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Report

Drugs of Abuse and Stress Trigger a Common Synaptic Adaptation in Dopamine Neurons

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Summary

Drug seeking and drug self-administration in both animals and humans can be triggered by drugs of abuse themselves or by stressful events. Here, we demonstrate that in vivo administration of drugs of abuse with different molecular mechanisms of action as well as acute stress both increase strength at excitatory synapses on midbrain dopamine neurons. Psychoactive drugs with minimal abuse potential do not cause this change. The synaptic effects of stress, but not of cocaine, are blocked by the glucocorticoid receptor antagonist RU486. These results suggest that plasticity at excitatory synapses on dopamine neurons may be a key neural adaptation contributing to addiction and its interactions with stress and thus may be an attractive therapeutic target for reducing the risk of addiction.

Introduction

Understanding the neurobiological basis of addiction has important implications not only for the treatment and prevention of this devastating and common illness but also as a model for how experiences modify neural circuitry and thereby behavior. Because humans abuse and become addicted to a wide array of legal (e.g., nicotine, ethanol) and illicit (e.g., cocaine, heroin) drugs, a valuable strategy has been to look for adaptations in the brain that are common to different classes of addictive substances. Such an approach led to the realization that one critically important component of the neural circuits mediating addiction is the mesolimbic dopamine (DA) system consisting of midbrain DA cells, located primarily in the ventral tegmental area (VTA) and the nucleus accumbens (NAc), which receives a dense projection from the VTA (Everitt and Wolf, 2002; Kelley and Berridge, 2002; Koob et al., 1998; Nestler, 2001; Wise, 1996). All drugs of abuse appear to act directly in the VTA and/or NAc to cause increases in DA levels in the NAc (Koob et al., 1998; Nestler, 2001; Wise, 1996). Although whether this is sufficient or even absolutely necessary for the reinforcing properties of drugs of abuse is the subject of debate (Berridge and Robinson, 1998), there is no doubt that the discovery of this common action of addictive drugs, despite their very different molecular actions, has had a major influence on thinking about both the neurobiological basis of addiction as well as the brain mechanisms mediating reinforcement and reward-based learning (Waelti et al., 2001).

Recently we found that a single in vivo exposure to cocaine caused an enhancement of synaptic strength at excitatory synapses on midbrain DA neurons (Ungless et al., 2001) and that this shared several features with long-term potentiation (LTP), the leading model for the cellular changes that mediate learning and memory (Martin et al., 2000). Here we examine whether in vivo administration of other commonly abused drugs causes a similar synaptic change. Furthermore, because in both humans and animals stress facilitates the initial acquisition and maintenance of drug self-administration and can elicit relapse (i.e., reinstatement of drug taking after abstinence) (Piazza and Le Moal, 1998; Shaham et al., 2000), we asked whether an acute stress also causes a change in excitatory synaptic strength on midbrain DA neurons analogous to that caused by cocaine. Our findings strengthen the idea that mechanisms involved in adaptive forms of experience-dependent plasticity such as learning may be pathologically usurped and contribute to the development of addiction (Hyman and Malenka, 2001; Nestler, 2001).

Results

The experimental approach was essentially identical to that used previously (Ungless et al., 2001). Twenty-four hours after in vivo administration of the drug (or stress), slices containing the VTA were prepared and whole-cell recordings were obtained from DA cells, identified by the presence of large I_h currents (Figure 1A). To assess synaptic strength, the ratio of AMPA receptor-mediated synaptic currents (AMPAR EPSCs) to NMDA receptor-mediated synaptic currents (NMDAR EPSCs) was calculated (Figure 1B). This measure offers the important advantage that it is independent of the number of synapses activated and therefore independent of such variables as the positioning of the electrodes or anatomy of the tissue. We first retested the effects of cocaine and compared its effects to those of amphetamine, a psychostimulant that shares some mechanistic features with cocaine in that it too enhances brain DA levels. Both psychostimulant drugs caused a similar increase in the ratio of AMPAR to NMDAR EPSCs (Figure 1C) (saline, $0.42 \pm .09$, $n = 14$ cells; cocaine, $0.70 \pm .08$, $n = 8$; amphetamine, $0.70 \pm .09$, $n = 12$), indicating that like cocaine, in vivo administration of amphetamine causes an increase in excitatory synaptic transmission onto DA neurons.

Is this in vivo drug-induced synaptic plasticity in midbrain DA neurons limited to psychostimulants or does it also occur in response to other addictive substances? We tested three other commonly abused drugs that have

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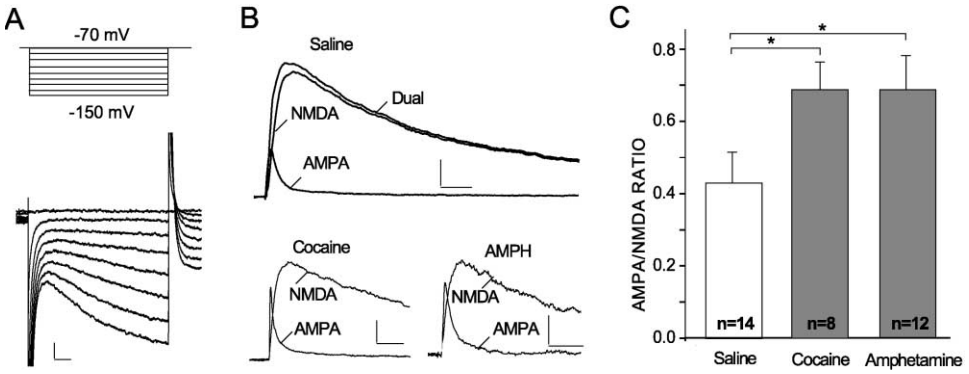

Figure 1. Psychostimulant Drugs Enhance the AMPA/NMDA EPSC Ratio at Excitatory Synapses on Midbrain DA Cells

(A) Example of I_h currents used to identify midbrain DA cells (calibration bars: 20 pA/50 ms).

(B) Top traces show example from a control cell of how AMPA/NMDA ratios were obtained. EPSCs were recorded at +40 mV (Dual trace), and then D-APV (50 μM) was applied to obtain the AMPA EPSC. The NMDA EPSC was obtained by digital subtraction of the AMPA EPSC from the dual EPSC. Bottom traces show examples of AMPA and NMDA EPSCs obtained from cocaine- and amphetamine-treated animals (calibration bars: 20 pA/15 ms).

(C) Summary of AMPA/NMDA ratios obtained from animals administered saline, cocaine, or amphetamine (asterisk indicates $p < 0.02$). In this and all subsequent figures, numbers within the bars indicate the number of cells examined.

very different molecular mechanisms of action: (1) morphine, which acts on μ opioid receptors; (2) nicotine, which acts on brain nicotinic receptors; and (3) ethanol, which acts on a number of different neurotransmitter receptors (Koob et al., 1998; Nestler, 2001). In vivo administration of all three of these substances caused significant increases in the ratio of AMPAR to NMDAR EPSCs measured 24 hr later (Figure 2) (saline, 0.46 ± .02, n = 22 cells; morphine, 0.68 ± .04, n = 9; nicotine, 0.67 ± .07, n = 14; ethanol, 0.64 ± .06, n = 10).

Thus far we have demonstrated that five different abused drugs (cocaine, amphetamine, morphine, nicotine, and ethanol) all cause a similar enhancement at excitatory synapses on midbrain DA cells. These are surprising results since these substances have very different molecular mechanisms of action. They also raise the important question of whether this modification has something to do with the addictive potential of these substances. Alternatively, it might simply be the consequence of administering any type of psychoactive substance. To address this issue, we asked whether administration of commonly used therapeutic drugs, which are known to have profound effects on emotional states in humans but no addictive liability, also caused a change at excitatory synapses on midbrain DA cells. Neither fluoxetine, a specific serotonin-reuptake inhibitor commonly used in the treatment of depression, nor carbamazepine, which is a common treatment for seizure disorders and bipolar disorder (i.e., manic-depressive illness) (Nestler et al., 2001), had an effect on the ratio of AMPAR to NMDAR EPSCs (Figure 3) (saline, 0.44 ± .02, n = 14 cells; fluoxetine, 0.44 ± .05, n = 9; carbamazepine, 0.41 ± .05, n = 8). These results provide further support for the hypothesis that changes in strength at excitatory synapses on midbrain DA cells may contribute to the addictive properties of multiple classes of abused substances.

The most difficult aspect of addiction treatment is the long-lasting risk of relapse (O'Brien, 1997); individuals can be abstinent for months or even years but still are susceptible to cravings that can stimulate renewed drug seeking and taking. In both humans and animals, reinstatement of drug seeking and self-administration can be triggered by single doses of drug, stimuli previously associated with drug taking, or stressful events (Piazza and Le Moal, 1998; Robinson and Berridge, 2000; Shaham et al., 2000). Stress also can facilitate the initial acquisition of drug self-administration, perhaps by enhancing the reinforcing efficacy of drugs of abuse (Piazza and Le Moal, 1998). We therefore tested whether an acute stress would affect excitatory synaptic strength on midbrain DA cells. Animals were subject to a modified

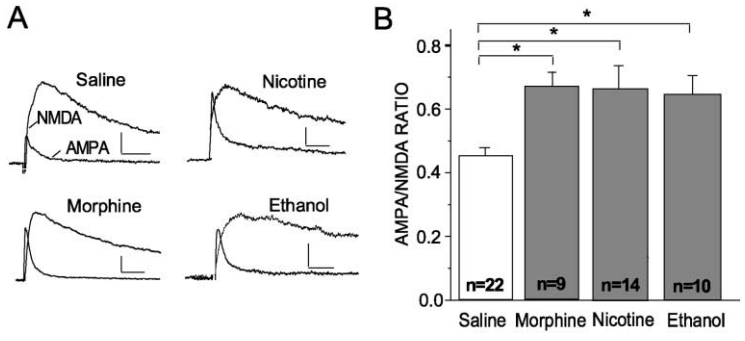

Figure 2. Commonly Abused Drugs Other than Psychostimulants also Increase the AMPA/NMDA Ratio

(A) Examples of AMPA and NMDA EPSCs obtained from animals given the indicated substance (calibration bars: 20 pA/15 ms).

(B) Summary of AMPA/NMDA ratios obtained from animals administered saline, morphine, nicotine, or ethanol (asterisk indicates $p < 0.03$).

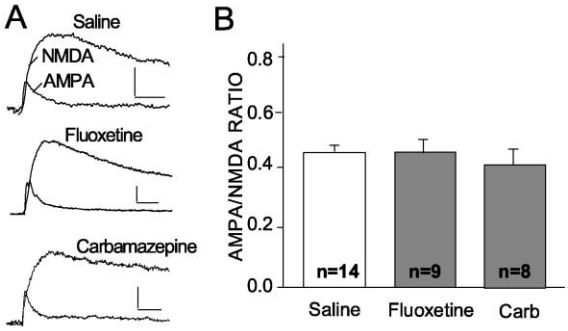

Figure 3. Commonly Used Psychoactive but Nonaddictive Drugs Do Not Affect the AMPA/NMDA Ratio

(A) Examples of AMPA and NMDA EPSCs obtained from animals given the indicated substance (calibration bars: 20 pA/15 ms).
(B) Summary of AMPA/NMDA ratios obtained from animals administered saline, fluoxetine, or carbamazepine.

Porsolt forced swim task, a commonly used acute stressor (Huber et al., 2001), and slices were prepared 1 day later. This resulted in an increase in the ratio of AMPAR to NMDAR EPSCs even larger than that elicited by drugs of abuse (Figures 4A and 4B) (saline, 0.46 ± .03, n = 11 cells; stress plus saline, 0.82 ± .04, n = 24). To begin to explore whether this stress-induced synaptic modification shares mechanisms with the cocaine-elicited synaptic enhancement, we administered the NMDAR antagonist MK-801 prior to the cold water swim. This stress protocol blocked the increase in the ratio of AMPAR to NMDAR EPSCs (Figures 4A and 4B) (stress plus MK801, 0.51 ± .06, n = 15), indicating that, like the effects of cocaine (Ungless et al., 2001), the synaptic changes due to the acute stress required activation of NMDARs.

One of the major consequences of acute stress is increased secretion of corticosteroids and activation of glucocorticoid receptors (GRs). To test whether GR activation played a role in mediating the effects of stress on synaptic strength in midbrain DA neurons, we administered the GR antagonist RU486 (Cadepond et al., 1997; Marinelli et al., 1998) prior to placing animals in the cold water. This completely blocked the synaptic change (Figures 4A and 4B) (stress plus RU486, 0.38 ± .04, n = 9 cells), implying a critical role for GR activation. The effectiveness of RU486 also allowed us to address the question of whether the synaptic effect of cocaine is due to an unrecognized drug-induced stress reaction rather than its reinforcing and/or addictive property. However, RU486 did not prevent the increase in the AMPAR to NMDAR EPSC ratio elicited by in vivo cocaine administration (Figure 4C) (saline, 0.45 ± .03, n = 14 cells; cocaine, 0.61 ± .13, n = 7; cocaine plus RU486, 0.69 ± .12, n = 9). This indicates that the synaptic consequences of in vivo cocaine exposure do not require GR activation and therefore are unlikely to be due to an acute stress response.

Discussion

We have demonstrated that in vivo administration of five different drugs of abuse with very different molecular mechanisms of action all elicit an enhancement of strength at excitatory synapses on midbrain DA neurons. Two psychoactive but therapeutic and nonaddictive medications did not cause such a change. This degree of specificity suggests that this in vivo, drug-induced synaptic plasticity in DA neurons may be one important component of the neural circuit adaptations that contribute to core features of addiction. Because of the profound effect of stress in facilitating the initiation and reinstatement of drug self-administration, we also examined whether an acute stress might have an effect similar to that of drugs of abuse and found that it too caused a large increase in synaptic strength at this same population of excitatory synapses. Based on these observations, we suggest that this enhancement of strength at excitatory synapses on midbrain DA neurons also is a key neural adaptation that contributes to the effects of stress on drug seeking and relapse.

The conclusion that the increases in the AMPAR to NMDAR EPSC ratio elicited by drugs of abuse and stress reflect an enhancement of AMPAR-mediated excitatory synaptic transmission is based on previous work that examined the mechanisms underlying the cocaine-induced increase in this ratio (Ungless et al., 2001). We cannot

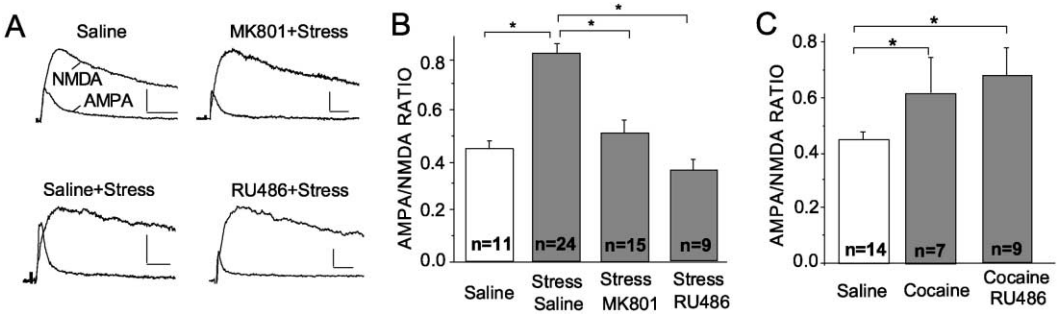

Figure 4. Acute Stress Increases the AMPA/NMDA Ratio

(A) Examples of AMPA and NMDA EPSCs obtained from a control animal and animals experiencing stress plus the indicated substance (calibration bars: 20 pA/15 ms).
(B) Summary of AMPA/NMDA ratios obtained from animals administered saline or experiencing stress along with administration of saline, the NMDA receptor antagonist MK801, or the glucocorticoid receptor antagonist RU486 (asterisk indicates $p < 0.01$).
(C) Summary of AMPA/NMDA ratios obtained from animals administered saline, cocaine, or cocaine plus RU486 (asterisk indicates $p < 0.05$).

rule out, however, additional mechanisms that could contribute to this increase in the AMPAR to NMDAR EPSC ratio. A decrease in the magnitude of the NMDAR EPSC due to changes in NMDAR function and/or number clearly would increase the ratio, as would decreases in the NMDAR EPSC due to the extent of glutamate spillover (Kullmann, 2000) should spillover occur normally in this brain region. In previous work an attempt was made to address the first of these possibilities by examining the inward currents generated by application of NMDA (Ungless et al., 2001). However, this manipulation activates extrasynaptic NMDARs and thus negative results are not conclusive. Another possibility is that if normally synaptic AMPARs on midbrain DA neurons exhibit inward rectification (i.e., pass more current in the inward than in the outward direction), a drug- or stress-induced decrease in the degree of inward rectification would result in an increase in the AMPAR to NMDAR EPSC ratio when measured at +40 mV (since the modified AMPARs will now pass more current at +40 mV although the same current at hyperpolarized membrane potentials). However, such a change in the biophysical properties of AMPARs cannot account for our results since a clear increase in the AMPAR to NMDAR EPSC ratio in response to cocaine administration was detected when AMPAR EPSCs were measured at −70 mV rather than +40 mV (unpublished observations).

There are several correlative findings that can be viewed as being consistent with the hypothesis that drugs of abuse enhance AMPAR-mediated synaptic transmission in midbrain DA cells. First, repeated administration of cocaine or amphetamine caused an increase in the single unit responses of VTA DA cells to AMPA but not to NMDA, an effect that was observed 3 but not 14 days after cessation of drug administration (Zhang et al., 1997). Second, both repeated administration of cocaine or morphine as well as repeated restraint stress increased levels of the AMPAR subunit GluR1 in the VTA (as well as the NMDAR subunit NR1) (Fitzgerald et al., 1996; but see Lu et al., 2002). Third, viral-mediated overexpression of GluR1 in the VTA enhanced the locomotor stimulating and rewarding properties of morphine (Carlezon et al., 1997). These sorts of findings have led to a hypothesis that elevated levels of GluR1 in the VTA are one primary trigger leading to the complex cascade of molecular and circuit adaptations that underlie sensitization to drugs of abuse (Carlezon and Nestler, 2002).

Accepting that drugs of abuse and stress do enhance the strength of excitatory afferent inputs to midbrain DA cells, how might this contribute to the development of addiction and/or relapse? There are three general hypotheses concerning the functions of midbrain DA cells and their projections to the NAc. DA cell firing and the consequent release of DA in the NAc may: (1) signify reward (Wise, 1996) or changes in the positive incentive value of stimuli (Koob, 1996), (2) serve as a reward prediction signal and thereby play an important role in reward-based learning (Waelti et al., 2001), or (3) be involved in signaling "incentive salience," which in the context of addiction suggests that adaptations in the mesolimbic DA system lead to "wanting" (i.e., craving) drugs although not necessarily "liking" them (Robinson and Berridge, 2000). These non-mutually exclusive hypotheses share the feature that external stimuli that are associated with DA cell firing are granted high appetitive or motivational significance. We suggest that by increasing synaptic strength at excitatory synapses on midbrain DA cells, drugs of abuse or stress enhance the motivational significance of drugs themselves as well as stimuli closely associated with drug seeking and self-administration.

Consistent with this hypothesis, there is considerable evidence that stress can enhance the rewarding efficacy of drugs, likely via influences on the mesolimbic DA system (Piazza and Le Moal, 1998). Indeed, stress, like drugs of abuse, causes an increase in DA levels in the NAc and prefrontal cortex (Horger and Roth, 1996; Piazza and Le Moal, 1998). Stress has also been reported to affect synaptic plasticity in the hippocampus. Specifically, acute stress appears to rapidly inhibit the generation of LTP and facilitate the generation of LTD (Shors et al., 1989; Kim et al., 1996; Xu et al., 1997, 1998). Whether this involves an increase in basal synaptic strength or rather some modification of the triggering mechanisms for synaptic plasticity is debated (Kim et al., 1996; Xu et al., 1997).

One important unanswered question is whether all or critical subsets of excitatory afferent inputs to midbrain DA cells are strengthened by in vivo drugs or stress. Strengthening of prefrontal cortical inputs may be particularly important, as this has been suggested to play a critical role in the development of behavioral sensitization, a prominent model for core features of addiction including craving (Everitt and Wolf, 2002; Robinson and Berridge, 2000). On the other hand, modifications of the recently described input from the bed nucleus of the stria terminalis (Georges and Aston-Jones, 2002) may be particularly important for mediating the effects of stress (Shaham et al., 2000). Whatever sets of synapses are affected, by demonstrating a synaptic modification commonly elicited by addictive drugs and stress, we provide a neural mechanism that may contribute to the clinically important interactions between addiction and stress as well as one potentially important target for therapeutic interventions in treatment of addictive disorders.

Experimental Procedures

Animals and In Vivo Manipulations

C57/Bl6 mice (21- to 30-days-old) were used for all experiments. For drug administration, animals were injected i.p. at the same time each day with saline (volume matched for experimental injections), cocaine (15 mg/kg), d-amphetamine (10 mg/kg), morphine (10 mg/kg), nicotine (0.5 mg/kg), ethanol (20 mg/kg), fluoxetine (10 mg/kg), or carbamazepine (15 mg/kg). Drugs were obtained from Sigma (except for ethanol, which was obtained from the Stanford Hospital pharmacy). Acute stress was elicited with a modified Porsolt forced swim task. Animals were placed in a 1 l graduated cylinder containing 400 ml of cold (6°C) water for 4–6 min. MK801 (1 mg/kg in saline) or RU486 (40 mg/kg in DMSO) was administered 15–30 min prior to the cold water swim. RU486 was administered 30 min prior to cocaine administration for the experiments illustrated in Figure 4C. The dose of RU486 was chosen based on published reports showing variable efficacy of 20–25 mg/kg (Pieretti et al., 1991; Douma et al., 1998; Xu et al., 1998) and the concern that because rodents lack a specific binding protein for RU486, brain concentrations of RU486 following peripheral administration are low (Heikinheimo and Kekkonen, 1993).

Electrophysiology

Animals were anesthetized with halothane and sacrificed 24–30 hr after the in vivo manipulation. All remaining procedures were essentially as described previously (Ungless et al., 2001). Briefly, a block of tissue containing midbrain was sliced in the horizontal plane (250 μm) in ice-cold low Ca^{2+} solution (containing in mM: 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 0.625 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose). Slices were transferred to a holding chamber containing artificial cerebrospinal fluid (ACSF, in mM: 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose) and equilibriated at 31°C –34°C for at least 1 hr. Picrotoxin (100 μM) was added to the ACSF for recording, to block $GABA_A$ receptor-mediated inhibitory postsynaptic potentials. All solutions were bubbled with 95% O_2/5% CO_2 and perfused over the slice at a rate of 2.5 ml/min. Cells were visualized with an upright microscope using infrared illumination, and whole-cell voltage-clamp recordings were made with an Axopatch 1D amplifier (Axon Instruments). Electrodes (2–6 MΩ) contained in mM: 117 cesium methansulfonic acid, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 2.5 MgATP, 0.25 MgGTP (pH 7.2–7.4), 275–285 mOsm. Dopamine cells were identified by the presence of a large I_h current, which was evoked by holding cells at −70 mV and stepping to −120 mV in 10 mV increments. While I_h is present in >90% of dopamine neurons (Cameron et al., 1997; Neuhoff et al., 2002), we recognize that its presence does not unequivocally identify dopamine cells in midbrain slices. However, both in previous work (Ungless et al., 2001) and in the present experiments, this criterion was sufficient to obtain clear differences between control and experimental cells.

A bipolar stimulating electrode was placed 100–300 μm rostral to the recording electrode and was used to stimulate excitatory afferents at 0.1 Hz. Cells were initially held at −70 mV for 5–15 min to ensure the stability of EPSCs and then were depolarized to +40 mV. Cells were again monitored for 5–15 min, at which point D-APV (50 μM) was applied for 10–20 min. To calculate the AMPAR/NMDAR ratio, a control dual component EPSC was obtained by averaging 20–30 consecutive responses immediately before application of D-APV. An average AMPAR EPSC was then obtained by averaging 20–30 consecutive responses beginning 5 min after application of D-APV. The average response in the presence of D-APV was subtracted from that seen in its absence to obtain an average NMDAR EPSC. The peak of the AMPAR EPSC was divided by the peak of the NMDAR EPSC to give an AMPAR/NMDAR ratio. Input and series resistances were monitored online throughout each experiment.

Over 65% of the data were collected and analyzed without knowledge of the treatment that the animals had received. There were no differences in the results from blinded and nonblinded experiments and therefore results were combined. Control, saline-injected animals were interleaved throughout the course of all experiments, and the saline controls presented in each bar graph represent the cells obtained during the time period when the experimental manipulations shown in that graph were performed. Only two slices were obtained from each animal and a single cell was examined from each slice. All values are expressed as mean and SEM. Statistical significance was assessed using two-tailed Student's t tests.

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