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Regional differences in dopamine release in the R6/2 mouse caudate putamen

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Abstract

Huntington's disease (HD) is a fatal neurodegenerative disorder that is characterized by degeneration of the striatum. Here, fast-scan cyclic voltammetry at carbon-fiber microelectrodes was used to uncover regional differences in dopamine (DA) release in the caudate putamen of R6/2 and wild-type control mice. We found a decreasing ventral-to-dorsal gradient in DA release, evoked by a single electrical stimulus pulse, in aged R6/2 mice. Moreover, under more intense stimulation conditions (120 pulses), DA release was significantly attenuated in the dorsal, but not in the ventral caudate. Autoradiography measurements using [³H]WIN 35,428 revealed that the overall density of DA transporter (DAT) protein molecules was significantly less in R6/2 mice compared to WT control mice; however, quadrants of the caudate putamen were not differentially altered in the R6/2 mice. These data collectively suggest that DA release in the dorsal caudate region is more vulnerable with age progression compared to the ventral region.

Keywords

Huntington's disease; R6/2 mice; fast-scan cyclic voltammetry; dopamine

1. Introduction

Huntington's disease (HD) is a fatal, autosomal dominant, neurodegenerative disorder characterized by motor dysfunction, altered behavior, and cognitive impairment. HD is caused by a CAG repeat expansion on chromosome 4 of the IT15 gene, which encodes the huntingtin protein (htt)^[1]. Mutation of this gene results in a polyglutamine (polyQ) expansion near the N-terminus of htt, leading to neurodegeneration. Healthy individuals possess 16–20 CAG repeats on the huntingtin gene, while those with 40 or more repeats will be afflicted with HD^[2]. The number of CAG repeats is directly correlated to the severity and onset age of HD. Symptoms typically begin between 35 to 40 years of age, but those with greater than 40 repeats often show symptoms significantly earlier^[2–3].

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While much of the pathology of HD is known, little is understood regarding neurotransmitter release mechanisms and how alterations of these mechanisms influence the expression of motor and psychological deficits. Recent work by our group and others has suggested that the release of dopamine, a neurotransmitter important for the proper execution of movement, cognition, and reward, is impaired in genetically-engineered HD model rodents, including R6/2 mice^[4], R6/1 mice^[5], YAC-128 mice^[6], Q171 mice^[7], and transgenic rats^[5b]. Moreover, these studies have collectively suggested that the expression of phenotypical motor deficits associated with HD are related to these release impairments^[3b, 3c, 8].

Our group has previously employed fast-scan cyclic voltammetry at carbon-fiber microelectrodes (FSCV) to identify dopamine release and uptake impairments in multiple strains and species of HD model rodents^[4b, 5b, 9]. A common thread of these studies is that release amplitudes were carried out in the dorsolateral caudate putamen (CPu), a brain region that receives dopaminergic innervation from the nigrostriatal pathway and plays a key role in the control of movement^[10].

In this work, we expand the scope of these measurements to include different regions of the CPu, divided into four quadrants. R6/2 mice, which are among the most widely used HD model rodents, were used here since dopamine release deficits in the dorsolateral CPu have already been well-documented^[4, 11]. We used coronal brain slice preparations to focus our analyses to the function of terminals and to make the data obtained consistent with our previous work in R6/2 mice. In agreement with previous studies^[4a], dopamine release was impaired in R6/2 mice at all ages. However, we also found that, as R6/2 mice aged, release was preferentially diminished in the dorsal CPu compared to the ventral CPu. This gradient was not present in WT control mice. A main effect of decreased DAT binding was found in the R6/2 CPu compared to WT. However, labeling between quadrants in R6/2 mice did not differ significantly, suggesting that this gradient is not a result of altered uptake.

2. Results and Discussion

2.1. Electrochemical determination of regional dopamine release

Representative raw data for 13-week-old R6/2 and WT mice obtained in the dorsolateral quadrant are shown in Figs. 1A and 1B. The cyclic voltammograms in these figures confirm that DA, evoked by a single stimulus pulse, is the analyte measured. The color plots show a series of unfolded and stacked CVs with a color-coded current response^[12]. The representative current responses taken from all regions show a decrease of dopamine release. Inspection of these plots suggests that other electroactive species, such as hydrogen peroxide^[13] and adenosine^[14], are not preferentially released as a result of the HD mutation.

Additionally, DA release ($[DA]_{\max}$), evoked with a single electrical pulse, was determined in the dorsolateral (DL), dorsomedial (DM), ventromedial (VM), and ventrolateral (VL) quadrants of the CPu in coronal brain slices (Fig. 2A). Four measurements were taken at random locations within each quadrant and averaged to account for possible heterogeneity of dopaminergic innervation between single measurements^[15]. Electrochemical measurements carried out on brain slices from multiple animals indicate a significant overall main effect of

the R6/2 mutation on $[DA]_{\max}$ (Fig. 2B; two-way ANOVA, $p < 0.01$, $n = 6$ WT and 6 R6/2 mice). Interestingly, within the R6/2 CPu, we found $[DA]_{\max}$ to be significantly greater in the ventral regions compared to the dorsal regions (DL vs. VL, $p < 0.01$, DM vs. VL, $p < 0.05$, DL vs. VM, $p < 0.01$, DM vs. VM, $p < 0.05$, one-way ANOVA).

2.2 Age progression and dopamine release

Due to the progressive nature of HD, we sought to identify how this ventral to dorsal gradient develops over time in R6/2 mice. Stimulated release was determined in slices from 8-, 11-, and 13-week-old R6/2 and WT mice. The progression of DA release attenuation by region is shown in Fig. 3. The data have been normalized to make the relative changes in DA release easier to see. These differences progress with age: at 8 weeks of age no significant differences were noted, at 11 weeks release in the VL was significantly greater than DL ($p < 0.05$, $n = 6$) while no other significant differences were found, and at 13 weeks release in the two ventral quadrants was significantly greater than release in the two dorsal quadrants (DL versus VL, DL versus VM, DM versus VL, DM versus VM, one-way ANOVA, $p < 0.01$, $n = 6$ WT and 6 R6/2 mice). No significant differences in release were found between the DL and DM quadrants or between VL and VM quadrants at any age. Thus, our results here suggest that, even though a general decrease of release occurs throughout the CPu, a ventral to dorsal gradient of decreasing release is established by 13 weeks of age. We found no statistically significant difference in DA release between quadrants in WT control mice.

This preferential impairment of DA release within the dorsal CPu may, in part, explain the expression of motor deficits in R6/2 mice. The nigrostriatal pathway, which plays a central role in the control of motor function, projects from cell bodies in the substantia nigra pars compacta primarily to the DL CPu^[16]. Thus, deficiencies in DA release in this region may have a detrimental effect on motor control. However, we emphasize that abnormalities in other neurotransmitter systems, such as glutamate^[17], GABA^[18], and acetylcholine^[19] may also contribute to the HD motor phenotype either directly or by influencing dopamine. For example, acetylcholine can exert a strong influence over dopamine release in the CPu^[20]; thus, it is conceivable that impaired acetylcholine levels, as has been found previously in R6/2 mice^[19] would result in diminished dopamine release.

2.3 Challenging the dopamine system with increased electrical stimulation

Previously, we have shown that DA storage within the reserve pool of R6/2 mice is impaired^[4b]. According to the generalized three-pool model, neurotransmitters, such as DA, exist in three distinct neurotransmitter pools: the readily releasable, recycling, and reserve pools^[21]. The readily releasable pool contains 1–2 % of stored vesicles and undergoes release upon mild stimulation. The recycling pool accounts for 5–20 % of vesicles and replenishes the readily releasable pool after its depletion^[22]. The reserve pool contains the majority of stored vesicles (80–90%) and is mobilized under prolonged neuronal stimulation. Electrical stimulation in conjunction with FSCV has been previously used to identify a diminished reserve pool in R6/2 mice^[4b].

To simulate periods of enhanced synaptic activity in the CPu, we measured DA release amplitudes in response to episodes of prolonged electrical stimulation. Sequences of 120

stimulation pulses were applied to the DL, DM, VM, and VL CPu of brain slices harvested from 13-week-old R6/2 and WT mice. Sample release plots are provided in Fig. 4. One measurement was taken at each frequency in each of the four quadrants of the CPu. Stimulated release (120 pulses) in the DL and DM quadrants of the CPu (Fig. 5) was significantly less in slices from R6/2 mice than those from WT mice (DL: $p < 0.01$, DM: $p < 0.05$, $n=4$ to 5 mice, two-way ANOVA); however, release in the VL and VM quadrants of R6/2 mice was not significantly impaired (VL: $p > 0.2$, VM: $p > 0.1$, $n=4$ to 5 mice, two-way ANOVA). Thus, the ventral to dorsal gradient of DA release in the CPu is also clearly revealed by application of multiple stimulus pulses.

Our prior work has also shown that a possible indicator of the relative amount of reserve pool DA is the ability of the tissue to release increased amounts of DA at higher stimulation frequencies. We had shown previously that DA release is significantly decreased in R6/2 mice compared to WT control mice when electrically evoked by high stimulation frequencies (50 and 60 Hz) in the dorsolateral CPu when applying 120 stimulation pulse trains^[4b]. This multiple stimulation pulse study along with pharmacological manipulations led to the conclusion that reserve pool DA storage is impaired in the DL CPu of R6/2 mice. Our results here are consistent with an impaired reserve pool in the dorsal region, but a less affected reserve pool in the ventral region.

2.4 Dopamine transporter binding measurements

Collectively, our data reveal a ventral-to-dorsal gradient of decreasing DA release in R6/2 mice, suggesting that the dorsal CPu is more vulnerable to neurological malfunction in HD. These data are consistent with the clinically-determined dorsal-to-ventral gradient of neurodegeneration in humans^[22c, 23]. However, it is not clear whether this gradient arises from altered uptake capacity or impairment in the ability of neurons to release DA.

To provide further insight into this release gradient, we measured the density of DAT by autoradiography with [³H]WIN 35,428 (Fig. 6). Representative striatal slices after [³H]WIN 35,428 binding are shown in Fig. 6A. Consistent with previous studies^[24] DAT was not of uniform density in the CPu of WT mice. In this study, where the density of DAT was averaged over the rostro-caudal extent of the CPu in the four quadrants, DAT in WT mice was 33% higher in the VL quadrant than the DM. Also in agreement with previous studies [27], the density of DAT labeling was markedly decreased in all quadrants of the CPu in HD R6/2 mice compared to WT (average decrease 43.9%; $p < 0.001$).

In 13-week-old R6/2 mice, the gradients between quadrants and within quadrants were not evident. Although there is a significant difference in the amount of DAT present in the CPu in comparison to their WT controls, there was no regional difference in DAT distribution in R6/2 mice (one-way ANOVA, $p > 0.05$, $n = 6$)(Fig. 6B).

Previous measurements in brain slices with FSCV have shown that the maximum rate of dopamine uptake (V_{max}) mediated by DAT is unchanged in R6/2 mice compared to WT control mice^[4a]. In agreement with these results, tritiated dopamine ([³H]-DA) uptake in brain slices from R6/2 mice was also the same as in slices from WT control mice^[25]. According to Michaelis-Menten enzyme kinetics, V_{max} is directly proportional to the total

enzyme concentration^[26]. These previous results might be consistent with degradation of dopaminergic terminals, with DAT protein molecules present at sufficient levels at the sites of release to efficiently take up DA. On the other hand, the sharp decrease in overall DAT levels in R6/2 mice may also indicate that re-assessment of DA uptake parameters is necessary.

Although DAT binding has been shown previously to be diminished in the R6/2 CPu^[27], our study is the first published, to our knowledge, that compares binding between regions within the R6/2 CPu. No differences in binding between quadrants were observed, even though a ventral to dorsal gradient of decreasing DA release formed. Thus, differences in $[DA]_{max}$ are likely not due to differences in the rate of dopamine uptake, but rather the ability of terminals to release dopamine.

2.5 Conclusions

In summary, we have shown that the progressive attenuation of DA release found in R6/2 model HD mice is more severe in the dorsal CPu than in the ventral CPu. Autoradiography measurements reveal that the density distribution of DAT is significantly less in R6/2 mice in comparison to their WT controls; however, there were no significant regional differences. These data collectively suggest that the genetic mutation involved in HD leads to the increased vulnerability of the dorsal CPu in comparison to the ventral CPu. This finding is in line with concept that impaired dopamine release may play a role in the expression motor dysfunction, given that dopaminergic innervation from the substantia nigra pars compacta is supplied to dorsal CPu via the nigrostriatal pathway. It is also possible, however, that diminished release is a response to overactivity of neuronal firing. In the future, understanding why certain populations of dopaminergic neurons are more affected than others could yield clues for disease progression and the expression of motor deficits in HD.

3. Experimental Procedures

3.1. Animals

All experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All procedures were approved by the University of Kansas Institutional Animal Care and Use Committee. Male R6/2 [B6CBA-Tg(HDexon1)62Gpb] and WT mice (Jackson Laboratories; Bar Harbor, ME, USA) were received at approximately 6 weeks of age and housed 5 per cage in the University of Kansas Animal Care Unit. Food and water was available *ad libitum*. Mice were maintained on a 12 hour light/dark cycle with lights on at 6:00 AM and lights out at 6:00 PM. A temperature of 70 ± 2 °C and humidity level of $50 \pm 20\%$ was maintained. Mice were used at 8, 11, and 13 weeks of age (± 1 week).

3.2. Brain slices

Brain slices were acutely harvested as previously described^[5b]. Briefly, mice were deeply anesthetized by isoflurane inhalation and decapitated. The brain was then immediately removed and placed into ice-cold artificial cerebral spinal fluid (aCSF). The aCSF solution contained the following components: 2.5 mM KCl, 126 mM NaCl, 1.2 mM NaH_2PO_4 , 25

mM NaHCO₃, 2.4 mM CaCl₂, 1.2 mM MgCl₂, 20 mM HEPES, 11 mM D-glucose. The pH was adjusted to 7.4. To ensure the tissue received ample oxygen, the aCSF was continuously bubbled with 95% O₂/5% CO₂ throughout the experiment. After chilling for one minute, the cerebellum was removed and the brain was then glued to a plate against a cube of agar for support. Several 300 µm coronal brain slices were then obtained using a vibratome (Leica Microsystems, Bannockburn, IL, USA). In a typical recording session, a single striatal brain slice was transferred to a perfusion chamber where oxygenated aCSF, maintained at 34 °C with a thermostatted perfusion chamber and in-line heater, flowed over the slice at 2 mL/min. Slices were equilibrated for at least one hour before collecting measurements. For autoradiography analyses, whole harvested brains were frozen at –80 °C and then subjected to the procedure described below.

3.3. Electrode fabrication

Carbon-fiber cylindrical microelectrodes were fabricated as previously described^[28]. Briefly, 7µm carbon-fiber purchased from Goodfellow Cambridge Ltd. (Huntingdon, England) was loaded into glass capillaries (4 in, 1.2 mm OD; A-M Systems, Inc. Carlsborg, WA, USA) and pulled using a heated coil puller (Narishige International USA, East Meadow, NY, USA). Carbon-fiber tips were then cut with a scalpel 25 µm from the end of the glass seal. Electrodes were then sealed by dipping into a well-mixed mixture of 0.24g EPI-CURE 3234 Curing Agent (lot FCXC4114/0886GG) and 2.00g EPON Resin 815C (lot HADN0003/1307GG). Excess resin was removed by dipping several times in toluene and electrodes were then baked for 1 hour at 100°C. The electrodes were back-filled with 0.5 M potassium acetate in order to establish an electrical connection between the carbon-fiber and the inserted silver wire.

3.4. Electrochemical measurements using FSCV

Procedures for measuring DA release with background-subtracted FSCV have been described in detail previously^[4b]. Briefly, a pre-calibrated cylindrical carbon-fiber microelectrode was inserted with micromanipulators 100µm into the brain slice. The electrode was positioned between two biphasic stimulating electrodes (A-M Systems Inc, Carlsborg, WA, USA) in the CPu. For DA detection, a triangular waveform starting at –0.4V, scanning up to 1.0V, and back to –0.4V, was applied to the carbon-fiber microelectrode at a scan rate of 300V/s and an update rate of 10 Hz. DA release was evoked both by applying a single, biphasic pulse (350 µA, 4 ms total duration) and by applying 120 pulse stimulations. For each slice, single pulse stimulated release amplitude was measured every five minutes. Once amplitudes were stable between consecutive stimulations, the recordings used in the analysis were obtained. For the 120-pulse stimulations, DA release was evoked at stimulation frequencies of 20, 30, 40, 50, and 60 Hz at each of the four quadrants of the CPu (DL, DM, VM, and VL), starting with the lowest stimulation frequency and going to the highest. The peak current after stimulation was used for all release measurements and a 5 minute recovery period was provided between each measurement. The current measured from DA oxidation was plotted versus potential and the successive voltammograms were plotted versus time.

3.5. Measurement of DAT by Autoradiography

Regional densities of DAT were determined by [³H]WIN 35,428 autoradiography according to a modification of the methods of Coulter et al.^[29]. Briefly, brains were collected, frozen in isopentane, and stored at –70 °C until sectioning. Coronal sections (20 µm) were cut on a cryostat. Duplicate sections were incubated with 4 nM [³H]WIN 35,428 (Perkin-Elmer, SA = 84 Ci/mmol) in buffer (50 mM NaHPO₄, 50 mM NaCl, pH 7.4) for one hour on ice. Non-specific binding was defined in the presence of 50 µM cocaine. After incubation, slides were dipped in ice-cold buffer, washed for two consecutive two minute periods in ice-cold buffer, dipped in ice-cold deionized water, and dried. Radiolabeled sections were then opposed to ³H-Hyperfilm (Amersham, Arlington Heights, IL) with [³H]methylmethacrylate autoradiography standards for 19 days. ³H-Hyperfilm was developed according to the manufacturer's instructions. Brain sections were then stained with cresyl violet.

Autoradiographic images were digitized and quantified using ImageJ. A Rodbard plot was used to describe the relationship between optical density and radioactivity. Measurements represent average pixel optical density by volume analysis. Brain regions were identified according to the atlas of Paxinos and Watson^[30]. The CPu was divided into quadrants and sampled bilaterally at five levels between Bregma 1.80 mm and –1.00 mm. Values, reported as specific binding (fmol/mg tissue equivalent), were averaged to yield a binding density for each animal.

3.6. Statistics

Statistical analyses of dopamine release were conducted using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). Autoradiographic data were analyzed with SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA). For all analyses, n = the number of mice. Data are given as mean ± SEM. For electrochemical and autoradiography measurements, n = 4 to 8 mice.

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Fig. 1.

Dopamine release comparison of wild-type control (A) and an R6/2 mouse (B) at 13 weeks of age. Representative color plots and stimulated DA release plots, sampled along the horizontal dashed lines on the color plots, are shown. Cyclic voltammograms, sampled along the vertical dashed lines on the color plots, are shown directly above the stimulated release plots to confirm the presence of DA. Dopamine was evoked by a single pulse.

Fig. 2.

Dopamine release concentration by striatal region in 13-week old R6/2 and WT mice. (A) Image of a mouse coronal brain slice containing the CPu. The four quadrants sampled from within the CPu are indicated. (B) [DA]_{max} for R6/2 mice in comparison to WT controls shown within each region of the CPu (* $p < 0.05$, ** $p < 0.01$, one-way ANOVA). Dopamine release was evoked by a single stimulus pulse. Image credit: R.W. Williams, University of Tennessee Health Science Center, with permission.

Fig. 3.

Age progression of striatal dopamine release in R6/2 and WT control mice. Dopamine release from each quadrant was normalized against the greatest release obtained from all quadrants. Release was evoked by a single stimulus pulse. Significant differences between quadrants were found in R6/2 mice at 11 and 13 weeks of age (one-way ANOVA, overall difference: 11 weeks, $p < 0.05$, 13 weeks, $p < 0.0001$. * $p < 0.05$, ** $p < 0.005$, $n = 6$ WT and 6 R6/2 mice).

Fig. 4. Representative raw data for a 13 week-old R6/2 and WT brain slices subjected to 120 stimulation pulses at 60Hz in the dorsolateral caudate. Cyclic voltammograms shown above the stimulated release plot confirm the presence of DA.

Fig. 5.

Dopamine release resulting from application of 120-pulse stimulations administered at multiple frequencies. Measurements were obtained in the dorsolateral (DL), dorsomedial (DM), ventromedial (VM), and ventrolateral (VL) CPu. Frequency is indicated on the x-axis of each panel. There was no significant effect of frequency on dopamine release in WT or R6/2 mice in any region (one-way ANOVA, $p > 0.05$, $n = 5$). Out of the four regions sampled, $[DA]_{\max}$ is significantly attenuated only in the dorsal CPu (DL, $p < 0.005$, DM, $p < 0.05$, two way ANOVA, $n = 5$ WT and 4 R6/2 mice).

Fig. 6. Autoradiographic localization of [^3H]WIN 35,428-labeled sites in striatal slices from WT and R6/2 mice. Data shown represent total binding. (A) Representative autoradiograms. (B) Bar graph of average binding within each striatal region of WT and R6/2 mice (two-way ANOVA, overall main effect, R6/2 versus WT: $p < 0.001$. Tukey post hoc test: $*p < 0.01$, $n = 8$ WT and 6 R6/2 mice).