Cognitive assessment of mice strains heterozygous for cell-adhesion genes reveals strain-specific alterations in timing

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We used a fully automated system for the behavioural measurement of physiologically meaningful properties of basic mechanisms of cognition to test two strains of heterozygous mutant mice, Bfc (batface) and L1, and their wild-type littermate controls. Both of the target genes are involved in the establishment and maintenance of synapses. We find that the Bfc heterozygotes show reduced precision in their representation of interval duration, whereas the L1 heterozygotes show increased precision. These effects are functionally specific, because many other measures made on the same mice are unaffected, namely: the accuracy of matching temporal investment ratios to income ratios in a matching protocol, the rate of instrumental and classical conditioning, the latency to initiate a cued instrumental response, the trials on task and the impulsivity in a switch paradigm, the accuracy with which mice adjust timed switches to changes in the temporal constraints, the days to acquisition, and mean onset time and onset variability in the circadian anticipation of food availability.

1. Introduction

A biochemical circadian clock enables animals (and other organisms) to modulate their physiology and behaviour by the internally signalled time of day and to anticipate events that occur at predictable phases of the daily cycle [1–3]. Fifty years ago, the neurobiology of this clock was so obscure that there were doubts in some quarters that it existed [4]. Circadian timing was thought by some to be an ‘emergent phenomenon’, not the consequence of an identifiable intracellular biochemical mechanism with a compact genetic specification. In this intellectual context, it was audacious of Seymour Benzer and his collaborators to search for mutant strains with heritable malfunctions in their behavioural circadian timing. The search was, however, famously successful, both in Drosophila [5] and later, by others, in mice [6]. The discovery of tau mutants—mutations that alter the period of the circadian clock—led to our current detailed, but still evolving understanding of the molecular biology and neurobiology of the circadian clock [7,8].

The circadian clock is a mechanism of cognition, as well as of physiology. It is among the mechanisms that enable animals to locate themselves in space and time [1]. In common with others, we believe that Benzer’s forward-genetics approach may be applied to other cognitive mechanisms that are currently known only by their behavioural manifestations, as the circadian clock was when Benzer began his search. In following this strategy, the behavioural...
screening technology is critical. The behavioural screens/tests used must be diagnostic of malfunctions or quantitative abnormalities in particular mechanisms; that is, they must have functional specificity, and they must be capable of being applied on a large scale with a modest amount of human labour. These properties of a good forward-genetics behavioural screening test are equally desirable in reverse-genetics behavioural testing.

The Gallistel laboratory developed a system for performing such screens/tests [9]. We here report the results obtained using it to test two strains of mice in their heterozygous mutant state: the L1 knockout and the β-catenin mouse mutant line (named Batface for its effect on facial morphology). Both proteins (L1 and β-catenin) play roles in cell adhesion and thus in the establishment, maintenance and modification of synaptic connections. The brain’s computational mechanisms are generally assumed to depend on patterns of synaptic connectivity, and its ability to store information is generally assumed to be mediated by modifications of those connections. Thus, it is not surprising that the genetic alterations in both strains have been implicated by previous research in learning and memory phenomena [10–12]. This and the availability of these strains to the Gallistel laboratory motivated their use in these experiments.

Our behavioural method measures the accuracy with which mice represent the ratios of the nutrient incomes they experience at two different feeding locations (the matching screen), the rates of instrumental and classical conditioning (the autoshaping screen), and the accuracy and precision with which they compute and remember temporal durations (the switch screen). The last protocol also measures impulsivity and time-on-task, which may be considered a measure of executive function. What distinguishes our measures from those most commonly used is the targeting of specific cognitive mechanisms (for example, the mechanism for measuring interval durations) rather than categories (e.g. spatial learning) and the lacking of behavioural measures of the quantitative content of what is learned (for example, the accuracy and precision with which durations are measured and remembered).

We find clear evidence of functionally selective genotypic alteration in the precision with which duration is represented. The Batface mutation in heterozygotes, however, shows a sizeable decrease in the precision with which the mice represent the duration of an elapsing interval. The L1 heterozygotic females show the opposite effect, an increase in the precision with which interval durations are timed. The evidence for specificity comes from our finding that the mutants in both lines do not differ from the wild-type controls on several other measures of learning and memory: in a concurrent variable-interval feeding protocol, both genotypes in both lines match equally well the ratio of their temporal investments in two feeding hoppers to the ratio of the incomes obtained from them. We find no genotypic difference in the rate at which an instrumental response is acquired, nor in the rate at which a Pavlovian response is acquired. There is no difference in the speed with which the genotypes initiate a learned instrumental response. Both genotypes in both lines adjust the distribution of their timed switch latencies appropriately when the temporal constraints are narrowed. Thus, we conclude that in both lines, the genotypic variation has a functionally specific effect on a basic mechanism of cognition: the genotypic variation alters either the precision with which interval durations are represented, or perhaps more generally the precision with which simple magnitudes are represented (e.g. distance, duration and number).

2. The mutant strains

Batface (Bfc+/+). The β-catenin protein is an intracellular anchor for membrane cadherins, which play an important role in modulating synaptic connectivity and synaptic plasticity [13–15]. The Batface (Bfc, MGI:2656734) mouse mutants carry a single point mutation within the β-catenin gene (Ctnnb1), which was identified in a large scale N-ethyl-N-nitrosourea mutagenized screen [16]. Tucci et al. [17] have extensively characterized the behavioural, neuroanatomical and neurophysiological phenotype. They mapped the linkage to a small interval of 0.56 Mb between 120.92 and 121.48 Mb on chromosome 9. Their sequencing of this small stretch revealed a single missense mutation, a C to A transition, in exon 13 of the β-catenin gene. Using the results from their studies on this mutation in the mouse as a guide, they have been able to define a novel clinically recognizable syndrome in humans, with a distinctive phenotype associated with de novo mutations in β-catenin. The parallels in the phenotypic features with the mouse model, Batface, suggest shared common pathological phenotype in morphologic and neurodevelopmental processes. These parallels strengthen previous suggestions that altered β-catenin signalling is a cause of cognitive dysfunction in humans.

The Bfc+/+ mice and littermate controls used in this study were females bred at the IIT in Italy. Mice were maintained on C57BL/6j x C3H mixed background for several generations. A cohort of adult mice was transferred to the Rutgers for the behavioural study. They were approximately six months old at the time of testing and they weighed 29–46 g. The Bfc/+ mice did not differ significantly in weight from their littermate controls. For genotyping, two independent PCRs were performed from the same DNA sample extracted from tail. Primers NF19.3 (5'-AACAGTAGCTGCAAGGTG-3') and NF19.1 (5'-GGCAGCAGCTGCGTATG-3') amplified a fragment of 238 bp from the Ctnnb1++ allele only. Primers NF19.3 (5'-AAGAGTAGCTGCAAGGTG-3') and NF19.2 (5'-AGGACCAGCTGCGTATG-3') amplified a fragment of 236 bp from the Ctnnb1Bfc allele only. Conditions were similar for the two PCRs: 94°C for 30 s, 60°C for 30 s, 72°C for 45 s for 35 cycles.

x/L1I. The L1 glycoprotein is expressed in neurons but not in astrocytes or oligodendrocytes in the central nervous system. It plays a role in neuronal migration, neurite outgrowth and guidance, fasciculation of axons, and myelination during development [12,18–22]. Some mutations in the L1 gene in humans produce the L1 syndrome, a severe and rare neurological disorder characterized by mental retardation, spasticity in the lower limbs, with hyperlexia, aphasia and abducted thumbs, dilated cerebral ventricles, stenosis of the aqueduct of Sylvia and hypoplasia or agenesis of the corticospinal tract and corpus callosum [23].

L1+/y mice (males with a mutation in the L1 gene, which is on the X chromosome) are hydrocephalic to varying degrees, depending on the genetic background, and are abnormal in the development of the corticospinal tract, cerebellar vermis and corpus callosum [24]. And they show impaired learning and memory [25]. Owing to the random inactivation of the X-chromosome, mammalian females express a mosaic cellular
program wrote the data to a text file every 10 min. Data environments and logged the data. The environment-control
ning on PCs in an adjacent room, which controlled the test at all times. The water bottle was in the nest tub.
were free to come and go between nest tub and test chamber second beginning 2 h before dawn (house light on). The mice
one beginning an hour before dusk (house light off) and the protocols during two 4 h daily feeding phases,
ever, they obtained their entire daily food ration performing in the protocols. The mice were never food deprived; how-
which delivered 20 mg pellets when mice performed in the hopper. The two flanking hoppers had pellet feeders, 
beam across the opening and illuminable by an LED within the reverse cycle). Each hopper was monitored by an infrared
and the rapidity with which it adjusts its visit behaviour to accord with changes in the income ratio may be visualized
expression pattern that derives either from the mother or the father. The inactivation of either X-chromosome occurs early
development and lasts a lifetime [26,27]. Therefore, female carriers of \( L1 \) mutations are heterozygous at the level of the \( L1 \)-expressing cells, in that each cell expresses either the normal or the mutated gene.

The mice used in this study were females from a hetero-
yzogously bred \( L1 \) knockout mouse line [28,29], that had been backcrossed for at least 10 generations onto a 129/SvJ background. For aspects of their unexpected neurobiological phenotype, see Schmid et al. [30]; these mutant females gen-
erate during development and maintain in the adulthood more neurons than the corresponding wild-type female litters-
ates and the mutation-carrying males. They were three to four months old at the beginning of testing. We tested 11 \( L1^{\text{−/−}} \) and 10 wild-type littermate controls. The weights at the onset of testing ranged from 23 to 37 g and did not differ significantly between the groups.

3. Apparatus and standard procedures

The testing system is described in detail in [9]. Briefly, the mice lived individually in test environments each consisting of a stan-
dard polypropylene nest tub connected via an acrylic tube to a Med Associates mouse test chamber with three hoppers (H) on the far wall. The two flanking hoppers have pellet feeders (P). (Adapted from [9].)

Figure 1. Schematic of the testing environment. Each fan-ventilated steel cabinet contains six to eight test environments. Each test environment consists of a polypropylene mouse tub connected via an acrylic tube to a Med Associates mouse test chamber with three hoppers (H) on the far wall. The two flanking hoppers have pellet feeders (P). (Adapted from [9].)

4. The behavioural protocols

We tested the mice with three experimental protocols: the investment-to-income matching protocol, the instrument-
al and classical-conditioning protocol and the timed-switching protocol. We were also able to test for circadian food antici-
pation in the \( L1 \) mice. We were not able to do this in the Batface mice owing to the 5-day power failure that terminated the testing of these mice.

(a) Investment-to-income ratio matching

In this protocol, which we call the matching protocol for short, the mouse obtains food pellets at two independent random rates by poking into two different hoppers. When it pokes into either hopper, it interrupts an infrared beam. If the protocol has armed the beam for a hopper, then interrupting it triggers the release of a pellet into that hopper. The two beams are armed on independent concurrently run-
ning variable-interval schedules of reinforcement: after each pellet delivery into a given hopper, a rearming interval for that hopper is drawn at random from an exponential distrib-
ution. Put another way, the arming intervals at the two hoppers are generated by independent Poisson processes. When an arming interval elapses, the beam for the hopper is rearmed. The next beam interruption delivers another pellet and restarts the Poisson process that will rearm that hopper’s beam.

Like rats, pigeons and humans, mice tend to match the ratio of the average durations of their visits to the two hoppers—the ratio of their temporal investments in the two locations—to the ratio of the incomes obtained from those locations [31]. Income is the number of pellets per unit time—not per unit time spent at the hopper, but rather per unit time full stop. The extent to which a mouse is matching and the rapidity with which it adjusts its visit behaviour to accord with changes in the income ratio may be visualized

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The income imbalance is \((F_1 - F_2)/(F_1 + F_2)\), where \(F_1\) is the number of pellets obtained from the \(i\)th hopper in a given interval. When computed on a pellet-by-pellet time scale, that is, at the end of each inter-pellet-delivery interval, this statistic takes on only the values \(+1\) and \(-1\), depending on whether the pellet delivery with which the interval ends is at hopper 1 or hopper 2, respectively. However, when the pellet-by-pellet values of this statistic are cumulated to make a cumulative record (cumulative income imbalance as a function of number of pellets obtained), then the slope of the cumulative record is the average imbalance. If, on average, the mouse obtains equal incomes from the two hoppers, then the slope is 0; if it obtains all of its income from hopper 1, then the slope is 1; if it obtains all of its income from hopper 2, the slope is \(-1\); and if it obtains, say three out of every four pellets from hopper 1, the slope of the cumulative record is \(((1 + 1 + 1 - 1))/(1 + 1 + 1 + 1)) = 0.75. Similarly, the investment imbalance statistic is \((T_1 - T_2)/(T_1 + T_2)\), where \(T_i\) is the time spent at the \(i\)th hopper. For a single inter-pellet interval, it can take on any value on the interval \([-1, 1]\), but, as for the income imbalance statistic, the slope of the cumulative record of the income imbalance over any interval is the average imbalance over that interval. Thus, when the slopes of the two cumulative records are the same, the mouse is matching its investment imbalance to the income imbalance that it is experiencing (see figure 2 for four examples).

The imbalance statistic is a linear transformation of the more common Herrnstein fractions, which are \(F_1/(F_1 + F_2)\) and \(T_1/(T_1 + T_2)\). The difference between two average imbalances (two cumulative-record slopes) is twice the difference in the corresponding Herrnstein fractions. Thus, for example, when the slope of the income imbalance exceeds the slope of the investment imbalance by 0.2, the corresponding difference in the Herrnstein fractions—\(F_1/(F_1 + F_2) - T_1/(T_1 + T_2)\)—is 0.1. When the slope of the cumulative record of the investment imbalance is less extreme than that of the income imbalance, the mouse is undermatching: when it is more extreme, the mouse is overmatching. (See figure 2 for illustrations of this calculation.)

The matching protocol measures the accuracy with which a mouse can estimate two rates and compute their ratio. Because rate is number divided by time, this protocol also tests whether the mechanisms for estimating number and duration are intact. If the mouse could not remember which hopper produced which income, then it could not adjust the ratio of its visit durations to match the ratio of the incomes, so this protocol also tests the mouse’s ability to remember a location and the events associated with that location. In other words, in addition to measuring the accuracy with which the mouse

Figure 2. Four examples of joint cumulative records of the pellet-by-pellet income (red plots) and the investment (black plots) imbalance statistics, two from heterozygotes \((a,c)\) and two from littermate wild-type controls \((b,d)\) for the two strains: \((a,b)\) Batface and \((c,d)\) L1. The mouse in \((a)\) was overmatching; the mouse in \((d)\) was undermatching. Note that the departures from matching in \((a,b)\) occurred only over a portion of the records. In general, mice from this strain, whether wild-type or heterozygotes matched as well as those from the \(L1\) strain. (Online version in colour.)
can estimate rates, the appearance of matching behaviour is
evidence that the fundamental mechanisms of cognition that
enable the animal to locate itself in space and time are intact.
Finally, matching behaviour appears immediately: as soon as
mice enter the test box and observe the rates at which they
may obtain pellets from the two hoppers, they begin to
match the ratio of their average visit durations to the ratio of
the incomes they have observed [31]. It is hard to improve on
this protocol when one wants a rapidly completed test of a
broad range of basic cognitive functions. We put the mice
into the environment completely naive; within 12 h we have
good matching data for most mice. They give us important
data while they are becoming accustomed to the environment.

(b) The instrumental- and classical-conditioning
protocol
The rate of conditioning is often measured in behavioural
work on the neurobiology of learning and memory. It is
usually measured in a group of subjects by averaging trial-
by-trial or block-of-trials by block-of-trials performance
across subjects. The resulting group-average learning curve
is generally concave downwards, and it may often be
approximated by the cumulative exponential, the parameter
of which may be taken as an estimate of the rate of learning.
The problem with this method for measuring the rate of
learning is that the relatively smooth concave downward
curve is an artefact of the averaging. When the trial-by-trial
performance of individual subjects is examined in most
of the common animal learning paradigms, the condi-
tioned response is commonly seen to appear in one or a
few abrupt steps [32–34]. For examples of the abrupt appear-
ance of classically conditioned hopper entry, see figure 4. The
steps occur after different numbers of trials in different sub-
jects, and they are of different heights, because some subjects
show much more vigorous conditioned responding than
do other subjects. Averaging across the steps in the individ-
ual records produces a function that seems to indicate an
underlying process that obeys first-order kinetics (an expo-
nential approach to asymptote), when in fact the underlying
phenomenon, the transition for unconditioned to conditioned
behaviour in a single subject, is discontinuous. Even when
the transition in some subjects appears more or less continuous
[35], it occurs after widely differing numbers of trials and
over widely differing trial spans in different subjects within
the same group. In such cases, averaging across the subjects in
a group or condition produces a function whose mathematical
form is not the same as the mathematical form of the function
that describes the transitions in any one subject [36–38]. For
example, the average across exponentials is not itself an expo-
nential. For these reasons, the parameter(s) of a group-average
learning curve are meaningless, except perhaps to students of
animal sociology. Thus, in our combined instrumental- and clas-
sical-conditioning protocol, we measure trials-to-acquisi-
tion for each conditioned response in each subject.

In our protocol, there are three hoppers arranged along
one wall of a standard MedAssociates mouse test chamber.
When the middle hopper lights up, it signals to the mouse
that it may initiate a simple sequence of events leading to
the release of a pellet: when the mouse interrupts the infrared
beam in the illuminated middle hopper, the light in that
hopper goes off and one or the other flanking hopper illumi-
nates. If it is the hopper on the left flank—the short-latency
hopper—that illuminates, then a pellet is released into that
hopper at the end of 4 s, regardless of what the mouse
does. If it is the hopper on the right flank—the long-latency
hopper—then the pellet is released into that hopper at the
end of 12 s, again regardless of what the mouse does. Thus,
the illumination of one or the other flanking hopper signals
the release of a pellet into that hopper at the end of a
hopper-specific delay.

In this protocol, the mouse’s poking into the middle hopper
initiates a simple sequence of events leading to reinforcement—
the illumination of a flashing hopper followed at a fixed hopper-
specific latency by the release of a pellet into that hopper.
Because the poking is instrumental—no poke, no pellet—the
emergence of trial-initiating pokes into the centre hopper is an
instance of instrumental conditioning (also known as operant
conditioning). On the other hand, once the trial has been
initiated, a pellet is released into whichever flashing hopper illu-
minates regardless of what the mouse does. Thus, anticipatory
poking into an illuminated flashing hopper (a conditioned
hopper-entry response) is an instance of classical conditioning
(also known as Pavlovian conditioning). Whether different
learning mechanisms mediate the appearance of instrumentally
conditioned and classically conditioned responses may be
doubted [39], but it is commonly assumed that there are two
different mechanisms [40]. If there are, and if it is possible to
knock out the classical mechanism without knocking out the
instrumental one, either genetically or pharmacologically, then
one would observe mice that learned to initiate trials (instru-
mentally conditioned behaviour) but did not learn to poke
into the illuminated flashing hopper in anticipation of a pellet
release (classically conditioned behaviour).

If there are two conditioning mechanisms, then our proto-
col measures rate of acquisition for both. As in our matching
protocol, we obtain our rate measures from cumulative
records of trial-by-trial statistics. For instrumental condi-
tioning, it is the cumulative record of trial-initiation speeds
(figure 3a). For classical conditioning, it is the cumulative
record of conditioned stimulus-intertrial interval (CS-ITI)
poke rate differences (figure 4).

The speed with which the mouse initiates a trial is the recip-
ocal of the latency of the trial-initiating poke. This latency is
the interval between the illumination of the centre hopper
and the beam interruption that signals the first post-illumin-
ation poke into that hopper. Because the mouse may be
otherwise occupied or not even present (see description of
the test environment below), very long trial-initiation latencies
occur with some frequency. They dominate the cumulative
record of the latencies. Taking reciprocals of these latencies to
get trial-initiation speed rather than latency maps these atypi-
cally long latencies to essentially zero, with the desirable
result that the much shorter and more typical trial-initiations
dominate the cumulative record. When the mouse is slow to
react to the illumination of the central hopper, the trial-
initiation speeds are small. When it learns to initiate trials,
the speeds increase, which produces a higher slope in the
cumulative record of trial-initiation speeds (see figure 3e for
an example). We take the trial at which the slope first shows
a statistically significant increase to be the acquisition trial for
the instrumental response (marked with an open circle in
figure 3e). The first statistically significant increase was defined
to be the first significant positive increment in average initiation
speed that is greater than the average in the first segment when
the cumulative record is segmented by the previously
The distribution is truncated at 0.7, because the extremely long latencies at which the horizontal solid line at 0.5 intersects the cumulative distribution.

The median latency (vertical dashed line) is the latency at which the slopes reverse from negative to positive. The point at which this reversal occurs is our measure of trials-to-acquisition for the classically conditioned responses.

Figure 4. Illustrative cumulative records of the trial-by-trial difference between the rate of poking during the illumination of a flanking hopper (\( \lambda_{\text{CS}} \)) and the rate of poking into that same hopper during the intertrial interval (\( \lambda_{\text{ITI}} \)), when it is not illuminated and not about to deliver a pellet. Heavy dots mark the trials where the slopes reverse from negative to positive. The point at which this reversal occurs is our measure of trials-to-acquisition for the classically conditioned responses. (Online version in colour.)

(c) The timed-switching protocol

This protocol measures the accuracy and precision with which a mouse can measure and remember the durations of intervals. Fetterman & Killeen [41] first employed a version of it with pigeons as subjects. Balci et al. [42] adapted it for the mouse. It is similar to the protocol just described, except that when the mouse pokes into the illuminated trial-initiation hopper, both flanking hoppers illuminate (and, as in the previous protocol, the light in the centre hopper goes off). As in the previous protocol, on any trial, a pellet can be released into only one hopper. That is, on any trial, only one hopper is the ‘hot’ hopper, but the mouse has no means of knowing which. In this protocol, the pellet is released only in response to the first poke into the hot hopper at or after the delivery latency peculiar to it. Thus, when the short-latency flanking hopper is hot, a pellet is released in response to the first poke there at or after 4 s. When the long-latency flanking hopper is hot, a pellet is released there in response to the first poke at or after 12 s. If on a given trial, the first poke at or after the critical latency (4 or 12 s, depending on which hopper is hot) is into the wrong hopper (the ‘cold’ hopper), then the mouse loses its pellet. The erroneous poke terminates the trial, turning out the lights in both flanking hoppers, without the release of a pellet.
The asymptote parameter is estimated to fall short of unity is an estimate of the fraction of trials on which the mouse was not on task. If the fit function is the cumulative distribution, it is necessary to add an asymptote parameter that allows the cumulative distribution to asymptote below unity. The thick red lines in (a,b) are the empirical cumulative distribution of the observed switch latencies, that is, they are a plot of the raw data. The thin red lines superimposed on them are the best fitting six-parameter Weibull–Gauss functions. Five of these parameters are the parameters of a Weibull–Gauss mixture distribution, while the sixth parameter (asymptote) is an estimate of the fraction of the trials on which the mouse was on task (see §4c for explanation). The thicker red curve in (a,b) is the probability density distribution for the Weibull–Gauss mixture distribution with the same parameters as the empirical cumulative distribution function (cdf) fitted to the cumulative data. It is an estimate of the probability density function (pdf) of the mouse’s switch latencies. (a) From the first block of trials, when the feed latency at the long hopper was 12 s. (b) From the second block of switch trials, when the longer feed latency was reduced to 8 s. Reducing the longer feed latency, that is, bringing the temporal goalposts (vertical black lines) closer together, caused this and almost all other mice to increase the precision with which they timed their switches. It also shifted the mean of the Gaussian portion of the distribution leftward, keeping it more or less centred between the temporal goalposts. The solid vertical lines on each plot show the nominal short and long feed latencies, which we refer to as the temporal goalposts, because rational, goal-oriented switch latencies should fall within the interval they delimit. (c) Shows a switch-latency histogram, with counts plotted against left axis. Dashed curve is the fitted mixture distribution, plotted against the right (probability density) axis. The heavy vertical lines are the short (4 s) and long (8 s) feed latencies (the temporal goalposts).  (d) To obtain a fit that accurately represents the observed steepness of the cumulative distribution, it is necessary to add an asymptote parameter that allows the cumulative distribution to asymptote below unity. If the fit function were required to asymptote at unity and to pass through the empirical censor point, it would either be too steep or too shallow. The amount by which the asymptote is estimated to fall short of unity is an estimate of the fraction of trials on which the mouse was not on task.

The rational thing to do on every trial in this protocol is to go first to the short-latency flanking hopper and poke there for the first 4 s. If it is a short-latency trial, poking there releases a pellet after 4 s. If a pellet is not released in response to pokes made at or after 4 s have elapsed, then the rational thing to do is to leave the short-latency flanking hopper and poke instead into the long-latency hopper on the other flank.

The latency to depart the short-latency hopper on a long-latency trial is a switch latency. In principle, the distribution of switch latencies might be thought to depend only on the duration of the short feeding latency (4 s). In fact, however, it depends on both this duration and the duration of the long feeding latency (12 s). These two feeding latencies are temporal goalposts. The mouse attempts to position its switch-latency distribution approximately optimally between these goalposts ([42,43]; figure 5a,b). Thus, the estimated mean ($\mu$) of the distribution of switch latencies measures the accuracy with which the mouse can target the centre of the temporal goalposts, while the coefficient of variation ($\sigma/\mu$) measures the precision with which it does so. Accuracy is a measure of how close the central tendency of directed responses comes to an objective target; precision is a measure of their dispersion about their central tendency. It is possible to be grossly inaccurate but highly precise and vice versa.) We begin with a 3:1 ratio between the short and long latencies (the goal posts). After at least 200 long trials (trials on which the mouse should switch), we shorten the long latency to 8 s and run the mouse for a minimum of another 200 long trials. For the mice with the L1 genotype, we ran two further blocks with at least 200 long trials, in which we shortened the longer delivery latency to 7 s and then to 6 s, keeping the shorter latency always at 4 s.
The switch latencies from a given subject in a given session tend to be bimodal [42,43]. There is an approximately Gaussian-distributed set of switches with mean near the optimal target switch latency. The second mode consists of switches that occur well before the short-latency pellet release (figure 5c). In these trials, the subject poked in the centre hopper to initiate a trial, poked in the short-latency hopper very briefly, and then quickly switched to the long-latency hopper. These quick departures (easily separable statistically from the typical, Gaussian-distributed timing behaviour) are not optimal—on short trials, they cost the mouse its pellet—so we refer to them as ‘impulsive switches’.

In some experiments, the impulsive switches have been best modelled by an exponential distribution [43], while in others they have been best modelled by a Weibull distribution [42]. Further work is required to tease apart the causes of these differences and their import. This is difficult because differences between the two models only become pronounced when the proportion of impulsive trials is large, and this is not often the case. We find that to fit the distributions obtained in the research we here report, we had to use the Weibull–Gauss mixture distribution, which has five parameters: the scale and shape parameters of the Weibull distribution, the mean and standard deviation of the Gaussian distribution and the proportion parameter which estimates the proportion of the switches attributable to the Weibull component.

Long trials, trials on which the subject should switch, automatically terminate if the subject is still poking into the short hopper when the long latency elapses. Thus, our switch times are censored at the longer feeding latency (figure 5c). We cannot record switch latencies longer than the longer delivery latency, so we do not know when the mouse would have switched on these censored long trials. We do, however, know the fraction of long trials on which they occur. A constraint on the distribution that we fit to the switch latencies is that it correctly predicts this fraction. Another constraint is that the steepness of the Gaussian component matches the steepness of the second rise in the observed cumulative distribution. The steepness of the second (and by far the greater) rise in the cumulative Weibull–Gauss mixture distribution is a function of the standard deviation of the Gaussian component. Our estimate of the precision of switch timing is the ratio between the estimated standard deviation of the Gaussian and the estimated mean of the Gaussian. Thus, systematic error in the estimation of the standard deviation leads to systematic error in the measure of precision.

We often find that the five-parameter Weibull–Gauss mixture distribution cannot simultaneously capture the fraction of switch trials on which the mouse fails to switch and the steepness of the Gaussian rise (see figure 5d for an example). A fit that accurately represents the steepness of the rise in the cumulative distribution of the observed switch latencies underpredicts the observed fraction of failures to switch. A fit that accurately predicts the observed fraction of failures to switch may grossly misrepresent the steepness of the rise in the Gaussian component of the observed distribution.

The problem arises from the fact that the algorithm for fitting a probability distribution to censored data assumes that the cumulative distribution asymptotes at unity, as do all cumulative distributions. In psychophysical work with human subjects, the empirical distributions often fail to asymptote at unity, because on some (usually small) fraction of the trials the subject is not on task. This creates empirical distributions with ‘fat tails’, also known as ‘outliers’. Thus, it is common to add an asymptote parameter, which allows the fitted distribution to come to an asymptote below unity. We follow that practice in fitting a Weibull–Gauss mixture distribution to our switch data. We add a sixth parameter that allows the fitted distribution to have an asymptote less than unity.

An asymptote parameter in psychophysical data fits is generally regarded as a necessary nuisance. That is what it is for us when we estimate the precision with which a mouse times its switches. In behavioural screening research, however, which is aimed at revealing the effects that a genetic or pharmacological manipulation may have on diverse underlying mechanisms of behaviour, the parameter may be of interest in its own right. Its additive inverse, $1 - A$, where $A$ is the estimated asymptote, measures the frequency with which a mouse is ‘not on task’ (figure 5d). A mouse may not be on task for a variety of reasons: it may, for example, have stopped to groom itself or left the test box for the nest-box and then returned much later to make another poke in the short hopper. Whatever the reason, it fails to time its switch to the longer hopper. For clinically oriented behavioural pharmacologists and the behavioural geneticists, this may be the measure of interest. For us, it is another way to assess the generality of the effects of a possibly deleterious genetic difference. Does the genetic difference cause the mice to be on task less often?

In summary, from the fitting of six-parameter Weibull–Gauss mixture distributions to switch latencies and failures to switch, we obtain four measures of interest: (i) the fraction of trials on which the mouse made an impulsive switch (a measure of impulsivity); (ii) the fraction of trials on which the mouse was not on task (a measure of distractibility); (iii) the Gaussian mean (a measure of the accuracy of interval timing) and (iv) the Gaussian coefficient of variation ($\sigma/\mu$, a measure of the precision of interval timing).

(d) Circadian food anticipation

Animals of many kinds learn the phase of the circadian cycle at which events occur [1]. A simple manifestation of the mechanism that records and anticipates daily times of occurrence is the appearance of anticipatory activity in the few hours preceding the onset of one or more fixed phases of daily food availability [24–47]. To assess whether the mechanisms that enable the animal to record and anticipate circadian phase (time of day) were intact, we restricted the daily phases during which the above-described food-providing protocols were operative. A 4 h dusk food-availability phase began 1 h before the house light went off (dusk) and terminated 3 h after dusk. A 4 h dawn phase began 2 h before the house light came on (dawn) and terminated 2 h after dawn. Figure 6 is a representative daily raster plot (24 h actograph).

As is evident in the representative plot in figure 6, there is more feeding during the dusk feeding phase than during the dawn feeding phase, and there is much more prominent anticipatory activity preceding the dusk phase than the dawn phase. Therefore, we only compute measures of circadian anticipatory activity for the dusk phase. On any given day, we record the occurrence of activity anticipating the onset of the dusk feeding phase only if three-fourths of the behavioural events occur during the final one-third
of the 9 h phase of no-food availability following the end of the dusk feeding phase and preceding the onset of the dusk feeding phase. This scoring treats the occurrence of anticipatory activity as an all or nothing event. Anticipatory activity thus scored does not occur during the first several days of testing. Our measure of ‘trials’ (days)-to-acquisition of circadian food-anticipatory activity is the day after which the frequency of anticipatory activity shows the first increase. The first positive change point in the probability of circadian anticipatory activity only occurs on some days. Those days become more frequent as testing progresses. The anticipatory activity is much more salient prior to the onset of the dusk feeding phase than prior to the dawn feeding phase. Feeding during the dusk phase often ceases abruptly when the house light comes on. All of these features are seen in most of the other 21 records from the testing of the L1 line, whether heterozygote or wild-type.

Figure 6. Representative 24 h actogram: raster plot of pokes (black dots) with one line per 24 h day. Cyan triangles with base to right mark the onset of the dusk feeding phase; base-left cyan triangles the offset; magenta triangles mark the onset and offset of the dawn feeding; black triangles mark house light on (dawn) and house light off (dusk). The light–dark cycle is also indicated by a bar at the top. Red and green dots mark pellet deliveries at the left and right hoppers, respectively. (Many green dots are overprinted with red dots.) The computed onsets of poking in anticipation of the onset of the 9 h phase of no-food availability following the end of the dusk feeding phase shows the first increase. The algorithm is in the electronic supplementary material.

5. Results

(a) Matching

There were no genetic effects on the accuracy with which mice matched (figure 7). From this, we can conclude that many of the most basic cognitive mechanisms mediating learning and memory are intact. Mice heterozygous for the Batface mutation and for the L1 mutation can estimate number and duration and compute the ratio of these estimates to obtain an estimate of rate (income) and they can adjust the ratio of their average visit durations to match the ratio of the average incomes. For this to be possible, they must correctly associate their estimates of the incomes (rates of pellet delivery) to the locations. Thus, matching implies that they can distinguish spatial locations.

(b) Learning rates in basic conditioning paradigms

Both groups of heterozygous mice learned our instrumental task at the same rates as their wild-type littermate controls (figure 8a), and they initiated trials with the same latency once they had learned to do so (figure 8b).

The same is true for the rate of learning to anticipate food delivery in classically conditioned hopper entry (figure 9): there is no significant effect of genotype (+/+ versus +/-mut or +/-) on trials to acquisition in either the Batface line or the L1 line. For both instrumental conditioning and classical conditioning, trials-to-acquisition measures vary by more than an order of magnitude within genotype, and some mice did not show anticipatory responding (classically conditioned hopper entry) within the span of the 250 trials that we put as the limit on this phase of the protocol. A large range of variation in trials to acquisition is commonly observed [32,34,49].

A measure that varies greatly between subjects drawn from highly inbred strains of laboratory animals may not be a promising measure from a genetic screening standpoint, because it is not under tight genetic control.

(c) Measures from the switch protocol

Figures 10 and 11 show the cumulative distributions of the four parameters of interest from fitting the Weibull–Gauss mixture distribution to the switch-latency data, as a function of trial block and genotype. Trials were run in blocks comprising at least 200 long trials, with the nominal pellet-delivery latency at the long-latency hopper reduced on successive blocks, from 12 to 8 to 7 to 6 s. Only blocks 1 (12 s) and 2 (8 s) were run with the Batface line.

Generally speaking, trial block (shortening the longer delivery latency) had an effect. It increased the per cent of trials on task. This may be simply an effect of increased experience with the task. Trial block also tended to increase impulsivity. This may well be because the difficulty of the task increased as the goalpost at the longer delivery latency moved closer to the goalpost at the shorter delivery. As expected, trial block had a strong effect on the Gaussian mean, the central tendency of the rationally timed switches. As the longer goalpost moved closer to the shorter, so did the central tendency of these switch latencies, keeping the Gaussian component of the mixture distribution approximately centred between the goalposts. Trial block also affected the precision of with which switches were timed: as the goalposts drew closer together, precision increased,
which is to say that the coefficient of variation decreased. Put another way, the measured Weber fraction got smaller as the task grew more demanding.

(d) Circadian food anticipation

Figure 12 shows for the L1 mice line, the cumulative distributions of day of acquisition, mean onset hour for...
anticipatory activity within the 9 h interval preceding the onset of the dusk phase of food availability, and standard deviation of the onset hour. There was no effect of genotype on any of these measures. The testing of the Batface line was cut short by a prolonged power failure after 11 days, which was too soon to estimate the onset of anticipatory activity.

Figure 9. (a, b) Cumulative distributions of trials to acquisition in the classically conditioned anticipatory hopper entry, as a function of genotype and short- versus long-duration of the conditioned stimulus (hopper illumination). The effects of genotype (+/+ versus +/mut or +/-) do not approach significance in either the Batface or the L1 lines. (Online version in colour.)

Figure 10. (a–d) Cumulative distributions of per cent trials on task, per cent impulsive trials, Gaussian mean switch latency and precision (CoV) in the Batface line, as a function of trial block and genotype. Difference in colour denotes difference in trial block (hence, in the nominal feed latency at the long hopper), while solid versus dashed denotes difference in genotype. The p-values (p$_g$ for genotype and p$_b$ for block) are for the main effects in two-way ANOVA. The only main effect of genotype was on the precision with which switches were timed (bottom right plot): the heterozygotes were less precise. (Online version in colour.)
Note that the median onset day in the L1 line is 20, which is consistent with what we have observed in other strains.

6. Discussion

The two genotypes Bfc(+/mut) and L1(female, +/-) have highly specific and opposing effects on the precision with which mice represent interval duration. The precision is reduced in the Bfc heterozygote, when compared with the wild-type control, and increased in the L1 heterozygote, when compared with the wild-type control. The functional specificity of these genetic effects is remarkable and important. Many other measures of learning, performance and cognition do not vary with these variations in genotype. These genotypic variations do not alter the rates of instrumental and classical conditioning. They do not alter the latency with which mice make an instrumentally conditioned response to the onset of a discriminative stimulus (the illumination of the central, trial-initiation hopper). They do not alter the accuracy with which mice match the ratio of their expected visit durations (their investment ratio) to the ratio of the incomes in a matching paradigm. They do not alter the accuracy with which mice adjust the mean of their switch-latency distribution to changes

Figure 11. (a–d) Cumulative distributions of per cent trials on task, per cent impulsive trials, Gaussian mean latency and the precision (CoV), in the L1 line, as a function of trial block and genotype. Difference in colour denotes difference in trial block, while solid versus dashed denotes difference in genotype. The p-values (p_g for genotype and p_b for trial block) are for the main effects in two-way ANOVAs. The interaction terms were in all cases insignificant. The only significant effect of genotype was on the precision with which trials were timed (bottom right plot): the heterozygotes were more precise.

Figure 12. (a–c) Cumulative distributions of day of acquisition, mean onset hour and standard deviation of the onsets of food-anticipatory activity in the L1 line.
in the temporal goalposts that delimit the interval of rational switch latencies. And, in the L1 line at least, they do not alter the development of circadian food-anticipitation, or the variability in the onset of this activity, or the length of the anticipatory interval (the interval between the onset of anticipatory activity and the onset of the anticipated phase of food availability). It will remain to be established how the behavioural phenotype of the heterozygous L1 mutant females relates to the observation that more neurons are generated and maintained in these mice when compared to their wild-type female littermates and the mutated male littermates. Thus, the histological phenotype of the heterozygous L1 knockout mutant is unexpectedly not intermediate between the wild-type and the homozygous mutant. This aspect together with the findings of the present—showing an improved functional parameter when compared with the wild-type littermates—deserves further investigations in humans carrying the L1 mutations in the heterozygous state, particularly in view of the notion that a surplus of neurons is not conducive to normal nervous system functions.

In short, the effect of these genetic variations is limited in its scope. We do not yet know how limited. It may be an effect on the precision with which quantities of all kinds are represented [50] or it may be specific to the representation of duration. To resolve that question, further behavioral testing is needed, including tests that assess the accuracy and precision of, for example, the representation of distance and direction in spatial tasks.

We also do not know whether these effects arise from the developmental effects of the genotypic variation or from the effects of the variations in the transcription of these genes in the adult brain. To determine that will require the development of facultative knockouts for these two genes. We also do not now know which altered physiological and/or neuroanatomical effects of these genotypic variations are responsible for the variation in the precision with which the mouse brain represents duration. That, too, must await the further investigation of these variations. The results we here report should motivate such investigations.

Forward behavioural genetics can play an important role in the establishment of linkage hypotheses [51], which is a central goal of behavioural and cognitive neuroscience [52–54]. It can best do so when behavioural screens target novel physiological measures of specific cognitive mechanisms. The precision with which a duration or any other simple magnitude, such as a distance, is represented in the brain is like the period of a behaviourally important intracellular biochemical oscillation (for example, the circadian clock) in that it can be measured both behaviourally and physiologically, that is, by electrophysiological, optical or by biochemical or biophysical methods for measuring physiological variables.

Finally, we stress the importance for future investigations of the fully automated, highly quantitative approach to behavioural and cognitive genetics made possible by the system used to make the measures we have here described. The system produces a wealth of meaningful measures with very little human effort. The mice were never handled in the course of as many as 86 days of continuous testing. The 21 mice of the L1 line were run simultaneously. The number of mice that can be tested simultaneously is limited only by the number of test environments. Eight of the environments fit in a 1.22 × 0.61 × 1.98 m cabinet. The code that controls the testing protocols and the data-analysis code that derives the measures here reported are available from the senior author on request. The data-analysis code is written in a custom software Matlab toolbox, TSystem, which greatly facilitates the creation of code that extracts complex biological structure from large scale databases consisting of time-stamped event records [9]. In the fully automated system, the data-analysis code is called automatically several times a day, providing the investigator with quasi-real-time graphic visualization of the progress of each mouse through the sequence of protocols, with summary statistics on each mouse, presented in graphic form.

The testing protocols and their application to these strains were approved by the Institutional Animal Care and Use Committee at Rutgers.

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Endnote

1°Precision’ in statistics commonly refers to the reciprocal of the variance, whereas ‘precision’ in measurement commonly refers to the coefficient of variation.

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