



## Stress-induced, glucocorticoid-dependent strengthening of glutamatergic synaptic transmission in midbrain dopamine neurons

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### ABSTRACT

Stress facilitates development of addictive behaviors in part by stress-induced increase in the strength of glutamatergic synapses at dopamine (DA) neurons within the ventral tegmental area (VTA). Here, we further demonstrate that this stress-induced synaptic adaptation is glucocorticoid-dependent and is progressively developed. Activation of glucocorticoid receptors (GRs) either by *in vivo* injection of dexamethasone (Dex) or incubation of the VTA slice with Dex potentiate the synaptic strength of glutamatergic synapses at VTA DA neurons, whereas preventing the activation of GRs by Ru486 abolishes this effect. These results suggest that the VTA GRs play a critical role in stress-induced cellular adaptations.

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Stress plays an important role in drug addiction as well as many other significant neurologically and psychiatrically relevant conditions. In animal models, exposure to stressful events induces locomotor sensitization, shortens the development period of cocaine-induced locomotor sensitization, and triggers reinstatement of cocaine administration during the extinction period [5,8,9,30]. Increasing evidence suggests that stress-induced cellular changes in the mesolimbic dopamine (DA) system may significantly contribute to these addiction-facilitating effects [11,13]. Excitatory synaptic transmission in the brain is mediated mainly by  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA)- and *N*-methyl-D-aspartate receptor (NMDAR)-mediated excitatory postsynaptic currents (EPSCs). AMPAR EPSCs comprise most of the fast excitatory synaptic transmission and represent excitatory synaptic strength. Given that NMDARs are relatively stable during experience-dependent synaptic plasticity [18,19], stress-induced increase in the AMPAR:NMDAR ratio in ventral tegmental area (VTA) DA neurons has been interpreted as an increase in AMPAR EPSCs, and thus a potentiation of the excitatory synaptic strength in these neurons [10,27]. Excitation of DA-signaling appears to be

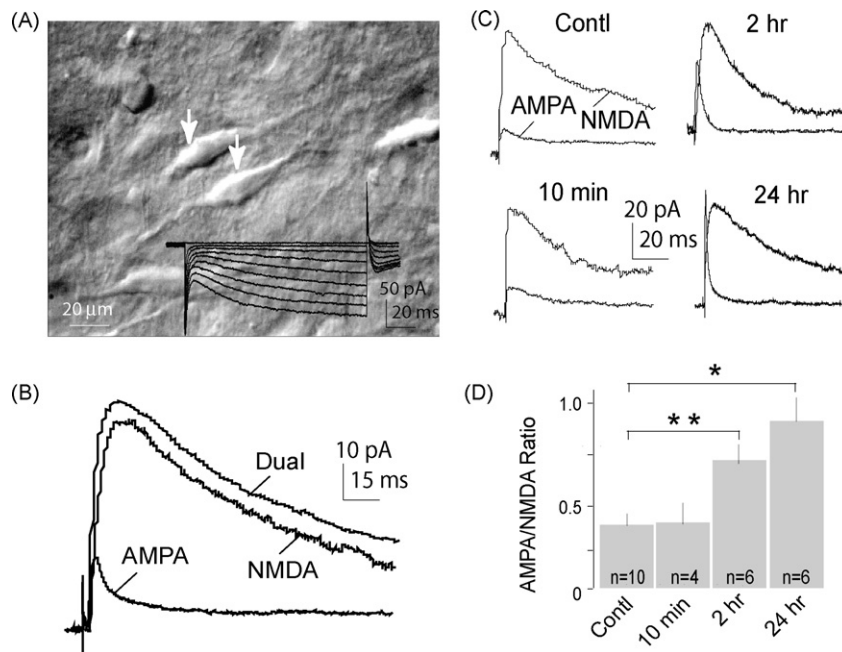
the first cellular step for drugs of abuse to initiate their addictive effects [11,13]. Therefore, stress-induced enhancement of excitatory synaptic transmission may function to prime the arousal of VTA DA neurons, thus facilitating the formation of drug-induced cellular adaptations within the mesolimbic DA system.

Although we previously demonstrated that the stress-induced increase in AMPAR/NMDAR ratio in VTA DA neurons requires activation of glucocorticoid receptors (GRs) [27], it remains unclear whether this synaptic adaptation is a local effect by intra-VTA GRs, or an event involving brain substrates located in other brain regions, and whether activation of GRs alone is sufficient to mediate the effect of stress. This study was specifically designed to address these questions.

In this study, we first determined the time course of stress-induced synaptic adaptation in VTA DA neurons. Using a modified Porsolt test (forced swimming test) [27], we acutely stressed the male Sprague–Dawley rats (21- to 30-day-old, Harlan Laboratories, Indianapolis, IN), followed by a 10 min, 2 or 24-h recovery period. We then performed whole-cell voltage-clamp recordings (using Multiclamp 700B amplifier, pClamp 9 software, and a DigiData 1332 A–D interface, Molecular Devices, Sunnyvale, CA; experimental procedures were identical to [10,27]) from the VTA DA neurons, which were identified by their morphology and the presence of large  $I_h$  currents (Fig. 1A) [25,27]. AMPAR/NMDAR ratio was measured and used to represent the strength of excitatory synapses on VTA DA neurons (Fig. 1B). As described previously, AMPAR/NMDAR ratio is independent of the number of synapses activated,

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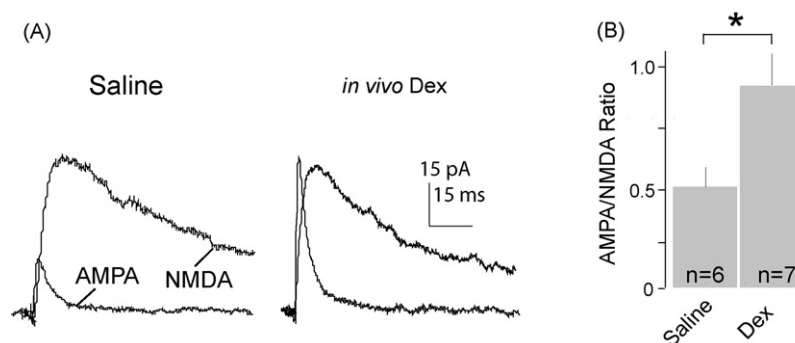


**Fig. 1.** Stress-induced synaptic adaptation in VTA DA neurons initially arises between 10 min and 2 h after stress exposure. (A) The VTA DA neurons (indicated by arrows) within the horizontal brain slice. *Inset* shows an example VTA DA neuron that exhibited the signature  $I_h$  currents, which was used to operationally distinguish DA neurons from non-DA neurons in this study. (B) An example of a VTA DA neuron, in which the AMPAR and NMDAR EPSCs were biophysically and pharmacologically isolated. (C) Examples showing AMPAR:NMDAR ratios of VTA DA neurons from a rat without stress treatment (control) and rats sacrificed at different time points (10 min, 2 h, and 24 h) following stress. (D) Summary showing that stress-induced increase in AMPAR:NMDAR ratio in VTA DA neurons arises after 10 min and before 2 h following the stress treatment. \* $p < 0.01$ .

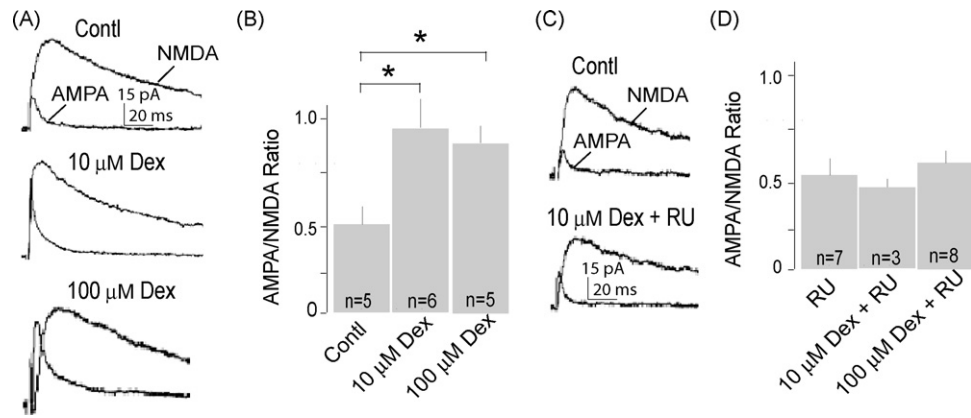
positioning of the stimulating electrode, and intensity of presynaptic stimulation [10,27]. We observed that at the time point 10 min, the AMPAR/NMDAR ratio of VTA DA neurons in stressed rats was not significantly different from that in saline-injected control rats. In contrast, at the time points 2 and 24 h this AMPAR/NMDAR ratio was significantly higher in stressed rats (control,  $0.35 \pm 0.05$ ,  $n = 10$  cells; 10 min,  $0.360 \pm 0.13$ ,  $n = 3$ ,  $p > 0.05$ ; 2 h,  $0.73 \pm 0.09$ ,  $n = 6$ ,  $p < 0.05$ ; 24 h,  $0.95 \pm 0.13$ ,  $n = 6$ ,  $p < 0.01$ ,  $t$ -test; Fig. 1C and D; note that the potential kainate receptor-mediated activities might be involved, possibly resulting in slower decay kinetics of EPSC as shown in the 10 min trace in C). Such temporal patterns indicate that stress-induced synaptic adaptation arises rapidly (h) but not instantaneously. Corticosteroid-signaling is essential in stress-induced synaptic adaptation in VTA DA neurons [10,27]. At the basal level, corticosteroids preferentially activate the high-affinity mineralocorticoid receptors (MRs); following stress, the level of corticosteroids is substantially increased for ~2 h, resulting in activation of the low-affinity GRs [21]. Taken together with

the arising time course of stress effect (Fig. 1), these observations lead to a hypothesis that stress-induced synaptic adaptation in VTA DA neurons is mediated by stress-induced activation of GRs. If this hypothesis is correct, activating GRs alone should be sufficient to induce the same or similar synaptic adaptation in VTA DA neurons. We experimentally activated GRs by injecting rats with 5 mg/kg dexamethasone (Dex), a potent synthetic GR agonist (intraperitoneally, or i.p. injection, saline injection with the matched volume for controls). When compared with saline-treated controls 24 h later, Dex-treated rats exhibited significantly higher AMPAR/NMDAR ratios in VTA DA neurons, with a magnitude similar to the effect of stress (saline,  $0.52 \pm 0.06$ ,  $n = 6$ ; Dex,  $0.93 \pm 0.08$ ,  $n = 7$ ,  $p < 0.01$ ; Fig. 2). Together with our previous observations [21], the above results suggest that activation of GRs alone is sufficient to mediate stress-induced synaptic adaptation in VTA DA neurons.

*In vivo* administration of Dex activates GRs that are distributed throughout the brain. Is the glucocorticoid-induced synaptic plasticity in DA neurons a local effect mediated by intra-VTA GRs, or



**Fig. 2.** Activation of glucocorticoid receptors is sufficient to increase the strength of excitatory synapses in VTA DA neurons. (A) Examples showing AMPAR and NMDAR EPSCs in VTA DA neurons from rats injected with saline and the GR agonist Dex (5 mg/kg). (B) Summary data showing that activation of GRs by *in vivo* injection of Dex is sufficient to increase the AMPAR:NMDAR ratio in VTA DA neurons. \* $p < 0.01$ .



**Fig. 3.** Ex vivo VTA activation of GRs is sufficient to increase the strength of excitatory synapses in VTA DA neurons. (A) Examples showing AMPAR and NMDAR EPSCs in VTA DA neurons from brain slices treated with nothing (control), 10 μM, and 100 μM Dex for ~30 min. (B) Summary showing that activation of the intra-VTA GRs by *in vitro* treatment of Dex is sufficient to increase the AMPAR:NMDAR ratio in VTA DA neurons. (C) Examples showing AMPAR and NMDAR EPSCs in VTA DA neurons from slices treated with RU486 (100 μM) alone and RU486 together with 10 μM. (D) Summary showing that inhibition of GRs does not significantly affect the AMPAR:NMDAR ratio but does prevent the *in vitro* Dex-induced increase in AMPAR:NMDAR ratio in VTA DA neurons. \* $p < 0.01$ .

a multi-factored effect involving GRs and brain substrates located in other brain regions? To address this question, we locally applied Dex (10 μM and 100 μM) to 230 μm thick VTA slices, which lack most of the afferent connection to VTA DA neurons. We observed that a brief activation (~30 min) of local GRs by Dex was sufficient to increase the AMPAR/NMDAR ratio in VTA DA neurons (control,  $0.37 \pm 0.06$ ,  $n = 5$ ; 10 μM Dex,  $0.85 \pm 0.13$ ,  $n = 5$ ,  $p < 0.01$ ; 100 μM Dex,  $0.80 \pm 0.04$ ,  $n = 5$ ,  $p < 0.01$ ; Fig. 3A and B). Because application of Dex activates both MRs and GRs, we next determined which type of corticosteroid receptors mediates the Dex-induced synaptic change in VTA DA neurons. We inhibited GRs with the GR-selective antagonist RU 486 (100 μM) and then incubated the VTA slice with Dex. Our results show that inhibiting GRs prevented the Dex-induced increase in AMPAR/NMDAR ratio in VTA DA neurons (RU486 alone,  $0.57 \pm 0.12$ ,  $n = 7$  cells; 10 μM Dex + RU486,  $0.4 \pm 0.05$ ,  $n = 8$ ; 100 μM Dex + RU486,  $0.59 \pm 0.06$ ,  $n = 3$ ; Fig. 3C and D). These observations indicate that local activation of GRs is sufficient to mediate stress-induced synaptic adaptation in VTA DA neuron.

Taken together, our current results extend our previous finding by demonstrating that the stress-induced synaptic adaptation in VTA DA neurons arises rapidly and involves intra-VTA activation of GRs. These results provide valuable information for understanding the role of stress-induced cellular changes in addictive behaviors.

In the present and a number of previous studies, the AMPAR/NMDAR ratio was used as an indirect measurement to compare excitatory synaptic strength between animals [10,27,31]. An ideal approach is to directly compare EPSCs evoked by the same presynaptic stimulation. However, this ideal approach is often technically unachievable. For example, subtle changes in the anatomy of brain slice and position of stimulator, which are unavoidable in slice electrophysiology, can introduce significant measurement errors in the absolute magnitude of EPSCs. Instead, the AMPAR/NMDAR ratio was used in the present study. This alternative approach is based on two assumptions: (1) drug- and stress-induced synaptic plasticity in VTA DA neurons share similar postsynaptic mechanisms with LTP and LTD in hippocampal CA1 neurons [2,4,10,27,32]; (2) in the postsynaptically expressed LTP- and LTD-like processes, AMPARs are dynamically modified whereas the function of NMDARs remains relatively stable [16–19]. Several lines of evidence support these two assumptions. First, similar to hippocampal LTP, drug- and stress-induced increase in AMPAR/NMDAR ratio in DA neurons is NMDAR-dependent [10,32]; second, the function of postsynaptic AMPARs in DA neurons is substantially increased following exposure to cocaine or stress, whereas the function of NMDARs is not [10,32]; third, in animals in which experience-dependent AMPAR

trafficking is blocked, neither stress nor cocaine causes an increase in AMPAR/NMDAR ratio [10]; fourth, it has been demonstrated that rapid synthesis and translocation of AMPARs mediate experience-dependent synaptic plasticity in VTA DA neurons [20]. These observations suggest that similar to hippocampal LTP, cocaine- and stress-induced changes in the AMPAR/NMDAR ratio in VTA DA neurons is likely mediated by regulations of postsynaptic AMPARs.

One caveat is related to the assumption that NMDARs are unchanged during synaptic plasticity. Indeed, more recent evidence indicates that NMDARs also undergo experience-dependent plastic changes; both the number and function of postsynaptic NMDARs can be up- or downregulated by *in vivo* and *in vitro* experience [22–24]. In theory, cocaine- or stress-induced increase in AMPAR/NMDAR ratio in VTA DA neurons may also be due to the downregulation of NMDARs without any change in AMPARs. However, the available evidence suggests that this is not the case. In contrast to substantial changes in AMPARs, no detectable change of NMDARs was observed in VTA DA neurons following exposure to either cocaine or stress [32]. Moreover, in GluR1 knockout mice, in which only experience-dependent AMPAR trafficking is abolished, the AMPAR/NMDAR ratio is not altered by exposure to either cocaine or stress [10]. Taken together, the available results suggest that drug- and stress-induced increase in AMPAR/NMDAR ratio in VTA DA neurons is an LTP-like process and is mediated by addition of new AMPARs on the postsynaptic membrane.

GRs are extensively expressed in the VTA DA neurons [1], inhibition of which prevents stress-induced synaptic adaptation [10,27]. Thus, a straightforward cellular model explaining stress-induced synaptic adaptation in VTA DA neurons is that activation of GRs upregulates the function/number of postsynaptic AMPARs in the same DA neurons. This hypothesis is supported by our present results that activation of GRs within the thin VTA slice is sufficient to induce a synaptic adaptation similar to the effect of stress (Fig. 3). Furthermore, recent evidence shows that activation of GRs upregulates postsynaptic AMPARs within the same neuron, which appears to be mediated by a GR-mediated transcriptional process [6,12].

It is important to note that stress induces differential synaptic adaptations within different brain regions. In contrast to that in VTA DA neurons, in hippocampal neurons, exposure to stress and the resulting elevation of the glucocorticoid level impairs LTP as well as enhances LTD, a counteracting process of LTP [14,26,29]. Furthermore, these stress- and glucocorticoid-induced synaptic changes in hippocampus are accompanied with atrophy of dendritic branches [33,34]. These differential cellular effects of stress may serve as a concep-

tual basis to understand the different behavioral consequences of stress. The deleterious effect of stress/glucocorticoids on hippocampal synapses may explain adverse effect of stress on hippocampus-dependent explicit memory and cognition [3,7], whereas the enhancing effect of stress/glucocorticoids on mesolimbic synapses may explain the facilitating effect of stress on the formation of DA-dependent implicit memories [15,28].

In summary, the present results provide valuable information for understanding the role of stress-induced cellular changes in addictive behaviors. These results suggest that acute environmental or endogenous stress can conditionally trigger cellular events that lead to GR-dependent increment of action potential firing of VTA DA neurons. It is well-known that chronic stress facilitates the acquisition of addictive behaviors and triggers relapse of drug use [5,8,9,30]. Given that DA in the prefrontal cortex and nucleus accumbens may function to arouse addiction-related incentives, a simplified cellular model based on our current finding would be that the stress-induced prolonged/repetitive enhancement of the activity in VTA DA neurons may facilitate the arousal of addiction-related incentives.

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### References

- [1] J.D. Baxter, G.G. Rousseau, Glucocorticoid hormone action: an overview, *Monogr. Endocrinol.* 12 (1979) 1–24.
- [2] C. Beurrier, R.C. Malenka, Enhanced inhibition of synaptic transmission by dopamine in the nucleus accumbens during behavioral sensitization to cocaine, *J. Neurosci.* 22 (2002) 5817–5822.
- [3] S.R. Bodnoff, A.G. Humphreys, J.C. Lehman, D.M. Diamond, G.M. Rose, M.J. Meaney, Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats, *J. Neurosci.* 15 (1995) 61–69.
- [4] S.L. Borgland, R.C. Malenka, A. Bonci, Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats, *J. Neurosci.* 24 (2004) 7482–7490.
- [5] E.L. Brown, C. Gardella, G. Malm, C.G. Prober, M. Forsgren, E.M. Krantz, A.M. Arvin, L.L. Yasukawa, K. Mohan, Z. Brown, L. Corey, A. Wald, Effect of maternal herpes simplex virus (HSV) serostatus and HSV type on risk of neonatal herpes, *Acta Obstet. Gynecol. Scand.* 86 (2007) 523–529.
- [6] K. Cho, H.J. Little, Effects of corticosterone on excitatory amino acid responses in dopamine-sensitive neurons in the ventral tegmental area, *Neuroscience* 88 (1999) 837–845.
- [7] C.D. Conrad, L.A. Galea, Y. Kuroda, B.S. McEwen, Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment, *Behav. Neurosci.* 110 (1996) 1321–1334.
- [8] H.E. Covington 3rd, K.A. Miczek, Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration “binges”, *Psychopharmacology (Berlin)* 158 (2001) 388–398.
- [9] A. Der-Avakian, S.T. Bland, R.R. Rozeske, J.P. Tamblin, M.R. Hutchinson, L.R. Watkins, S.F. Maier, The effects of a single exposure to uncontrollable stress on the subsequent conditioned place preference responses to oxycodone, cocaine, and ethanol in rats, *Psychopharmacology (Berlin)* 191 (2007) 909–917.
- [10] Y. Dong, D. Saal, M. Thomas, R. Faust, A. Bonci, T. Robinson, R.C. Malenka, Cocaine-induced potentiation of synaptic strength in dopamine neurons: behavioral correlates in GluRA(–/–) mice, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004) 14282–14287.
- [11] S.E. Hyman, R.C. Malenka, E.J. Nestler, Neural mechanisms of addiction: the role of reward-related learning and memory, *Annu. Rev. Neurosci.* (2006).
- [12] H. Karst, M. Joels, Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells, *J. Neurophysiol.* 94 (2005) 3479–3486.
- [13] J.A. Kauer, R.C. Malenka, Synaptic plasticity and addiction, *Nat. Rev. Neurosci.* 8 (2007) 844–858.
- [14] J.J. Kim, M.R. Foy, R.F. Thompson, Behavioral stress modifies hippocampal plasticity through N-methyl-D-aspartate receptor activation, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 4750–4753.
- [15] S.M. Korte, Corticosteroids in relation to fear, anxiety and psychopathology, *Neurosci. Biobehav. Rev.* 25 (2001) 117–142.
- [16] R.C. Malenka, Synaptic plasticity and AMPA receptor trafficking, *Ann. N.Y. Acad. Sci.* 1003 (2003) 1–11.
- [17] R.C. Malenka, M.F. Bear, LTP and LTD: an embarrassment of riches, *Neuron* 44 (2004) 5–21.
- [18] R.C. Malenka, R.A. Nicoll, Long-term potentiation—a decade of progress? *Science* 285 (1999) 1870–1874.
- [19] R. Malinow, R.C. Malenka, AMPA receptor trafficking and synaptic plasticity, *Annu. Rev. Neurosci.* 25 (2002) 103–126.
- [20] M. Mameli, B. Balland, R. Lujan, C. Luscher, Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area, *Science* 317 (2007) 530–533.
- [21] B.S. McEwen, E.R. De Kloet, W. Rostene, Adrenal steroid receptors and actions in the nervous system, *Physiol. Rev.* 66 (1986) 1121–1188.
- [22] J.M. Montgomery, D.V. Madison, State-dependent heterogeneity in synaptic depression between pyramidal cell pairs, *Neuron* 33 (2002) 765–777.
- [23] J.M. Montgomery, J.C. Selcher, J.E. Hanson, D.V. Madison, Dynamin-dependent NMDAR endocytosis during LTD and its dependence on synaptic state, *BMC Neurosci.* 6 (2005) 48.
- [24] W. Morishita, H. Marie, R.C. Malenka, Distinct triggering and expression mechanisms underlie LTD of AMPA and NMDA synaptic responses, *Nat. Neurosci.* 8 (2005) 1043–1050.
- [25] H. Neuhoff, A. Neu, B. Liss, J. Roeper, I(h) channels contribute to the different functional properties of identified dopaminergic subpopulations in the mid-brain, *J. Neurosci.* 22 (2002) 1290–1302.
- [26] C. Pavlides, Y. Watanabe, B.S. McEwen, Effects of glucocorticoids on hippocampal long-term potentiation, *Hippocampus* 3 (1993) 183–192.
- [27] D. Saal, Y. Dong, A. Bonci, R.C. Malenka, Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons, *Neuron* 37 (2003) 577–582.
- [28] R.J. Servatius, T.J. Shors, Exposure to inescapable stress persistently facilitates associative and nonassociative learning in rats, *Behav. Neurosci.* 108 (1994) 1101–1106.
- [29] T.J. Shors, T.B. Seib, S. Levine, R.F. Thompson, Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus, *Science* 244 (1989) 224–226.
- [30] B.A. Sorg, P.W. Kalivas, Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum, *Brain Res.* 559 (1991) 29–36.
- [31] M.J. Thomas, C. Beurrier, A. Bonci, R.C. Malenka, Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine, *Nat. Neurosci.* 4 (2001) 1217–1223.
- [32] M.A. Ungless, J.L. Whistler, R.C. Malenka, A. Bonci, Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons, *Nature* 411 (2001) 583–587.
- [33] Y. Watanabe, E. Gould, B.S. McEwen, Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons, *Brain Res.* 588 (1992) 341–345.
- [34] C.S. Woolley, E. Gould, B.S. McEwen, Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons, *Brain Res.* 531 (1990) 225–231.