

# Supplementary Material – Wright, Gilmour, Dwyer

#### **1** Details of social and environmental stressors

**Table S1** Social and environmental stressors. Three of the five stressors were randomly applied each week in addition to social isolation and unenriched home cages. Stressor identity and the day that the stressor was given were randomly allocated to minimise habituation to the stress procedure over time.

Stressor	Duration	Description	
Wet Bedding < 4 Hours		Rats were transferred to a different cage where the sawdust had been dampened with approximately 300 ml of cold water.	
Overnight Illumination Light-dark cycle v manipulation was		Light-dark cycle was temporarily reversed. This manipulation was never done on consecutive days.	
Cage Swap		The cages of two rats were randomly swapped including water bottles. Rats remained in the cage of the unfamiliar rat until their cages were next cleaned (maximum of 7 days).	
Pair-Up	< 12 Hours	Rats were paired at random, within their strain, and left housed together overnight. All pair-ups included a defending male and an intruder male, as one rat was placed into the home cage of another instead of into clean 'neutral' home cage. Which rat was to be the intruder/defending male was randomly allocated. *	
Brief-Swim Test	< 2 Minutes	Rats were carefully lowered into a black container of water, measuring 33.5 cm by 23 cm (H × D). The temperature of the water was maintained at 20-22 ° C. To minimize escape, the water surface was kept at 16-17 cm from the lip of the container. Any rat which escaped was placed back in the water for a maximum of four times after which the trial was terminated. As two rats (1 Wistar and 1 WKY) were run simultaneously in separate containers, trials were terminated for both rats. Upon trial termination or once 2-min had elapsed, rats were removed from the water and carefully dried off before being replaced in their home cage. Water was replaced after four rats had been tested.	

\*Rats were carefully monitored for signs of fighting. One rat received bite marks after being paired up and so no longer underwent this manipulation.

### 2 Dates of stressor application and experimental procedures

Table S2 Timing of stress and experimental procedures.

Date	Stress /Husbandry	Experiment details
17/10		Arrival - rats weight-matched and separated into Stress and No-Stress conditions
18/10 -		
23/10	Adapt to home cage environment	
24/10	Cage Swap	
25/10	Pair Up	
26/10	Overnight Illumination	Food restriction started
27/10		
28/10		Habituate to test equipment (all)
29/10		Habituate to test equipment (all)
30/10	Overnight Illumination	Habituate to test equipment (all)
31/10	Brief Swim test 1	Pre-train_NAC
01/11		NAC_1 – session block 1
02/11		NAC_2 – session block 1
03/11	Pair up	NAC_3 – session block 1
04/11	Overnight Illumination	NAC_4 – session block 1
05/11		NAC_5 – session block 2
06/11		NAC_6 – session block 2
07/11	Wet Cage	NAC_7 – session block 2
08/11	Wet Cage	NAC_8 – session block 2
09/11		NAC_9 – session block 3
10/11		Rest
11/11		NAC_10 – session block 3
12/11	Pair up	NAC_11 – session block 3
13/11		NAC_12 – session block 3
14/11	Cage Swap	NAC_13 – session block 4
15/11		NAC_14 – session block 4
16/11	Brief Swim Test 2	Rest
17/11		NAC_15 – session block 4
18/11		NAC_16 – session block 4
19/11	Overnight Illumination	NAC_17 – session block 5
20/11	*	NAC_18 – session block 5
21/11	Brief Swim test 3	NAC 19 – session block 5

22/11	Overnight Illumination	NAC_20 – session block 5
23/11		NAC_21 – session block 6
24/11		Rest
25/11		NAC_22 – session block 6
26/11	Cage Swap	NAC_23 – session block 6
27/11	Wet Cage	NAC_24 – session block 6
28/11	Pair up	NAC_25 – session block 7
29/11		NAC_26 – session block 7
30/11		Rest
01/12		Rest
02/12	Overnight Illumination	NAC_27 – session block 7
03/12		NAC_28 – session block 7
04/12	Cage Swap	NAC_29 – session block 8
05/12		NAC_30 – session block 8
06/12	Brief Swim test 4	NAC_31 – session block 8
07/12		NAC_32 – session block 8
08/12		
09/12	Pair Up	
10/12		
11/12	Wet cage	Cons_T1
12/12	Wet cage	Cons_T2
13/12		Cons_T3
14/12		
15/12		
16/12	Pair Up	Cons_T4
17/12	Cage Swap	Cons_T5
18/12		Cons_T6
19/12		
20/12	Brief swim Test 5	
21/12		Cons_T7
22/12		Cons_T8
23/12		Cons_T9

## 3 Animal weight data and analysis during the Anticipatory Contrast Study

**Table S3** Weights and percentage of free-feeding weights of animals during the anticipatory contraststudy period. Data is shown as means across the period (with SEM).

	Wistar No-Stress	Wistar Stress	WKY No-Stress	WKY Stress
Mean weight (g)	260.5 (4.1)	260.9 (5.8)	230.3 (3.5)	223.3 (5.6)

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Percentage of				
free-feeding	100.5 (1.1)	101.6 (0.8)	101.3 (0.7)	102.0 (0.9)
weight				

Both the weight (g) and percentage of free-feeding weights were analysed with two-way between subjects ANOVA with factors of strain (WKY v Wistar) and stress (Stress v No-Stress). For the weight data, this revealed a main effect of strain (F(1, 44) = 44.44, p < 0.001,  $\eta^2_p = 0.524$ ), and no significant effect of stress (F(1, 44) = 0.46, p = .503,  $\eta^2_p = 0.010$ ) or interaction between the two factors (F(1, 44) = 0.58, p = .451,  $\eta^2_p = 0.013$ ). For the percentage of free-feeding weight data, this revealed no significant effects of strain (F(1, 44) = 0.50, p = .485,  $\eta^2_p = 0.011$ ) or stress (F(1, 44) = 1.034, p = .315,  $\eta^2_p = 0.023$ ), nor an interaction between the two factors (F(1, 44) = 0.09, p = .765,  $\eta^2_p = 0.002$ ). Thus, although the WKY and Wistar strains differed in overall bodyweight, they were matched in terms of their percentage of free-feeding weights, and there was no effect of stress (on interaction between strain and stress) on either measure.

#### 4 Animal weight data and analysis during the Consumption Study

**Table S4** Weights and percentage of free-feeding weights of animals during the consumption study period. Data is shown as means across the period (with SEM).

	Wistar No-Stress	Wistar Stress	WKY No-Stress	WKY Stress
Mean weight (g)	290.4 (5.5)	287.3 (5.4)	253.9 (3.8)	245.9 (4.9)
Percentage of free-feeding weight	112.0 (1.6)	112.0 (1.2)	111.8 (1.2)	112.5 (1.6)

Both the weight (g) and percentage of free-feeding weights were analysed with two-way between subjects ANOVA with factors of strain (WKY v Wistar) and stress (Stress v No-Stress). For the weight data, this revealed a main effect of strain (F(1, 44) = 61.01, p < 0.001,  $\eta_p^2 = 0.581$ ), and no significant effect of stress (F(1, 44) = 1.25, p = .270,  $\eta_p^2 = 0.028$ ) or interaction between the two factors (F(1, 44) = 0.24, p = .630,  $\eta_p^2 = 0.005$ ). For the percentage of free-feeding weight data, this revealed no significant effects of strain (F(1, 44) = 0.01, p = .924,  $\eta_p^2 < 0.001$ ) or stress (F(1, 44) = 0.07, p = .788,  $\eta_p^2 = 0.002$ ), nor an interaction between the two factors (F(1, 44) = 0.06, p = .806,  $\eta_p^2 = 0.001$ ). Thus, although the WKY and Wistar strains differed in overall bodyweight, they were matched in terms of their percentage of free-feeding weights, and there was no effect of stress (on interaction between strain and stress) on either measure.