Deficits in a visual go/no-go discrimination task in two mouse models of Huntington's disease.

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Abstract

Huntington's disease (HD), a devastating neurological disorder caused by a CAG repeat expansion located on chromosome 4, is associated with a characteristic pattern of progressive cognitive dysfunction known to involve early deficits in executive function. A go/no-go discrimination task, requiring mouse subjects to withhold instrumental responding when cued to do so by a light stimulus, was designed to tap into the type of online response control/executive function known to be disrupted in patients. The present studies show that this simple discrimination assay revealed early and robust deficits in two mouse models of HD, the z_Q175 KI mouse (deficits from 16 weeks of age) and the R6/2 Tg mouse, carrying ~240-CAG repeats (deficits from 9 weeks of age). These deficits are not due to motor dysfunction in the test animals, but instead appear to measure some inability to inhibit responding in the HD mouse models, suggesting this assay may measure deficits in underlying attentional and/or behavioral inhibition processes. Accordingly, this assay may be well suited to evaluation of simple deficits in cognitive function in mouse HD models, providing a potential platform for preclinical screening.

General methods

Colony procedures

Animals were maintained throughout on a standard 12:12 light cycle with free access to water. Prior to experimentation, the animals were reduced to 85% of their *ad libitum* weights. Animals were caged in optiMOUSE housing (Animal Care Systems, CO), enriched with play tunnels, shredded paper, and plastic bones.

Equipment

Mice were tested in standard narrow mouse operant chambers (Med Associates, VT) with the floor area measuring 6.25" long x 5.5" wide and 5.0" high walls. Each chamber contained a nosepoke recess, which could be illuminated by a small embedded bulb, located centrally on the wall opposite the food magazine. Reinforcement was provided by time-limited access to a dipper containing evaporated milk (Carnation™) delivered via a dipper. The hardware was controlled and all events were recorded by the Med-PC IV software package.

Nosepoke training procedure

Animals were initially magazine trained for two sessions and then trained to nosepoke via a simple fixed interval 20-s (FI20) free operant procedure, where nosepoking was reinforced with access to an evaporated milk reinforcer. No reinforcement was delivered without a nosepoke. Animals were trained to a criterion, requiring them to obtain 40 reinforcers across 2 consecutive 40 min sessions.

Discrimination training procedure

Discrimination training sessions followed completion of instrumental pre-training. These sessions were also 40 mins in duration, presenting the animals with both potentially reinforced and unreinforced periods, with the availability of reinforcement signaled by the illumination state of the nosepoke recess. In all cases, the light condition presented in pre-training served as the reinforced state, such that the animals were required here to learn to avoid responding in the novel condition. The sessions were unbalanced, with 30 min of potentially reinforced time presented pseudorandomly in blocks of 30, 60, 90, 120 or 150 seconds, interspersed with 10 min of unreinforced time presented pseudorandomly in blocks of 10, 20, 30 or 60 seconds. Nosepoking was reinforced during the potentially reinforced periods on a response-initiated variable-interval 5 second (VI5) schedule with 3 seconds of access to the milk reinforcer.

Discrimination performance

Discrimination performance was indexed by a discrimination ratio (DR) calculated by dividing the response rate in the reinforced condition by the average of the response rates in the reinforced and the unreinforced conditions for each mouse for each session. Raw response rates were also collected.

Conclusions

The experiments described here reveal clear deficits in discrimination ratios in a visual discrimination go/no-go task in both the R6/2 transgenic and the CAG 175 knock-in mouse models of HD. Overall, this assay seems a promising and relatively simple method to measure some aspects of executive dysfunction in these HD mouse models.

The nature of the underlying deficit here appears to stem from a reduced ability in the HD mice to control a learned response, with the HD mice more prone than controls to continue making responses even when clear cues are present to indicate that reinforcement is not available.

This task, which requires around 5 weeks of total training time, might prove valuable in preclinical investigations of response control in these HD mouse models.

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R6/2 mice provided courtesy of King's College, London.

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Statistical analysis conducted with SAS and StatView software (SAS Institute), alpha level of 0.05 adopted throughout.

References

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW and Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* ***87,*** 493-506.

R6/2 testing

Subjects

R6/2 mice (Mangiarini et al., 1996), carrying ~240-CAG repeats on a congenic C57Bl6 background (R62 CHDI-004-1 and R62 CHDI-004-(1)(6)), were bred in our facility.

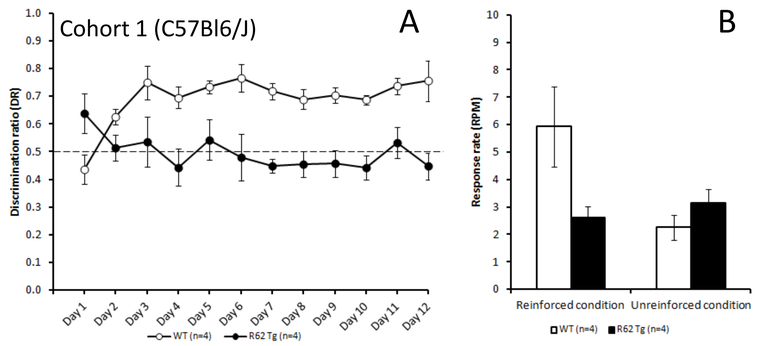
Two separate groups of animals were trained on different genetic backgrounds. A congenic C57Bl6/J cohort (cohort 1) were bred by crossing male R6/2 mice with WT females, producing offspring with a mean CAG repeat length of 247.5 repeats (ranging from 245 to 249). A C57Bl6/J x CBACaJ F1 cohort (cohort 2) were generated by crossing WT CBACaJ males with C57Bl6/J OT females, producing offspring with a mean CAG repeat length of 234 (ranging from 228 to 247 repeats). All mice were 65 ± 5 days old at the start of discrimination training.

Pretraining results

Only a subset of the animals successfully passed the reinforcement criterion in this experiment sufficiently rapidly to be included in the discrimination training phase, leaving significantly reduced cohorts of animals. In cohort 1, only 4 of an initial 6 mice per genotype progressed to discrimination training, leaving a final sample size per genotype of 4 animals. In cohort 2, only 9 of 16 WT mice and 5 of 16 R6/2 Tg were included in the final phase.

Amongst those mice which did acquire the response, there were no genotype differences in their rate of instrumental acquisition.

Results (discrimination training, 9 w R62 mice)

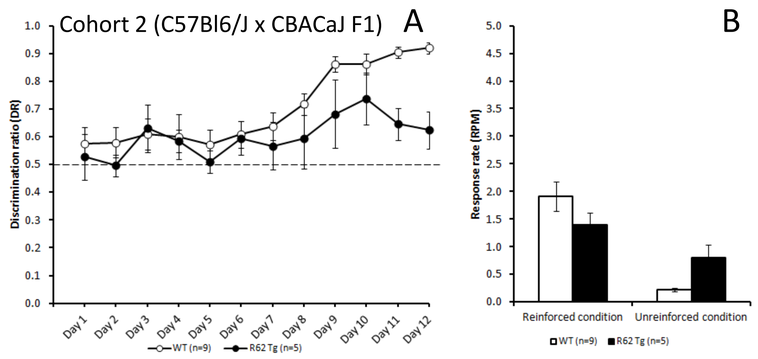


Discrimination training data across the training period (panel A) clearly indicates that the R62 Tg mice (filled symbols) are significantly impaired in acquisition of this discrimination task relative to WT controls (open symbols).

Response rate data taken from the final four sessions (panel B) indicates that this deficit is not solely driven by reduced responding, with the R62 Tg mice responding more rapidly than controls in the unreinforced condition.

This impression was confirmed by analysis, revealing a significant overall effect of genotype, $F(1,6) = 38.7$, $p < 0.001$, along with a significant interaction between genotype and test day, $F(11,66) = 3.32$, $p < 0.01$, but no overall effect of test day, $F(11,66) = 1.21$, $p > 0.25$. Follow-up analysis of the significant interaction indicated that there was a significant effect of test day only in the WT mice, $F(11,66) = 2.87$, $p < 0.01$, with R6/2 Tg mice failing to improve with training, $F(11,66) = 1.65$, $p > 0.1$.

Over the final four days of discrimination training, no reliable differences were observed in response rate in either condition, reinforced $t(6) = 2.19$, $p > 0.07$, unreinforced $t(6) = 1.33$, $p > 0.2$.



Discrimination training data across the training period (panel A) again indicates that the R62 Tg mice (filled symbols) are significantly impaired in acquisition of this discrimination task relative to WT controls (open symbols).

As with cohort 1, response rate data taken from the final four sessions (panel B) indicates that this deficit is not a consequence of slower overall responding, with the R62 Tg mice responding more rapidly than controls in the unreinforced condition.

Statistical analysis was consistent with this impression, revealing a significant interaction between genotype and test day along with a significant overall effect of test day, smaller $F(11,132) = 2.09$, $ps < 0.03$, along with a weak trend towards an overall effect of genotype, $F(1,12) = 3.17$, $p > 0.1$. Follow-up analysis of the significant interaction revealed significant learning over the course of testing only in the WT animals, $F(11,132) = 12.3$, $p < 0.001$. In contrast to the previous experiment, however, there was a clear trend towards improvement in the R6/2 Tg mice, $F(11,132) = 1.74$, $p < 0.08$. The two genotypes did not significantly differ early in training (days 1 to 8) or on day 10, largest $F(1,132) = 1.92$, all $ps > 0.15$, but WT mice performed significantly better on days 9, 11 and 12, smallest $F(1,132) = 4.29$, $ps < 0.05$.

Inspection of raw response rate data from the final four days of training revealed that the R6/2 Tg animals did not differ from WT controls in their responding to the reinforced stimulus, $t(12) = 1.29$, $p > 0.2$, but responded significantly more rapidly than control animals in the unreinforced condition, $t(12) = 3.38$, $p < 0.01$.

z_Q175 KI testing

Subjects

z_Q175 knock-in (KI) animals (CHDI-15-1), on a congenic C57Bl/6J background, were bred either in our facility (cohort 1) or at the Jackson laboratory (Bar Harbor, ME).

Two cohorts of z_Q175 knock-in (KI) mice (CHDI-15-1) were tested.

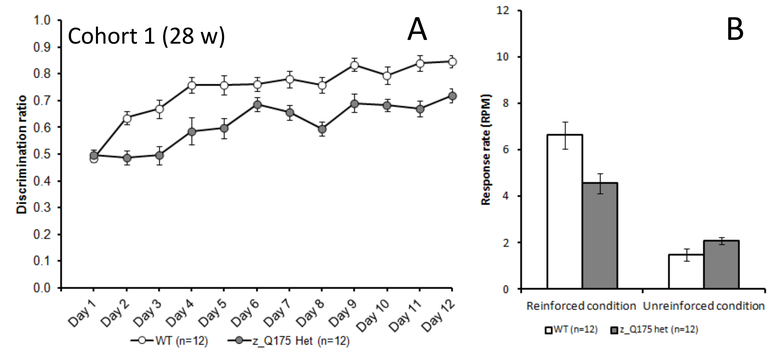
Cohort 1, a mixed sex group of 24 mice (6 mice per sex per genotype, z_Q175 KI vs. WT) were 28 ± 1 weeks old at the start of discrimination training. The heterozygous mice in this study carried a mean of 192.9 CAG repeats, ranging from 187 to 205 repeats.

Cohort 2, a mixed sex group of 63 mice (z_Q175 homo vs. z_Q175 het vs. WT, 8 animals per sex per genotype except female z_Q175 homo where n=6), were 18 ± 1 weeks old at the start of discrimination training.

Pretraining results

All mice successfully acquired the nosepoke response in both cohorts, though there were genotype differences. In both cohorts, WT animals were significantly quicker to acquire than were z_Q175 het mice in both cohorts, while all three genotypes significantly differed in cohort 2. Two z_Q175 homo mice acquired the nosepoke too slowly to participate in the discrimination training phase of experiment 2.

Results (discrimination training, 28 w z_Q175 KI mice)

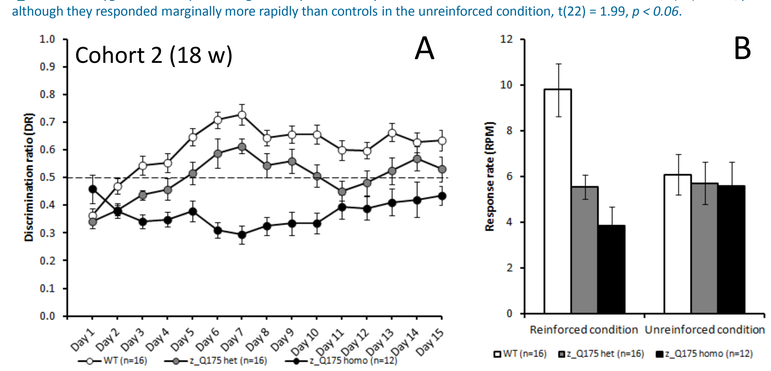


Discrimination training data across the training period (panel A) indicates that z_Q175 het mice (grey symbols) are significantly impaired in acquisition of this discrimination task relative to WT controls (open symbols).

Response rate data taken from the final four sessions (panel B) indicates that this deficit is not driven by reduced responding, with the z_Q175 het mice responding more rapidly than controls in the unreinforced condition.

Analysis was consistent with this impression, revealing a significant effect of genotype, $F(1,16) = 31.5$, $p < 0.001$, and a significant overall effect of training day, $F(11,176) = 26.4$, $p < 0.001$, along with a significant interaction between genotype and test day, $F(11,176) = 2.31$, $p < 0.02$. Follow-up of this significant interaction revealed that there was significant learning in both genotypes, smaller $F(11,176) = 11.39$, $ps < 0.0001$, while the two genotypes were significantly different at all days except day 1, $F < 1$, and day 6, where there was a trend towards a difference, $F(1,176) = 3.23$, $p < 0.08$, smallest remaining $F(1,176) = 7.49$, all $ps < 0.01$.

z_Q175 heterozygous mice responded significantly more slowly than did WT controls in the reinforced condition, $t(22) = 2.88$, $p < 0.01$, although they responded marginally more rapidly than controls in the unreinforced condition, $t(22) = 1.99$, $p < 0.06$.



Discrimination training data (panel A) here indicates that both z_Q175 homo (filled symbols) and z_Q175 het (grey symbols) mice are significantly impaired in acquisition of this discrimination task relative to WT controls (open symbols), with the impairment more pronounced in the homozygous mice.

Response rate data taken from the final four sessions (panel B) here suggests that discrimination performance is largely driven by response rates in the reinforced condition.

Analysis of these data via ANOVA confirmed this impression, with significant main effects of genotype, $F(2,32) = 43.4$, $p < 0.0001$, and of test day, $F(14,448) = 3.77$, $p < 0.0001$, and a significant interaction between genotype and test day, $F(28,448) = 3.02$, $p < 0.0001$. Follow-up analysis of this interaction confirmed that there were significant effects of test day in both WT and z_Q175 het mice, smaller $F(14,448) = 5.55$, $p < 0.001$, but not in the z_Q175 homo animals, $F(14,448) = 1.40$, $p > 0.1$, while there were significant differences between the genotypes at days 3 to 15, smallest $F(2,448) = 7.59$, all $ps < 0.001$, but not on days 1 and 2, larger $F(2,448) = 2.76$, $p > 0.05$. This analysis also indicated that WT animals differed from both z_Q175 het and z_Q175 homo mice on all these days except day 14, where WT and z_Q175 het mice did not significant differ.

Separate analyses of the response rate data indicated that there were significant overall effects of genotype in the reinforced, $F(2,32) = 10.7$, $p < 0.001$, but not the unreinforced, $F < 1$, $p > 0.9$, condition.