

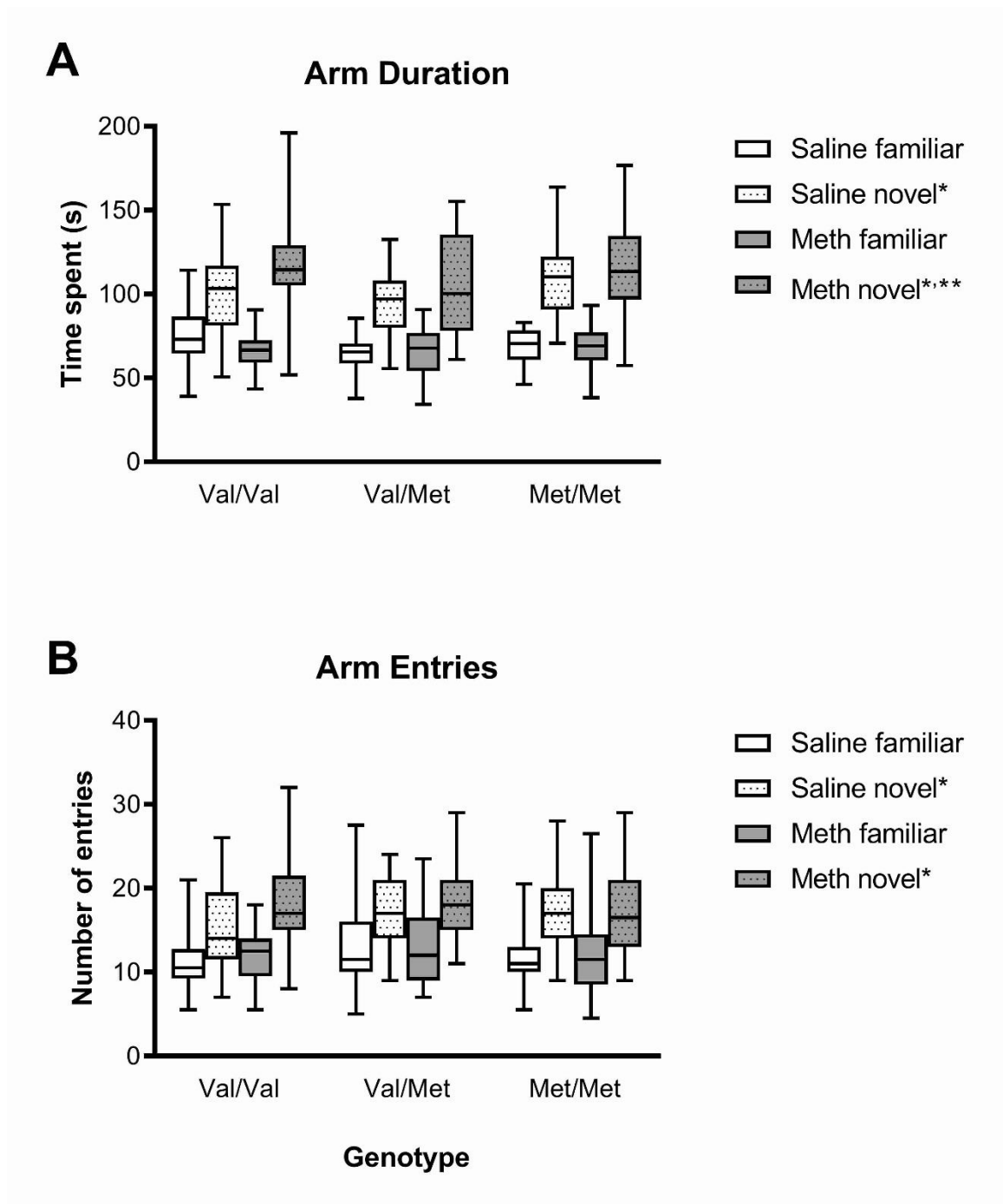
Methamphetamine-induced locomotor sensitization in mice is not associated with deficits in a range of cognitive, affective and social behaviours: interaction with brain-derived neurotrophic factor (BDNF) Val66Met genotype.

Michelle Corrone¹, Ruvee Ratnayake¹, Nicole de Oliveira¹, Emily Jaehne¹ and Maarten van den Buuse¹

(1) School of Psychology and Public Health, La Trobe University, Melbourne, Victoria 3086, Australia

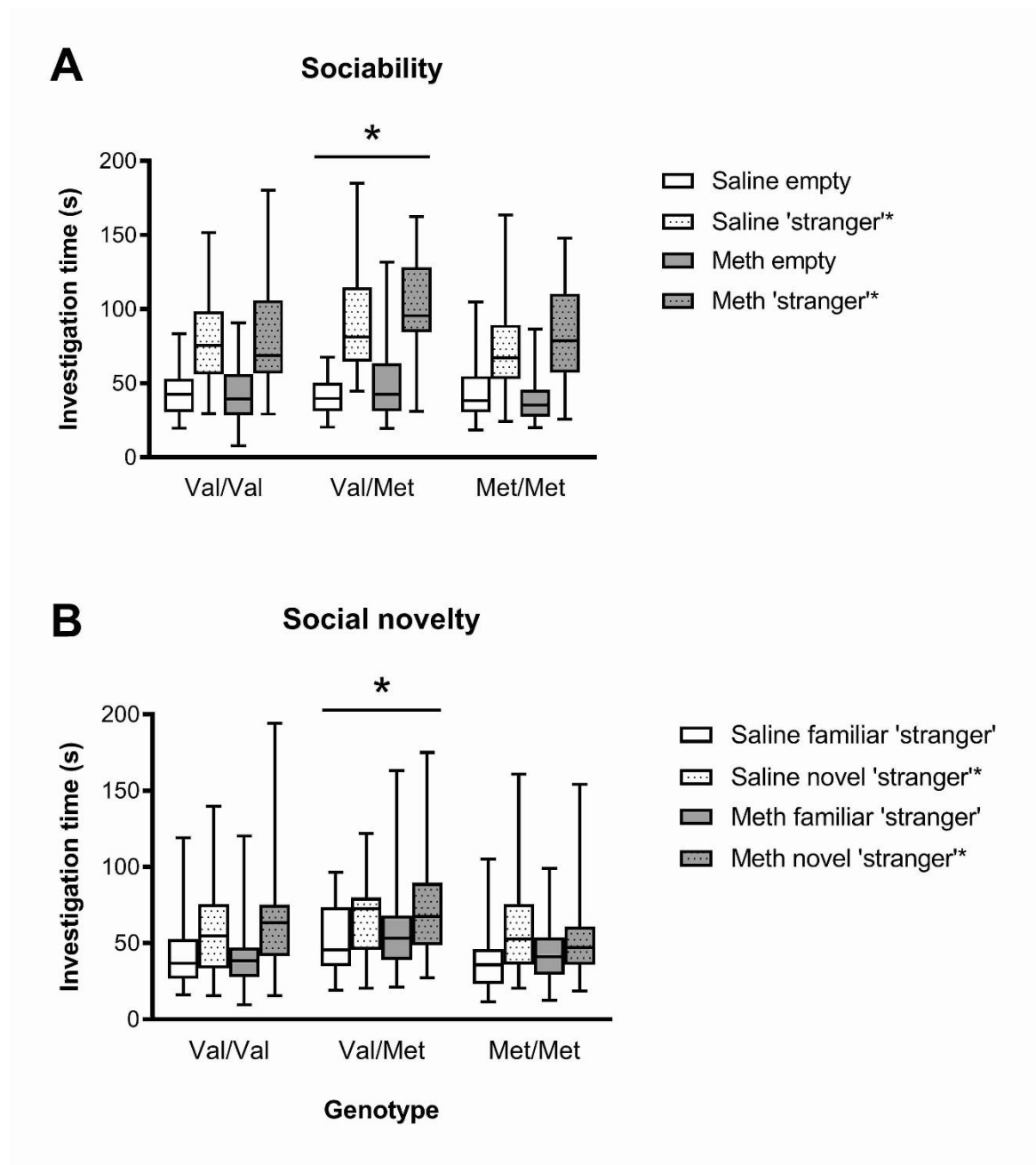
Supplementary results

Supplementary figure 1: Y-maze time in arms and number of arm entries.



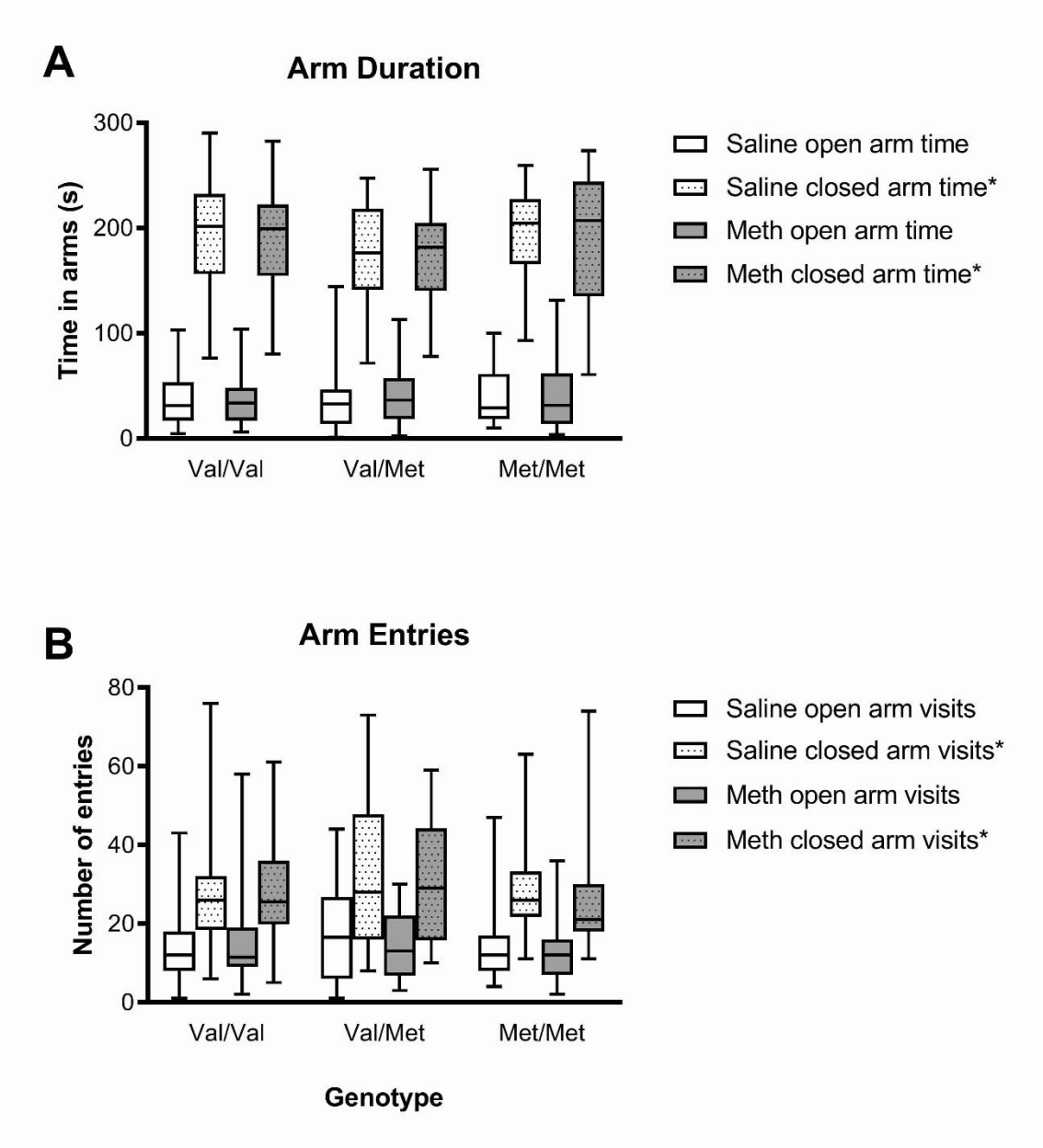
Supplementary Figure 1. Mice displayed a preference to explore the Y-maze novel arm, as shown by significantly greater duration of time spent in (A) and higher number of visits to (B) the novel arm compared to the familiar arms (* $P < 0.001$). There was a significantly higher preference for the novel arm in Meth-pretreated mice independent of genotype (** $P < 0.05$ for Arm x pretreatment interaction). This was not observed for the number of arm entries (B). For number of mice per treatment group, see Supplementary Table 1.

Supplementary figure 2: social behaviour investigation time.



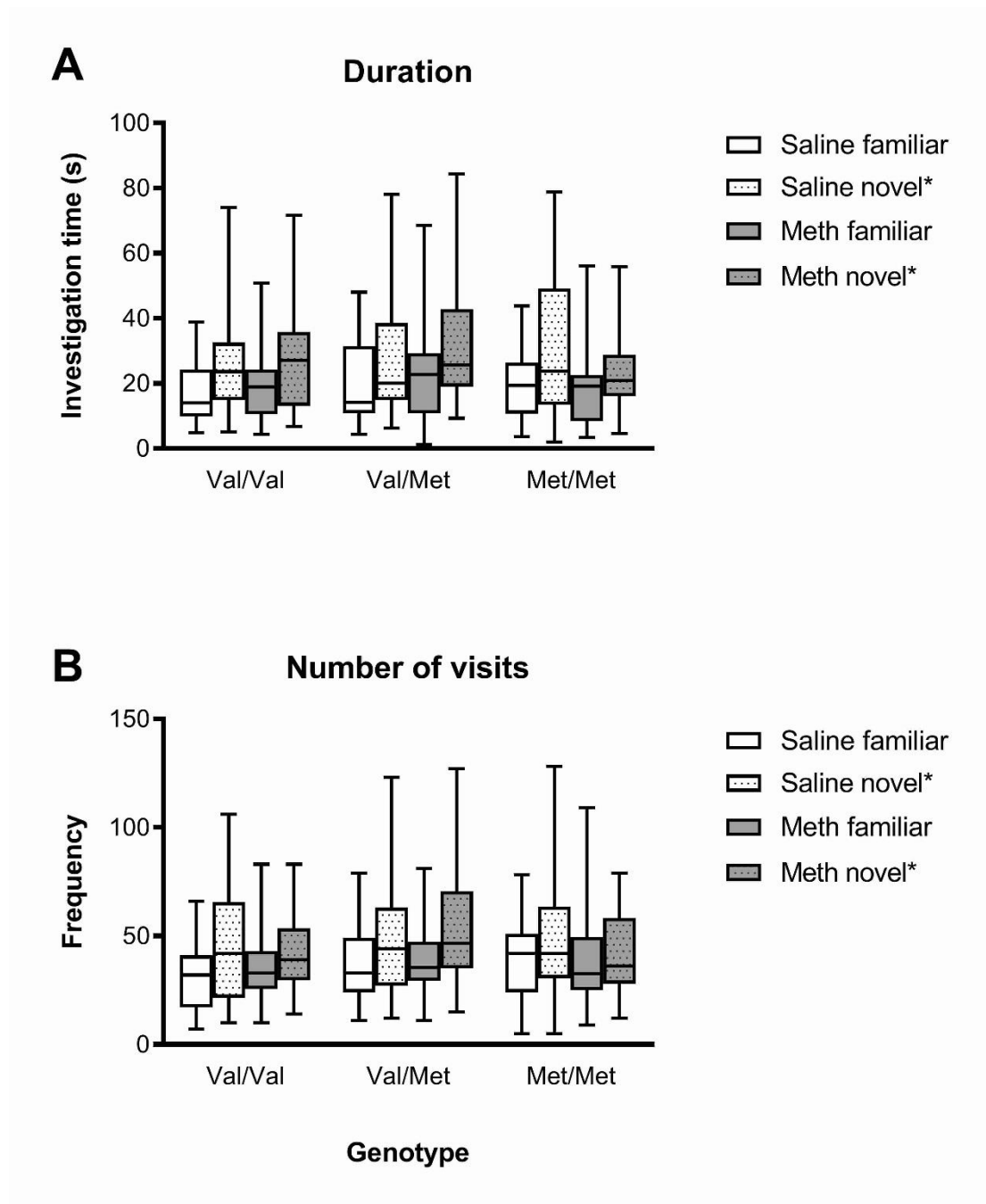
Supplementary Figure 2. Mice displayed a preference to investigate a 'stranger' mouse, as shown by significantly higher investigation time (* $P < 0.001$) in the social behaviour test. This preference was similar in Meth-pretreated and saline-pretreated Val/Val, Val/Met and Met/Met mice. Panels show investigation time of the stranger mouse vs. empty enclosure in the sociability phase (A) and investigation time of the stranger mouse vs. the familiar mouse in the social preference phase of the test (B). In both phases of the test, Val/Met mice showed significantly higher overall investigation time (* $P < 0.001$ for main effect of genotype) than Val/Val mice and Met/Met mice. For number of mice per treatment group, see Supplementary Table 1.

Supplementary figure 3: elevated plus maze time in arms and number of arm entries.



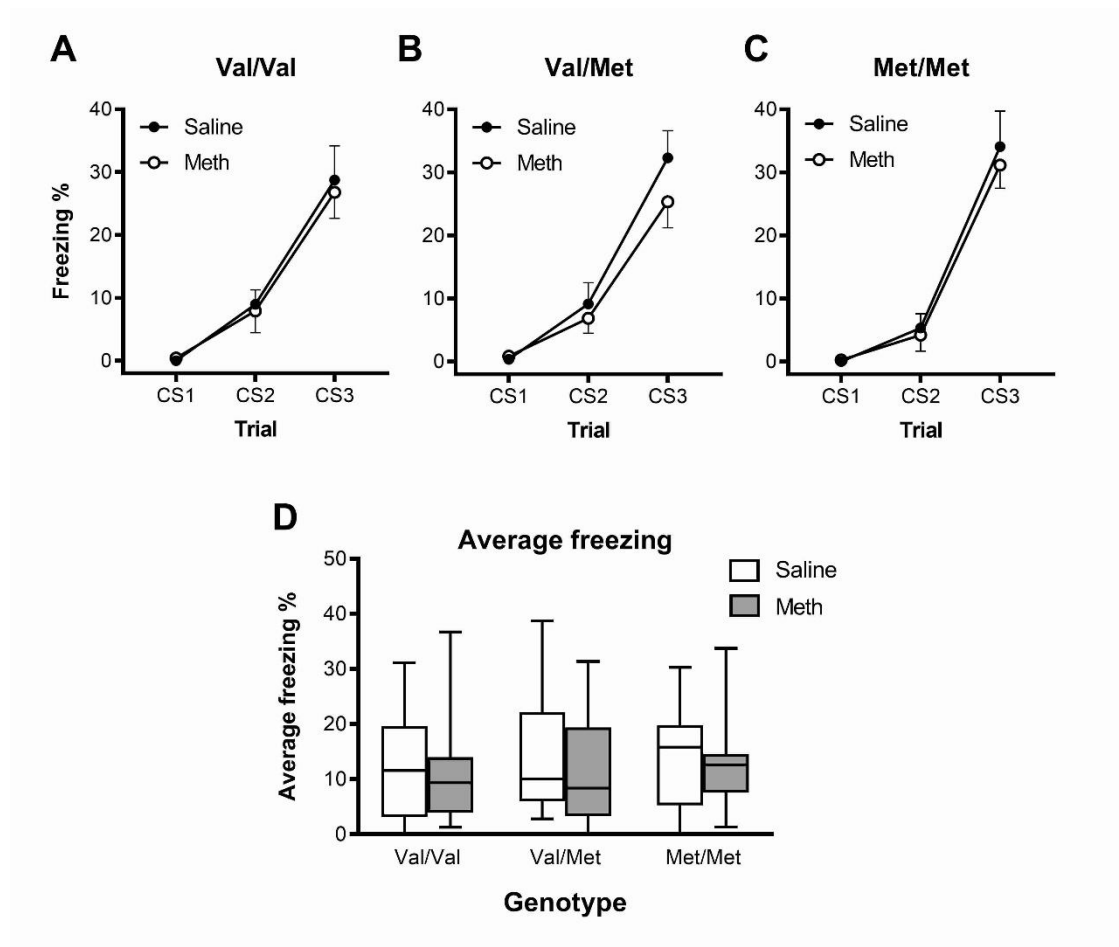
Supplementary Figure 3. Mice displayed a significant preference (* $P < 0.001$) to spend time in (A) and enter (B) the closed arms vs. the open arms on the elevated plus-maze (EPM). This preference was similar in Meth-pretreated and saline-pretreated Val/Val, Val/Met and Met/Met mice. Panels show time spent in the arms (A) and number of entries (B). For number of mice per treatment group, see Supplementary Table 1.

Supplementary figure 4: novel-object recognition investigation time and frequency.



Supplementary Figure 4. Mice displayed a preference to investigate a novel object vs. a familiar object (* $P < 0.001$). This preference was similar in Meth-pretreated and saline-pretreated Val/Val, Val/Met and Met/Met mice. Panels show investigation time (A) and number of investigative bouts (frequency) of the novel object vs. familiar object. For number of mice per treatment group, see Supplementary Table 1.

Supplementary figure 5: fear acquisition.



Supplementary Figure 5. In the learning phase of the fear conditioning protocol, Val/Val mice (A), Val/Met mice (B) and Met/Met mice (C) all displayed significantly increased freezing over the three trials, independent of Meth pretreatment. Panel D shows average freezing over the three learning trials. For number of mice per treatment group, see Supplementary Table 1.

Supplementary Table 1: Number of mice per treatment group for each behavioural test. Different cohorts of mice had overlapping behavioural tests done (Figure 1) which were combined as indicated.

| | Pre-injection locomotor activity (Figure 2) | Meth-induced locomotor hyperactivity (Figure 3) | Y-maze (Figure 4) | Social behaviour (Figure 5) | Plus maze (Figure 6) | NORT* (Figure 7) | Fear conditioning (Figure 8) | PPI (Figure 9) |
|---------------------|--|--|----------------------|--------------------------------|-------------------------|---------------------|---------------------------------|-------------------|
| Cohorts | 2 + 3 | 2 + 3 | 1 + 2 | 1 + 3 | 1 + 3 | 2 + 3 | 2 + 3 | 1 + 3 |
| Male Val/Val Sal | 20 | 17 | 17 | 21 | 22 | 14 | 16 | 22 |
| Male Val/Val Meth | 21 | 19 | 17 | 20 | 21 | 17 | 19 | 21 |
| Male Val/Met Sal | 25 | 22 | 9 | 12 | 13 | 16 | 18 | 11 |
| Male Val/Met Meth | 21 | 18 | 7 | 12 | 12 | 15 | 19 | 12 |
| Male Met/Met Sal | 19 | 16 | 19 | 20 | 20 | 12 | 15 | 20 |
| Male Met/Met Meth | 16 | 16 | 19 | 19 | 19 | 9 | 14 | 20 |
| Female Val/Val Sal | 18 | 18 | 16 | 21 | 19 | 11 | 12 | 21 |
| Female Val/Val Meth | 18 | 16 | 16 | 20 | 21 | 12 | 13 | 20 |
| Female Val/Met Sal | 22 | 16 | 8 | 11 | 11 | 15 | 18 | 11 |
| Female Val/Met Meth | 20 | 20 | 8 | 10 | 10 | 17 | 18 | 10 |
| Female Met/Met Sal | 20 | 14 | 16 | 17 | 18 | 13 | 16 | 18 |
| Female Met/Met Meth | 21 | 19 | 19 | 17 | 20 | 15 | 18 | 19 |

* = outliers with <10 sec investigation time removed.

Supplementary Table 2A: PPI at the 100 msec inter-stimulus interval (ISI).

| Group (n) | % PPI 100 msec ISI | | | | |
|--------------------------|--------------------|------------|------------|------------|-------------|
| | PP2 | PP4 | PP8 | PP16 | Avg |
| Male Val/Val Sal (22) | 7.9 ± 2.8 | 14.8 ± 4.6 | 35.9 ± 2.7 | 55.7 ± 2.5 | 28.6 ± 2.2 |
| Male Val/Val Meth (21) | 11.5 ± 3.5 | 21.4 ± 3.4 | 35.8 ± 3.5 | 59.5 ± 2.6 | 32.0 ± 2.4 |
| Male Val/Met Sal (11) | 1.3 ± 7.8 | 16.5 ± 4.9 | 30.6 ± 4.5 | 53.0 ± 3.2 | 25.3 ± 2.8* |
| Male Val/Met Meth (12) | 1.2 ± 6.5 | 15.1 ± 4.5 | 35.2 ± 5.7 | 53.1 ± 5.0 | 26.1 ± 3.7* |
| Male Met/Met Sal (20) | 2.2 ± 4.6 | 10.6 ± 3.0 | 28.4 ± 3.2 | 49.3 ± 4.0 | 22.6 ± 2.8 |
| Male Met/Met Meth (20) | 5.1 ± 4.2 | 18.5 ± 1.9 | 35.8 ± 2.5 | 55.3 ± 2.2 | 28.7 ± 1.8 |
| Female Val/Val Sal (21) | 7.5 ± 5.0 | 15.0 ± 3.8 | 33.8 ± 3.7 | 60.6 ± 3.3 | 29.2 ± 3.1 |
| Female Val/Val Meth (20) | 0.6 ± 2.9 | 16.6 ± 2.6 | 32.6 ± 3.9 | 55.1 ± 3.6 | 26.2 ± 2.4 |
| Female Val/Met Sal (11) | -5.8 ± 6.5 | 8.5 ± 5.6 | 29.2 ± 5.6 | 47.8 ± 4.3 | 19.9 ± 3.5* |
| Female Val/Met Meth (10) | 13.9 ± 6.4 | 8.7 ± 4.9 | 23.9 ± 3.5 | 51.7 ± 4.1 | 24.5 ± 3.2* |
| Female Met/Met Sal (18) | 10.9 ± 4.3 | 14.1 ± 2.0 | 35.5 ± 4.3 | 54.6 ± 3.6 | 28.8 ± 2.3 |
| Female Met/Met Meth (19) | 10.0 ± 3.8 | 12.4 ± 3.0 | 31.4 ± 3.1 | 51.0 ± 3.1 | 26.2 ± 2.3 |

PP= prepulse intensity at 2 (PP2), 4 (PP4), 8 (PP8) and 16 (PP16) dB over the 65 dB background.

Avg = average PPI of all PPs combined.

Sal = saline pretreatment; Meth = Meth pretreatment.

Data are mean ± standard error of the mean (SEM).

* P<0.05 for difference between Val/Met mice vs. Val/Val mice independent of Meth pretreatment.

Supplementary Table 2B: PPI at the 30 msec inter-stimulus interval (ISI).

| Group (n) | % PPI 30 msec ISI | | | | |
|--------------------------|-------------------|------------|------------|------------|-------------|
| | PP2 | PP4 | PP8 | PP16 | Avg |
| Male Val/Val Sal (22) | 2.2 ± 3.8 | 9.3 ± 5.7 | 33.3 ± 4.1 | 57.2 ± 3.4 | 25.5 ± 2.9 |
| Male Val/Val Meth (21) | -0.4 ± 6.1 | 23.2 ± 3.1 | 41.2 ± 4.0 | 60.8 ± 4.2 | 31.2 ± 2.7 |
| Male Val/Met Sal (11) | -5.3 ± 3.6 | 8.6 ± 7.2 | 22.2 ± 4.7 | 52.8 ± 2.8 | 19.6 ± 3.1* |
| Male Val/Met Meth (12) | -5.8 ± 4.4 | 13.8 ± 5.3 | 28.7 ± 5.6 | 59.3 ± 4.7 | 24.0 ± 3.1* |
| Male Met/Met Sal (20) | -23.3 ± 6.8 | 4.9 ± 4.8 | 23.0 ± 4.3 | 51.9 ± 3.9 | 14.1 ± 3.2 |
| Male Met/Met Meth (20) | 2.9 ± 4.2 | 20.1 ± 4.4 | 34.5 ± 3.2 | 61.1 ± 2.2 | 29.6 ± 2.7 |
| Female Val/Val Sal (21) | -0.5 ± 6.0 | 27.5 ± 3.9 | 38.6 ± 4.6 | 62.5 ± 3.1 | 32.0 ± 3.5 |
| Female Val/Val Meth (20) | 0.1 ± 3.4 | 26.0 ± 4.1 | 34.6 ± 4.5 | 56.5 ± 3.7 | 29.3 ± 2.8 |
| Female Val/Met Sal (11) | -2.1 ± 4.8 | 9.0 ± 3.4 | 20.9 ± 4.5 | 48.7 ± 4.8 | 19.1 ± 2.5* |
| Female Val/Met Meth (10) | 1.9 ± 5.3 | 9.7 ± 6.3 | 33.7 ± 3.9 | 54.3 ± 3.0 | 24.9 ± 3.2* |
| Female Met/Met Sal (18) | -7.1 ± 4.1 | 24.7 ± 3.0 | 31.5 ± 2.5 | 59.5 ± 2.7 | 27.2 ± 1.7 |
| Female Met/Met Meth (19) | 7.1 ± 5.9 | 15.0 ± 6.8 | 35.8 ± 3.6 | 56.2 ± 3.0 | 28.5 ± 3.8 |

PP= prepulse intensity at 2 (PP2), 4 (PP4), 8 (PP8) and 16 (PP16) dB over the 65 dB background.

Avg = average PPI of all PPs combined.

Sal = saline pretreatment; Meth = Meth pretreatment.

Data are mean ± SEM.

* P<0.05 for difference between Val/Met mice vs. Val/Val mice independent of Meth pretreatment.

Supplementary Table 2C: Startle amplitude in four consecutive blocks of 10 stimuli.

| Group (n) | Startle amplitude (arbitrary units) | | | | |
|--------------------------|-------------------------------------|----------|----------|----------|----------|
| | Block 1 | Block 2 | Block 3 | Block 4 | Avg |
| Male Val/Val Sal (22) | 477 ± 29 | 437 ± 26 | 388 ± 35 | 358 ± 35 | 421 ± 25 |
| Male Val/Val Meth (21) | 467 ± 44 | 417 ± 36 | 374 ± 40 | 379 ± 36 | 410 ± 37 |
| Male Val/Met Sal (11) | 413 ± 39 | 341 ± 29 | 350 ± 38 | 306 ± 38 | 352 ± 22 |
| Male Val/Met Meth (12) | 394 ± 36 | 388 ± 32 | 362 ± 38 | 338 ± 37 | 371 ± 31 |
| Male Met/Met Sal (20) | 437 ± 40 | 388 ± 29 | 369 ± 29 | 277 ± 27 | 393 ± 28 |
| Male Met/Met Meth (20) | 429 ± 28 | 415 ± 26 | 387 ± 27 | 333 ± 34 | 400 ± 25 |
| Female Val/Val Sal (21) | 327 ± 31 | 284 ± 23 | 255 ± 23 | 245 ± 20 | 279 ± 22 |
| Female Val/Val Meth (20) | 320 ± 29 | 281 ± 20 | 251 ± 17 | 231 ± 21 | 269 ± 20 |
| Female Val/Met Sal (11) | 361 ± 32 | 284 ± 28 | 265 ± 24 | 279 ± 36 | 297 ± 25 |
| Female Val/Met Meth (10) | 364 ± 23 | 360 ± 31 | 297 ± 20 | 275 ± 36 | 324 ± 23 |
| Female Met/Met Sal (18) | 320 ± 25 | 278 ± 23 | 245 ± 25 | 223 ± 24 | 267 ± 22 |
| Female Met/Met Meth (19) | 356 ± 26 | 339 ± 25 | 279 ± 24 | 246 ± 27 | 308 ± 22 |

Avg = average startle amplitude of all blocks combined.

Sal = saline pretreatment; Meth = Meth pretreatment.

Data are mean ± SEM.