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Possible breakdown of dopamine receptor synergism in a mouse model of Huntington's Disease

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Abstract

The model of basal ganglia function proposed by Albin, Young and Penney (1989) describes two anatomically independent motor pathways, the direct and indirect. However, under normal conditions striatal dopamine (DA) is required for the expression of motor behavior, and DAergic control of the two pathways (via D1 and D2 receptors, respectively) is dependent on co-activation. We tested for a possible breakdown of D1/D2 synergism using transgenic R6/1 mice bearing the human *huntingtin* allele (Htt). Motor stereotypy, observed prior to the onset of HD-related symptoms, was rated on a 5-point scale following activation of: A) D1 receptors alone, B) D2 receptors alone, and C) stimulation of both D1 and D2 receptors. Results revealed that mice with the HD allele, like their WT litter mates, depend on the co-activation of the indirect and direct motor pathways to facilitate deliberate behavior.

Keywords: dopamine, synergism, motor pathway, Huntington's Disease, basal ganglia, striatum

Introduction

Huntington's Disease (HD) is a progressive, neurodegenerative disorder in which diagnostic symptomology results primarily from cell death in the striatum. First described as a heritable choreiform syndrome by George Huntington in 1872, HD manifests itself in mid-adulthood and results in the deterioration of cognitive ability and motor function as well as abrupt and unpredictable changes in mood and behavior. (Huntington's Disease Collaborative Research Group, 1993). With the advent of genetic sequencing methods in the early 1980's, James Guesella and his team at Massachusetts General Hospital identified the genetic contribution to HD in 1983 (Guesella et al. 1983). This fatal condition results from an autosomal dominant mutation located on chromosome 4. The normal gene codes for the protein huntingtin (HTT) which is ubiquitous in the brain and the body (Sharp et al., 1995). The mutated huntingtin gene (*mHTT*) found in HD patients includes an unstable expansion of the polyglutamine (CAG) region towards its 5' terminus. (Huntington's Disease Collaborative Research Gorup, 1993). Due to the polymorphic nature of the normal *HTT*, these glutamine repeats naturally vary at lower frequencies. The threshold for an HD diagnosis ranges between 36 and 41 glutamine repeats (Andrew et al., 1993; Kremer et al., 1994; Rubinszetein et al., 1996). Individuals with a comparatively greater number of repeats show more severe symptoms and an earlier age of onset than those individuals who just reach the diagnosable threshold. (Duyao et al., 1993).

Prevalence

According to Rawlins et. al. (2016), prevalence of the huntingtin mutation differs dramatically depending on ethnicity and geographic location. Areas with higher populations of Western European decent showed the highest prevalence between 4 and 10 cases per 100,000

people. The United States falls at the higher end of this range where approximately 30,000 people are diagnosed with HD and over 150,000 more have a 50% chance of developing HD in their lifetime. Predominantly Asian populations, however, showed the lowest prevalence rates that averaged less than 1 case per 100,000 people.

Symptoms

The genetic variability of the HD genotype presents with a myriad assortment of afflicted phenotypes such that age of onset and severity of symptoms is dependent but not exclusively determined by the number of trinucleotide (CAG) additions. For example, the average HD patient begins to present with symptoms between 30 and 50 years of age (Langbehn, Brinkman, Falush, Paulsen, & Hayden, 2004); however, cases have been reported as early as age 5 and as late as age 85 (Andrew et al., 1993; S. Chen, Ferrone, & Wetzel, 2002). The 10% of cases that present before age 20 result from a juvenile variant of the disorder known to typically have repeat lengths >60 and be considerably more aggressive (Conneally, 1984; Beighton & Hayden, 1981). Likewise, those beginning to present with symptoms relatively later in life typically have repeat lengths closer to 40 and more manageable symptoms.

Patients in the early stages of HD, averaging between the ages of 30 and 45, typically present with changes in behavior and impaired cognition as well as minor psychiatric disturbances. For example, changes in weight, sexual behavior, and sleeping cycles are some of the most commonly reported symptoms early on. These symptoms grow progressively worse over the course of the patient's remaining life (Bates & Jones, 2002).

In the later stages of HD, patients gradually lose the ability to maintain control of their motor function. An involuntary series of spontaneous, dance-like movements classified as chorea

characteristically manifest first, followed by dystonia and possibly paralysis to some muscle groups. Tasks such as walking, talking, and consuming food become increasingly more difficult and eventually impossible without continuous aid and supervision (Bates & Jones, 2002; Rosenblatt, 2007).

Cognitive and motor impairment diagnostic in both early and late stages of HD continue to worsen until culminating in the patient's untimely death. The severity of this progression, however, adheres to a unique timeline for each individual. Studies have shown that roughly half of the variation in this aspect of the clinical phenotype can be attributed to the length of the mutated polyglutamine (Andrew et. al, 1993). Premature death typically results not from the disease itself but as a result of life-threatening complications due to the aforementioned symptoms. It is not uncommon for patients to suffer nutritional deficiencies from not eating, infections from fall related injuries, and choking. The most common cause of death is aspiration pneumonia due to the combination of motor difficulties in the esophagus and high susceptibility to infection. (Bates & Jones, 2002).

Currently, there is no therapy available that can treat, prevent, or delay the progression of HD. However, medications such as antidepressants, multivitamins and antipsychotics have been given to patients to alleviate the severity of secondary symptoms; i.e. severe depression, weight loss, and psychosis. (Zuccato, Valenza, & Cattaneo, 2010)

Neuropathology

Neurodegeneration in HD results in a significant loss in volume of the basal ganglia due to the death of targeted cells in the striatum (Dunlap, 1927; Vonsattel el al., 1985; de la Monte, Vonsattel, & Richardson, 1988). Despite widespread expression of the huntingtin allele, cell

atrophy is observed exclusively in the brain and predominately in the striatum (Sharp et al., 1995). The most affected cells, composing 95% of this structure, are identified as GABA-ergic, medium-sized, spiny neurons (Sapp et al., 2004; Saudou, Finkbeiner, Devys, & Greenberg, 1998; Graybiel, Ragsdale, & Edley, 1979). Within this phenotype, striatal matrix neurons expressing enkephalin and D2 receptors appear disproportionally affected in the early stages of HD (Saudou et al., 1998).

Although the striatum appears to be a principally targeted region of cell death in HD, studies reveal a number of other brain regions affected in later stages of the disease. These include but are not limited to layers III, V, and VI of the cerebral cortex, globus pallidus, thalamus, subthalamic nucleus, substantia nigra, white matter, hypothalamus, and cerebellum. The variation in damage to these additional areas across diagnosed individuals could largely account for the complexity and heterogeneity observed in the HD phenotype (Zuccato, Valenza, & Cattaneo, 2010).

Structure of the Basal Ganglia (Humans vs. Mice)

The structures comprising the basal ganglia in humans include the striatum, internal and external globus pallidus, substantia nigra pars reticulata, and subthalamic nucleus (Figure 1). The primary afferent structure is the striatum which is subdivided by a large white matter tract, the internal capsule, into a distinct caudate and putamen. These structures are fundamentally the same and only distinguishable by name and location. The primary output structures of the basal ganglia are the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). Much like the caudate and the putamen, the GPi and the SNr are virtually the same structure only divided by white matter of the cerebral peduncle, an extension of the internal capsule.

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The neuronal landscape in general is very similar between the human and rodent basal ganglia. The major distinction between the two is how the structures are named, and by variation in the white matter tracts subdividing the basal ganglia (Figure 2). In contrast to the human striatum, which is neatly bisected by the internal capsule, a distinct caudate nucleus and putamen are not distinguishable in rodents. Instead, the internal capsule courses through the rodent striatum, or caudate-putamen (CPu), in small bundles. Before reaching the output structures of the basal ganglia, the white matter aggregates and forms the cerebral peduncle. This tract then forks around the entopeduncular nucleus (EN), or the functional equivalent of a human's GPi. With no need to differentiate a similarly named structure by its location, the human external globus pallidus (GPe) is simply referred to as the globus pallidus (GP) in the rodent brain.

Motor Pathways

The current model for basal ganglia function that accounts for the symptomology of movement disorders, including HD, was presented in 1989 by Albin, Penny and Young. Through a series of necropsies as well as brain stimulation and lesion experiments, they proposed that assimilating motor information into a fluid series of actions was the primary function of the basal ganglia. Disrupting the major motor pathways running through this structure results in one of three outcomes: over stimulation, under stimulation, or lack of stimulation to motor cortices that facilitate action.

Cortico-striatal afferents projecting from the isocortex excite the striatum by releasing the neurotransmitter glutamate. Projections from the striatum to the IGP/EN and the SNr (the output structures of the basal ganglia) can follow one of two pathways. First, a direct projection to the IGP/EN and the SNr from the striatum is possible. Signals can also reach the IGP/EN and the

SNr from the striatum via an indirect pathway through the EGP/GP and the subthalamic nucleus (STN). The striatum, GP, and SNr all release the inhibitory neurotransmitter, GABA. The STN and the substantia nigra pars compacta (SNc) are also capable of creating negative feedback loops by projecting back onto the striatum. The STN releases the excitatory neurotransmitter glutamate. The SNc releases dopamine which has an excitatory effect on the "direct" output pathway (Figure 4, and see below) and an inhibitory effect on the "indirect" output pathway (Figure 3 and see below). Thus, dopamine has a major influence on the release of GABA from the axon terminals of striatal projection neurons. The motor pathway continues from the basal ganglia, i.e. the IGP/EN and the SNr, to the thalamus and terminates in the motor cortex which are both glutamatergic structures. Disinhibition of the thalamus leads to motor activity and other behaviors.

The striatum itself is composed mostly of medium spiny GABAergic projection neurons (90-95%). The remaining 5-10% are large cholinergic and small aspiny GABAergic interneurons. The projection neurons directly inhibit the GP, SNr, and SNc. Two types of macroscopic compartments can be discerned in the striatum on the basis of neurochemical phenotype: large striosomes on a background matrix. Afferent connections to the striosomes project from the prefrontal and limbic cortices while their efferent connections project onto the SNc. Dopaminergic axons from the SNc project back onto the striatum, creating one of the two negative feedback loops. Afferent connections to the matrix project from the primary motor, somatosensory, frontal, parietal, and occipital cortices. Their efferent connections differentiate matrix neurons even further. Those neurons containing the co-transmitter Substance P project onto the IGP/EN or the SNr, along the direct motor pathway; while those containing the co-transmitter enkephalin project mainly upon the EGP/GP, along the indirect motor pathway.

These enkephalin-positive matrix projection neurons are preferentially targeted in HD, causing an initial breakdown in the indirect motor pathway. The interneurons, containing somatostatin and neuropeptide Y, link these two striatal compartments and allow for communication between the striosomes and the matrix.

Dopamine Synergism

Projections from the SNc directly stimulate two kinds of neurons in the striatum: medium-spiny GABAergic neurons with D1-like dopamine receptors and medium spiny GABAergic neurons with D2-like dopamine receptors. These G-protein coupled receptors are differentiated primarily by their amino acid sequence, their affinity for various synthetic drugs, and ultimately by their physiology upon activation. When bound with dopamine, a striatal D1-like receptor will couple with the $G_{\alpha s}$ protein which has an overall excitatory effect on the cell. These striatal matrix neurons contain Substance P and project onto the IGP/EN and the SNr as part of the direct motor pathway. Neurons with D2 like receptors use the $G_{\alpha i}$ protein which has an overall inhibitory effect on the cell. These striatal matrix neurons contain enkephalin and project along the indirect motor pathway. These are the same cells differentially targeted by early stages of HD.

Drug influence of D1 and D2 class receptors is known to modify unconditioned motor behavior. For example, antagonists blocking either or both DA receptor types will prevent noncompulsory movement (Christensen et al., 1984; Honda et al., 1977) while administration of dual agonists at low to moderate doses will induce movement. At high doses, these same agonists able to stimulate both D1 and D2 receptor types will induce continuous and focused stereotypy (Ernst, 1967). Interestingly, DA motor activation occurs only with stimulation of both D1 and D2-like receptors simultaneously. Stimulation of either receptor type in isolation produces no behavioral

effect. Thus combined activation of both D1 and D2 receptors are required for the manifestation of dopamine's effects, a phenomenon termed D1/D2 receptor synergism (LaHoste and Marshall, 1996). Repeated evidence of dopamine synergism in normal rodents suggests a dependency on interplay between the two motor pathways (direct and indirect).

Motor Dysfunction in HD

There are three types of movement disorders that result from interruptions in the motor pathways through the basal ganglia: hyperkinetic disorders, hypokinetic disorders, and dystonia. HD patients are afflicted with both diagnostic chorea, a hyperkinetic symptom, and dystonia due to an imbalance between synaptic dopamine concentrations and the declining number of available corresponding receptors.

Hyperkinetic symptomology is defined by excessive activity with rapid, uncontrolled movements that hinder normal motor functions. These symptoms manifest in earlier stages of HD due to an excessive amount of dopamine in the synapses between afferent SNc axons and efferent striatal neurons containing D2-like receptors. As the differentially targeted, enkephalin-containing striatal neurons diminish in number, so does the initiation of the indirect motor pathway. However, the substance P containing matrix neurons with afferent projections to the GPi and SNr (direct motor pathway) and the striosome neurons with afferent projections to the SNc remain largely unaffected. This creates a neuronal network in which the normal functioning feedback loop from striatal neurons with D1-like receptors to the SNc releases "normal" concentrations of dopamine on to fewer D2-like receptors in the striatum. Overstimulation of these striatal neurons results in increased inhibition of the indirect motor pathway and ultimately an overstimulation of the motor cortex which is behaviorally manifested as chorea. Hyperkinetic

symptoms are subdued by dopamine antagonists which block the highly concentrated dopamine from binding with striatal neurons.

Dystonia is defined by involuntary fixed postures that prevent voluntary movement for seconds to minutes at a time. This symptom is commonly witnessed in patients suffering later stages of HD due to the breakdown of the direct pathway. By the later stages of the disease, more cell types become susceptible to death including the Substance P producing striatal matrix neurons with D1 receptors whose axonal efferents connect to the IGP/EN and SNr. With the decreasing number of D1 receptors, synapses become flooded with dopamine and over stimulate the remaining striatal cells. This indirectly decreases stimulation to the motor cortex and inhibits voluntary movement.

Figures

Figure 1: Schematic diagram of the human basal ganglia

Thesis statement

The Albin, Penny, and Young model for Huntington's Disease describes two independent motor pathways, each initiated by stimulation of its own dopamine receptor type. We now understand, however, that in normal, healthy systems simultaneous stimulation of both D1 and D2 receptors is required to elicit a behavioral response. Therefore, the direct and indirect motor pathways should not be able to function independently as their model suggests. This synergistic property, however, has not been tested under the conditions of HD.

In this study, we independently targeted D1 and D2 dopamine receptors in a mouse model of HD mice to determine whether HD is associated with a breakdown in D1/D2 synergism.

Approach/ Methods

All procedures performed in this study were approved by the University of New Orleans Institutional Animal Care and Use Committee beforehand (Approval # UNO-16-004) and were on strict adherence to the guidelines established by the U.S. Public Health Service. Every effort was made to minimize animal discomfort and reduce the number of experimental mice.

Animals

We chose the R6/1 transgenic murine model of Huntington's Disease originally developed by Mangiarini et al. in 1996. An R6/1 line is created by using the human promoter and exon-1 of the HD gene. cDNA is generated using E.coli and is subsequently injected into single cell CBAxC57B/L6 embryos. Integration of this transgene allows for ubiquitous expression of an N-terminal huntingtin fragment containing the extended glutamine repeat. Our P1 generation, consisting of two HD males and four wild-type females, was purchased from Jackson Laboratory

(JAX) in Bar Harbor, Maine, USA. HD males and females from subsequent generations bred within our own lab at the University of New Orleans were used for this study.

Tail Biopsy and Identification

All offspring of the purchased P1 generation mice underwent a tail biopsy to obtain DNA for genotyping. This procedure was done within three weeks after birth, prior to weaning, to maximize the potential for DNA collection and avoid cutting through the ossified vertebrae. Adhering to this time frame did not require the use of anesthesia and was also less traumatic for the subject.

The mouse was restrained between the thumb and forefinger to prevent excessive movement. Each individual received ear punch markings that correspond to a specific identifying number. Once identified, the distal 2-5 mm of the tail was removed using a sterile straight razor. Bleeding was then controlled using local pressure with a clean cotton ball. The tail clip was then placed in 300µl of Direct PCR lysis reagent and 11.6 µl of proteinase K solution (Viagen BioTech, Los Angeles, California, USA). Samples were placed on rotation in an incubator at 55ºC overnight to allow for complete cell lysis.

PCR

Once incubated, the proteinase K was deactivated by placing the samples in a hot water bath 90ºC for approximately 1 hour. 2.0µl of each sample was mixed with 12.5µl of GoTaq Green Mater Mix, 3µl of both forward (5'-CCGCTCAGGTTCTGCTTTT-3') and backward (5'-TGGAAGGACTTGAGGGAC-3') primers, and 9.5µl of nuclease free water. The samples were then placed into a thermal cycler programed to anneal at 55ºC for 1 minute with a 1-minute extension time for 31 cycles. Samples were then preserved at 4ºC until retrieved the following day.

Gel Electrophoresis

Molecular separation is carried out using a 3% agarose gel. The first well contains a 50bp ladder as a standard to determine the length of the presenting alleles. Remaining wells contain 5µl of concentrated DNA samples obtained from PCR. A 65v electrical current was run through the gel for approximately an hour (until the marker dyes approached the farther edge). The DNA was post-stained within the gel using a 0.5µg/ml solution of ethidium bromide and then imaged using a standard florescent light box. Well lines fluorescing at the 305bp mark identify transgenic mice. Wells containing samples from wild type mice will not fluoresce.

Later Edit to Genotyping Process

Due to the contamination of the working primers as well as time constraints, 53 of the total 71 mice used in this experiment were genotyped externally (Mouse Genotype, Escondito, California, USA). Nevertheless, standard PCR and gel electrophoresis methods were still used to isolate, amplify, and distinguish the DNA.

Motor Performance

The Rotarod apparatus (Med Associates Inc., Georgia, VT) was used to measure motor performance in all experimental mice. Its speed was set at 16 rotations per minute for the duration of the experiment. All mice were given 1 day of habituation at two months of age in addition to 1 day of testing prior to each drug trial date. To measure motor function, each mouse was placed on the rotarod and the tendency to fall off the rod was timed and scored. Scores for each trial corresponded to the number of seconds the animal remained on the rotating rod, allowing for the highest total score of 60 per trial. This was repeated 3 times with at least a 1-minute break between each trial, allowing for a maximum score of 180 per day (Stack et al., 2005).

Experimental Design

Experimental mice will be housed in same-sex cages with free access to food and water. Artificial lighting is provided on a 12-hour cycle. A total of $N = 71$ mice are used (25 = HD). 24 hours prior to experimental testing, all mice were tested on the Rotarod to ensure that symptomatic motor deterioration had not progressed enough to interfere with behavioral results.

Each mouse in the study was treated identically. Between 2 and 2.5 months of age, all were exposed to each of the following 3 trial conditions separated by 48-72 hours: A) stimulation of D1 receptor alone using a D2 specific antagonist (0.3mg/kg of eticlopride) followed by a D1 agonist (3.0mg/kg SKF-38393); B) stimulation of D2 receptor alone using a D1 specific antagonist (0.1mg/kg SCH-23390) followed by a D2 agonist (3.0mg/kg quinpirole); C) stimulation of both D1 and D2 receptors using a saline injection followed by a mixed solution of D1 and D2 agonist (3.0mg/kg SKF-38393 and 3.0mg/kg quinpirole respectively). The initial antagonist injection effectively prevents endogenous dopamine from stimulating the heterotypic receptor. To control for order effects, the sequence of administered conditions was counterbalanced.

Each mouse was placed in a Plexiglas cube lined with a thin layer of bedding and backed by a large mirror. After 30 minutes of habituation, the first injection of a condition (either an antagonist or saline) was administered. Fifteen minutes after the first injection, the second injection (either a D1 or D2 agonist alone, or the combination) was administered. The subject's behavior was recorded following the agonist injection and continued for 60 minutes. The entire duration of this process was recorded using an HD video camera. Upon subsequent review of the recording, the investigator observed each mouse's behavior and rated the behavior during a 30-second interval according to an established scale of 0-5 (Nolan et al, 2007). Observations were

repeated every 5 minutes from 0-60 minutes. Time 0 is considered the point at which the second injection, the agonist(s), is administered. The behavioral scale is defined as follows: 0 = still, 1 = grooming or normal exploration, 2 = unfocused stereotypy behavior (brief episodes of strong sniffing), 3 = continuous unfocused stereotypy (behavior directed towards multiple objects), 4 = continuous focused sniffing, 5 = continuous focused oral stereotypy (licking or chewing one object).

Results

Rotarod data immediately prior to drug testing showed no difference between HD and WT mice. Therefore, the results were not influenced by potential bias between the two genotypes due to differences in motor abnormalities.

We conducted a 2 (Genotype: HD versus WT) X 3 (Drug Condition: A) stimulation of only D1-like receptors, B) stimulation of only D2-like receptors, C) stimulation of both D1- and D2-like receptors) mixed design ANOVA on behavior. Due to the violation of sphericity of covariance matrix assumption, as indicated by a significant Mauchly's Test (p < .001); Greenhouse-Geisser values are used. Results reveal a main effect of drug condition [F (1.53, 96.31) = 1200.79, p < .001, η^2 = 1] as well as a main effect of genotype [F (1,63) = 5.39, p = .02, η^2 = .08]; but no interaction between drug condition and genotype [F (1.53,96.31) = 1.53, p = .28, η^2 = .22]. Bonferoni contrasts reveal that condition C, the simultaneous stimulation of both D1- and D2-like receptors, induced significantly greater stereotypy than condition A (p < .001) or condition B (p < .001), the discrete stimulation of D1-like and D2 like receptors respectively. In effect, D1/D2 synergism remains intact regardless of genotype; however, stereotypy appears to be greater in HD mice.

Discussion

Evidence of D1/D2 receptor synergism as a feature of normal motor function is explored in several behavioral studies though the mechanism of this phenomenon remains abstruse. Only a few conditions are known in which purposeful movements are possible without their simultaneous activation. In Parkinson's Disease, for example, dopamine depletion results in acute dopamine receptor supersensitivity. In this state, behavioral responses can be elicited from stimulation of D1-like receptors alone, suggesting a breakdown in dopamine receptor synergism. The fact that Huntington's Disease depletes enough striatal neurons to effectively alter the ratio between dopamine and it's D1-like receptor type suggests a breakdown of D1/D2 receptor synergism could be possible. Induced stereotypy from either of DA receptor types alone would suggest that the direct and indirect motor pathways can function independently as a result of neurodegeneration, supporting the current models of HD proposed by Albin, Penny, and Young.

We tested the synergistic property of dopamine receptors in HD mice by comparing their behavior when D1 and D2 receptor types are independently stimulated versus co-stimulated. Results indicate that HD mice maintain DA receptor synergism and display behavior patterns similar to WT mice (Figure 5).

Figure 5

Behavioral scores plotted in Figure 5 clearly illustrate D1/D2 receptor synergism in both WT and HD mice. It is visually evident that all three drug conditions deviate from normal behavior, or a score of 1 on this scale. Drug condition B is defined as the isolated stimulation of

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the D2-mediated, indirect motor pathway. Both WT and HD mice, plotted as green and maroon respectively, show very little to no movement following the administration of the D1 antagonist and D2 agonist. Therefore, we can conclude that activation of the indirect pathway alone will not produce deliberate behavior. Similarly, drug condition A resulted in reduced movement due to isolated stimulation of the D1-mediated, direct motor pathway. Both WT and HD mice, plotted as orange and yellow respectively, show mean scores closer to 1 than condition B because grooming is a D2 independent D1-mediated behavior. Therefore, injection of the D1 agonist SKF 38393 induces excessive grooming even after the administration of a D2 antagonists. Nevertheless, stimulation of the direct motor pathway alone results in mean scores reflecting far less movement than observed in normal behavior. Behavioral scores of mice treated with condition C, however, show an entirely unique pattern when compared with conditions A and B. Following the administration of a D1 and a D2 agonist, the mice show a gradual increase in behavior until activity peaks between approximately 15 and 25 minutes and then slowly resumes to normal behavior. This is evident in both HD and WT mice plotted in dark green and brown respectively, suggesting that the early stages of neurodegeneration caused by HD do not result in a breakdown of D1/D2 synergism.

Behaviors scored 5 minutes ($T = -5$) prior to the second injection, between the antagonists and agonists in conditions A and B and between the saline injection and the mixed agonists injection in condition C, also give evidence to D1/D2 synergism in HD mice. In conditions A and B, the heterotype antagonists prevent any activation of its respective pathway leaving only one motor pathway stimulated by endogenous dopamine. Likewise, in both conditions mean behavioral scores are less than 1. This indicates a decrease in motor activity from normal behavior. In condition C, however, the initial injection is saline. Without the

antagonistic effects on either dopamine type, all mice display normal activity at time T = -5. Therefore, we can again conclude that HD mice, like their WT litter mates, depend on the co-activation of the indirect and direct motor pathways to facilitate deliberate motor behavior

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Vita

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