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DOPAMINE-DEPENDENT LONG TERM POTENTIATION IN THE DORSAL STRIATUM IS REDUCED IN THE R6/2 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Abstract—The striatum is critically important in motor, cognitive and emotional functions, as highlighted in neurological disorders such as Huntington's disease (HD) where these functions are compromised. The R6/2 mouse model of HD shows progressive motor and cognitive impairments and alterations in striatal dopamine and glutamate release. To determine whether or not dopamine-dependent neuronal plasticity is also altered in the dorsolateral striatum of R6/2 mice, we compared long term potentiation (LTP) and long term depression (LTD) in striatal slices from R6/2 mice with that seen in slices from wild type (WT) mice. In adult WT mice (aged 8–19 weeks), frequency-dependent bidirectional plasticity was observed. High frequency stimulation (four 0.5 s trains at 100 Hz, inter-train interval 10 s) induced LTP (134±5% of baseline), while low frequency stimulation (4 Hz for 15 min) induced LTD (80±5% of baseline). LTP and LTD were significantly blocked by the *N*-methyl-D-aspartic acid (NMDA) receptor antagonist D(−)-2-amino-5-phosphonopentanoic acid (D-AP5) (to 93±6% and 103±8% of baseline respectively), indicating that they are both dependent on NMDA glutamate receptor activation. LTP was significantly blocked by the dopamine D_1 receptor antagonist R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH-23390) (98±8% of baseline), indicating that LTP is dependent on activation of dopamine D_1-type receptors, whereas LTD was not significantly different (90±7%). In adult R6/2 mice (aged 8–19 weeks), LTP was significantly reduced (to 110±4% of baseline), while LTD was not significantly different from that seen in WT mice (85±6%). These data show that R6/2 mice have impaired dopamine-dependent neuronal plasticity in the striatum. As dopamine-dependent plasticity is a proposed model of striatum-based motor and cognitive functions, this impairment could contribute to deficits seen in R6/2 mice.

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Abbreviations: ACSF, artificial cerebrospinal fluid; D-AP5, D(−)-2-amino-5-phosphonopentanoic acid; HD, Huntington's disease; HFS, high frequency stimulation; LFS, low frequency stimulation; LTD, long term depression; LTP, long term potentiation; NMDA, *N*-methyl-D-aspartic acid; SCH-23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; WT, wild type.

Key words: synaptic plasticity, dorsolateral striatum, glutamate, NMDA receptor, dopamine D_1 receptor, habit formation.

The basal ganglia are a collection of forebrain nuclei involved in goal-directed actions, motor learning, and habit formation (Packard and Knowlton, 2002; Graybiel, 2004; Yin and Knowlton, 2006). The importance of the basal ganglia is highlighted in neurological disorders where function is compromised, such as Huntington's disease (HD), which is characterized by motor, cognitive and psychiatric impairments (Bates et al., 2002).

Glutamatergic inputs from almost all areas of the cerebral cortex as well as dopaminergic inputs from the midbrain converge on the striatum of the basal ganglia (Graybiel, 2004; Yin and Knowlton, 2006). The dorsolateral striatum is implicated in procedural learning. For example, dorsolateral striatal neurons change their activity during a procedural learning task in rats (Jog et al., 1999; Graybiel, 2004; Barnes et al., 2005) and monkeys (Schultz et al., 2003), and dopamine agonists and *N*-methyl-D-aspartic acid (NMDA) glutamate receptor antagonists influence procedural learning task performance in rats (Packard and Knowlton, 2002). Activity changes in dorsal striatum are also detected during procedural learning tasks in human subjects, and notably HD patients are impaired in procedural learning tasks (Packard and Knowlton, 2002).

Dopamine-dependent forms of glutamatergic synaptic plasticity in the striatum are proposed as cellular models of procedural learning (Wickens et al., 2003; Mahon et al., 2004). In rats, long term potentiation (LTP) has been observed at corticostriatal synapses *in vivo* (Charpier and Deniau, 1997), while LTP as well as long term depression (LTD) has been observed in the dorsolateral striatum *in vitro* in response to high frequency stimulation (HFS) (Calabresi et al., 1996; Partridge et al., 2000). Recently, low frequency stimulation (LFS) has been shown to evoke LTD (Ronesi and Lovinger, 2005), and frequency-dependent bidirectional synaptic plasticity has been demonstrated in striatal slices from young rats (Fino et al., 2005). Together, these studies indicate that, as in other brain regions, corticostriatal synapses can increase or decrease their strength in response to different patterns of afferent input activity. Corticostriatal LTP and LTD have not been extensively studied in the mouse striatum, although there is increasing interest in deficits in corticostriatal synaptic plasticity in transgenic mice lacking key corticostriatal proteins (for example, Allen et al., 2006; Wang et al., 2006).

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R6/2 mice are transgenic for exon 1 of the human HD gene carrying an expanded CAG repeat (Mangiarini et al., 1996; Bates et al., 2002). They show progressive motor and cognitive impairments (Carter et al., 1999; Lione et al., 1999), and alterations in neuronal plasticity in the hippocampal formation (Murphy et al., 2000; Gibson et al., 2005). One feature of HD is a dramatic loss of medium spiny neurons in the striatum (Bates et al., 2002), although increasing evidence suggests that a progressive decline in neuronal function precedes the overt symptoms and neuronal death in HD patients and in R6/2 mice (Lione et al., 1999; Li et al., 2003; Smith et al., 2006). Neuronal function has been examined in pre- and post-symptomatic R6/2 mice, and changes include alterations in corticostriatal synaptic transmission (Levine et al., 1999; Lafloret et al., 2001; Cepeda et al., 2001; Zeron et al., 2002; reviewed by Levine et al., 2004) and compromised dopaminergic function (Cha et al., 1998; Reynolds et al., 1999; Bibb et al., 2000; Hickey et al., 2002; Johnson et al., 2006). Alterations in glutamatergic and dopaminergic function in the striatum are likely to disrupt both synaptic activity and plasticity in the corticostriatal network, and impaired synaptic plasticity at corticostriatal synapses could contribute to the progressive motor and cognitive symptoms seen in R6/2 mice and in HD patients. Therefore, in this study we have compared LTP and LTD in wild type (WT) and R6/2 mice. We found that dopamine-dependent LTP, but not LTD, is impaired in R6/2 mice compared with that seen in WT mice.

EXPERIMENTAL PROCEDURES

Animals

R6/2 transgenic mice were taken from a colony established in the Department of Pharmacology, University of Cambridge and maintained by backcrossing R6/2 males to CBA×C57BL/6 F1 females. Genotyping was confirmed by polymerase chain reaction as reported in Gibson et al. (2005). A common set of mice was used by Gibson et al. (2005) and in the present study both for electrophysiology and for measurement of the repeat lengths of the mutation carried by R6/2 mice, which were 243±3 (n=26 mice). The present experiments were carried out using 67 slices from 41 WT mice (23 slices from 20 mice for HFS in control conditions; 7 slices from 7 mice for HFS in D(−)-2-amino-5-phosphonopentanoic acid (D-AP5); 7 slices from 7 mice for HFS in R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH-23390); 12 slices from 12 mice for LFS in control conditions; 8 slices from 8 mice for LFS in D-AP5; 10 slices from 9 mice for LFS in SCH-23390), and 31 slices from 24 R6/2 mice (22 slices from 19 mice for HFS in control conditions; 9 slices from 9 mice for LFS in control conditions). All experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and conformed to UK Home Office and local guidelines on the ethical use of animals. All attempts were made to minimize the suffering and the number of animals used for this study.

Brain slice preparation

Brain slices were prepared from adult male WT and R6/2 littermates aged between 8 and 19 weeks. Mice were killed by cervical dislocation and the brains were rapidly removed into ice-cold sucrose-based Ringer of the following composition (mM): sucrose 75, NaCl 70, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 0.5, $MgCl_2$ 14, and D-glucose 10. Horizontal slices (400 μm) containing the dorsal striatum were prepared (DTK-1000 microslicer, Dosaka, Kyoto, Japan) and held after cutting on a net in a submersion chamber containing artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 119, KCl 2.5, NaH_2PO_4 1.0, $NaHCO_3$ 26, $CaCl_2$ 2.5, $MgCl_2$ 7, D-glucose 10 at 30 °C and saturated with 95% O_2/5% CO_2.

Electrophysiological recordings

Slices were transferred to a submersion recording chamber 1–6 h after preparation, suspended between two nylon nets and continuously perfused at 2.5–3.5 ml min^{-1} with ACSF as detailed above but containing 1.3 mM $MgCl_2$, at 29–31 °C, and saturated with 95% O_2/5% CO_2. All experiments were carried out in brain slices taken dorsal to the anterior commissure and in the middle third of the anterior–posterior axis. A bipolar stainless steel electrode (Frederick Haer and Co., Bowdoin, ME, USA) was placed in the white matter on the lateral edge of the striatum to stimulate glutamatergic afferents to striatum. Test stimuli (100 μs duration) were applied at 10 s intervals at an intensity that evoked approximately 50% of the initial maximum compound field potential response. Stable field potential responses were monitored for at least 10 min to ensure there was no movement of the recording electrode or variability in the response. Movement of the electrodes occurred infrequently and was readily detected by sudden changes in field potential amplitude. HFS (four 0.5 s trains at 100 Hz, inter-train interval 10 s) was used to induce LTP and LFS (4 Hz for 15 min) was used to induce LTD; separate slices were used to study the effect of HFS and LFS. LTP and LTD experiments were carried out in the absence of GABA receptor blockers in order to study plasticity under nominally physiological conditions. Glass microelectrodes (2–6 MΩ when filled with ACSF) were placed in the dorsolateral striatum to record compound field potentials. Field potentials were amplified using an Axoprobe 1A amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA), low-pass filtered at 1–2 kHz and digitally sampled to a PC at 10–20 kHz using a Micro1401 interface (Cambridge Electronic Design, Cambridge, UK).

The amplitude of the field potential was measured using the computer program Spike 2 (version 4, Cambridge Electronic Design). To determine whether or not a significant change in field potential amplitude followed HFS or LFS in individual experiments, field potentials were analyzed using ANOVA with a post hoc Dunnett's test (GraphPad Prism, version 4, San Diego, CA, USA); the criterion for stating that LTP or LTD had occurred was a significant increase or decrease ($P<0.05$) in amplitude in more than two 10 min periods occurring at least 20 min after HFS or LFS. To determine whether or not significant LTP or LTD had occurred within the combined data set for each experimental group, paired t-tests were used to compare field potential amplitudes during the 10 min baseline period with field potential amplitudes 30–40 min post-HFS/LFS. To compare the magnitude of LTP and LTD between slices from different experimental groups, the field potential amplitudes were measured 30–40 min post-HFS/LFS and compared across groups using the unpaired t-test (with Welch's correction if the variances between the groups were unequal). The 'n' values reported refer to the number of slices. All combined data are shown as mean±the standard error of the mean (S.E.M.). A critical P value of $P<0.05$ was considered significant for the statistical tests used throughout this study.

Materials

All standard laboratory salts were obtained from BDH Laboratory Supplies (Poole, Dorset, UK). Drugs were obtained from Sigma-Aldrich (Gillingham, Dorset, UK).