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# Temporal Control Deficits in Murine Models of Huntington's Disease

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Timing is a ubiquitous process that underlies a great variety of human activities and depends on highly conserved neuronal circuitry, the cortico-striatal loops. The peak interval (PI) task is an operant task that conditions subjects to initiate and terminate behavioral responses bracketing a fixed interval associated with reinforcement. Performance in this task depends on the efficacy of temporal control processes that coordinate interval encoding and decoding, instrumental response innitiation, cessation and maintenance, and motor control. Here, we used the PI procedure to characterize temporal control in zQ175 knockin (KI) and BAC HD transgenic (Tg) mice generated to model Huntington's Disease (HD), and contrast the result with previously published R6/2 Tg PI data. HD is a progressive neurodegenerative disorder that involves degeneration of the same neural circuits underlying temporal information processing and control of motor output. Our results indicate that temporal control is disrupted in R6/2 Tg and zQ175 KI mice but intact in BAC HD Tg mice. Trial-by-trial analysis of break-run patterns in response rates indicated that shifts in zQ175 KI response curves were driven by significant delays in response initiation and cessation. Similar temporal control deficits were previously reported in HD patients and R6/2 transgenic HD mice. These findings support the use of zQ175 mice in preclinical studies of HD-related cognitive deficits. They provide evidence of a strong homology between the human and rodent neural bases of temporal information processing, temporal response control, and their pathology in neurodegeneration.

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an autosomal CAG repeat expansion in the *huntingtin (HTT)* gene. Cognitive, motor, and psychiatric symptoms typically manifest in adulthood along the progessive degeneration of the striatum and corticostriatal circuits (Cepeda, Wu, Andre, Cummings, & Levine, 2007; Kung, Hassam, Morton, & Jones, 2007; Paulsen, Ready, Hamilton, Mega, & Cummings, 2001). The disruption of striatal networks early in the disorder is associated with the failure of temporal processing mechanisms critical to interval timing, associative learning, and motor control (Hinton et al., 2007; Paulsen et al., 2004; Rao, Marder, Uddin, & Rakitin, 2014; Wolf et al., 2008). Work in rat and mouse models of HD has revealed abnormalities in corticostriatal information processing (Hohn et al., 2011; Walker, Miller, Fritsch, Barton, & Rebec, 2008), which are thought to underlie deficits in temporal processing (Buhusi & Meck, 2005). Consistent with this, Hohn et al. (2011) showed that abnormal corticostriatal processing in a transgenic rat HD model correlated with timing deficits assayed with the temporal bisection task.

Genetic HD models differ in the genetic constructs used to mimic the HD genetic insult, as well as in the genetic background. Balci, Day, Rooney, and Brunner (2009) demonstrated for the first time a timing-related deficit in two *fragment* R6/2 mouse lines, with approximately 115 and 250 CAG repeats each, respectively, expressed on a

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pure C57BL/6J or mixed C57BL/6J x CBA/J background strain. It is critical to explore processing deficits in alternative model systems to ensure that they do not simply reflect idiosynchratic model features not particularly representative of HD pathology per se, and therefore not conducive to predictive testing of putative therapeutic treatments.

In the present study we used the peak interval procedure to assess temporal processing in two alternative *full-length Htt* models. We focused on the BAC HD and zQ175 KI Het mice because both model systems exhibit reliable and robust behavioral deficits attributable to selective neurodegeneration, but were generated using very different genetic manipulations (bacterial artificial chromosome and knockin techniques, respectively). The ages of zQ175 and BAC mice studied, 26 and 45 weeks, respectively, were chosen based on prior studies indicating robust cognitive and mild motor deficits at these timepoints (Balci et al., 2013; Oakeshott et al., 2011; Oakeshott et al., 2013). Our results identify robust temporal processing deficits in zQ175KI mice that appear similar to deficits previously observed in the two lines of R6/2 mice, whereas no evidence of temporal processing deficits was found in BAC HD mice.

## Method

### Subjects

**zQ175 KI Het mice.** Two cohorts of zQ175 KI Het (CHDI-81003003) and WT littermate mice were obtained from the CHDI colony at Jackson laboratories. The first cohort consisted of 23 zQ175 KI Het (11 male and 12 female) and 21 WT (10 male and 11 female) mice on a C576L/J background. The second cohort included 29 zQ175 Het (14 male, 15 female) and 27 WT (14 male, 13 female) mice, again on a C57BL/6 background. Tail samples were taken on arrival of each cohort to confirm genotypes (Laragen, Ca) and quantify the length of CAG repeat sequences carried by zQ175 KI Het mice (cohort 1: range: 188-203, $M$ = 194.6 ± 1; cohort 2: range: 185-208, $M$ = 200 ± 1). Mice were pair-housed with littermates of the same sex and genotype in OptiMice cages (Animal Care Systems, CO) with Betachip bedding (Nepco, NY). Enrichment provided included Enviro-dri nesting material (Fibercore, OH), a transparent cylindrical tunnel, and a bone-shaped chew-toy. Standard 5001 lab chow (LabDiet, MO) and water were available ad libitum except in periods of testing and food-restriction (see below). Rooms were kept on a 12:12 light:dark cycle and all testing was conducted during the light period of the cycle. Mice in both cohorts began behavioral training at 26 weeks.

**BAC HD mice.** A cohort of eight female BAC HD FVD (CHDI-81001010) and eight female littermate WT mice on a FVB/NJ background strain (Gray et al., 2008) were obtained from Jackson Laboratories for use in these experiments. BAC HD mice carried the full-length human mutant huntingtin gene with ~97 CAGCAA repeats (Gray et al., 2008). Mice were paired-housed in homogenous OptiMice cages (Animal Care Systems, CO) with identical enrichment, diet, and light cycle conditions as the zQ175 KI Het cohorts, though food-restriction procedures differed slightly (described below). Mice were 45 week old when they began behavioral training.

All mice were treated in accordance with the Guide for the Animal Care and Use of Laboratory Animals (National Research Council, 1996), and all procedures were approved by the Institutional Animal Care and Use Committee at PsychoGenics, Inc.

### Food Restriction Procedures

Food restriction procedures were implemented to reduce bodyweights to 85% of a free feeding baseline. Mice were weighed daily during food-restriction to determine appropriate food-allocation. These procedures were consistent for weekdays and weekends, though testing was only conducted on weekdays.

For zQ175 KI Het cohorts lab chow was removed from cages one week prior to testing, and animals were fed limited quantities of 500 mg Bioserve pellets. Food quantities were adjusted per free feeding and actual bodyweights. The BAC HD Tg mice are overweight as compared to the WT mice (Gray et al., 2006; Oakeshott et al., 2011, 2013), requiring a different food restriction procedure. Since baseline BAC HD mice were already overweight, target bodyweights were determined as 85% of WT baselines, and 12 weeks were allowed to achieve a gradual weight loss. At their target bodyweights, BAC HD and WT mice consumed comparable quantities when tested with 30 min of ad libitum food access (not shown).

**Apparatus**

Mice were tested in eight operant chambers (Med Associates, VT; Model ENV-307W) placed in sound-attenuating cubicles. The floor area of chambers measured 8.5" long by 7.0" wide, with 5.0" high walls. On one wall of the operant chambers, two retractable levers were installed, with a food magazine and liquid dipper between them. The dipper was programmed to allow 3 s access to 0.01 ml of liquid reinforcer (evaporated Carnation$^{tm}$ milk). A fan mounted at one end of the operant chamber was continuously on throughout the experiments. Operant chambers were controlled with the MED-PC IV software package.

**Protocol**

**Magazine training.** The first stage of operant testing trained mice to locate food reinforcement in the operant chamber. Mice were placed in the operant chamber for 40-min sessions each day (excluding weekends) with the house light illuminated and no levers extended, and reinforcement was provided on a variable-interval 30 s (VI30) schedule of reinforcement (average of 30 s). Retrieval of the reinforcer was detected by the infrared magazine head-entry detector, and this event triggered the next trial. Sessions continued for 40 trials (reinforcers) or until 40 min had elapsed. Magazine training continued for two consecutive daily sessions.

**Fixed-Interval 20 s training (FI20).** The second stage of training conditioned mice to press a designated lever after a 20 s delay. Mice were placed in the unlit operant chamber and houselights were switched on at the beginning and off at the end of each trial. The first lever-press made 20 s after trial-onset was rewarded with 3 s access to the liquid dipper. Following the 3 s reinforcement interval, the houselight was extinguished and levers were retracted until the next trial. The inter-trial interval had an average duration of 25 (fixed) ± 15 s (with a uniform distribution). There was no time limit on individual trials but mice needed to lever-press to advance to the next trial. The same response lever was continually used across trials. Each session consisted of 50 40-min sessions. Mice continued training in the FI20 phase until their performance reached a criterion of 50 reinforcers over two consecutive sessions.

**Peak-Interval 20 s training.** This stage of training used the same protocol from the FI20 phase but included unreinforced peak interval (PI) trials, which were uniformly distributed among FI20 trials. In peak trials no reinforcement was provided when animals lever-pressed at the 20 s interval (the reinforcement window in FI20 trials), and house lights remained illuminated throughout the trial. PI trials lasted 80 (fixed) ± 10 s (with a uniform distribution), and sessions included 40 FI20 trials and eight PI trials. This phase continued for 27 sessions for the first zQ175 cohort and for 20 sessions with the second zQ175 cohort. The BAC cohort completed 18 sessions in this phase.

**Fixed- and Peak-Interval 45 s training.** In these stages mice were trained to respond to the alternative lever 45 s after trials began. As in prior stages, trials begin with the illumination of the house light, but the lever previously reinforced was retracted, and the opposing lever extended. In fixed-interval 45 s (FI45) trials, mice were given reinforcement for the first lever press following a 45 s interval from trial initiation. Sessions included 30 FI45 trials with inter-trial intervals of 56 (fixed) ± 34 s, uniformly distributed. Six peak interval trials were distributed among FI45 trials, with peak interval trials extended to 180 (fixed) ± 20 s in length. As in the prior PI task no reinforcement was provided on peak interval trials, and the house lights stayed lit throughout the trial. Both zQ175 cohorts completed 20 sessions in this phase, and the BAC cohort completed 10 sessions.

**Analysis**

**Absolute and Relative Response Rates.** Absolute response rates (lever press/s) were first measured in 1-s bins within-trials, and then trials were averaged to determine mean response rate of each mouse in each session. Data from the final five testing sessions, when response rates had reached a steady-state, were averaged. Data were analyzed in SAS (v9.4) with mixed model (PROC MIXED) tests of Type III main effects Genotype, Sex, Acquition Phase, Trial Latency, and Sessions. Genotype, Sex, and Acquisition Phase were categorical factors with two possible values: WT or zQ175, Male or Female, and Pre- or Post-Acquisition. Sex was excluded from factors tested with BAC datasets as all mice were femaleThe sessions factor is a direct encoding of the number of training sessions, used primarily in analysis of start-stop data, and values ranged from 10-27, depending on the number of sessions a cohort completed for a given phase (see notes on peak interval training). Trial latency refers to the 1-s time bins within trials. Relative response rates were processed and analyzed similarly, except that absolute responses rates for each animal were normalized by dividing the rate recorded in each bin by the mean response rate in the FI bin (i.e., 19 to 21 s for the 20 s trials, 43 to 48 s for the 45 s trials). Excepting this normalization procedure, the analysis of steady-state relative response rates (individual averages for the last five sessions) using mixed-models was the same as for absolute response rates. All statistical hypotheses were tested against an alpha criterion of 0.05 to determine significance.

**Detection of response start and stop change-points within trials.** Contrary to the smooth response curves resulting from the averaging of several trials, which suggest a gradual responding onset and offset, the response rates of individual mice on a trial-by-trial basis appear to follow a break-run-break pattern (Gallistel et al., 2004; Balci, Day, et al., 2009). Figure 1 illustrates how start and stop times are parsed from the pattern of responding within a trial. To capture this, we applied a relative-likelihood change-point algorithm to identify break-points in response patterns reflecting the abrupt onset/offset (start/stop) of the responding within a trial. For each data point, this method evaluates the relative likelihood of two models (change and no-change model) given the inter-response times up to and including a given datum. The *no-change model* assumes that inter-response times up to a given datum come from a single exponential distribution whereas the *change model* assumes that inter-response times up to an earlier datum and following that datum come from two different exponential distributions. If the odds in favor of the change model (after penalizing for the extra parameter) exceeds a predetermined threshold (i.e. odds ratio of 10:1), the data are truncated at that point and the algorithm described above is then applied to the rest of the data.

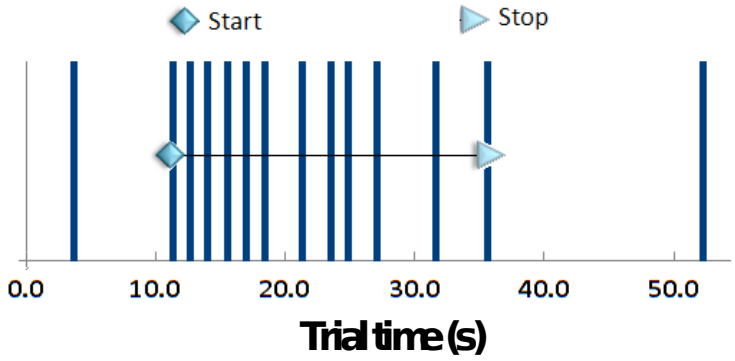


*Figure 1.* Visualization of start-stop analysis. Vertical bars represent lever-presses distributed over time; the algorithm detects the point where the pattern of responses *breaks* from one response state to another (see Gallistel, 2004).

The start/stop times were analyzed in SAS with repeated-measures MIXED models testing Type III fixed effects of Genotype, Sex, Session Number, and the interaction of those factors. The same general model was used to test differences in BACHD mice except sex was not included as a factor.

**Detection of temporal control acquisition.** The change-point algorithm is most effective at identifying change-points in clearly delineated response patterns. Particularly in early trials and sessions, the algorithm was frequently unable to identify a clear shift in within-trial response rates. Conversely, in later sessions start/stop break-run patterns were reliably detected in the vast majority of trials (60-95% of

trials, depending on genotype). In order to capture a possible increase in temporal control over behavior, we first coded each individual peak interval trial as being a *success* or *failure*, with success indicating a detected stop time that was later than 2.5x the FI value, and failure indicating no stop time detected.

We used this classification to identify the trial number where there was a reliable increase in the rate of *success trials*, as in Balci, Day, et al. (2009), indicating the acquisition of temporal control in the response. We also used the success: failure ratio for simple comparisons (2-way ANOVA, Prism) of timing performance across genotypes and response-acquisition phases (pre- vs. post-acquisition).

## Results

### Temporal processing deficits in the R6/2 and BAC models

In a prior study, Balci, Day, et al. (2009) demonstrated that both R6/2 CAG 120 and R6/2 CAG 240 Tg mice showed reduced absolute response rates and impaired ability to stop responding. These effects were apparent in steady state peak response curves, with R6/2 response rates exhibiting an elevated tail to the right of the FI.

In these experiments we found no evidence of comparable deficits in BAC HD mice relative to corresponding WT mice, either on measures of absolute (Figure 2A) or normalized response rates (Figure 2C). Comparison of absolute response rates of WT and BAC HD mice in the 20 s peak interval task (Figure 2A) indicated neither a significant Genotype x Trial Latency interaction, $F(69, 965) = 0.75$, $p = 0.94$, nor any overall Genotypie effect on absolute response rates, $F(1, 965) = 1.54$, $p = 0.22$.

Comparison of the relative response curves (Figure 2C) also revealed no significant effects in the interaction of Genotype x Trial Latency factors, $F(69, 966) = 0.88$, $p = 0.75$, nor an overall effect of Genotype,


*Figure 2.* Absolute (A, B) and relative (C, D) response rates for the FI20 peak for the two models studies here (blue lines) and corresponding WT mice (gold lines). Data shown are the mean (± *SEM*) absolute rate of lever presses (top panels) or normalized rate (bottom panels) for each time bin of peak (unreinforced) trials.

$F(1, 966) = 0.33$, $p = 0.57$. The absolute and relative response functions for WT and BAC mice were thus statistically undistinguishable in the 20 s peak interval task.

Consistent temporal control among both WT and BAC mice was again apparent in the 45 s peak interval task (not shown). Comparison of WT versus BAC absolute response functions in this task revealed neither a significant Genotype x Trial Latency interaction, $F(159, 2226) = 0.96$, $p = 0.63$, nor an overall Genotype effect, $F(1, 2226) = 0.04$, $p = 0.84$.

Similarly, neither a Genotype x Genotype x Trial Latency interaction, $F(159, 2226) = 0.92$, $p = 0.74$, nor a significant overall Genotype effect, $F(1, 2226) = 2.52$, $p = 0.11$, was apparent in the comparison of WT versus BAC relative response functions. In sum, the results of testing with both peak interval values indicate that, in contrast to the R6/2 HD mice deficits, temporal control processes engaged in the 0-160 s range are intact in BAC HD mice.

**Temporal processing deficits in the zQ175 KI model**

The absolute response rate of zQ175 Het mice (Figure 2B) in the 20 s peak interval task was generally flatter than that of WT mice, whereas the relative response rate (Figure 2D) showed a failure to inhibit responding after the FI had elapsed. Differences in the WT and zQ175 absolute response functions were driven by a significant interaction of Genotype x Trial Latency, $F(89, 3560) = 1.69$, $p < 0.0001$, with the absolute response rates differing consistently from 4 to 26 s (post-hoc comparisons: largest $p = 0.047$), and from 40 to 50 s (post hoc comparisons: largest $p = 0.019$). The directionality of the significant differences in response rate was time-dependent, with WT mice responding more prior to the FI (4-26 s), and zQ175 responding more after the FI had elapsed (40-50 s). Surprisingly, although we detected no significant overall difference between males and females, $F(1, 3560) = 0.08$, $p = 0.78$, the Genotype x Trial Latency interaction entered into a significant three-way interaction with Sex (Genotype x Trial Latency x Sex), $F(90, 3560) = 2.15$, $p < 0.0001$. This higher-order interaction is visualized in Figure 3, reflecting relatively pronounced deficits in zQ175 Het females relative to female WT mice, while deficits in male zQ175 were comparatively mild.


*Figure 3.* Sex-specific temporal control deficits in the zQ175 KI Het model. Plots show mean (± *SEM*) relative response rates for WT and zQ175 KI Het mice in FI20 (2A, 2B) and FI45 (2C, 2D) peak (unreinforced) trials in the last block (steady-state) of training. Dashed lines indicate the target peak reinforcement interval. Data for males and females are plotted separately to show the effects of zQ175 KI Het genotype were different in males and females.

Similar effects were apparent in a comparison of the WT versus zQ175 relative response rates for the 20 s peak task, with differential responses rates being driven by the interaction of Genotype x Trial Latency, $F(89, 3557) = 2.30$, $p < 0.0001$, entering in a higher-order interaction with Sex effects (Sex x Genotype x Trial Latency), $F(90, 3557) = 1.93$, $p < 0.0001$. This interaction was driven by the dissimilar effects observed in males and females, with female zQ175 mice exhibiting elevated responding consistently relative to female controls from 30-65 s (Figure 3; post-hoc comparisons, largest $p = 0.025$), while male zQ175 mice showed similar normalized response rates relative to male WT mice.

Robust temporal control deficits in zQ175 Het mice were also apparent in the 45 s peak interval task, but these were less overtly sex-specific than in the 20 s task. Differences in absolute response rates (not shown) were primarily driven by the

interaction of Genotype x Trial Latency factors, $F(159, 6360) = 3.80$, $p < 0.0001$, with consistent differences between WT and zQ175 mice emerging from the 29-62 s range (post-hoc comparisons, largest $p = 0.033$). Unlike effects observed in the shorter peak interval task, this interaction did not result in a significant higher-order interaction with Sex effects, $F(161, 6360) = 0.59$, $p > 0.99$.

A similar pattern was observed in the relative response rates (Figure 3), with differences primarily driven by the interaction of Genotype x Trial Latency factors, $F(159, 6360) = 2.23$, $p < 0.0001$, which did not result in any Sex-dependent higher-order interaction, $F(161,6360) = 1.04$, $p = 0.36$. Comparison of overall response rates (Genotype x Trial Latency interaction) with post-hoc tests revealed the longest chain of sequential differences between WT and zQ175 response rates emerged from the 66-95 s range (post-hoc comparisons, largest $p = 0.043$), reflecting increased responding in zQ175 Het mice after the FI had elapsed.

**Replication of the temporal processing deficits in the zQ175 KI model**

To confirm the deficits identified in the zQ175 Het KI model were robust and reproducible, we replicated those experiments with another cohort of zQ175 Het KI mice.

For the 20 s peak interval task, we found that absolute response rates (not shown) were driven by the interaction of Genotype x Trial Latency factors, $F(89, 4628) = 1.28$, $p = 0.042$, reflecting significantly reduced response rates in zQ175 Het mice in the 8 to 31s range (post-hoc comparisons, largest $p = 0.025$). Unlike the results of experiment 1, we found no overall Sex effects, $F(1, 4628) = 0.47$, $p = 0.49$, nor any higher-order interaction among sex, genotype, and session factors, $F(90, 4628) = 0.83$, $p = 0.88$.

Comparison of relative response rates (Figure 4) yielded similar results, with a significantly reduced response rate in zQ175 Het KI mice, overall, $F(1, 4539) = 25.14$, $p < 0.0001$. A significant Genotype x Trial Latency interaction, $F(89, 4539) = 2.98$, $p < 0.0001$, was driven by significantly increased responding in zQ175 Het KI mice in the 32 to 72 s interval (post-hoc comparisons, largest $p = 0.030$), reflecting a failure to terminate responding (see start-stop analysis).

Similar effects were observed in the 45 s peak task, with absolute response rates, overall, reduced in zQ175 Het mice relative to WT mice, $F(1, 8268) = 12.68$, $p = 0.0004$, and overall response rate curves differing due to a significant Genotype x Trial Latency interaction, $F(159, 8268) = 2.38$, $p < 0.0001$. Unlike in experiment 1, no overall Sex effect was detected, $F(1, 8268) = 2.69$, $p = 0.10$, nor did Sex interact with Genotype x Trial Latency effects, $F(160, 8268) = 0.94$, $p = 0.70$. Post-hoc comparisons indicated the Genotype x Trial Latency interaction was driven by significant reductions in zQ175 Het response rates in the 10 to 70 s interval (largest $p = 0.032$).

# Males

*Figure 4.* Replication of temporal control deficits in zQ175 KI Het model. Plots show mean (± *SEM*) normalized response rates (100* [rate per bin/rate at peak interval]) for WT and zQ175 KI Het mice in FI20 (2A, 2B) and FI45 (2C, 2D) peak (unreinforced) trials in the last block (steady-state) of training. Dashed lines indicate the target peak reinforcement interval. Data for males and females are plotted separately to show the effects of zQ175 KI Het genotype were different in males and females.

Analysis of relative response rates detected significant Sex effects, consistent with experiment 1, which entered in a higher order interaction with Genotype and Trial Latency factor (Sex x Genotype x Session), $F(160, 8268) = 1.24$, $p = 0.022$. This effect was primarily driven by the differential genotypic effects observed in both sexes, with the normalized response rates of female zQ175 mice being higher than female WT mice in the 58 to 96 s range, while male zQ175 responded more than WT mice in the 89 to 116 s, and from 118 to 139 s.

**Start-Stop analysis of the BAC and zQ175 Het KI HD models**

In contrast to the average response curves, responding in individual peak trials follows a break-run-break pattern (Gibbon & Church, 1990), which can be analyzed with a trial-by-trial analysis. To capture the trial time of the first robust increase (start) and decrease (stop) in the response rate we used a relative-likelihood change-point algorithm (Balci, Gallistel, et al., 2009; Gallistel et al., 2004). This method was used previously to show that R6/2 CAG 120 mice exhibit significantly delayed start and stop times (Balci, Day, et al., 2009). R6/2 CAG 240 mice also exhibited delayed stop times, in particular the males, but similar start times (Balci, Day, et al., 2009).

Figure 5 plots start and stop times parsed from data collected from the BAC experiments. There was no overall difference in mean start times between BAC and WT mice, $F(1, 230) = 1.15$, $p = 0.29$, in 20 s interval PI trials (Figure 5A), however we detected a significant Genotype x Session interaction, $F(17, 230) = 2.47$, $p = 0.001$, in start times, indicating a session-dependent genotype effect. Post-hoc comparisons indicate this effect was driven by delayed start times in BAC mice in sessions 2, 4, and 5 ($ps < 0.020$); except for these initial differences, however, start times in BAC and WT mice were comparable.

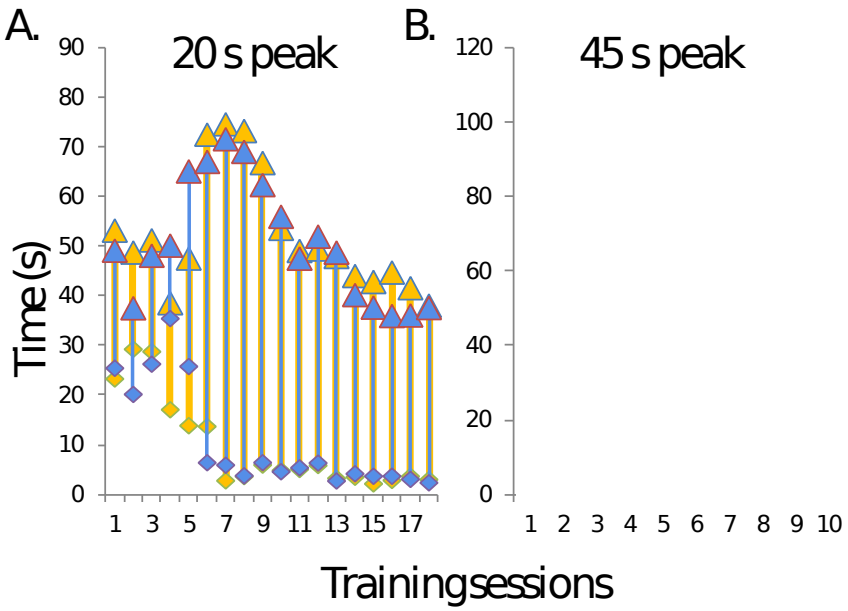


*Figure 5.* Start-stop analysis of the BAC model. Data show mean within-trial start (diamonds) and stop (triangles) times for WT (yellow, $N = 8$) and BAC (blue, $N = 8$) mice across testing sessions. Note the connecting lines between start and stop points provide an indicator of the start-stop interval.

There were no overall differences in stop times between BAC and WT mice, $F(1, 230) = 0.25$, $p = 0.62$, nor session-specific effects, i.e. no Session x Genotype interaction, $F(17, 230) = 1.42$, $p = 0.13$. These findings suggest similar temporal control capacity in WT and BAC mice in the 20 s peak task, with some slight differences in the acquisition of control.

Data collected in the 45 s peak task showed similarly consistent performance among BAC and WT mice, with no overall genotypic differences in start times, $F(1,126) = 1.85$, $p = 0.18$, or stop times, $F(1, 126) = 0.18$, $p = 0.68$, or session-specific effects (Genotype x Session interactions) in start, $F(9, 126) = 0.95$, $p = 0.49$, or stop times, $F(9, 126) = 1.66$, $p = 0.11$.

We next applied this method to the analysis of data collected in the zQ175 experiments (Figure 6). Start times for the 20 s peak task in experiment 1 were driven primarily by simple main effects, rather than by higher-order interactions. zQ175 Het mice, overall, started responding significantly later than WT mice, $F(1, 1038) = 12.91$, $p = 0.0003$, and start times for male mice, independent of genotype, were significantly later than for females, $F(1, 1038) = 6.85$, $p = 0.009$.

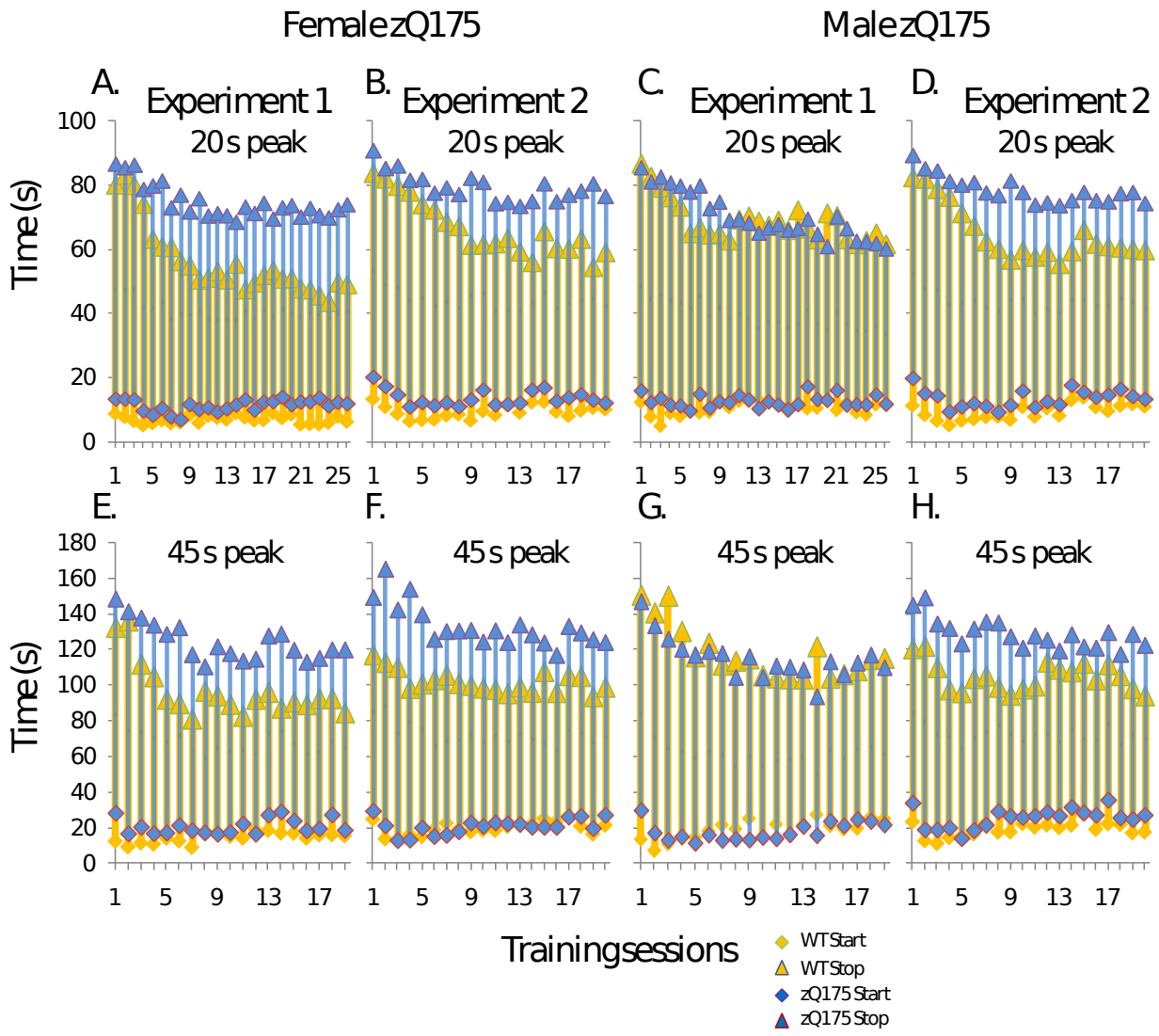


*Figure 6.* Start-stop analysis of the zQ175 KI Het HD model. Each panel plots the mean within-trial time (y-axes) of start (diamonds) and stop (triangles) parameters across training sessions (x-axes), with WT data in yellow and zQ175 in blue. Datasets were split by sex for comparison with the sex-specific effects found in the averaged peak response curves.

Stop times, in contrast, were driven by the three-way interaction of Sex, Genotype, and Session factors, $F(27, 1038) = 2.23$, $p = 0.0003$, reflecting the prominent deficits (delayed stop times) in female zQ175 Het mice (Figure 6A), compared to the relatively consistent stop times of male zQ175 Het and WT mice (Figure 6C).

In experiment 2 we found a similar overall delay in zQ175 start times for the 20 s peak task (Figure 6B; $F(1, 987) = 26.52$, $p < 0.0001$), but no overall difference between males and females, $F(1, 987) = 0.10$, $p = 0.76$). We additionally found a significant Genotype x Session interaction, $F(19, 987) = 1.62$, $p = 0.045$, reflecting the

convergence of start times between genotypes in later sessions compared to a relatively broad difference in early sessions.

Stop times for the 20 s peak task in experiment 2 were driven by a Session x Genotype interaction, $F(19, 987) = 4.57$, $p < 0.0001$, reflecting the consistent overall delay in zQ175 Het stop times in sessions 8 to 20 (post-hoc comparisons, largest $p = 0.002$). These effects were further complicated by a significant three-way Sex x Genotype x Session interaction, $F(20, 987) = 2.72$, $p < 0.0001$, reflecting the earlier emergence of significant differences in male zQ175 Het (vs. male WT mice) by session 6 ($p < 0.0001$), as compared to the significant deficits in zQ175 Het females (relative WT female mice), which did not emergence until session 9 ($p = 0.0004$).

Start-stop analysis of the 45 s peak interval task revealed similar temporal control deficits in the zQ175 Het model. Start times were significantly delayed in zQ175 Het mice, overall, $F(1, 1011) = 6.57$, $p = 0.011$, but, unlike in the shorter peak task, there was no overall difference in start times between males and females, $F(1, 1011) = 1.36$, $p = 0.24$. The interaction of Genotype x Session factors was significant, $F(25, 1011) = 1.63$, $p = 0.027$, reflecting significant delays in the starts of zQ175 Het mice in sessions 1 ($p < 0.0001$) and 2 ($p = 0.006$), although genotypic differences subsided with more training sessions.

Stop times in experiment 1 were significantly delayed in zQ175 Het mice (Genotype main effect: $F(1, 1099) = 4.65$, $p = 0.031$). Genotype effects entered a significant interaction with Session (Genotype x Session: $F(27, 1099) = 1.62$, $p = 0.024$), reflecting significant delays in zQ175 Het stop times in sessions 5-8, 10-13, 16, and 21 (post-hoc comparisons, largest $p = 0.048$).

The results of experiment 2 were generally similar, with an overall delay in zQ175 Het start times, $F(1, 987) = 24.31$, $p < 0.0001$, but no overall sex differences, $F(1, 987) = 0.10$, $p = 0.76$. As in the first experiment, the Genotype x Session interaction was significant, $F(19, 987) = 1.62$, $p = 0.045$, but, in experiment 2, zQ175 Het mice start times were delayed in all sessions except 8, 11-12, 15, and 19-20 (largest $p$ in other sessions: 0.046). Stop times for the 45 s peak interval task in experiment 2 were driven by the simple main effect of Genotype, $F(1, 985) = 22.52$, $p < 0.0001$, but this effect did not enter any significant higher-order interactions, nor was the main effect of Sex significant, $F(1, 985) = 0.30$, $p = 0.59$.

In sum, these analyses identify consistent temporal control deficits in zQ175 Het KI mice using two different peak intervals, which may be primarily attributable to delayed onset of response initiation and failure of response inhibition.

**Acquisition of Temporal Control in BAC and zQ175 KI Het mice**

We next examined how the frequency of trials with identifiable stop points (successful trials), and trials where stops were not detected (failure trials; see Methods for criterion), changed over the course of training. A change-point algorithm was used to identify the number of trials each mouse needed to transition from an initial phase of

high failure rates (pre-acquisition phase) to a stage with low failure rates (post-acquisition phase). This approach was chosen for its sensitivity to abrupt shifts in response patterns that are thought to underlie the apparently-gradual shifts presented in averaged response curves (Balci et al., 2008; Balci, Ludvig, & Brunner., 2010; Cheng & Westwood, 1993; Church et al., 1994).

For BAC HD and WT mice we found similar patterns of learning and temporal precision in both the 20 s and 45 s task, shown in Figure 7. There were no differences in the number of trials needed for BAC HD and WT mice to acquire temporal control in the 20 s, $t(14) = 0.22$, $p = 0.83$ (Figure 7A), or the 45 s task, $t(14) = 0.09$, $p = 0.93$ (Figure 7C). The proportion of failure trials in the post-acquisition phase was consistently reduced among both BAC HD and WT mice in the 20 s task, reflecting acquisition of temporal control (main effect of Acquisition Phase, pre- vs. post-; $F(1, 14) = 69.93$, $p < 0.0001$; Figure 7B), but there were no significant effects or interactions related to Genotype.

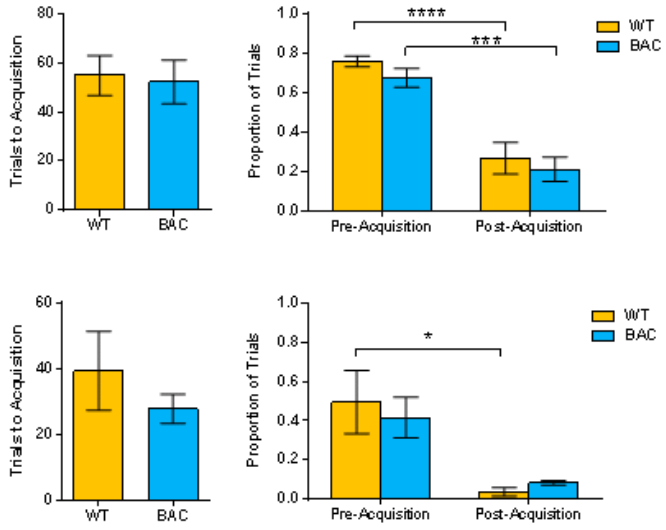


*Figure 7.* Acquisition and reliability of temporal control in BAC HD and WT mice. Bars plot mean values ± *SEM*. In panels B and D the y-axis reflects the failure:success proportion of trials, so higher values should be interpreted as poor performance. Figure annotations refer to statistically significant differences, where indicated, such that a single * indicates $p$ values < 0.05, *** means $p < 0.001$, and **** indicates $p < 0.0001$.

Similarly, in the 45 s task, reduced failure rates in the post-acquisition phase were driven by significant acquisition effects, $F(1, 14) = 7.13$, $p = 0.017$ (Figure 7D), with no significant effects attributable to Genotype.

In contrast to the robust performance of the BAC HD model, we found a consistent pattern of deficits in the acquisition and reliability of temporal control in zQ175 KI Het mice (we pooled experiments 1 and 2 for this analysis as the separated analysis yielded equal results), shown in Figure 8 (females) and Figure 9 (males). In the

20 s peak task, female zQ175 needed significantly more trials than female WT mice to acquire reliable temporal control (Figure 8A; $t(43) = 2.10$, $p = 0.039$; note one WT and 6 zQ175 mice were excluded for failing to reach acquisition criteria). Male zQ175 also required significantly more trials to reach acquisition criterion (Figure 9A; $t(38) = 4.40$, $p < 0.0001$; four WT and eight zQ175 mice excluded).

The proportion of well-timed stops in 20 s trials significantly improved following acquisition in females, $F(1, 44) = 31.88$, $p < 0.0001$ (Figure 8B, pre- vs. post-acquisition phases), and in males, $F(1, 36) = 183.2$, $p < 0.0001$ (see Figure 9B, pre- vs. post-acquisition phases), reflecting robust acquisition of temporal control in both sexes and genotypes. Interestingly, among female zQ175 KI Het we found significant main effects of Genotype, $F(1, 44) = 34.66$, $p < 0.0001$, and the interaction of Acquisition Phases (pre- vs. post-acquisition) x Genotype factors, $F(1, 44) = 31.88$, $p < 0.0001$, indicating deficient temporal control, while among males those effects were not significant. Post-hoc analysis (Sidak) of female performance indicated these effects were attributable to the significantly worse temporal control (higher ratio of poorly-timed stops) in zQ175 females relative to WT mice in the post-acquisition phase ($p < 0.0001$). Thus for the 20 s task we found deficits in the rate of temporal control acquisition (number of trials) for male and female zQ175 mice relative to WT mice, but the end-point measure of temporal control in the post-acquisition phase was only significantly worse in female zQ175 relative to WT mice.

We also found deficits in the rate of acquisition (number of trials) for zQ175 female mice (Figure 8C; $t(37) = 2.82$, $p = 0.008$; note five WT and 8 zQ175 mice were excluded for failing to reach criteria), but not male mice (Figure 9C; $t(30) = 1.64$, $p = 0.11$; note 11 WT and 9 zQ175 mice excluded for failing to reach criteria) in learning the 45 s response interval.

As in the 20 s task, for the 45 s interval we found the main effects of Acquisition Phases (pre- vs. post-acquisition) were significant for females, overall (Figure 8D), $F(1, 37) = 93.82$, $p < 0.0001$, but female zQ175 performed worse than female WT, $F(1, 37) = 15.44$, $p = 0.0004$, with no significant Genotype x Acquisition Phase effect. The overall effect of Aquisition Phase was also significant for male mice (Figure 9D), $F(1, 30) = 131.40$, $p < 0.0001$, confirming both sexes ultimately learned the task. Among male mice, however, we found no significant main effects of Genotype, $F(1, 30) = 2.28$, $p = 0.14$, nor the interaction of Genotype x Acquisition Phase (Figure 9D), $F(1, 30) = 1.59$, $p = 0.22$. In sum, these findings emphasize that WT and zQ175 mice were capable of learning a longer peak interval task, but, first, male and female zQ175 mice exhibit significant deficits in the acquisition of temporal control, and, second, the strength of temporal control, once acquired, is significantly worse among females zQ175 than female WT mice.

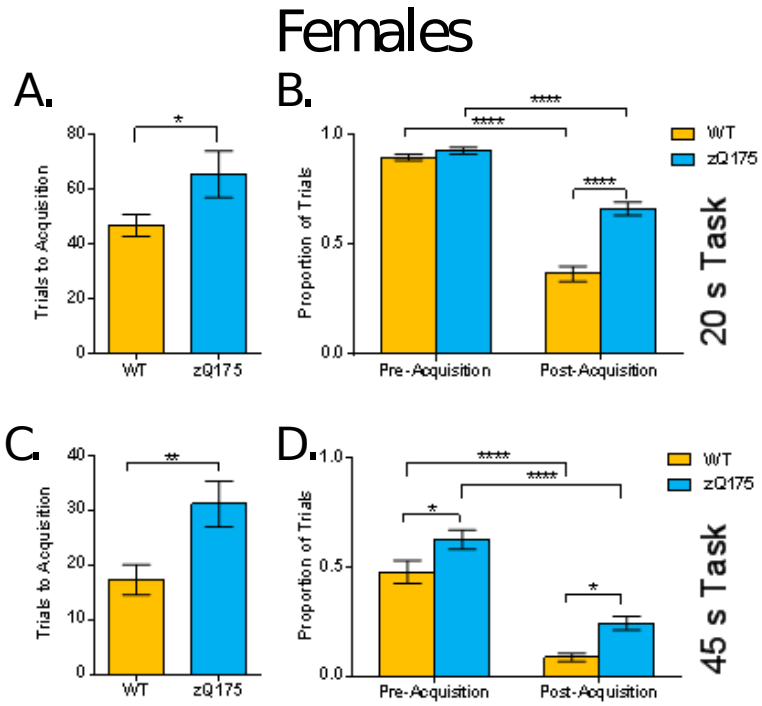


*Figure 8.* Acquisition and reliability of temporal control in female zQ175 KI Het and WT Mice. Data in initial experiments and in the replication study were pooled in these figures and analyses. Bars plot mean values ± *SEM*. In panels B and D the y-axis reflects the failure:success proportion of trials, so higher values should be interpreted as poor performance. Figure annotations refer to statistically significant differences, where indicated,

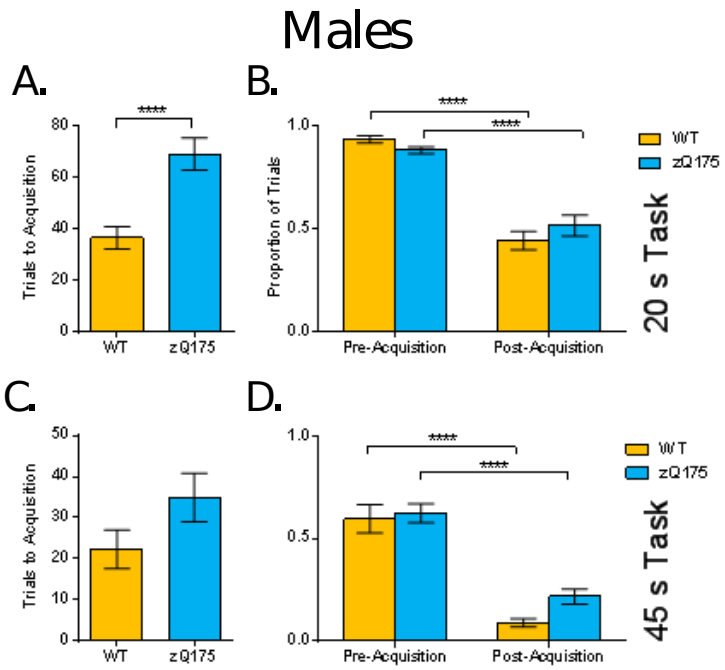


*Figure 9.* Acquisition and reliability of temporal control in male zQ175 KI Het and WT mice. Data in initial experiments and in the replication study were pooled in these figures and analyses. Bars plot mean values ± *SEM*. In panels B and D the y-axis reflects the failure:success proportion of trials, so higher values should be interpreted as poor performance. Data in initial experiments and in the replication study were pooled in these figures and analyses. Figure annotations refer to statistically significant differences, where indicated, such that **** means $p < 0.0001$.

## Discussion

Organisms need a way to encode temporal characteristics of their environment. Some activities requiring motor coordination, speech recognition, decision making and others (Buhusi & Meck, 2005), are mediated through interval timing, defined as the ability to perceive, remember and organize behavior around periods in the range of seconds to minutes. Interval timing has been studied in the context of foraging in the wild (Brunner, Kacelnik, & Gibbon, 1992), and in complex decisions such as discounting future reward or choosing between reward sequences (Brunner & Gibbon, 1995; Mazur, 1984). We focus on this process as the postulated neural mechanism underlying time perception is the same that undergoes pathological changes in HD.

This study assessed temporal control in BAC HD Tg and zQ175 KI Het mice with 20 and 45 s peak interval durations. Our experiments with BAC HD mice found little evidence of any difference with WT mice in absolute response or relative response rates, response patterns within-trials, or shifts in response patterns with training. Transient delays in response *start* times were detected in the first few training sessions, but these resolved within a few training sessions, and were not apparent in steady state

comparisons of absolute or relative response rates. Importantly, the BAC HD cohort (and control animals) studied here was a small sample size and exclusively female; sex-specific deficits in this model thus cannot be ruled out.

Among the zQ175 KI het mice, in contrast, prominent timing deficits were apparent in comparison with WT absolute and relative response rates, and response start and stop times. Impaired temporal control of response initiation (as measured by the starts) and cessation (meassured by the stops) in zQ175 were consistently found with both 20 and 45 s intervals, and reproduced across two different test cohorts. These findings provide a functional explanation for the significant shifts apparent in the timing of relative response rates. The delayed onset of start in zQ175 is also consistent with the reduction in absolute response rates prior to the reinforcement interval for zQ175 relative to WT, as well as the reduced absolute response rates in R6/2 mice reported by Balci, Day et al. (2009), although this could also indicate a more fundamental inability to accurately estimate time.

Notably, this pattern of findings reflects previously reported results, in which R6/2 and zQ175, but not BAC HD mice, exhibited response inhibition deficits in a Go/No-go operant task (Oakeshott et al., 2013). Indeed, the present findings indicate that zQ175 HET KI het mice display deficits in acquisition of of a timed response that are characterized by inability to accurately time duration onset and inhibite responding after the FI has elapsed. Impaired acquisition of reliable response timing did not, however, prevent zQ175 KI het mice from learning the PI task, as temporal control in pre- vs post-acquisition phases was significantly improved for male and female zQ175, as well as WTs.

Given the disruption in the cessation of responding we chose not to pursue other typical analyses for timing tasks, for example, exploration of the scalar property (which requires that the shape of the relative response for the 20 s peak overlap with the shape of the 45 s peak curves, for each group). Although we did not quantify the actual shape of the response curves apart from the causal start and stop measures, it is clear that the WT curves, in the 20 s peak interval, showed a second peak of responding a little after 3 times the FI (in both zQ175 experiments). This second peak is typical of the peak procedure but depends on the particular FI and associated intertrial intervals (ITIs) used (Brunner, Fairhurst, Stolovitzky, & Gibbon, 1997). The 45 s curves did not show the second peak. It is possible that the second peak reflects a harmonic of the FI (a little shifted from a 3*FI harmonic) although this may simply reflect responding to the *next* trial (current trial + ITI + next trial FI = 20 s + 25 s + 20 s = 65 s. However, note for the 45 s then there should be a second peak at 45 s +56 s + 45 s = 146 s, thus more experimentation would be needed to explain this pattern of results). Importantly, independently of the interpretation, there seems to be a shoulder at about the same time into the trial for the zQ175 Het mice, suggesting that the underlying process is somehow intact.

Our present results, in combination with those from prior timing (Balci, Day, et al., 2009) and Go/no-Go studies (Oakeshott et al., 2013) indicate that deficits in response inhibition are present in both R6/2 and zQ175 KI mice. The consistency of these findings in two very different HD models underscores the crucial point that these

deficits are not simply idiosyncratic model features, but rather are pathological HD features, lending considerable construct validity in support of the use of these preclinical mouse models in HD research. In contrast, the relatively robust temporal control we find in BAC HD mice indicates this model is less useful for studies of temporal processing and/or response inhibition deficits in HD.

Some aspects of temporal control deficits in zQ175 were sex-dependent. While temporal control deficits were apparent in female zQ175 mice for both cohorts, deficits among male mice were more pronounced in the second cohort. The different number of training sessions given for cohort 1 (27 sessions) vs. cohort 2 (20 sessions) is a potential source of experimental error that could have contributed to these dissimilar effects. Analysis of male start and stop times, however, indicated that significant deficits in the stops emerged as early as the 8th session in the second cohort; if similar effects had emerged in the 1st cohort but receded with additional training, it would have been apparent as a Genotype x Session interaction. Thus while our results present a clear pattern of reproducable temporal control deficits related to CAG KI, further research is needed to identify the role of sex in disrupted temporal control.

We considered if performance deficits in zQ175 KI Het mice could be related to dysfunctional learning processes, rather than a timing deficit per se. Supporting this, WT mice acquired reliable temporal control in fewer trials than zQ175 KI Het mice; zQ175 KI Het nonetheless showed significant improvement in all measures with training.

Considerable work has been done using the peak procedure (Balci et al., 2008; Balci, Day, et al., 2009; Balci, Gallistel, et al., 2009; Balci, Ludvig, Abner, et al., 2010; Balci, Ludvig, & Brunner, 2010; Buhusi & Meck, 2002; 2005; Matell, Bateson & Meck, 2006; Meck, 2006; Paule et al., 1999) to investigate underlying circuitry and pharmacology. Research looking into the dependency between start and stops has shown that the two decisions are separable by genetic (as in this paper and Balci, Day, et al., 2009) or phamacological manipulation (Abner, Edwards, Douglas, & Brunner, 2001). Macdonald and colleagues have shown, using anisomycin to inhibit protein synthesis, that the acquisition of the start response depends on the dorsal striatum whereas the stop response depends on the ventral striatum (Macdonald, Cheng, & Meck, 2012). Our results therefore are consistent with a more aggressive degeneration of ventral striatum in the mouse models, and fit evidence of altered ventral striatal activation during reinforcement in premanifest HD (Enzi et al., 2012). On the other hand, the robust performance of BAC HD mice in this striatal-dependent interval timing task, while exhibiting poor circadian rhythms (Oakeshott et al., 2011), emphasizes anatomical as well as functional separation in discrete timing mechanism across different temporal scales.

Our work provides strong support for the use of mouse models of disease, HD in particular, for the development of novel therapeutics. The similarities of the functional changes between mice and HD patients in the same paradigm (reviewed in Buhusi & Meck, 2005; see also Rao et al., 2014) are undeniable. As the endpoint measures can now be tracked to the same brain circuits affected in HD, the homologies luckily go beyond trivial face validity arguments.

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