

## SUPPORTING INFORMATION

### **Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test**

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## **EXPANDED MATERIAL AND METHODS**

### **Procedure**

The rats were handled during the week before testing began. The handling procedure was similar to that of previous experiments (Roman and Colombo, 2009; Roman et al., 2007) and consisted of individual tail marking, handling, weighing, and adaptation to the transportation bucket that was used to take the animals from the home cage to the testing rooms. The animals were 12–14 weeks old when the behavioral testing started. All testing was conducted during the period of September – October. The MCSF was the main test for this investigation. The OF and EPM tests served as reference tests to permit comparisons with other studies. In accordance with previous experiments, (Roman et al., 2007) each animal was tested only once in each of the three tests over three consecutive days with the MCSF test completed on the first day, the OF test on the second, and finally the EPM test on the third day. The sequential order of the three tests was based on a pilot study in which it was shown that the MCSF test was most sensitive to previous experience and should be performed as the first test in order to reduce the likelihood of any carry-over effects (Augustsson, 2004). The rats were tested using a counterbalanced running schedule to avoid time of day and order biases and tested in the following order: AA/ANA, P/NP, HAD1-2/LAD1-2 and N/Nih, and sP/sNP and Wistar. All testing was performed in a room separate from the housing room, with a masking background noise. The conditions in the testing room were similar with regard to temperature and humidity to those in the animal room. The illumination in each specific test is given below. The animal to be tested was transferred in a bucket from the home cage to the respective apparatus. After each test, the test apparatus was wiped with a cloth containing 10% alcohol solution and sufficient time was allowed to dry before the next animal was placed in the test. All behavioral observations were carried out during the dark period of the light/dark cycle.

### **The MCSF test**

The MCSF test has been described in detail elsewhere (Meyerson et al., 2006; Roman and Colombo, 2009). The MCSF arena (Fig. 1) used in the present study was similar to the rat arena used in other studies (Alsiö et al., 2009; Roman and Colombo, 2009). AA and ANA rats have previously been tested using a smaller arena (Roman et al., 2007), which today is used for mice (Wallén-Mackenzie et al., 2009). The apparatus consists of a square field (100 x 100 cm) with a smaller square field (70 x 70 cm) located in the center of the larger one. The walls are 25 cm high except for the walls surrounding the BRIDGE (see below), which are 40 cm high. The arena is divided into zones, which forms the basis of the description and the

variables of the animals' performance in this test. The defined zones (Fig. 1) were: CENTER, the center field of the arena; CORRIDORs, the corridors surrounding the center field; DCR, the dark corner room where the animal can seek shelter; HURDLE, a high passage to a hole board with a photocell device located under the hole board floor, allowing recording of head dips into the holes; BRIDGE, the elevated and illuminated bridge construction, associated with risk-taking behavior; SLOPE, the slope leading up to the BRIDGE, associated with risk assessment; BRIDGE ENTRANCE, the initial part of the BRIDGE used as an additional risk-assessment area; CENTRAL CIRCLE, the circular zone (diameter 25 cm) in the middle of the CENTER, used for assessment of risk-taking behavior in the central part of CENTER versus thigmotaxis. The sum of frequencies to the three CORRIDORs (FRQ TOTCORR) and to all zones (TOTACT) was used for assessment of general locomotor activity. The total time spent in the three CORRIDORs was given the denomination DUR TOTCORR. An operational categorization of the various parameters generated from the MCSF with regard to function is used in the interpretation of results (Roman and Colombo, 2009). The shelter/risk index is calculated from the difference in time spent in the dark corner room (DCR) and on the BRIDGE, relative to the total time spent in the two zones and is used as one way of interpreting anxiety-like behavior. A positive value indicates that the animals spent more time in the DCR than on the BRIDGE and is interpreted as higher anxiety-like behavior. The SLOPE/BRIDGE interval reveals how long time it takes the animals to enter the BRIDGE in relation to first entering the SLOPE and is here used as one way of interpreting impulsive-like behavior. Thus, a value close to zero indicates less risk assessment and a fast risk-taking response.

### **The OF test**

The OF arena consisted of a brown painted square field (90 x 90 cm with 30 cm high walls). White lines divided the arena into a 6 x 6 array of small squares (15 x 15 cm) used to score number of crossings. Duration and frequency of visits into a peripheral zone, 15 cm from each of the four walls of the arena (the width of one of the small squares) was used for assessment of thigmotaxis whereas duration and frequency of visits into the central part (60 x 60 cm) revealed center activity. Thus, the center portions of the OF and MCSF were of different shapes and relative sizes. The total number of lines crossed (CROSSINGS) and the sum of visits to the periphery and center (TOTACT) were used as measures of general locomotor activity. Each rat was released in the peripheral zone and the test session lasted 10 min.

### **The EPM test**

The EPM apparatus (AccuScan, Columbus, OH) was white acrylic plastic and consisted of two open arms (50 x 10 cm) at right angles to two wall-enclosed and covered arms (50 x 10 x 50 cm). The apparatus was raised 90 cm off the floor. The behavior on the open and closed arms, and in the center was recorded. The sum of visits to the open and closed arms (TOTACT) was used for assessment of general locomotor activity. Each rat was released in the center of the EPM facing an open arm and the test session lasted 5 min.

### **Behavioral recordings**

The animals were monitored by video cameras. In the MCSF test, the numbers of stretched attend postures (SAPs; from the CORRIDORS into the CENTER and from the CORRIDOR into the SLOPE) and rearings were recorded by direct observation, and the number of head dips into the hurdle hole board of the MCSF was noted. The observer watched remotely via a video monitor from a room adjacent to the one in which the test apparatus was located.

Manual scoring of the videotaped behavior in the MCSF, OF and the EPM tests was performed using the Observer XT 8.0 (Noldus Information Technology, Wageningen, the Netherlands). Visits to the defined zones were only scored as such if both hind legs had crossed over into that section. The latency (LAT, s) of first visiting a zone, frequency (FRQ) of visits, and duration (DUR, s) of time spent in a certain zone, and also the number of animals visiting each zone (OCCURRENCE) were registered for the defined zones in the respective test. The mean duration per visit to a zone (DUR/FRQ, s), the percentage duration and the percentage number of visits in each zone (in the MCSF and EPM) were calculated.

When an animal did not enter a zone this was considered to be a missing value in the statistical analysis, except for the comparisons of percentage number of visits to each zone (in the MCSF and EPM tests) and percentage duration of time spent in each zone.

**Supporting Table 1.** Results from the multivariate concentric square field™ (MCSF) test in adult male Alko, alcohol (AA) and Alko, non-alcohol (ANA) rats.

Functional categories	Parameters	AA	ANA	
General activity	TOTACT	84.7 ± 5.7	118.4 ± 12.0	*
	FRQ TOTCORR	23.5 ± 2.1	37.6 ± 4.0	*
	FRQ CENTER	23.1 ± 1.3	21.6 ± 2.5	
	DUR CENTER	396.8 ± 41.6	214.7 ± 47.9	***
	DUR/FRQ CENTER	17.6 ± 2.0	10.6 ± 1.6	*
Exploratory activity	LAT LEAVE	99.7 ± 35.9	18.2 ± 3.0	****
	DUR TOTCORR	390.0 ± 17.7	417.1 ± 31.4	
	DUR/FRQ TOTCORR	18.4 ± 2.1	13.0 ± 2.0	*
	LAT HURDLE	207.5 ± 41.0	76.0 ± 8.0	**
	FRQ HURDLE	5.4 ± 0.7	9.8 ± 1.0	**
	DUR HURDLE	93.8 ± 9.6	102.2 ± 10.8	
	DUR/FRQ HURDLE	19.2 ± 2.7	10.4 ± 0.5	**
	OCC HURDLE	10/12	11/12	
	PHOTOCELL COUNTS	3.3 ± 0.7	6.0 ± 0.9	*
	# ZONES VISITED	9.5 ± 0.3	9.4 ± 0.6	
	OCC VIST ALL ZONES	10/12	11/12	
	REARING	73.8 ± 2.6	78.6 ± 8.7	
Risk assessment	LAT SLOPE	303.9 ± 81.4	82.7 ± 11.9	*
	FRQ SLOPE	8.0 ± 1.1	15.2 ± 1.6	**
	DUR SLOPE	46.3 ± 6.2	54.5 ± 8.5	
	DUR/FRQ SLOPE	6.0 ± 0.5	3.4 ± 0.4	***
	OCC SLOPE	12/12	11/12	
	LAT BRIDGE ENTRANCE	338.5 ± 82.3	98.2 ± 15.7	*
	FRQ BRIDGE ENTRANCE	8.9 ± 1.4	15.8 ± 1.8	**
	DUR BRIDGE ENTRANCE	65.8 ± 14.1	64.7 ± 10.7	
	DUR/FRQ BRIDGE ENTRANCE	7.4 ± 1.7	4.1 ± 0.6	*
	OCC BRIDGE ENTRANCE	12/12	11/12	
	SAP TO CENTER	- ± -	- ± -	
	OCC SAP CENTER	1/12	2/12	
	SAP TO SLOPE	0.7 ± 0.3	0.7 ± 0.3	
	OCC SAP TO SLOPE	12/12	11/12	
	Risk taking	LAT BRIDGE	341.0 ± 82.4	99.5 ± 15.7
FRQ BRIDGE		4.5 ± 0.7	8.2 ± 0.9	**
DUR BRIDGE		161.3 ± 29.7	188.8 ± 33.1	
DUR/FRQ BRIDGE		33.0 ± 3.4	21.9 ± 2.3	*
OCC BRIDGE		12/12	11/12	
LAT CTRCI		122.3 ± 40.5	134.9 ± 53.5	
FRQ CTRCI		9.4 ± 0.8	6.8 ± 1.6	*
DUR CTRCI		19.3 ± 4.2	12.9 ± 8.6	**
DUR/FRQ CTRCI		1.9 ± 0.3	1.2 ± 0.3	**
Shelter seeking	LAT DCR	291.0 ± 47.1	86.8 ± 36.5	***
	FRQ DCR	3.3 ± 0.3	7.6 ± 1.0	***
	DUR DCR	50.5 ± 8.9	179.2 ± 59.0	*
	DUR/FRQ DCR	15.1 ± 2.1	25.3 ± 9.7	
	OCC DCR	10/12	12/12	
Other	BODY WEIGHT	313.3 ± 5.3	379.8 ± 8.5	****
Anxiety-like behavior	DUR SHELTER/RISK INDEX	-0.49 ± 0.14	-0.18 ± 0.16	
Impulsive-like behavior	SLOPE/BRIDGE INTERVAL	-0.26 ± 0.22	-0.28 ± 0.23	

Behavioral parameters recorded during the 20-min trial of the MCSF test. Values represent mean  $\pm$  SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 12 rats/group). No values are shown if OCC  $\leq$  3 animals. \*p  $\leq$  0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 comparing AA and ANA rats (Mann-Whitney U-test); #p  $\leq$  0.05, ##p < 0.01, ###p < 0.001 comparing AA and ANA rats (Chi-Square test).

*Abbreviations:* CTRCI, central circle; DCR, dark corner room; DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; SAP, stretched attend posture; TOTACT, total activity, i.e. the sum of all frequencies; TOTARENA, total arena; TOTCORR; total corridor, i.e. the sum of all corridors.

**Supporting Table 2.** Results from the open field (OF) and elevated plus maze (EPM) tests in adult male Alko, alcohol (AA) and Alko, non-alcohol (ANA) rats.

Parameters	AA	ANA	
<b>A. OF test</b>			
Lat center	31.3 ± 7.3	23.4 ± 8.1	
Frq center	12.2 ± 1.9	12.0 ± 1.5	
Dur center	45.7 ± 6.0	36.6 ± 7.0	
Dur/Frq center	4.0 ± 0.4	2.9 ± 0.3	*
%Dur center	7.6 ± 1.0	6.1 ± 1.2	
Frq periphery	12.9 ± 1.7	13.3 ± 1.6	
Dur periphery	554.3 ± 6.0	563.4 ± 7.0	
Dur/Frq periphery	53.8 ± 9.5	51.1 ± 7.7	
Crossings	159.2 ± 9.5	152.4 ± 13.2	
TOTACT	25.1 ± 3.6	25.3 ± 3.1	
<b>B. EPM test</b>			
Lat leave	8.1 ± 2.4	7.5 ± 1.6	
Lat open	60.7 ± 16.3	31.7 ± 8.5	
Frq open	3.2 ± 0.5	3.2 ± 0.7	
Dur open	47.0 ± 8.1	22.9 ± 4.5	*
Dur/Frq open	14.8 ± 2.1	7.5 ± 1.1	**
%Dur open	15.7 ± 2.7	7.6 ± 1.5	*
%Frq open	25.8 ± 3.7	16.5 ± 3.3	
Lat closed	19.2 ± 6.4	15.3 ± 5.9	
Frq closed	8.8 ± 0.4	15.1 ± 0.7	****
Dur closed	126.3 ± 15.7	181.8 ± 10.2	*
Dur/Frq closed	14.0 ± 1.5	12.5 ± 0.9	
%Dur closed	42.1 ± 5.2	60.6 ± 3.4	*
Frq center	12.3 ± 0.5	18.4 ± 0.9	****
Dur center	126.7 ± 10.6	95.3 ± 6.8	*
Dur/Frq center	10.3 ± 0.9	5.2 ± 0.4	****
%Dur center	42.2 ± 3.5	31.8 ± 2.3	*
TOTACT	12.0 ± 0.5	18.3 ± 0.9	****

Behavioral parameters recorded during the 10-min trial of the OF test (A) and 5-min trial of the EPM test (B). Values represent mean ± SEM. Occurrence (OCC) is shown for the behaviors that were not performed by all animals in each group (n = 11-12 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 comparing AA and ANA rats (Mann-Whitney U-test); ###p < 0.001, ####p < 0.00001 comparing AA and ANA rats (Pearson Chi-Square test).

*Abbreviations:* DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; TOTACT, total activity, i.e. the sum of all frequencies.

**Supporting Table 3.** Results from the multivariate concentric square field™ (MCSF) test in adult male Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats.

Functional categories	Parameters	sP	sNP		
General activity	TOTACT	51.7 ± 7.1	99.4 ± 5.6	****	
	FRQ TOTCORR	25.1 ± 2.3	34.7 ± 1.8	**	
	FRQ CENTER	10.9 ± 2.1	22.4 ± 1.7	***	
	DUR CENTER	401.3 ± 83.7	254.8 ± 15.0		
	DUR/FRQ CENTER	208.9 ± 100.0	13.0 ± 2.1	**	
	OCC LEAVE CENTER	14/16	16/16		
Exploratory activity	LAT LEAVE	201.3 ± 35.8	69.3 ± 14.4	***	
	DUR TOTCORR	612.5 ± 27.0	488.3 ± 10.9	***	
	DUR/FRQ TOTCORR	27.7 ± 3.2	14.7 ± 0.8	****	
	LAT HURDLE	489.3 ± 73.7	231.1 ± 38.0	**	
	FRQ HURDLE	7.2 ± 1.1	7.1 ± 0.5		
	DUR HURDLE	103.8 ± 19.0	130.0 ± 8.7	*	
	DUR/FRQ HURDLE	14.2 ± 1.4	18.6 ± 0.8	**	
	OCC HURDLE	13/16	16/16		
	PHOTOCELL COUNTS	4.6 ± 1.0	7.6 ± 1.7		
	# ZONES VISITED	7.3 ± 0.8	10.0 ± 0.0	**	
	OCC VIST ALL ZONES	6/16	16/16	###	
	REARING	56.3 ± 6.1	104.5 ± 4.4	****	
	Risk assessment	LAT SLOPE	769.9 ± 82.0	307.3 ± 44.0	***
FRQ SLOPE		3.5 ± 0.6	7.8 ± 0.4	****	
DUR SLOPE		20.8 ± 2.9	40.1 ± 2.2	****	
DUR/FRQ SLOPE		7.2 ± 1.7	5.4 ± 0.4		
OCC SLOPE		10/16	16/16	##	
LAT BRIDGE ENTRANCE		827.1 ± 66.4	377.3 ± 51.1	***	
FRQ BRIDGE ENTRANCE		3.0 ± 0.6	8.8 ± 0.5	****	
DUR BRIDGE ENTRANCE		19.8 ± 4.6	44.9 ± 4.5	**	
DUR/FRQ BRIDGE ENTRANCE		7.6 ± 1.8	5.2 ± 0.5		
OCC BRIDGE ENTRANCE		9/16	16/16	##	
SAP TO CENTER		8.0 ± 1.0	2.9 ± 0.6	**	
OCC SAP CENTER		14/16	7/16	##	
SAP TO SLOPE		7.5 ± 0.9	1.7 ± 0.3	****	
OCC SAP TO SLOPE		13/16	16/16		
Risk taking		LAT BRIDGE	793.1 ± 77.4	380.7 ± 51.3	**
		FRQ BRIDGE	1.7 ± 0.3	5.0 ± 0.4	****
	DUR BRIDGE	40.1 ± 8.2	113.8 ± 8.1	****	
	DUR/FRQ BRIDGE	23.0 ± 2.8	23.6 ± 1.8		
	OCC BRIDGE	7/16	16/16	###	
	LAT CTRCI	546.8 ± 113.1	231.3 ± 62.8		
	FRQ CTRCI	3.3 ± 0.8	6.1 ± 0.8	*	
	DUR CTRCI	8.1 ± 3.7	12.2 ± 1.8	*	
	DUR/FRQ CTRCI	2.5 ± 0.9	2.5 ± 0.5		
	OCC CTRCI	9/16	16/16	##	
Shelter seeking	LAT DCR	377.2 ± 63.8	291.2 ± 43.0		
	FRQ DCR	7.8 ± 0.6	7.6 ± 0.6		
	DUR DCR	162.1 ± 24.9	116.3 ± 6.4		
	DUR/FRQ DCR	20.2 ± 2.5	16.1 ± 1.2		
	OCC DCR	13/16	16/16		
Other	BODY WEIGHT	432.3 ± 7.8	414.4 ± 6.2		
Anxiety-like behavior	DUR SHELTER/RISK INDEX	0.57 ± 0.09	0.02 ± 0.04	***	



Impulsive-like behavior	SLOPE/BRIDGE INTERVAL	-0.09 ± 0.08	-0.39 ± 0.19	**
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Behavioral parameters recorded during the 20-min trial of the MCSF test. Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 16 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 comparing sP and sNP rats (Mann-Whitney U-test); #p ≤ 0.05, ##p < 0.01, ###p < 0.001 comparing sP and sNP rats (Chi-Square test).

*Abbreviations:* CTRCI, central circle; DCR, dark corner room; DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; SAP, stretched attend posture; TOTACT, total activity, i.e. the sum of all frequencies; TOTARENA, total arena; TOTCORR; total corridor, i.e. the sum of all corridors.

**Supporting Table 4.** Results from the open field (OF) and elevated plus maze (EPM) tests in adult male Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats.

Parameters	sP		sNP		
<b>A. OF test</b>					
Lat center	53.1	± 35.5	80.9	± 32.6	
Occ center		10/16		15/15	##
Frq center	1.8	± 0.4	8.7	± 1.2	***
Dur center	6.5	± 1.4	25.3	± 3.6	***
Dur/Frq center	4.4	± 1.1	3.0	± 0.2	
%Dur center	0.7	0.2	4.2	0.6	****
Frq periphery	2.1	± 0.3	9.7	± 1.2	****
Dur periphery	595.9	± 1.2	574.7	± 3.6	****
Dur/Frq periphery	376.6	± 47.0	86.4	± 18.7	****
Crossings	43.3	± 13.0	223.7	± 11.1	****
TOTACT	3.3	± 0.7	18.3	± 2.4	****
<b>B. EPM test</b>					
Lat leave	34.0	± 14.7	7.6	± 1.7	
Occ leave		15/16		16/16	
Lat open	71.2	± 25.9	43.6	± 10.3	
Occ open		6/16		16/16	###
Frq open	2.0	± 0.5	4.6	± 0.6	*
Dur open	20.0	± 6.7	48.6	± 5.8	*
Dur/Frq open	10.5	± 2.0	11.2	± 0.8	
%Dur open	2.5	± 1.1	16.2	± 1.9	****
%Frq open	6.6	2.5	27.8	2.8	****
Lat closed	36.9	± 14.6	19.3	± 7.2	
Frq closed	8.6	± 1.0	11.4	± 0.6	*
Dur closed	171.5	± 14.7	118.5	± 5.5	**
Dur/Frq closed	25.9	± 5.0	10.9	± 0.9	***
%Dur closed	53.6	± 5.8	39.5	± 1.8	**
Frq center	9.4	± 1.3	16.4	± 0.8	***
Dur center	131.8	± 17.3	132.8	± 5.0	
Dur/Frq center	34.4	± 18.3	8.4	± 0.5	**
%Dur center	43.9	± 5.8	44.3	± 1.7	
TOTACT	8.8	± 1.2	16.1	± 0.9	****

Behavioral parameters recorded during the 10-min trial of the OF test (A) and 5-min trial of the EPM test (B). Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 15-16 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 comparing sP and sNP rats (Mann-Whitney U-test); #p ≤ 0.05, ##p < 0.01, ###p < 0.001 comparing sP and sNP rats (Pearson Chi-Square test).

*Abbreviations:* DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; TOTACT, total activity, i.e. the sum of all frequencies.

**Supporting Table 5.** Results from the multivariate concentric square field™ (MCSF) test in adult male Indiana preferring (P) and Indiana non-preferring (NP) rats.

Functional categories	Parameters	P	NP	
General activity	TOTACT	103.0 ± 11.5	132.7 ± 6.4	*
	FRQ TOTCORR	34.3 ± 4.0	46.8 ± 2.6	*
	FRQ CENTER	21.1 ± 4.1	33.7 ± 2.0	*
	DUR CENTER	242.3 ± 32.4	285.8 ± 14.6	
	DUR/FRQ CENTER	19.1 ± 4.3	8.9 ± 0.9	*
Exploratory activity	LAT LEAVE	66.8 ± 7.7	37.0 ± 5.8	**
	DUR TOTCORR	532.1 ± 16.8	452.8 ± 22.9	*
	DUR/FRQ TOTCORR	19.2 ± 3.1	9.9 ± 0.6	**
	LAT HURDLE	238.3 ± 53.0	170.9 ± 36.6	
	FRQ HURDLE	9.1 ± 1.1	9.8 ± 1.0	
	DUR HURDLE	124.3 ± 16.5	118.5 ± 12.2	
	DUR/FRQ HURDLE	15.2 ± 2.7	12.8 ± 1.5	
	PHOTOCELL COUNTS	7.2 ± 2.7	7.4 ± 1.0	
	# ZONES VISITED	9.5 ± 0.3	10.0 ± 0.0	
	OCC VIST ALL ZONES	9/12	12/12	
	REARING	79.8 ± 6.7	70.3 ± 3.0	
Risk assessment	LAT SLOPE	268.3 ± 48.1	262.0 ± 77.1	
	FRQ SLOPE	10.1 ± 1.0	9.8 ± 0.9	
	DUR SLOPE	41.0 ± 4.8	47.6 ± 5.9	
	DUR/FRQ SLOPE	4.3 ± 0.5	4.8 ± 0.4	
	LAT BRIDGE ENTRANCE	281.8 ± 49.3	319.7 ± 76.2	
	FRQ BRIDGE ENTRANCE	10.9 ± 1.1	10.0 ± 0.8	
	DUR BRIDGE ENTRANCE	50.7 ± 9.0	39.2 ± 3.7	
	DUR/FRQ BRIDGE ENTRANCE	4.7 ± 0.5	4.1 ± 0.4	
	SAP TO CENTER	- ± -	- ± -	
	OCC SAP CENTER	3/12	0/12	
	SAP TO SLOPE	1.7 ± 0.4	2.0 ± 0.6	
Risk taking	LAT BRIDGE	285.0 ± 49.3	321.9 ± 76.3	
	FRQ BRIDGE	5.9 ± 0.6	5.3 ± 0.4	
	DUR BRIDGE	123.6 ± 9.8	122.7 ± 8.9	
	DUR/FRQ BRIDGE	22.1 ± 1.8	23.5 ± 1.3	
	LAT CTRCI	293.0 ± 100.1	59.1 ± 17.0	
	FRQ CTRCI	8.1 ± 2.2	9.3 ± 1.0	
	DUR CTRCI	10.7 ± 2.3	13.5 ± 1.5	
	DUR/FRQ CTRCI	1.6 ± 0.2	1.5 ± 0.2	
	OCC CTRCI	9/12	12/12	
Shelter seeking	LAT DCR	316.5 ± 66.5	201.7 ± 56.6	
	FRQ DCR	6.1 ± 1.0	8.0 ± 0.7	
	DUR DCR	85.5 ± 13.7	119.8 ± 14.2	
	DUR/FRQ DCR	14.2 ± 1.0	15.9 ± 2.3	
	OCC DCR	11/12	12/12	
Other	BODY WEIGHT	421.8 ± 9.9	425.9 ± 5.4	
Anxiety-like behavior	DUR SHELTER/RISK INDEX	-0.22 ± 0.09	-0.03 ± 0.06	
Impulsive-like behavior	SLOPE/BRIDGE INTERVAL	-0.07 ± 0.02	-1.15 ± 1.03	

Behavioral parameters recorded during the 20-min trial of the MCSF test. Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 12 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*p < 0.01 comparing P and NP rats (Mann-Whitney U-test); ##p < 0.01 comparing P and NP rats (Chi-Square test).

*Abbreviations:* CTRCI, central circle; DCR, dark corner room; DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; SAP, stretched attend posture; TOTACT, total activity, i.e. the sum of all frequencies; TOTARENA, total arena; TOTCORR; total corridor, i.e. the sum of all corridors.

**Supporting Table 6.** Results from the open field (OF) and elevated plus maze (EPM) tests in adult male Indiana preferring (P) and Indiana non-preferring (NP) rats.

Parameters	P		NP		
<b>A. OF test</b>					
Lat center	76.2	± 20.1	49.3	± 27.8	
Occ center		11/12		12/12	
Frq center	16.3	± 2.2	10.4	± 1.5	
Dur center	70.8	± 14.3	45.4	± 8.8	
Dur/Frq center	4.0	± 0.5	4.2	± 0.7	
%Dur center	10.8	2.4	7.6	1.5	
Frq periphery	16.0	± 2.5	11.5	± 1.6	
Dur periphery	535.1	± 14.3	554.6	± 8.8	
Dur/Frq periphery	86.0	± 47.2	79.4	± 24.0	
Crossings	279.3	± 13.6	174.9	± 9.8	***
TOTACT	30.9	± 4.9	21.9	± 3.2	
<b>B. EPM test</b>					
Lat leave	12.6	± 2.4	6.5	± 1.4	*
Lat open	46.0	± 17.3	45.9	± 18.1	
Occ open		11/12		10/12	
Frq open	3.1	± 0.5	3.9	± 0.8	
Dur open	29.0	± 4.9	41.3	± 7.5	
Dur/Frq open	9.8	± 0.8	11.2	± 1.1	
%Dur open	8.9	± 1.7	11.5	± 2.6	
%Frq open	19.5	3.4	20.8	4.5	
Lat closed	27.1	± 6.2	14.3	± 5.4	
Frq closed	11.2	± 0.7	11.5	± 0.7	
Dur closed	119.8	± 8.1	134.6	± 10.3	
Dur/Frq closed	11.0	± 0.7	11.8	± 0.9	
%Dur closed	39.9	± 2.7	44.9	± 3.4	
Frq center	14.8	± 0.9	15.1	± 0.7	
Dur center	153.4	± 6.1	131.3	± 5.9	*
Dur/Frq center	11.1	± 1.0	9.0	± 0.5	
%Dur center	51.1	± 2.0	43.8	± 2.0	*
TOTACT	14.0	± 0.8	14.8	± 0.8	

Behavioral parameters recorded during the 10-min trial of the OF test (A) and 5-min trial of the EPM test (B). Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 12 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*\*p < 0.001 comparing P and NP rats (Mann-Whitney U-test); #p ≤ 0.05 comparing P and NP (Pearson Chi-Square test).

*Abbreviations:* DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; TOTACT, total activity, i.e. the sum of all frequencies.

**Supporting Table 7.** Results from the multivariate concentric square field™ (MCSF) test in adult male Indiana high alcohol drinking 1-2 (HAD1-2) and Indiana low alcohol drinking 1-2 (LAD1-2) rats.

Functional categories	Parameters		HAD	LAD		
General activity	TOTACT	H1/L1	98.5 ± 3.7	99.6 ± 7.6		
		H2/L2	83.3 ± 6.3	68.8 ± 5.3		
	FRQ TOTCORR	H1/L1	31.6 ± 1.5	29.6 ± 2.7		
		H2/L2	30.1 ± 1.9	24.3 ± 1.8	*	
	FRQ CENTER	H1/L1	17.6 ± 1.4	21.2 ± 2.9		
		H2/L2	16.3 ± 2.1	12.3 ± 1.5		
	DUR CENTER	H1/L1	184.0 ± 16.3	221.0 ± 22.2		
		H2/L2	229.8 ± 18.0	230.3 ± 35.4		
DUR/FRQ CENTER	H1/L1	10.9 ± 1.1	11.5 ± 0.9			
	H2/L2	16.2 ± 1.7	25.8 ± 9.0			
Exploratory activity	LAT LEAVE	H1/L1	48.0 ± 7.6	42.4 ± 7.6		
		H2/L2	76.6 ± 7.1	86.3 ± 25.9		
	DUR TOTCORR	H1/L1	448.1 ± 24.5	466.5 ± 25.3		
		H2/L2	487.8 ± 32.7	447.1 ± 30.6		
	DUR/FRQ TOTCORR	H1/L1	14.6 ± 1.2	17.0 ± 1.5		
		H2/L2	17.3 ± 2.0	19.5 ± 2.1		
	LAT HURDLE	H1/L1	145.3 ± 37.4	199.1 ± 75.2		
		H2/L2	223.8 ± 34.3	403.0 ± 57.9	*	
	FRQ HURDLE	H1/L1	7.9 ± 0.7	6.9 ± 1.0		
		H2/L2	6.8 ± 1.0	5.1 ± 0.5		
	DUR HURDLE	H1/L1	90.0 ± 12.7	119.8 ± 14.1		
		H2/L2	100.5 ± 17.0	108.9 ± 14.3		
	DUR/FRQ HURDLE	H1/L1	11.3 ± 1.0	18.5 ± 1.4	***	
		H2/L2	14.8 ± 1.0	22.2 ± 2.5	**	
	PHOTOCELL COUNTS	H1/L1	7.8 ± 1.4	6.6 ± 1.7		
		H2/L2	6.6 ± 1.4	7.2 ± 1.0		
	# ZONES VISITED	H1/L1	9.9 ± 0.1	9.8 ± 0.1		
		H2/L2	9.4 ± 0.3	9.3 ± 0.4		
OCC VIST ALL ZONES	H1/L1		11/12	10/12		
	H2/L2		7/12	9/12		
REARING	H1/L1	90.1 ± 5.6	80.1 ± 5.5			
	H2/L2	45.1 ± 2.6	42.3 ± 3.1			
Risk assessment	LAT SLOPE	H1/L1	153.6 ± 31.7	141.8 ± 22.1		
		H2/L2	258.5 ± 67.9	441.8 ± 56.6	*	
	FRQ SLOPE	H1/L1	11.4 ± 0.8	10.7 ± 0.9		
		H2/L2	9.1 ± 1.2	7.7 ± 0.9		
	DUR SLOPE	H1/L1	52.9 ± 5.6	56.1 ± 8.3		
		H2/L2	75.2 ± 11.6	80.2 ± 15.1		
	DUR/FRQ SLOPE	H1/L1	4.6 ± 0.4	5.2 ± 0.6		
		H2/L2	8.2 ± 0.4	10.9 ± 1.3		
	OCC SLOPE	H1/L1		12/12	12/12	
		H2/L2		11/12	12/12	
	LAT BRIDGE ENTRANCE	H1/L1	172.8 ± 32.3	173.8 ± 36.7		
		H2/L2	271.9 ± 69.0	524.7 ± 64.4	**	
FRQ BRIDGE ENTRANCE	H1/L1	12.7 ± 0.9	12.3 ± 1.2			
	H2/L2	9.0 ± 1.4	8.2 ± 1.0			
DUR BRIDGE ENTRANCE	H1/L1	80.7 ± 17.0	70.0 ± 13.8			

		H2/L2	40.0 ± 4.4	54.0 ± 11.5	
	DUR/FRQ BRIDGE	H1/L1	6.6 ± 1.5	5.7 ± 1.2	
	ENTRANCE				
		H2/L2	5.1 ± 0.6	6.1 ± 0.8	
	OCC BRIDGE ENTRANCE	H1/L1	12/12	12/12	
		H2/L2	11/12	11/12	
	SAP TO CENTER	H1/L1	– ± –	– ± –	
		H2/L2	1.8 ± 0.3	3.7 ± 1.8	
	OCC SAP CENTER	H1/L1	2/12	2/12	
		H2/L2	6/12	6/12	
	SAP TO SLOPE	H1/L1	0.8 ± 0.3	1.0 ± 0.3	
		H2/L2	1.1 ± 0.5	1.8 ± 0.3	*
Risk taking	LAT BRIDGE	H1/L1	174.0 ± 32.3	175.8 ± 36.7	
		H2/L2	274.9 ± 69.6	538.3 ± 62.0	**
	FRQ BRIDGE	H1/L1	6.8 ± 0.5	6.7 ± 0.7	
		H2/L2	4.5 ± 0.7	4.3 ± 0.6	
	DUR BRIDGE	H1/L1	200.4 ± 22.2	154.3 ± 18.1	
		H2/L2	113.9 ± 18.1	136.9 ± 26.8	
	DUR/FRQ BRIDGE	H1/L1	29.8 ± 2.8	23.5 ± 2.3	
		H2/L2	26.0 ± 2.5	30.6 ± 2.7	
	OCC BRIDGE	H1/L1	12/12	12/12	
		H2/L2	11/12	11/12	
	LAT CTRCI	H1/L1	200.7 ± 52.8	316.8 ± 106.0	
		H2/L2	363.6 ± 93.2	195.2 ± 109.4	
	FRQ CTRCI	H1/L1	5.0 ± 0.6	8.3 ± 1.5	*
		H2/L2	4.0 ± 1.5	3.0 ± 0.6	
	DUR CTRCI	H1/L1	5.6 ± 1.0	14.2 ± 2.7	**
		H2/L2	8.8 ± 3.3	9.6 ± 3.2	
	DUR/FRQ CTRCI	H1/L1	1.1 ± 0.1	1.8 ± 0.3	**
		H2/L2	2.6 ± 0.4	2.8 ± 0.5	
	OCC CTRCI	H1/L1	11/12	11/12	
		H2/L2	9/12	9/12	
Shelter seeking	LAT DCR	H1/L1	206.6 ± 39.6	253.3 ± 90.5	
		H2/L2	273.5 ± 52.5	256.1 ± 105.4	
	FRQ DCR	H1/L1	6.0 ± 0.4	5.2 ± 0.6	
		H2/L2	6.5 ± 0.7	6.3 ± 0.9	
	DUR DCR	H1/L1	133.8 ± 21.7	108.5 ± 22.1	
		H2/L2	165.0 ± 24.1	164.6 ± 33.7	
	DUR/FRQ DCR	H1/L1	21.7 ± 2.8	20.8 ± 3.8	
		H2/L2	24.9 ± 2.7	24.9 ± 3.4	
	OCC DCR	H1/L1	12/12	11/12	
		H2/L2	12/12	11/12	
Other	BODY WEIGHT	H1/L1	280.3 ± 5.3	311.3 ± 5.5	***
		H2/L2	273.9 ± 5.9	310.2 ± 5.9	***
Anxiety-like behavior	DUR SHELTER/RISK INDEX	H1/L1	-0.21 ± 0.12	-0.20 ± 0.09	
		H2/L2	0.10 ± 0.14	0.08 ± 0.16	
Impulsive-like behavior	SLOPE/BRIDGE INTERVAL	H1/L1	-0.27 ± 0.23	-0.23 ± 0.15	
		H2/L2	-0.09 ± 0.03	-0.33 ± 0.14	

Behavioral parameters recorded during the 20-min trial of the MCSF test. Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals

in each group or the behaviors that were not performed by all animals in each group (n = 12 rats/group). No values are shown if OCC  $\leq$  3 animals. \*p  $\leq$  0.05, \*\*p < 0.01, \*\*\*p < 0.001 comparing the respective replicate line of HAD and LAD rats (Mann-Whitney U-test).

*Abbreviations:* CTRCI, central circle; DCR, dark corner room; DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; SAP, stretched attend posture; TOTACT, total activity, i.e. the sum of all frequencies; TOTARENA, total arena; TOTCORR; total corridor, i.e. the sum of all corridors.



**Supporting Table 8.** Results from the open field (OF) and elevated plus maze (EPM) tests in adult male Indiana high alcohol drinking 1-2 (HAD1-2) and Indiana low alcohol drinking 1-2 (LAD1-2) rats.

Parameters		HAD		LAD		
<b>A. OF test</b>						
Lat center	H1/L1	105.3	± 49.3	52.4	± 12.0	
	H2/L2	183.7	± 31.8	186.4	± 63.7	
Occ center	H1/L1		11/11		11/11	
	H2/L2		11/12		8/11	
Frq center	H1/L1	4.1	± 0.8	15.1	± 1.8	***
	H2/L2	5.5	± 0.9	2.6	± 0.5	*
Dur center	H1/L1	15.2	± 4.3	76.1	± 13.5	****
	H2/L2	21.8	± 5.9	14.1	± 3.6	
Dur/Frq center	H1/L1	3.4	± 0.5	5.3	± 0.7	*
	H2/L2	3.7	± 0.5	5.5	± 0.9	
%Dur center	H1/L1	2.5	± 0.7	12.7	± 2.2	****
	H2/L2	3.3	± 0.9	1.7	± 0.5	
Frq periphery	H1/L1	5.3	± 0.9	15.8	± 1.7	***
	H2/L2	5.9	± 0.9	3.0	± 0.6	*
Dur periphery	H1/L1	584.8	± 4.3	525.7	± 13.7	****
	H2/L2	580.0	± 5.7	589.7	± 3.3	
Dur/Frq periphery	H1/L1	154.5	± 29.4	47.2	± 14.9	***
	H2/L2	154.6	± 44.6	302.8	± 62.1	*
Crossings	H1/L1	96.5	± 8.5	174.1	± 15.8	***
	H2/L2	161.1	± 14.1	67.5	± 13.0	***
TOTACT	H1/L1	9.4	± 1.7	30.9	± 3.5	***
	H2/L2	10.9	± 1.9	4.9	± 1.1	*
<b>B. EPM test</b>						
Lat leave	H1/L1	8.2	± 2.9	14.7	± 3.1	
	H2/L2	17.1	± 5.2	10.0	± 3.1	
Lat open	H1/L1	51.6	± 15.0	77.2	± 21.7	
	H2/L2	130.9	± 32.5	44.1	± 26.5	*
Occ open	H1/L1		11/12		11/12	
	H2/L2		9/12		7/12	
Frq open	H1/L1	2.6	± 0.5	2.9	± 0.5	
	H2/L2	1.8	± 0.2	2.1	± 0.5	
Dur open	H1/L1	30.1	± 7.6	33.4	± 6.6	
	H2/L2	18.9	± 3.8	35.6	± 4.9	*
Dur/Frq open	H1/L1	11.1	± 1.1	10.7	± 0.6	
	H2/L2	10.2	± 1.4	20.1	± 3.1	*
%Dur open	H1/L1	9.2	± 2.5	10.2	± 2.2	
	H2/L2	4.7	± 1.3	6.9	± 2.0	
%Frq open	H1/L1	22.0	± 3.9	21.3	± 3.9	
	H2/L2	10.9	± 2.3	12.0	± 3.6	
Lat closed	H1/L1	18.8	± 6.6	29.6	± 9.4	
	H2/L2	19.7	± 6.0	27.3	± 7.6	
Frq closed	H1/L1	7.8	± 0.8	9.5	± 0.6	
	H2/L2	10.2	± 0.4	9.2	± 1.1	
Dur closed	H1/L1	172.9	± 15.5	124.7	± 11.6	*
	H2/L2	164.5	± 9.5	175.4	± 9.6	
Dur/Frq closed	H1/L1	26.4	± 5.3	13.5	± 1.4	**
	H2/L2	16.4	± 1.1	22.5	± 3.3	
%Dur closed	H1/L1	57.6	± 5.2	41.6	± 3.9	*

Frq center	H2/L2	54.8 ± 3.2	58.5 ± 3.2	
	H1/L1	10.4 ± 1.0	12.6 ± 0.7	
Dur center	H2/L2	11.7 ± 0.5	10.4 ± 1.1	
	H1/L1	99.6 ± 12.2	144.6 ± 7.9	**
Dur/Frq center	H2/L2	121.3 ± 9.9	103.9 ± 9.3	
	H1/L1	9.9 ± 1.0	11.8 ± 0.9	
%Dur center	H2/L2	10.8 ± 1.1	10.3 ± 0.7	
	H1/L1	33.2 ± 4.1	48.2 ± 2.6	**
TOTACT	H2/L2	40.4 ± 3.3	34.6 ± 3.1	
	H1/L1	10.3 ± 1.0	12.2 ± 0.7	
	H2/L2	11.5 ± 0.5	10.4 ± 1.1	

Behavioral parameters recorded during the 10-min trial of the OF test (**A**) and 5-min trial of the EPM test (**B**). Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 11-12 rats/group). No values are shown if OCC ≤ 3 animals. \*p ≤ 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 comparing the respective replicate line of HAD and LAD rats (Mann-Whitney U-test); #p ≤ 0.05 comparing the respective replicate line of HAD and LAD rats (Pearson Chi-Square test).

*Abbreviations:* DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; TOTACT, total activity, i.e. the sum of all frequencies.

**Supporting Table 9.** Results from the multivariate concentric square field™ (MCSF) test in adult male Wistar and N/Nih rats.

Functional categories	Parameters	Wistar	N/Nih
General activity	TOTACT	137.0 ± 7.1	108.1 ± 7.7
	FRQ TOTCORR	45.4 ± 3.5	35.0 ± 2.1
	FRQ CENTER	21.5 ± 3.6	18.4 ± 2.0
	DUR CENTER	168.8 ± 24.1	164.3 ± 10.7
	DUR/FRQ CENTER	11.2 ± 1.9	9.4 ± 0.5
Exploratory activity	LAT LEAVE	18.2 ± 4.0	23.4 ± 3.9
	DUR TOTCORR	458.4 ± 12.3	401.9 ± 23.4
	DUR/FRQ TOTCORR	11.0 ± 1.1	12.0 ± 1.1
	LAT HURDLE	84.7 ± 16.7	218.7 ± 31.7
	FRQ HURDLE	13.8 ± 1.1	8.6 ± 0.6
	DUR HURDLE	151.5 ± 14.1	107.5 ± 7.9
	DUR/FRQ HURDLE	11.2 ± 0.8	12.9 ± 0.9
	OCC HURDLE	12/12	12/12
	PHOTOCELL COUNTS	10.1 ± 1.5	6.3 ± 1.2
	# ZONES VISITED	9.4 ± 0.3	9.9 ± 0.1
	OCC VIST ALL ZONES	9/12	11/12
	REARING	81.9 ± 3.1	87.0 ± 4.5
Risk assessment	LAT SLOPE	109.6 ± 22.1	199.7 ± 35.4
	FRQ SLOPE	17.0 ± 1.5	12.6 ± 1.1
	DUR SLOPE	68.1 ± 10.7	64.3 ± 4.5
	DUR/FRQ SLOPE	3.9 ± 0.3	5.4 ± 0.5
	OCC SLOPE	12/12	12/12
	LAT BRIDGE ENTRANCE	134.4 ± 23.9	205.3 ± 35.3
	FRQ BRIDGE ENTRANCE	17.6 ± 1.7	13.1 ± 1.3
	DUR BRIDGE ENTRANCE	82.3 ± 12.2	71.9 ± 8.0
	DUR/FRQ BRIDGE ENTRANCE	4.7 ± 0.5	5.7 ± 0.6
	OCC BRIDGE ENTRANCE	12/12	12/12
	SAP TO CENTER	7.7 ± 2.5	2.1 ± 0.4
	OCC SAP CENTER	6/12	7/12
	SAP TO SLOPE	0.3 ± 0.1	0.5 ± 0.2
Risk taking	LAT BRIDGE	135.8 ± 23.9	207.5 ± 35.6
	FRQ BRIDGE	9.3 ± 1.0	6.6 ± 0.7
	DUR BRIDGE	185.7 ± 20.4	167.3 ± 13.9
	DUR/FRQ BRIDGE	20.1 ± 1.0	26.4 ± 1.9
	LAT CTRCI	132.8 ± 53.9	396.2 ± 105.0
	FRQ CTRCI	5.7 ± 1.1	6.1 ± 1.3
	DUR CTRCI	7.6 ± 1.9	11.8 ± 1.7
	DUR/FRQ CTRCI	1.4 ± 0.2	2.3 ± 0.2
	OCC CTRCI	11/12	11/12
Shelter seeking	LAT DCR	43.0 ± 13.3	171.4 ± 55.3
	FRQ DCR	9.6 ± 1.0	8.3 ± 0.8
	DUR DCR	103.8 ± 14.3	211.9 ± 32.4
	DUR/FRQ DCR	10.9 ± 0.8	26.4 ± 4.4
	OCC DCR	9/12	12/12
Other	BODY WEIGHT	476.9 ± 6.0	327.5 ± 11.8
Anxiety-like behavior	DUR SHELTER/RISK INDEX	-0.20 ± 0.07	0.07 ± 0.10
Impulsive-like behavior	SLOPE/BRIDGE INTERVAL	-0.40 ± 0.20	-0.06 ± 0.02

Behavioral parameters recorded during the 20-min trial of the MCSF test. Values represent mean  $\pm$  SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 12 rats/group). No values are shown if OCC  $\leq$  3 animals.

*Abbreviations:* CTRCI, central circle; DCR, dark corner room; DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; SAP, stretched attend posture; TOTACT, total activity, i.e. the sum of all frequencies; TOTARENA, total arena; TOTCORR; total corridor, i.e. the sum of all corridors.

**Supporting Table 10.** Results from the open field (OF) and elevated plus maze (EPM) tests in adult male Wistar and N/Nih rats.

Parameters	Wistar	N/Nih
<b>A. OF test</b>		
Lat center	56.1 ± 45.1	82.4 ± 27.2
Frq center	18.3 ± 3.0	7.7 ± 1.4
Dur center	45.4 ± 6.7	30.2 ± 6.0
Dur/Frq center	2.8 ± 0.2	3.9 ± 0.1
%Dur center	7.6 ± 1.1	5.0 ± 1.0
Frq periphery	19.3 ± 3.1	8.9 ± 1.5
Dur periphery	554.6 ± 6.7	569.8 ± 6.0
Dur/Frq periphery	56.1 ± 19.3	86.1 ± 14.9
Crossings	352.8 ± 11.3	144.2 ± 15.1
TOTACT	37.6 ± 6.1	16.6 ± 3.0
<b>B. EPM test</b>		
Lat leave	4.9 ± 1.7	6.0 ± 1.4
Lat open	34.7 ± 22.1	50.7 ± 25.9
Occ open	10/10	7/12
Frq open	4.7 ± 0.7	3.1 ± 0.7
Dur open	42.0 ± 6.6	30.1 ± 7.4
Dur/Frq open	8.6 ± 0.7	10.0 ± 1.4
%Dur open	14.0 ± 2.2	5.9 ± 2.0
%Frq open	21.0 ± 2.7	13.5 ± 4.2
Lat closed	16.3 ± 5.5	17.4 ± 8.2
Frq closed	17.0 ± 0.5	11.6 ± 0.8
Dur closed	135.6 ± 9.9	174.1 ± 14.3
Dur/Frq closed	8.1 ± 0.6	15.9 ± 2.0
%Dur closed	45.2 ± 3.3	58.0 ± 4.8
Frq center	21.8 ± 0.7	13.7 ± 0.8
Dur center	122.4 ± 4.0	108.4 ± 10.8
Dur/Frq center	5.7 ± 0.2	7.8 ± 0.6
%Dur center	40.8 ± 1.3	36.1 ± 3.6
TOTACT	21.7 ± 0.8	13.4 ± 0.7

Behavioral parameters recorded during the 10-min trial of the OF test (A) and 5-min trial of the EPM test (B). Values represent mean ± SEM. Occurrence (OCC) is shown for the zones that were not visited by all animals in each group or the behaviors that were not performed by all animals in each group (n = 10-12 rats/group). No values are shown if OCC ≤ 3 animals. *Abbreviations:* DUR, duration (s); DUR/FRQ, duration per visit (s); FRQ, frequency; LAT, latency (s); OCC, occurrence; TOTACT, total activity, i.e. the sum of all frequencies.

## **EXPANDED DISCUSSION**

### **Consistency of results comparing the MCSF, OF and EPM tests**

The present results add to previous findings (Augustsson et al., 2005; Augustsson and Meyerson, 2004; Roman and Colombo, 2009; Roman et al., 2006; Roman et al., 2007) demonstrating that the MCSF provides more information compared to the OF and EPM tests. The MCSF contains two risk areas, i.e. the illuminated, elevated bridge construction and the central part (CENTRAL CIRCLE) of the open center field (Meyerson et al., 2006; Roman and Colombo, 2009). These two areas correspond to the open arms in the EPM test and the center of the OF, respectively. In the interpretations, the construct of anxiety-like behavior combines a number of measures, including activity, risk taking and shelter seeking. Similarly, interpretations as to impulsive-like behavior are made based on the relation between exploration, risk assessment and risk taking. There is a different approach with the MCSF test compared to other commonly used tests (EPM, OF, light-dark box, operant paradigms etc.). The diversity of choices in the MCSF allows the expression of a greater behavioral repertoire in the animals. The multivariate strategy, using statistical approaches including the PLS and trend analysis, emphasizes the overall behavioral profile of the animals rather than a single construct such as anxiety.

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