like and D2-like receptors, reduced the EPSP to 58±8% of baseline (n=4, p<.02, Figure 23). In contrast, the D2-specific agonist quinpirole (20 μM) did not significantly depress EPSPs (92±7% of baseline, n=6, p>.2, Figure 24). These results are consistent with the involvement of a D1-like receptor. However, the results obtained with D1 agonists were less clear. Neither the partial D1 agonist (+)-SKF38393 (30 μM, n=6, Figure 25) nor the full D1 agonist (±)-SKF81297 (30 μM, n=11, Figure 26) significantly reduced EPSPs (103±5% of baseline, p>.5 and 93±5% of baseline, p>.1; respectively), despite the fact that these agonists were used at concentrations exceeding their EC_{50} values for activation of D1 receptors as determined by cAMP assays in striatal tissue (Andersen and Jansen, 1990). A third D1 agonist, dihydrexidine (Brewster et al., 1990; Kohli et al., 1993; Mottola et al., 1992), was also incapable of reducing the EPSP recorded in whole-cell current clamp when applied at 10 μM (97±5%, n=4, p>.5, Figure 27A) or 40 μM (91±5%, n=5, p>.1, Figure 27B), despite the ability of dopamine to depress the EPSP in the same cells (63±9%, n=5, p<.05, Figure 27C). However, 100 μM (±)-SKF38393 did reduce synaptic responses to 77±3% of baseline (n=6, p<.01, Figure 28), and this effect was significantly antagonized (p<.02) by SCH23390 (10 μM, 94±10% of baseline, n=4, Figure 29).

Serotonin and norepinephrine. Because psychostimulants can increase the extracellular levels of monoamines other than dopamine (Ritz et al., 1987; Seiden et al., 1993) and dopamine can act on non-dopamine receptors (Goldberg, 1972; Malenka and Nicoll, 1986), one concern was that non-dopamine receptors may have contributed

to the observed effects of psychostimulants and dopamine agonists. The effects of two monoamine neurotransmitters which might be expected to contribute to the actions of psychostimulants were therefore examined. Serotonin (2 μM) caused a decrease in NAc synaptic field responses (69±8% of baseline, n=11, p<.005, Figure 30) and in whole cell EPSPs (56±10% of baseline, n=3, p<.003, Figure 31). In the same cells, the serotonin-induced decrease of the EPSP was not reduced by SCH23390 (10 μM, 59±13% of baseline, n=4, Figure 31) at the concentration that antagonized the effects of amphetamine and dopamine (e.g., Figure 21 and Figure 22). Norepinephrine (100 μM) also reduced excitatory synaptic responses to 73±3% of baseline (n=11, p<.0001, Figure 32), an effect that was abolished by the α-adrenergic antagonist phentolamine (10 μM, 98±2% of baseline, n=5, p>.05, Student Newman Keuls test, Figure 32C,D,E) but not affected by the β-adrenergic antagonist propranolol (10 μM, 73±4% of baseline, n=7, p<.05, Student Newman Keuls test, Figure 32A,B,E). However, phentolamine (10 μM) did not antagonize the reduction in excitatory synaptic responses caused by amphetamine (10 μM, 51±7% of baseline for amphetamine alone, n=7; 63±10% of baseline for amphetamine in the presence of phentolamine, n=7; p>.05 for the effect of phentolamine on amphetamine, Figure 33). It is therefore unlikely that activation of adrenergic or serotonergic receptors are important in mediating the psychostimulant-induced depression of excitatory synaptic transmission in the NAc.

6-OHDA lesion studies. As a further test of the importance of dopamine in mediating the effects of the psychostimulants, the consequences of lesioning the dopaminergic pathway from the VTA into the NAc by injecting 6-OHDA into the medial forebrain bundle of one hemisphere were examined. Such lesions cause dopaminergic axons and terminals to degenerate within 4 days after the injection (Ungerstedt, 1971b). If amphetamine causes a synaptic depression by releasing dopamine from dopaminergic terminals, then eliminating these terminals should block the synaptic actions of amphetamine. Before preparing slices from 6-OHDA lesioned animals, the apomorphine-induced increase in rotation behavior was examined. Dopamine agonist-induced rotation is a standard behavioral assay which tests for the effectiveness of a unilateral lesion in the

nigrostriatal pathway (Hefti et al., 1979). All three of the 6-OHDA treated animals exhibited a strong tendency to rotate to the side opposite the lesion, with the average turning rate being 3.1 turns/min for the 5 min beginning 15 min after the injection (i. p.) of 1 mg/kg apomorphine. Unlesioned control rats treated with the same dose of apomorphine did not rotate in either direction (n=2). These results indicate that the 6-OHDA injections were effective in lesioning a significant proportion of dopamine-containing fibers (Hefti et al., 1979).

Figure 34A and B illustrates that the effect of amphetamine on synaptic transmission could be drastically reduced in slices prepared from the lesioned hemisphere when compared to its effects in slices prepared from the contralateral, control hemisphere of the same animal. On average, amphetamine (5 – 10 μM) caused a significantly greater reduction ($p<.03$) in the synaptic responses in slices prepared from unlesioned hemispheres (27±12% of baseline synaptic responses, n=6, Figure 34C) than in slices prepared from 6-OHDA lesioned hemispheres (58±10% of baseline, n=8, Figure 34C). These results provide additional evidence indicating that the effects of amphetamine on cortico-accumbens synaptic transmission are in large part due to the release of dopamine from dopamine-containing terminals.

D1a knockout mice. To begin to test more directly the role of specific D1-like receptors, the effects of dopamine on cortico-accumbens synaptic transmission in slices prepared from mutant mice lacking D1a receptors were examined (Xu et al., 1994a, b). As illustrated in Figure 35, there was no difference in the depressant effects of dopamine in slices prepared from the mutant mice when compared to slices prepared from wildtype control mice ($p>.9$). Dopamine (75 μM) reduced the EPSP to 73±14% of baseline in the wildtype mice (n=5, $p<.003$, Figure 35A,C) and to 73±6% of baseline in the mice lacking D1a receptors (n=9, Figure 35B,C). Thus, D1a receptors are not essential for the dopamine-induced reduction in excitatory synaptic transmission in the NAc.

B. Inhibitory Synaptic Transmission

D1 and D2 receptor antagonists. To begin to determine which receptor subtype is responsible for the depressant effects of dopamine on IPSPs, the effects of the D2 antagonist sulpiride were examined. As shown in Figure 36, sulpiride (20 μM) had no significant effect on the depression elicited by dopamine (60 μM). Dopamine caused a depression of 20±5% when initially applied and a depression of 16±4% when reapplied to the same cells (n=8) in the presence of sulpiride ($p>.34$). In contrast, SCH23390, a D1 antagonist, was very effective in blocking the effects of dopamine on IPSPs (Figure 37). In these experiments, dopamine (75 μM) initially caused a depression of 18±1% but no significant depression (3±2%) when reapplied to the same cells in the presence of SCH23390 (10 μM, n=7, $p<.001$). SCH23390 (10 μM) also antagonized the depressant effect of amphetamine (10 μM) which reduced the IPSPs by 28±7% in control conditions but by only 9±4% in the same cells (n=8) in the presence of SCH23390 ($p<.02$; Figure 38). Thus, the comparative ability of D1 and D2 antagonists to attenuate synaptic depression is similar for EPSPs and IPSPs.

D1 and D2 receptor agonists. To further characterize the receptor subtype mediating the actions of dopamine on inhibitory synaptic transmission, the effects of a number of different agonists were examined. The broad-spectrum dopamine agonist 6,7-ADTN (50 μM) mimicked the effects of dopamine and amphetamine by causing a significant (21±3%) reduction in the IPSPs (n=4, $p<.02$; Figure 39). In contrast, the specific D2 receptor agonist quinpirole (10 μM) had no effect (3±2% depression, n=14, $p>.1$; Figure 40). As was the case with EPSPs, neither the partial D1 agonist SKF38393 nor the full D1 agonist dihydrexidine affected the amplitude of the IPSP. SKF38393 (20 μM) depressed IPSPs by 0±1% (n=9, $p>.5$; Figure 41), and dihydrexidine depressed the IPSP by 5±1% at 10 μM (n=3, $p>.05$, Figure 42A) and by 6±6% at 40 μM (n=3, $p>.5$, Figure 42B). In the same cells, dopamine was capable of depressing the IPSP by 21±4% (n=3, $p<.005$, Figure 42C). However, when applied prior to and during the application of dopamine (75 μM), SKF38393 (20 μM) was able to significantly attenuate the actions of dopamine (22±3% depression induced by dopamine alone,

11±3% depression induced by dopamine in the presence of SKF38393, n=9, p<.001; Figure 41). These results suggest that D1 agonists bind to the dopamine receptor responsible for depressing inhibitory synaptic transmission but are not capable of activating it.

Serotonin and norepinephrine. To determine whether other monoamines affect the IPSP, serotonin and norepinephrine were applied during recordings of IPSPs. Serotonin (5 – 10 μM) depressed the IPSP amplitude by 29±5% (n=9, p<.001, Figure 43A-C), similar to its action on EPSPs (Figure 30 and Figure 31). As with excitatory responses, the D1 antagonist SCH23390 did not reduce the ability of serotonin to depress the IPSP, since serotonin (5 – 10 μM) had similar effects when applied in the same cells in the absence and then in the presence of 10 μM SCH23390 (29±4% depression for control, 29±5% depression in SCH23390, p>.3, n=3, Figure 43D). In contrast to the norepinephrine-induced depression observed with EPSPs (Figure 32), norepinephrine did not affect IPSPs (.6±4.7% depression, n=4, p>.5, Figure 44). It is therefore unlikely that serotonin, norepinephrine or their receptors are involved in the effects of dopamine and amphetamine on inhibitory synaptic transmission.

Discussion

The similar pharmacological features of the dopamine-induced depression of EPSPs and IPSPs suggest that the same receptor type is responsible for dopamine's effects on inhibitory and excitatory synaptic transmission. The dopamine-induced depression of both EPSPs and IPSPs is blocked by the D1 antagonist SCH23390, not affected by D2 antagonists, mimicked by amphetamine and the broad-spectrum dopamine agonist 6,7-ADTN, and, remarkably, not mimicked by either D1 or D2 agonists. Furthermore, D1 agonists appear to bind the receptor, probably with relatively low affinity, without strongly activating it. Very high concentrations of SKF38393 are required to observe a depression of excitatory responses, and this depression is antagonized by SCH23390, suggesting that it results from the binding of the SKF38393 to the dopamine receptor

responsible for dopamine-induced depression rather than to a different type of receptor. In the case of IPSPs, SKF38393 applied concurrently with dopamine reduces the amount of depression elicited by dopamine, indicating again that SKF38393 probably binds to the dopamine receptor responsible for synaptic depression without activating it. Taken together, these results are consistent with the hypothesis that the same receptor type is responsible for depression of both EPSPs and IPSPs.

The ability of neither D1 nor D2 agonists to activate the receptor responsible for dopamine-induced synaptic depression raises the question of whether this receptor is a dopamine receptor at all. Dopamine is capable of binding to and activating other monoamine receptors, such as adrenergic receptors (Goldberg, 1972; Malenka and Nicoll, 1986). However, a number of experiments yielded results that would not be expected were the receptor indeed not a dopamine receptor. First, amphetamine causes a depression of both EPSPs and IPSPs that is blocked by SCH23390, the same antagonist that blocks the effects of dopamine. While amphetamine causes the release of both dopamine and other monoamines by interacting with their transporters (Ritz et al., 1987; Seiden et al., 1993), the extremely dense dopaminergic innervation of the NAc (Ungerstedt, 1971b) suggests that dopamine terminals far outnumber those of any other monoamine system. Any effect of amphetamine in the NAc is therefore likely to be mediated by dopamine, although it is possible that other monoamines could be involved.

Second, the broad-spectrum dopamine agonist 6,7-ADTN mimics the synaptic depression caused by dopamine and psychostimulants. This result is most consistent with an action at dopamine receptors. (This result also suggests that binding of both D1 and D2 receptors may be required to observe the effect; however, EPSPs were not depressed by co-application of the D1 agonist SKF81297 and the D2 agonist quinpirole; not shown.) Third, while both serotonin and norepinephrine reduced synaptic transmission, several experiments indicate that these monoamines are not involved to any great extent in the dopamine-induced depression. SCH23390 was incapable of antagonizing the depressant effects of serotonin on EPSPs and IPSPs at

concentrations that were very effective in antagonizing the effects of dopamine and amphetamine. Norepinephrine reduced excitatory synaptic transmission, but not inhibitory transmission, indicating that activation of adrenergic receptors is probably not responsible for dopamine-induced depression of IPSPs. The effects of norepinephrine on excitatory responses were fully blocked by the α-adrenergic antagonist phentolamine, but phentolamine had little, if any effect on the ability of amphetamine to reduce excitatory responses. Thus, the effects of amphetamine are likely to be due to the release of dopamine that acts at dopamine receptors, and not the release of other monoamines or the binding of dopamine to other monoamine receptors.

The fourth experiment that suggests that dopamine receptor activation is responsible for the effects of dopamine and amphetamine involved the unilateral lesioning of the medial forebrain bundle (MFB) with 6-OHDA, a toxin that is specific for catecholaminergic neurons. The MFB carries dopaminergic fibers from the midbrain to the forebrain, including from the VTA to the NAc. Amphetamine produced smaller effects in slices taken from the lesioned hemisphere when compared with slices taken from the intact hemisphere of the same animals. This result is unlikely to be due to differences in the health of slices from the lesioned hemisphere, since the toxin injections were made into the MFB, not into the NAc itself; slices from both hemispheres yielded equally robust synaptic fields. While a 6-OHDA lesion in the MFB is likely to destroy adrenergic fibers as well as dopaminergic fibers, adrenergic innervation of the NAc is quite sparse in comparison with dopaminergic innervation (Ungerstedt, 1971b) and therefore the observed reduction in the effectiveness of amphetamine, which induces catecholamine release at the nerve terminal, is likely to be due to the destruction of dopaminergic fibers. Consistent with this idea, norepinephrine levels in the striatum are reduced to a lesser extent than dopamine levels after 6-OHDA lesions of the substantia nigra (Hefti et al., 1980). The residual depression by amphetamine in slices from lesioned hemispheres is likely to be due mostly to denervation supersensitivity (Ungerstedt, 1971a), although activation of a non-

dopamine receptor cannot be completely ruled out. The simplest interpretation of the results of the lesion experiment is that the number of dopaminergic terminals is reduced in NAc slices from the lesioned hemisphere, resulting in the smaller effect of amphetamine.

The exact identity of this receptor, however, remains unknown. None of the dopamine receptors cloned from mammals so far fits the pharmacological profile reported here, since those receptors that bind SCH23390 with high affinity (e.g., the D1a and D1b receptors) are also activated by D1 agonists such as SKF38393 (Jensen et al., 1996). The cloned D1 receptors are positively coupled to adenylate cyclase, but activation of this enzyme with forskolin does not mimic the effects of dopamine on either inhibitory or excitatory synaptic transmission (see Figure 60 through Figure 62 in Chapter 5). Furthermore, slices taken from mice lacking the D1a receptor (Xu et al., 1994a,b) were as capable of exhibiting dopamine-induced depression of EPSPs as slices from wildtype mice, suggesting that the D1a receptor does not normally contribute to the effects of dopamine on synaptic transmission. The D1b receptor may instead be involved, or another receptor of the D1 class that has yet to be cloned from mammals. Because of the high affinity of the D1b receptor for benzazepine D1 agonists (such as SKF38393), it is perhaps more likely that a different dopamine receptor is involved.

There is, in fact, increasing evidence for the existence of D1-like dopamine receptors with properties unaccounted for by the cloned receptors (Waddington and Deveney, 1996). For instance, there is no correlation between the relative ability of D1 agonist compounds to elevate cAMP levels (the classical effect of D1 receptor activation) and their effectiveness in producing behaviors that are usually associated with systemic administration of D1 agonists (Arnt et al., 1992; Deveney and Waddington, 1995; Downes and Waddington, 1993; Waddington and Deveny, 1996). Furthermore, there is evidence for a dissociation between the location within the brain of binding sites for radiolabeled SCH23390 and the location of dopamine-stimulated adenylate cyclase (Andersen et al., 1990; Mailman et al., 1986). And several studies

have suggested that there exists a D1-like receptor that causes an increase in phosphoinositide turnover (Mahan et al., 1990; Undie and Friedman, 1990, 1992; Undie et al., 1994; Wang et al., 1995) although none of the cloned D1 receptors appear to exhibit this activity (Jensen et al., 1996). This putative phosphoinositide turnover-coupled receptor is reported to be bound by D1 agonists such as SKF38393 with much lower affinity than the cloned receptors, and is similar in this regard to two D1-like receptors that have been cloned from *Drosophila* (Feng et al., 1996; Sugamori et al., 1995) and to the dopamine receptor that is apparently responsible for the effects on synaptic transmission reported here. The apparent existence of many varied D1-like receptors coupled to various second messenger systems and with various abilities to bind classical D1 agonists seems to indicate, at least circumstantially, that mammalian D1-like dopamine receptors are not limited to the two that have been cloned to date. While a more trivial explanation (such as cross-reactivity of dopamine with a non-dopamine receptor) cannot be completely ruled out, an unidentified dopamine receptor should also be considered as a possible explanation for the atypical agonist binding and activation properties of the receptor that is responsible for the depressant effects of dopamine on synaptic transmission reported here.

The existence of multiple D1-like receptors may help explain the confusion in the literature regarding the identity of the receptor responsible for synaptic depression in the NAc. Early studies found that D1 receptor activation depresses excitatory synaptic transmission only in slices from amphetamine-pretreated animals (Higashi et al., 1989), but this was disputed by a later study reporting D1-mediated effects on EPSPs in slices from naïve animals (Pennartz et al., 1992a). A subsequent study found no effect of D1 receptor activation, and reported instead that D2 receptors are responsible for synaptic depression (O'Donnell and Grace, 1994). Expression of different receptors with varying agonist and antagonist binding properties across different stages of development and in different strains of rats may explain the apparent discrepancies.

Figure 19. The D2 antagonist sulpiride does not affect the synaptic depression resulting from application of amphetamine to excitatory responses in the NAc. ***A***, A typical field recording experiment in which 10 μM amphetamine was first applied in the absence of sulpiride and then in the presence of 10 μM sulpiride. ***B***, Summary of 8 field experiments in which 10 μM amphetamine was applied in the absence of sulpiride. ***C***, In the same 8 experiments, amphetamine (10 μM) was again applied in the presence of 10 μM sulpiride; the amphetamine-induced reduction in the synaptic response was not diminished by the sulpiride.

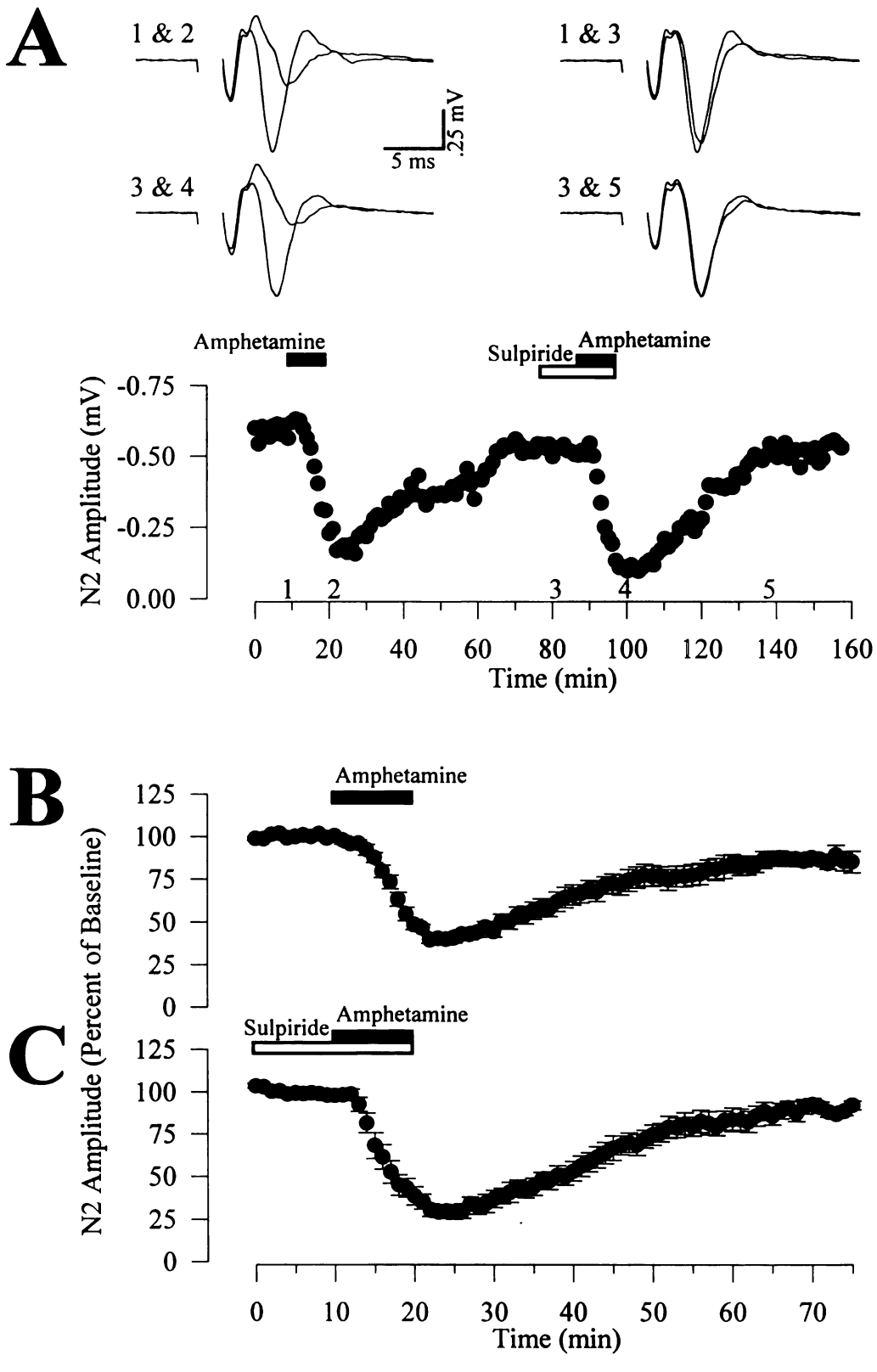
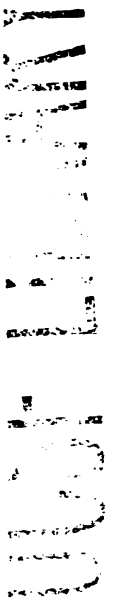

A
1 & 2
1 & 3
3 & 4
3 & 5
.25 mV
5 ms
Amphetamine
Sulpiride
Amphetamine
N2 Amplitude (mV)
-0.75
-0.50
-0.25
0.00
1
2
3
4
5
0
20
40
60
80
100
120
140
160
Time (min)
B
Amphetamine
C
Sulpiride
Amphetamine
N2 Amplitude (Percent of Baseline)
125
100
75
50
25
0
0
10
20
30
40
50
60
70
Time (min)

Figure 20. The D2 antagonist sulpiride does not affect the synaptic depression resulting from application of dopamine to excitatory responses in the NAc.
A, A representative field recording in which 75 μM dopamine was applied first in the absence, and then in the presence, of sulpiride (10 μM). ***B***, Summary of 8 experiments, demonstrating the effects of dopamine (75 μM) in the absence of sulpiride. ***C***, In 12 experiments (5 of which were performed in the same slices as those shown in E, and the remainder of which were interleaved with those shown in E), dopamine (75 μM) was applied in the presence of 10 μM sulpiride; the sulpiride did not block the reduction of the synaptic response caused by dopamine.



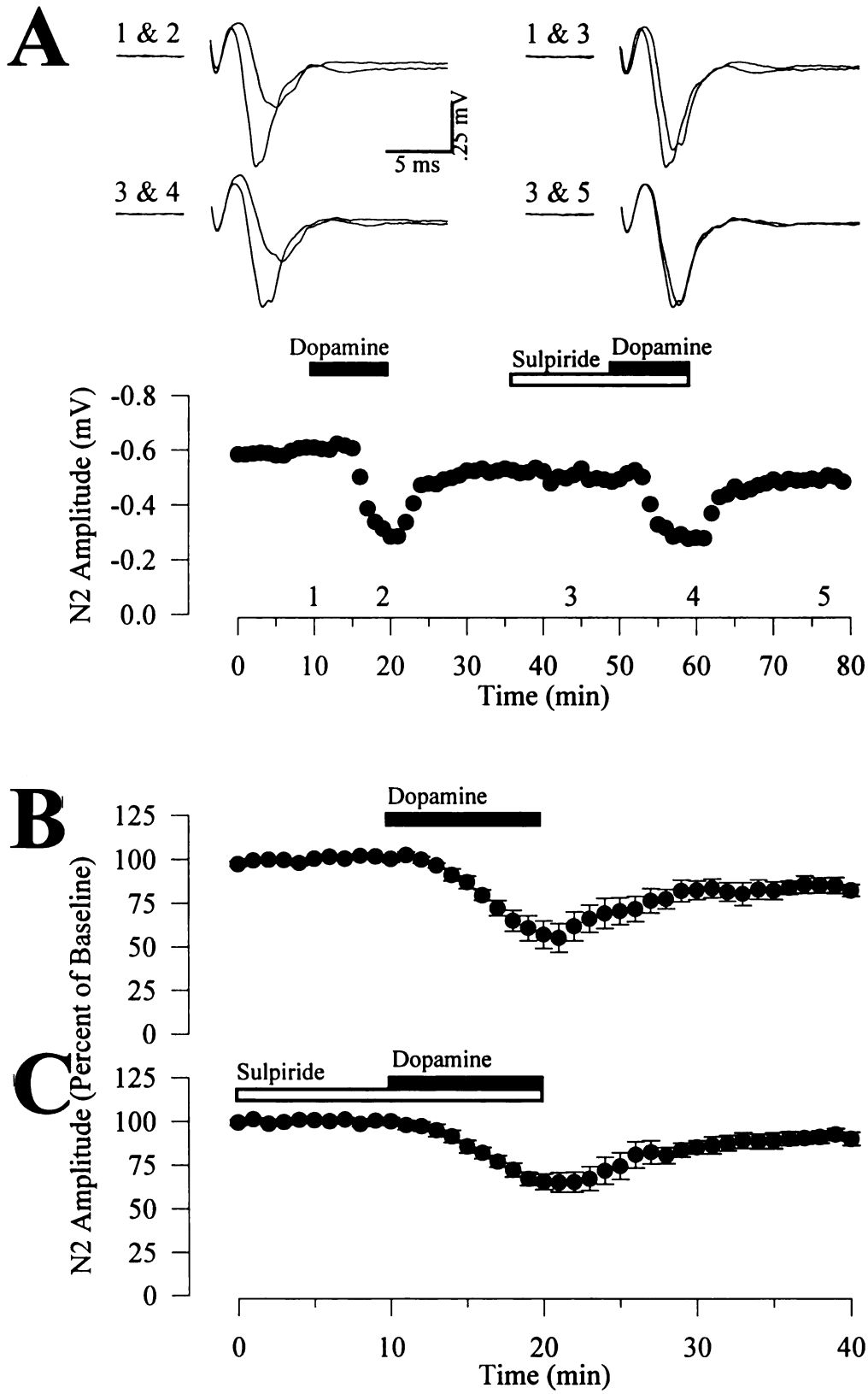

A
1 & 2
1 & 3
3 & 4
3 & 5
.25 mV
5 ms
Dopamine
Sulpiride
Dopamine
N2 Amplitude (mV)
-0.8
-0.6
-0.4
-0.2
0.0
1
2
3
4
5
0
10
20
30
40
50
60
70
80
Time (min)
B
Dopamine
125
100
75
50
25
0
C
Sulpiride
Dopamine
N2 Amplitude (Percent of Baseline)
125
100
75
50
25
0
0
10
20
30
40
Time (min)

Figure 21. The D1 antagonist SCH23390 inhibits the actions of amphetamine on EPSPs in the NAc.

A, A representative current clamp experiment in which 10 μM amphetamine was first applied in the presence of 2 μM SCH23390, and again after washout of the SCH23390. ***B***, Summary (n=8) of experiments in which 10 μM amphetamine was applied. ***C***, Summary of experiments in which 10 μM amphetamine was applied in the presence of 2 μM SCH23390, showing that SCH23390 blocked the effects of dopamine (n=8, 6 of which were performed in the same cells as those shown in B, and the remainder of which were interleaved with them).

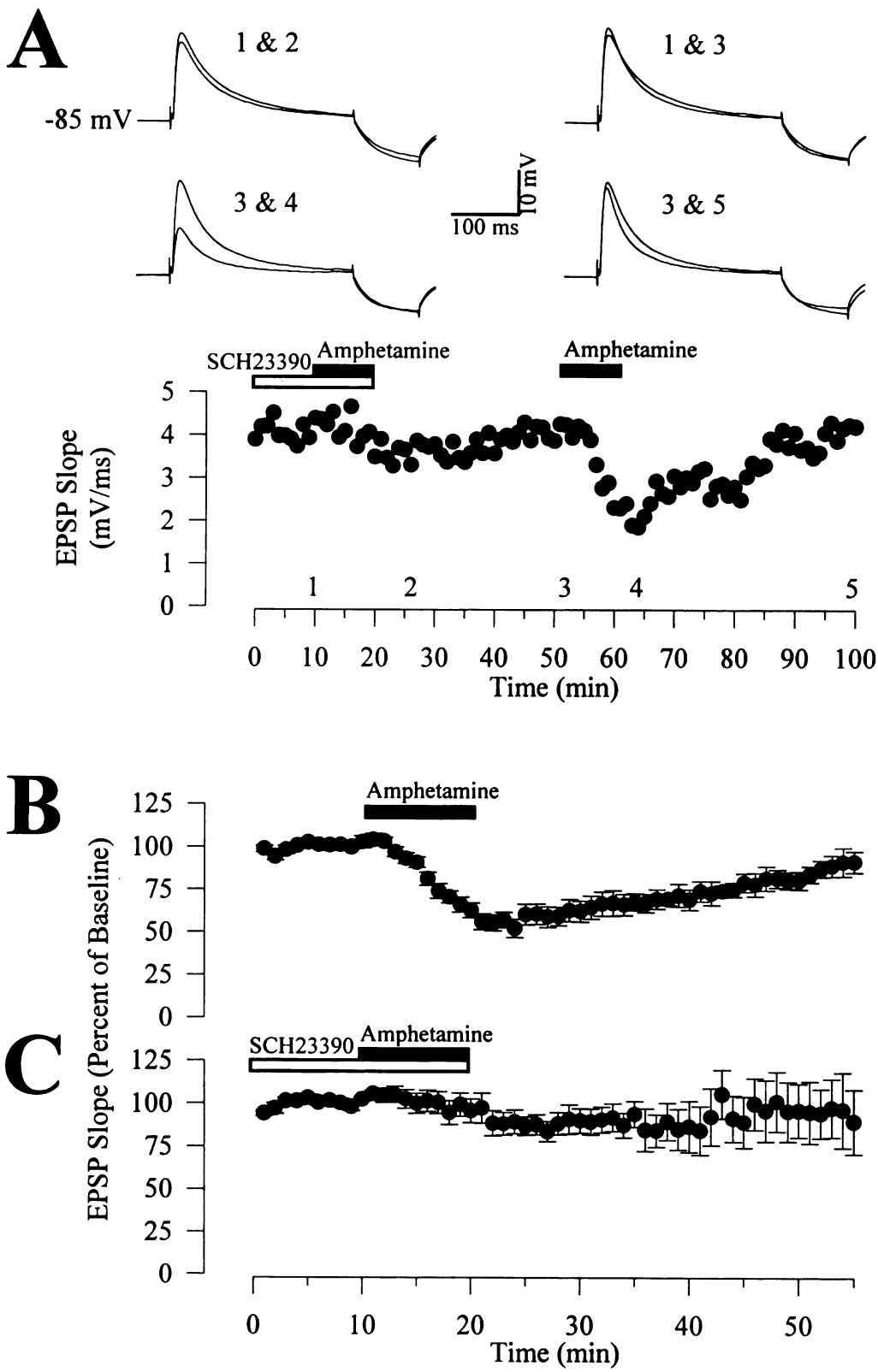

A
1 & 2
1 & 3
-85 mV
10 mV
100 ms
3 & 4
3 & 5
SCH23390
Amphetamine
Amphetamine
EPSP Slope (mV/ms)
0
1
2
3
4
5
1
2
3
4
5
0
10
20
30
40
50
60
70
80
90
100
Time (min)
B
Amphetamine
EPSP Slope (Percent of Baseline)
125
100
75
50
25
0
C
SCH23390
Amphetamine
125
100
75
50
25
0
0
10
20
30
40
50
Time (min)

Figure 22. The D1 antagonist SCH23390 inhibits the actions of dopamine on excitatory synaptic transmission in the NAc.

A, A field recording experiment in which 75 μM dopamine elicited a depression of the synaptic response that was sensitive to SCH23390 (10 μM). ***B***, Summary (n=14) of the effects of 75 μM dopamine on the synaptic response. ***C***, In the same 14 experiments as those shown in E, dopamine (75 μM) was applied in the presence of 10 μM SCH23390, which significantly reduced the effects of dopamine.

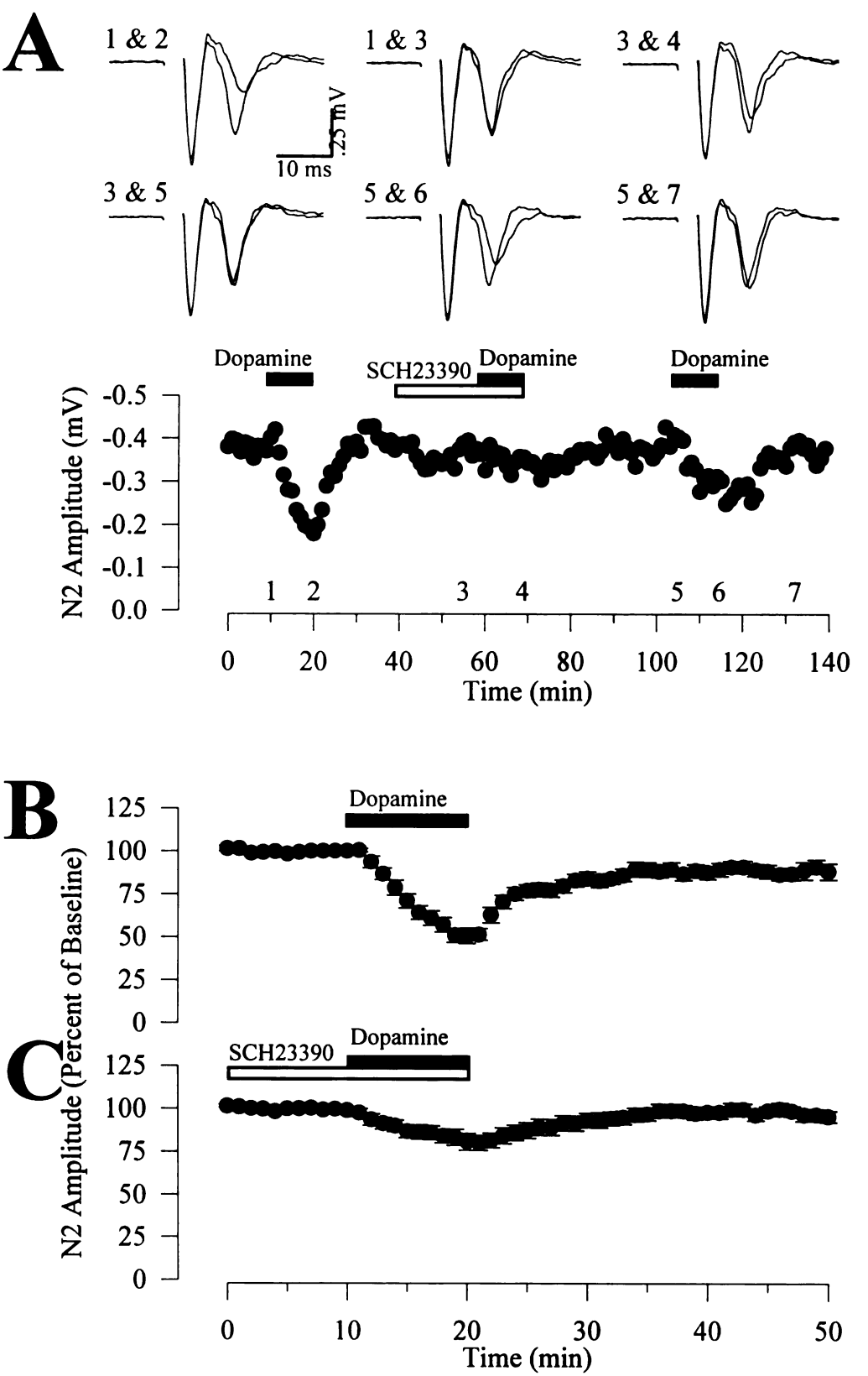
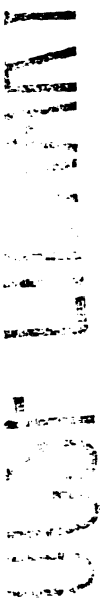

A
1 & 2
1 & 3
3 & 4
3 & 5
5 & 6
5 & 7
.25 mV
10 ms
Dopamine
SCH23390
Dopamine
Dopamine
N2 Amplitude (mV)
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
1 2 3 4 5 6 7
0 20 40 60 80 100 120 140
Time (min)
B
Dopamine
125
100
75
50
25
0
C
SCH23390
Dopamine
N2 Amplitude (Percent of Baseline)
125
100
75
50
25
0
0 10 20 30 40 50
Time (min)

Figure 23. The broad-spectrum dopamine agonist 6,7-ADTN depresses NAc EPSPs. ***A*** and ***B***, An experiment demonstrating the reduction of EPSPs as result of the application of 6,7-ADTN (50 μM). ***C***, In an average of 4 experiments, 6,7-ADTN (50 μM) reversibly reduced the initial slope of the EPSP.



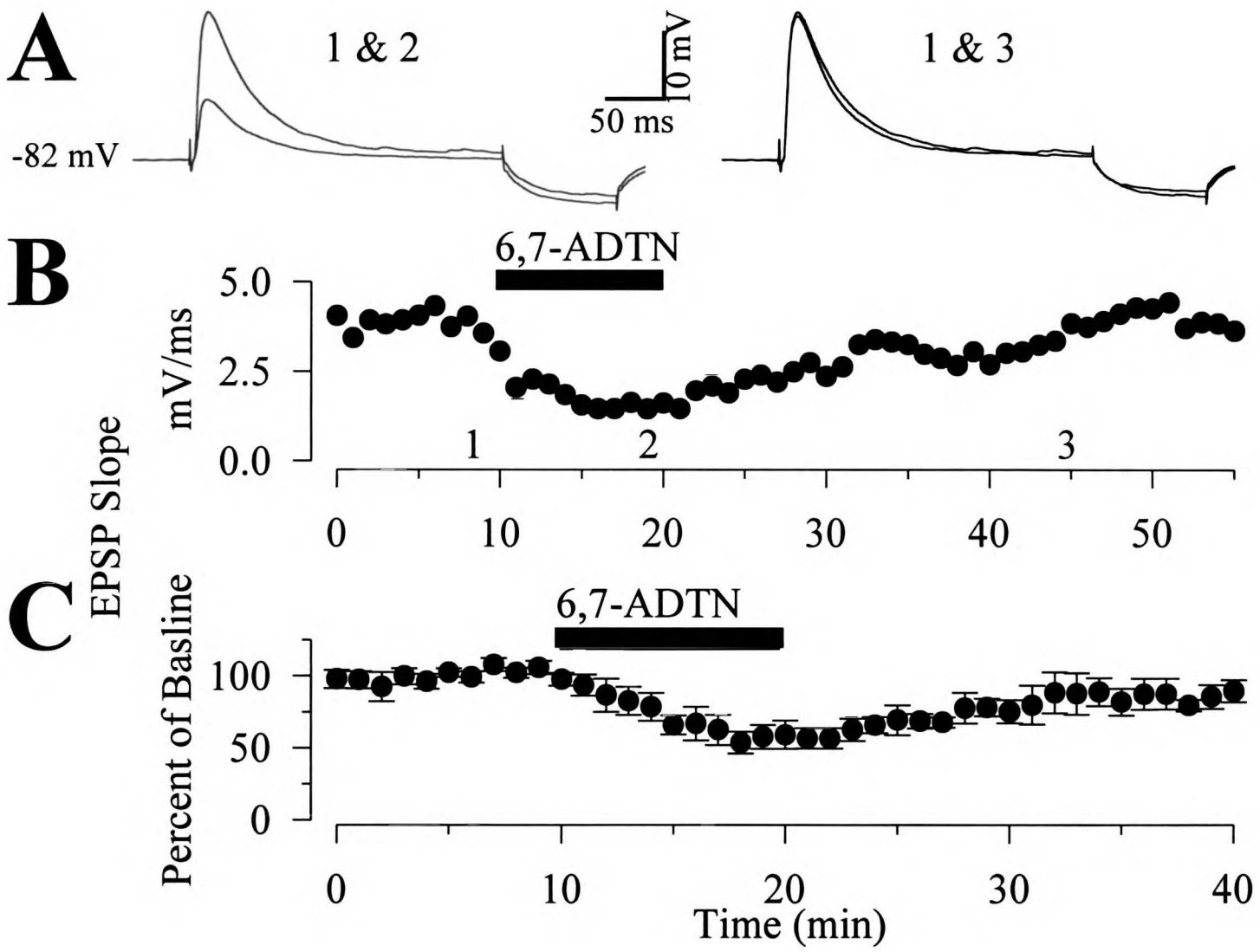
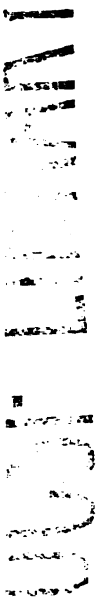


Figure 24. The D2 agonist quinpirole has no effect on the NAc EPSP.
A and ***B***, An experiment in which dopamine (75 μM) depressed the EPSP, but not quinpirole (20 μM). ***C***, An average graph (n=6) demonstrating the lack of effect of quinpirole.



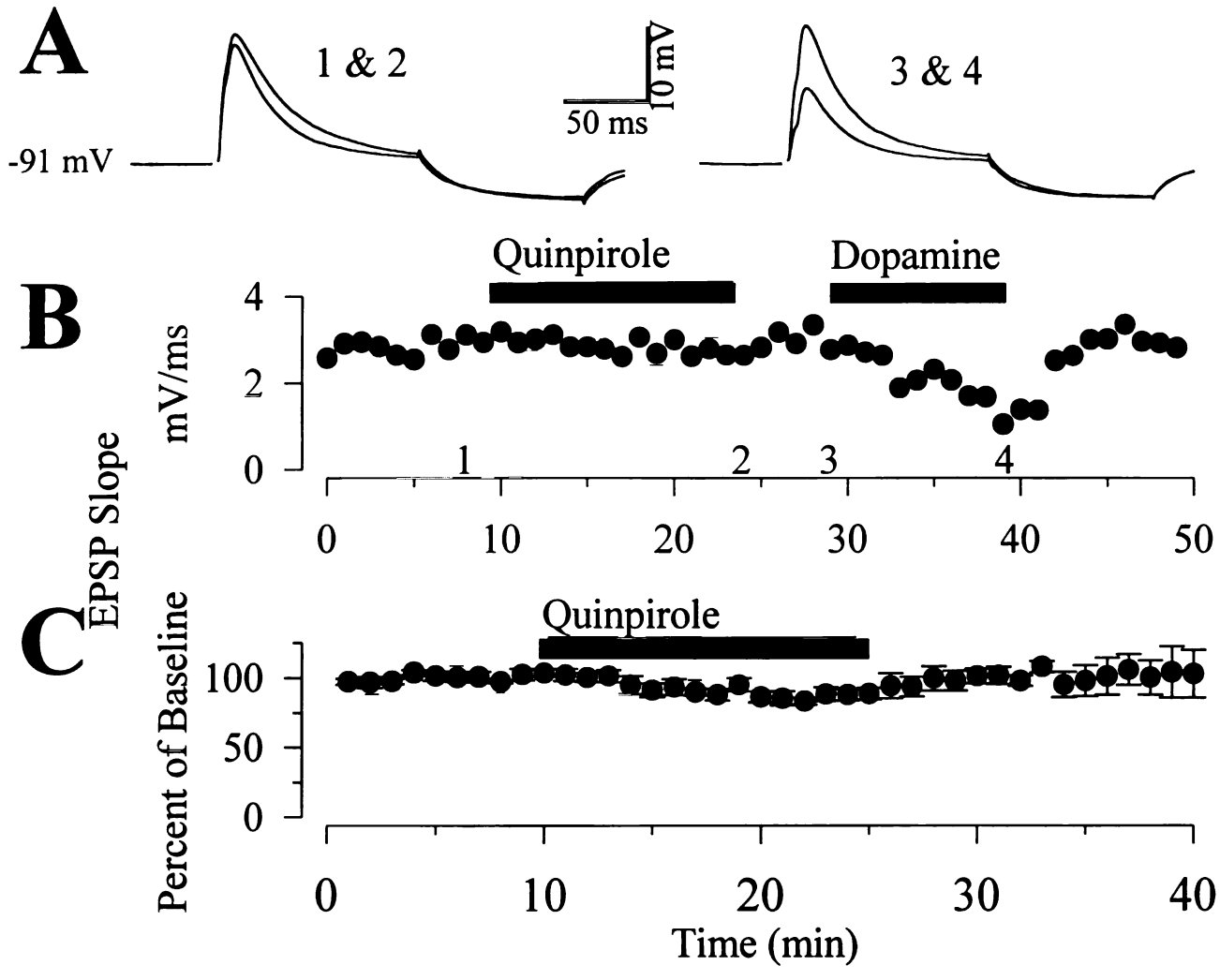

A
1 & 2
10 mV
50 ms
3 & 4
-91 mV
B
Quinpirole
Dopamine
EPSP Slope
mV/ms
4
2
0
1
2
3
4
0
10
20
30
40
50
C
Quinpirole
Percent of Baseline
100
50
0
0
10
20
30
40
Time (min)

Figure 25. The partial D1 agonist SKF38393 has no effect on the NAc EPSP.

A and ***B***, An experiment in which dopamine (75 μM) depressed the EPSP, but not (+)-SKF38393 (30 μM). ***C***, An average graph (n=6) demonstrating the lack of effect of (+)-SKF38393.

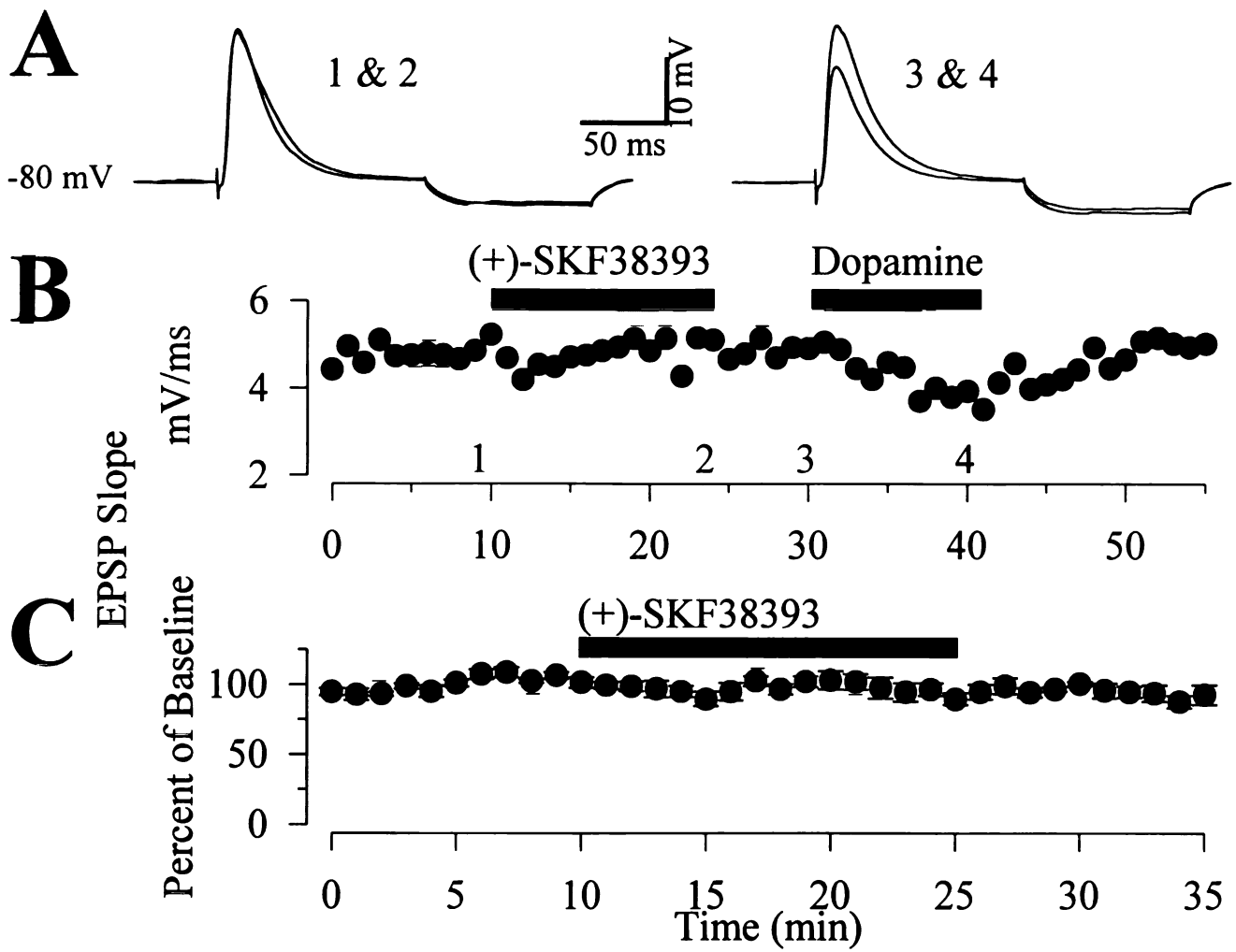

A
1 & 2
10 mV
50 ms
3 & 4
-80 mV
B
(+)-SKF38393
Dopamine
EPSP Slope
mV/ms
6
4
2
1
2
3
4
0
10
20
30
40
50
C
(+)-SKF38393
Percent of Baseline
100
50
0
0
5
10
15
20
25
30
35
Time (min)

Figure 26. The full D1 agonist SKF81297 has no effect on the NAc EPSP.

A and ***B***, An experiment in which dopamine (75 μM) depressed the EPSP, but not (±)-SKF81297 (30 μM). ***C***, An average graph (n=11) demonstrating the lack of effect of (±)-SKF81297.

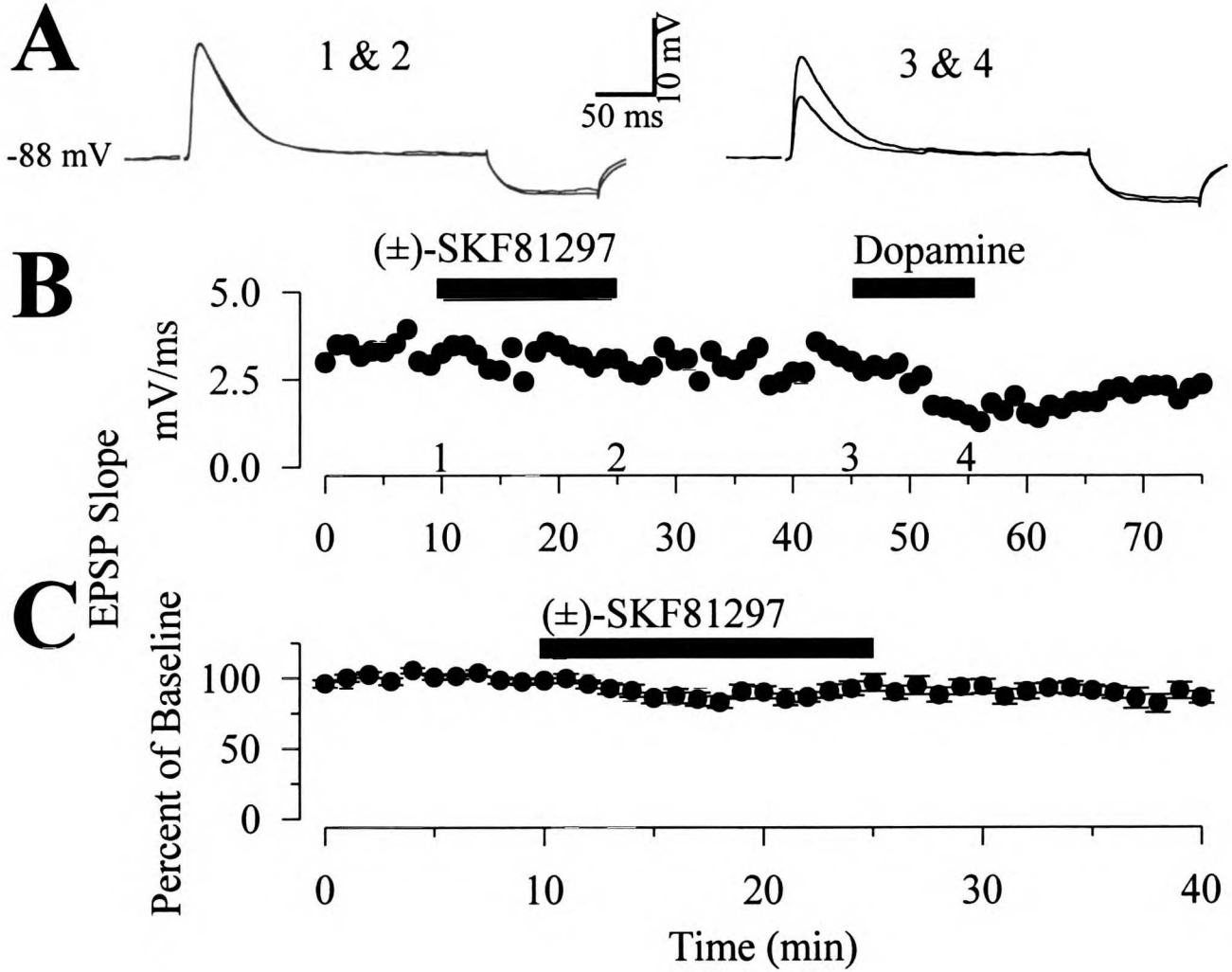


Figure 27. The full D1 agonist dihydrexidine has no effect on NAc EPSPs. ***A***, Dihydrexidine at 10 μM was incapable of depressing the EPSP (n=4). ***B***, At 40 μM dihydrexidine still had no effect on the EPSP (n=5). ***C***, Dopamine (75 μM) depressed the EPSP in the same cells (n=5).

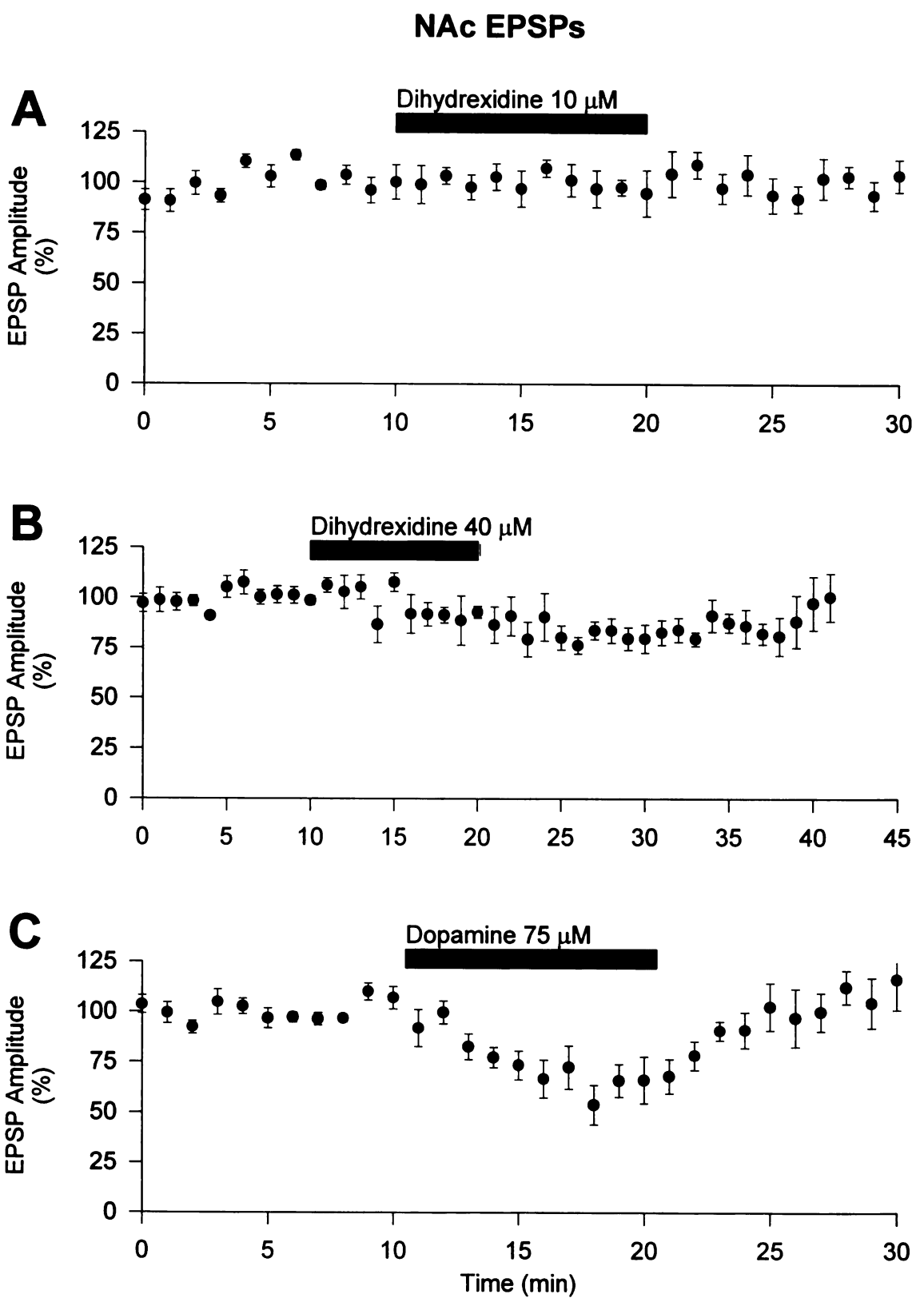

NAc EPSPs
A
Dihydrexidine 10 μM
EPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
B
Dihydrexidine 40 μM
EPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
35
40
45
C
Dopamine 75 μM
EPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
Time (min)

Figure 28. The D1 agonist SKF38393 at higher concentrations depresses excitatory synaptic transmission in the NAc.

A and ***B***, An example experiment in which (±)-SKF38393 (100 μM) caused a depression of the excitatory field response. ***C***, A summary (n=6) of similar experiments.

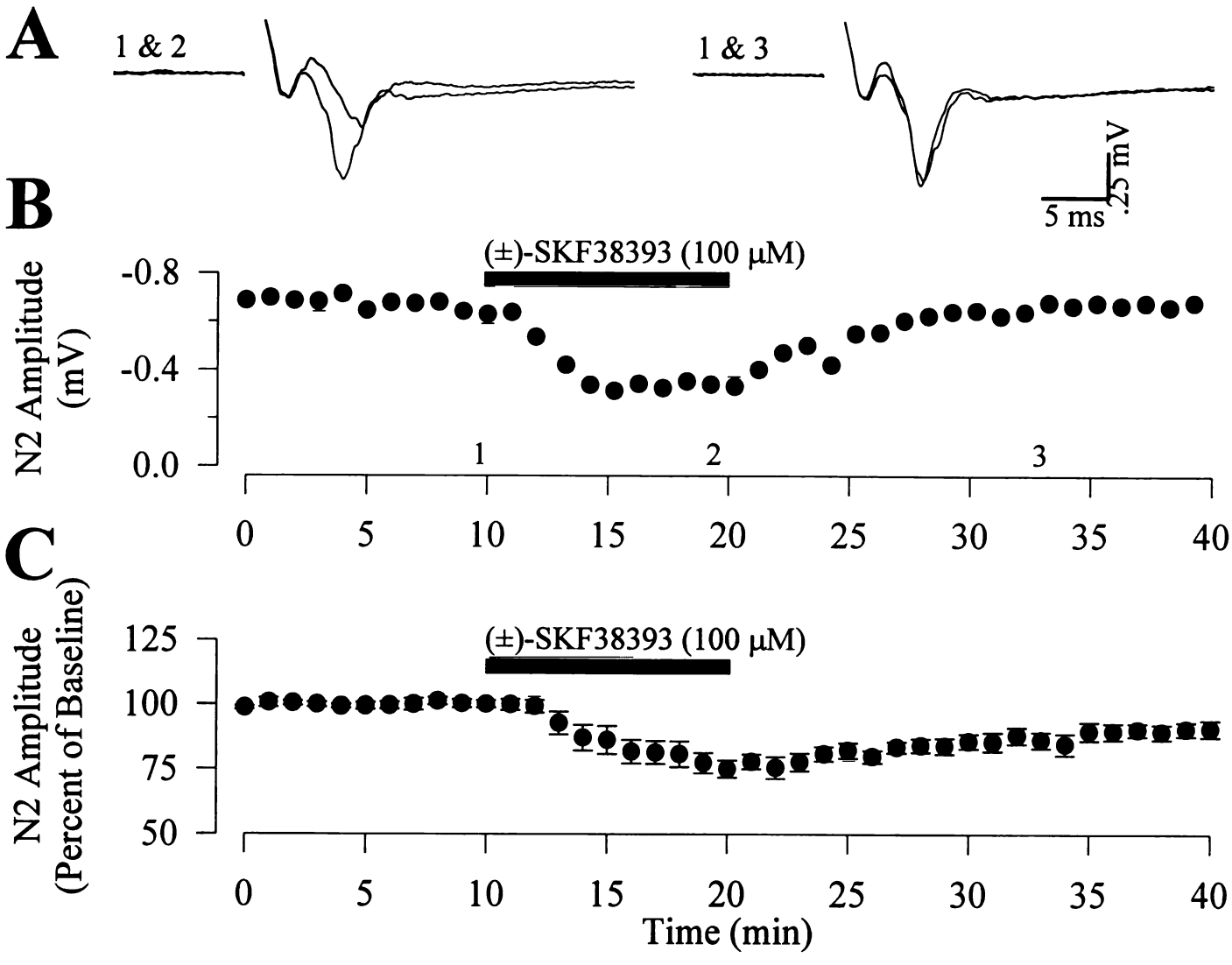

A
1 & 2
1 & 3
.25 mV
5 ms
B
(±)-SKF38393 (100 μM)
N2 Amplitude (mV)
-0.8
-0.4
0.0
1
2
3
0
5
10
15
20
25
30
35
40
C
N2 Amplitude (Percent of Baseline)
125
100
75
50
(±)-SKF38393 (100 μM)
0
5
10
15
20
25
30
35
40
Time (min)

Figure 29. The D1 antagonist SCH23390 blocks the effects of SKF38393 on excitatory NAc responses.

A and ***B***, An example experiment in which (±)-SKF38393 (100 μM) had no effect when applied in the presence of SCH23390 (10 μM). ***C***, A summary of the antagonism by SCH23390 of the depression elicited by 100 μM (±)-SKF38393 (n=4); these experiments were done in the same slices as those of Figure 28.

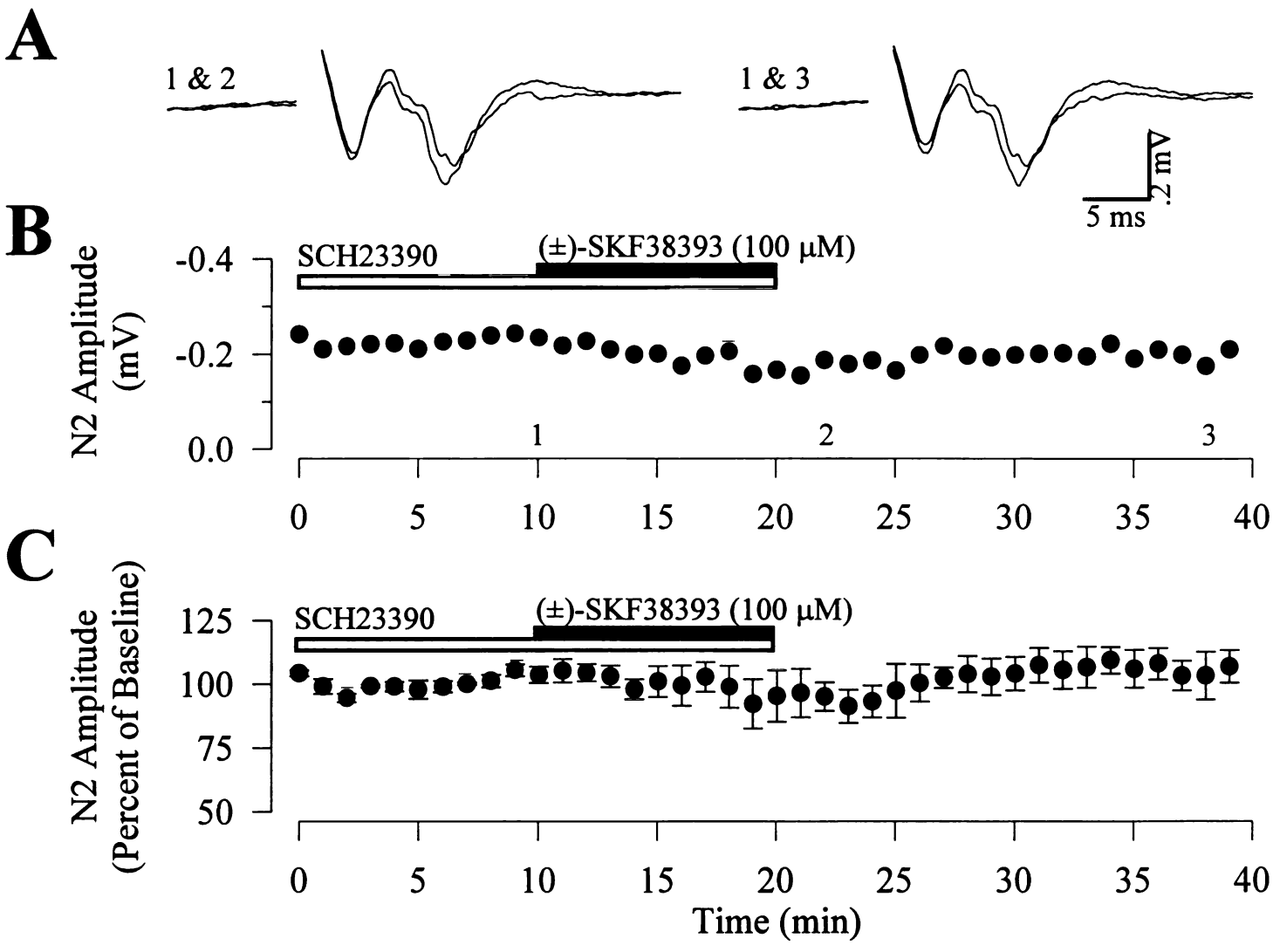

A
1 & 2
1 & 3
.2 mV
5 ms
B
N2 Amplitude (mV)
-0.4
-0.2
0.0
SCH23390
(±)-SKF38393 (100 µM)
1
2
3
0
5
10
15
20
25
30
35
40
C
N2 Amplitude (Percent of Baseline)
125
100
75
50
SCH23390
(±)-SKF38393 (100 µM)
0
5
10
15
20
25
30
35
40
Time (min)

Figure 30. Serotonin depresses excitatory synaptic transmission in the NAc. ***A*** and ***B***, A typical field experiment demonstrating the effects of serotonin (2 μM) on the amplitude of the synaptic component. ***C***, In 11 experiments serotonin (2 μM) depressed the excitatory response.

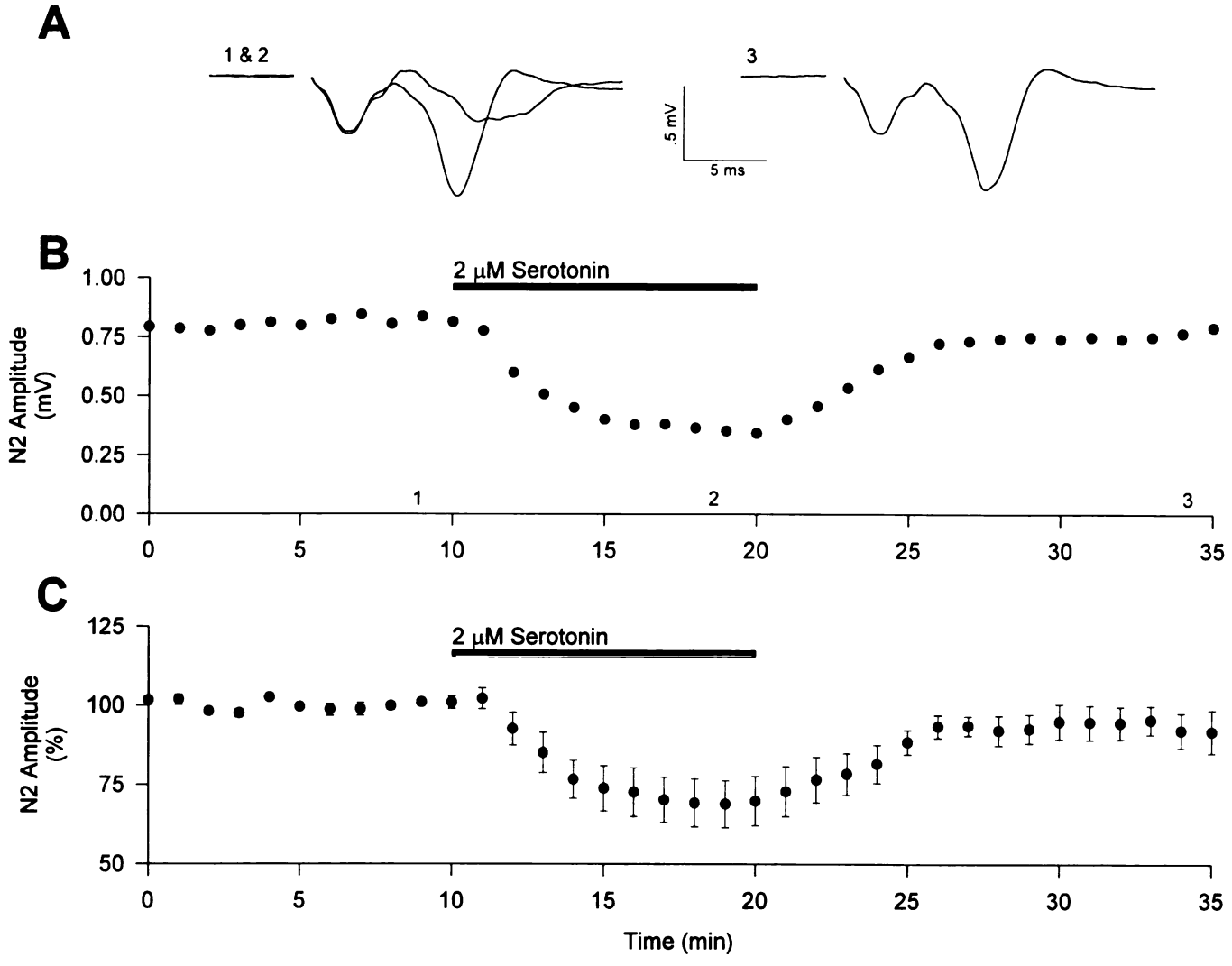

A
1 & 2
3
.5 mV
5 ms
B
2 μM Serotonin
N2 Amplitude (mV)
1.00
0.75
0.50
0.25
0.00
1
2
3
0
5
10
15
20
25
30
35
C
2 μM Serotonin
N2 Amplitude (%)
125
100
75
50
0
5
10
15
20
25
30
35
Time (min)

Figure 31. SCH23390 does not antagonize the effects of serotonin on EPSPs in the NAc.

A and ***B***, An experiment in which serotonin (2 μM) was applied first in the absence and then in the presence of SCH23390 (10 μM). ***C***, A comparison of the average effects of serotonin on NAc EPSPs in the absence (n=3) and presence (n=4) of SCH23390. The 3 serotonin controls were done in cells in which SCH23390 was applied.

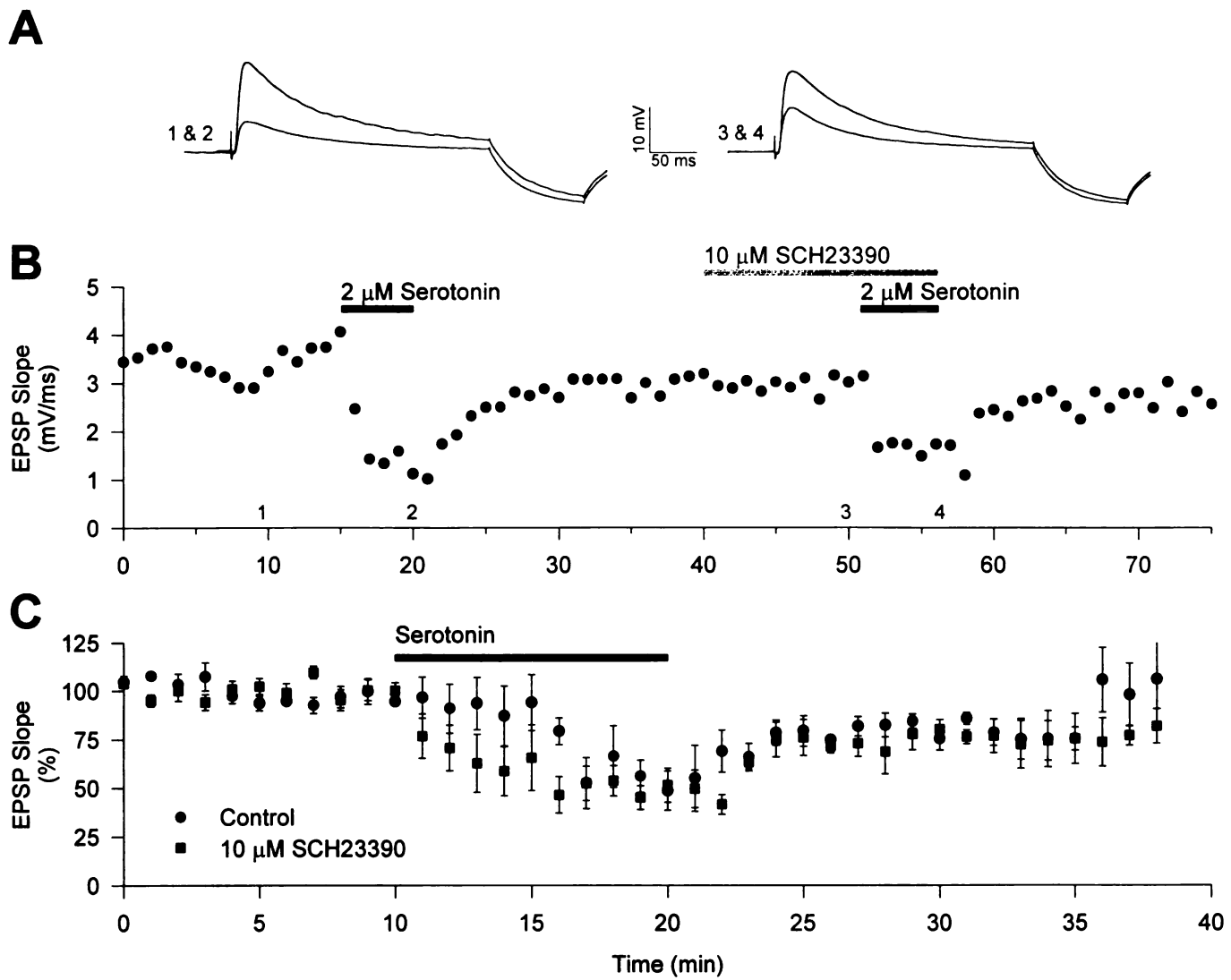

A
1 & 2
10 mV
50 ms
3 & 4
B
10 μM SCH23390
2 μM Serotonin
2 μM Serotonin
EPSP Slope (mV/ms)
1
2
3
4
C
Serotonin
EPSP Slope (%)
Control
10 μM SCH23390
Time (min)

Figure 32. Norepinephrine depresses excitatory synaptic transmission in the NAc by activating an α-adrenergic receptor.

A and ***B***, A typical field experiment in which norepinephrine (100 μM) depressed the response, and the β-adrenergic antagonist propranolol (10 μM) had little effect on the ability of norepinephrine to cause the depression. ***C*** and ***D***, A similar experiment in which norepinephrine (100 μM) again depressed the excitatory response, and this depression was blocked by the α-adrenergic antagonist phentolamine (10 μM). ***E***, A summary of the effects of propranolol and phentolamine on the depressant actions of norepinephrine. Norepinephrine depressed the response (n=11), and the depression was blocked by phentolamine (n=5) but not propranolol (n=7).

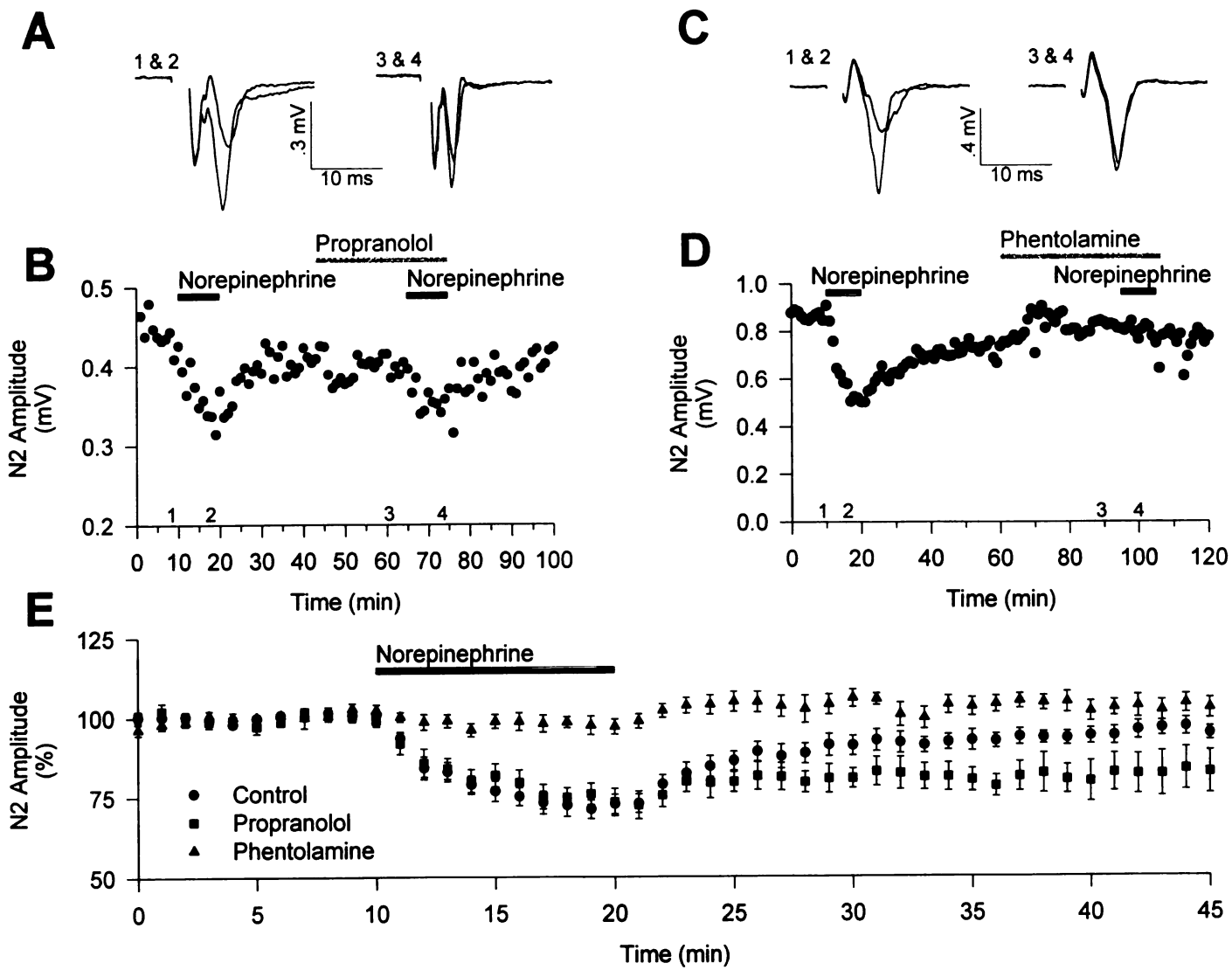

A
1 & 2
3 & 4
.3 mV
10 ms
C
1 & 2
3 & 4
.4 mV
10 ms
B
Propranolol
Norepinephrine
Norepinephrine
N2 Amplitude (mV)
0.5
0.4
0.3
0.2
1
2
3
4
0 10 20 30 40 50 60 70 80 90 100
Time (min)
D
Phentolamine
Norepinephrine
Norepinephrine
N2 Amplitude (mV)
1.0
0.8
0.6
0.4
0.2
0.0
1 2
3 4
0 20 40 60 80 100 120
Time (min)
E
Norepinephrine
N2 Amplitude (%)
125
100
75
50
Control
Propranolol
Phentolamine
0 5 10 15 20 25 30 35 40 45
Time (min)

Figure 33. Antagonism of α-adrenergic receptors does not block the depressant effects of amphetamine on excitatory synaptic transmission in the NAc.
A and ***B***, In one experiment, amphetamine (10 μM) was applied in the absence and then in the presence of phentolamine (10 μM). ***C***, An average of experiments similar to the example in B demonstrates that phentolamine only slightly (nonsignificantly) reduced the effects of amphetamine (n=7).

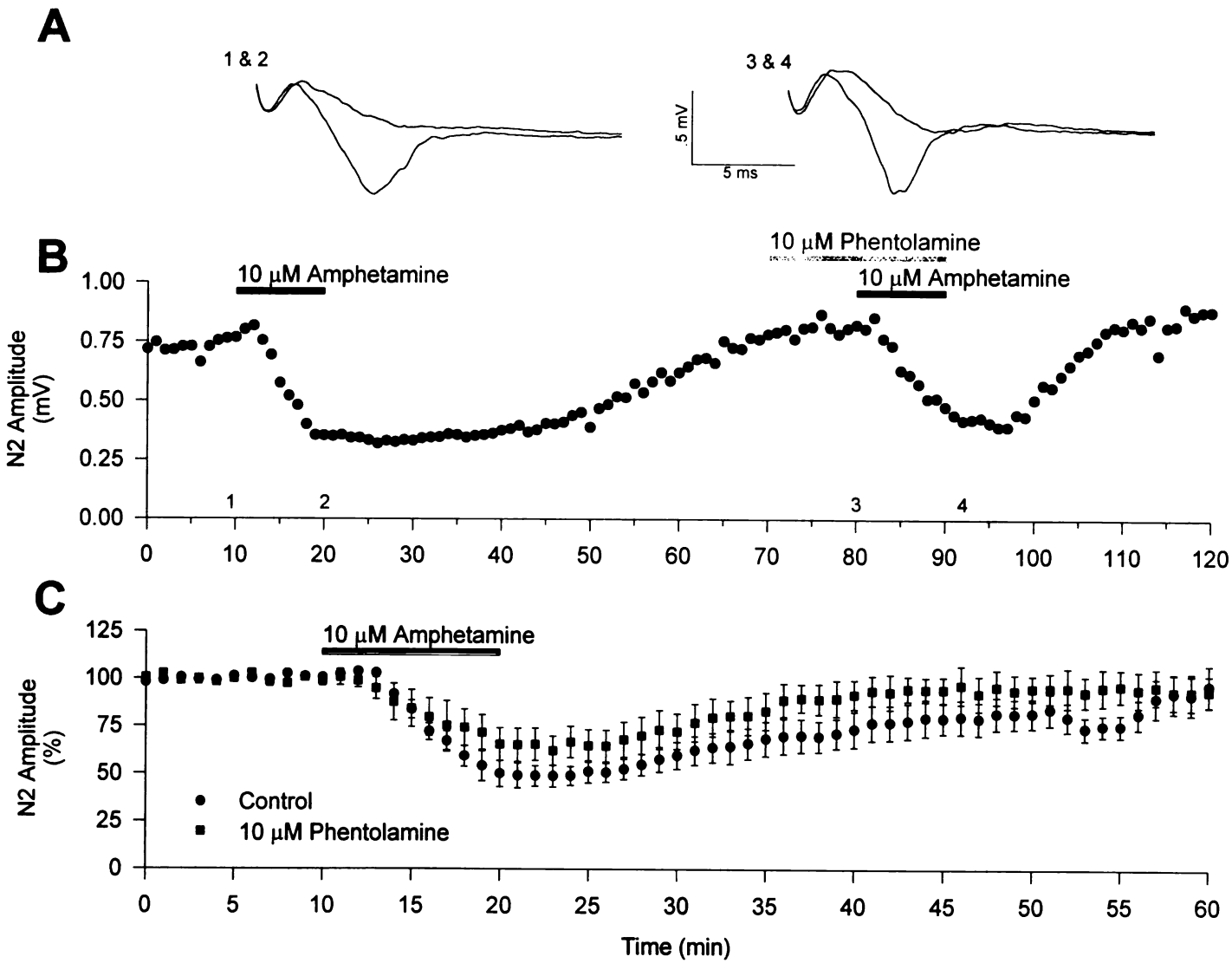

A
1 & 2
3 & 4
.5 mV
5 ms
B
10 μM Amphetamine
10 μM Phentolamine
10 μM Amphetamine
N2 Amplitude (mV)
1.00
0.75
0.50
0.25
0.00
1
2
3
4
0
10
20
30
40
50
60
70
80
90
100
110
120
C
10 μM Amphetamine
N2 Amplitude (%)
125
100
75
50
25
0
Control
10 μM Phentolamine
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (min)

Figure 34. Unilateral lesions of dopaminergic afferents to the NAc diminish the actions of amphetamine on excitatory synaptic transmission in the NAc.
A, In a slice taken from the unlesioned hemisphere, 5 μM amphetamine caused a large reduction in the synaptic response. ***B***, In a slice taken from the same rat, but from the hemisphere that had sustained a 6-OHDA lesion in the medial forebrain bundle, 5 μM amphetamine had little effect. ***C***, Summary of experiments in which amphetamine was applied to slices from the unlesioned hemispheres (5 μM, n=3; 10 μM, n=3) and 6-OHDA lesioned hemispheres (5 μM, n=4; 10 μM, n=4) of 3 rats.

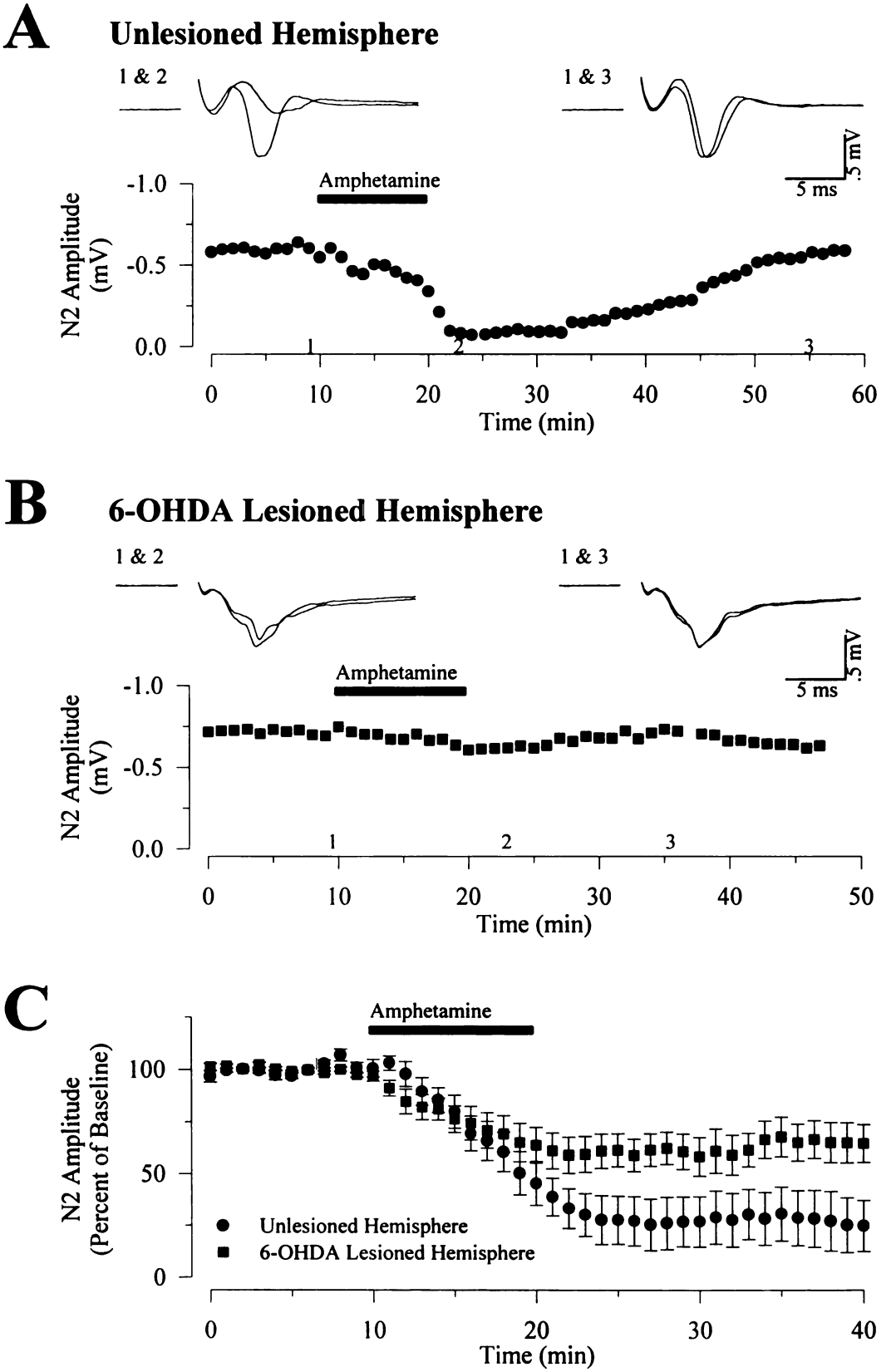

A
Unlesioned Hemisphere
1 & 2
1 & 3
.5 mV
5 ms
Amphetamine
N2 Amplitude (mV)
-1.0
-0.5
0.0
1
2
3
0
10
20
30
40
50
60
Time (min)
B
6-OHDA Lesioned Hemisphere
1 & 2
1 & 3
.5 mV
5 ms
Amphetamine
N2 Amplitude (mV)
-1.0
-0.5
0.0
1
2
3
0
10
20
30
40
50
Time (min)
C
Amphetamine
N2 Amplitude (Percent of Baseline)
100
50
0
Unlesioned Hemisphere
6-OHDA Lesioned Hemisphere
0
10
20
30
40
Time (min)

Figure 35. Dopamine reduces the EPSP to the same extent in NAc slices taken from mutant mice lacking D1a receptors and in slices taken from control mice.
A, In a cell from a wildtype control mouse, 75 μM dopamine reduced the initial slope of the EPSP. ***B***, Dopamine (75 μM) had similar effects in a cell from a D1a mutant mouse. ***C***, A summary of current clamp recordings in cells from wild type mice (n=5) and D1a knock-out mice (n=9), demonstrating that the reduction in EPSP initial slope was nearly identical in mutant and control mice.

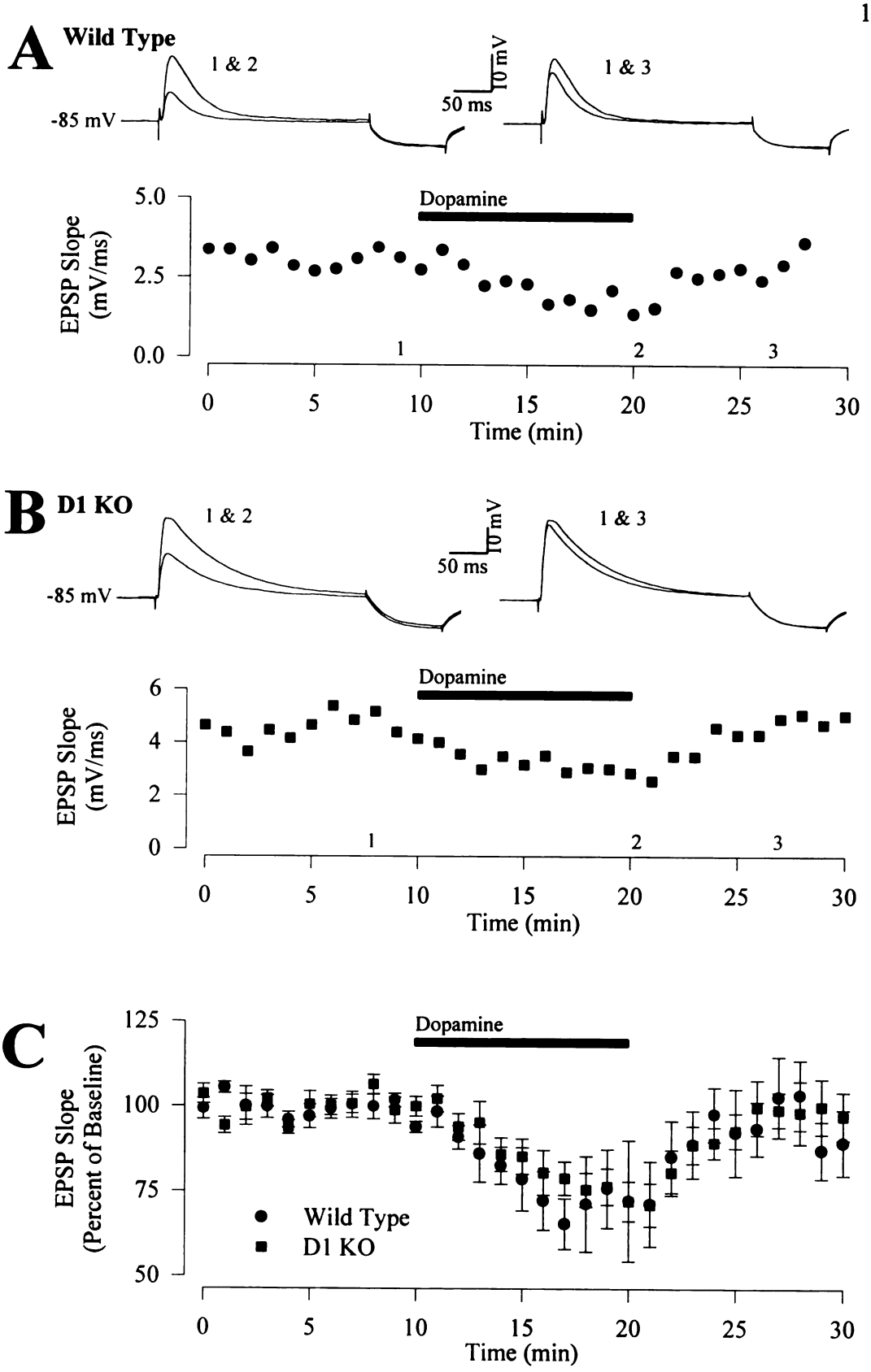

A
Wild Type
1 & 2
1 & 3
10 mV
50 ms
-85 mV
Dopamine
EPSP Slope (mV/ms)
5.0
2.5
0.0
1
2
3
0
5
10
15
20
25
30
Time (min)
B
D1 KO
1 & 2
1 & 3
10 mV
50 ms
-85 mV
Dopamine
EPSP Slope (mV/ms)
6
4
2
0
1
2
3
0
5
10
15
20
25
30
Time (min)
C
Dopamine
EPSP Slope (Percent of Baseline)
125
100
75
50
Wild Type
D1 KO
0
5
10
15
20
25
30
Time (min)

Figure 36. A D2 antagonist does not reduce the effects of dopamine on IPSPs in the NAc.

A, IPSP traces were taken at the times indicated in the example experiment shown in ***B***, in which dopamine (60 μM) was applied first in the absence and then in the presence of the D2 antagonist sulpiride (20 μM). ***C***, A summary (n=8) comparing the effects of dopamine on IPSPs in the presence and absence of sulpiride.

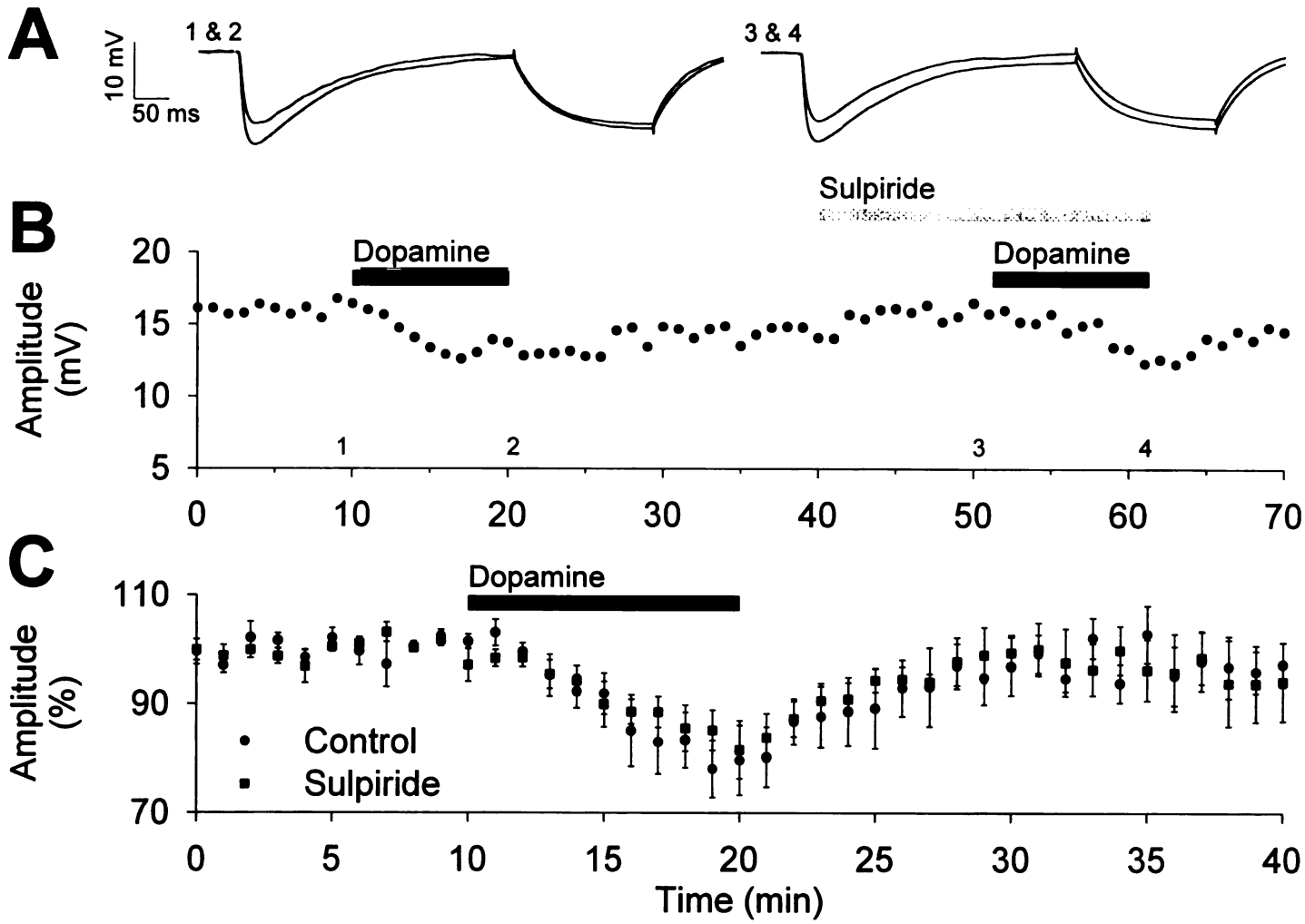

A
1 & 2
3 & 4
10 mV
50 ms
B
Sulpiride
Dopamine
Dopamine
Amplitude (mV)
20
15
10
5
1
2
3
4
0
10
20
30
40
50
60
70
C
Dopamine
Amplitude (%)
110
90
70
Control
Sulpiride
0
5
10
15
20
25
30
35
40
Time (min)

Figure 37. A D1 antagonist reduces the effects of dopamine on IPSPs in the NAc. ***A***, Traces taken from an example experiment shown in ***B***, in which dopamine (75 μM) was applied first in the absence then in the presence of the D1 antagonist SCH23390 (10 μM). ***C***, A summary (n=7) comparing the effects of dopamine in the presence and absence of SCH23390.

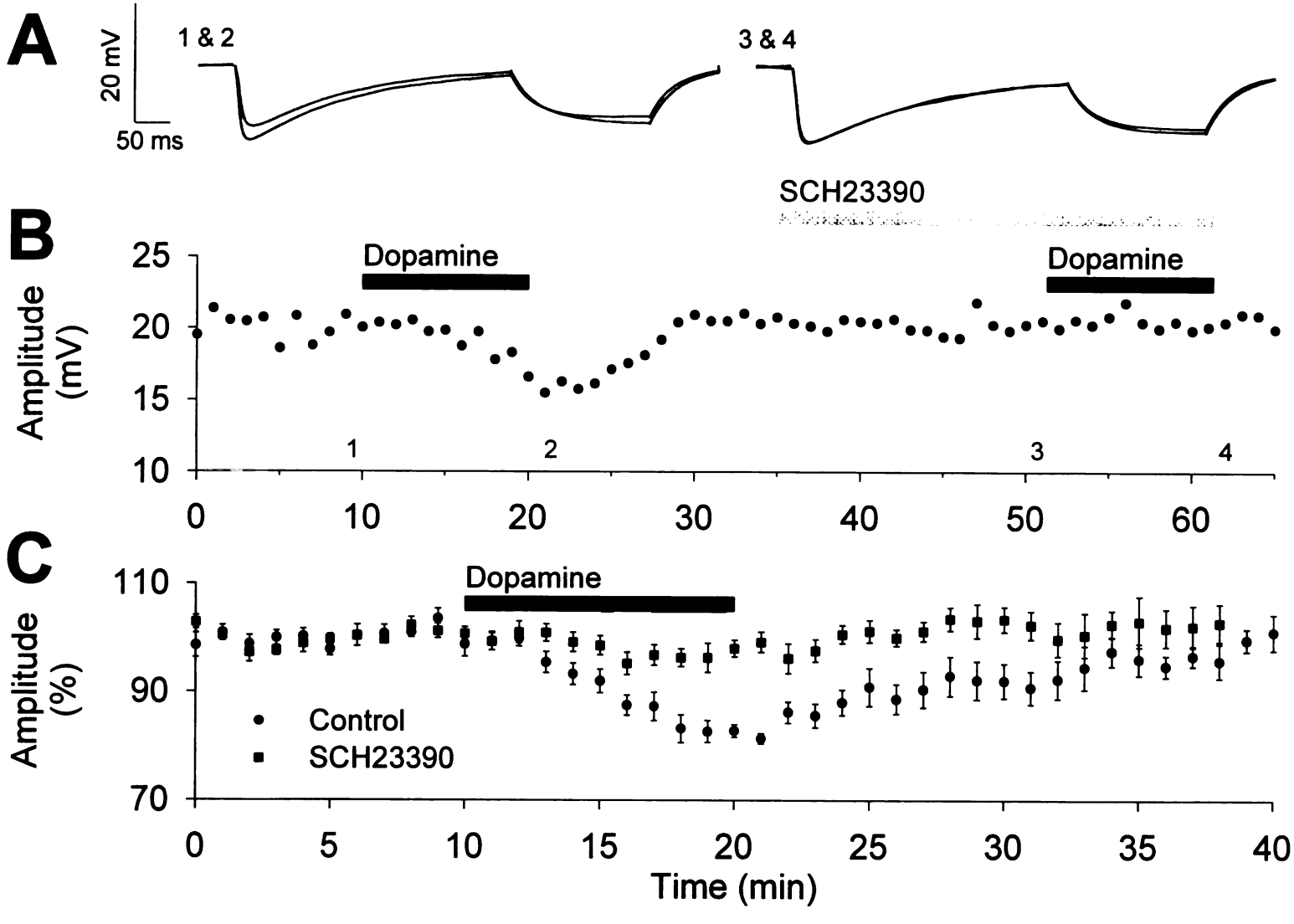

A
20 mV
50 ms
1 & 2
3 & 4
SCH23390
B
Amplitude (mV)
Dopamine
Dopamine
25
20
15
10
1
2
3
4
0
10
20
30
40
50
60
C
Amplitude (%)
Dopamine
110
90
70
Control
SCH23390
0
5
10
15
20
25
30
35
40
Time (min)

Figure 38. SCH23390 antagonizes the effects of amphetamine on IPSPS. ***A***, Traces from the experiment in B. ***B***, A representative experiment in which amphetamine (10 μM) was first applied in the absence and then in the presence of SCH23390 (10 μM). ***C***, A summary (n=6) comparing the effects of amphetamine in the presence and absence of SCH23390.

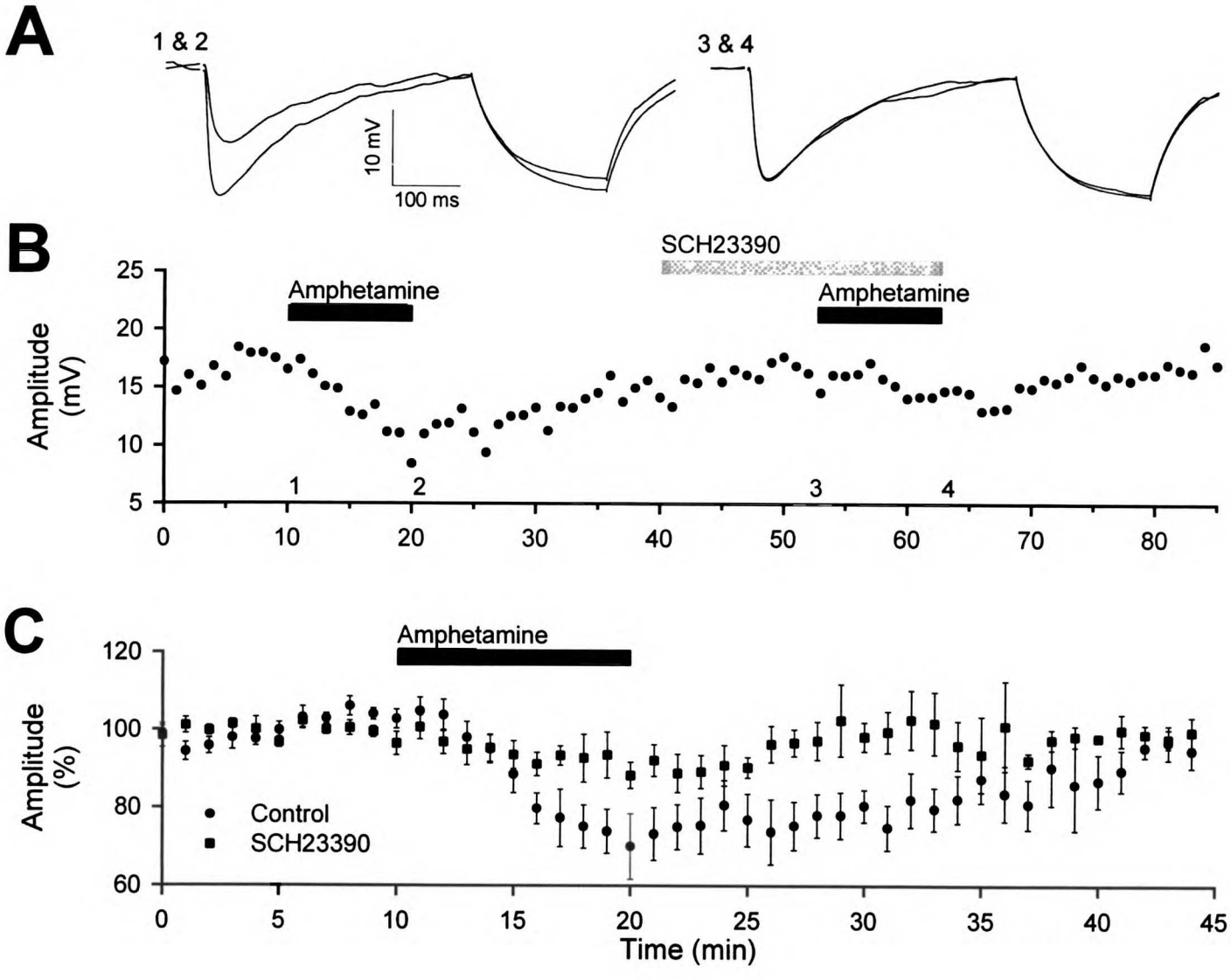

A
1 & 2
3 & 4
10 mV
100 ms
B
SCH23390
Amphetamine
Amphetamine
Amplitude (mV)
25
20
15
10
5
1
2
3
4
0
10
20
30
40
50
60
70
80
C
Amphetamine
Amplitude (%)
120
100
80
60
Control
SCH23390
0
5
10
15
20
25
30
35
40
45
Time (min)

Figure 39. The broad-spectrum dopamine agonist 6,7-ADTN depresses NAc IPSPs. ***A***, The traces are taken from the single experiment shown in B during baseline, during the application of 6,7-ADTN (50 μM) and during wash-out. ***B***, The time course of the effect of 6,7-ADTN on the amplitude of the NAc IPSP. ***C***, A summary of 4 experiments in which 6,7-ADTN was applied to IPSPs.

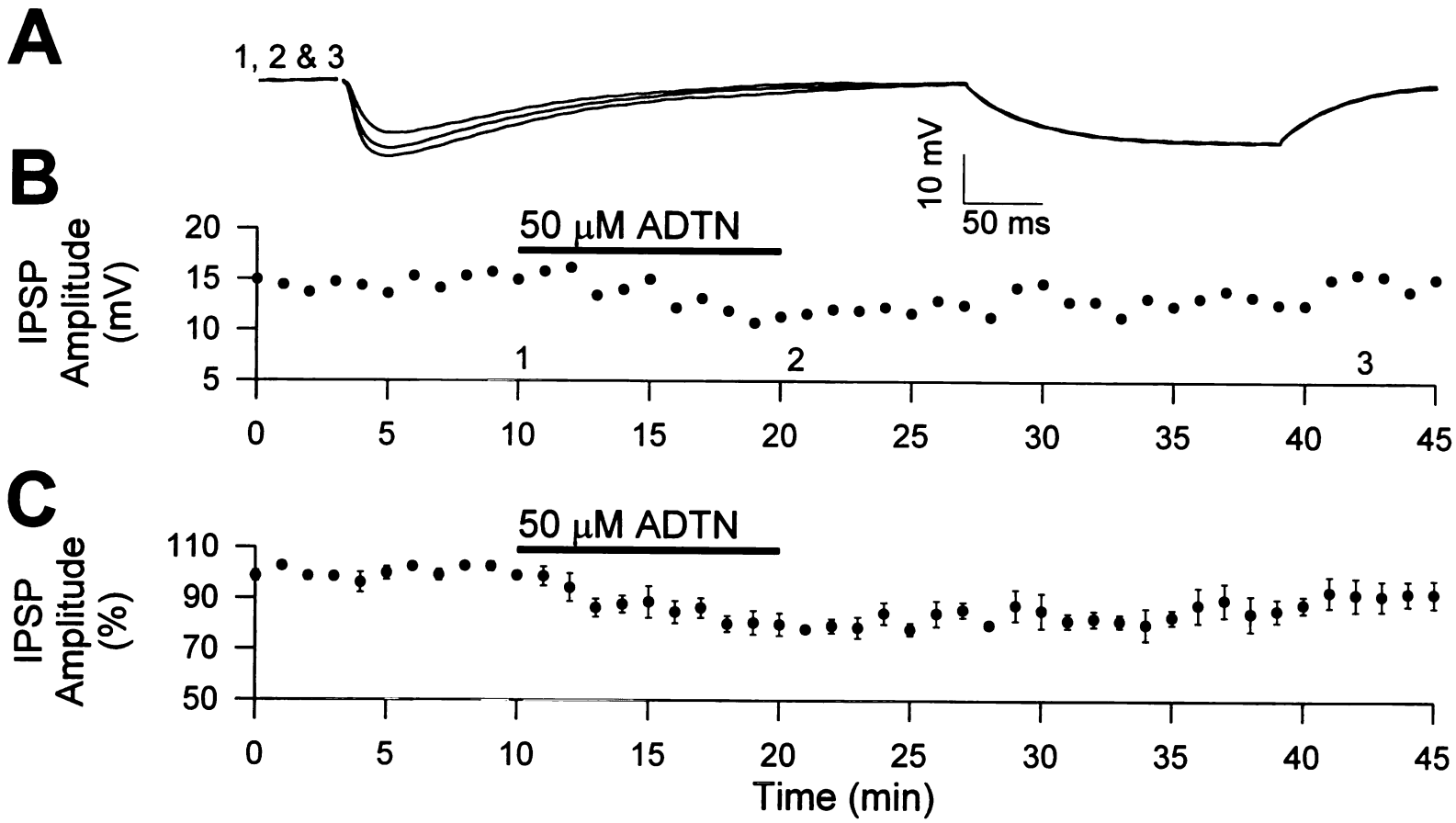

A
1, 2 & 3
10 mV
50 ms
B
IPSP Amplitude (mV)
50 μM ADTN
20
15
10
5
1
2
3
0
5
10
15
20
25
30
35
40
45
C
IPSP Amplitude (%)
50 μM ADTN
110
90
70
50
0
5
10
15
20
25
30
35
40
45
Time (min)

Figure 40. Quinpirole does not depress the IPSP in the NAc.

A, Traces taken from the experiment shown in ***B***, in which dopamine (75 μM) depressed the IPSPs, whereas in the same cell application of the D2 agonist quinpirole (10 μM) was without effect. ***C***, A summary of 14 experiments in which quinpirole was applied.

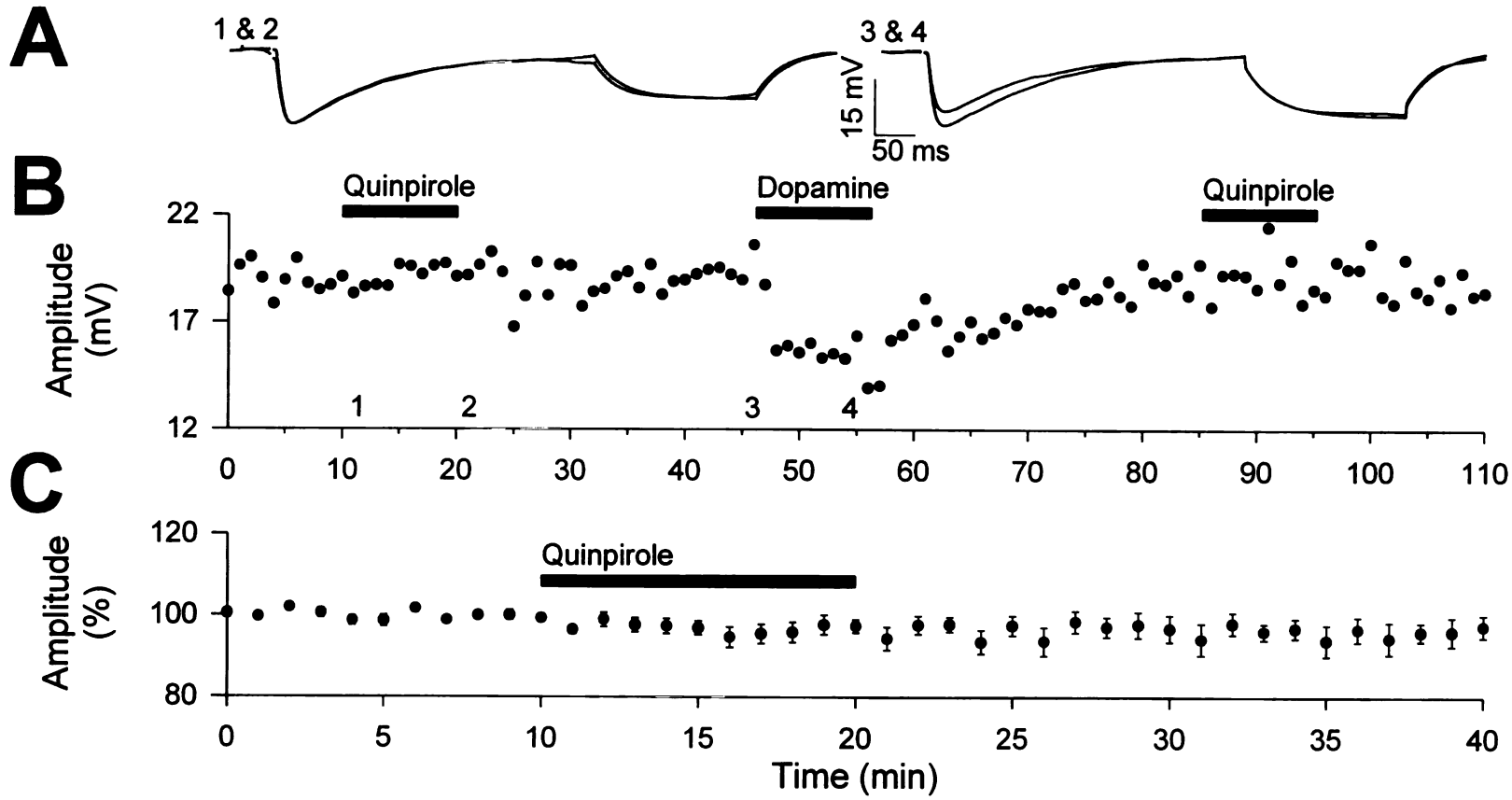

A
1 & 2
3 & 4
15 mV
50 ms
B
Quinpirole
Dopamine
Quinpirole
Amplitude (mV)
22
17
12
1
2
3
4
0
10
20
30
40
50
60
70
80
90
100
110
C
Amplitude (%)
120
100
80
Quinpirole
0
5
10
15
20
25
30
35
40
Time (min)

Figure 41. The D1 agonist (+)-SKF38393 does not depress the NAc IPSP, but does antagonize the effects of concomitantly applied dopamine.

A and ***B***, A typical experiment in which (+)-SKF38393 (20 μM) had no effect on the amplitude of the IPSP, but did reduce the ability of dopamine to depress the IPSP. ***C***, A summary (n=9) of experiments in which (+)-SKF38393 (20 μM) was applied to IPSPs. ***D***, A comparison of the effectiveness of dopamine (75 μM) applied first in the presence and then in the absence of (+)-SKF38393 (20 μM).

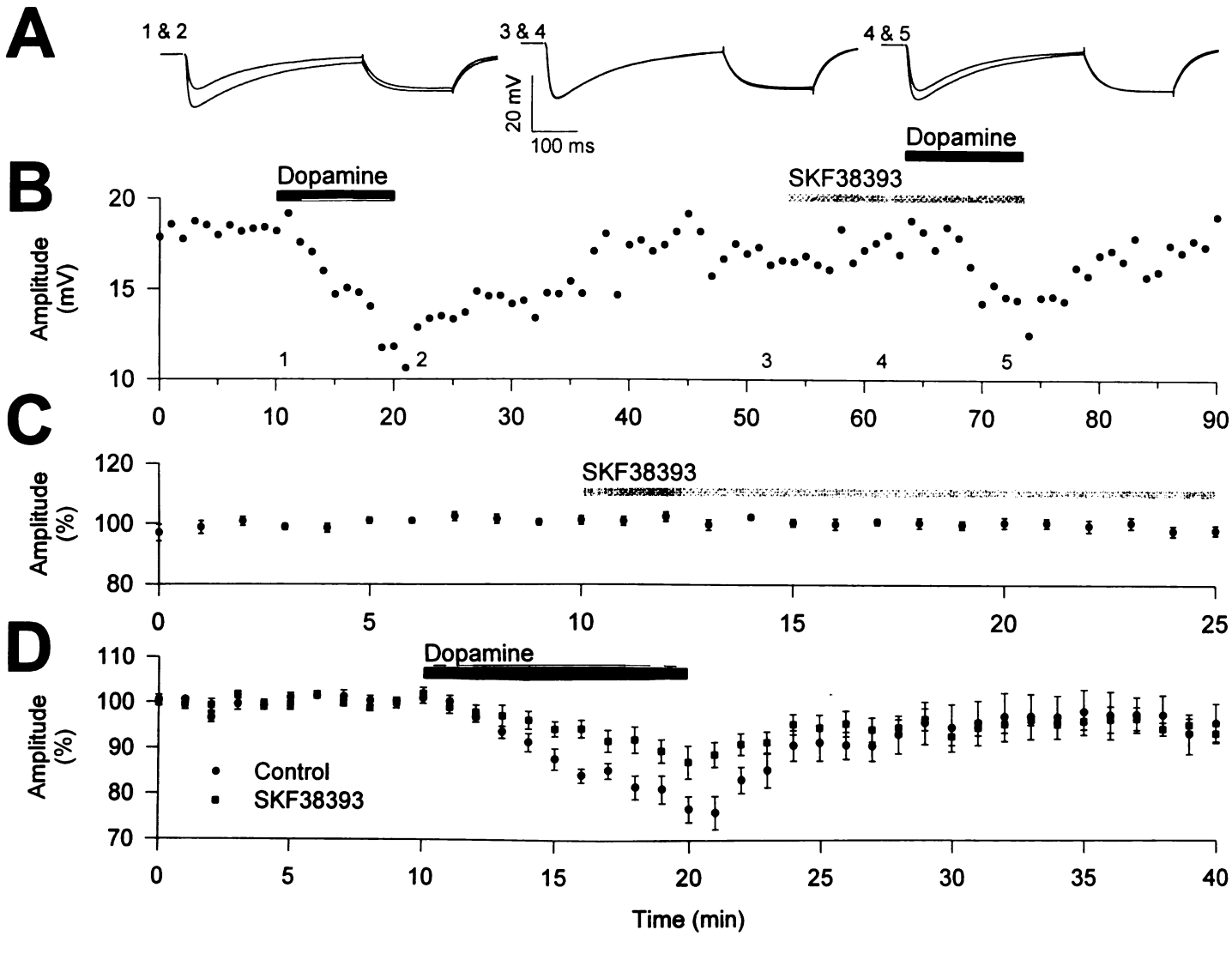

A
1 & 2
3 & 4
4 & 5
20 mV
100 ms
B
Dopamine
SKF38393
Dopamine
Amplitude (mV)
20
15
10
1
2
3
4
5
0
10
20
30
40
50
60
70
80
90
C
SKF38393
Amplitude (%)
120
100
80
0
5
10
15
20
25
D
Dopamine
Amplitude (%)
110
100
90
80
70
Control
SKF38393
0
5
10
15
20
25
30
35
40
Time (min)

Figure 42. The full D1 agonist dihydrexidine has no effect on NAc IPSPs. ***A***, Dihydrexidine at 10 μM was incapable of depressing the IPSP (n=3). ***B***, At 40 μM dihydrexidine still had no effect on the IPSP (n=5). ***C***, Dopamine (75 μM) depressed the IPSP in the same cells (n=3).

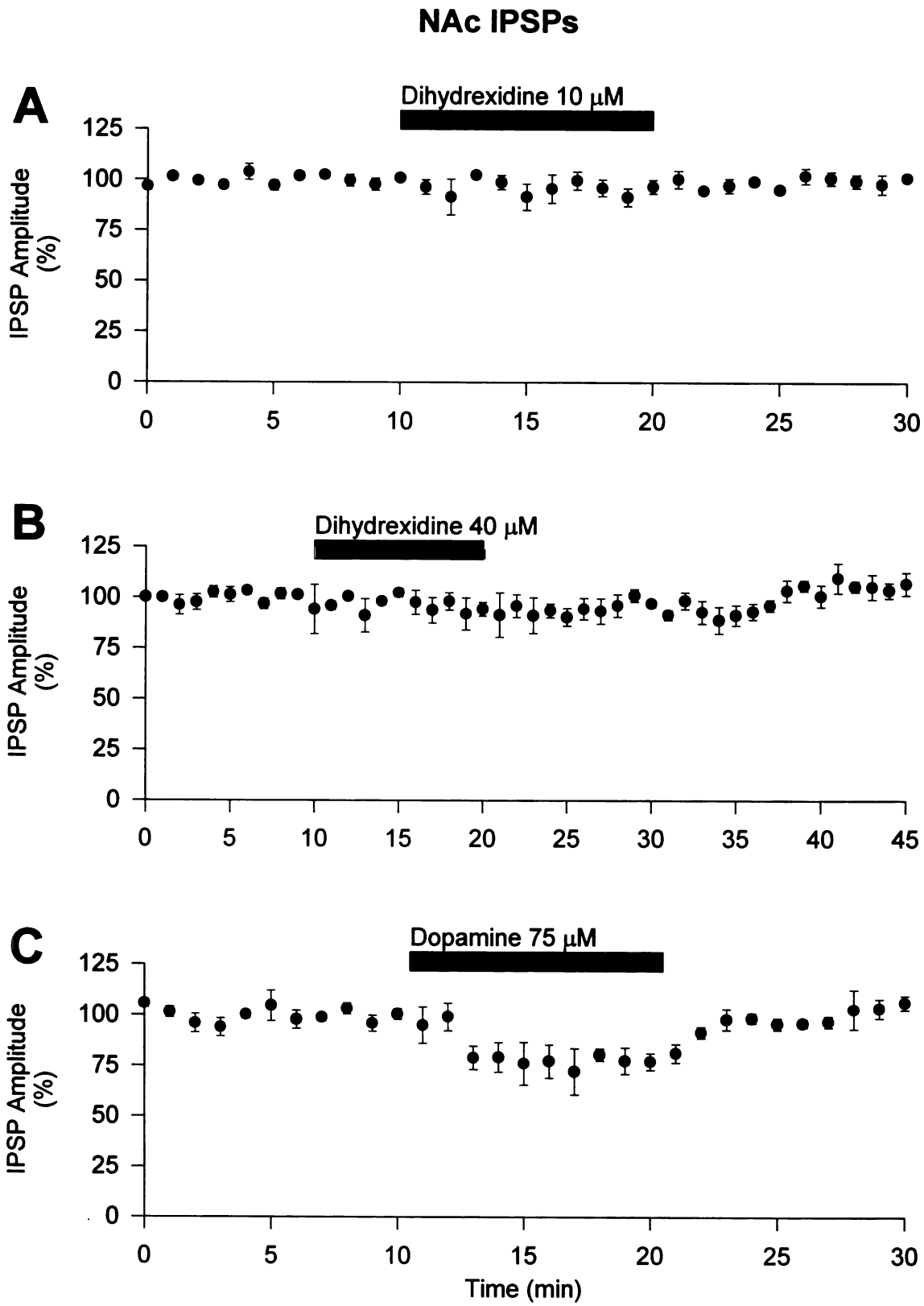

NAc IPSPs
A
Dihydrexidine 10 μM
IPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
B
Dihydrexidine 40 μM
IPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
35
40
45
C
Dopamine 75 μM
IPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
Time (min)

Figure 43. Depression of IPSPs in the NAc by serotonin is insensitive to SCH23390. ***A*** and ***B***, an experiment in which serotonin (10 μM) was applied to NAc IPSPs first in the absence and then in the presence of SCH23390 (10 μM). ***C***, A summary of 9 experiments in which 5 – 10 μM serotonin was applied. ***D***, On average, the D1 antagonist SCH23390 (10 μM) did not reduce the ability of serotonin (5 – 10 μM) to depress the IPSP in cells in which serotonin was applied both in the absence and the presence of SCH23390 (n=3).

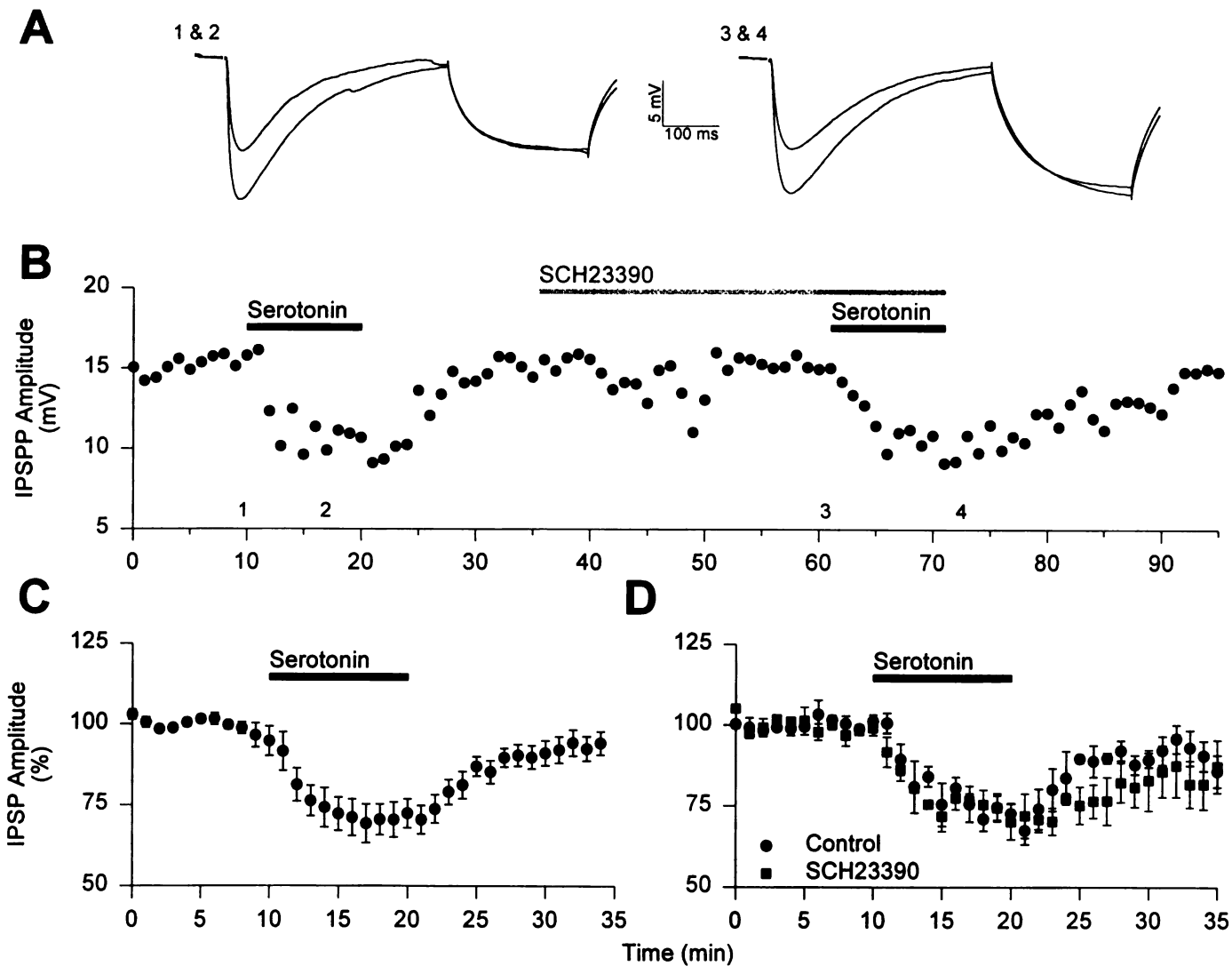
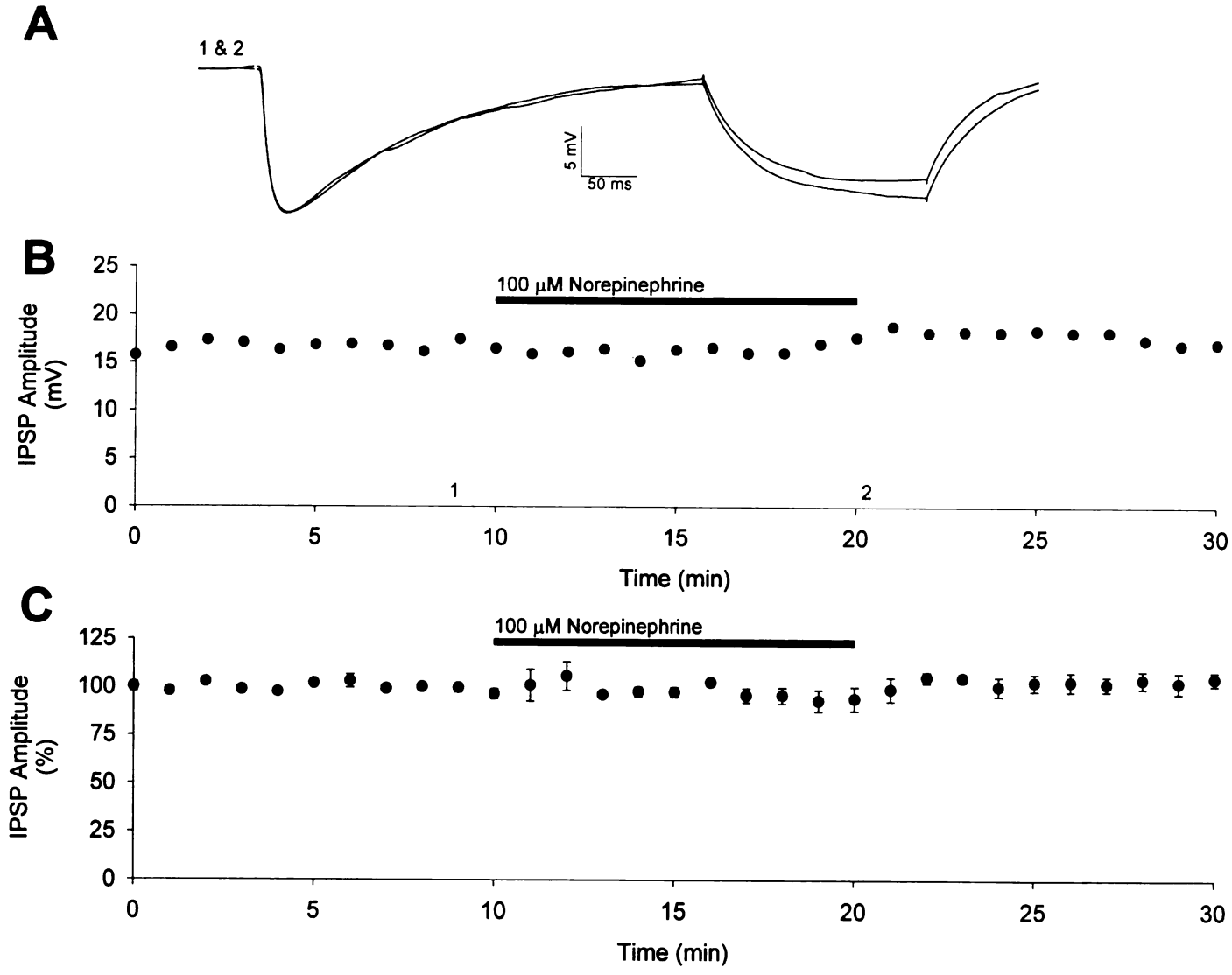

A
1 & 2
3 & 4
5 mV
100 ms
B
SCH23390
Serotonin
Serotonin
IPSPP Amplitude (mV)
1
2
3
4
C
Serotonin
IPSP Amplitude (%)
D
Serotonin
Control
SCH23390
Time (min)

Figure 44. Norepinephrine has no effect on NAc IPSPs.

A and ***B***, an experiment in which norepinephrine (100 μM) was applied to NAc IPSPs.

C, A summary of 4 similar experiments.


A
1 & 2
5 mV
50 ms
B
100 μM Norepinephrine
IPSP Amplitude (mV)
25
20
15
10
5
0
1
2
0
5
10
15
20
25
30
Time (min)
C
100 μM Norepinephrine
IPSP Amplitude (%)
125
100
75
50
25
0
0
5
10
15
20
25
30
Time (min)

Chapter 5

Mechanisms of Modulation of Synaptic Transmission by Dopamine in the Nucleus Accumbens

Introduction

The modulation of fast synaptic transmission that results from activation of metabotropic receptors can occur by several different hypothetical mechanisms. While all of them are likely to involve the binding of the agonist to the receptor and the subsequent activation of a second messenger system, these mechanisms can be divided into those that act presynaptically and those that act postsynaptically. Postsynaptic mechanisms, in which the sensitivity of a cell to the neurotransmitter is altered, include the direct modulation of the ionotropic receptors that mediate the synaptic potential and current, as well as the modulation of conductances (often voltage-dependent) that affect the resistance or rectification properties of the cell membrane. Presynaptic mechanisms include changes in the number of vesicles released per action potential in the axon terminal (that is, the probability of release or the number of vesicles available to be released in the terminal), or in the amount of neurotransmitter per vesicle.

A number of electrophysiological techniques have been devised to determine which of these mechanisms are at work when modulation of synaptic transmission is observed. The first task is usually to determine whether a modulatory effect is pre- or postsynaptic. One means by which this is done is to examine the effects of the modulator on the paired-pulse ratio (Manabe et al., 1993; Zucker, 1989). In this approach the synapse is stimulated twice in quick succession (usually 10 to 100 ms interval), and the ratio of the second response to the first is computed. The second response is often consistently larger than the first (paired-pulse facilitation, or PPF), and this facilitation is attributed to the presence of residual Ca^{2+} in the presynaptic terminal (Katz and Miledi, 1968; Zucker, 1989). In some cases paired-pulse depression (PPD) is observed, an effect that can be attributed to depletion of the releasable pool of synaptic vesicles in the terminal (del Castillo and Katz, 1954b; Zucker, 1989) although at some synapses feedback inhibition of the released neurotransmitter acting at receptors on the presynaptic terminal is responsible for PPD (e.g., Davies et al., 1990). Because both PPF and PPD occur in the presynaptic terminal and affect the probability of release or the number of vesicles available for release for the second pulse,

neuromodulators that presynaptically affect synaptic transmission by altering the number of vesicles released per stimulus can be expected to alter the measured paired-pulse ratio. In general, manipulations that can be shown by other means to have presynaptic effects can usually be shown to alter the paired-pulse ratio (Creager et al., 1980; Katz and Miledi, 1968; Mallart and Martin, 1968; Manabe et al., 1993).

An additional means by which to determine whether a pre- or postsynaptic mechanism is involved is to examine the distribution of spontaneous miniature synaptic events. Most synapses spontaneously release vesicles, resulting in miniature potentials or currents observable while recording from the postsynaptic cell (Fatt and Katz, 1952; Katz, 1966). One can measure the frequency and amplitude of the minis to determine which of these parameters a neuromodulator influences. Changes in the frequency of minis are likely to reflect changes in the probability of release or in the number of vesicles available for release: the higher the probability of release or the number available, the higher the frequency of spontaneous release events (del Castillo and Katz, 1954a). On the other hand, changes in the mini amplitude distribution are likely to result from changes in the postsynaptic sensitivity to released vesicles (Fatt and Katz, 1952). There are, however, exceptions to this rule. First, changes in the amount of neurotransmitter per vesicle will result in an altered distribution of mini amplitudes (and no change in the paired-pulse ratio), although the change is a presynaptic change. Second, the small size of minis and the use of voltage-clamp recording to collect them means that a change in the mini amplitude distribution will not necessarily be observed if evoked synaptic potentials are altered by the neuromodulator as a result of a change in a voltage-dependent conductance. Thus, a lack of change in the amplitude distribution cannot be taken as evidence that there is no postsynaptic change at all; further analysis of the voltage dependence of the membrane conductance is required to address this issue. And third, minis are recorded under conditions in which voltage-dependent currents in the presynaptic terminal are minimized (and, ideally, abolished completely). This means that changes in the probability of release that arise from an alteration in the amount of Ca^{2+} entry into the presynaptic terminal per action potential

are likely not to be reflected in the mini frequency.

Several experiments can be performed to determine whether a change in presynaptic Ca^{2+} entry contributes to the observed modulation of synaptic transmission. An elegant experiment by Dittman and Regehr (1996), for instance, used Ca^{2+} imaging to measure changes in Ca^{2+} concentration during the action potential in presynaptic terminals at the parallel fiber to Purkinje cell synapse. Using manipulations (such as changing the extracellular Ca^{2+} concentration) that are known to alter Ca^{2+} influx, a curve relating the magnitude of the postsynaptic response and the measured presynaptic Ca^{2+} signal was generated. The effects of several different concentrations of the presynaptic modulators chloroadenosine (an adenosine A1 agonist) and baclofen (a $GABA_B$ agonist) on the presynaptic Ca^{2+} signal and the postsynaptic EPSC were then obtained. By determining whether the plot of the EPSC reduction *vs* the Ca^{2+} signal reduction fell on the previously determined curve, the authors could discern whether the effects of these drugs were due entirely to a reduction in presynaptic Ca^{2+} influx or whether some other process must be required to explain some or all of the synaptic depression. Their results indicated that chloroadenosine-induced synaptic depression can be entirely accounted for by a reduction of Ca^{2+} influx into the presynaptic terminal, while the negative modulation induced by baclofen could be only partly accounted for by a reduction in Ca^{2+} influx. Consistent with these findings, the frequency of miniature EPSCs (mEPSCs) recorded in the Na^+ channel blocker tetrodotoxin (TTX) was unaltered by chloroadenosine but reduced by baclofen; the amplitude distribution was unchanged by either drug (Dittman and Regehr, 1996).

Electrophysiological methods without Ca^{2+} imaging can also be used to address this question. An extensive literature has analyzed the presynaptic mechanisms by which baclofen reduces excitatory and inhibitory synaptic transmission in hippocampal pyramidal cells; this literature will be discussed here in detail. Recordings of CA3 and CA1 cells in acute slices or slice culture found that baclofen depressed the frequency of mEPSCs (Scanziani et al., 1992) but not mIPSCs (Cohen et al., 1992; Doze et al., 1995; Scanziani et al., 1992); the amplitude distributions of neither mEPSCs nor

mIPSCs were changed. Both mEPSCs and mIPSCs were recorded in TTX and therefore in the absence of voltage changes in the presynaptic terminals that would activate Ca^{2+} channels; this was confirmed by application of the Ca^{2+} channel blocker Cd^{2+}, which produced no change in mEPSC or mIPSC frequency (Doze et al., 1995; Scanziani et al., 1992). The hypothesis was therefore proposed that baclofen modulates excitatory transmission by interacting with a Ca^{2+}-independent step of the release mechanism (i.e., occurring after the increase in intracellular Ca^{2+} has been detected by the release machinery), while the same drug modulates inhibitory transmission by reducing Ca^{2+} entry into the presynaptic terminal during the presynaptic action potential (Scanziani et al., 1992; Thompson et al., 1993). Modulation of Ca^{2+} entry could have occurred in both types of terminal, but the absence of an effect on mIPSC frequency suggested that inhibitory transmission is regulated *only* by a change in Ca^{2+} influx, whereas excitatory transmission is regulated by a post-Ca^{2+} mechanism in addition to *possible* regulation of Ca^{2+} influx. In fact, a later Ca^{2+} imaging study in area CA1 found that baclofen reduced the Ca^{2+} transient resulting from stimulation of *stratum radiatum*, although no effort was made to distinguish between Ca^{2+} signals from excitatory and inhibitory terminals (Wu and Saggau, 1995).

Two experiments provided further evidence that the modulation of inhibitory synaptic transmission by baclofen occurs at a Ca^{2+}-dependent step in the release process. First, the K^+ channel blocker Ba^{2+} reduced the ability of baclofen to reduce stimulation-evoked IPSPs, but not EPSPs (Thompson and Gähwiler,1992). Blockade of presynaptic K^+ channels should prolong the time course of the action potential in the axon terminal, thereby resulting in increased Ca^{2+} entry. Alternately, Ba^{2+} may have replaced some of the Ca^{2+} entering the terminal, which would result in a decrease in the probability of release (Augustine and Eckert, 1984). Both of these consequences of Ba^{2+} application would alter Ca^{2+} concentrations in presynaptic terminals, albeit in different directions, and thereby alter the probability of release. The most likely explanation for the finding that the effects of baclofen were reduced in Ba^{2+} is that the synaptic release mechanism becomes oversaturated with Ca^{2+}, and therefore reduction

of Ca^{2+} influx by baclofen has little effect. The replacement of Ca^{2+} ions with Ba^{2+} ions would work against such saturation, and indeed it is possible that Ca^{2+} influx is *reduced* in Ba^{2+} instead of increased. If this were the case, one explanation for the observed decreased effectiveness of baclofen might be that Ca^{2+} influx that supports transmitter release occurs through a subpopulation of Ca^{2+} channels that are less permeant to Ba^{2+}, and these channels might be less susceptible to modification by baclofen. While both of these hypotheses remain speculative, alteration of Ca^{2+} influx is the simplest means by which Ba^{2+} could reduce the effectiveness of baclofen. Thus, a reduction of the effectiveness of a presynaptic neuromodulator by Ba^{2+} may be used as one indicator that the neuromodulator acts, at least in part, by reduction of Ca^{2+} influx into the presynaptic terminal.

Ba^{2+} was capable of attenuating baclofen's effects only on inhibitory terminals, not excitatory ones (Thompson and Gähwiler,1992). This suggests that modulation of excitatory transmission by baclofen occurs independently of Ca^{2+}, whereas modulation of inhibitory transmission occurs by an interaction with Ca^{2+} dynamics in the presynaptic terminal. It should be noted that Lambert et al. (1991) found that modulation of IPSPs by baclofen was *resistant* to Ba^{2+}; however, a recent study (Jarolimek and Misgeld, 1997) has found differences in the ability of baclofen to reduce mIPSC frequency in the hippocampus of different species of rodents and strains of rats. These differences may well explain this discrepancy.

The second experiment to test the Ca^{2+} dependence of baclofen's effects on inhibitory transmission was to examine the effect of baclofen on the frequency of mIPSCs that are dependent on the influx of Ca^{2+} into the presynaptic terminal (Doze et al., 1995). The external K^+ concentration was elevated from 5 mM to 20 mM, which resulted in a greatly increased mIPSC frequency (see also Scanziani et al., 1995). Furthermore, the frequency of mIPSCs was reduced to baseline values (e.g., those in low K^+) by application of the Ca^{2+} channel blocker Cd^{2+}, indicating that the new (high K^+) mIPSCs resulted from Ca^{2+} entry into the presynaptic terminal. Application of baclofen caused a reduction in the frequency of these Ca^{2+}-dependent mIPSCs, in

contrast to baclofen's lack of effect when applied in normal K^+ (Doze et al., 1995). The results of these experiments suggest that modulation of inhibitory synaptic transmission by baclofen in hippocampal cells occurs through the modulation of voltage-dependent currents (such as K^+ or Ca^{2+} currents) in GABAergic terminals; however, this conclusion does not appear to be valid for all species of rodents (Jarolimek and Misgeld, 1997) or for GABAergic terminals in other brain nuclei (Jarolimek and Misgeld, 1992; Ulrich and Huguenard, 1996). Furthermore, inhibition of inhibitory synaptic transmission in hippocampal pyramidal cells by activation of receptors other than $GABA_B$ receptors (e.g., adenosine and opioid) does not necessarily occur by a Ca^{2+}-dependent mechanism (Cohen et al., 1992; Lupica, 1995; Rekling, 1993; Thompson et al., 1993; Scanziani et al., 1992, 1995). Therefore, at least two mechanisms (Ca^{2+}-dependent and Ca^{2+}-independent) exist for the presynaptic modulation of transmitter release, and both can be found in the same brain nucleus and probably even in the same presynaptic terminals.

Few studies have addressed the question of whether dopamine's effects in the NAc are the result of a pre- or a postsynaptic mechanism. *In vivo* iontophoresis studies found that NAc cell firing evoked by glutamate could be reduced by application of dopamine and dopamine agonists (Johansen et al., 1991; White, 1987; White and Wang, 1986). The mechanism of this depression is likely to be postsynaptic, since exogenous glutamate was applied; an alternate possibility, however, is that if the glutamate activated neighboring NAc cells that inhibited the recorded cell, an increase in inhibition by dopamine could account for the observed reduction in action potential firing evoked by glutamate. Although details of the mechanism by which dopamine reduces the effects of glutamate have not been worked out in NAc cells *in vitro*, later findings in the striatum of dopamine-induced reductions of inward rectification at potentials between –50 and –80 mV (Calabresi et al., 1987, 1988), as well as of the response to iontophoretically applied glutamate or AMPA (Cepeda et al., 1993; but see Calabresi et al., 1995) are consistent with a postsynaptic mechanism. However, Pennartz et al. (1992a) found that a dopamine-induced reduction of the EPSP in NAc

cells was accompanied by increased PPF, which indicates a presynaptic mechanism in addition to or instead of a postsynaptic mechanism. No studies have examined the site of action in the case of the dopaminergic modulation of NAc inhibitory synaptic transmission. Thus, the mechanistic details of the modulation of synaptic transmission in the NAc have not yet been fully explored.

Another issue is the identity of the second messenger system responsible for dopamine-induced synaptic depression. D1 receptors were originally defined based on their ability to increase cAMP levels by activating adenylate cyclase (Kebabian and Calne, 1979), and both of the D1 receptors cloned from mammals activate this enzyme (Jensen et al., 1996). However, elevation of cAMP levels usually results in an *increase* in the size of synaptic responses, most often by a presynaptic mechanism (Bonci and Williams, 1997; Capogna et al., 1995; Chavez-Noriega and Stevens, 1994; Kandel and Schwartz, 1982; Llano and Gerschenfeld, 1993; Salin et al., 1996; Weisskopf et al., 1994; Zhong and Wu, 1991) although direct modulation of postsynaptic receptors (most likely through protein kinase A activation and phosphorylation of the receptor proteins) may be involved in some instances as well (Greengard et al., 1991; Knapp and Dowling, 1987; Knapp et al., 1990; Wang et al., 1991). Thus, cAMP elevation generally potentiates synaptic transmission, in contrast to the effects of D1 receptor activation in the NAc (Pennartz et al., 1992a; this work). It is therefore of interest to compare the effects of cAMP elevation with those of D1 receptor activation.

Results

Depression of excitatory synaptic transmission is presynaptic. To begin the analysis of the site of action of dopamine, the effect of dopamine on paired pulse facilitation (PPF) was examined. PPF is a presynaptic phenomenon, the magnitude of which inversely correlates with the probability of neurotransmitter release (Creager et al., 1980; Mallart and Martin, 1968; Manabe et al., 1993; Zucker, 1989). A pair of synaptic responses was elicited with an interstimulus interval of 50 to 100 ms and the magnitude of

increase of the second response relative to the first one was monitored continuously during the experiment. To obviate any effect of non-linear summation on the magnitude of PPF (Martin, 1955), experiments were performed in voltage clamp. Figure 45 demonstrates that an increase in the PPF ratio occurs simultaneously with the reduction in EPSC amplitude induced by dopamine (75 μM). Dopamine increased the PPF ratio to an average of 129±7% of the baseline PPF ratio (n=8, p≤.024, Wilcoxon Rank Sum Test, Figure 45C top), an increase that accompanied a decrease in the amplitude of the first pulse to 71±10% of the baseline amplitude (n=8, p≤.054, Wilcoxon Rank Sum Test, Figure 45C bottom). The results of these experiments suggest that psychostimulants and dopamine act at cortico-accumbens synapses to reduce transmitter release, thereby decreasing the efficacy of excitatory transmission between the cortex and the NAc.

To further confirm this result, spontaneous miniature excitatory postsynaptic currents (mEPSCs) in the presence of TTX (1.5 μM) were recorded in whole-cell voltage clamp (V_h = –80 mV). A decrease in the frequency of spontaneous events recorded in these conditions is classically attributed to a reduction in the probability of neurotransmitter release or in the number of quanta available for release, while a decrease in the amplitude of these events normally indicates a reduction in the postsynaptic sensitivity to the released quanta (Katz, 1966). All recordings were initially made in the absence of TTX; TTX was then applied and complete abolition of evoked EPSCs was observed prior to beginning the experiments summarized in Figure 46 through Figure 49. The frequency of mEPSCs in NAc cells was quite high, often 10 – 15 Hz, and the range of amplitudes was large (4 – 70 pA). To confirm that the recorded events were true mEPSCs arising from glutamate receptor activation, CNQX was applied (10 μM) at the end of 6 experiments and in each of these cases, mEPSCs were blocked (Figure 48A, far right).

A typical experiment in which the effect of amphetamine (10 μM) on mEPSC frequency and amplitude was examined is shown in Figure 46. Representative consecutive current traces taken before (1), during (2), and after (3) amphetamine application (Figure 46A) demonstrate that mEPSC frequency, but not amplitude, was

reversibly reduced by the presence of amphetamine. This result is summarized for this cell in Figure 46B and C, which shows that mEPSC amplitude was unchanged throughout the entire course of the experiment (Figure 46C) while amphetamine reduced mEPSC frequency with a time course of recovery similar to that observed for evoked responses (Figure 46B). The distribution of mEPSC amplitudes was unaffected by amphetamine (Figure 46E,G) while the distribution of the time intervals between successive mEPSCs was shifted towards longer intervals following application of amphetamine (Figure 46D,F). These experiments were repeated in 6 cells, in which mEPSC frequency was reduced to 57±2% of the baseline value ($p<.05$, Figure 47A) while mEPSC amplitude remained at 95±3% of baseline ($p>.1$, Figure 47B).

Similar results were obtained when the effects of dopamine on mEPSCs were examined (Figure 48 and Figure 49). Representative consecutive current traces from a typical cell (Figure 48A) demonstrate that mEPSC frequency, but not amplitude, was reversibly depressed by the application of dopamine (100 μM, Figure 48B,C). As was the case for amphetamine, dopamine did not cause a shift in the distribution of mEPSC amplitudes (Figure 48E,G), but did cause a shift towards longer intervals between consecutive mEPSCs (Figure 48D,F). Similar results were observed in 15 cells, in which dopamine application resulted in an average reduction in mEPSC frequency to 61±4% of baseline values ($p<.001$, Figure 49A) whereas mEPSC amplitude in dopamine was 100±3% of baseline ($p>.05$, Figure 49B). Thus, both dopamine and amphetamine reduce mEPSC frequency without affecting mEPSC amplitude, suggesting that they both depress excitatory synaptic transmission via a presynaptic mechanism.

Presynaptic modulation of synaptic transmission may occur by one of two general mechanisms: by the reduction of Ca^{2+} entry into the presynaptic terminal, or by the reduction of some part of the release process that is independent of Ca^{2+} entry (Thompson et al., 1993). To determine by which of these mechanisms dopamine acts to reduce transmitter release in excitatory terminals in the NAc, mEPSCs were recorded in the presence of the Ca^{2+} channel blockers Cd^{2+} (100 μM, n=4) or Co^{2+} (5 mM, n=4).

In these experiments, TTX was not used and complete abolition of the evoked EPSC by the Ca^{2+} channel blocker was observed prior to beginning the experiment. When amphetamine (10 μM) was applied, mEPSC frequency was reduced by 41±7% ($p<.005$) while mEPSC amplitude remained unchanged (2±3% depression, $p>.2$, n=8; Figure 50). Thus, amphetamine, and most likely dopamine as well, can presynaptically decrease excitatory synaptic transmission in the NAc by a mechanism that does not involve decreasing Ca^{2+} entry into the presynaptic terminal through voltage dependent Ca^{2+} channels.

Depression of inhibitory synaptic transmission is presynaptic. To examine the effects of dopamine on the paired-pulse ratio for inhibitory synapses, cells were voltage-clamped at 0 mV and paired pulses (50 ms interstimulus interval) were delivered alternately with single pulses. Because of the long decay time course of the IPSC, the second pulse was superimposed on the decay phase of the first (Figure 51), unlike for EPSCs (Figure 45). Therefore, single pulses were delivered alternately with paired pulses, and, for illustration purposes (Figure 51A), averages of single pulse sweeps were subtracted from averages of paired pulse sweeps to demonstrate the true amplitude of the second pulse. For computation of the amplitude, a moving average of the amplitude of the single pulse IPSCs taken at the point of maximal amplitude of the second IPSC was computed and subtracted from the amplitude of the second IPSC to determine its true amplitude (Figure 51B). Dopamine (100 – 150 μM) significantly increased the paired-pulse ratio to 180±22% ($p<.05$) of the baseline ratio (.74±.11, n=6, Figure 51B upper) while decreasing the amplitude of the first pulse by 66±7% ($p<.001$, Figure 51B lower). This result is consistent with a dopamine-induced decrease in the probability of GABA release although it does not rule out contributions of additional postsynaptic mechanisms.

Different mechanisms of modulation of excitatory and inhibitory synaptic transmission. To determine whether dopamine may also depress $GABA_A$ receptor function or number, miniature IPSCs (mIPSCs) in the presence of tetrodotoxin (1.5 μM) were recorded. The electrode solution for these experiments contained either

cesium gluconate (n=6, holding potential of 0 mV) or CsCl (n=4, holding potential of –80 mV). Picrotoxin (100 – 200 μM) reduced mIPSC frequency to zero under both recording conditions (not shown), indicating that the mIPSCs were not contaminated by excitatory or nonsynaptic events. As shown in Figure 52, dopamine (75 – 100 μM) did not change the mIPSC amplitude distribution (Figure 52B) or mean mIPSC amplitude (Figure 52C) (6±3% depression, $p>.05$). These findings indicate that the effects of dopamine on synaptic transmission are unlikely to involve a postsynaptic reduction in the sensitivity of the cell to synaptically released GABA. However, in contrast to the robust depression in mEPSC frequency (Figure 48 and Figure 49), mIPSC frequency was not reduced by dopamine (113±8%, $p>.05$; Figure 52A,C).

Given the dichotomy in the effects of dopamine on mEPSC and mIPSC frequency, it was important to ensure that slices in which no change in mIPSC frequency was observed were competent to express dopamine-induced changes in mEPSC frequency. To accomplish this, mEPSCs and mIPSCs were simultaneously recorded in the same cell (Figure 53) by maintaining the holding potential at –15 to –25 mV using a cesium gluconate electrode solution and bathing the slice in TTX (1.5 μM) and D-APV (50 μM) (but no AMPA or GABA receptor antagonists). Under these conditions, both inward and outward miniature synaptic currents could be resolved (Figure 53A). Application of DNQX (10 – 20 μM) selectively abolished the inward currents, and picrotoxin (200 μM) abolished the outward currents, indicating that inward currents were mEPSCs and outward currents were mIPSCs (not shown). Application of dopamine (100 μM, n=3) caused a clear decrease in the frequency of mEPSCs (49±4% reduction, $p<.01$) while the frequency of mIPSCs remained unchanged (8±14% reduction, $p>.5$; Figure 53A,C). Also consistent with the previous results, no change in either mEPSC or mIPSC amplitude was elicited by dopamine (Figure 53). This clear differential effect of dopamine on the frequency of miniature events suggests that dopamine depresses excitatory and inhibitory synaptic transmission by different presynaptic mechanisms.

Presynaptic modulation of transmitter release may occur through at least two

general mechanisms: the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels in the synaptic terminal may be reduced (for instance, by modulation of Ca^{2+} or K^+ channels), or the release machinery may be altered at some point after Ca^{2+} entry into the terminal (Thompson et al., 1993). Blockade of presynaptic K^+ channels with Ba^{2+} will broaden the presynaptic action potential waveform and may therefore interact with the former mechanism, but is unlikely to interact with the latter (Thompson and Gähwiler, 1992). Therefore, the effects of dopamine on evoked IPSPs and EPSPs in the presence and absence of Ba^{2+} were examined to determine whether this manipulation differentially changes the dopamine-mediated inhibition of inhibitory and excitatory synaptic transmission (Figure 54 and Figure 55). For monosynaptic IPSPs (recorded in DNQX and APV), dopamine was first applied in normal external solution and then in solution containing 1 mM Ba^{2+} (Figure 54). In the absence of Ba^{2+} dopamine (75 μM) depressed the IPSP by 42±9%, whereas in the presence of Ba^{2+}, dopamine reduced the IPSP by 16±6%, a significant reduction in the effectiveness of dopamine ($p<.01$, n=5). For EPSPs (recorded in picrotoxin), however, dopamine's effects were identical whether or not Ba^{2+} was present (Figure 55). In control conditions, dopamine (75 μM) reduced the EPSP by 27±6%, and in 1 mM Ba^{2+}, dopamine reduced the EPSP by 26±6% ($p>.1$, n=5). These results provide evidence that dopamine modulates inhibitory synaptic transmission by modification of a presynaptic ionic conductance, whereas dopamine modulates excitatory transmission by interference with a process that is independent of the entry of Ca^{2+}.

To further test this hypothesis, the external K^+ concentration was elevated from 1.6 mM to 22 – 25 mM and mIPSCs recorded in TTX were examined. In high K^+, mIPSC frequency was increased by 4 – 8 fold over the frequency in normal K^+ (Figure 56A,B). Brief application of the Ca^{2+} channel blocker Cd^{2+} (100 μM) reversibly reduced the increased mIPSC frequency to its value in normal K^+, while Cd^{2+} application in normal K^+ had no effect on the frequency of mIPSCs recorded in the same cells (n=3; Figure 56B). This indicates that the increased mIPSC frequency in elevated K^+ was due to Ca^{2+} influx through voltage-dependent Ca^{2+} channels, while mIPSCs recorded in

normal K^+ are independent of Ca^{2+} channel activity (Doze et al., 1995; Scanziani et al., 1995). Dopamine (100 μM) reduced the frequency of Ca^{2+}-dependent mIPSCs recorded in high K^+ by 18±4% ($p<.05$, n=4) while leaving their amplitude unchanged (3±4% depression, $p>.5$; Figure 56). In the same cells, the frequency of Ca^{2+}-independent mIPSCs recorded in normal K^+ was not reduced by dopamine (Figure 56B). Thus, dopamine is capable of modulating only those inhibitory synaptic events that are dependent on Ca^{2+} entry, suggesting that dopamine depresses the degree of Ca^{2+} influx into GABAergic terminals. This result is in contrast to that observed for mEPSCs, the frequency of which can be depressed even during the blockade of Ca^{2+} channels (Figure 50).

$GABA_B$ and adenosine receptors are not involved. In the VTA, activation of D1 receptors causes the potentiation of synaptically released GABA (Cameron and Williams, 1993). If dopamine has a similar effect in the NAc, it is conceivable that GABA released during dopamine application could activate $GABA_B$ receptors on inhibitory and excitatory terminals. $GABA_B$ receptor activation presynaptically inhibits synaptic transmission in many brain nuclei (Misgeld et al., 1995), including the NAc (Uchimura and North, 1991). It is therefore possible that the presynaptic effects of dopamine observed here were due to $GABA_B$ receptor activation. To test this, the effects of the $GABA_B$ antagonist CGP35348 on the dopamine-induced depression of excitatory and inhibitory synaptic transmission were examined (Figure 57 and Figure 58). Dopamine (75 μM) was applied to six field recordings first in the absence and then in the presence of the $GABA_B$ antagonist CGP35348 (500 μM). Excitatory synaptic responses were depressed by dopamine to the same extent whether or not the antagonist was present (35±6% depression for control, 34±6% depression in the presence of CGP35348, $p>.5$, Figure 57). The dopamine-induced depression of IPSPs was similarly unaffected by CGP35348 (23±4% depression for control, 21±4% depression in 500 μM CGP35348, n=3, $p>.5$, Figure 58). Thus, activation of $GABA_B$ receptors is apparently not a part of the mechanism of dopamine-induced depression of synaptic transmission.

Another transmitter which may mediate the effects of dopamine on synaptic transmission is adenosine. In VTA slices prepared from animals chronically administered cocaine or morphine, dopamine depresses inhibitory synaptic transmission via a mechanism involving adenosine acting at presynaptic adenosine A1 receptors (Bonci and Williams, 1996). A similar mechanism involving the NMDA receptor-dependent release of adenosine has recently been suggested to underlie the synaptic effects of dopamine in the NAc (Harvey and Lacey, 1996a). However, the adenosine receptor antagonist 8-cyclopentyltheophylline (CPT, 20 μM) did not reduce the depressant action of dopamine on either EPSPs or IPSPs. Dopamine depressed IPSPs by 23±7% in control conditions and by 26±4% in the same cells in the presence of CPT ($p>.9$, $n=3$; Figure 59B,D). Similarly, dopamine depressed the EPSP by 26±1% in control conditions and by 35±4% in the presence of CPT ($p>.3$, $n=2$; Figure 59A,D). Furthermore, under the conditions employed here, depression of neither EPSPs nor IPSPs requires the activation of NMDA receptors, since dopamine was capable of depressing the EPSP by 32.3±1% in the presence of d,l-APV (75 μM; Figure 59C,D), and all of the experiments involving IPSPs are performed with this concentration of d,l-APV in the bathing medium. Thus, the effects of dopamine on inhibitory and excitatory synaptic transmission in the NAc do not appear to require activation of $GABA_B$, adenosine or NMDA receptors.

Activation of adenylate cyclase does not depress synaptic transmission. The second messenger most commonly associated with D1 dopamine receptors is cAMP (Kebabian and Calne, 1979). Each of the cloned D1 receptors activates adenylate cyclase, the enzyme that catalyzes the synthesis of cAMP (Jensen et al., 1996). In order to determine whether activation of this enzyme and the consequent elevation of cAMP levels could depress synaptic transmission in the NAc, the effects of the adenylate cyclase activator forskolin (applied in conjunction with the phosphodiesterase inhibitor IBMX) on excitatory and inhibitory synaptic transmission were examined (Figure 60 through Figure 62). Because IBMX can inhibit adenosine receptors, most experiments were done in the presence of CPT (20 μM); those that were not done in CPT

demonstrated similar results and were therefore combined with those that were done in CPT. Forskolin potentiated the EPSPs to 150±16% of baseline (n=12, p<.002, Figure 60) while leaving IPSPs unaffected (106±8% of baseline, n=9, p>.5, Figure 61). To determine whether forskolin acts pre- or postsynaptically, its effects on mEPSCs were examined (Figure 62). The frequency of mEPSCs was increased by forskolin to 450±88% of the baseline value while the amplitude remained at 112±4% of baseline (n=3, Figure 62C). The results of these experiments indicate that elevation of cAMP levels in excitatory presynaptic terminals potentiates glutamate release, while having little if any effect in inhibitory terminals.

Discussion

Dopamine increased the paired-pulse ratio while decreasing the synaptic current for both EPSCs and IPSCs. These results are most consistent with a presynaptic locus of action for dopamine (Katz and Miledi, 1968; Mallart and Martin, 1968; Manabe et al., 1993), although they do not rule out an additional postsynaptic mechanism. In retinal horizontal cells, dopamine acts at a postsynaptic D1 receptor to alter the kinetics of ionotropic glutamate receptor channels to favor the open state, thus potentiating excitatory synaptic transmission (Knapp and Dowling, 1987; Knapp et al., 1990). To examine whether a similar direct interaction with postsynaptic glutamate or GABA receptors could contribute to the observed depression of synaptic transmission in the NAc, spontaneous miniature synaptic events (mEPSCs and mIPSCs) were recorded. Dopamine and amphetamine elicited no change in the amplitude distribution of either mEPSCs or mIPSCs. This result is most consistent with the absence of an effect on postsynaptic glutamate and GABA receptors (Fatt and Katz, 1952). However, a number of factors should be considered before concluding that dopamine has no postsynaptic effect on synaptic transmission. First, the whole-cell recording method used for these studies often allows the dialysis out of the cell of molecules critical for the observation of postsynaptic effects. Postsynaptic effects of dopamine may therefore

be present when other means of measuring the synaptic potential are used. Second, the voltage-clamp procedure would have precluded the interaction between dopamine and voltage-dependent conductances in the postsynaptic cell which may contribute to the synaptic depression observed when cells are not voltage-clamped. Further studies are required to determine whether dopamine has such an effect in NAc cells. These factors may explain why the results reported here differ from those of Cepeda et al. (1993), who used sharp electrode intracellular current-clamp recording in striatal cells to observe postsynaptic effects of dopamine on the response to exogenously applied glutamate. However, another group (Calabresi et al., 1995) using intracellular current-clamp did not replicate the results of Cepeda et al.

Analysis of the frequency of spontaneous events revealed a difference between the effects of dopamine on mEPSCs and mIPSCs. The frequency of mEPSCs was reduced by dopamine, consistent with the PPF results for EPSCs that suggest a presynaptic mechanism. The frequency of mIPSCs, however, was unaffected by dopamine despite the increase in the paired-pulse ratio observed when dopamine was applied to IPSCs. The difference between the results for mEPSCs and mIPSCs was not due to the inability of slices in which mIPSCs were recorded to exhibit depression of miniature event frequency, since simultaneous recording of mEPSCs and mIPSCs at a membrane potential intermediate between their reversal potentials revealed that dopamine could cause the reduction of mEPSCs frequency while mIPSC frequency remained unaltered. These results are analogous to those of Scanziani et al. (1992), who found in hippocampal CA3 cells that the $GABA_B$ agonist baclofen reduced the frequency of mEPSCs while leaving mIPSC frequency unchanged, and reduced the amplitudes of neither mEPSCs nor mIPSCs. The conclusion from this result was that modulation of excitatory transmission by baclofen occurs at some point in the release process after Ca^{2+} has entered the cell and has been sensed by the release apparatus, while in inhibitory terminals, baclofen causes a reduction of the amount of Ca^{2+} that enters the terminal during each action potential (Thompson et al., 1993). Similarly, distinct mechanisms in excitatory and inhibitory terminals may be in operation in the case of

dopamine-induced modulation of synaptic transmission in the NAc.

Several further experiments confirm that dopamine causes a reduction in presynaptic Ca^{2+} entry in inhibitory terminals, and that such a reduction is not necessary for depression of excitatory transmission. In the first of these experiments, mIPSCs that were dependent on Ca^{2+} entry into the presynaptic terminal were recorded after elevation of the extracellular K^+ concentration, and shown to be dependent on the entry of Ca^{2+} through voltage-dependent Ca^{2+} channels by the reduction in their frequency with the application of the Ca^{2+} blockers Co^{2+} or Cd^{2+}. The frequency of these mIPSCs was reduced by dopamine, in contrast to the lack of effect of dopamine on the frequency of Ca^{2+} entry-independent mIPSCs. This result is similar to the finding that baclofen can reduce mIPSC frequency in hippocampal pyramidal cells only in elevated K^+ (Doze et al., 1995). In the case of NAc mEPSCs, however, amphetamine reduced their frequency even when Co^{2+} or Cd^{2+} were used to block synaptic transmission instead of TTX. Thus, reduction of NAc inhibitory transmission can occur only when Ca^{2+} can enter the presynaptic terminal, whereas Ca^{2+} entry is not required for the reduction of excitatory transmission.

This distinction between modulation of excitatory and inhibitory transmission was further analyzed by recording evoked EPSPs and IPSPs in the presence of the K^+ channel antagonist Ba^{2+}. While Ba^{2+} most likely reduced K^+ currents and broadened the action potential in both excitatory and inhibitory terminals, Ba^{2+} reduced only the dopamine-induced depression of IPSPs. Ba^{2+} may also have reduced Ca^{2+} entry into the presynaptic terminal by competition with Ca^{2+} at Ca^{2+} channels (Augustine and Eckert, 1984). Both of these effects of Ba^{2+} are on the dynamics (time course and magnitude) of presynaptic Ca^{2+}, and the impact of Ba^{2+} on the dopamine-induced depression only of IPSPs, but not of EPSPs, suggests that dopaminergic modulation occurs by an interaction with Ca^{2+} entry in inhibitory terminals, but not in excitatory terminals. Similar conclusions were reached for baclofen in hippocampal pyramidal cells (Thompson and Gähwiler, 1992).

The exact identity of the conductance modulated by dopamine in inhibitory

terminals is unknown, since modulation of K^+, Na^+ or Ca^{2+} channels could conceivably result in a reduction in Ca^{2+} influx. Modulation by dopamine of each of these ion channels has been observed in NAc or striatal cells: a D1 receptor on NAc cells activates a K^+ conductance (Uchimura et al., 1986), and in striatal cells, a D1 receptor-dependent reduction was observed for Na^+ currents (Schiffmann et al., 1995; Surmeier et al., 1992) and N and P-type Ca^{2+} currents (Surmeier et al., 1995). Because of the recording conditions employed in the present study, effects on Na^+, K^+ and Ca^{2+} conductances were not detected, but these D1-mediated conductance changes may have been present in the axon terminals of other NAc cells that form GABAergic synapses with the NAc cells from which recordings were made. These changes in Na^+ currents, K^+ currents, or N or P-type Ca^{2+} currents (which contribute to transmitter release; Olivera et al., 1994) would serve to reduce transmitter release in the absence of a post-Ca^{2+} interaction with the release machinery, which must exist in excitatory terminals.

One reason for the similarity between dopamine's actions on synaptic transmission in the NAc and those of baclofen in the hippocampus may be that $GABA_B$ receptors themselves are somehow involved in the effects of dopamine. This possibility is particularly intriguing given the potentiation of GABA release that results from D1 receptor activation in the VTA (Cameron and Williams, 1993). If such a mechanism were at work in the NAc, the increased release of GABA at some inhibitory terminals could bind to $GABA_B$ receptors on other inhibitory terminals and on excitatory terminals, where the main function of $GABA_B$ receptors in the NAc (Uchimura and North, 1991) and many other brain nuclei (Misgeld et al., 1995) appears to be to reduce transmitter release. The result might then be the observed presynaptic reduction of synaptic transmission. However, the depression of excitatory and inhibitory transmission elicited by dopamine was not affected by the $GABA_B$ antagonist CGP35348, making this mechanism unlikely.

Another transmitter with presynaptic depressant effects is adenosine (Greene and Haas, 1991; Nicoll et al., 1990). In the VTA of animals chronically treated with cocaine or morphine, D1 receptor activation causes an increase in extracellular

adenosine levels, which inhibit synaptic transmission (Bonci and Williams, 1996). Harvey and Lacey (1996a) have suggested a similar mechanism for the effects of dopamine in the NAc. They propose that activation of NMDA receptors causes the release of adenosine (Manzoni et al., 1994) which results in presynaptic inhibition of the EPSP. However, neither an adenosine antagonist (CPT) nor an NMDA receptor antagonist (APV) reduces the magnitude of the dopamine-induced depression of either EPSPs or IPSPs, consistent with the findings of others (O. Manzoni, personal communication). It appears, therefore, that the effects of dopamine are the result of the activation of a dopamine receptor on excitatory and inhibitory axon terminals.

Two lines of anatomical evidence complicate this interpretation. The first is the finding that in the NAc and striatum there are relatively small numbers of axo-axonic synapses between dopaminergic and other types of terminals; dopaminergic terminals appear instead to synapse mostly onto dendrites and spines (Bouyer et al., 1984a; Freund et al., 1984; Johnson et al., 1994; Pickel et al., 1981; Sesack and Pickel, 1990, 1992). This evidence seems to leave little room for presynaptic actions of dopamine. However, several further observations argue against this interpretation. First, a consideration of the probability of detecting axo-axonic synapses (which requires observation of two synaptic specializations as opposed to just one for axodendritic or axosomatic synapses) from single sections without serial reconstruction has lead to the conclusion that the number of axo-axonic synapses is likely to be greatly underestimated (Kornhuber and Kornhuber, 1983, 1986). Even if this were not the case, Descarries et al. (1996) observed that many dopaminergic synapses in the striatum have no identifiable target, and may therefore elevate local dopamine concentrations at sites relatively distant from the terminal ending. Diffusion of dopamine across distances much longer than a synaptic cleft has been observed in the retina (Pereda et al., 1992; Dowling, 1991) and in fact dopamine released in the hypothalamus acts as a hormone to influence the pituitary several millimeters away (Ben-Jonathan, 1985). Furthermore, a calculation of the diffusion distance within the NAc of dopamine released by stimulation of dopaminergic afferents suggests that

dopamine diffuses greater than 10 μm away from its original release site before being reduced to half its concentration in the synaptic cleft; in a sphere of radius 10 to 12 μm there are about 200 dopaminergic synapses (Garris et al., 1994). Therefore, the anatomical evidence against axo-axonic synapses between dopaminergic and non-dopaminergic terminals does not rule out the possibility that synaptically released dopamine can diffuse to excitatory and inhibitory terminals where it can inhibit glutamate and GABA release.

The second anatomical finding that is at odds with presynaptic modulation by dopamine of synaptic transmission comes from ultrastructural studies of the NAc and striatum using antibodies directed against the cloned D1a and D2 receptors, which have found that the vast majority of labeling for these receptors is on postsynaptic structures (dendrites and spines) as opposed to axon terminals (Caillé et al., 1996; Delle Donne et al., 1996; Fisher et al., 1994; Hersch et al., 1995; Huang et al., 1992; Levey et al., 1993; Sesack et al., 1994; Yung et al., 1993). While some of these studies found small numbers of labeled terminals, the much more prevalent postsynaptic labeling seems to suggest that the role of these receptors is not likely to include modulation of transmitter release. However, if D1b receptors are more commonly expressed on presynaptic terminals, or if the few D1a receptors seen on terminals are sufficient for producing synaptic depression (which is unlikely given the equivalent effects of dopamine in D1a knockout and wildtype mice reported here), the most likely means by which these receptors could modulate transmitter release is by the activation of adenylate cyclase and consequent elevation of cAMP levels in the presynaptic terminal.

To determine whether such a presynaptic elevation in cAMP could account for the observed effects of dopamine, the adenylate cyclase activator forskolin was applied to slices during recording of EPSPs and IPSPs. EPSPs were potentiated by forskolin while IPSPs were unaffected by it. The effects of forskolin on excitatory transmission appear to be the result of a presynaptic mechanism, since forskolin also dramatically increased the frequency of spontaneous mEPSCs while leaving their amplitude distribution unchanged. These results are consistent with those observed when cAMP

levels are increased at other presynaptic terminals, which almost always results an increase in synaptic transmission (Bonci and Williams, 1997; Capogna et al., 1995; Chavez-Noriega and Stevens, 1994; Kandel and Schwartz, 1982; Llano and Gerschenfeld, 1993; Salin et al., 1996; Weisskopf et al., 1994; Zhong and Wu, 1991). However, the effects of forskolin in the NAc are the opposite of what one would expect if activation of the dopamine receptor responsible for synaptic depression causes the elevation of cAMP in excitatory and inhibitory terminals. Perhaps the most likely explanation for this discrepancy is that D1 receptors (or a subset of them) are coupled to a different second messenger system in addition to or instead of the cAMP pathway. There is some evidence for the notion that there exist D1 receptors that have not yet been cloned and which are coupled to second messenger systems other than cAMP (see the Discussion in Chapter 4, p. 96). One of these receptors may be expressed on presynaptic terminals in the NAc and may be responsible for synaptic depression.

Figure 45. Dopamine increases paired-pulse facilitation (PPF) of NAc EPSCs. ***A***, Representative current traces from the voltage-clamped cell shown in B. Two stimuli were administered with an interpulse interval of 75 ms. The current deflection beginning 400 ms after the first stimulus was the result of a –10 mV voltage pulse. For clarity, capacitive transients were truncated. ***B***, The ratio of the amplitude of the second EPSC to that of the first increases with the application of 75 μM dopamine (*upper*), which simultaneously reduces the amplitude of the first EPSC (*lower*). ***C***, Summary (n=8) of voltage clamp experiments similar to the one shown in B.

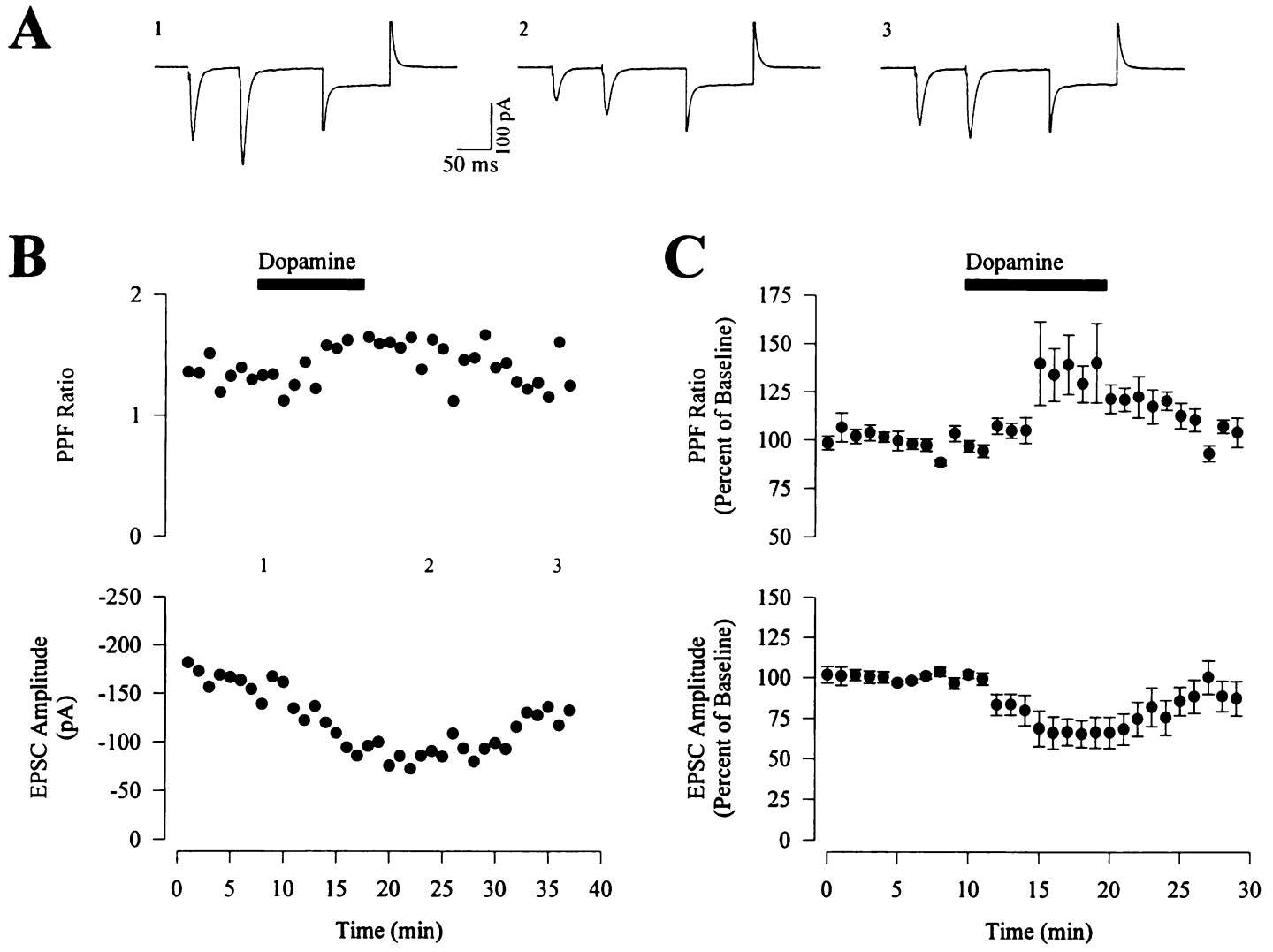

A
1
2
3
100 pA
50 ms
B
Dopamine
PPF Ratio
EPSC Amplitude (pA)
Time (min)
C
Dopamine
PPF Ratio (Percent of Baseline)
EPSC Amplitude (Percent of Baseline)
Time (min)

Figure 46. Analysis of the depressant effects of amphetamine on mEPSC frequency but not amplitude in the NAc.

A, Representative consecutive 1 s current sweeps (taken at the times shown in B) from a voltage-clamped cell in which spontaneous mEPSCs were recorded in the presence of TTX. ***B***, The average mEPSC frequency (1 min bins) was reduced by application of 10 μM amphetamine. ***C***, The average mEPSC amplitude (1 min bins) was unchanged by the amphetamine. ***D***, The cumulative probability distribution of the time intervals between successive mEPSCs before and during the application of 10 μM amphetamine. ***E***, The cumulative probability distribution of mEPSC amplitudes before and during application of amphetamine. ***F*** and ***G***, The same distributions shown in D and E, respectively, but with absolute probability along the ordinate instead of cumulative probability.

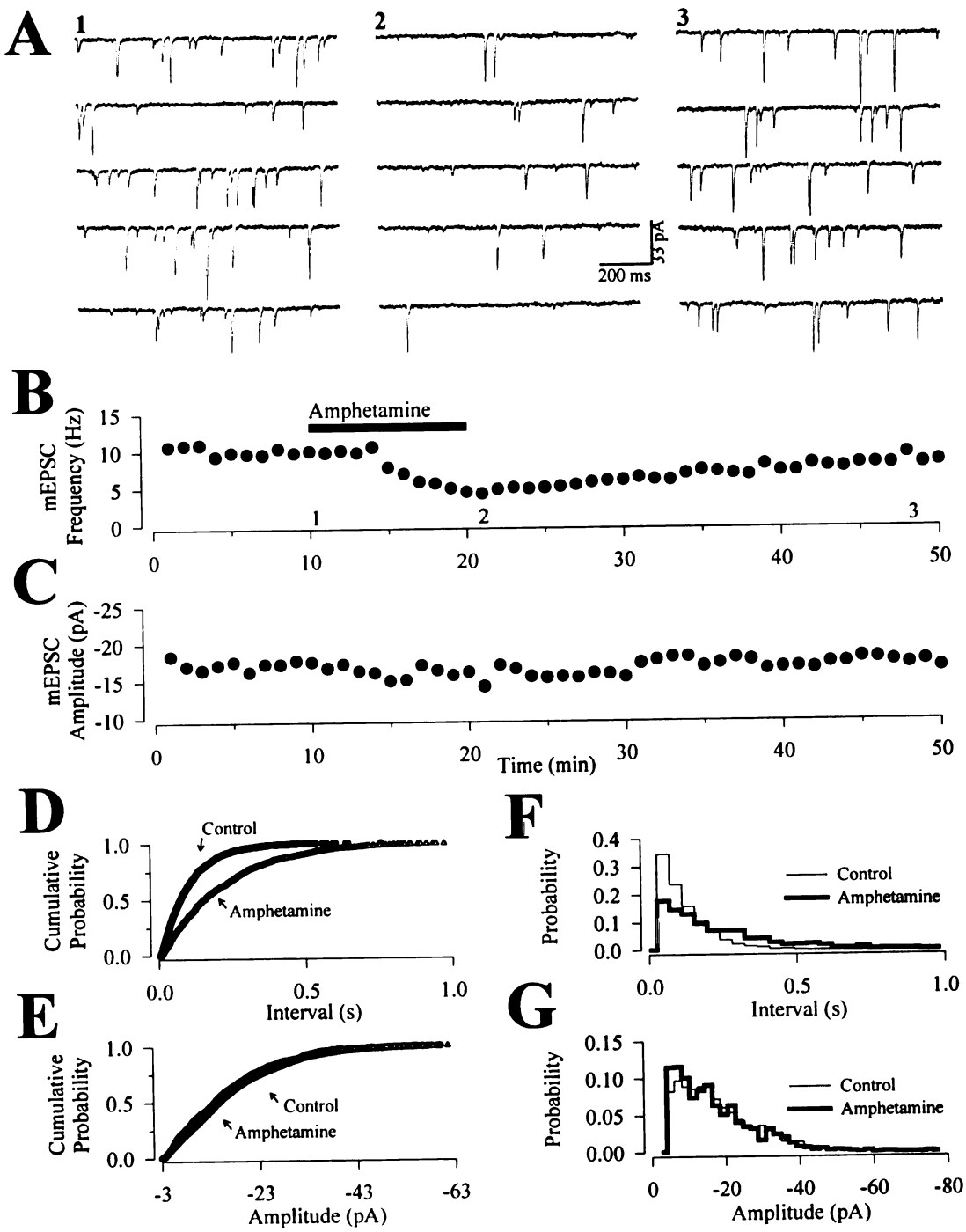

A
1
2
3
35 pA
200 ms
B
Amphetamine
mEPSC Frequency (Hz)
15
10
5
0
1
2
3
0
10
20
30
40
50
C
mEPSC Amplitude (pA)
-25
-20
-15
-10
0
10
20
Time (min)
30
40
50
D
Control
Amphetamine
Cumulative Probability
1.0
0.5
0.0
0.0
0.5
1.0
Interval (s)
E
Cumulative Probability
1.0
0.5
0.0
Control
Amphetamine
-3
-23
-43
-63
Amplitude (pA)
F
Probability
0.4
0.3
0.2
0.1
0.0
Control
Amphetamine
0.0
0.5
1.0
Interval (s)
G
Probability
0.15
0.10
0.05
0.00
Control
Amphetamine
0
-20
-40
-60
-80
Amplitude (pA)

Figure 47. Summary of the depressant effects of amphetamine on mEPSC frequency but not amplitude in the NAc.

A, Summary of 6 experiments in which mEPSCs were recorded in the presence of 10 μM amphetamine, demonstrating that mEPSC frequency was reversibly reduced. ***B***, In the same 6 experiments, mEPSC amplitude was unchanged by the amphetamine.

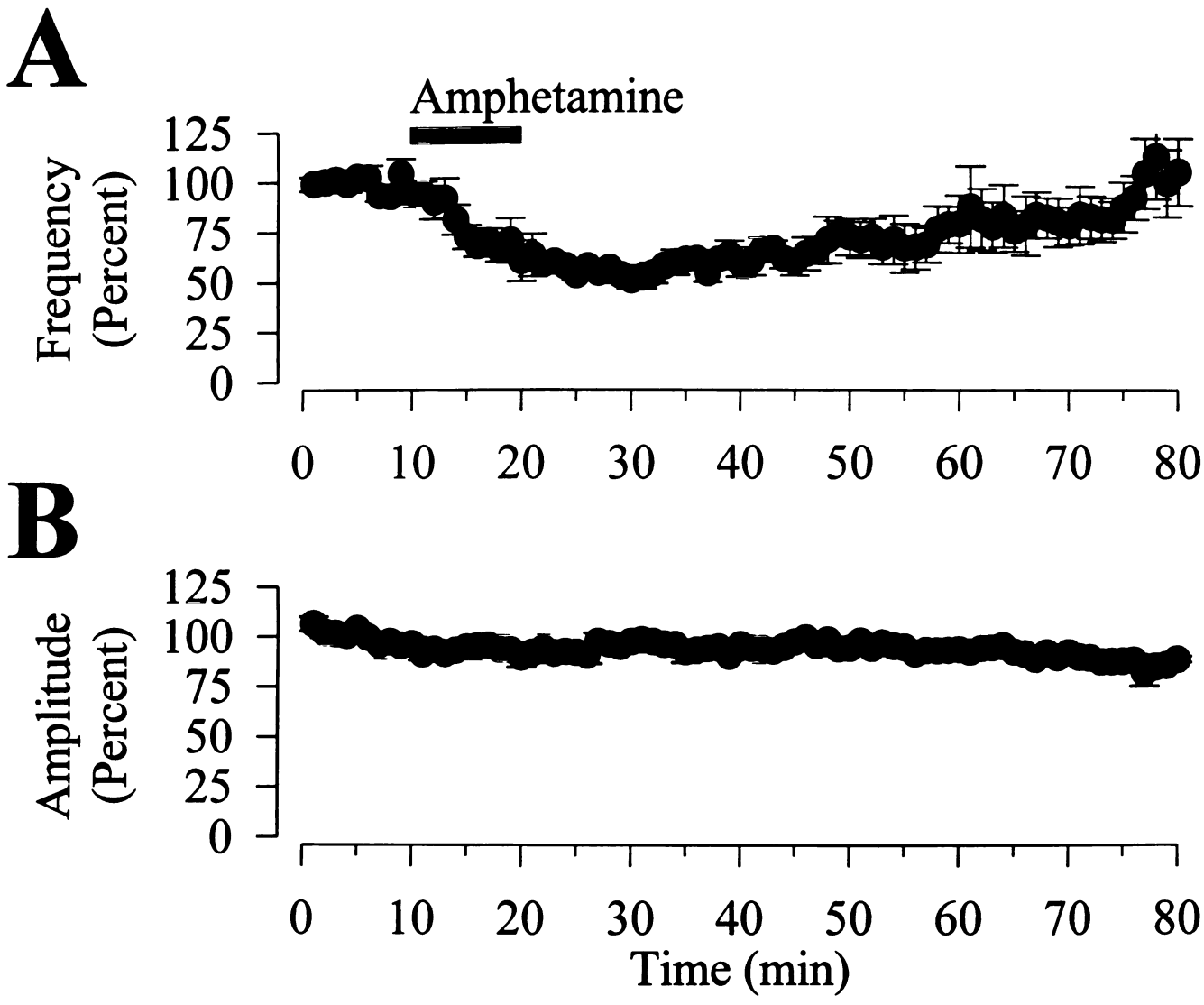

A
Amphetamine
Frequency (Percent)
125
100
75
50
25
0
0
10
20
30
40
50
60
70
80
B
Amplitude (Percent)
125
100
75
50
25
0
0
10
20
30
40
50
60
70
80
Time (min)

Figure 48. Analysis of the depressant effects of dopamine on mEPSC frequency but not amplitude in the NAc.

A, Representative consecutive 1 s current sweeps from a voltage-clamped cell in which spontaneous mEPSCs were recorded in the presence of TTX. Sweeps were taken before application of dopamine (1), in the presence of dopamine (2) and after wash-out (3); the last set of sweeps was taken at the end of the experiment, after application 10 μM CNQX. ***B***, The average mEPSC frequency (1 min bins) was reduced by application of 100 μM dopamine. ***C***, The average mEPSC amplitude (1 min bins) was unchanged by the dopamine. ***D***, The cumulative probability distribution of the time intervals between successive mEPSCs before and during the application of 100 μM dopamine. ***E***, The cumulative probability distribution of mEPSC amplitudes before and during application of dopamine. ***F*** and ***G***, The same distributions shown in D and E, respectively, but with absolute probability along the ordinate instead of cumulative probability.

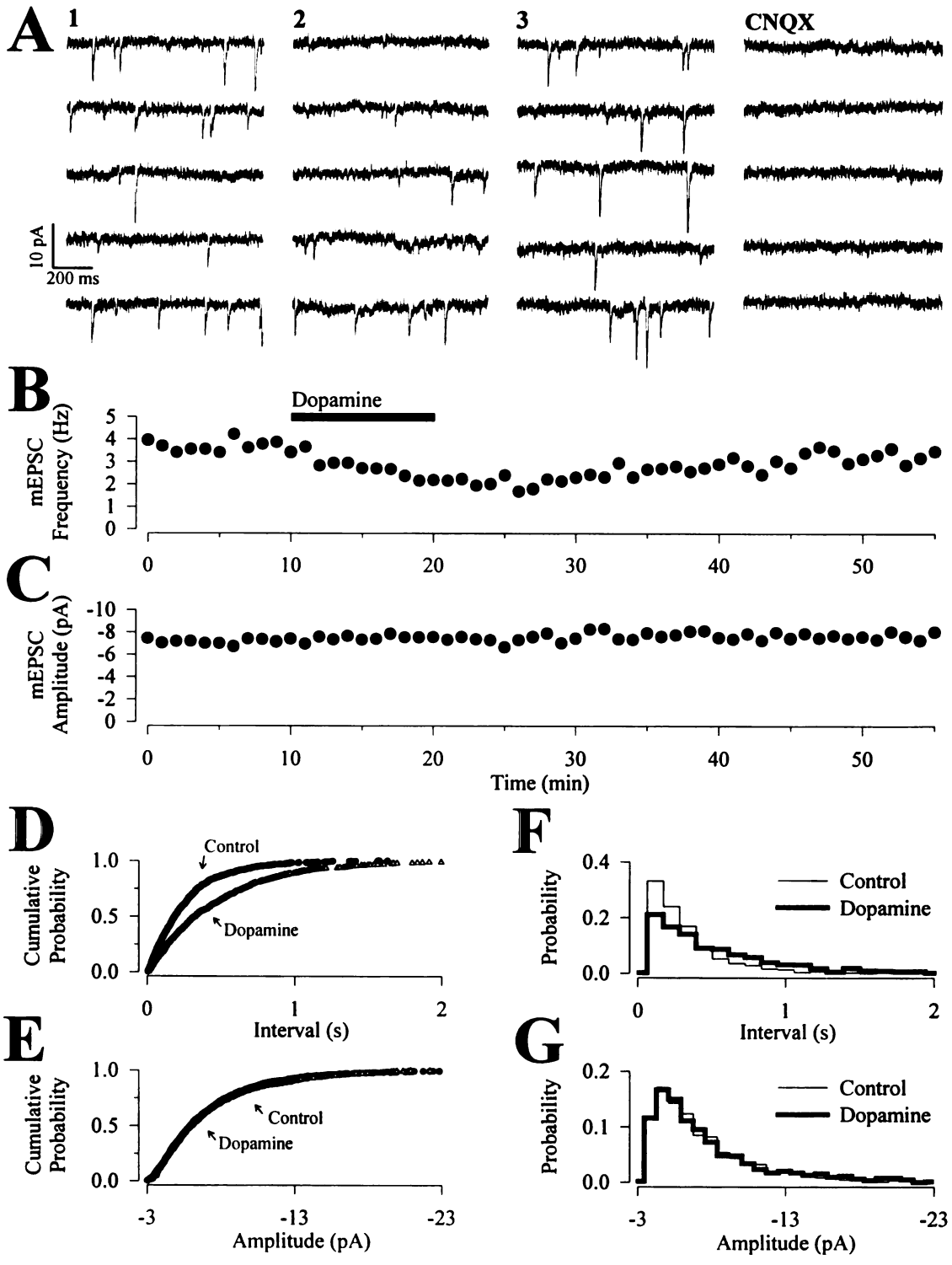

A
1
2
3
CNQX
10 pA
200 ms
B
Dopamine
mEPSC Frequency (Hz)
C
mEPSC Amplitude (pA)
Time (min)
D
Cumulative Probability
Control
Dopamine
Interval (s)
E
Cumulative Probability
Control
Dopamine
Amplitude (pA)
F
Probability
Control
Dopamine
Interval (s)
G
Probability
Control
Dopamine
Amplitude (pA)

Figure 49. Summary of the depressant effects of dopamine on mEPSC frequency but not amplitude in the NAc.

A, Summary of 15 experiments in which mEPSCs were recorded during application of 100 μM dopamine, demonstrating that mEPSC frequency was reversibly reduced. ***B***, In the same 15 experiments, mEPSC amplitude was unchanged by the dopamine.

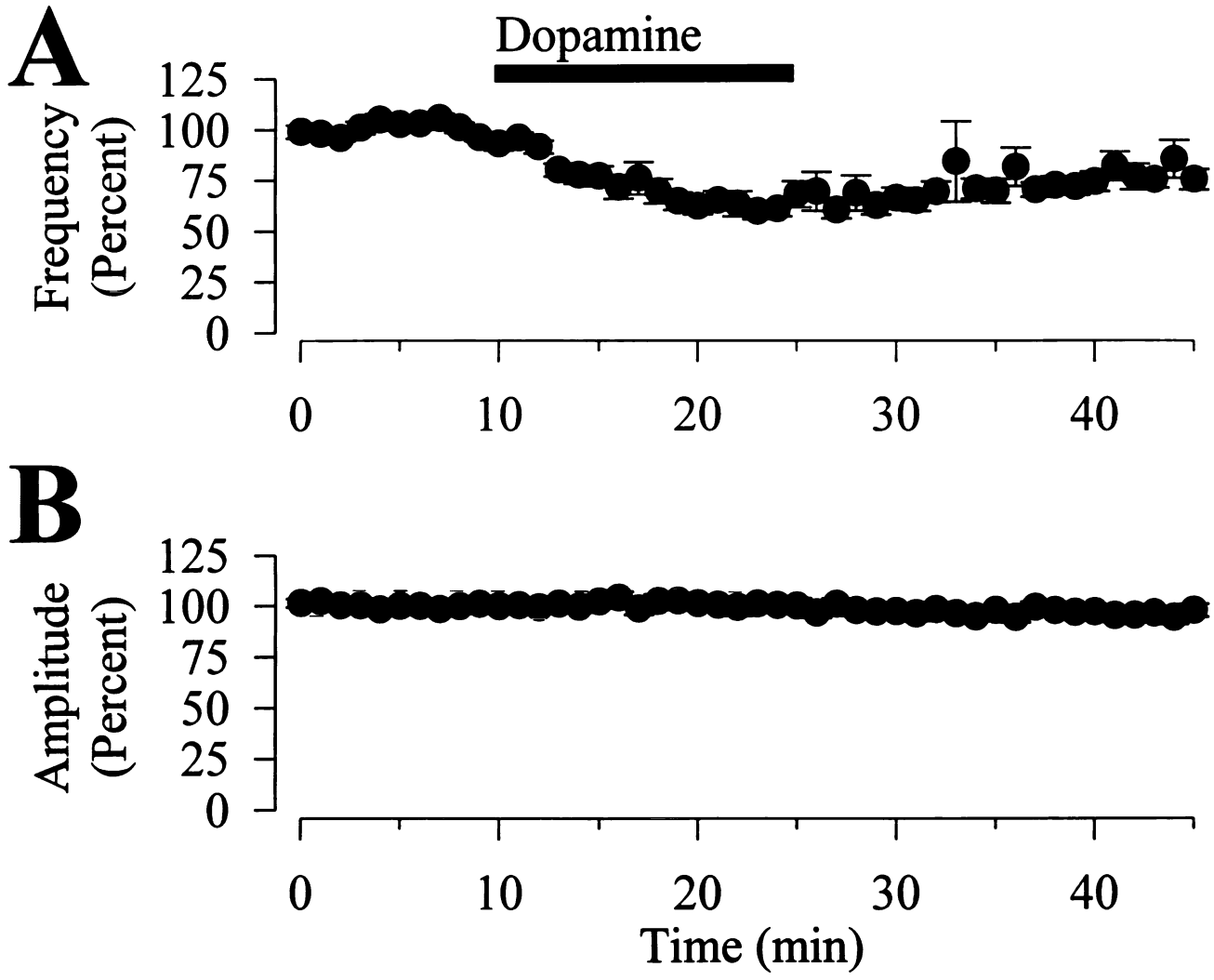

A
Dopamine
Frequency (Percent)
125
100
75
50
25
0
0
10
20
30
40
B
Amplitude (Percent)
125
100
75
50
25
0
0
10
20
30
40
Time (min)

Figure 50. Amphetamine reduces NAc mEPSC frequency in the presence of the Ca^{2+} channel blockers Co^{2+} and Cd^{2+}.

A, Consecutive traces taken from an experiment in which pharmacologically isolated mEPSCs were recorded in the presence of the Ca^{2+} channel antagonist Co^{2+} at a concentration (5 mM) sufficient to abolish evoked EPSCs. Compared with baseline (*left*), 10 μM amphetamine (*right*) reduced the frequency but not the amplitude of mEPSCs. ***B***, A summary graph (n=8) showing the effects of amphetamine (10 μM) on the frequency and amplitude of mEPSCs recorded either in 5 mM Co^{2+} (n=4) or 100 μM Cd^{2+} (n=4). The * indicates a statistically significant difference from baseline ($p<.005$).

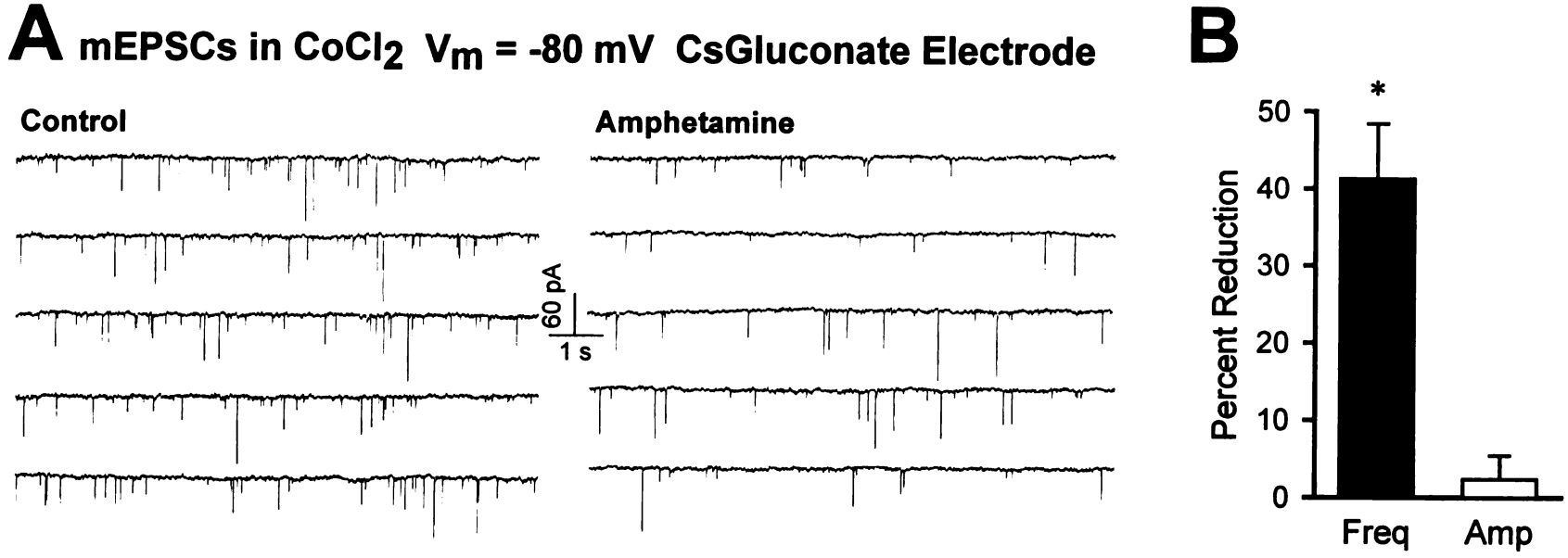

A mEPSCs in CoCl2 Vm = -80 mV CsGluconate Electrode
Control
Amphetamine
60 pA
1 s
B
*
50
40
30
20
10
0
Percent Reduction
Freq
Amp

Figure 51. Dopamine increases the paired-pulse ratio for IPSCs in the NAc. ***A***, IPSCs in response to single stimuli (*top*) and paired stimuli (*bottom*) (50 ms interstimulus interval) before and during application of dopamine (150 μM). The single and paired stimuli were alternately applied to the slice. In the paired pulse traces the IPSCs to the second pulse were obtained after subtracting an averaged IPSC in response to the single stimuli. In the paired-pulse trace in dopamine (*bottom right*) the first IPSC was scaled to the first IPSC in the absence of dopamine. ***B***, Summary (n=6) of the time course of the effects of dopamine (100 – 150 μM) on the paired-pulse ratio (*upper graph*) and the amplitude of the IPSC in response to the first pulse (*lower graph*).

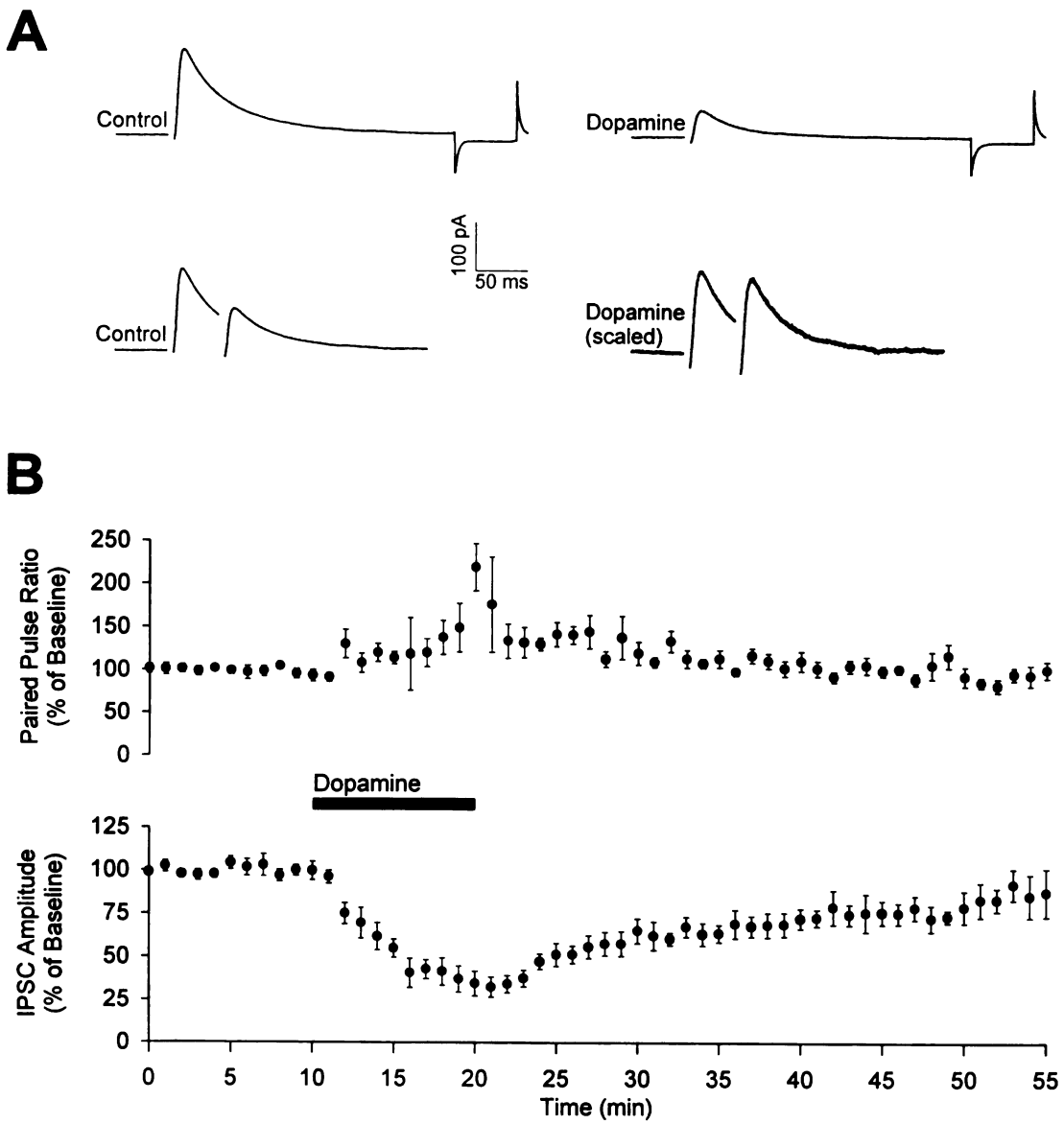

A
Control
Dopamine
100 pA
50 ms
Control
Dopamine
(scaled)
B
Paired Pulse Ratio
(% of Baseline)
250
200
150
100
50
0
Dopamine
IPSC Amplitude
(% of Baseline)
125
100
75
50
25
0
0
5
10
15
20
25
30
35
40
45
50
55
Time (min)

Figure 52. Dopamine does not affect mIPSC frequency or amplitude in the NAc. ***A***, Consecutive 10 s traces before (*left*) and during (*right*) application of dopamine (100 μM). Spontaneous mIPSCs were recorded with a CsCl-based electrode solution at –80 mV, and with DNQX and APV in the external bathing solution. ***B***, Cumulative probability histograms of mIPSC amplitudes taken from the cell shown in A showing the lack of effect of dopamine on mIPSC amplitude. ***C***, Normalized averages (1 minute bins) of the mIPSC frequency (*top*) and amplitude (*bottom*) from 10 experiments demonstrate that dopamine (75 – 100 μM) does not affect mIPSC frequency or amplitude.

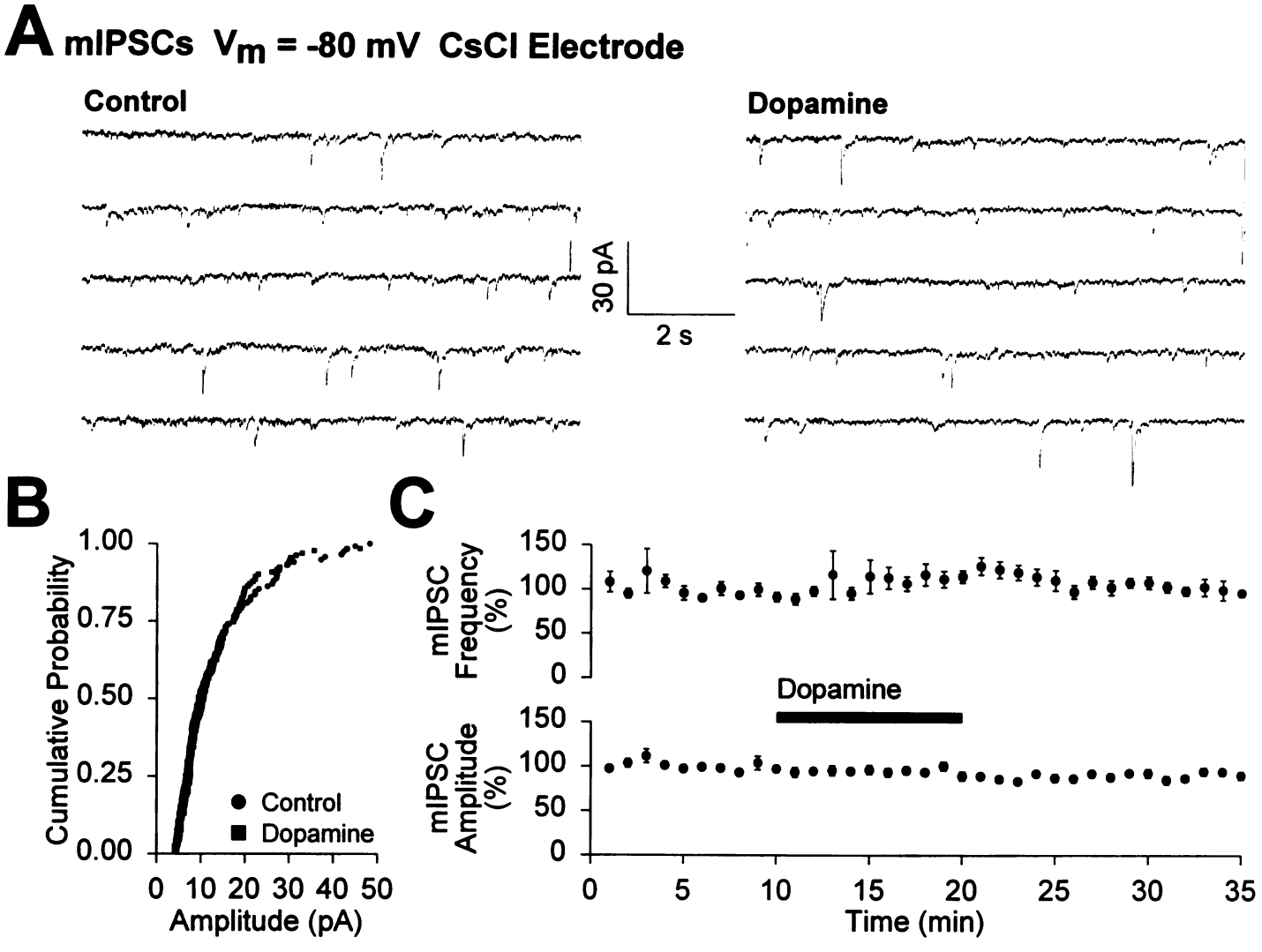

A mIPSCs V_m = -80 mV CsCl Electrode
Control
Dopamine
30 pA
2 s
B
Cumulative Probability
1.00
0.75
0.50
0.25
0.00
Control
Dopamine
0 10 20 30 40 50
Amplitude (pA)
C
mIPSC Frequency (%)
150
100
50
0
Dopamine
mIPSC Amplitude (%)
150
100
50
0
0 5 10 15 20 25 30 35
Time (min)

Figure 53. Dopamine does not reduce mIPSC frequency in NAc cells in which an effect of dopamine on mEPSC frequency can be observed.

A, Consecutive 10 s traces before (*left*) and during application of dopamine (100 μM) taken from a cell recorded with a cesium gluconate-based electrode solution and held at –25 mV. The external bathing solution contained D-APV (50 μM) but no DNQX or picrotoxin. *Inward currents* are mEPSCs and their frequency is reduced in dopamine. Dopamine had no effect on the *outward currents* which are mIPSCs. ***B***, Cumulative probability histograms of mEPSC amplitudes (*left*) and mIPSC amplitudes (*right*) show that neither amplitude distribution was affected by dopamine. ***C***, Summary (n=3) of the effects of dopamine on simultaneously monitored mEPSC and mIPSC frequency and amplitude. The * indicates a statistically significant difference from baseline ($p<.01$).

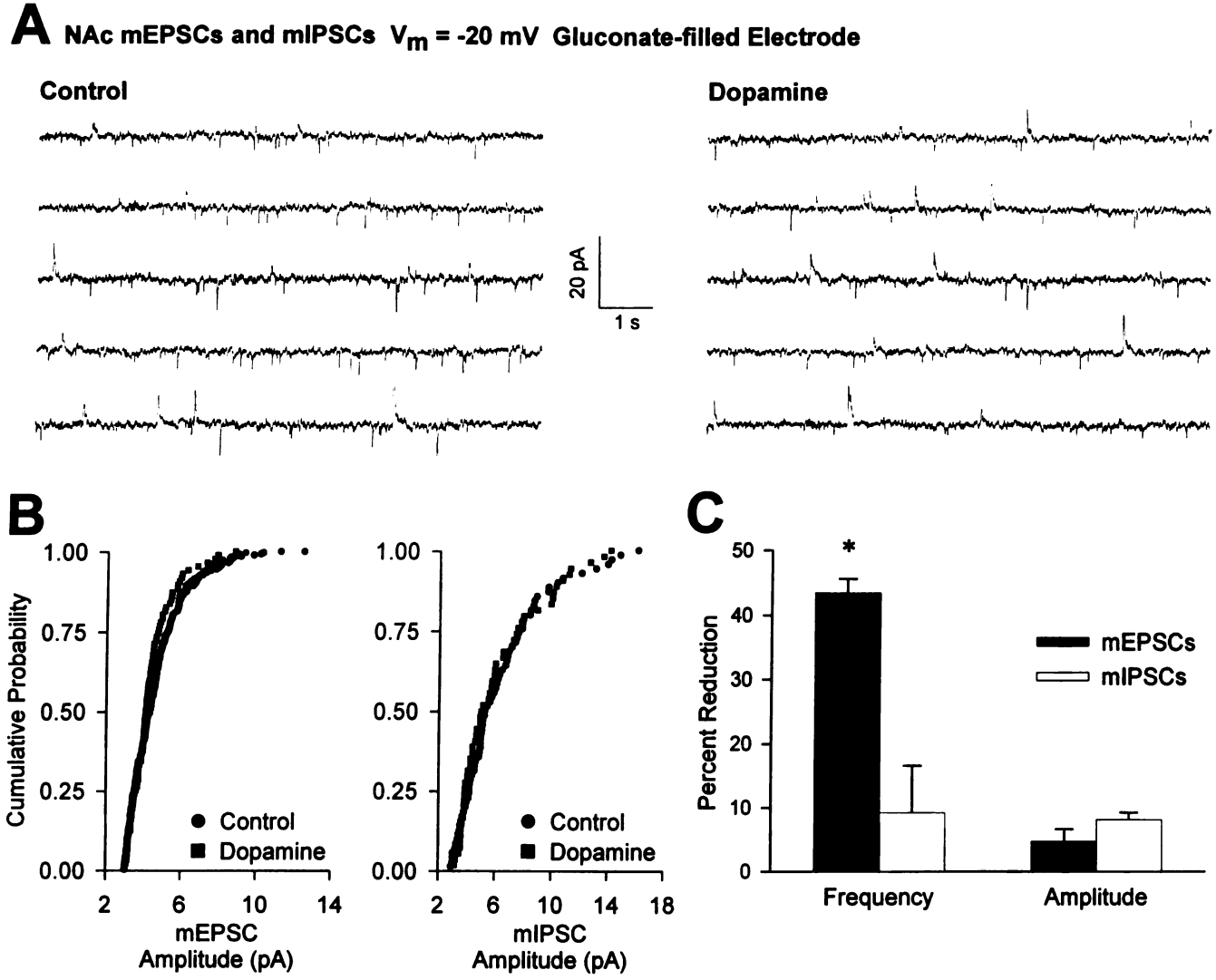

A
NAc mEPSCs and mIPSCs V_m = -20 mV Gluconate-filled Electrode
Control
Dopamine
20 pA
1 s
B
Cumulative Probability
1.00
0.75
0.50
0.25
0.00
Control
Dopamine
2
6
10
14
mEPSC
Amplitude (pA)
18
mIPSC
Amplitude (pA)
C
Percent Reduction
50
40
30
20
10
0
*
mEPSCs
mIPSCs
Frequency
Amplitude

Figure 54. Ba^{2+} reduces the effects of dopamine on inhibitory synaptic transmission in the NAc.

A, Traces taken from an example experiment (B) at the times indicated. ***B***, Dopamine (75 μM) was applied to pharmacologically isolated IPSPs in cells held at 0 mV, and then reapplied in the presence of $BaCl_2$ (1 mM). ***C***, A summary graph (n=5) compares the dopamine-induced depression of IPSP amplitude in the absence and presence of Ba^{2+}.

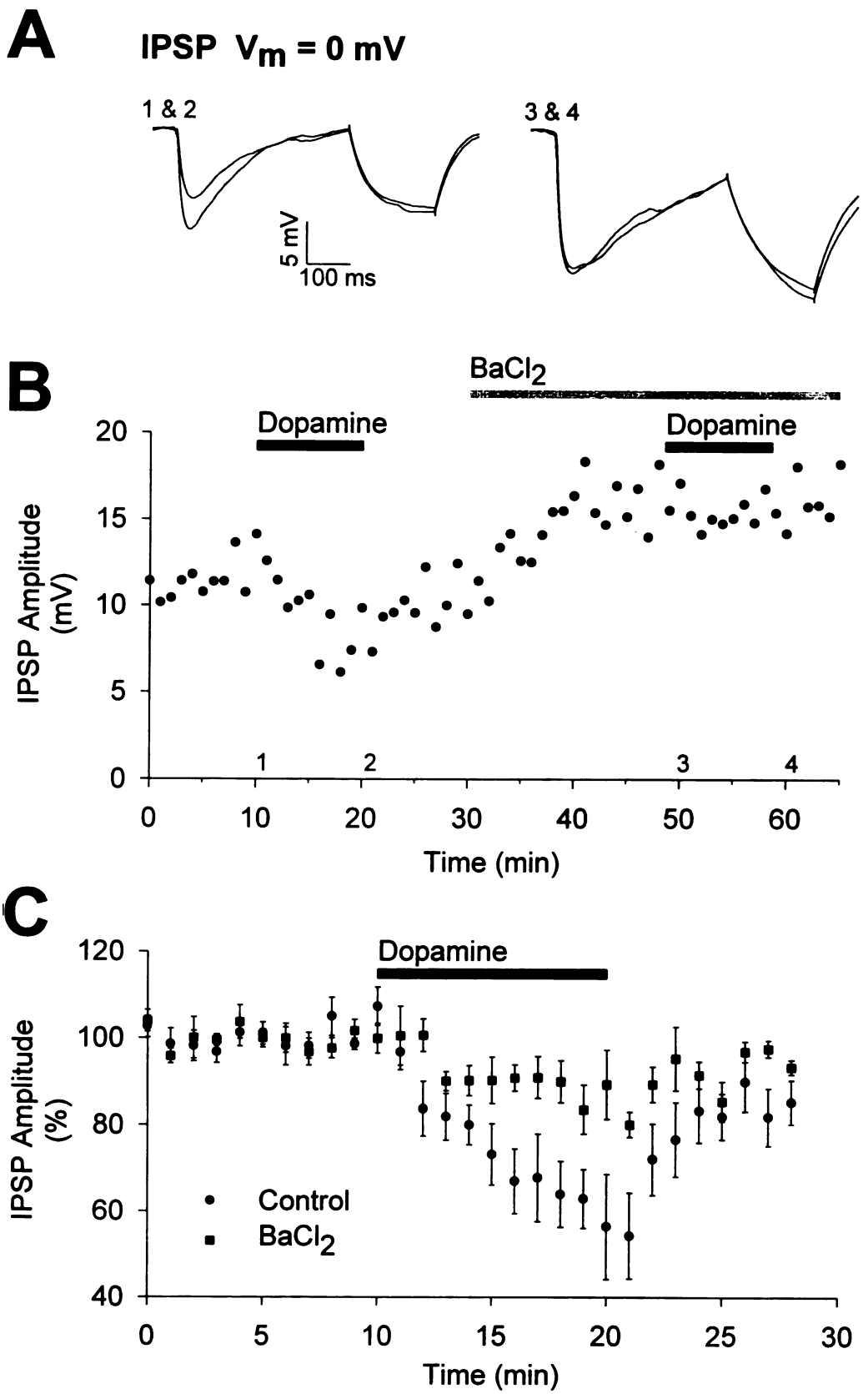

A
IPSP V_m = 0 mV
1 & 2
3 & 4
5 mV
100 ms
B
$BaCl_2$
Dopamine
Dopamine
IPSP Amplitude (mV)
20
15
10
5
0
1
2
3
4
0
10
20
30
40
50
60
Time (min)
C
Dopamine
IPSP Amplitude (%)
120
100
80
60
40
Control
$BaCl_2$
0
5
10
15
20
25
30
Time (min)

Figure 55. Ba^{2+} does not reduce the effects of dopamine on excitatory synaptic transmission in the NAc.

A, Traces of reversed EPSPs taken from the example experiment (B) at the times indicated. ***B***, The effects of dopamine (75 μM) on EPSPs was examined first in the absence then in the presence of Ba^{2+}. The cell was held at +25 mV in the presence of D-APV (50 μM) to prevent action potentials which often occurred during the EPSP in the presence of Ba^{2+}. ***C***, A summary graph (n=5) compares the dopamine-induced depression of EPSP slope in the absence and presence of Ba^{2+}.

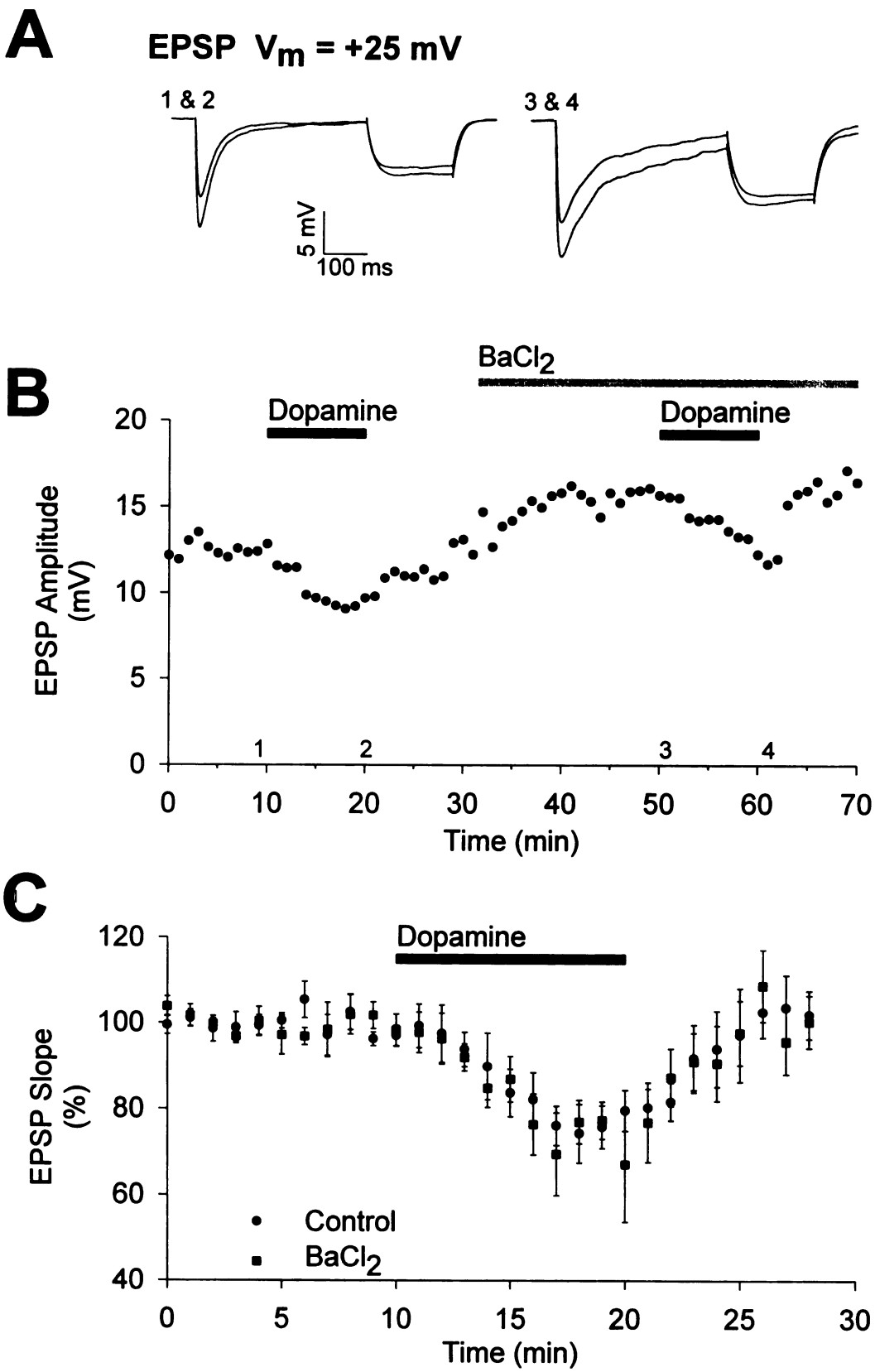

A
EPSP V_m = +25 mV
1 & 2
3 & 4
5 mV
100 ms
B
$BaCl_2$
Dopamine
Dopamine
EPSP Amplitude (mV)
20
15
10
5
0
1
2
3
4
0
10
20
30
40
50
60
70
Time (min)
C
Dopamine
EPSP Slope (%)
120
100
80
60
40
Control
$BaCl_2$
0
5
10
15
20
25
30
Time (min)

Figure 56. Dopamine depresses Ca^{2+}-dependent mIPSCs in the NAc.

A, Consecutive traces taken from the experiment in B show that the frequency of mIPSCs (holding potential=0 mV, cesium gluconate-based electrode solution) was greatly increased by raising extracellular KCl from 1.6 mM to 22 mM. ***B***, The time course of the frequency of mIPSCs is shown for one experiment. In 22 mM KCl, the Ca^{2+} channel antagonist $CdCl_2$ reduced the frequency of mIPSCs, whereas in 1.6 mM KCl, Cd^{2+} was without effect. Dopamine (100 μM) decreased the frequency of Ca^{2+}-dependent mIPSCs recorded in high KCl but not the frequency of Ca^{2+}-independent mIPSCs recorded in low KCl. ***C***, Neither Cd^{2+} nor dopamine influenced the amplitude of mIPSCs in either condition. ***D***, Summary graph (n=4) demonstrating the effects of dopamine (100 μM) on the frequency and amplitude of mIPSCs in high KCl (22-25 mM). The * indicates a statistically significant difference from baseline ($p<.01$). Solutions containing high K^+ were prepared by equimolar substitution of KCl for NaCl.

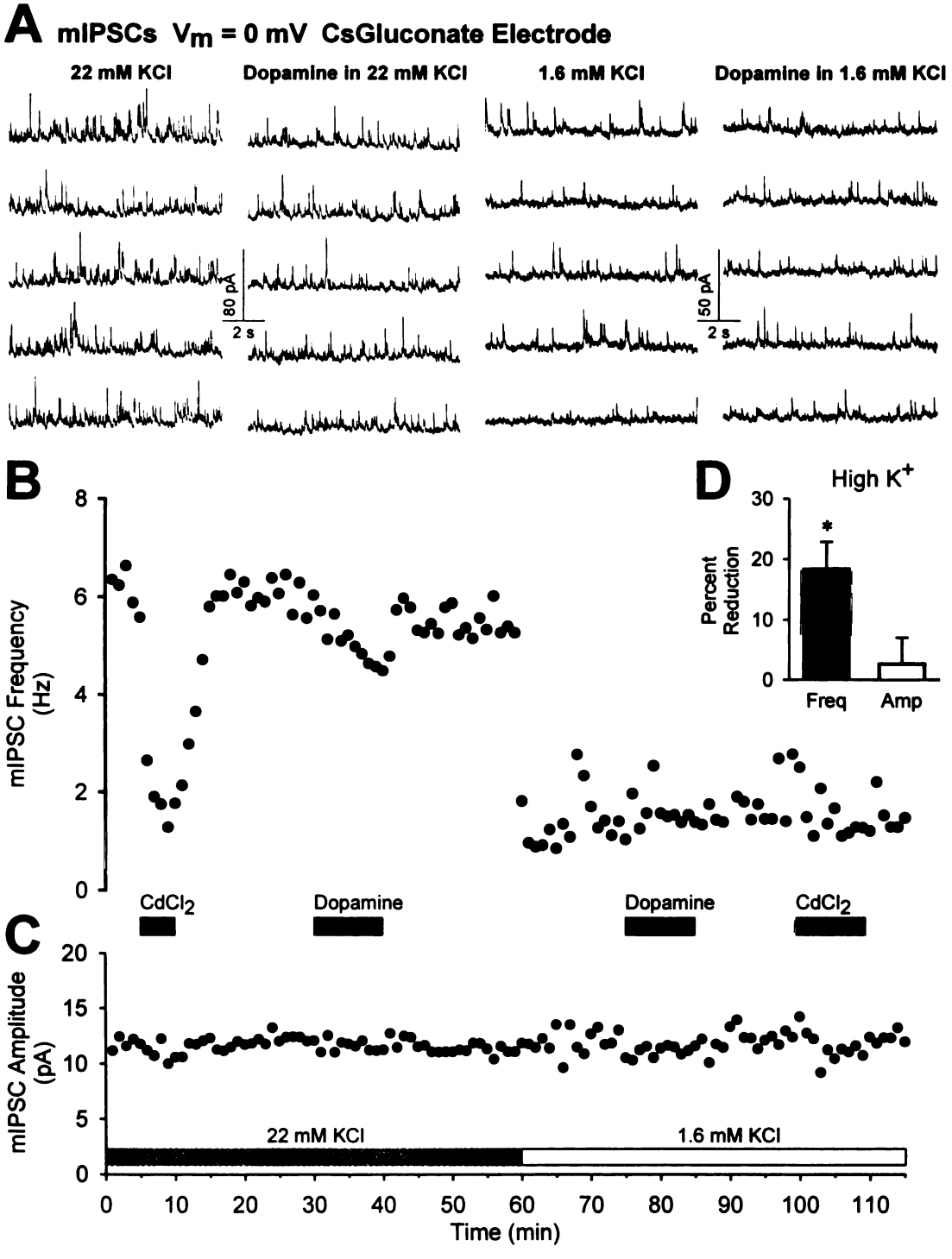

A
mIPSCs V_m = 0 mV CsGluconate Electrode
22 mM KCl
Dopamine in 22 mM KCl
1.6 mM KCl
Dopamine in 1.6 mM KCl
80 pA
2 s
50 pA
2 s
B
mIPSC Frequency (Hz)
CdCl2
Dopamine
Dopamine
CdCl2
D
High K+
Percent Reduction
Freq
Amp
C
mIPSC Amplitude (pA)
22 mM KCl
1.6 mM KCl
Time (min)

Figure 57. The $GABA_B$ antagonist CGP35348 does not affect the ability of dopamine to depress excitatory synaptic transmission in the NAc.
A and ***B***, An example experiment demonstrating the effects of dopamine (75 μM) applied to the field first in the absence and then in the presence of CGP35348 (500 μM). ***C***, Summary of similar experiments (n=6) demonstrates the lack of effect of CGP35348.

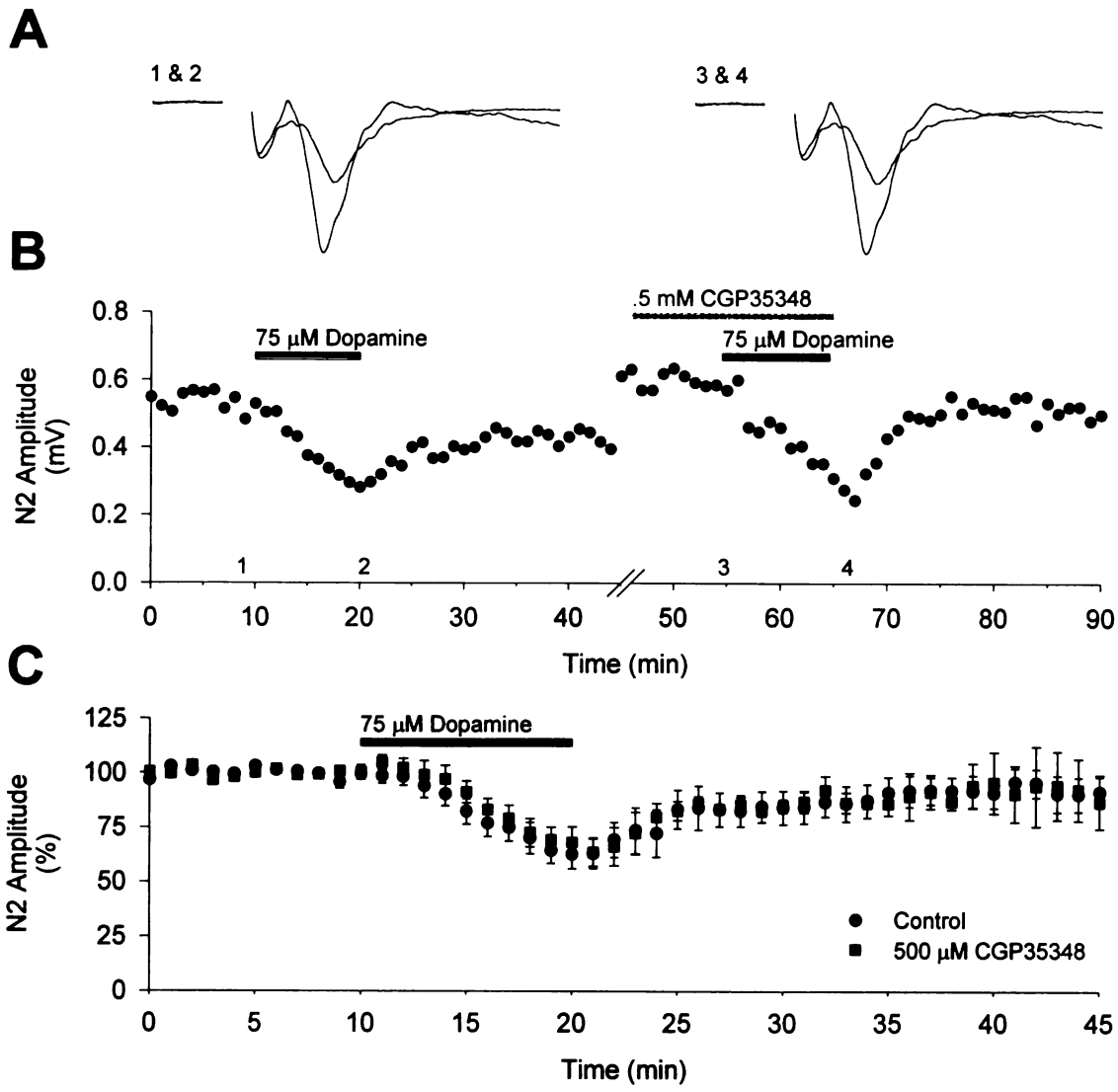

A
1 & 2
3 & 4
B
.5 mM CGP35348
75 μM Dopamine
75 μM Dopamine
N2 Amplitude (mV)
0.8
0.6
0.4
0.2
0.0
1
2
3
4
0
10
20
30
40
50
60
70
80
90
Time (min)
C
75 μM Dopamine
N2 Amplitude (%)
125
100
75
50
25
0
Control
500 μM CGP35348
0
5
10
15
20
25
30
35
40
45
Time (min)

Figure 58. The $GABA_B$ antagonist CGP35348 does not affect the ability of dopamine to depress inhibitory synaptic transmission in the NAc.
A and ***B***, An example experiment demonstrating the effects of dopamine (75 μM) applied to the IPSP first in the absence and then in the presence of CGP35348 (500 μM). ***C***, Summary of similar experiments (n=3) demonstrates the lack of effect of CGP35348.

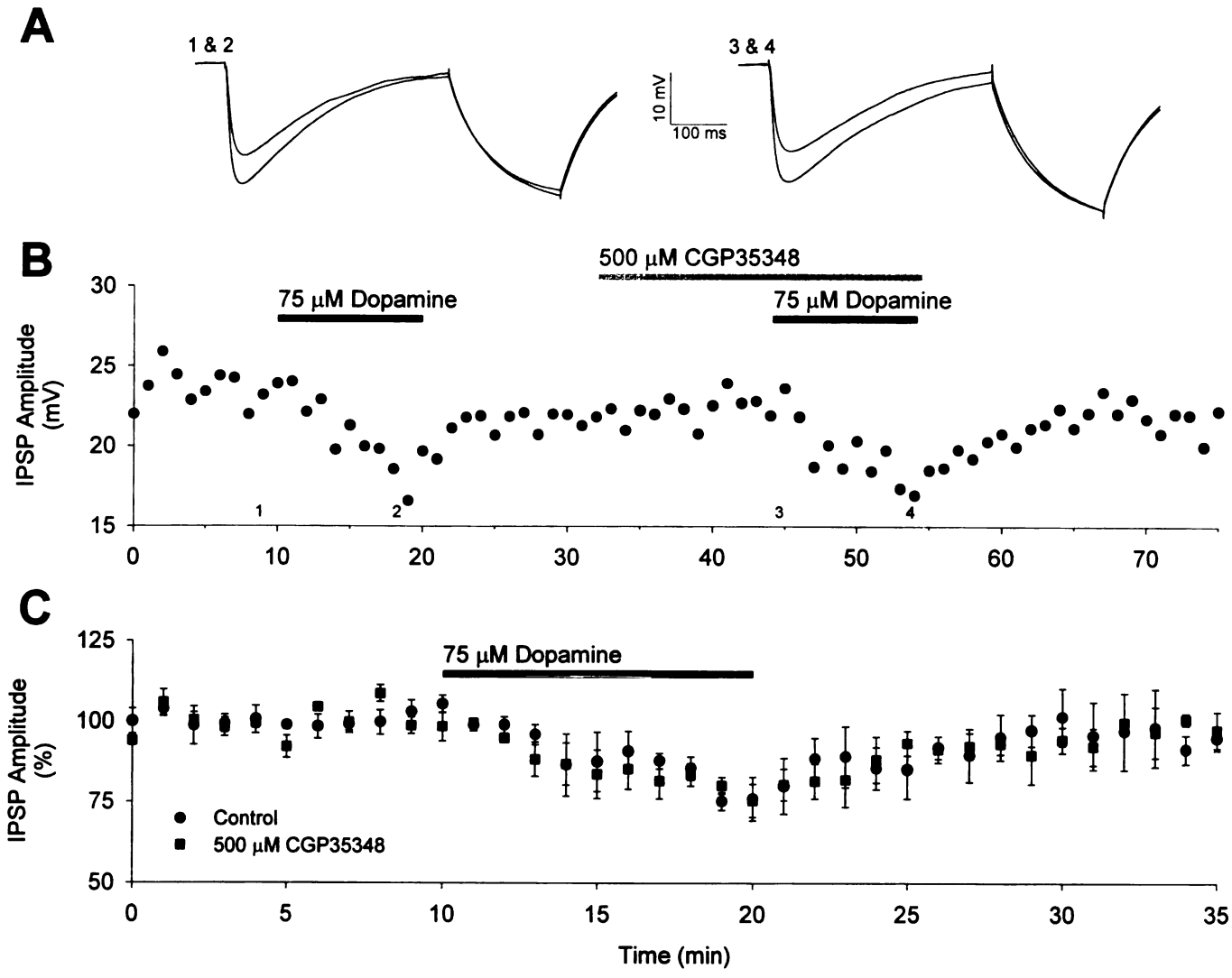

A
1 & 2
3 & 4
10 mV
100 ms
B
500 µM CGP35348
75 µM Dopamine
75 µM Dopamine
IPSP Amplitude (mV)
30
25
20
15
1
2
3
4
0
10
20
30
40
50
60
70
C
75 µM Dopamine
IPSP Amplitude (%)
125
100
75
50
Control
500 µM CGP35348
0
5
10
15
20
25
30
35
Time (min)

Figure 59. Adenosine and NMDA receptor antagonists do not reduce the effects of dopamine on NAc EPSPs and IPSPs.

A, As illustrated by the traces (*top*) taken from an example experiment (*bottom*), the adenosine antagonist 8-CPT (20 μM) does not reduce the effects of dopamine (75 μM) on IPSPs. ***B***, An experiment identical to that in A, except that EPSPs were examined instead of IPSPs. ***C***, The NMDA receptor antagonist d,l-APV (75 μM) does not reduce the magnitude of the depression of EPSPs caused by dopamine (75 μM). ***D***, A summary graph illustrates that none of these antagonists reduced the ability of dopamine to depress EPSPs and IPSPs ($n \geq 2$ for each antagonist). Dopamine was applied twice in each cell, once prior to application of antagonist and once in the presence of the antagonist.

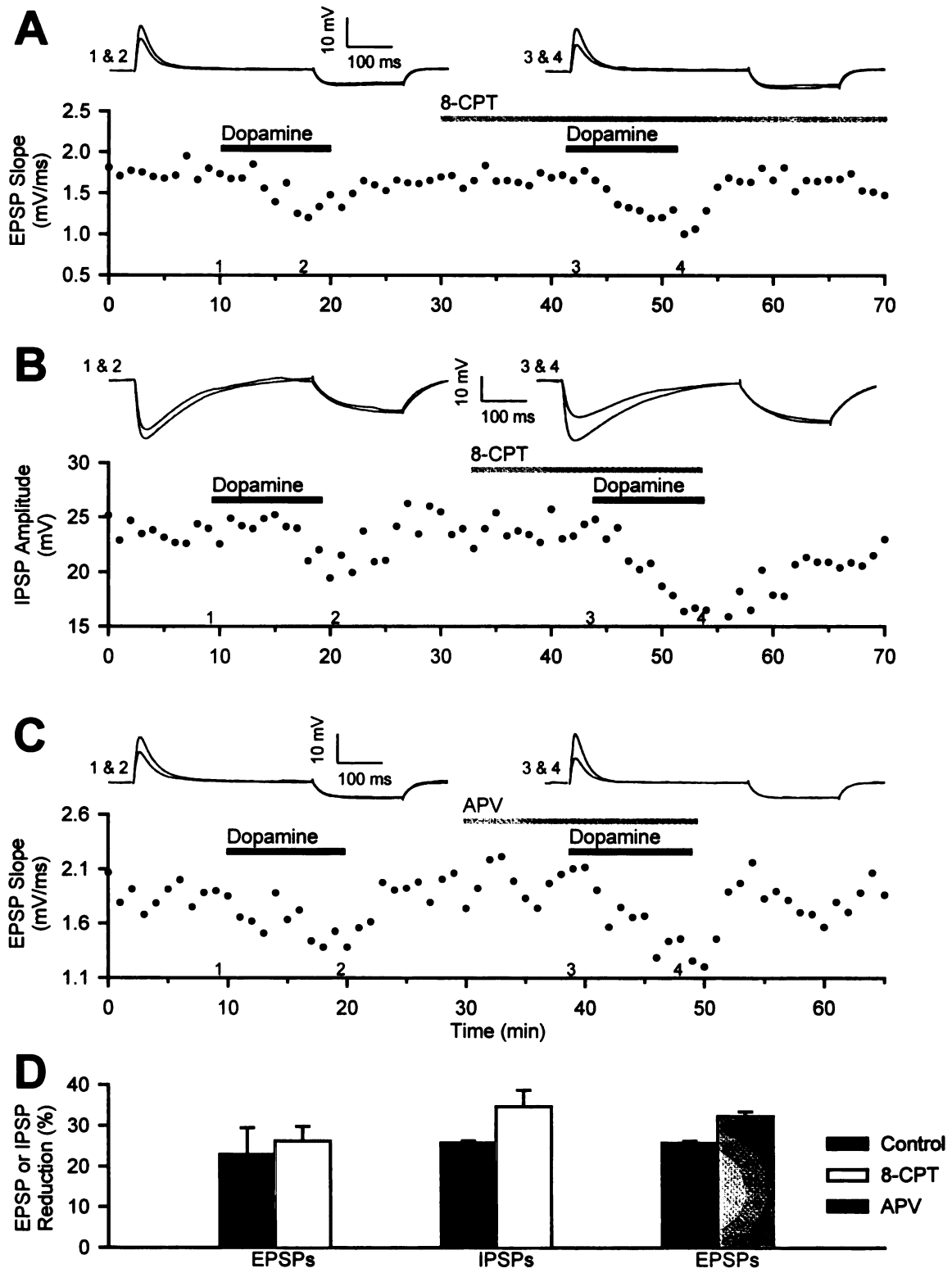

A
1 & 2
10 mV
100 ms
3 & 4
8-CPT
Dopamine
Dopamine
EPSP Slope (mV/ms)
2.5
2.0
1.5
1.0
0.5
1
2
3
4
0
10
20
30
40
50
60
70
B
1 & 2
10 mV
100 ms
3 & 4
8-CPT
Dopamine
Dopamine
IPSP Amplitude (mV)
30
25
20
15
1
2
3
4
0
10
20
30
40
50
60
70
C
1 & 2
10 mV
100 ms
3 & 4
APV
Dopamine
Dopamine
EPSP Slope (mV/ms)
2.6
2.1
1.6
1.1
1
2
3
4
0
10
20
30
40
50
60
Time (min)
D
EPSP or IPSP Reduction (%)
40
30
20
10
0
EPSPs
IPSPs
EPSPs
Control
8-CPT
APV

Figure 60. Forskolin potentiates excitatory synaptic transmission in the NAc. ***A*** and ***B***, An example of an experiment, conducted in 8-CPT (20 μM), in which application of forskolin (50 μM) and IBMX (50 μM) increased the size of the EPSP. ***C***, A summary illustrates the mean effect of forskolin and IBMX (n=12). Seven of these experiments were conducted with 8-CPT (20 μM) in the bathing medium throughout the experiment, and 5 were conducted without 8-CPT.

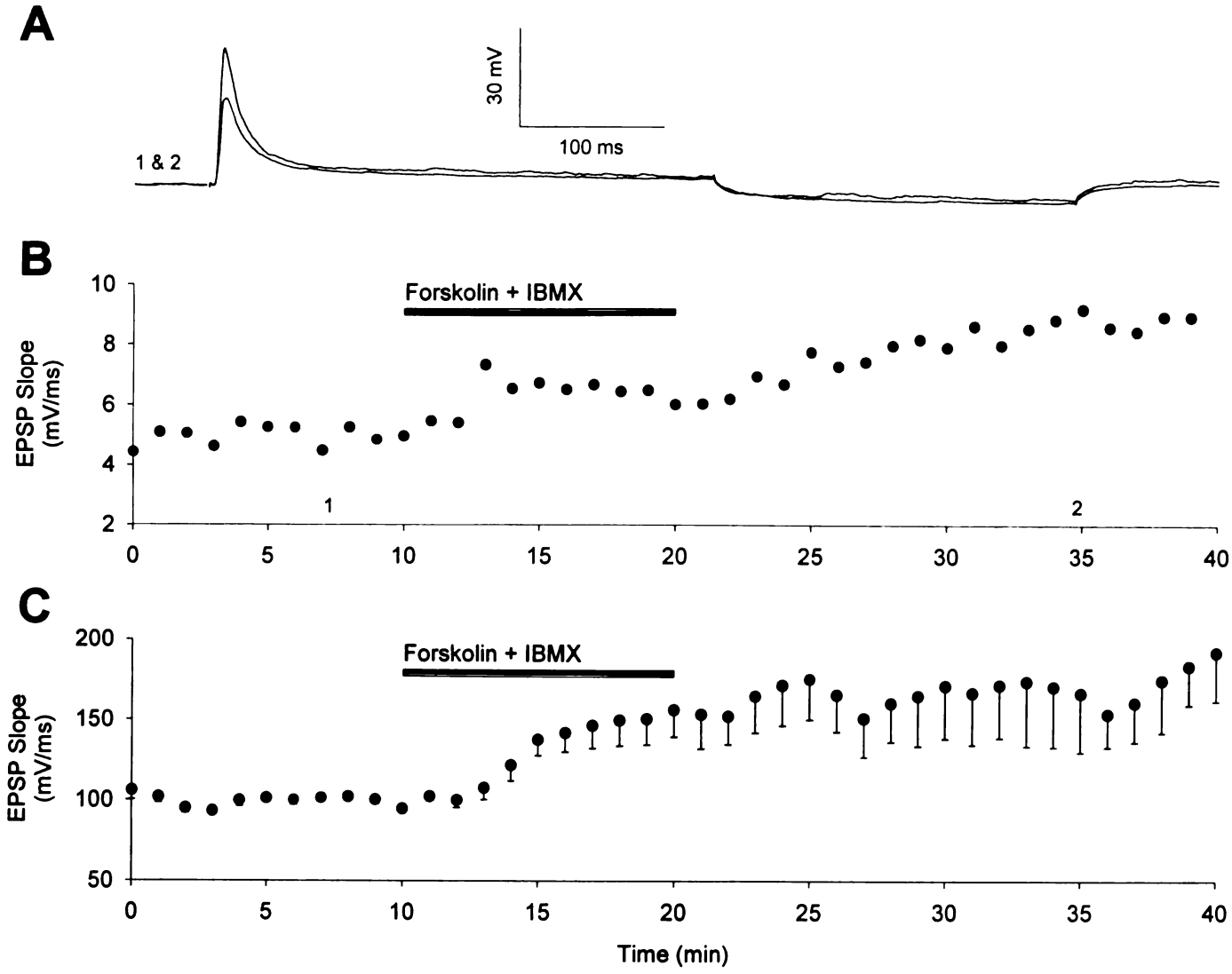

A
30 mV
100 ms
1 & 2
B
Forskolin + IBMX
EPSP Slope (mV/ms)
10
8
6
4
2
1
2
0
5
10
15
20
25
30
35
40
C
Forskolin + IBMX
EPSP Slope (mV/ms)
200
150
100
50
0
5
10
15
20
25
30
35
40
Time (min)

Figure 61. Forskolin has no effect on inhibitory synaptic transmission in the NAc. ***A*** and ***B***, An example of an experiment, conducted in 8-CPT (20 μM), in which application of forskolin (50 μM) and IBMX (50 μM) had no effect on the IPSP. ***C***, A summary illustrates the mean effect of forskolin and IBMX (n=9). Four of these experiments were conducted with 8-CPT (20 μM) in the bathing medium throughout the experiment, and 5 were conducted without 8-CPT.

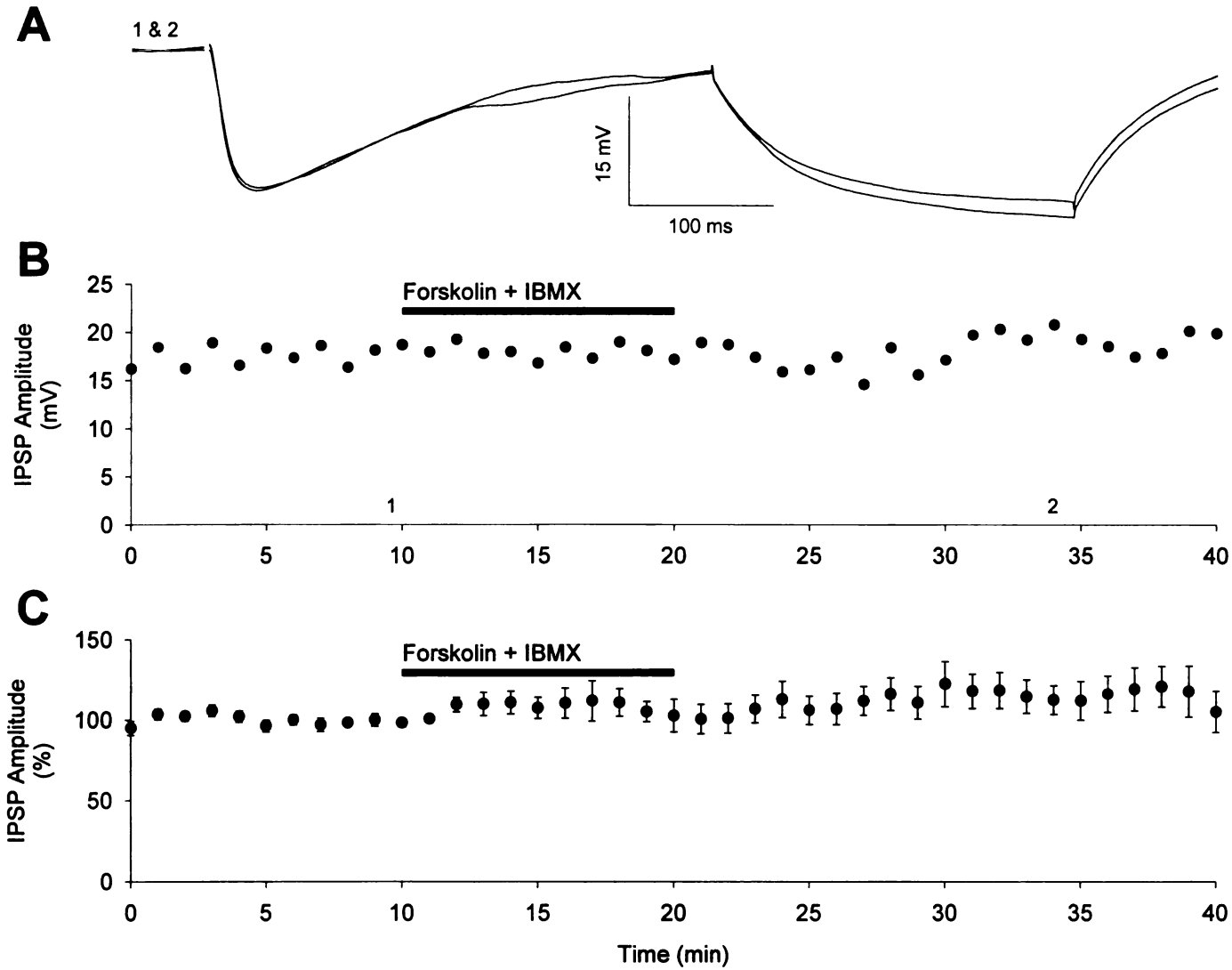
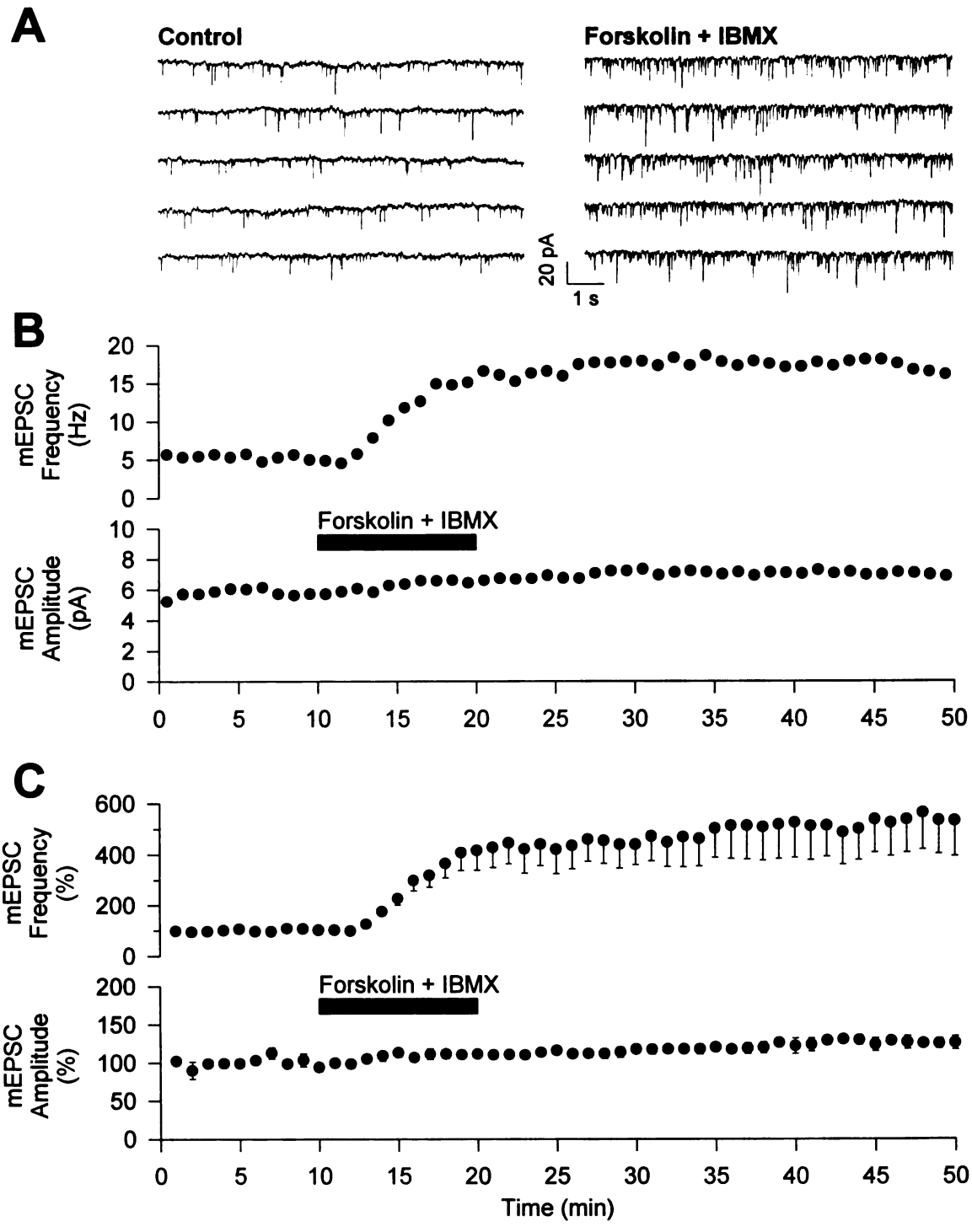

A
1 & 2
15 mV
100 ms
B
Forskolin + IBMX
IPSP Amplitude (mV)
25
20
15
10
5
0
1
2
0
5
10
15
20
25
30
35
40
C
Forskolin + IBMX
IPSP Amplitude (%)
150
100
50
0
0
5
10
15
20
25
30
35
40
Time (min)

Figure 62. Forskolin increases the frequency of mEPSCs in the NAc.
A, Consecutive 10 s sweeps demonstrate the frequency and amplitude of mEPSCs in one cell recorded in 8-CPT before the application (*left*) of 50 μM forskolin and 50 μM IBMX and in the presence of these drugs (*right*). ***B***, In this cell forskolin and IBMX caused an increase in the frequency (*top*) but not the amplitude (*bottom*) of mEPSCs. ***C***, An average from three cells, all of which were recorded in 8-CPT, of the effects of forskolin and IBMX on mEPSC frequency (*top*) and amplitude (*bottom*).


A
Control
Forskolin + IBMX
20 pA
1 s
B
mEPSC Frequency (Hz)
mEPSC Amplitude (pA)
Forskolin + IBMX
C
mEPSC Frequency (%)
mEPSC Amplitude (%)
Forskolin + IBMX
Time (min)

Chapter 6

Conclusions

This work demonstrates that, by acting at presynaptic terminals, dopamine and psychostimulants depress both glutamatergic excitatory and GABAergic inhibitory synaptic transmission in the NAc, but not in the striatum. The receptor responsible for this depression appears to be the same for both excitatory and inhibitory transmission, and its pharmacological properties, while not typical of any dopamine receptor cloned to date, are most consistent with a D1-like receptor. The effects of dopamine are antagonized by the D1 antagonist SCH23390, but D1 and D2 agonists applied at concentrations specific for their receptors have no effect by themselves. However, these concentrations of D1 agonists are capable of antagonizing the effects of dopamine, and higher concentrations can cause synaptic depression that is blocked by SCH23390. The lack of a depressant effect of adenylate cyclase activation is further evidence that the dopamine receptor is atypical. Additional pharmacological experiments suggest that other inhibitory neuromodulators and their receptors, such as norepinephrine, serotonin, adenosine, and GABA acting at $GABA_B$ receptors are not responsible for the effects of dopamine and psychostimulants. Finally, the mechanism of synaptic depression is likely to involve only the reduction of Ca^{2+} influx into GABAergic terminals, while in glutamatergic terminals, a modification of a step in the release process occurring after Ca^{2+} entry and detection is responsible for depression in addition to or instead of a reduction in Ca^{2+} entry.

A number of studies of the actions of dopamine on synaptic transmission have yielded results quite similar to those reported here. An investigation of the effects of dopamine on NAc cells found that dopamine could depress excitatory synaptic transmission while increasing PPF, and that this was blocked by SCH23390 (Pennartz et al., 1992a). An additional observation that dopamine is effective only in the shell, not in the core region of the NAc (Pennartz et al., 1992b) was not replicated in the present work, in which most recordings were made in the core region. A recent more extensive examination of dopamine's effects on excitatory synaptic transmission in the NAc has also revealed a SCH23390-sensitive depression, with no discernable difference between results in the core and shell (Harvey and Lacey, 1996b). The

studies by Pennartz et al. and Harvey and Lacey differ from the present results in one major finding: both of these groups found that D1 agonists (such as SKF38393 and SKF81297) mimic the effects of dopamine, whereas no such effect is found here. One possible explanation for this difference is that the agonists, which are sensitive to oxidation, have broken down in solution before they could reach the slice. Arguing against this possibility, however, are the following: 1) the antioxidant sodium metabisulphite (50 mM in drug stock solutions, 50 μM final concentration in the bathing medium) was included in solutions of all catechol-derived drugs; 2) no fewer than three D1 agonists were used in the present study (SKF38393, SKF81297 and dihydrexidine), none of which, at low concentrations, had significant effects on synaptic transmission by themselves; 3) the oxidizable receptor agonists 6,7-ADTN and norepinephrine were capable of affecting synaptic transmission; and 4) SKF38393 at high concentrations was effective in producing a depression, and at lower concentrations was capable of antagonizing the effects of dopamine, indicating that the agonists most likely binds to the receptor with low affinity without activating it. One difference between the present work and previous studies is that the age of the animals used here was younger (< 3 weeks postnatal) than those of Pennartz et al. (1992a) and Harvey and Lacey (1996b). It is possible that in older animals a different dopamine receptor is expressed in addition to or instead of the one that is present in younger animals. One possibility is that in older animals, activation of a different, postsynaptic D1 receptor (activated by D1 agonists) causes the release of adenosine, which then presynaptically inhibits transmitter release (Harvey and Lacey, 1996a).

The effect of dopamine on excitatory transmission described by Harvey and Lacey (1996b) may also be due to an atypical D1-like receptor. This group found that forskolin potentiated EPSCs, and that dopamine depressed the EPSC to the same extent whether or not forskolin was present. These results are consistent with those reported here, and suggest that the D1-like receptor responsible for the presynaptic effects of dopamine in the NAc is not coupled to adenylate cyclase. However, another group has found D1 receptor-mediated presynaptic inhibition of excitatory and inhibitory

synaptic transmission in magnocellular basal forebrain cells, and that application of forskolin mimics and occludes the depressant effects of dopamine (Momiyama and Sim, 1996; Momiyama et al., 1996). These results are surprising given the enhancement of synaptic transmission by forskolin observed at many different synapses (Bonci and Williams, 1997; Capogna et al., 1995; Chavez-Noriega and Stevens, 1994; Kandel and Schwartz, 1982; Llano and Gerschenfeld, 1993; Salin et al., 1996; Weisskopf et al., 1994; Zhong and Wu, 1991). Novel cAMP-dependent inhibitory mechanisms may therefore be at work in the basal forebrain; apparently this is the case for both GABAergic and glutamatergic terminals.

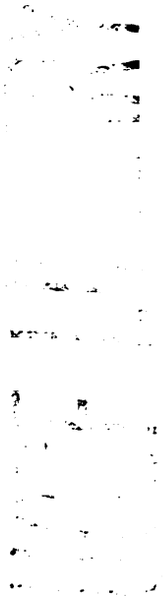
Another interesting finding from this group was that dopamine depressed the frequency of mEPSCs that were Ca^{2+} entry-dependent, but not of those that were Ca^{2+} entry-independent (Momiyama et al., 1996). Ca^{2+} entry was not required, however, to observe depression of mIPSC frequency (Momiyama and Sim, 1996). These results are the converse of those found for activation of the D1-like receptor in the present work: depression of mIPSC frequency occurred only for those mIPSCs that were dependent on Ca^{2+} entry, whereas Ca^{2+} entry was not required for depression of mEPSC frequency. The difference in these findings may be the result of a multiplicity of mechanisms by which D1 receptor activation can depress synaptic transmission among different areas of the brain.

The results described here are also consistent with examinations of dopamine's effects in cortical slices, which have found SCH23390-sensitive depression of excitatory synaptic transmission in the prefrontal cortex (Law-Tho et al., 1994) and entorhinal cortex (Pralong and Jones, 1993). The mechanisms and site of action of dopamine in these cortical areas were not assessed, and could be due to presynaptic mechanisms as reported in the NAc (Pennartz et al., 1992a; this work) and basal forebrain (Momiyama and Sim, 1996; Momiyama et al., 1996); or to postsynaptic effects on voltage-dependent membrane conductances as described in the striatum (Calabresi et al., 1987, 1988; Schiffmann et al., 1995; Surmeier et al., 1992); or to the increased release of GABA as reported in the VTA (Cameron and Williams, 1993).

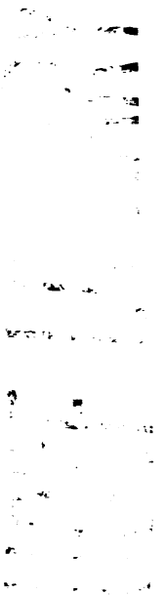
The widespread findings that D1 receptor activation depresses synaptic transmission and cell excitability in many brain nuclei seem to indicate that one primary role of D1 receptors is to inhibit neuronal activity. Furthermore, the findings in the NAc of presynaptic inhibitory actions that are not mimicked by adenylate cyclase activation provides further evidence either for the coupling of additional second messenger systems to D1 receptors, or for the existence of D1 receptors that to date have not yet been cloned.

Clarification of the effects of dopamine and psychostimulants on excitatory and inhibitory synaptic transmission in the NAc has important consequences for theories of drug addiction. Robinson and Berridge (1993) propose that addiction is a consequence of sensitization of the salience of objects present in the environment while the animal is under the influence of the drug. In other words, over time and repeated use, psychostimulants somehow cause any object associated with the drug and the mental state it induces to become recognized as extremely important. Evidence for this contention comes from the fact that even after years of abstinence, addicts who attempt to give up the drug often find themselves unable to resist the temptation to take the drug when exposed even briefly to people, surroundings or paraphernalia associated with earlier use of the drug. Because of the known involvement of the NAc in natural reward (Le Moal and Simon, 1991), it is reasonable to posit that one of the functions of the NAc may be to provide the information that particular stimuli in the environment are salient because of their potential for providing reward. The role of dopamine, which is released by the axon terminals of VTA cells that fire when the animal receives a reward (Mirenowicz and Schultz, 1996), may then be to "inform" the NAc that a rewarding event is occurring.

One means by which this information may be delivered is by the enhancement of the signal-to-noise ratio for stronger excitation of the NAc, perhaps in part as a result of the reduction of both excitatory and inhibitory synaptic transmission. Such an enhancement might cause those NAc cells that are most strongly activated to respond more vigorously to stimuli that would otherwise be lost in the background noise firing.



Thus, in the presence of psychostimulants, the enhanced effects of dopamine may result in inappropriate stimuli becoming recognized as salient. Repeated use of the drug in circumstances that are of necessity similar to previous circumstances (e.g., a crack pipe is always required, the place of use is often the same from binge to binge, the dealer is often the same, etc.) would cause those repeated stimuli to take on vastly increased importance. This might be the result of a contribution of the NAc or its outputs to the formation of long-term associations. While this theory of the role of dopamine in the NAc in drug addiction is by necessity speculative, many of its elements are currently testable with *in vivo* recording in the NAc and other relevant nuclei of drug-seeking animals.



References

Abercrombie, ED and Jacobs, BL (1985) Dopaminergic modulation of sensory responses of striatal neurons: single unit studies. *Brain Res.* 358:27-33.

Agnati, LF, Zoli, M, Strömberg, I and Fuxe, K (1995) Intercellular communication in the brain: wiring versus volume transmission. *Neurosci.* 69:711-726.

Akaike, A, Ohno, Y, Sasa, M and Takaori, S (1987) Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons in vitro. *Brain Res.* 418:262-272.

Albin, RL, Young, AB and Penney, JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12:366-375.

Alexander, GE and Crutcher, MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci.* 13:266-271.

Altemus, KL and Levine, MS (1996) Potassium channel blockade does not alter the modulatory effects of dopamine in neostriatal slices. *Brain Res.* 718:212-216.

American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders.* Washington, DC:American Psychiatric Association.

Andén, N-E, Fuxe, K, Hamberger, B and Hökfelt, T (1966) A quantitative study on the nigro-neostriatal dopamine neuron system in the rat. *Acta Physiol. Scand.* 67:306-312.

Andersen, PH, Gingrich, JA, Bates, MD, Dearry, A, Falardeau, P, Senogles, SE and Caron, MG (1990) Dopamine receptor subtypes: beyond the D_1/ D_2 classification. *Trends Pharmacol. Sci.* 11:231-236.

Andersen, PH and Jansen, JA (1990) Dopamine receptor agonists: selectivity and dopamine D_1 receptor efficacy. *Eur. J. Pharmacol. – Mol. Pharmacol. Sect.* 188:335-347.

Arnt, J, Hyttel, J and Sánchez, C (1992) Partial and full dopamine D_1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity in vitro. *Eur. J. Pharmacol.* 213:259-267.

Ascher, P and Nowak, L (1988) Quisqualate- and kainate-activated channels in mouse central neurones in culture. *J. Physiol.* 399:227-245.

Augustine, GJ and Eckert, R (1984) Divalent cations differentially support transmitter release at the squid giant synapse. *J. Physiol.* 346:257-271.

Baxter, BL, Gluckman, MI, Stein, L and Scerni, RA (1974) Self-injection of

apomorphine in the rat: positive reinforcement by a dopamine receptor stimulant. *Pharmacol, Biochem. Behav.* 2:387-391.

Beebe, DK and Walley, E (1995) Smokable methamphetamine ('ice'): an old drug in a different form. *Amer. Fam. Physician* 51:449-453.

Ben-Jonathan, N (1985) Dopamine: a prolactin-inhibiting hormone. *Endocrine Rev.* 6:564-589.

Bernardi, G, Marciani, MG, Morocutti, C, Pavone, F and Stanzione, P (1978) The action of dopamine on rat caudate neurones intracellularly recorded. *Neurosci. Lett.* 8:235-240.

Blanton, M, LoTurco, J and Kriegstein, A (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Meth.* 30:203-210.

Bonci, A and Williams, JT (1996) A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine. *Neuron* 16:631-639.

Bonci, A and Williams, JT (1997) Increased probability of GABA release during withdrawal from morphine. *J. Neurosci.* 17:796-803.

Boundy, VA, Luedtke, RR, Gallitano, AL, Smith, JE, Filtz, TM, Kallen, RG and Molinoff, PB (1993) Expression and characterization of the rat D3 dopamine receptor: pharmacologic properties and development of antibodies. *J. Pharmacol. Exp. Ther.* 264:1002-1011.

Bouyer, JJ, Joh, TH and Pickel, VM (1984a) Ultrastructural localization of tyrosine hydroxylase in rat nucleus accumbens. *J. Comp. Neurol.* 227:92-102.

Bouyer, JJ, Park, DH, Joh, TH and Pickel, VM (1984b) Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylase-containing terminals in rat neostriatum. *Brain Res.* 302:267-275.

Bozarth, MA and Wise, RA (1985) Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. *J. Amer. Med. Assoc.* 254:81-83.

Brewster, WK, Nichols, DE, Riggs, RM, Mottola, DM, Lovenberg, TW, Lewis, MH and Mailman, RB (1990) *trans*-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine: a highly potent selective dopamine D_1 full agonist. *J. Med. Chem.* 33:1756-1764.

Britton, DR, Curzon, P, MacKenzie, RG, Kebabian, JW, Williams, JEG and Kerkman, D (1991) Evidence for involvement of both D1 and D2 receptors in maintaining

cocaine self-administration. *Pharmacol. Biochem. Behav.* 39:911-915.

Brown, JR and Arbuthnott, GW (1983) The electrophysiology of dopamine (D_2) receptors: a study of the actions of dopamine on corticostriatal transmission. *Neurosci.* 10:349-355.

Bunney, BS and Aghajanian, GK (1976) Dopamine and norepinephrine innervated cells in the rat prefrontal cortex: pharmacological differentiation using microiontophoretic techniques. *Life Sci.* 19:1783-1792.

Bunzow, JR, Van Tol, HHM, Grandy, DK, Albert, P, Salon, J, Christie, M, Machida, CA, Neve, KA and Civelli, O. (1988) Cloning and expression of a rat D_2 dopamine receptor cDNA. *Nature* 336:783-787.

Caillé, I, Dumartin, B and Bloch, B (1996) Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. *Brain Res.* 730:17-31.

Caine, SB, Heinrichs, SC, Coffin, VL and Koob, GF (1995) Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res.* 692:47-56.

Caine, SB and Koob, GF (1994a) Effects of dopamine D-1 and D-2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. *J. Pharmacol. Exp. Ther.* 270:209-218.

Caine, SB and Koob, GF (1994b) Effects of mesolimbic dopamine depletion on responding maintained by cocaine and food. *J. Exp. Anal. Behav.* 61:213-221.

Calabresi, P, Benedetti, M, Mercuri, NB and Bernardi, G (1988) Endogenous dopamine and dopaminergic agonists modulate synaptic excitation in neostriatum: intracellular studies from naive and catecholamine-depleted rats. *Neurosci.* 27:145-157.

Calabresi, P, De Murtas, M, Mercuri, NB and Bernardi, G (1992) Chronic neuroleptic treatment: D2 dopamine receptor supersensitivity and striatal glutamatergic transmission. *Ann. Neurol.* 31:366-373.

Calabresi, P, De Murtas, M, Pisani, A, Stefani, A, Sancesario, G, Mercuri, NB and Bernardi, G (1995) Vulnerability of medium spiny striatal neurons to glutamate: role of Na^+/K^+ ATPase. *Eur. J. Neurosci.* 7:1674-1683.

Calabresi, P, Mercuri, NB, Sancesario, G and Bernardi, G (1993) Electrophysiology of dopamine-denervated striatal neurons. *Brain* 116:433-452.

Calabresi, P, Mercuri, N, Stanzione, P, Stefani, A and Bernardi, G (1987) Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons *in vitro:* evidence for D1 receptor involvement. *Neurosci.* 20:757-771.

Calabresi, P, Pisani, A, Mercuri, NB and Bernardi, G (1996) the corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. *Trends Neurosci.* 19:19-24.

Cameron, DL and Williams, JT (1993) Dopamine D1 receptors facilitate transmitter release. *Nature* 366:344-347.

Capogna, M, Gähwiler, BH and Thompson, SM (1995) Presynaptic enhancement of inhibitory synaptic transmission by protein kinases A and C in the rat hippocampus *in vitro*. 15:1249-1260.

Carelli, RM and Deadwyler, SA (1994) A comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats. *J. Neurosci.* 14:7735-7746.

Carelli, RM and Deadwyler, SA (1996a) Dose-dependent transitions in nucleus accumbens cell firing and behavioral responding during cocaine self-administration sessions in rats. *J. Pharmacol. Exp. Ther.* 277:385-393.

Carelli, RM and Deadwyler, SA (1996b) Dual factors controlling activity of nucleus accumbens cell-firing during cocaine self-administration. *Synapse* 24:308-311.

Carelli, RM, King, VC, Hampson, RE and Deadwyler, SA (1993) Firing patterns of nucleus accumbens neurons during cocaine self-administration in rats. *Brain Res.* 626:14-22.

Castañeda, E, Becker, JB and Robinson, TE (1988) The long-term effects of repeated amphetamine treatment *in vivo* on amphetamine, KCl and electrical stimulation evoked striatal dopamine release *in vitro*. *Life Sci.* 42:2447-2456.

del Castillo, J and Katz, B (1954a) Changes in end-plate activity produced by pre-synaptic polarization. *J. Physiol.* 124:586-604.

del Castillo, J and Katz, B (1954b) Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol.* 124:574-585.

Cepeda, C, Buchwald, NA and Levine, MS (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proc. Natl. Acad. Sci. U. S. A.* 90:9576-9580.

Cepeda, C, Chandler, SH, Shumate, LW and Levine, MS (1995) Persistent Na^+ conductance in medium-sized neostriatal neurons: characterization using infrared videomicroscopy and whole cell patch-clamp recordings. *J. Neurophysiol.* 74:1343-1348.

Chabert, C, Cavegn, C, Bernard, A and Mills, A (1994) Characterization of the functional activity of dopamine ligands at human recombinant dopamine D_4 receptors. *J. Neurochem.* 63:62-65.

Chang, HT and Kitai, ST (1985) Projection neurons of the nucleus accumbens: an intracellular labeling study. *Brain Res.* 347:112-116.

Chang, J-Y, Sawyer, SF, Lee, R-S and Woodward, DJ (1994) Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats. *J. Neurosci.* 14:1224-1244.

Chavez-Noriega, LE and Stevens, CF (1994) Increased transmitter release at excitatory synapses produced by direct activation of adenylate cyclase in rat hippocampal slices. *J. Neurosci.* 14:310-317.

Chiodo, LA and Berger, TW (1986) Interactions between dopamine and amino acid-induced excitation and inhibition in the striatum. *Brain Res.* 375:198-203.

Christie, MJ, Summers, RJ, Stephenson, JA, Cook, CJ and Beart, PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[^{3}H]aspartate and [^{3}H]GABA. *Neurosci.* 22:425-439.

Civelli, O, Bunzow, JR and Grandy, DK (1993) Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* 32:281-307.

Cohen, GA, Doze, VA and Madison, DV (1992) Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons. *Neuron* 9:325-335.

Conn, EE, Stumpf, PK, Bruening, G and Doi, RH (1987) *Outlines of biochemistry.* New York: John Wiley and Sons

Chronister, RB, Sikes, RW, Trow, TW and DeFrance, JF (1981) The organization of the nucleus accumbens. In *The Neurobiology of the Nucleus Accumbens*, RB Chronister and JF DeFrance, eds. Brunswick, ME: Haer Institute.

Cornish, JW and O'Brien, CP (1996) Crack cocaine abuse: an epidemic with many public health consequences. *Annu. Rev. Public Health* 17:259-273.

Corrigall, WA and Coen, KM (1991) Cocaine self-administration is increased by both D1 and D2 dopamine antagonists. *Pharmacol. Biochem. Behav.* 39:799-802.

Creager, R, Dunwiddie, T and Lynch, G (1980) Paired-pulse and frequency facilitation in the CA1 region of the *in vitro* rat hippocampus. *J. Physiol.* 299:409-424.

Creese, I, Burt, DR and Snyder, SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:481-483.

Dahlström, A and Fuxe, K (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta Physiol. Scand. Suppl.* 232:1-55.

Das, G (1993) Cocaine abuse in North America: a milestone in history. *J. Clin. Pharmacol.* 33:296-310.

Davies, CH, Davies, SN and Collingridge, GL (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol.* 424:513-531.

Dearry, A, Gingrich, JA, Falardeau, P, Fremeau, RT Jr, Bates, MD and Caron, MG (1990) Molecular cloning and expression of the gene for a human D_1 dopamine receptor. *Nature* 347:72-76.

DeFrance, JF, Marchand, JJ, Sikes, RW, Chronister, RB and Hubbard, JI (1985a) Characterization of fimbria input to nucleus accumbens. *J. Neurophysiol.* 54:1553-1567.

DeFrance, JF, Sikes, RW and Chronister, RB (1985b) Dopamine action in the nucleus accumbens. *J. Neurophysiol.* 54:1568-1577.

Delle Donne, KT, Sesack, SR and Pickel, VM (1996) Ultrastructural immunocytochemical localization of neurotensin and the dopamine D_2 receptor in the rat nucleus accumbens. *J. Comp. Neurol.* 371:552-566.

Demchyshyn, LL, Sugamori, KS, Lee, FJS, Hamadanizadeh, SA and Niznik, HB (1995) The dopamine D1D receptor. *J. Biol. Chem.* 270:4005-4012.

Deneau, G, Yanagita, T, and Seevers, MH (1969) Self-administration of psychoactive substances by the monkey. *Psychopharmacologia* 16:30-48.

Derlet, RW and Heischober, B (1990) Methamphetamine: stimulant of the 1990s? *West. J. Med.* 153:625-628.

Descarries, L, Watkins, KC, Garcia, S, Bosler, O and Doucet, G (1996) Dual character,

asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *J. Comp. Neurol.* 375:167-186.

Deveney, AM and Waddington, JL (1995) Pharmacological characterization of behavioural responses to SK&F 83959 in relation to 'D_1-like' dopamine receptors not linked to adenylyl cyclase. *Brit. J. Pharmacol.* 116:2120-2126.

De Wit, H and Wise, RA (1977) Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Canad. J. Psychol.* 31:195-203.

Dowling, JE (1991) Retinal neuromodulation: the role of dopamine. *Vis. Neurosci.* 7:87-97.

Downes, RP and Waddington, JL (1993) Grooming and vacuous chewing induced by SK&F 83959, and agonist of dopamine 'D_1-like' receptors that ***inhibits*** dopamine-sensitive adenylyl cyclase. *Eur. J. Pharmacol.* 234:135-136.

Doze, VA, Cohen, GA and Madison, DV (1995) Calcium channel involvement in $GABA_B$ receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *J. Neurophysiol.* 74:43-53.

Dray, A (1980) The physiology and pharmacology of mammalian basal ganglia. *Prog. Neurobiol.* 14:221-335.

Dunwiddie, TV, Proctor, WR and Tyma, J (1988) Local anaesthetic actions of cocaine: effects on excitatory and inhibitory synaptic responses in the hippocampus *in vitro*. *Brit. J. Pharmacol.* 95:1117-1124.

Ettenberg, A, Pettit, HO, Bloom, FE and Koob, GF (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. *Psychopharmacol.* 78:204-209.

Fatt, P and Katz, B (1952) Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* 117:109-128.

Feng, G, Hannan, F, Reale, V, Hon, YY, Kousky, CT, Evans, PD and Hall, LM (1996) Cloning and functional characterization of a novel dopamine receptor from *Drosophila melanogaster*. *J. Neurosci.* 16:3925-3933.

Ferron, A, Thierry, AM, Le Douarin, C and Glowinsky, J (1984) Inhibitory influence of the mesocortical dopaminergic system on spontaneous activity or excitatory response induced from the thalamic mediodorsal nucleus in the rat medial prefrontal

cortex. *Brain Res.* 302:257-265.

Fibiger, HC, LePiane, FG, Jakubovic, A and Phillips, AG (1987) The role of dopamine in intracranial self-stimulation of the ventral tegmental area. *J. Neurosci.* 7:3888-3896.

Fisher, RS, Levine, MS, Sibley, DR and Ariano, MA (1994) D_2 dopamine receptor protein location: golgi impregnation-gold toned and ultrastructural analysis of the rat neostriatum. *J. Neurosci. Res.* 38:551-564.

Fonnum, F and Walaas, I (1981) Localization of neurotransmitters in nucleus accumbens. In *The Neurobiology of the Nucleus Accumbens*, RB Chronister and JF DeFrance, eds. Brunswick, ME: Haer Institute.

Freedman, JE and Weight, FF (1988) Single K^+ channels activated by D_2 dopamine receptors in acutely dissociated neurons from rat corpus striatum. *Proc. Natl. Acad. Sci. U. S. A.* 85:3618-3622.

Freedman, SB, Patel, S, Marwood, R, Emms, F, Seabrook, GR, Knowles, MR and McAllister, G (1994) Expression and pharmacological characterization of the human D_3 dopamine receptor. *J. Pharmacol. Exp. Ther.* 268:417-426.

Freund, TF, Powell, JF and Smith, AD (1984) Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. *Neurosci.* 13:1189-1215.

Garcia-Munoz, M, Young, SJ and Groves, PM (1991) Terminal excitability of the corticostriatal pathway. I. Regulation by dopamine receptor stimulation. *Brain Res.* 551:195-206.

Garris, PA, Ciolkowski, EL, Pastore, P and Wightman, RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J. Neurosci.* 14:6084-6093.

Geijo-Barrientos, E and Pastore, C (1995) The effects of dopamine on the subthreshold electrophysiological responses of rat prefrontal cortex neurons *in vitro*. *Eur. J. Neurosci.* 7:358-366.

Gerfen, CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. *J. Neural Transm. Suppl.* 36:43-59.

Gingrich, JA, Dearry, A, Falardeau, P, Fremeau, RT Jr, Bates, MD and Caron, MG (1991) Molecular characterization of G-protein coupled receptors: isolation and cloning of a D1 dopamine receptor. *J. Receptor Res.* 11:521-534.

Gnanalingham, KK, Hunter, AJ, Jenner, P and Marsden, CD (1995) Stimulation of adenylate cycalse activity by benzazepine D-1 dopamine agonists with varying efficacies in the 6-hydroxydopamine lesioned rat – relationship to circling behaviour. *Biochem. Pharmacol.* 49:1185-1193.

Goeders, NE, Dworkin, SI and Smith, JE (1986) Neuropharmacological assessment of cocaine self-administration into the medial prefrontal cortex. *Pharmacol. Biochem. Behav.* 24:1429-1440.

Goeders, NE and Smith, JE (1983) Cortical dopaminergic involvement in cocaine reinforcement. *Science* 221:773-775.

Goeders, NE and Smith, JE (1985) Reinforcing properties of cocaine in the medial prefrontal cortex: primary action on presynaptic dopaminergic terminals. *Pharmacol. Biochem. Behav.* 25:191-199.

Goldberg, LI (1972) Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol. Rev.* 24:1-29.

Goldman-Rakic, PS and Selemon, LD (1990) New frontiers in basal ganglia research. *Trends Neurosci.* 13:241-244.

Grandy, DK, Zhang, Y, Bouvier, C, Zhou, Q-Y, Johnson, RA, Allen, L, Buck, K, Bunzow, JR, Salon, J and Civelli, O (1991) Multiple human D_5 dopamine receptor genes: a functional receptor and two pseudogenes. *Proc. Natl. Acad. Sci. U. S. A.* 88:9175-9179.

Gratton, A (1996) In vivo analysis of the role of dopamine in stimulant and opiate self-administration. *J. Psychiat. Neurosci.* 21:264-279.

Graybiel, AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* 13:244-254.

Graybiel, AM, Aosaki, T, Flaherty, AW and Kimura, M (1994) The basal ganglia and adaptive motor control. *Science* 265:1826-1831.

Greene, RW and Haas, HL (1991) The electrophysiology of adenosine in the mammalian central nervous system. *Prog. Neurobiol.* 36:329-341.

Greengard, P, Jen, J, Nairn, A and Stevens, CF (1991) Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science* 253:1135-1138.

Greif, GJ, Lin, Y-J, Liu, J-C and Freedman, JE (1995) Dopamine-modulated potassium

channels on rat neostriatal neurons: specific activation and cellular expression. *J. Neurosci.* 15:4533-4544.

Groves, PM (1983) A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. *Brain Res. Rev.* 5:109-132.

Harvey, J and Lacey, MG (1996a) Dopamine depresses EPSCs in the nucleus accumbens via NMDA receptor-dependent release of adenosine. *Soc. Neurosci. Abstr.* 22:1739.

Harvey, J and Lacey, MG (1996b) Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens *in vitro* via D_1 receptor activation. *J. Physiol.* 492:143-154.

Hefti, F, Melamed, E, Sahakian, BJ and Wurtman, RJ (1979) Circling behavior in rats with partial, unilateral nigro-striatal lesions: effect of amphetamine, apomorphine, and DOPA. *Pharmacol. Biochem. Behav.* 12:185-188.

Hefti, F, Melamed, E and Wurtman, RJ (1980) Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterization. *Brain Res.* 195:123-137.

Heimer, L, Switzer, RD and Van Hoesen, GW (1982) Ventral striatum and ventral pallidum: components of the motor system? *Trends Neurosci.* 5:83-87.

Heimer, L and Wilson, RD (1975) The subcortical projections of the allocortex: similarities in the neural associations of the hippocampus, the piriform cortex, and the neocortex. In *Golgi Centennial Symposium: Perspectives in Neurobiology*, M Santini, ed. New York: Raven Press.

Herkenham, M (1987) Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. *Neurosci.* 23:1-38.

Hernández-López, S, Bargas, J, Reyes, A and Galarrage, E (1996) Dopamine modulates the afterhyperpolarization in neostriatal neurones. *Neuroreport* 7:454-456.

Herrling, PL and Hull, CD (1980) Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. *Brain Res.* 192:441-462.

Hersch, SM, Ciliax, BJ, Gutekunst, C-A, Rees, HD, Heilman, CJ, Yung, KKL, Bolam, JP, Ince, E, Yi, H and Levey, AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J. Neurosci.* 15:5222-5237.

Hestrin, S, Nicoll, RA, Perkel, DJ and Sah, P (1990) Analysis of excitatory synaptic

action in pyramidal cells using whole-cell recording from rat hippocampal slices. *J. Physiol.* 422:203-225.

Higashi, H, Inanaga, K, Nishi, S and Uchimura, N (1989) Enhancement of dopamine actions on rat nucleus accumbens neurones *in vitro* after methamphetamine pre-treatment. *J. Physiol.* 408:587-603.

Himmler, A, Stratowa, C and Czernilofsky, AP (1993) Functional testing of human D_1 and D_5 receptors expressed in stable cAMP-responsive luciferase reporter cell lines. *J. Receptor Res.* 13:79-94.

Hoebel, BG, Monaco, AP, Hernandez, L, Aulisi, EF, Stanley, BG and Lenard, L (1983) Self-injection of amphetamine directly into the brain. *Psychopharmacol.* 81:158-163.

Horne, AL, Woodruff, GN and Kemp, JA (1990) Synaptic potentials mediated by excitatory amino acid receptors in the nucleus accumbens of the rat, *in vitro. Neuropharmacol.* 29:917-921.

Hsu, K-S, Huang, CC, Yang, C-H and Gean, P-W (1995) Presynaptic D_2 dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. *Brain Res.* 690:264-268.

Hu, X-T and Wang, RY (1988) Comparison of effects of D-1 and D-2 dopamine receptor agonists on neurons in the rat caudate putamen: an electrophysiological study. *J. Neurosci.* 8:4340-4348.

Huang, Q, Zhou, D, Chase, K, Gusella, JF, Aronin, N and DiFiglia, M (1992) Immunohistochemical localization of the D_1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. *Proc. Natl. Acad. Sci. U. S. A.* 89:11988-11992.

Iorio, LC, Barnett, A, Leitz, FH, Houser, VP and Korduba, CA (1983) SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. *J. Pharmacol. Exp. Ther.* 226:462-468.

Israel, JM, Kirk, C and Vincent, JD (1987) Electrophysiological responses to dopamine of rat hypophysial cells in lactotroph-enriched primary cultures. *J. Physiol.* 390:1-22.

Jablensky, A (1995) Schizophrenia: recent epidemiological issues. *Epidemiol. Rev.* 17:10-20.

Jaeger, D, Kita, H and Wilson, CJ (1994) Surround inhibition among projection neurons is weak or nonexistent in the rat neostriatum. *J. Neurophysiol.* 72:2555-2558.

Jarolimek, W and Misgeld, U (1997) $GABA_B$ receptor-mediated inhibition of tetrodotoxin-resistant GABA release in rodent hippocampal CA1 pyramidal cells. *J. Neurosci.* 17:1025-1032.

Jarolimek, W and Misgeld, U (1992) On the inhibitory actions of baclofen and γ-aminobutyric acid in rat ventral midbrain culture. *J. Physiol.* 451:419-443.

Jarvie, KR, Tiberi, M, Silvia, C, Gingrich, JA and Caron, MG (1993) Molecular cloning, stable expression and desensitization of the human dopamine D1B/D5 receptor. *J. Receptor Res.* 13:573-590.

Javitch, JA, Kaback, J, Li, X and Karlin, A (1994) Expression and characterization of human dopamine D_2 receptor in baculovirus-infected insect cells. *J. Receptor Res.* 14:99-117.

Jay, TM, Glowinski, J and Thierry, AM (1995) Inhibition of hippocampo-prefrontal cortex excitatory responses by the mesocortical DA system. *Neuroreport* 6:1845-1848.

Jellinger, KA (1995) Neurodegenerative disorders with extrapyramidal features – a neuropathological overview. *J. Neural Transm. Suppl.* 46:33-57.

Jensen, AA, Pedersen, UB, Din, N and Andersen, PH (1996) The dopamine D_1 receptor family: structural and functional aspects. *Biochem. Soc. Trans.* 24:163-169.

Johansen, PA, Hu, X-T and White, FJ (1991) Relationship between D1 dopamine receptors, adenylate cyclase, and the electrophysiological responses of rat nucleus accumbens neurons. *J. Neural. Transm. Gen. Sect.* 86:97-113.

Johanson, CE, Balster, RL and Bonese, K (1976) Self-administration of psychomotor stimulant drugs: the effects of unlimited access. *Pharmacol. Biochem. Behav.* 4:45-51.

Johnson, LR, Aylward, RLM, Hussain, Z and Totterdell, S (1994) Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. *Neurosci.* 61:851-865.

Johnson, SW, Palmer, MR and Freedman, R (1983) Effects of dopamine on spontaneous and evoked activity of caudate neurons. *Neuropharmacol.* 22:843-851.

Juhl, RP, Tsuang, MT and Perry, PJ (1977) Concomitant administration of haloperidol and lithium carbonate in acute mania. *Dis. Nervous Sys.* 38:675-676.

Kalivas, PW and Duffy, P (1988) Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. *J. Neurochem.* 50:1498-1504

Kalivas, PW and Stewart, J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res. Rev.* 16:223-244.

Kandel, ER and Schwartz, JH (1982) Molecular biology of learning: modulation of transmitter release. *Science* 218:433-443.

Katz, B (1966) *Nerve, muscle and synapse.* New York: McGraw Hill.

Katz, B and Miledi, R (1968) The role of calcium in neuromuscular fascilitation. *J. Physiol.* 195:481-492.

Kawaguchi, Y, Wilson, CJ, Augwood, SJ and Emson, PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. *Trends Neurosci.* 18:527-535.

Kebabian, JW and Calne, DB (1979) Multiple receptors for dopamine. *Nature* 277:93-96.

Kelly, PH, Seviour, PW and Iversen, SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* 94:507-522.

Kelly, PH and Iversen, SD (1976) Selective 6OHDA-inducded destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *Eur. J. Pharmacol.* 40:45-56.

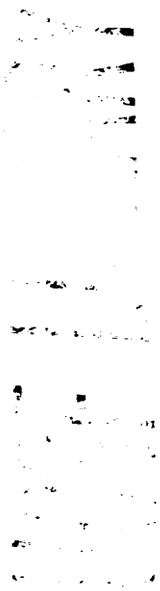
Kim, K-M, Nakajima, Y and Nakajima, S (1995) G protein-coupled inward rectifier modulated by dopamine agonists in cultured substantia nigra neurons. *Neurosci.* 69:1145-1158.

Kimble, GA (1961) *Hilgard and Marquis' Conditioning and Learning.* New York: Appleton-Century-Crofts.

Kitai, ST, Sugimori, M and Kocsis, JD (1976) Excitatory nature of dopamine in the nigro-caudate pathway. *Exp. Brain Res.* 24:351-363.

Kitai, ST and Surmeier, DJ (1993) Cholinergic and dopaminergic modulation of potassium conductances in neostriatal neurons. *Adv. Neurol.* 60:40-52.

Kiyatkin, EA (1995) Functional significance of mesolimbic dopamine. *Neurosci. Biobehav. Rev.* 19:573-598.



Kiyatkin, EA and Rebec, GV (1996) Dopaminergic modulation of glutamate-induced excitations of neurons in the neostriatum and nucleus accumbens of awake, unrestrained rats. *J. Neurophysiol.* 75:142-153.

Knapp, AG and Dowling, JE (1987) Dopamine enhances excitatory amino acid-gated conductances in cultured retinal horizontal cells. *Nature* 325:437-439.

Knapp, AG, Schmidt, KF and Dowling, JE (1990) Dopamine modulates the kinetics of ion channels gated by excitatory amino acids in retinal horizontal cells. *Proc. Natl. Acad. Sci. U. S. A.* 87:767-771.

Kohli, JD, Horn, PT, Glock, D, Brewster, WK and Nichols, DE (1993) Dihydrexidine: a new potent peripheral dopamine D_1 receptor antagonist. *Eur. J. Pharmacol.* 235:31-35.

Kolta, MG, Shreve, P, De Souza, V and Uretsky, NJ (1985) Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. *Neuropharmacol.* 24:823-829.

Kolta, MG, Shreve, P and Uretsky, NJ (1989) Effect of pretreatment with amphetamine on the intreraction between amphetamine and dopamine neurons in the accumbens. *Neuropharmacol.* 28:9-14.

Kombian, SB and Malenka, RC (1994) Simultaneous LTP of non-NMDA and LTD of NMDA-receptor mediated responses in the nucleus accumbens. *Nature* 368:242-246.

König, JFR and Klippel, RA (1963) The rat brain, a stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore, MD: Williams and Wilkins.

Koob, GF (1992a) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* 13:177-184.

Koob, GF (1992b) Neural mechanisms of drug reinforcement. *Annals N.Y. Acad. Sci.* 654:171-191.

Koob, GF, Le, HT and Creese, I (1987) The D_1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. *Neurosci. Lett.* 79:315-320.

Kornhuber, J and Kornhuber, ME (1983) Axo-axonic synapses in the rat striatum. *Eur. Neurol.* 22:433-436.

Kornhuber, J and Kornhuber, ME (1986) Presynaptic dopaminergic modulation of cortical input to the striatum. *Life Sci.* 39:669-674.

Kubota, Y, Inagaki, S, Kito, S and Wu, J-Y (1987) Dopaminergic axons make synapses with GABAergic neurons in the rat neostriatum. *Brain Res.* 406:147-156.

Kuhar, MJ, Ritz, MC and Boja, JW (1991) The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* 14:299-302.

Lacey, MG, Mercuri, NB and North, RA (1987) Dopamine acts on D_2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. *J. Physiol.* 392:397-416.

Lacey, MG, Mercuri, NB and North, RA (1988) On the potassium conductance increase activated by $GABA_B$ and dopamine D_2 receptors in rat substantia nigra neurones. *J. Physiol.* 401:437-453.

Lacey, MG, Mercuri, NB and North, RA (1989) Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *J. Neurosci.* 9:1233-1241.

Lambert, NA, Harrison, NL and Teyler, TJ (1991) Baclofen-induced disinhibition in area CA1 of rat hippocampus is resistant to extracellular Ba^{2+}. *Brain Res.* 547:349-352.

Law-Tho, D, Hirsch, JC and Crepel, F (1994) Dopamine modulation of synaptic transmission in rat prefrontal cortex: an in vitro electrophysiological study. *Neurosci. Res.* 21:151-160.

Le Moal, M and Simon, H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol. Rev.* 71:155-234.

Levey, AI, Hersch, SM, Rye, DB, Sunahara, RK, Niznik, HB, Kitt, CA, Price, DL, Maggio, R, Brann, MR and Ciliax, BJ (1993) Localization of D_1 and D_2 dopamine receptors in brain with subtype-specific antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 90:8861-8865.

Levine, MS, Altemus, KL, Cepeda, C, Cromwell, HC, Crawford, C, Ariano, MA, Drago, J, Sibley, DR and Westphal, H (1996a) Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D_{1A}-deficient mutant mice. *J. Neurosci.* 16:5870-5882.

Levine, MS, Li, Z, Cepeda, C, Cromwell, HC and Altemus, KL (1996b) Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. *Synapse* 24:65-78.

Liman, ER, Knapp, AG and Dowling, JE (1989) Enhancement of kainate-gated currents in retinal horizontal cells by cyclic AMP-dependent protein kinase. *Brain Res.*

481:399-402.

Lin, Y-J, Greif, GJ and Freedman, JE (1996) Permeation and block of dopamine-modulated potassium channels on rat striatal neurons by cesium and barium ions. *J. Neurophysiol.* 76:1413-1422.

Llano, I and Gerschenfeld, HM (1993) β-Adrenergic enhancement of inhibitory synaptic activity in rat cerebellar stellate and purkinje cells. *J. Physiol.* 468:201-224.

Lledo, PM, Homburger, V, Bockaert, J and Vincent, J-D (1992) Differential G protein-mediated coupling of D_2 dopamine receptors to K^+ and Ca^{2+} currents in rat anterior pituitary cells. *Neuron* 8:455-463.

Lledo, P-M, Israel, J-M and Vincent, J-D (1990a) A guanine nucleotide-binding protein mediates the inhibition of voltage-dependent calcium currents by dopamine in rat lactotrophs. *Brain Res.* 528:143-147.

Lledo, P-M, Legendre, P, Israel, J-M and Vincent, J-D (1990b) Dopamine inhibits two characterized voltage-dependent calcium currents in identified rat lactotroph cells. *Endocrinol.* 127:990-1001.

Lledo, P-M, Legendre, P, Zhang, J, Israel, J-M and Vincent, J-D (1990) Effects of dopamine on voltage-dependent potassium currents in identified rat lactotroph cells. *Neuroendocrinol.* 52:545-555.

Loh, EA and Roberts, DCS (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. *Psychopharmacol.* 101:262-266.

Lupica, CR (1995) δ and μ Enkephalins inhibit spontaneous GABA-mediated IPSCs via a cyclic AMP-indpendent mechanism in the rat hippocampus. *J. Neurosci.* 15:737-749.

Lyness, WH, Friedle, NM and Moore, KE (1979) Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacol. Biochem. Behav.* 11:553-556.

Lyness, WH, Friedle, NM and Moore, KE (1980) Increased self-administration of d-amphetamine after destruction of 5-hydroxytryptaminergic neurons. *Pharmacol. Biochem. Behav.* 12:937-941.

MacVicar, BA and Pittman, QJ (1986) Novel synaptic responses mediated by dopamine and γ-aminobutyric acid in neuroendocrine cells of the intermediate pituitary. *Neurosci. Lett.* 64:35-40.

Mahan, LC, Burch, RM, Monsma, FJ and Sibley, DR (1990) Expression of striatal D_1 dopamine receptors coupled to inositol phosphate production and Ca^{2+} mobilization in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U. S. A.* 87:2196-2200.

Mailman, RB, Schulz, DW, Kilts, CD, Lewis, MH, Rollema, H and Wyrick, S (1986) Biochemical and functional studies of D-1 dopamine receptors. *Psychopharmacol. Bull.* 22:593-598.

Maldonado, R, Robledo, P, Chover, AJ, Caine, SB and Koob, GF (1993) D_1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacol. Biochem. Behav.* 45:239-242.

Malenka, RC and Kocsis, JD (1988) Presynaptic actions of carbachol and adenosine on corticostriatal synaptic transmission studied *in vitro*. *J. Neurosci.* 8:3750-3756.

Malenka, RC and Nicoll, RA (1986) Dopamine decreases the calcium-activated afterhyperpolarization in hippocampal CA1 cells. *Brain Res.* 379:210-215.

Mallart, A and Martin, AR (1968) The relation between quantum content and facilitation at the neuromuscular junction of the frog. *J. Physiol.* 196:593-604.

Manabe, T, Wyllie, DJA, Perkel, DJ and Nicoll RA (1993) Modulation of synaptic transmission and long-term potentiation: effects of paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J. Neurophysiol.* 70:1451-1459.

Manzoni, OJ, Manabe, T and Nicoll, RA (1994) Release of adenosine by activation of NMDA receptors in the hippocampus. *Science* 265:2098-2101.

Martin, AR (1955) Further study of the statistical composition of the end plate potential. *J. Physiol.* 130:114-122.

Martin-Iverson, MT, Szostak, C and Fibiger, HC (1986) 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. *Psychopharmacol.* 88:310-314.

McGeorge, AJ and Faull, RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. *Neurosci.* 29:503-537.

McGregor, A and Roberts, DCS (1993) Dopaminergic antagonist within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain Res.* 624:245-252.

Mercuri, N, Bernardi, G, Calabresi, P, Cotugno, A, Levi, G and Stanzione, P (1985) Dopamine decreases cell excitability in rat neostriatal neurons by pre- and postsynaptic mechanisms. *Brain Res.* 358:110-121.

Mills, A, Allet, B, Bernard, A, Chabert, C, Brandt, E, Cavegn, C, Chollet, A and Kawashima, E (1993) Expression and characterization of human D4 dopamine receptors in baculovirus-infected cells. *FEBS Lett.* 320:130-134.

Mirenowicz, J and Schultz, W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379:449-451.

Misgeld, U, Bijak, M and Jarolimek, W (1995) A physiological role for $GABA_B$ receptors and the effects of baclofen in the mammalian central nervous system. *Prog. Neurobiol.* 46:423-462.

Momiyama, T and Sim, JA (1996) Modulation of inhibitory transmission by dopamine in rat basal forebrain nuclei: activation of presynaptic D_1-like dopaminergic receptors. *J. Neurosci.* 16:7505-7512.

Momiyama, T, Sim, JA and Brown, DA (1996) Dopamine D_1-like receptor-mediated presynaptic inhibition of excitatory transmission onto rat magnocellular basal forebrain neurones. *J. Physiol.* 495:97-106.

Monsma, FJ Jr, Mahan, LC, McVittie, LD, Gerfen, CR and Sibley, DR (1990) Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation. *Proc. Natl. Acad. Sci. U. S. A.* 87:6723-6727.

Mora, F, Sweeney, KF, Rolls, ET and Sanguinetti, AM (1976) Spontaneous firing rate of neurones in the prefrontal cortex of the rat: evidence for a dopaminergic inhibition. *Brain Res.* 116:516-522.

Mottola, DM, Brewster, WK, Cook, LL, Nichols, DE and Mailman, RB (1992) Dihydrexidine, a novel full efficacy D_1 dopamine receptor agonist. *J. Pharmacol. Exp. Ther.* 262:383-393.

Murphy, DL, Brodie, HKH, Goodwin, FK and Bunney, WE (1971) Regular induction of hypomania by L-dopa in "bipolar" manic-depressive patients. *Nature* 229:135-136.

Murray, AM and Waddington, JL (1989) The induction of grooming and vacuous chewing by a series of selective D-1 dopamine receptor agonists: two directions of D-1:D-2 interaction. *Eur. J. Pharmacol.* 160:377-384.

Nedergaard, S, Hopkins, C and Greenfield, SA (1988) Do nigro-striatal neurones possess a discrete dendritic modulatory mechanism? *Exp. Brain Res.* 69:444-448.

Nicola, SM, Kombian, SB and Malenka RC (1996) Psychostimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D1-like dopamine receptors. *J. Neurosci.* 16:1591-1604.

Nicola, SM and Malenka, RC (1997) Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. *J. Neurosci.*, in press.

Nicoll, RA (1988) The coupling of neurotransmitter receptors to ion channels in the brain. *Science* 241:545-551.

Nicoll, RA, Malenka, RC and Kauer, JA (1990) Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol. Rev.* 70:513-565.

Nisenbaum, ES, Orr, WB and Berger, TW (1988) Evidence for two functionally distinct subpopulations of neurons in the rat striatum. *J. Neurosci.* 8:4138-4150.

O'Donnell, P and Grace, AA (1993) Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. *Synapse* 13:135-160.

O'Donnell, P and Grace, AA (1994) Tonic D_2-mediated attenuation of cortical excitation in nucleus accumbens neurons recorded in vitro. *Brain Res.* 634:105-112.

O'Donnell, P and Grace, AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J. Neurosci.* 15:3622-3639.

Olivera, BM, Miljanich, GP, Ramachandran, J and Adams, ME (1994) Calcium channel diversity and neurotransmitter release: the ω-conotoxins and ω-agatoxins. *Annu. Rev. Biochem.* 63:823-867.

Parent, A (1990) Extrinsic connections of the basal ganglia. *Trends Neurosci.* 13:254-258.

Paxinos, G and Watson, C (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Penit-Soria, J, Audinat, E and Crepel, F (1987) Excitation of rat prefrontal cortical neurons by dopamine: an in vitro electrophysiological study. *Brain Res.* 425:263-274.

Pennartz, CMA, Boeijinga, PH, Kitai, ST and Lopes da Silva, FH (1991) Contribution of NMDA receptors to postsynaptic potentials and paired-pulse facilitation in identified

neurons of the rat nucleus accumbens in vitro. *Exp. Brain Res.* 86:190-198.

Pennartz, CMA, Dolleman-Van der Weel, MJ, Kitai, ST and Lopes da Silva, FH (1992a) Presynaptic dopamine D1 receptors attenuate excitatory and inhibitory limbic inputs to the shell region of the rat nucleus accumbens studied in vitro. *J. Neurophysiol.* 67:1325-1334.

Pennartz, CMA, Dolleman-Van der Weel, MJ and Lopes da Silva, FH (1992b) Differential membrane properties and dopamine effects in the shell and core of the rat nucleus accumbens studied in vitro. *Neurosci. Lett.* 136:109-112.

Pennartz, CMA, Groenewegen, HJ and Lopes da Silva, FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. *Prog. Neurobiol.* 42:719-761.

Pennartz, CMA and Kitai, ST (1991) Hippocampal inputs to identified neurons in an *in vitro* slice preparation of the rat nucleus accumbens: evidence for feed-forward inhibition. *J. Neurosci.* 11:2838-2847.

Pereda, A, Triller, A, Korn, H and Faber, DS (1992) Dopamine enhances both electrotonic coupling and chemical excitatory postsynaptic potentials at mixed synapses. *Proc. Natl. Acad. Sci. U. S. A.* 89:12088-12092.

Peris, J, Boyson, SJ, Cass, WA, Curella, P, Dwoskin, LP, Larson, G, Lin, L-H, Yasuda, RP and Zahniser, NR (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. *J. Pharmacol. Exp. Ther.* 253:38-44.

Peris, J and Zahniser, NR (1989) Persistent augmented dopamine release after acute cocaine requires dopamine receptor activation. *Pharmacol. Biochem. Behav.* 32:71-76.

Peroutka, SJ and Snyder, SH (1980) Relationship of neuroleptic drug effects at brain dopamine, serotonin, α-adrenergic, and histamine receptors to clinical potency. *Am. J. Psychiat.* 137:1518-1522.

Peterson, SL, Olsta, SA and Mathews, RT (1990) Cocaine enhances medial prefrontal cortex neuron response to ventral tegmental area activation. *Brain Res. Bull.* 24:267-273.

Pettit, HO, Ettenberg, A, Bloom, FE and Koob, GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacol.* 84:167-173.

Phillips, AG and Fibiger, HC (1978) The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum, nucleus accumbens and prefrontal cortex.

Canad. J. Psychol. 32:58-66.

Phillips, AG, Mora, F and Rolls, ET (1981) Intracerebral self-administration of amphetamine by rhesus monkeys. *Neurosci. Lett.* 24:81-86.

Pickel, VM, Beckley, SC, Joh, TH and Reis, DJ (1981) Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. *Brain Res.* 225:373-385.

Pickel, VM, Towle, AC, Joh, TH and Chan, J (1988) Gamma-aminobutyric acid in the medial rat nucleus accumbens: ultrastructural localization in neurons receiving monosynaptic input from catecholaminergic afferents. *J. Comp. Neurol.* 272:1-14.

Pierce, RC and Rebec, GV (1995) Iontophoresis in the neostriatum of awake, unrestrained rats: differential effects of dopamine, glutamate and ascorbate on motor- and nonmotor-related neurons. *Neurosci.* 67:313-324.

Post, RM, Jimerson, DC, Bunney, WE and Goodwin, FK (1980) Dopamine and mania: behavioral and biochemical effects of the dopamine receptor blocker pimozide. *Psychopharmacol.* 67:297-305.

Pralong, E and Jones, RSG (1993) Interactions of dopamine with glutamate- and GABA-mediated synaptic transmission in the rat entorhinal cortex *in vitro*. *Eur. J. Neurosci.* 5:760-767.

Rekling, JC (1993) Effects of met-enkephalin on GABAergic spontaneous miniature IPSPs in organotypic slice cultures of the rat hippocampus. *J. Neurosci.* 13:1954-1964.

Risner, ME and Jones, BE (1976) Role of noradrenergic and dopaminergic processes in amphetamine self-administration. *Pharmacol. Biochem. Behav.* 5:477-482.

Ritz, MC, Lamb, RJ, Goldberg, SR and Kuhar, MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237:1219-1223.

Roberts, DCS and Koob, GF (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol. Biochem. Behav.* 17:901-904.

Roberts, DCS, Corcoran, ME and Fibiger, HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol. Biochem. Behav.* 6:615-620.

Roberts, DCS, Koob, GF, Klonoff, P and Fibiger, HC (1980) Extinction and recovery

of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav.* 12:781-787.

Roberts, DCS, Loh, EA and Vickers, G (1989) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. *Psychopharmacol.* 97:535-538.

Robinson, TE and Becker, JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. *Eur. J. Pharmacol.* 85:253-254.

Robinson, TE and Berridge, KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* 18:247-291.

Rodrigues, PDS and Dowling, JE (1990) Dopamine induces neurite retraction in retinal horizontal cells via diacylglycerol and protein kinase C. *Proc. Natl. Acad. Sci. U. S. A.* 87:9693-9697.

Rolls, ET (1984) Responses of neurons in different regions of the striatum of the behaving monkey. In *The Basal Ganglia: Structure and Function.* JS McKenzie, RE Kemm and LN Wilcock, eds. New York: Plenum.

Rolls, ET, Thorpe, SJ, Boytim, M, Szabo, I and Perrett, DI (1984) Responses of striatal neurons in the behaving monkey. 3. Effects of iontophoretically applied dopamine on normal responsiveness. *Neurosci.* 12:1201-1212.

Rutherford, A, Garcia-Munoz, M and Arbuthnott, GW (1988) An afterhyperpolarization recorded in striatal cells 'in vitro': effect of dopamine administration. *Exp. Brain Res.* 71:399-405.

Salin, PA, Malenka, RC and Nicoll, RA (1996) Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. *Neuron* 16:797-803.

Scanziani, M, Capogna, M, Gähwiler, BH and Thompson, SM (1992) Presynaptic inhibition of miniature excitatory synaptic currents by baclofen and adenosine in the hippocampus. *Neuron* 9:919-927.

Scanziani, M, Gahwiler, BH and Thompson SM (1995) Presynaptic inhibition of excitatory synaptic transmission by muscarinic and metabotropic glutamate receptor activation in the hippocampus: are Ca^{2+} channels involved? *Neuropharmacol.* 34:1549-1557.

Schenk, S, Horger, BA, Peltier, R and Shelton, K (1991) Supersensitivity to the reinforcing effects of cocaine following 6-hydroxydopamine lesions to the medial

prefrontal cortex in rats. *Brain Res.* 543:227-235.

Schiffmann, SN, Lledo, P-M and Vincent, J-D (1995) Dopamine D_1 receptor modulates the voltage-gated sodium current in rat striatal neurones through a protein kinase A. *J. Physiol.* 483:95-107.

Schneider, JS, Levine, MS, Hull, CD and Buchwald, NA (1984) Effects of amphetamine on intracellular responses of caudate neurons in the cat. *J. Neurosci.* 4:930-938.

Seiden, LS, Sabol, KE and Ricaurte, GA (1993) Amphetamine: effects on catecholamine systems. *Annu. Rev. Pharmacol. Toxicol.* 32:639-677.

Sesack, SR, Aoki, C and Pickel, VM (1994) Ultrastructural localization of D_2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. *J. Neurosci.* 14:88-106.

Sesack, SR, Deutsch, AY, Roth, RH and Bunney, BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *phaseolus vulgaris* leucoagglutinin. *J. Comp. Neurol.* 290:213-242.

Sesack, SR and Pickel, VM (1990) In the rat nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. *Brain Res.* 527:266-279.

Sesack, SR and Pickel, VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.* 320:145-160.

Seutin, V, Johnson, SW and North, RA (1994) Effect of dopamine and baclofen on *N*-methyl-D-aspartate-induced burst firing in rat ventral tegmental neurons. *Neurosci.* 58:201-206.

Shinonaga, Y, Takada, M and Mizuno, N (1994) Topographic organization of collateral projections from the basolateral amygdaloid nucleus to both the prefrontal cortex and nucleus accumbens in the rat. *Neurosci.* 58:389-397.

Siggins, GR (1978) Electrophysiological role of dopamine in the striatum: excitatory or inhibitory? In *Psychopharmacology: A Generation of Progress*. MA Lipton and KF Killam, eds. New York: Raven.

Smith, AD and Bolam, JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci.* 13:259-

265.

Snyder, SH (1972) Catecholamines in the brain as mediators of amphetamine psychosis. *Arch. Gen. Psychiat.* 27:169-179.

Stack, J and Surprenant, A (1991) Dopamine actions on calcium currents, potassium currents and hormone release in rat melanotrophs. *J. Physiol.* 439:37-58.

Starr, MS and Starr, BS (1993) Seizure promotion by D_1 agonists does not correlate with other dopaminergic properties. *J. Neural. Transm. Parkinson's Dis. Sect.* 6:27-34.

Sugamori, KS, Demchyshyn, LL, Chung, M and Niznik, HB (1994) D_{1A}, D_{1B}, and D_{1C} dopamine receptors from *Xenopus laevis*. *Proc. Natl. Acad. Sci. U. S. A.* 91:10536-10540.

Sugamori, KS, Demchyshyn, LL, McConkey, F, Forte, MA and Niznik, HB (1995) A primordial dopamine D1-like adenylyl cyclase-linked receptor from *Drosophila melanogaster* displaying poor affinity for benzazepines. *FEBS Lett.* 362:131-138.

Sulzer, D, Chen, T-K, Lau, YY, Kristensen, H, Rayport, S and Ewing A (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci.* 15:4102-4108.

Sunahara, RK, Guan, HC, O'Dowd, BF, Seeman, P, Laurier, LG, Ng, G, George, SR, Torchia, J, Van Tol, HHM and Niznik, HB (1991) Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. *Nature* 350:614-619.

Sunahara, RK, Niznik, HB, Weiner, D, Stormann, TM, Brann, MR, Kennedy, JL, Gelernter, JE, Rozmahel, R, Yang, Y, Israel, Y, Seeman, P and O'Dowd, BF (1990) Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. *Nature* 347:80-83.

Surmeier, DJ, Bargas, J, Hemmings, HC, Nairn, AC and Greengard, P (1995) Modulation of calcium currents by a D_1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14:385-397.

Surmeier, DJ, Eberwine, J, Wilson, CJ, Cao, Y, Stefani, A and Kitai, ST (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proc. Natl. Acad. Sci. U. S. A.* 89:10178-10182.

Surmeier, DJ and Kitai, ST (1993) D_1 and D_2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. *Prog. Brain Res.* 99:309-324.

Swanson, LW and Cowan, WM (1975) A note on the connections and development of

the nucleus accumbens. *Brain Res.* 92:324-330.

Swerdlow, NR and Koob, GF (1987) Dopamine, schizophrenia, mania, and depression: toward a unified hypothesis of cortico-striato-pallido-thalamic function. *Behav. Brain Sci.* 10:197-245.

Thompson, SM, Capogna, M, and Scanziani M (1993) Presynaptic inhibition in the hippocampus. *Trends. Neurosci.* 16:222-227.

Thompson, SM and Gähwiler, BH (1992) Comparisons of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus *in vitro*. *J. Physiol.* 451:329-345.

Tiberi, M, Jarvie, KR, Silvia, C, Falardeau, P, Gingrich, JA, Godinot, N, Bertrand, L, Yang-Feng, TL, Fremeau, RT Jr and Caron, MG (1991) Cloning, molecular characterization and chromosomal assignment of a gene encoding a second D_1 receptor subtype: differential expression pattern in rat compared with the D_{1A} receptor. *Proc. Natl. Acad. Sci. U. S. A.* 88:7491-7495.

Trulson, ME and Arasteh, K (1986) Effects of dopamine and amphetamine on mouse caudate neurons recorded in vitro. *Eur. J. Pharmacol.* 124:161-165.

Uchimura, N, Higashi, H and Nishi, S (1986) Hyperpolarizing and depolarizing actions of dopamine via D-1 and D-2 receptors on nucleus accumbens neurons. *Brain Res.* 375:368-372.

Uchimura, N and North, RA (1990) Actions of cocaine on rat nucleus accumbens neurones *in vitro*. *Brit. J. Pharmacol.* 99:736-740.

Uchimura, N and North, RA (1991) Baclofen and adenosine inhibit synaptic potentials mediated by γ-aminobutyric acid and glutamate release in rat nucleus accumbens. *J. Pharmacol. Exp. The.r* 258:663-668.

Ulrich, D and Huguenard, JR (1996) $GABA_B$ receptor-mediated responses in GABAergic projection neurones of rat nucleus reticularis thalami *in vitro*. *J. Physiol.* 493:845-854.

Undie, AS and Friedman, E (1990) Stimulation of a dopamine D_1 receptor enhances inositol phosphates formation in rat brain. *J. Pharmacol. Exp. Ther.* 253:987-992.

Undie, AS and Friedman, E (1992) Selective dopaminergic mechanism of dopamine and SKF38393 stimulation of inositol phosphate formation in rat brain. *Eur. J. Pharmacol – Mol. Pharmacol. Sect.* 226:297-302.

Undie, AS, Weinstock, J, Sarau, HM and Friedman, E (1994) Evidence for a distinct D_1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J. Neurochem.* 62:2045-2048.

Ungerstedt, U (1971a) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand. Suppl.* 367:69-93.

Ungerstedt, U (1971b) Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand. Suppl.* 367:1-48.

Waddington, JL and Deveney, AM (1996) Dopamine receptor multiplicity: 'D_1-like' – 'D_2-like' interactions and 'D_1-like' receptors not linked to adenylate cyclase. *Bicochem. Soc. Trans.* 24:177-182.

Wang, H-Y, Undie, AS and Friedman, E (1995) Evidence for the coupling of G_q protein to D_1-like dopamine sites in rat striatum: possible role in dopamine-mediated inositol phosphate formation. *Mol. Pharmacol.* 48:988-994.

Wang, L-Y, Salter, MW and MacDonald, JF (1991) Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. *Science* 253:1132-1135.

Wang, Z and Rebec, GV (1993) Neuronal and behavioral correlates of intrastriatal infusions of amphetamine in freely moving rats. *Brain Res.* 627:79-88.

Weinshank, RL, Adham, N, Macchi, M, Olsen, MA, Branchek, TA and Hartig, PR (1991) Molecular cloning and characterization of a high affinity dopamine receptor ($D_{1\beta}$) and its pseudogene. *J. Biol. Chem.* 266:22427-22435.

Weisskopf, MG, Castillo, PE, Zalutsky, RA and Nicoll, RA (1994) Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. *Science* 265:1878-1882.

White, FJ (1987) D-1 dopamine receptor stimulation enables the inhibition of nucleus accumbens neurons by a D-2 receptor agonist. *Eur. J. Pharmacol.* 135:101-105.

White, FJ and Wang, RY (1986) Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. *J. Neurosci.* 6:274-280.

Wilcox, RA, Robinson, TE and Becker, JB (1986) Enduring enhancement in amphetamine-stimulated striatal dopamine release in vitro produced by prior exposure to amphetamine or stress in vivo. *Eur. J. Pharmacol.* 124:376-376.

Williams, GV and Goldman-Rakic, PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* 376:572-575.

Williams, PJ, MacVicar, BA and Pittman, QJ (1989) A dopaminergic inhibitory postsynaptic potential mediated by an increased potassium conductance. *Neurosci.* 31:673-681.

Wilson, CJ and Groves, PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular injection of horseradish peroxidase. *J. Comp. Neurol.* 194:599-615.

Wise, RA, Murray, A and Bozarth, MA (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing rats. *Psychopharmacol.* 100:355-360.

Wise, RA, Newton, P, Leeb, K, Burnette, B, Pocock, D and Justice, JB (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacol.* 120:10-20.

Withers, NW, Pulvirenti, L, Koob, GF and Gillin, JC (1995) Cocaine abuse and dependence. *J. Clin. Psychopharmacol.* 15:63-78.

Wu, L-G and Saggau, P (1995) $GABA_B$ receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca^{2+} influx. *J. Physiol.* 485:649-657.

Xu, M, Moratalla, R, Gold, LH, Hiroi, N, Koob, GF, Graybiel, AM and Tonegawa, S (1994a) Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. *Cell* 79:729-742.

Xu, M, Hu, X-T, Cooper, DC, Moratalla, R, Graybiel, AM, White, FJ and Tonegawa, S (1994b) Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. *Cell* 79:945-955.

Yamada, S, Kojima, H, Yokoo, H, Tsutsumi, T, Takamuki, K, Anraku, S, Nishi, S and Inanaga, K (1988) Enhancement of dopamine release from striatal slices of rats that were subchronically treated with methamphetamine. *Biol. Psychiat.* 24:399-408.

Yang, CR and Mogenson, GJ (1984) Electrophysiological responses of neurones in the nucleus accumbens to hippocampal stimulation and the attenuation of the excitatory responses by the mesolimbic dopaminergic system. *Brain Res.* 324:69-84.

Yim, CY and Mogenson, GJ (1982) Response of nucleus accumbens neurons to amygdala stimulation and its modification by dopamine. *Brain Res.* 239:401-415.

Yim, CY and Mogenson, GJ (1986) Mesolimbic dopamine projection modulates amygdala-evoked EPSP in nucleus accumbens neurons: an in vivo study. *Brain Res.* 369:347-352.

Yim, CY and Mogenson, GJ (1988) Neuromodulatory action of dopamine in the nucleus accumbens: an *in vivo* intracellular study. *Neurosci.* 26:403-415.

Yokel, RA and Pickens, R (1973) Self-administration of optical isomers of amphetamine and methylamphetamine by rats. *J. Pharmacol. Exp. Ther.* 187:27-33.

Yokel, RA and Wise, RA (1975) Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. *Science* 187:547-549.

Yokel, RA and Wise, RA (1976) Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacol.* 48:311-318.

Yokel, RA and Wise, RA (1978) Amphetamine-type reinforcement by dopaminergic agonists in the rat. *Psychopharmacol.* 58:289-296.

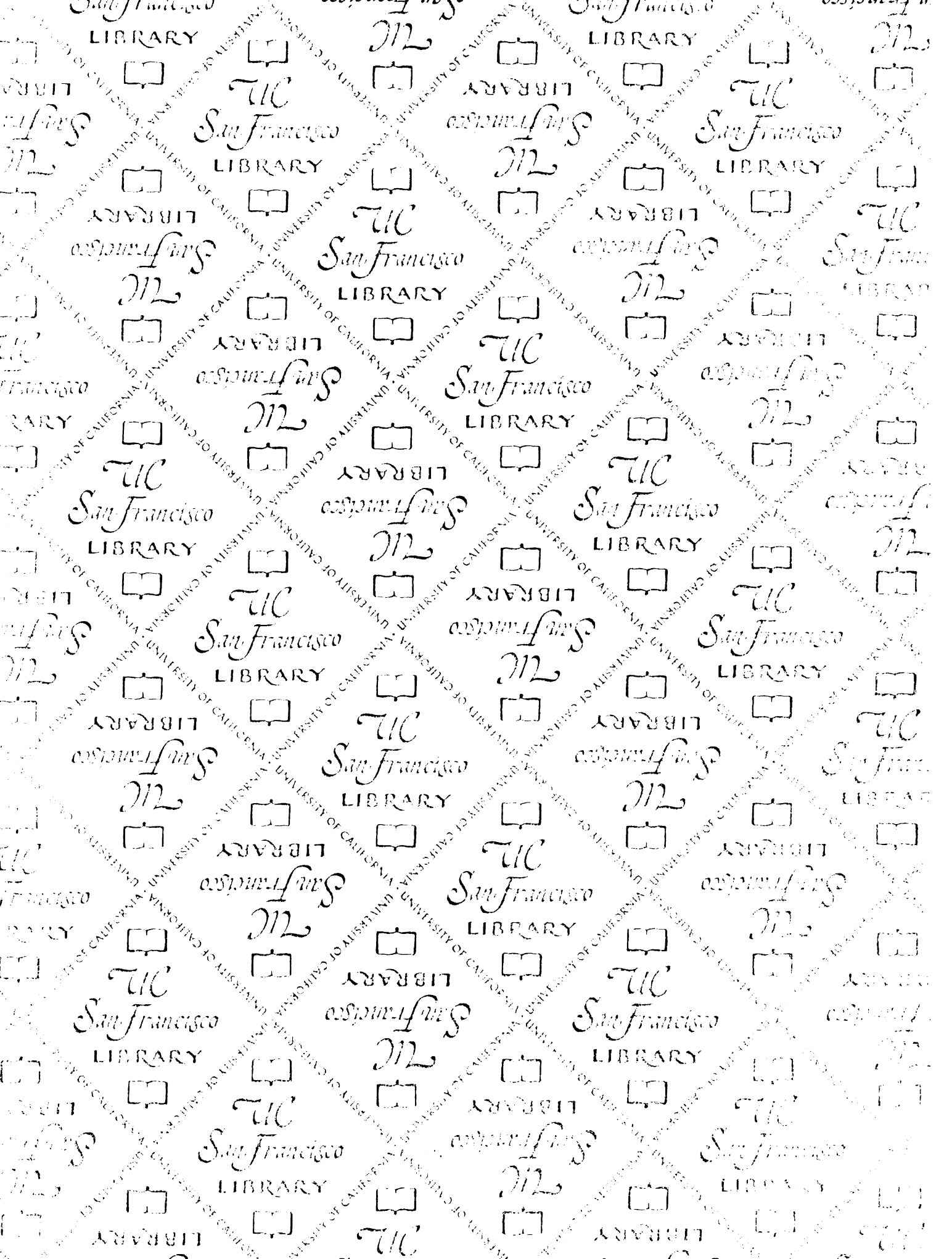
Yung, KKL, Bolam, JP, Smith, AD, Hersch, SM, Ciliax, BJ and Levey, AI (1995) Immunocytochemical localization of D_1 and D_2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neurosci.* 65:709-730.

Zhong, Y and Wu, C-F (1991) Altered synaptic plasticity in *Drosophila* memory mutants with a defective cyclic AMP cascade. *Science* 251:198-201.

Zhou, Q-Y, Grandy, DK, Thambi, L, Kushner, JA, Van Tol, HHM, Cone, R, Pribnow, D, Salon, J, Bunzow, JR and Civelli, O (1990) Cloning and expression of human and rat D_1 dopamine receptors. *Nature* 347:76-80.

Zito, KA, Vickers, G and Roberts, DCS (1985) Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav.* 23:1029-1036.

Zucker, RS (1989) Short-term plasticity. *Annu. Rev. Neurosci.* 12:13-31.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