An analysis of the genetics of alcohol intoxication in inbred mice

By: John C. Crabbe, Pamela Metten, Andy J. Cameron, Douglas Wahlsten

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Abstract:

We compared the behaviors of eight inbred mouse strains across 18 variables, using 11 behavioral assays, and gave ethanol (EtOH) as an intoxicant. Genetic influences on behavior and sensitivity to EtOH were pronounced, but strain sensitivities were generally only modestly correlated across tasks. Certain well-correlated clusters of responses suggested that some genes affect similar neurobiological substrates. No strains of mice were generally sensitive or resistant to intoxication across tasks. Anthropomorphically appealing concepts like 'muscle strength' had little explanatory power across tasks. A battery of selected tests was proposed for future studies. Overall, the results show that each mouse behavioral assay captures only a portion of ataxia, a genetically complex behavioral domain. Conversely, multiple behavioral capacities are apparently required for performance in each specific assay. Thus, if only one or two tests are used to evaluate motor function in genetically engineered mutant mice, only a small portion of the domain will be assessed and results may be misleading. This caveat likely extends to many behavioral domains (e.g. learning and memory, anxiety).

Keywords: Inbred mouse strains; Pharmacogenetics; Ataxia; Ethanol; Intoxication; Acute tolerance; Rotarod; Balance; Grip strength; Activity; Genetic correlations; Pleiotropy; Coordination; Loss of righting reflex

Article:

1. Background

Many neurological disorders have been targeted using genetically engineered mice, where a single gene has been caused to be over or under expressed or to direct production of an inactive protein. Motor system function is studied in mice with a large number of laboratory measures, and dysfunction in these assays is often broadly described as 'ataxia'. The most commonly used task is probably some variant of the rotarod, where the ability of a mouse to remain on a rotating rod by continuously adjusting its position is assessed [1]. A partial list of other assays includes tests of loss of the righting reflex [2], hanging from a screen or wire or coat hanger [3,4], grip strength (GRIP) [5], balancing on a beam or dowel or narrow unsteady platform without falling, or traveling across them without foot slips [4,6,7], traversing an apparatus without foot slips through a grid floor or a floor with randomly placed holes [8,9], climbing a pole or knotted rope [9], analysis of gait based on treadmill performance [10] or analysis of the patterning of footprints after dipping the animal's feet in paint [1 1]. New tasks are always being developed. For example, mice have been placed in a box with a wire mesh floor, which is turned upside down. Rather than measuring latency to fall (as in the screen test, which is performed with the screen at right angles to the table top), these investigators videotaped the mice and reported that mice treated with MPTP to mimic Parkinsonian symptoms showed increased forepaw placement errors (like the errors measured in the grid test (GT)) and shorter step lengths than controls [12]. Occasionally, a test appears to have been developed expressly to assess a specific function, e.g. the staircase test of skilled reaching [13]. More often, the tests appear to have been developed heuristically. We presume that performance on these widely varied tests probably reflects the convergence of competencies in multiple underlying physiological substrates. Motor performance is behaviorally complex, and on any given task, the animal may be required to employ vision, balance, accurate proprioceptive feedback and gait coordination, locomotion, adequate muscle strength, and attention. For tasks where multiple trials are given, motivation and ability to learn and remember may be called into play.

We have been interested in the genetic determinants of task performance in the broadly defined domain of motor coordination. Specifically, we have employed some of these tasks to assess genetic differences in sensitivity to EtOH intoxication by testing multiple inbred strains of mice for their behavioral competence with and without EtOH [14–21]. While our ultimate goal is to understand which genes might lead to increased or decreased sensitivity to EtOH intoxication, our data have revealed striking complexity in the genetic determinants of undrugged performance as well as in sensitivity to EtOH. In this report, we synthesize the results of several of the above, previously published analyses. For most tasks employed, our previous analyses of strain differences used multiple task parameters (e.g. widths of balance beams (BBs) or doses of EtOH) and we have selected from those published reports representative variables to index each task based on their reliability and the magnitude of strain differences. The tasks employed and variables selected are described briefly below, and the reader can find full details in the published papers. Some data we include are from similar analyses of unpublished data, not yet submitted for publication. For these variables, we report the methods used more completely.

Our data show that the pattern of strain differences in performance depends on exactly how a given task is performed (e.g. details of the apparatus and procedures used), and that different tasks appear to call into play effector systems engaged by different constellations of genes. Strain-specific sensitivity to EtOH is by no means uniform across tasks, and can depend as well on EtOH dose and/or how long after administration the behavioral assay is performed. While the tasks employed do not supply redundant information about genetic influences on behavior, it is possible to select some tasks that, considered in the aggregate, represent a reasonably wide spectrum of genetic influences. We end by proposing a test battery constructed from several tests analyzed here.

2. Methods

2. 1. Animals, husbandry, and general procedures

A trade-off between financial considerations and statistical power to detect genetic correlations led us to limit our studies to mice from eight inbred strains. Seven of the strains were selected from the 10 on the 'A' list of the Mouse Phenome Project (http://aretha.jax.org/pub-cgi/phenome/ mpdcgi?rtn = docs/pristrains). The Mouse Phenome Project is a large effort to compile a relational database describing many physiological and behavioral characteristics of up to 40 standard inbred mouse strains. Mouse strains are assigned to one of four lists (A–D). A-list designation reflects wide use, high fertility, relative inexpensiveness (average cost of the strains we studied= $15.38/mouse; for the entire A-list, cost was $26.23/mouse), and A-list strains have been derived from a reasonably diverse set of pedigrees [22]. We also included the BTBR strain ($41.30/mouse), which once, briefly, was on the A-list, but is now on the D-list, because by the time we began this project we already had data on them for several other-related tasks. We elected not to use CAST/EiJ or SPRET/EiJ from the A-list because we have found them to be so wild and hard to handle that meaningful behavioral data could not be obtained from them in many tasks [28]. We also did not test SJL/J from the A-list. The following eight inbred strains were used in all tests discussed here: 129S1/SvImJ (129), A/J (A), BALB/cByJ (BALB), BTBR T^{+tf}/J (BTBR), C3H/HeJ (C3H), C57BL/6J (B6), DBA/2J (D2), and FVB/NJ (FVB). All mice were obtained from The Jackson Laboratory (Bar Harbor, Maine) at 5–6 weeks of age.

Mice were housed in polycarbonate (some in poly-sulfone) cages lined with Bed-o-Cob™ bedding, usually three mice per cage, with others of their strain and sex. Food (Purina 5001™) and water were provided ad libitum. Ambient temperature was 21 ± 1 ºC, and colony lights were on for 12h (06:00–18:00). Bedding was changed weekly, but always after testing. Multiple shipments of mice were received for many tests, each generally comprising mice of all strains. Mice were generally used in only one test (but see specific references). All housing and test procedures were approved by the Institutional Animal Care and Use Committee at the VA Medical Center and were in accordance with USPHS Guidelines.

2.2. Variables from previously published tasks examined for genetic correlations

All studies used adult mice of the eight strains listed above, given EtOH ip (20%, v/v in saline) or saline at the stated doses. Ages ranged from 7 to 17 weeks across all studies. Housing and testing environment, apparatus and procedures were as described above. For further details, see each cited reference. For nearly all tests,

animals were moved from the colony to the testing room an hour before testing and left undisturbed in their home cage or a holding cage until being weighed, injected, and tested. As noted below, all variables were rescaled so larger positive numbers reflected greater sensitivity to EtOH.

2.2.1. Observer-rated ataxia

Using newly devised rating scales, mice were scored for several signs of ambulatory ataxia 10 min after ip injection of EtOH. Two variables were included here. The severity of hind limb splaying (SPLAY) after 3 g/kg EtOH was scored from 0 to 5 (e.g. 3, constant splaying with limited coordination, often moving in circles; 5, constant splaying, no attempts to move). We also report severity of wobbling (WOBBLE) after 2.75–3.25g/kg EtOH from 0 to 4 (e.g. 2, both wobbles and falls, at least 2 feet are off the platform; 4, falls onto side and cannot get up) [18].

2.2.2. Hypothermia (HT)

Mice were placed in small individual chambers for 1 h habituation, and their rectal temperature was taken. After ip injection of 3 g/kg EtOH, temperatures were again taken at 30, 60, and 120 min. The change from baseline temperature at 60 min after injection was used to index acute hypothermic response (HT) (Crabbe et al., in preparation). HT was included for two reasons. First, earlier studies had found a negative genetic correlation between HT and loss of righting reflex (LORR) duration [19]. Furthermore, core body temperature has been found to modulate behavioral sensitivity to EtOH using the LORR measure [23].

2.2.3. Loss of righting reflex

The mice tested for acute HT were tested again 2 week later employing a variant of the classic method [24] for assessing LORR. After 1 h habituation, a mouse was weighed and injected ip with 4 g/kg EtOH and restrained by the tail until it appeared to have become intoxicated. At this point, it was placed on its back in a V-shaped plastic trough. LORR was declared and recorded if the mouse failed to right itself for at least 30 s within 3 min after injection. The 30 s criterion insures that a spasmodic kick or twitch is not scored as a righting. The 3 min criterion was employed because at least 15% of ip injections, even administered by a highly experienced experimenter, do not fully reach the peritoneal cavity [25]. In our experiment, 23/96 mice failed this criterion and were excluded from the experiment. Mice were then observed until they righted themselves, at which point the recovery time was noted. The mouse was placed again on its back, and recovery was noted after two successive rightings within 30 s. Sensitivity was indexed as the duration of LORR (LORR DUR) =time at LORR recovery —time at LORR loss.

We have also recently reported LORR sensitivity for these strains using a new and more sensitive method. Mice were restrained in a cylinder immediately after a 3 g/kg EtOH injection and the cylinder was rotated 90º each 5 s until the mouse failed to right. Mice were then retested periodically until they regained righting reflex. LORR NEW BEC (blood EtOH concentration (BEC) at time of LORR) was included in the analysis [21,25]. With this new method, animals lose righting reflex sooner (i.e. at lower BEC), and after lower doses of EtOH, than when tested by the classic method. Because lower BEC values are associated with greater sensitivity, LORR NEW BEC values shown in Table 4 were multiplied by (— 1) for correlational analyses.

2.2.4. Acute functional tolerance

Using the new LORR method, mice regain righting reflex at a higher BEC than the BEC at LORR (LORR NEW BEC). The difference between these values represents within-session, acute functional tolerance (AFT) development and was used as the index for correlations [21,25].

2.2.5. Screen test sensitivity

Latency to fall from a screen 30 min after 2.25 g/kg EtOH was tested. The index of sensitivity (SCREEN) included is latency to fall X (— 1), as more sensitive animals have shorter latencies [15].

2.2.6. Grid test (GT)
Mice were habituated to an apparatus with a wire mesh floor after ip saline injection for 3 days and tested the next day for 15 min starting immediately after a 1.5 g/kg EtOH injection. The number of foot fall errors divided by the distance traveled during 6–10 min after injection was used to index sensitivity in this task, as more activated animals have more opportunities to slip through the grid than inactive subjects [16].

2.2.7. Accelerating rotarod decrease (ARR DECR)
The reduction from baseline latency to fall (i.e. impaired performance) from a 6.5 cm rotarod accelerated at a constant rate of 20 rpm/min was tested 30 min after 2 g/kg EtOH [14].

2.2.8. Accelerating rotarod increase (ARR INCR)
The increase from baseline latency to fall (i.e. improved performance) from a 6.5 cm rotarod accelerated at a constant rate of 20 rpm/min was tested 30 min after 1.0 g/kg EtOH [14].

2.2.9. Fixed speed rotarod
The same rotarod was turned at a constant speed of 6.5 rpm and the variable used to index sensitivity was latency to fall immediately after 1.0 g/kg EtOH, multiplied by (— 1) (FSRR-0). A second assessment, in different mice, used a constant 3 rpm speed. Under these conditions, the percent mice of a given strain that failed a 3 min test 30 min after 1.75 g/kg EtOH was the index (FSRR-30). [14].

2.2.10. Balance beam
Number of hind limb foot slips off the surface during transition of a 19 mm wide, flat BB starting 10 min after 1.2 g/kg EtOH or saline was recorded [16]. The strain sensitivity index was the difference between number of foot slips in the EtOH-treated and saline-treated groups for each strain.

2.2.11. Grip strength
Force of grip using a strain gauge was assessed before and 30 min after 2.0 g/kg EtOH [15]. The index was baseline force minus post-EtOH force.

2.2.12. Dowel test (DT)
It was indexed as latency to fall from a 15.8 mm round dowel immediately after 1.5 g/kg EtOH (DT-15.8-0) and, in differentmice,froma9.6 mm dowel 30 min (DT-9.6-30) after 1.5 g/kg EtOH [15]. Both latencies were multiplied by (— 1).

2.2.13. Basal locomotor activity
These variables were available from multiple tasks. The activity (cm traveled) following the third daily saline test in the GT (BASAL ACT) was used in these analyses [16].

2.2.14. EtOH-induced activity (ETACT INCR)
This variable was taken from the GT as cm travelled during 6–10 min after 1.5 g/kg EtOH, as this time period was most sensitive to strain differences [16].

2.2.15. Pharmacokinetic measures
BEC were taken in several studies to index strain differences in effective EtOH dose. Variables included in these analyses were BEC 15 min after 1.0 g/kg EtOH (BEC 1.0–15, unpublished data) and 30 min (BEC 1.0–30) after 1.0 g/kg EtOH [20]. The 15 min data were in exactly the same substrains, while the 30 min data included some different substrains (e.g. BALB/cJ instead of BALB/cByJ). Other blood levels analyzed were BEC 35 min after 1.5 g/kg (BEC 1.5–35) or 2.0 g/kg (BEC 2.0–35) EtOH [15], and BEC 5 min (BEC 3.0–5) after 3 g/kg EtOH [42]. Several other BEC values appear in the published papers. We also included Widmark's β, the rate of EtOH metabolism in mg/ml/h, estimated from linear regression on BEC values 60, 105, and 150 min after a 2.0 g/kg EtOH injection [15].

2.3. Genetic correlations

The extent to which one can infer the influence of a common set of genes on two traits can be estimated from the correlation of inbred strain means if the strains have been maintained and tested in equivalent environments [27]. With only eight strains, a correlation of $|r| \geq 0.71$ is required to achieve the usual level of statistical significance ($P<0.05$). However, we were interested in the overall pattern of relationships among the many tasks we explored, so we discuss some smaller correlations as well as those that were significant. As has always been our practice in such analyses [19,20], we also examined scatterplots for all correlations. For some variables, individual strains were unable to provide meaningful data because they were so sensitive or insensitive to EtOH, but we selected measures of sensitivity for each task on which we had data for all eight strains.

With such a small number of strains, even if they are not extreme responders for either correlated trait, a single outlier strain can have a marked effect on the value of a correlation, producing either false positive or false negative results. We did not want to overlook potentially interesting relationships (i.e. commit false negative errors). Therefore, in an attempt to search for outlier influences in a systematic way, we recomputed the intercorrelation matrix eight times, eliminating a single strain each time. We then explored each correlation where elimination of a strain had made a noticeable change in the *r* value. In Sections 3 and 4, we have occasionally chosen to interpret the relationships among variables based on elimination of an outlier strain, and we indicate this when it occurs. Of the 153 correlations reported in Table 3, we believe that in 28 (18%) cases, interpretation is stronger with an outlier deleted. Any interested reader may obtain any of these values in a data file upon request. The range of a genetic correlation is technically — 1.0 to + 1.0, but the magnitude of each correlation is actually bounded by the true reliabilities of each correlated task, and of the correlation itself. Reliability estimates, when available, were presented for the individual variables in the publications from which they were drawn.

Table 1
Tasks, task-related parameters and variables, and abbreviations employed

Task	Task Abbreviation	Dose (g/kg)	Time (min)	Variable description	Variable Abbreviation	Reference
Observer-rated ataxia	ORA	3.00	10	Splayed hind limb	SPLAY	[18]
		2.75–3.25	10	Wobbling gait	WOBBLE	[18]
Hypothermia	HT	3.00	60	Change from basal temperature	HT	Unpublished
Loss of righting reflex	LORR	4.00	0	Duration	LORR DUR	Unpublished
		3.00	0	[Blood EtOH] at LORR	LORR NEW BEC[a]	[21]
Acute functional tolerance	AFT	3.00	30–113	LORR BEC at recovery minus LORR NEW BEC	AFT	[21]
Screen test	SCREEN	2.25	30	Latency to fall	SCREEN[a]	[15]
Grid test	GT	1.50	6–10	Foot slips/cm traveled	GT	[16]
Accelerating rotarod	ARR	2.00	30	Reduction from baseline latency to fall (impaired performance)	ARR DECR	[14]
		1.00	30	Increase from baseline latency to fall (improved performance)	ARR INCR	[14]
Fixed speed rotarod	FSRR	1.00	0	Latency to fall (impairment)	FSRR-0[a]	[14]
		1.75	30	Percent failing test	FSRR-30	[14]
Balance beam	BB	1.20	10	Foot slip errors	BB	[16]
Grip strength	GRIP	2.00	30	Fore limb grip force reduction	GRIP	[15]
Dowel test	DT	1.50	0	Latency to fall (15.8 mm)	DT-15.8-0[a]	[15]
		1.50	30	Latency to fall (9.6 mm)	DT-9.6-30[a]	[15]
Activity	BASAL ACT	0	6–10	Distance traveled in GT (cm)	BASAL ACT	[16]
EtOH-induced activity	ET ACT INCR	1.50	6–10	Distance traveled in GT (cm)	ET ACT INCR	[16]
Blood EtOH concentration (mg/ml)	BEC	1.00	15	Blood ethanol levels from several studies	BEC 1.0–15	Unpublished
		1.00	30		BEC 1.0–30	[20]
		1.50	35		BEC 1.5–35	[15]
		2.00	35		BEC 2.0–35	[15]
		3.00	5		BEC 3.0–5	[42]
		2.00	60–150	Widmark's β, EtOH metabolism rate mg/ml per h	BETA	[15]

Only those EtOH doses and times after injection used in the correlational analyses are shown. For the full range of doses, times, and variables, see the original papers.
[a] Variables for which data from Table 4 were multiplied by (−1) to perform correlational analyses (Table 3).

3. Results

Table 1 lists the tasks, tasks-related variables, and their abbreviations used throughout the paper. Each task had a somewhat different sensitivity to EtOH in terms of effective dose and time after injection. For each task where multiple variables and/or apparatus configurations and/or EtOH doses were employed, we selected for correlational analysis one or two combinations to best represent strain sensitivity on that task, as described in

the primary publications. To simplify presentation, the variables were generally scaled such that high positive numbers represent high sensitivity to EtOH intoxication. Where this was not possible, the values were multiplied by (— 1) for the correlational analyses. Some tasks were much more sensitive to low-doses of ethanol than others; consequently, doses used for this analysis differed greatly across tasks. Whenever possible, these choices were based on allowing meaningful data to be collected for as many strains as possible, on maximizing the contribution of genotype (strain) to individual differences, and/or on the reliability of the variable. Occasionally, additional variables for a given task are discussed, for which further information can be found in the published papers.

Table 2
Inbred strains tested

Strain	Abbreviation
129S1/SvImJ	129
A/J	A
BALB/cByJ	BALB
BTBR T^{+tf}/J	BTBR
C3H/HeJ	C3H
C57BL/6J	B6
DBA/2J	D2
FVB/NJ	FVB

Table 2 gives the list of inbred strains studied, and the abbreviations used to refer to them throughout. Table 3 gives the correlations among strain means for the variables. Our approach to interpretation of correlations is discussed in Section 2. Positive correlations indicate similar ethanol sensitivity of the strains in both measures. Correlations significant at $p \leq 0.05$ are shown in bold type, and those at $p \leq 0.01$ in underlined bold italics. Table 3 includes a separate entry where elimination of a strain rendered a correlation significant, and the deleted strain is indicated. Table 4 shows the mean values for all variables discussed, and Table 5 gives the rank order strain sensitivities for each variable. Although many variables show relatively strong patterns of positive or negative association, no single variable seemed to dominate the overall patterns. We discuss selected groups of variables chosen to organize the illustration of some of the complexity apparent in the results rather than because we believe them to be mechanistically distinct.

As noted in the primary report of the observer-rated ataxia (ORA) data [18], splaying scores and wobbling scores recorded after equivalent doses of EtOH tended to be negatively related (r = -0.62, P = 0.10). These two responses were, in turn, associated with several other responses. The negative relationship between SPLAY and WOBBLE seemed to reflect the fact that relatively insensitive strains scored high on WOBBLE, but more sensitive strains scored high on SPLAY. These two responses were incompatible—an animal showing uncontrollable splaying of its hind feet was no longer capable of displaying a wobbling gait. Fig. 1 gives a schematic rendering of one cluster of variables, each of which was related to SPLAY. The companion figure (Fig. 2) shows a group of variables related to WOBBLE. As would be expected from the negative correlation between SPLAY and WOBBLE, these two clusters of variables are partially overlapping.

Figs. 1 and 2 reveal some clusters of systematically related responses. SPLAY, GT, and BB scores were positively interrelated ($0.52 \leq r \leq 0.81$, see Fig. 3), and all three responses employ the hind limb foot slip to index intoxication. The correlation between SPLAY and BB was r = 0.56 across the eight strains, but when the FVB strain was eliminated because of its extremely low splay scores, this correlation was much higher (r = 0.81, P = 0.03). Strains with high splaying scores also tended to be highly intoxicated on ARR DECR (r = 0.61), a correlation that became r = 0.81 when the BTBR strain was eliminated because it was extremely impaired on the ARR. Both SPLAY and ARR DECR were associated with low BASAL ACT scores. SPLAY was strongly associated with low EtOH activation (r = -0. 84, see Fig. 4a), and with low intoxication in the screen test (r = -0.7 1). The strains with the highest splaying scores were 129 and A, and with the lowest, FVB and C3H.

Wobbling scores tended to be associated with some of the same and some different variables (see Fig. 2).

WOBBLE, HT, and GRIP were related. Strains scoring high in WOBBLE tended to show low GRIP intoxication scores (r = -0.49) and upon removal of the A strain, which scored very low on both variables, this correlation was quite large (r = -0.78). Wobbling strains also showed low HT responses (r = -0.77), and HT and GRIP were highly related when the A strain was deleted (r = 0. 86). WOBBLE, HT, SPLAY, and ET ACT INCR also were related. WOBBLE and AFT to LORR were also related. The A strain showed the highest AFT score of the eight strains, but nearly the lowest WOBBLE score, partly because mice that are splaying cannot wobble at the same time. When it was left in the correlation, the value was r = 0.25, but we believe that the more plausible relationship between these variables is represented by the other seven strains (r = 0.78). The highest scoring strains for WOBBLE were C3H and FVB, and the lowest scoring were BTBR and BALB.

Table 3
Strain mean correlations among variables, based on eight strains

	SPLAY	WOBBLE	HT	LORR DUR	LORR NEW BEC	AFT	SCREEN	GT	ARR DECR	ARR INCR	FSRR-0	FSRR-30	BB	GRIP	DT-15.8-0	DT-9.6-30	BASAL ACT	ET ACT INCR
WOBBLE	−0.62	1.00																
HT	0.46	**−0.77**	1.00															
LORR DUR	0.21	−0.35	0.24	1.00														
LORR NEW BEC	0.16	0.21 (0.54)[a]	0.19 (**0.76**)[b]	−0.60 (**−0.81**)[c]	1.00													
AFT	−0.09	0.25 (**0.78**)[a]	0.10	−0.62 (**−0.82**)[a]	***0.84***	1.00												
SCREEN	**−0.71**	**0.73**	−0.69 (**−0.81**)[a]	−0.15	0.10	0.19	1.00											
GT	0.52	−0.42	**0.74**	−0.07	0.66 (***0.93***)[b]	0.64	−0.44	1.00										
ARR DECR	0.61 (**0.81**)[d]	−0.50 (**−0.76**)[c]	0.18	0.33	−0.01 (0.64)[c]	−0.36	−0.11	0.10	1.00									
ARR INCR	−0.02	0.30	−0.09	−0.18	0.25	0.58 (**0.78**)[e]	−0.05	0.32	−0.67 (**−0.83**)[f]	1.00								
FSRR-0	0.32	−0.03	0.48	−0.001	0.68 (***0.91***)[b]	0.70 (**0.85**)[b]	−0.16	***0.86***	−0.15	0.61	1.00							
FSRR-30	0.37	−0.21	0.05	0.17	0.22 (**0.82**)[d]	−0.04	0.15	0.24	**0.81**	−0.56	−0.01	1.00						
BB	0.56 (**0.81**)[f]	−0.45	0.70 (**0.80**)[c]	0.12	0.44	0.08	−0.47	0.67	0.49	−0.37	0.40	0.58	1.00					
GRIP	0.33	−0.49 (**−0.78**)[a]	0.34 (**0.84**)[a]	0.06	−0.42	−0.55 (−0.66)[d]	**−0.79**[g]	−0.13	0.03	−0.30	−0.40	−0.23	0.24	1.00				
DT-15.8-0	−0.13	0.03	−0.23	−0.07	−0.24	−0.48	0.11	−0.43	0.38	**−0.81**	**−0.71**	0.51	0.26	0.36	1.00			
DT-9.6-30	−0.10	−0.21	0.22	0.44	0.02	−0.10	0.41	0.09	0.56 (**0.79**)[f]	−0.55	0.04	0.59	0.28 (**0.79**)[h]	−0.47	0.16	1.00		
BASAL ACT	**−0.80**	0.36	−0.43	0.02	−0.62 (**−0.76**)[d]	−0.25	0.31 (0.71)[c]	−0.67	−0.68 (***−0.97***)[d]	0.15	−0.51	−0.62 (**−0.77**)[d]	**−0.76**	0.08	0.04	−0.24	1.00	
ET ACT INCR	***−0.84***	0.63	**−0.76**	−0.03	−0.48	−0.17	0.69 (**0.84**)[c]	**−0.76**	−0.48 (***−0.87***)[d]	0.07	−0.54	−0.28	**−0.78**	−0.27	0.20	−0.06	***0.86***	1.00
BEC 1.0–15	0.12	−0.03	−0.13	0.64	−0.31	−0.13	0.14	0.08	0.19	0.15	0.14	0.44	0.01	−0.32	−0.03	0.26	0.00	0.21
BEC 1.0–30	0.22	−0.11	−0.06	***0.85***	**−0.74**	−0.51	−0.20	−0.18	−0.29	0.66	0.10	−0.41	−0.35	0.04	−0.59	−0.19	0.37	0.25
BEC 1.5–35	0.28	−0.14	0.25	**0.82**	−0.22	−0.45	−0.07	0.03	0.35	−0.13	0.24	0.10	0.24	−0.08	−0.23	0.43	−0.25	−0.26
BEC 2.0–35	0.07	0.20	−0.26	0.63	−0.40	−0.35	0.13	−0.28	−0.05	0.35	0.12	−0.29	−0.42	−0.23	−0.49	−0.01	0.16	0.19
BEC 3.0–5	0.66	−0.09	0.28	0.16	0.31	0.07	−0.49	0.53	0.20	0.14	0.47	0.35	0.69	0.22	0.04	−0.25	−0.61	−0.56
BETA	−0.40	0.63	−0.47	0.46	−0.15	−0.05	0.64	−0.25	−0.18	0.23	0.14	0.06	−0.28	−0.61	−0.13	0.26	0.27	0.54

Correlations significant at $p \leq 0.05$ are shown in bold type, and those at $p \leq 0.01$ in bold italics. Correlations significant with seven strains are shown in parentheses beneath. A few seven strain correlations not meeting statistical significance, but passing the scatterplot test, are shown.

[a] Without A.
[b] Without C3H.
[c] Without BALB.
[d] Without BTBR.
[e] Without D2.
[f] Without FVB.
[g] Only one of six doses on SCREEN showed a significant correlation with GRIP. Although this dose of SCREEN was chosen as representative for correlations with other variables, the correlation with GRIP shown here is not representative of the apparent lack of relationship between these two variables (see Ref. [15]).
[h] Without 129S1.

The relationship between EtOH-stimulated activity (ET ACT INCR) and the disruption of rotarod performance (ARR DECR) offers a good example of how we sometimes chose to disregard an outlier strain in interpreting a correlation (see Fig. 4b). The correlation was r = -0.48 with all eight strains, but was r = -0.88 when the outlier, BTBR, was eliminated.

A third cluster of variables involving the rotarod tasks is shown in Fig. 5. We have previously discussed how strain sensitivity to EtOH on the same apparatus, the rotarod, differs as a function of whether the accelerating or fixed speed variant of the task is used, whether testing occurs immediately after EtOH injection or 30 min later, and as a function of EtOH dose [14]. Sensitivity to the performance-enhancing effects of 1.0 g/kg EtOH (ARR INCR) was negatively correlated with the performance decrement after 2.0 g/kg (ARR DECR), both tested 30 min after injection. This negative genetic correlation of r= K0.67 was r = K 0. 83 when the FVB strain was eliminated because it showed no impairment at all after 2 g/kg. Fixed speed rotarod performance immediately after administration of EtOH was not significantly related to either FSRR 30 min later (r=0.01) or to ARR DECR (r= K0.15). However, if FSRR performance was assessed 30 min after injection, it correlated well with

ARR DECR (r=0.81). FSRR-30 was also highly correlated with BEC at onset of LORR (LORR NEW BEC: r= 0.82), when the BTBR strain was eliminated because of its very low LORR onset BEC and because none of them could perform the FSRR task. Sensitivity on the smallest dowel tested 30 min after 1.5 g/kg (DT-9.6-30) was significantly correlated with ARR DECR (r=0.79), again after elimination of FVB. ARR INCR was significantly negatively correlated with sensitivity to 1.5 g/kg EtOH on a larger dowel (DT-15.8-0, r=K0.81) and tended to be negatively related to DT-9.6-30 (r= K0.55)—the two DT measures were selected for this analysis explicitly because they were uncorrelated [15].

Table 4
Strain means

Trait	[129]	[A]	[BALB]	[BTBR]	[C3H]	[B6]	[D2]	[FVB]
SPLAY	4.29	3.25	2.62	3.13	2.50	3.00	3.08	1.13
WOBBLE	0.23	0.17	0.14	0.09	1.21	0.53	0.87	1.13
HT	2.93	3.20	2.27	1.68	0.54	2.17	0.81	1.56
LORR DUR	90.07	79.63	70.87	101.36	49.92	92.06	95.85	89.14
LORR NEW BEC[a]	2.58	2.29	2.92	3.00	2.32	2.69	2.87	2.78
AFT	0.40	1.5	0.52	−0.05	1.11	0.39	0.62	0.58
SCREEN[a]	171.50	130.73	158.08	118.00	98.56	126.00	121.44	98.00
GT	0.19	0.29	0.07	0.04	0.07	0.08	0.08	0.05
ARR DECR	22.82	12.48	−11.03	48.02	9.60	14.30	−1.70	−15.40
ARR INCR	11.15	22.43	17.94	−3.68	14.37	14.67	31.57	13.20
FSRR-0[a]	99.22	54.25	148.15	165.86	129.30	107.86	104.36	125.21
FSRR-30[b]	66.70	66.70	0.00	100.00	60.00	16.70	33.30	33.30
BB	4.83	3.31	0.70	2.21	1.44	1.37	0.07	1.60
GRIP	68.42	36.55	69.69	51.74	42.63	48.67	42.82	45.74
DT-15.8-0[a]	115.00	202.86	148.00	77.33	111.63	195.38	196.38	118.50
DT-9.6-30[a]	178.13	59.86	228.50	15.67	171.88	96.00	198.25	91.75
BASAL ACT	45.60	68.80	265.50	142.90	126.30	141.00	203.80	283.80
ET ACT INCR	57.50	143.50	372.25	370.00	386.13	238.88	466.00	571.88
BEC 1.0–15	0.94	0.98	0.84	1.03	0.84	0.83	1.10	0.97
BEC 1.0–30	0.64	0.61	0.64	–	0.53	0.67	0.77	–
BEC 1.5–35	1.19	1.09	0.96	1.16	0.96	1.28	1.16	1.14
BEC 2.0–35	1.48	1.39	1.37	1.52	1.34	1.86	1.94	1.55
BEC 3.0–5	4.66	4.19	3.93	4.02	4.11	3.98	4.27	4.05
Widmark's β-2.0	0.54	0.57	0.42	0.60	0.57	0.61	0.76	0.76

[a] The values shown for these variables were multiplied by (−1) before calculating the correlations shown in Table 3. For LORR NEW BEC, animals with lower blood ethanol concentrations are more sensitive. For the other four variables, values shown are latencies to fall, and longer latencies indicate less sensitivity to EtOH.
[b] FSRR-30 is expressed as the proportion of animals failing the 3 min test.

Table 5
Strain rank sensitivity

Trait	129	A	BALB	BTBR	C3H	B6	D2	FVB
SPLAY	1	2	6	3	7	5	4	8
WOBBLE	5	6	7	8	1	4	3	2
HT	2	1	3	5	8	4	7	6
LORR DUR	4	6	7	1	8	3	2	5
LORR NEW BEC	3	1	7	8	2	4	6	5
AFT	6	1	5	8	2	7	3	4
SCREEN	8	6	7	3	2	5	4	1
GT	2	1	5	8	6	4	3	7
ARR DECR	2	4	7	1	5	3	6	8
ARR INCR	7	2	3	8	5	4	1	6
FSRR-0	2	1	7	8	6	4	3	5
FSRR-30	2.5	2.5	8	1	4	7	5.5	5.5
BB	1	2	7	3	5	6	8	4
GRIP	2	8	1	3	7	4	6	5
DT-NEW-0	3	8	5	1	2	6	7	4
DT-NEW-30	6	2	8	1	5	4	7	3
BASAL ACT	8	7	2	4	6	5	3	1
ET ACT INCR	8	7	4	5	3	6	2	1
Mean rank±SD	4.03±2.52	3.75±2.70	5.50±2.12	4.39±2.91	4.67±2.22	4.72±1.23	4.47±2.10	4.47±2.21

1, most sensitive; 8, least sensitive.

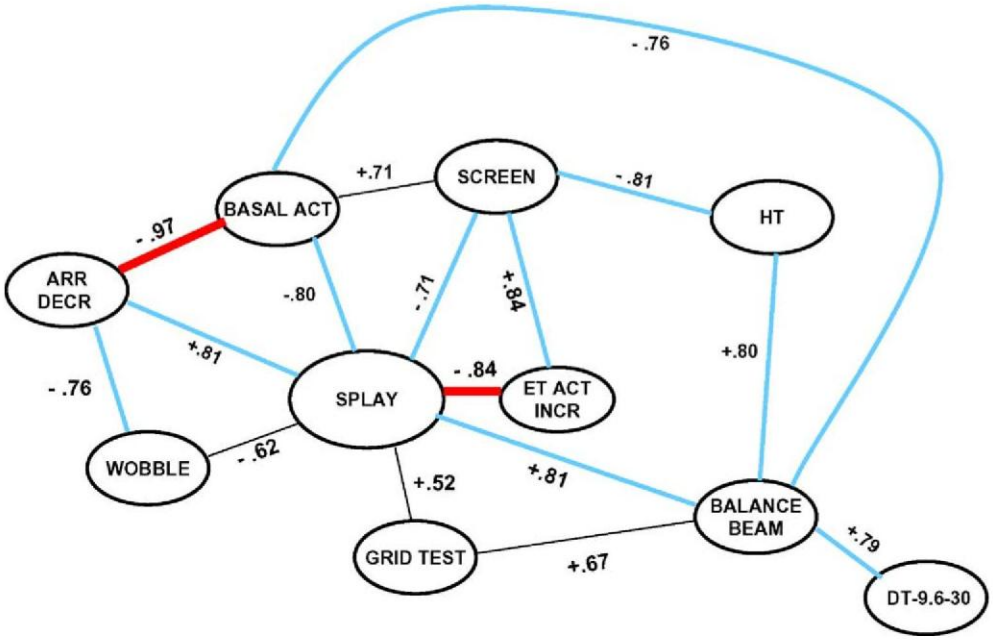

Fig. 1. Schematic representation of selected genetic correlations among strain mean EtOH sensitivity scores. Variables associated positively or negatively with splaying (SPLAY) are shown connected by lines, along which the genetic correlation is shown (see Table 3). SPLAY is represented with a larger oval simply because this variable was selected as a central reference point for the figure. Heavy red lines, $p \leq 0.01$; medium blue lines, $p \leq 0.05$; light black lines, $0.05 \leq p \leq 0.15$. Variable names and test conditions are as described in Section 2. This visual display enables the reader to see, for example, that SPLAY is negatively correlated with both SCREEN and ET ACT INCR, and that the latter variables are themselves positively correlated.

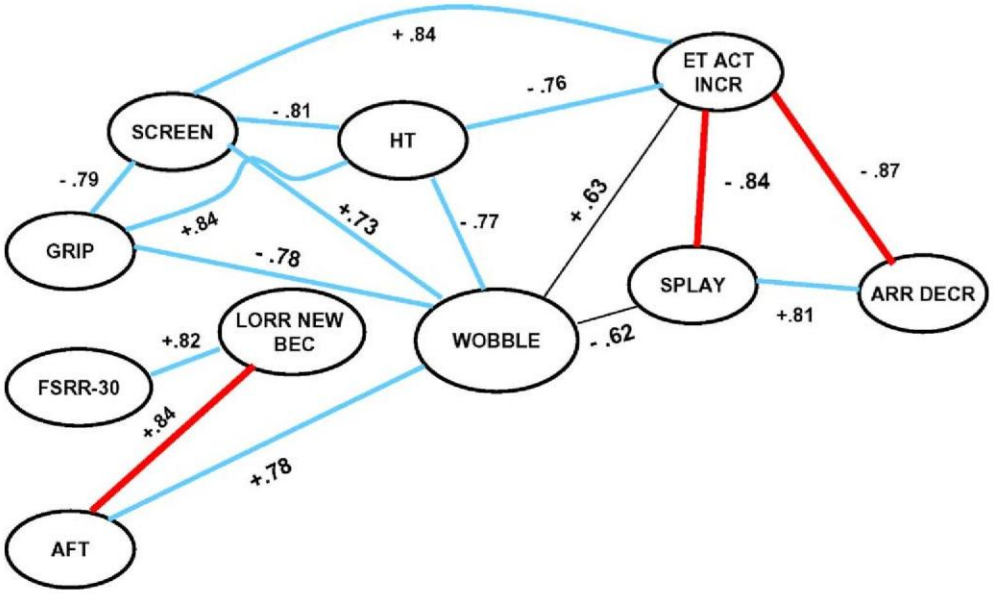

Fig. 2. Schematic representation of selected genetic correlations among strain mean EtOH sensitivity scores. Variables associated positively or negatively with WOBBLE are shown.

4. Discussion

What is to be made of this pattern of results? Most tasks tended to show modest correlations with at least several other tasks, with the possible exception of the classic version of the LORR task (LORR DUR). However, only a handful of correlations were very high, suggesting that the tasks are not redundant in their capacity for reflecting genetic influences on behavior. Here, an exception would be the ARR DECR and FSRR-30, which showed extremely similar patterns of strain sensitivity to EtOH [14]. The pattern of widespread, modest correlation suggests that the behavioral domain is complex and multiple tasks are required to map its genetic features. As was already suggested by the analyses of relationships among smaller groups of tasks reported in the individual papers, few simplifying generalizations appeared from this analysis. Had all mice been tested on all tasks, we could have used some form of statistical clustering analysis to search for patterns of similarity. However, our interest was in the average strain response in each task, leaving us with a maximum of

eight 'subjects' and fewer than 20 variables, rendering such techniques as principal component analysis invalid. Because these studies were conducted over a several year period, and because tasks were refined along the way, we cannot realistically claim to have stated any truly a priori hypotheses. Nonetheless, some conjectures that seemed reasonable from a common sense perspective could be ventured, and we consider several in turn.

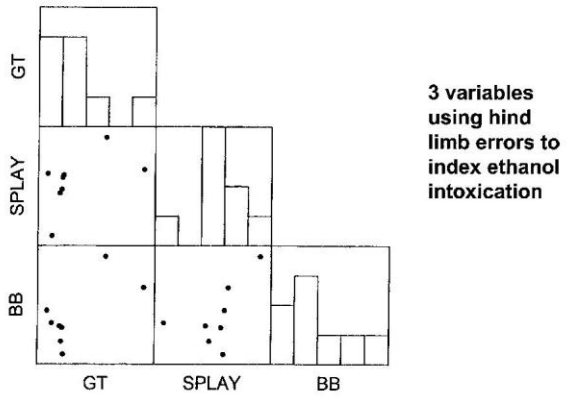

Fig. 3. Correlations among three variables using hind limb errors as the index of EtOH intoxication are shown. The 'splom plot' from Systat 10 output is a quick way to assess the patterns of correlations among a limited number of variables. Each dot represents an inbred strain's mean value along the (unlabeled) axes, scaled to capture the range of strain means. In each case, the axes run from lesser (left, bottom) to greater (right, top) EtOH sensitivity. The three panels with bars represent the frequency distribution of strains within the range captured by the width of the bar along the abscissa. For example, for SPLAY, there were 1, 0, 4, 2, and 1 strain in each of the five bins from least to most sensitive. GT, grid test; SPLAY, splayed gait; and BB, balance beam.

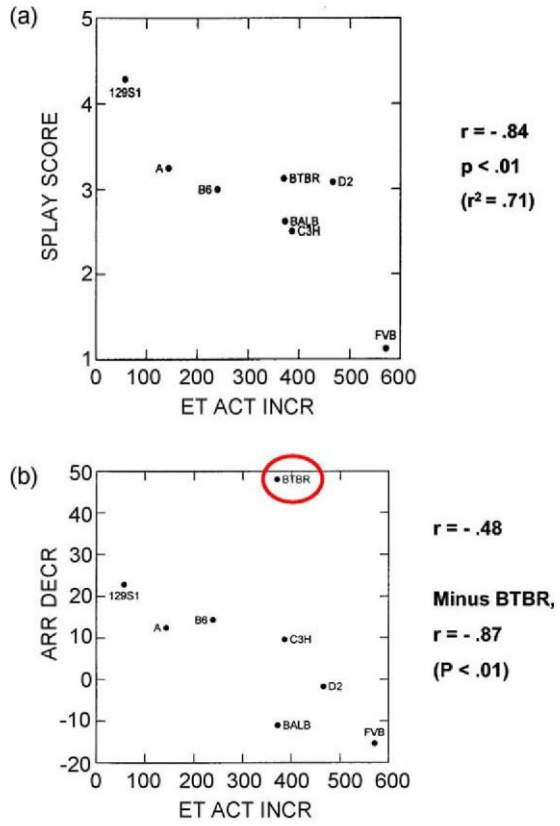

Fig. 4. Panel (a) SPLAY scores are plotted vs. EtOH-stimulated activity (ET ACT INCR). Each data point represents the mean value for an inbred strain on each trait. Panel (b) ARR DECR scores are plotted vs. EtOH-stimulated activity (ET ACT INCR). Each data point represents the mean value for an inbred strain on each trait.

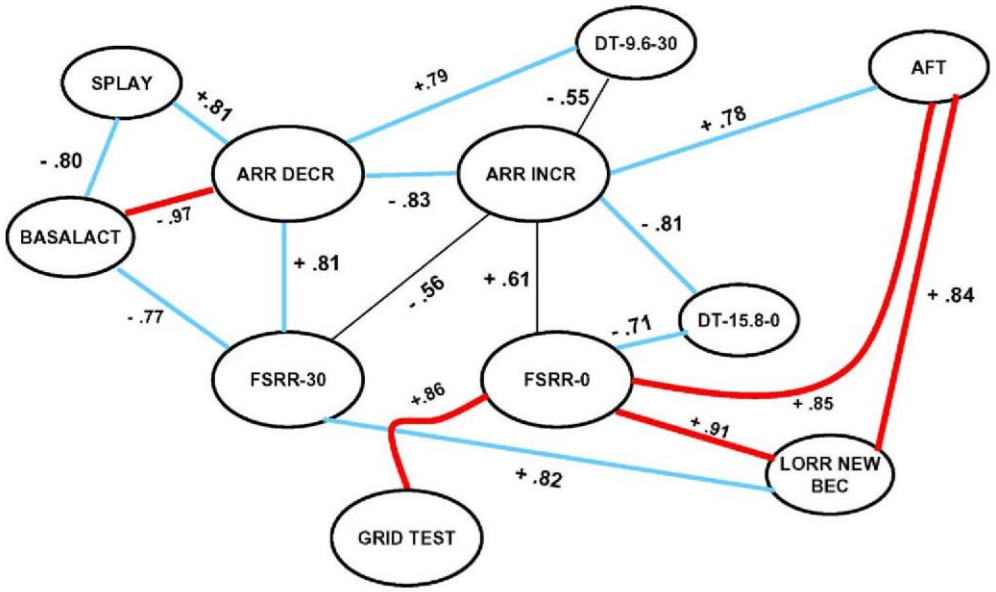

Fig. 5. Schematic representation of selected genetic correlations among strain mean EtOH sensitivity scores. The targeted variables were accelerating (ARR DECR and ARR INCR) and fixed speed (FSRR-0 and FSRR-30) rotarod (see Section 2).

4. 1. Individual strain contributions

Table 5 gives the rank order of strain sensitivities on each task. One possibility was that some strain or strains could be generally sensitive or resistant to EtOH across many tasks. BALB came the closest to being such a strain, as it was ranked seventh or eighth most sensitive on half the variables (9/18).

On the other end of the scale, 129 and A each scored first or second most sensitive in about half of the variables. BTBR was interesting in that it scored either most or least sensitive on 11 of the variables, split evenly between extreme sensitivity (5/11) and insensitivity (6/11). A contrasting picture was presented by the B6 strain, which was between third and sixth most sensitive on every variable save two (it was second least sensitive on AFT to LORR and on the FSRR-30). It was also never an outlier for the correlational analyses. Reliable, middle-of-the-road performance in many behavioral domains is consistent with the popularity of this strain for behavioral studies. However, B6 mice have an unusual pedigree compared to that of other common inbred strains, and it would not be wise to assume that they were average performers (e.g. that they were a suitable background strain for transgenic studies) in the absence of comparative strain data.

As reported earlier, for certain tasks, a strain or strains seemed to be unable to provide interpretable data under most test conditions. For example, 129 mice were extremely sensitive in the GT, and their data were only useful under the lowest dose conditions tested [16]. The 129, A, and C3H strains all produced uninterpretable data for many BB conditions [16]. However, our systematic analysis of the correlations with each strain in turn deleted (see Section 2) supported the general impression that no strain was sufficiently odd that its elimination from the entire data set would have improved the overall interpretability of the correlation matrix. Rather, certain strains were deviant for certain tasks, or for particular inter-task correlations. In other circumstances, some known physiological attribute of a strain could suggest that it was reasonable not to consider its data. For example, if we had been studying spatial learning and memory-related tasks here, we would have considered deleting strains known to possess the rd allele for retinal degeneration, reasoning that blind mice would be unable to parse visual spatial cues. Here, we did not attempt to collect data from wild-derived strains because our previous experience suggested they would be non-compliant [28].

In summary, no strain appeared to be generally sensitive or insensitive to EtOH-induced incoordination (see Table 5).

4.2. Task characteristics

It was very tempting to attempt to classify the tasks employed according to what we presumed to be the neural systems that may underlie them. This is made difficult by the fact that we have no real idea what the behavioral demands facing the mouse are for most tasks. For example, an intuitively appealing outcome would have been that the strain sensitivity to EtOH in the screen test and GRIP test were highly correlated, as both tasks seem to the experimenter to require first and foremost muscle strength. In fact, the strain means were not meaningfully correlated [15]. Although the correlation reported in Table 3 is statistically significant, it is negative, and we do not consider it representative. Correlations of GRIP with responses on the screen test at higher and lower doses were not strong and sometimes were positive [15]. There-fore, we indicated in Table 3 that we did not consider it meaningful. The response to the 2.25 g/kg EtOH dose on the screen test was selected for this analysis because it was representative of performance on this test, near the middle of the dose–effect curve for all strains, and its choice did not affect interpretation of the correlations between the screen test and other variables. Plausibly, the BB and DTs both require the ability to maintain balance, and BB was correlated r=0.79 with DT-9.6-30 if the 129 strain was eliminated due to its extremely high number of BB missteps, but BB was not significantly correlated with DT-15.8-0. Perhaps only the narrower of the dowels invokes balance mechanisms.

On the other hand, three tasks employed the same physiological response (hind limb foot slips) as an indication of EtOH intoxication (BB, GT, and SPLAY). These tasks showed a reasonably consistent pattern of positive correlation (see Figs. 1 and 3). Most tasks required locomotion, while others did not, but there was no apparent pattern of general correlation among those that did (or those that did not). However, the strain correlations for different measures of locomotion, whether within a task or across tasks, were very high (data not shown).

A useful outcome would have been to find that two tasks were so strongly correlated that they might be considered to be redundant insofar as identifying specific genetic influences. Given that only eight genotypes were examined, any such conclusion would be limited to these genotypes, but could be tested by examining additional inbred strains. FSRR-30 and ARR DECR represent one such case (r=0.81). As noted previously, the similarity of genetic contributions to EtOH sensitivity in these two versions of the rotarod task persists across a battery of 20 inbred strains but vanishes when FSRR performance is examined immediately after EtOH injection [14]. However, we also have data in separate groups of mice from these eight strains 30 min after several other doses of EtOH for the ARR. Furthermore, these mice were also tested at acceleration rates other than 20 rpm/min. Sensitivity scores to several other doses and at other rates also correlate well with the FSRR data, so we are confident that these two tasks indeed yield similar outcomes. The DT immediately after injection and the increased performance after low-dose EtOH on the accelerating rotarod were also rather similar genetically, albeit negatively correlated (r= —0.81). SPLAY and ET ACT INCR were substantially negatively correlated (r= —0.84), suggesting that activated strains tended not to show the SPLAY response to higher EtOH doses; this is probably partly because mice that are sufficiently impaired to splay cannot ambulate. Also, GT and FSRR-0 were highly correlated (r=0.86). The other high genetic correlations we found were seen only when a strain was deleted from the relationship. Although we believe these relationships to be substantial, having found one outlier out of a sample of eight strains suggests to us that the tasks should not be substituted cavalierly. More strains (data points) would be needed to assure that the relation-ships were fairly generalizable.

In summary, correlations among tasks did not emerge along anthropomorphically appealing parameters (such as balance, muscle strength, or locomotor demand). Although some individual correlations were very strong, the general impression was that inter-task correlations were idiosyncratic.

4.3. Role of EtOH dose and timing

Another possible way strains could sort differently on different tasks would be according to dose–effect curves with different slopes. For example, in the screen test, multiple doses were tested and strains appeared to show one of two general patterns, either relatively steep or relatively shallow dose–effect curves. FVB showed particularly steep dose sensitivity in this task [15] and for SPLAY [18]. Perhaps some strains might be sensitive to lower-dose EtOH effects, while others were more sensitive to higher-dose effects. And, high-dose and low-dose tasks might tend to be negatively correlated. Little evidence for this can be found in Tables 3–5. Three tasks can be considered to reflect relatively high-dose (i.e. ≥ 3 g/kg EtOH) sensitivity—LORR, HT, and the ambulatory ataxia reflected in SPLAY and WOBBLE. Low-dose tasks (about 1–1.5 g/kg) include ET ACT INCR, GT, ARR INCR, FSRR-0, BB, DT-15.8-0, and DT-9.6-30. While there are correlations that fit the proposed pattern based on dose, there are many more that do not.

Some tasks were performed immediately after alcohol was injected (FSRR-0, LORR NEW BEC), others shortly after administration (BB, GT, ET ACT INCR, SPLAY, WOBBLE), while the rest were 30 min after injection (see Table 1). These time points represent, respectively, a period of rapid absorption of EtOH into brain, reaching peak levels by 5 min [25,42]; a period of redistribution among body compartments, during which time blood and brain EtOH levels equilibrate (5–30 min); and a period of relatively stable blood and brain concentrations that decline according to pseudolinear kinetics at the rate of about 60–80 mg EtOH/ml blood/h depending on dose and genotype [15,26]. It would be plausible, for example, to find that the immediate post-EtOH responses were somehow similar to one another and were, therefore, correlated. However, the best test of this hypothesis is to compare loss of function on the dowel and the FSRR immediately after EtOH at T0. Yet, these two responses were negatively correlated (r = — 0.7 1, Fig. 5). Extending the concept to include those tests performed between 5 and 10 min after injection offers some support for the idea. SPLAY, GT, and BB were all positively intercorrelated, and SPLAY was negatively correlated with WOBBLE and ET ACT INCR, which themselves were positively correlated (see Fig. 2). The possible reason for the negative relationship between SPLAY and WOBBLE or ET ACT INCR was mentioned earlier. If activity is conceived as an even lower dose response along the same dose continuum, these relation-ships might reflect a sorting of strains according to a shift in dose–effect curves. ET ACT INCR would not be expected to be correlated with GT scores because they were explicitly corrected for activity.

In summary, there is limited overall support for the idea that responses assessed within a few minutes after an ip EtOH injection share some similar genetic influences. However, there is less support for the idea that these 'early' responses are negatively genetically related to 'late' responses to EtOH, or even that the later responses tended themselves to be positively correlated.

4.4. Role of acute functional tolerance

One contribution to the difference between early and late responses to EtOH is likely the fact that AFT develops during a single exposure to the drug. This was first noted by Mellanby [29] studying ambulatory ataxia in dogs, and has been studied more recently using mouse models for LORR [6,30]. We recently reported the degree of AFT by assessing BEC at loss and regaining of righting reflex using the new method briefly described above [21,25]. Previously published studies had attempted to use the classic LORR method to assess AFT by comparing blood EtOH levels at loss and regaining righting reflex, and had sometimes reported AFT in different mouse genotypes. However, these studies were flawed by the delay required to assess initial onset to LORR (and, therefore, the BEC at which this occurred) in a mouse when the paradigm requires waiting 30 s [31,32]. Thus, these studies systematically under-estimated the sensitivity of mice to lose righting reflex, falsely concluding that some strains did not develop significant AFT. Deitrich worked out one method for avoiding this problem, and we adapted the LORR method to obtain an assessment of LORR very shortly after injection, making it possible to see large and reliable strain differences in AFT [21,24].

Although the data reported here are for AFT to the LORR response, it is conceivable that AFT occurs to many different responses to EtOH. If this is true, then any measure taken 30 min after injection could represent a combination of the 'true' initial sensitivity of the subject diminished by any AFT that had developed. For LORR, we found that AFT was detectable 5 min after beginning EtOH exposure and was likely complete within 20 min [21]. Studies that have used comparisons of serial recoveries of the ability to balance in the DT have inferred the development of significant AFT, and have in fact selectively bred mice to score high or low on this trait [30,33]. In the current analysis, we found a few meaningful relationships between AFT and other variables (see Figs. 2 and 5). GRIP at 30 min was negatively correlated with AFT (r= — 0.66 minus the BTBR strain), while WOBBLE at 10 min was positively associated (r=0.78 without the A strain). ARR INCR at 30 min was also positively associated, such that strains showing greater EtOH enhancement of ARR performance were those with greater AFT in LORR (r=0.78 minus the D2 strain). Conceivably, those strains showing high sensitivity to EtOH-induced WOBBLE had already developed a substantial amount of AFT, while those that had not developed substantial AFT tended to score highly on SPLAY instead. Similar reasoning could be used to suggest that high-AFT strains might show a shift from intoxication indexed by ARR DECR toward the lower-dose enhancement of performance seen as ARR INCR. And, strains with less affected GRIP at 30 min after EtOH might have been displaying great AFT on this measure as well as to LORR. These conjectures (that AFT contributes to the WOBBLE vs. SPLAY distribution of strains, to ARR performance, and to GRIP) could possibly be tested using a paradigm employed by Ponomarev [21]. He administered a relatively low-dose of EtOH (i.e. a dose insufficient in itself to affect the LORR end point studied) and showed that administration of a second dose a few minutes later caused animals to lose righting reflex at a higher BEC than animals pretreated with saline. By administering divided doses of EtOH and studying the other end points, an assessment of AFT for many of the traits reported here could be made.

In another instance, FSRR performance at T0, but not 30 min later, was positively associated with AFT (r = 0.70), an association that was striking after deletion of the C3H strain (r=0.85). Because the correlations of essentially the same variable with AFT were different at two different post-EtOH times, AFT could have played a role at the later time point. However, this would imply that if measured directly, AFT to FSRR would not be expected to be highly correlated with AFT to LORR. AFT was also associated with the LORR variables from which it was calculated, but these correlations are computationally forced and are not discussed.

In summary, the potential role of acute tolerance should not be discounted in any measure of EtOH sensitivity taken even a few minutes after ip injection. To the extent that a group (or genotype) is prone to develop tolerance rapidly, it will show what appears to be attenuated sensitivity to EtOH.

4.5. Role of EtOH pharmacokinetics

An obvious way in which strain differences could occur, if administration of fixed EtOH doses on a g/kg body weight basis led to different brain EtOH concentrations at the time of testing. Data reported in previous papers show that strains do differ significantly at or near the time of testing in brain and/or BEC after fixed doses of EtOH [15,20]. Furthermore, their EtOH elimination rates are significantly different [15,26]. The correlational analyses (See Table 3) show that the differences in the dose of EtOH that reaches the brain in these strains cannot predict behavioral response, because very few correlations between BEC and any behavioral endpoint were of substantial magnitude. Thus, EtOH pharmacokinetics does not appear to contribute substantially to EtOH sensitivity for any of these behavioral assays. In many prior inbred strain analyses of genetically influenced responses to EtOH, pharmacokinetics has never appeared to play a major role in either EtOH sensitivity or tolerance [20,34].

4.6. Comparison with historical results

Before the current studies were undertaken, we had previously published EtOH sensitivity data from multiple inbred strains, and have often used similar tasks (e.g. HT, LORR) as well. How do the current results compare with earlier attempts to examine patterns of genetic correlation? This question is difficult to answer for three reasons. First, in the older studies, different substrains of mice were often used. Although we often studied 15–20 strains, we routinely obtained some strains through a collaborative NIH—Veterans Administration agreement, so our earliest studies examined C57BL/6N, DBA/2N, C3H/HeN, A/HeN, and BALB/cAnN instead of the Jax substrains studied here. While the N substrains of C57, C3H, and DBA were likely very genetically similar to the J substrains we used in our studies during the 1990s and here, A/HeN and BALB/cAnN had long diverged from the A/J and BALB/cByJ substrains used here. The importance of substrain is idiosyncratic, sometimes small but sometimes extreme. For example, we found that 129 substrains 129P3 and 129S1 (the substrain used here) differed markedly in their sensitivity to EtOH-induced SPLAY across several doses [18]. Still, BALB substrains of mice are more similar genetically to each other than to C57 substrains, so it is useful to consider the older data even though the genetic subline differences might mitigate against finding a high degree of similarity. A second caveat is that in the older work, we typically used a single dose of EtOH and often a single variant of each behavioral assay studied. Now that we have taken the trouble to explore some of the tasks used earlier in more detail, it has become clear that small differences in apparatus and procedure can often reveal different patterns of genetic influence. In some systems, the individual experimenter collecting the data has been shown to affect patterns of mouse genetic differences substantially [35,36]. Finally, even when strain and many procedural variants are well controlled, the pattern of strain differences can vary across multiple testing laboratories due to unknown, laboratory-specific environmental influences [37].

Comparison of the current data with the older data sets revealed that sometimes the individual tasks did correlate well with variants of the same task. For example, EtOH HT values from 1983 (change from baseline temperature 30 min after 3 g/kg EtOH), 1994 (BEC required to lower body temperature by 2 ºC), and here (change from baseline temperature 60 min after 3 g/kg EtOH) were well correlated ($0.82 \leq r \leq 0.97$, $n = 6–8$ strains [19,20] (Crabbe et al., in preparation). Activity was also reported at all three periods. Manually scored activity (line crossings) in an open plastic box for 2 min starting 10 min after a saline injection was highly correlated (r=0.73, six strains) with activity (distance traveled) automatically recorded by infrared beam interruptions during a 15 min period starting immediately after a saline injection [19,20]. While the 1994 measure was reasonably correlated with the BASAL ACT data reported here (r = 0.47), the older data were essentially uncorrelated with the current data (r = -0. 11).

In 1983, we reported that sensitivity immediately after 3 g/kg EtOH ip (10%, v/v) on a 10 mm dowel was positively correlated (r=0.79) with increased activity in an open arena under dim light studied between 10 and 12 min after ip injection of 1 g/kg EtOH, (10%, v/v) [19]. DT sensitivity 30 min after 3 g/kg EtOH (20%, v/v) also tended to be negatively correlated with a crude, home cage measure of ambulatory ataxia that probably resembles the current SPLAY measure [18,19]. The current analysis saw no such relationship between DT and ET ACT INCR, but none of the strains from the 1983 analysis was exactly the same as those reported here. The DT—ataxia correlation seen earlier in 16 inbred strains was also not detected here. A negative relationship

between LORR duration and HT sensitivity across 20 strains in 1983 was not found here, and the positive relationship between ambulatory ataxia and HT from 1983 was accompanied by significant negative relationship between HT and WOBBLE in the current data.

A more systematic analysis was undertaken with 15 inbred strains in the early 1990s [20]. Multiple EtOH doses were employed to study activity increase and HT. A variant of the FSRR test immediately after injection with 2 g/kg EtOH was used, and the up-and-down method was used to establish the ED_{50} for each strain to lose the righting reflex using the classic criteria. This study also failed to find a negative relationship between HT and classic LORR sensitivity. There were few points of direct comparison between the 1994 study and the current analysis, but the general lack of relationships among FSRR-0, LORR DUR, HT, and ET ACT INCR was seen in both analyses. The exception was our current finding of a significant negative correlation between ET ACT INCR and HT. The prior analysis estimated from regression the effective dose of EtOH to induce HT, while the current studies used the magnitude of the change after a fixed dose.

The take home message from the comparisons with historical data is that giving a response a name that implies that it represents a behavioral domain is foolhardy because it represents only a single set of conditions. Without knowledge of the limits of the generalization to other conditions, it is premature to name a test as if it were a representation of a presumed, theoretical domain. While it is certainly true that motor activity had something to do with the ‘activity’ responses as we measured them in 1983, 1994, and recently, and that genetic influences were apparent in each case, it is not so clear that these phenotypes really represented the output of the same set of neurobiological systems.

In summary, it appears that the patterns of correlations among similar variables across historical studies reflect the particular task variants, laboratories, mice, and conditions studied to a substantial degree. A realistic comparative test across studies would require using the same mouse strains (and substrains) at least.

4.7. Sex and body weight differences

The data reported here included both male and female mice, generally of approximately equal numbers per strain. Males are heavier than females, strains differ markedly in body weight, and both sex and body weight can affect performance in some tasks [38]. In the original papers, we discussed sex and body weight differences where they occurred. In a few cases, sex and genotype interacted, and whether or not the sex differences could be accounted for by body weight differences also depended on the particular task. However, we generally did not have sufficient numbers of males and females of each strain to explore the interactions of sex and other experimental factors with a great deal of statistical power. Therefore, in the interests of simplification, we have ignored the sex differences and body weights for the correlational analyses presented here.

4.8. A proposed intoxication test battery

One might be interested in constructing a battery of tests to index genetic sensitivity to intoxication, and the current data show that one would need to employ multiple tasks. The presumed advantage of such a test battery would be to capture a complex domain by reducing it to independent component assays that together span the domain. An alternative use, however, is that different tests may in fact capture etiologically dissociable aspects of the behavior [39]. In a few cases, two tasks appear to be so well correlated that they may be considered to be largely redundant, in that strains sensitive on one are also similarly sensitive to another. For example, the researcher interested in constructing a battery could choose either the FSRR or the ARR tested 30 min after 2–3 g/kg EtOH and detect the same pattern of genetic influence. This particular finding is of limited practical value, however, as the same apparatus can just as easily measure the one effect as the other. The negative genetic coupling between locomotor stimulation and SPLAY scores would allow elimination of one response from a battery. However, it may be just as easy to measure both at the same time. An additional hurdle to be faced would be the potential effect of multiple testing. The large savings that could be effected by testing mice serially for multiple responses might lead to different patterns of responding for tests later in the series [40]. Most of the data analyzed here, but not all, were collected in naïve mice.

Nonetheless, considering the patterns of genetic correlation, the reliability of the tasks where such estimates are available, and data from other doses and variants of the tasks, we venture the following as a simple combination of tests that would probe a wide range of the genetic landscape revealed by the correlational analysis. Where possible, we propose tests that do not require expensive, specialized apparatus. We have also selected tests that were not highly correlated genetically, as well as some that were. It will be necessary to perform this battery as proposed in multiple genotypes to determine its reliability, studies which we plan to undertake. Order effects can be ascertained by correlating the means generated with those already collected in individual tests. Given our previous experience it would be prudent to test this battery in multiple laboratories, as reliability within a laboratory does not necessarily predict replicability between laboratories [17,37].

We believe that to explore a range of intoxication responses across a range of EtOH doses and times after injection would ideally require three sets of tests. We propose that these be conducted serially, leaving 2–3 days between alcohol challenges to minimize carry-over effects (such as tolerance). However, as stated above, formal tests of order effects and carry-over will need to be conducted. Two alternatives are proposed. One assumes that the laboratory has available a rotarod, and the other depends entirely on equipment that can be inexpensively constructed locally as described in the individual papers. Our suggestions about the requirements for an adequate rotarod are discussed elsewhere [41]. The proposed battery might be a useful way to screen novel genotypes for their behavioral sensitivity to alcohol, and likely to other intoxicants, and may also be of value in screening mutants with deficits in gait, coordination or balance. Inference from the pattern of deficits seen in the proposed battery might then be useful in directing further analyses with these genotypes.

4.8.1. Day 1
Train animals for four trials on the 19 cm BB (If using a rotarod, follow this training by 10 trials on a 6.5 cm rotarod accelerating at 20 rpm/min).

4.8.2. Day 2
Weigh all animals (if using a rotarod, give each animal up to three trials to reach the criterion latency to fall (3 min) on the FSRR (6.5 rpm)). Inject animals with 1.2 g/kg EtOH (if using a rotarod, test them immediately with one trial at a fixed speed of 6.5 rpm and record latency to fall). Starting 10 min after injection, give each animal a single trial on the BB (if using a rotarod, test for enhanced performance at 30 min on the accelerating rotarod with three trials at 20 rpm/min).

4.8.3. Days 3, 4
Rest in home cage.

4.8.4. Day 5
Weigh animals and give each animal up to three trials on the 9.6 mm dowel to reach the criterion latency to fall of 2 min. Give animals 1.75 g/kg EtOH and test immediately for latency to fall from a 15.8 mm dowel with a maximum latency of 5 min. Starting 5 min after injection, place in any small, dimly illuminated activity monitor, or plastic bin with lines on the floor and record line crossing and rearing for 5 min. At 30 min after injection, test on the vertical screen and record latency to fall, with a maximum latency of 3 min. Immediately thereafter, test on a 9.6 mm dowel for up to 5 min (if using a rotarod, test on fixed speed rotarod at 6.5 rpm for up to 3 min, recording latency to fall but using pass/fail as the index of intoxication).

4.8.5. Days 6, 7
Rest. Divide the animals into two groups for testing on Day 8.

4.8.6. Day 8
Group 1: individually house mice for at least 30 min. Take a baseline rectal temperature, weigh, and inject with 3 g/kg EtOH. Place on a platform and score for WOBBLE and SPLAY during the period 10–11 min after injection [18]. At 30 min after injection, and again at 120 min, take another body temperature, returning mice to individual holding cages between assessments. Group 2: individually house mice for at least 30 min. Weigh and

inject with 3 g/kg EtOH. Place immediately in the LORR apparatus described elsewhere [25] and score until the animal loses righting reflex, at which point a retroorbital sinus blood sample is taken (about 2–3 min later). Immediately replace in the LORR apparatus. Score periodically in the LORR apparatus as described until the animal regains RR and record time (about 30–120 min later). Take a second retroorbital blood sample for AFT assessment (n.b., with a very experienced technician, retroorbital sinus sampling is not as disruptive of behavior assessed immediately thereafter as one might think).

Of course, it may be possible that fewer tests than proposed here would suffice, but additional data would be required to reach this conclusion. Because assessments of HT and LORR/AFT are incompatible, note that separate groups of mice are necessary to assess both phenotypes, so assignment of sufficient numbers of mice will be necessary to assess group differences (the advantage is that twice the number of mice strictly necessary will therefore have been used to test the several traits tested during Days 1–5).

5. Conclusions

We have used eight inbred strains in the majority of the work reported here. This is a bare minimum for correlational work, as a single genetic (strain) outlier can cloud interpretation of relationships between traits, and it is the minimum number of genotypes required for entry of strain difference data into the Mouse Phenome Project's relational database [22]. A much more reasonable goal for assessing relationships, and for testing other hypotheses regarding genotype × environment interactions, would be to use 15–20 strains. For example, to test whether the same pattern of strain sensitivity is seen in multiple laboratories, we plan to employ the test battery proposed using all 20 strains from the Mouse Phenome Project A and B list, excepting those we already know are too wild to cooperate in some of the tests [28]. As more strains are added, some will probably be found to be extremely sensitive or insensitive using a particular task, but the preliminary work with eight strains has narrowed the range of test parameters with the goal of strain inclusiveness, so this problem should be minimized. A list of our conclusions from this analysis follows:

1. Ataxia, and the manifestation of alcohol intoxication as motor incoordination, appear to reflect the activities of complex sets of functional systems. The patterns of genetic codetermination among different behavioral assays did not often reveal groups of several tasks that appeared to measure the same thing. Ataxia is a complex behavioral domain.

2. The modest correlations across tasks further our under-standing of EtOH intoxication by reminding us that it is not a monolithic state. Rather, it is better operationally defined by responses to the drug using multiple apparatus and procedures that call into play many different neural systems.

3. An individual's genotype clearly influences its sensitivity to EtOH, but an individual strain sensitive to one effect of EtOH is not necessarily likely to be sensitive to another. There were no generally sensitive or resistant strains.

4. It would have been convenient that we had found that tasks X and Y were essentially interchangeable and appeared to reflect disturbances of balance, while tasks W and Z seemed rather to reveal effects on muscle strength. However, anthropomorphizing did not help.

5. An exception to the notion that a multiplex array of EtOH-responsive tasks is needed to index intoxication is that we can tentatively conclude that tests performed during the phase of EtOH absorption and rapid distribution into brain may reveal the sensitivity of certain genotypes that are not similarly sensitive to effects measured after blood and brain levels have equilibrated.

6. The differences in sensitivity to EtOH cannot be explained by delivery of different effective doses to different strains, regardless of when the data were taken. EtOH sensitivity differences must reflect brain sensitivity rather than access of the drug to the effective brain regions.

7. Strains differ in the rapid development of AFT to EtOH. Behavioral sensitivity measures taken even a few minutes after an ip injection may underestimate the sensitivity of a group (or genotype) to the extent that tolerance has developed.

8. If only one or two tests are used to evaluate motor function in genetically engineered mutant mice, only a small portion of the domain will be assessed and results may be misleading. To characterize a novel genotype will require systematic exploration of a number of end points. No task that we have explored seems particularly better suited to this end than others, and each has its advantages and disadvantages, discussed in the individual reports analyzed here.

9. There is no reason to suppose that the behavioral domain, ataxia, is unique. The findings reported here likely extend to many behavioral domains (e.g. learning and memory, anxiety).

6. Notes added in proof

Since submission of this manuscript, we have collected some data on the proposed Ethanol Intoxication Battery. We tested 28 WSC mice plus six mice each from inbred strains BALB/cByJ and 129S1/SvlmJ following EtOH injection to see whether the phenotypic correlations among individual scores on the 11 variables measured in this genetically segregating population of mice was similar to the pattern of genetic correlations reported here. Broadly speaking, there was a reasonable concordance between the two studies. To see whether a multivariate summary of the intoxication data was informative, we used principal components analysis (Systat 10 without varimax rotation or restriction of number of factors). Three components were extracted with eigen-values > 1.0 that together accounted for more than 60% of the variance. The first component reflected the negative correlation between splay and wobble scores reported here. The second component captured the positive correlations among sensitivity scores on the balance beam and dowel test at 30 min, and the third factor was dominated by the correlation reported here between ethanol-induced loco-motor activation and screen test sensitivity. These data still demand much further scrutiny, as we have yet to examine the variables for normality, verify the robustness of the correlations by examining scatterplots, explore factor rotations, etc. Because the full battery requires two groups of mice, we did not perform half of the tests following the highest dose of EtOH (LORR, AFT). Nonetheless, they encourage us that we may be able to employ the Ethanol Intoxication Battery usefully as proposed.

We therefore proceeded to test 6 male mice per strain using the Intoxication Test Battery. Comparison of the pattern of genetic correlations from mice tested serially in the Intoxication Test Battery corresponded reasonably well with the patterns reported here, which were largely tested between-groups. In continuing studies, more mice will be tested from these 8 strains, and the battery will be extended to additional strains.

Finally, since submitting this manuscript, we have completed analysis of data using a test conceptually similar to the grid test. In the parallel rod floor apparatus, we also found that the 129 strain was exceptionally sensitive to EtOH-induced foot slip errors, as they were in the grid test. Data across the 8 strain panel in the parallel rod floor apparatus correlated quite well with grid test strain sensitivity data (r=0.72). Additionally, the parallel rod floor apparatus was quite insensitive to a range of rod diameters and spacings, yielding no meaningful changes in strain sensitivity rank orders across conditions. Thus, this new test may prove to be a more useful version of the grid test for future studies.

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